The impact of water temperature on the transmission of aquatic parasites

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Daniel Rainer Dangel

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Angaben zur Prüfung

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1. Gutachter: Prof. Dr. Bernd Sures

2. Gutachter: Prof. Dr. Daniel Hering

3. Gutachter:

Vorsitzender des Prüfungsausschusses: Prof. Dr. Jens Boenigk

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The whole is more than the sum of its parts

(Aristoteles)

to

Max, Emma & Kerstin

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A	abundance
A. auricollis	*Allogamus auricollis* (PICTET, 1834); Trichoptera
AI	aggregation index
BM	fish body mass
BMI	benthic macroinvertebrate
C. ephemeridarum	*Cystidicoloides ephemeridarum* (Linstow, 1872); Nematoda
C. farionis	*Cystidicola farionis* Fischer, 1798, Nematoda
C. rivulorum	*Caenis rivulorum* EATON, 1884; Ephemeroptera
C. truncatus	*Cyatocephalus truncatus* (Pallas, 1781); Cestoda
C. truttae	*Cucullanus truttae* Fabricius, 1794; Nematoda
CF	condition factor; the ratio of the fish body mass ∗ 100/fish total length³
CI	confidence interval
CTI	community temperature index
d	Berger-Parker index
DD>6	degree-days > 6 °Celsius
DD>17	degree-days > 17 °Celsius
E. truttae	*Echinorhynchus truttae* (Schrank, 1788); Acanthocephala
EH	Shannon evenness
EPT	Ephemeroptera, Plecoptera and Trichoptera
GSI	gonadosomatic index; ratio of fish gonad mass/fish body mass ∗ 100
GM	fish gonad mass
H	Shannon-index
HB	Brillouin index
HSI	hepatosomatic index; ratio of fish liver mass/fish body mass ∗ 100
hsp	heat shock protein
hsp70	heat shock protein with a molecular weight of 70 kDa
H_2SO_4	sulfuric acid
H. siltalai	*Hydropsyche siltalai* DÖHLER, 1963; Trichoptera
KOH	potassium hydroxide
L1c	river Lenne; cold sampling site at Lenhausen
L1w	river Lenne; warm sampling site at Germaniahütte
L2c	river Lenne; cold sampling site at Dresel

L2w	river Lenne; warm sampling site at Elverlingsen
LM	fish liver mass
MI	mean intensity
N. rutili	*Neoechinorhynchus rutili* (Müller, 1780); Acanthocephala
NaCl	sodium chloride solution
Na_2SO_4	sodium sulphate
P	prevalence (%)
P. flavomaculatus	*Polycentropus flavomaculatus* (PICTET, 1834); Trichoptera
P. laevis	*Pomphorhynchus laevis* (Zoega in Müller, 1776); Acanthocephala
P. salvelini	*Pseudocapillaria salvelini* (Polyansky, 1952); Nematoda
pi	relative abundance (%)
R. acus	*Raphidascaris acus* (Bloch, 1779); Nematoda
Rc	river Ruhr; cold sampling site at Meschede
rpm	revolutions per minute of rotor
Rw	river Ruhr; warm sampling site at Heinrichsthal
S. salar	*Salmo salar* Linnaeus, 1758
S. trutta fario	*Salmo trutta fario* Linnaeus, 1758; brown trout
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SI	saprobic index
sp.	species
spp.	species pluralis
STI	species temperature index
T. clavata	*Tylodelphis clavata* (Nordmann, 1832); Trematoda
TL	fish total length
WQC	water quality class
WT	water temperature
09a	autumn 2009
10a	autumn 2010
10s	spring 2010
1-D	Simpson's index

General Introduction

1.1 Background

Biodiversity of natural ecosystems is strongly threatened by significant anthropogenic climate change worldwide (Walther et al., 2002; Stuart, 2004; Parmesan, 2006; Schneider et al., 2007; Vörösmarty et al., 2010). An efficient planning of maintenance and support measures for the protection of biodiversity requires a sound knowledge about genetic diversity, ecological functions and interactions in and between natural habitats.

Freshwater ecosystems are directly threatened by human activities (Meybeck, 2003) and current scenarios predict a substantial impact on biodiversity of freshwater ecosystems (Sala et al., 2000). Although 10 % of all worldwide known and described species occur in freshwater ecosystems, the impact of environmental factors on biodiversity of freshwater ecosystems and its processes has been widely investigated. Especially freshwater ecosystem research has started to take into account the multitude of its interactions with parasites in and subsisting on freshwater ecosystems.

One of the most notable anthropogenic impacts of increasing concern is temperature rising, i.e. global warming. The effects of climate change on biota and ecosystem functioning are confidently projected. Apart from studies on free-living organisms, climate effects on infectious diseases attain growing interest (Harvell et al., 2002; Cattadori et al., 2005; IPCC, 2007; IPCC, 2014).

As air temperature rises in association with anthropogenic climate change, additional indirect effects may occur. For instance, across the German federal state North Rhine-Westphalia, warming air temperatures have been linked to increasing precipitation during winter months accompanied by decreasing snow accumulations and earlier snowmelt (Kropp et al., 2009).

Air temperature affect water temperature via several mechanisms that include solar radiation and direct sensible heat transfer. Water temperature is one of the key factors in aquatic ecosystems which decisively affect the composition, dispersion and distribution of biotic communities. Thermal stress and coupled changes in water temperature poses new challenges to watercourses which will enforce in the course of climate change. This will lead to a progressive loss of cold water habitats in the upper reaches of rivers in the temperate zones. In particular cold-stenotherm species will be affected by the loss of suitable retreat areas (Mohseni et al., 2003; Melcher et al., 2013).

For an interpretation of independent environmental influences and interactions, the application of multiple taxa instead of a single taxon of freshwater organisms is necessary (Bowman et al., 2008; Warfe

et al., 2013). Benthic macroinvertebrates (BMI) provide important information on the ecological status of watercourses (Vannote et al, 1980; Jacobsen et al, 1997; Chinnayakanahalli et al., 2011) and BMI assemblages are often used as indicators for ecosystem integrity (Brown et al., 2012; Kilgour & Barton, 1999). Furthermore, insects with aquatic larval stages like mayflies, stoneflies and caddis flies are important parts of biocenosis due to their high abundance and diversity in freshwater habitats.

The impact of water temperature increase on freshwater fish communities is well documented, such as a decrease of cold-adapted species (mainly salmonids), while thermophilous fish species (mainly cyprinids) benefit (Daufresne et al, 2004). Similarly, predicted changes in water temperatures are expected to have implications for the migrations, ontogeny, growth and life-history traits of cold-water fish species including brown trout (*Salmo trutta fario* L.) (Jonsson & Jonsson, 2009). Additionally, seasonality is particularly caused by variations in water temperature, which immediately affects the metabolism of fish (Martinez et al., 1994).

Increasing water temperatures can additionally promote the transmission of parasites and raise their local abundance (Marcogliese, 2001; Poulin, 2006). Their use as indicators of biodiversity seems logical, as they respond to environmental stressors and reflect food webs via their transmission route (Marcogliese, 2004). Due to sensitivity of some pathogens and vectors to climate conditions, the severity of threats associated with climate change could increase due to links between temperature increase and disease. The effect of climate change on parasites can be numerical, functional or microevolutionary and may involve cascading changes.

Harvell et al. (2002) formulated a special need to study effects of climate change on parasites with multi-host life cycles, ideally under complex environmental conditions following experimental manipulation. However, Brooks & Hoberg (2007) stressed the still existing paucity of empirical data on numerical- and density-dependent impacts of global warming on host-parasite associations.

Surprisingly, the role of benthic macroinvertebrate species and fish as hosts for parasites has not been investigated in detail so far. In an ecological context, parasites are of importance as they might influence host abundance due to decreased vitality or increased predation rate of infected individuals. This will have an effect on species composition in an ecosystem with parasites compared to one without. Additionally, parasites often have complex life cycles and thus indicate the presence of more than one host species in the habitat. For this reason, considering parasites, will improve the validity of biological water quality assessments.

1.2 Aims of the present study

Environmental assessments are often based on the response of a single taxonomic group to stressors like changes in temperature. Thus, protective protective measures are often based only on single elements of the whole aquatic ecosystem. Furthermore, the implementation of such measures is often conducted at a relatively small-scale. Understanding potential differences in the response of benthic macroinvertebrates, fish and parasites to stressors, as well as the scale at which stressors originate is important for an integrative ecosystem approach improving aquatic ecosystem assessment and

management. Monitoring ecosystem change allow us to track response of species and communities to temperature alterations and identify those organisms that are particularly vulnerable to change.

Based on this, the present study will evaluate and compare the response of benthic macroinvertebrate, fish and parasite communities to water temperature alterations across two mid-sized mountain rivers in the south-eastern part of the German federal state North Rhine-Westphalia (NRW).

Since water temperature increases due to global warming, it is important to obtain quantitative information on the thermal implications on brown trout and its parasites so that potential problems can be anticipated by those responsible for the fisheries management, and the biodiversity maintenance in freshwater ecosystems.

Even knowing preferred temperature ranges of free-living species, predictions from experimentally based hypotheses on the impact of an increase in water temperature are difficult to confirm in field studies. Therefore, artificial water temperature modifications, such as cooling water discharge or outflow from reservoirs, are particularly suitable for tackling this problem. Here, in contrast to many other studies, brown trout and its parasite communities were studied in the field following an integrative ecosystem approach by using artificial modified temperature regimes.

The results could be a first step to explain temperature caused alterations in brown trout´s parasite communities and population structures in freshwater ecosystems in order to assess the usefulness of parasite community changes as an early warning system. Additionally, the findings increase the knowledge on the thermal implications on the host-parasite-interactions of brown trout and its parasites in general.

General Materials and Methods

2

2.1 Introduction

Since the sampling sites as well as temperature data are basic data for all subsequent analyses of the present study, they are described in this general chapter. In addition, specific materials and methods are described in detail in the respective chapters.

2.2 Study Area and Sampling Sites

The study area was located in the south-eastern part of the German federal state North Rhine-Westphalia. This region, known as Sauerland is a part of the Variscan mountain belt in central Europe called Rhenish Massif. In this region the two mid-sized mountain rivers Ruhr and Lenne have been investigated at comparable river sections. The water temperature in the middle reaches of both rivers is influenced by the cold hypolimnetic water from reservoirs. The river Ruhr (catchment area 4.485 km²) is affected by the inflow of the Henne-reservoir (Ruhr). The river Lenne (catchment area 1.353 km²) is affected by the inflow of the Bigge-reservoir (Lenne1). At the river Lenne, there is an additional impact by cooling water released from a power station at Werdohl-Elverlingsen in the further river course (Lenne2).
Sampling sites with similar stream width, flow rate and substrate were selected at each river on the basis of this specific thermal regime. Each sampling stretch covered about 200 m. The cold sampling sites were located at Lenhausen (L1c), near Dresel (L2c) on the river Lenne and below Meschede (Rc) on the river Ruhr. The warm sampling sites were situated at Germaniahütte (L1w), below Elverlingsen (L2w) on the river Lenne and nearby Heinrichsthal (Rw) on the river Ruhr. An overview of the study area indicating the sampling sites is given in Table 2-1 and Figure 2-1.

Table 2-1: Locality and sampling site designation.

(c: cold; w: warm)

Locality **Sampling site**	**Code**	**Longitude [decimal,WGS84]**	**Latitude [decimal,WGS84]**
Ruhr (R)			
Heinrichsthal	Rw	8.31393	51.35157
Meschede	Rc	8.26575	51.34983
Lenne 1 (L1)			
Germaniahütte	L1w	8.03405	51.13051
Lenhausen	L1c	7.96679	51.20505
Lenne 2 (L2)			
Dresel	L2c	7.71581	51.27953
Elverlingsen	L2w	7.70079	51.26845

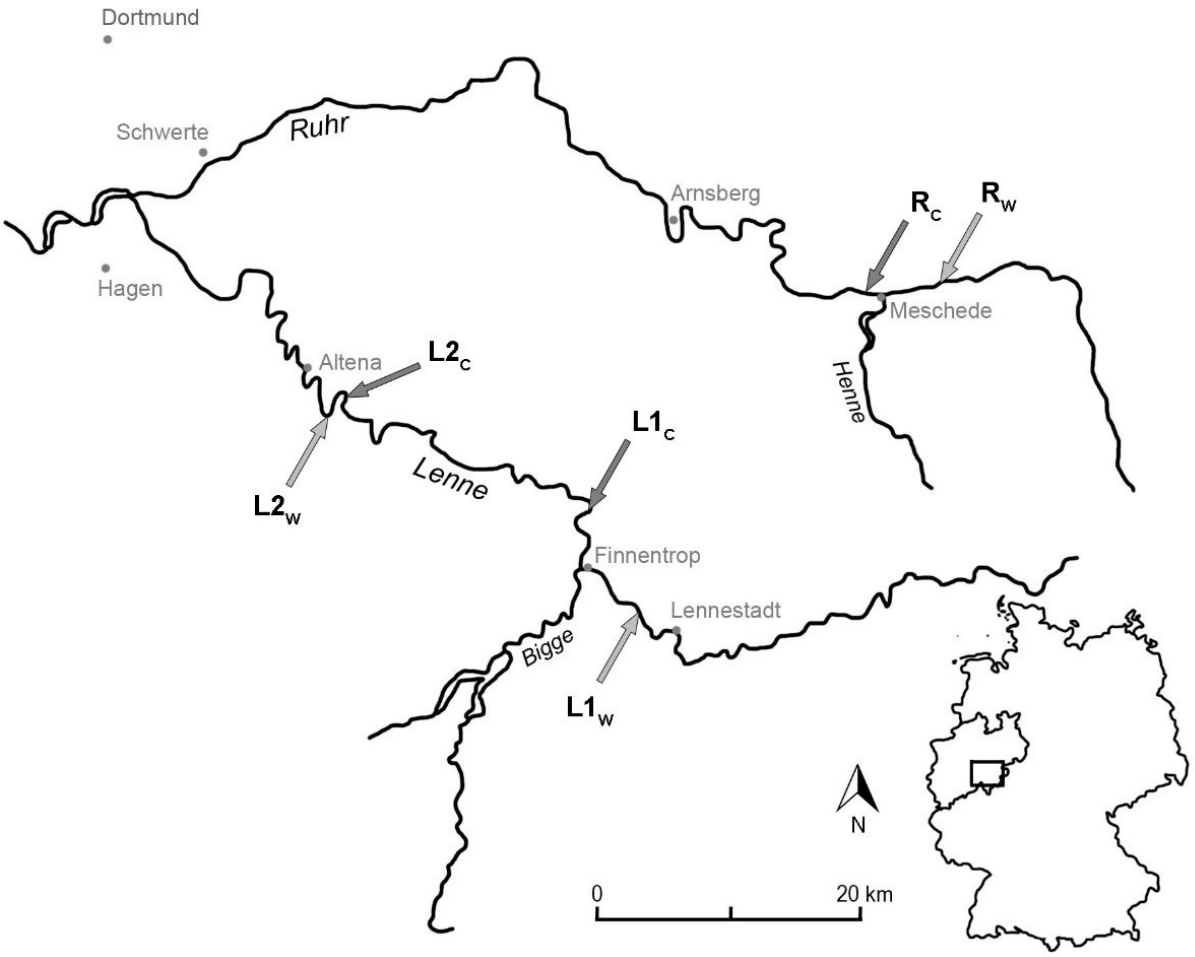

Figure 2-1: Location of the sampling sites. Site designation according to Table **2-1**.

2.3 Recording and Processing of Water Temperature Data

Water temperature (WT) was recorded every 30 min in the period from May 2009 to September 2010 using data loggers (Testo AG, 175-T1) with a resolution of 0.1 °C. In order to avoid exposure to direct solar radiation as well as sediment coverage the loggers were fixed at shady places in the river bed using stainless steel ground sleeves. This kind of fixation also guaranteed that the loggers remain under flowing water at all water levels.

In February and March 2010, no continuous recordings were made at Ruhr cold site (Rc) due to technical defects. Therefore, missing temperature data were computed by adding together the mean temperature difference of the measured months from site Rc itself and the water temperature data on hourly basis from records at adjacent gauge "Meschede" of the district government of Arnsberg.

Additionally, the annual mean water temperature and the cumulative number of degree-days >4 °C (DD>4) and >17 °C (DD>17) were calculated, to point out differences in water temperature between sampling sites.

The influence of water temperature on benthic macroinvertebrate communities

3

3.1 Introduction

Changes in water temperature have considerable impacts on aquatic ecosystems. Water temperature exerts direct influence on river ecology, because most aquatic organisms are ectothermic. Among other things, changes in water temperature have effects on the growth, survival and spatial distribution of aquatic organisms of all trophic levels (e.g. Ward & Stanford, 1982; Daufresne et al., 2004; Caissie, 2006; Ficke et al., 2007).

For an interpretation of independent environmental influences and interactions, the application of multiple taxa instead of a single taxon of freshwater organisms seems necessary (Bowman et al., 2008; Warfe et al., 2013). Benthic macroinvertebrate (BMI) assemblages are often used as indicators for ecosystem integrity (Kilgour & Barton, 1999; Brown et al., 2012).

Species abundances and distributions are changing due to local temperature alterations. Moreover different species or whole aquatic communities will not necessarily be affected equally. In addition, consequences of temperature increase are likely to vary from place to place for individual species and communities.

Benthic macroinvertebrates (BMI) provide important information on the ecological status of watercourses (Jacobsen et al., 1997; Vannote et al., 1980; Chinnayakanahalli et al., 2011) and play a fundamental role in applied procedures in the organic water quality assessment (see Hering et al., 2004). Furthermore, insects with aquatic larval stages like mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddis flies (Trichoptera) are important parts of biocenoses due to their high abundance and diversity in freshwater habitats. Also, many species are important indicators for water quality assessment (Rosenberg & Resh, 1993). Several species have been identified to be restricted in their distribution by the temperature range (e.g. Haidekker & Hering, 2008; Durance & Ormerod, 2009; Hering et al., 2009) and species richness is found to be temperature dependent as well (e.g. Hogg & Williams, 1996; Durance & Ormerod, 2009).

Numerous studies revealed that thermal pollution can lead to detrimental effects on the BMI community. On the other hand, it may cause no alterations or even have a positive influence on BMI

(e.g. Kamler, 1965; Ward & Stanford, 1982; Rader & Ward, 1988; Brussock & Brown, 1991; Durance & Ormerod, 2007).

Laboratory studies are generally conducted in order to directly assess short or medium-term effects of several constant water temperatures on single species. Additionally, no long-term effects of various water temperature regimes on BMI community structure could be analyzed. In order to capture these long-term effects on community structures, field studies provide an opportunity to fill this gap of knowledge. Given the importance of water temperature to the ecology of rivers, it is of considerable interest to identify how future changes in water temperature affect BMI communities. This chapter focuses on changes in BMI communities caused by an increase in water temperature in combination with seasonality.

3.2 Materials and Methods

Detailed information on study area and sampling sites, as well as explanation on water temperature data investigations are given in chapter 2.

3.2.1 Macroinvertebrate Sampling and Data Processing

Benthic macroinvertebrates (BMI) were collected by using multi-habitat sampling technique (Hering et al., 2004). Sampling was performed in autumn 2009 and spring and autumn 2010. All samples were taken at comparable periods of time for seasonal and annual comparison. The sampling at cold-warm site pairs of the particular river were performed at the same day. All benthic macroinvertebrates were taken with a 0.25 x 0.25 m shovel-sampler (500 µm mesh size). A sample consisted of 20 sampling units taken from all the microhabitat/substrate types with at least 5 % coverage at the sampling site. The relative share of the microhabitat types defined the 20 sampling units. Those units were pooled and the recovered macroinvertebrates were subsequently identified to species level when possible.

The species temperature index (STI) was determined for each species using the species´ river zonation index (Hering et al., 2009). The community temperature index (CTI) was calculated by averaging the STI of the constituent species weighted by their abundances. CTI calculation has been effected in accordance with Devictor et al. (2012).

A comparative assessment of the impact of water temperature on macroinvertebrate community structure dynamics was carried out using non-metric multi-dimensional scaling (NMDS) to ordinate cold and warm sampling sites using macroinvertebrate abundance. The similarity in species composition between sampling sites was measured by the Bray-Curtis coefficient (Bray & Curtis, 1957) on square root transformed species abundance data. Contributions to similarity by abundant species were reduced by square root transformation, and the importance of rare or uncommon species in the dataset (Clarke, 1993). NMDS was performed using 50 random starting configurations of sample points, while for cold vs. warm comparison two-dimensional solution is presented.

A similarity percentage analysis (SIMPER) was applied to identify the percentage contribution of each taxon to any observed differences (Clarke, 1993; Clarke & Warwick, 2001). In addition, an analysis of

similarities (ANOSIM) with a one-way layout for each locality (Ruhr, Lenne1 and Lenne2) was performed to test for differences between cold-warm sampling site groups. All analyses were performed using the software PRIMER (Version 6.1.13, PRIMER-E Ltd, 2009).

Results of the cold-warm site pair of each locality were tested for significant differences by the nonparametric Mann-Whitney U Test. The results were considered to be statistically significant when the p-value was less than or equal to 0.05. Levels of significance were set to $p \leq 0.0001$ (****), $0.0001 < p \leq 0.001$ (***), $0.001 < p \leq 0.01$ (**), and $0.01 < p \leq 0.05$ (*). Statistics were conducted using Statistica 12 (StatSoft 2013).

3.3 Results

3.3.1 Water Temperature

Water temperature results were summarized in Table 3-1. Differences in water temperature from May 2009 to September 2010 were highly significant for all cold-warm site pairs ($p < 0.0001$). The highest difference in water temperature was found for the Lenne1 site pair (1.9 K), followed by the Lenne2 (0.8 K) and the Ruhr site pair (0.7 K). The same ranking applies for differences in cumulative number of degree-days >4 °C, which was significantly different for all site pairs. Cumulative number of degree-days >17 °C did not occur at cold sites of Ruhr and Lenne1, therefore differences could only be tested significantly for the Lenne2 site pair.

Table 3-1: Water temperature parameters May 2009 – September 2010.

(Mean ± SD. WT: water temperature in °C; ΔWT: difference mean WT (warm-cold) in Kelvin;
DD>4: cumulative number of degree-days >4 °C; DD>17: cumulative number of degree-days >17 °C;
*: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001, significance tested with Mann-Whitney U test)

Site	WT	ΔWT	DD>4	ΔDD>4	DD>17	ΔDD>17
Rc	9.60 ± 3.31	0.68****	2579.80	289.70***	0.00	121.05
Rw	10.28 ± 4.96		2869.50		121.05	
L1c	9.40 ± 3.28	1.92****	2609.65	865.25****	0.00	127.35
L1w	11.32 ± 5.61		3474.90		127.35	
L2c	10.91 ± 4.61	0.80****	3623.10	350.85**	17.45	62.85*
L2w	11.71 ± 4.70		3973.95		80.30	

3.3.2 Macroinvertebrate Fauna

The complete macroinvertebrate taxalists are given in Appendix III.1.

The ordination through NMDS revealed no clear separation of Ruhr, Lenne1 and Lenne2 samples according to temperature exposure (Figure 3-1 to Figure 3-3).

The ANOSIM test based on Bray-Curtis similarity confirmed that the water temperature exhibits no significant effects on macroinvertebrate abundance.

The SIMPER analysis showed that the average dissimilarity between cold and warm groups was highest at Lenne1 (51.9 %), followed by Ruhr (43.1 %) and Lenne2 (41.6 %) (Table 3-2).

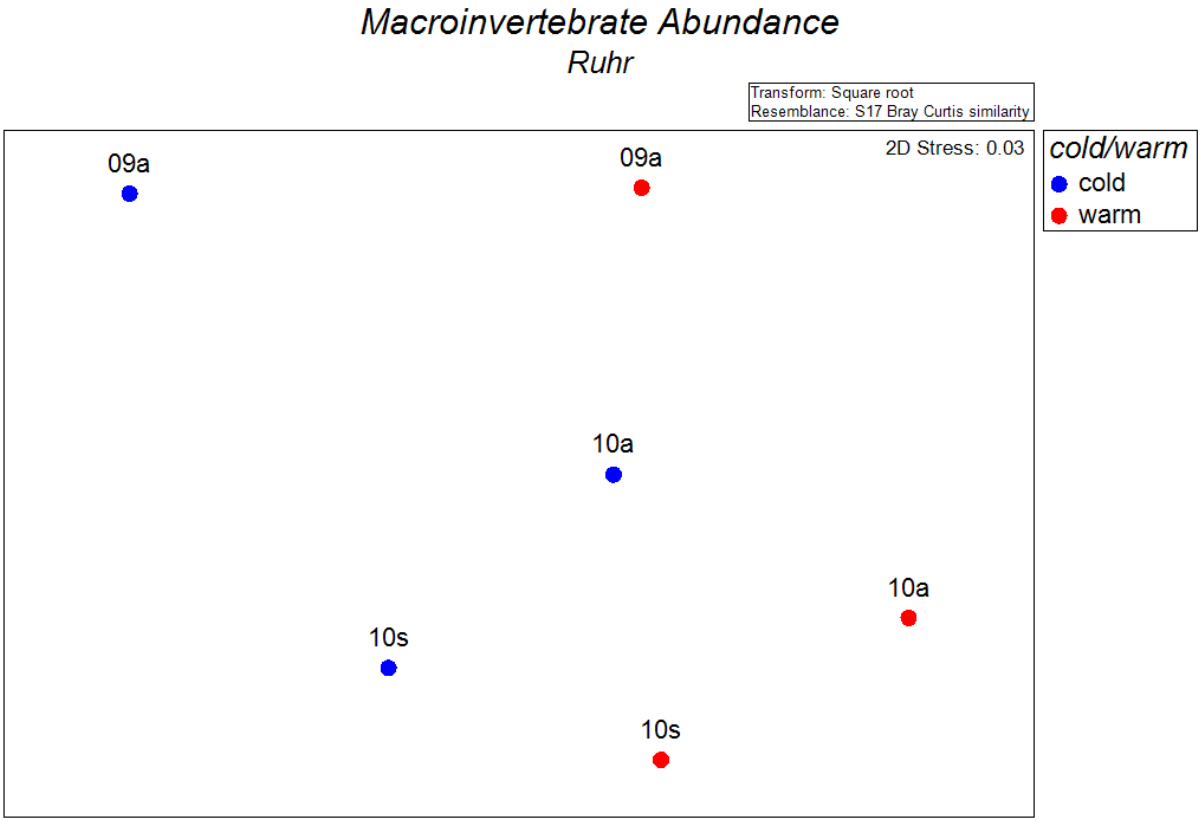

Figure 3-1: Non-metric multidimensional scaling of temperature impact on macroinvertebrate abundance at Ruhr.

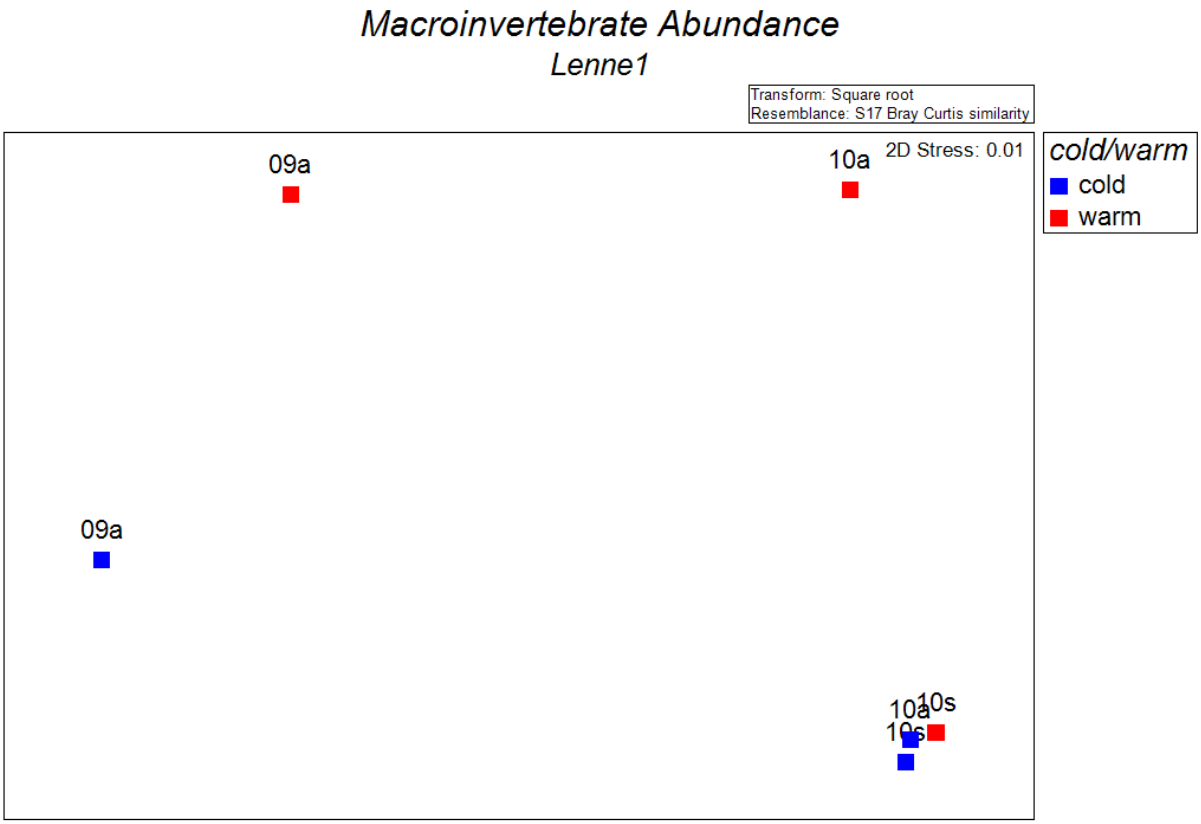

Figure 3-2: Non-metric multidimensional scaling of temperature impact on macroinvertebrate abundance at Lenne1.

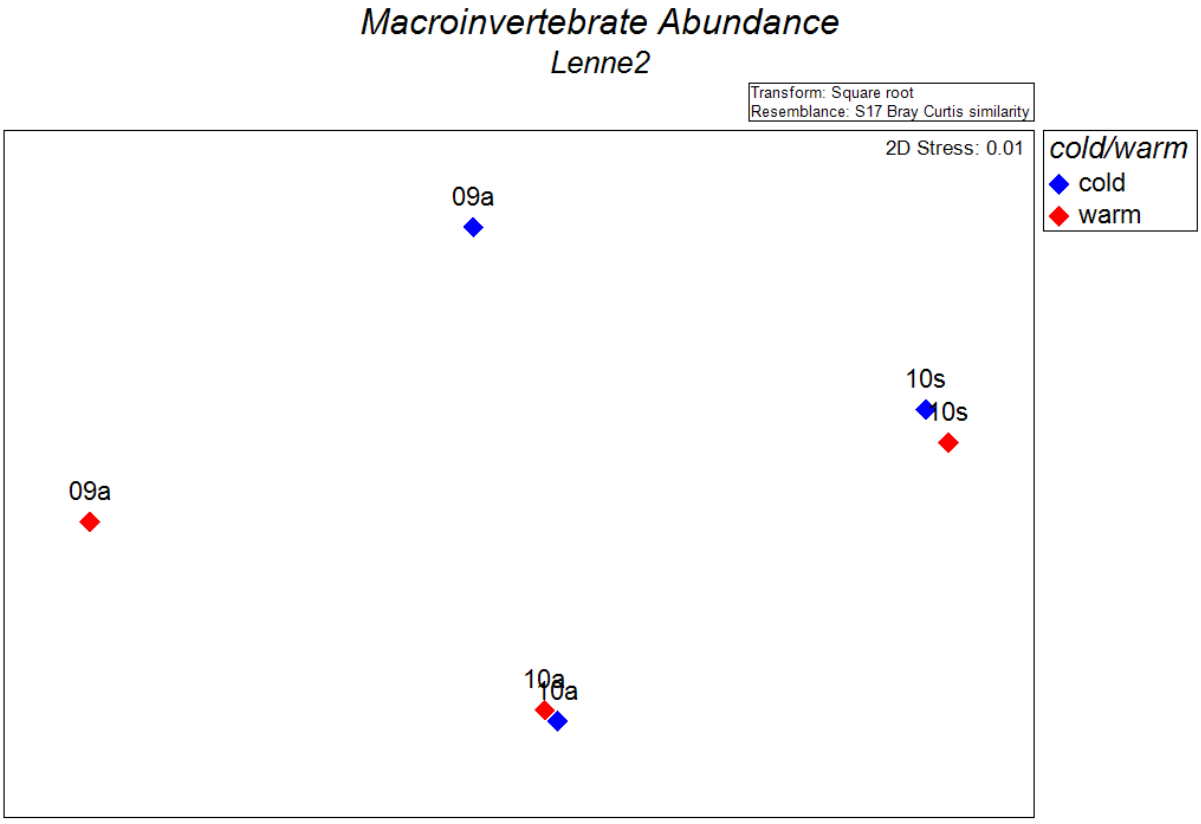

Figure 3-3: Non-metric multidimensional scaling of temperature impact on macroinvertebrate abundance at Lenne2.

Table 3-2: Results of one-way ANOSIM tests (R and p values) and SIMPER analyses on macroinvertebrate abundance. (Av. Dissimilarity = average dissimilarity between cold and warm group)

Locality	Ruhr	Lenne1	Lenne2
Global R-value	0.037	0.111	-0.037
p-value	0.5	0.5	0.6
Av. Dissimilarity (%)	43.11	51.87	41.62

3.3.2.1 EPT community

The ordination through NMDS revealed no clear separation of Ruhr, Lenne1 and Lenne2 samples according to temperature exposure (Figure 3-4 to Figure 3-6).

The ANOSIM test based on Bray-Curtis similarity confirmed only unclear separation, showing no significant effects of temperature on EPT-taxa abundance.

However, SIMPER analysis showed the highest average dissimilarity between cold and warm groups at Lenne1 (62.7 %), followed by Ruhr (48.6 %) and Lenne2 (44.8 %) (Table 3-3).

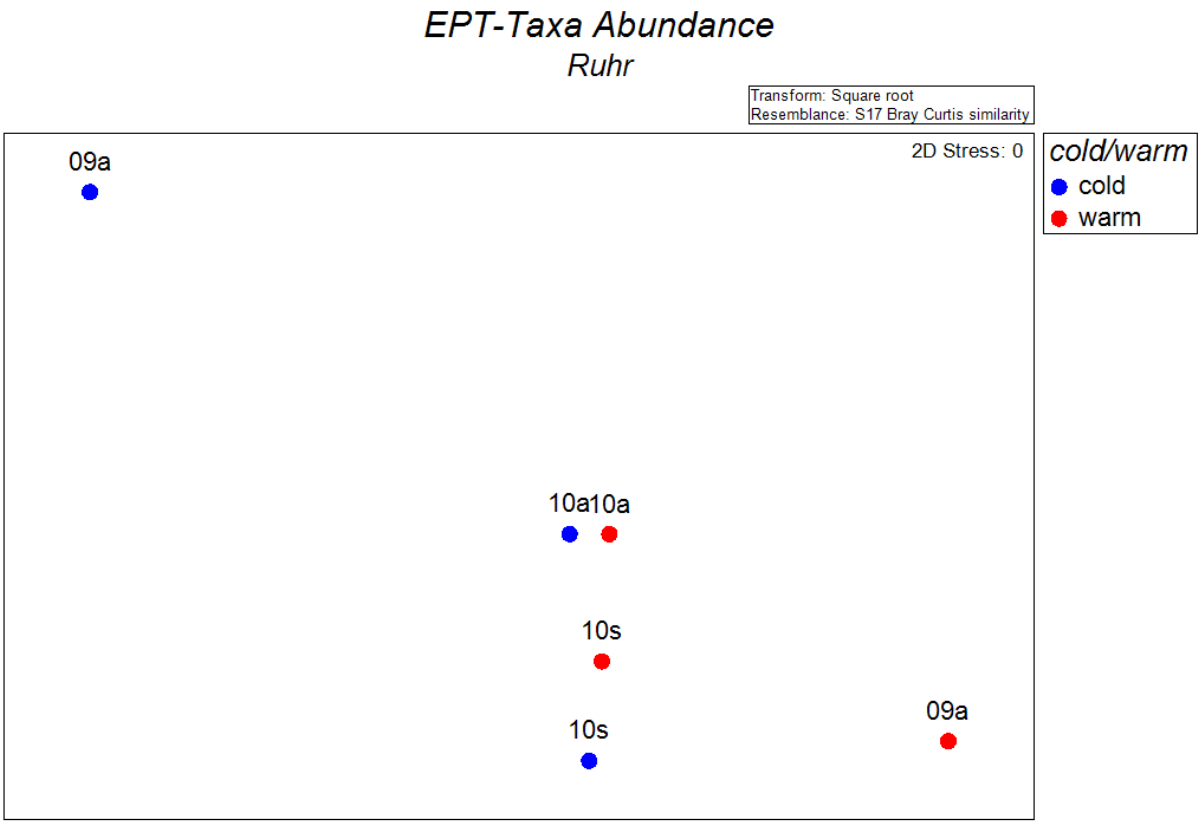

Figure 3-4: Non-metric multidimensional scaling of temperature impact on EPT-Taxa abundance at Ruhr.

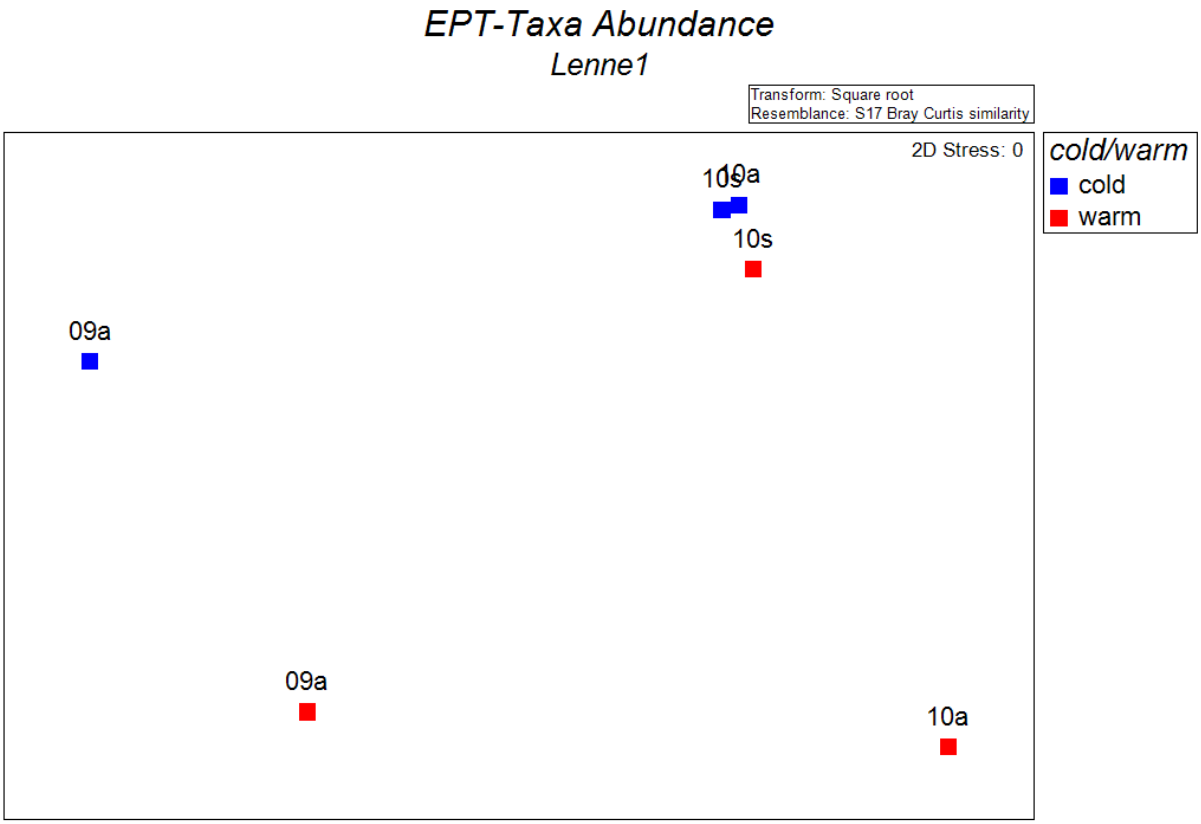

Figure 3-5: Non-metric multidimensional scaling of temperature impact on EPT-Taxa abundance at Lenne1.

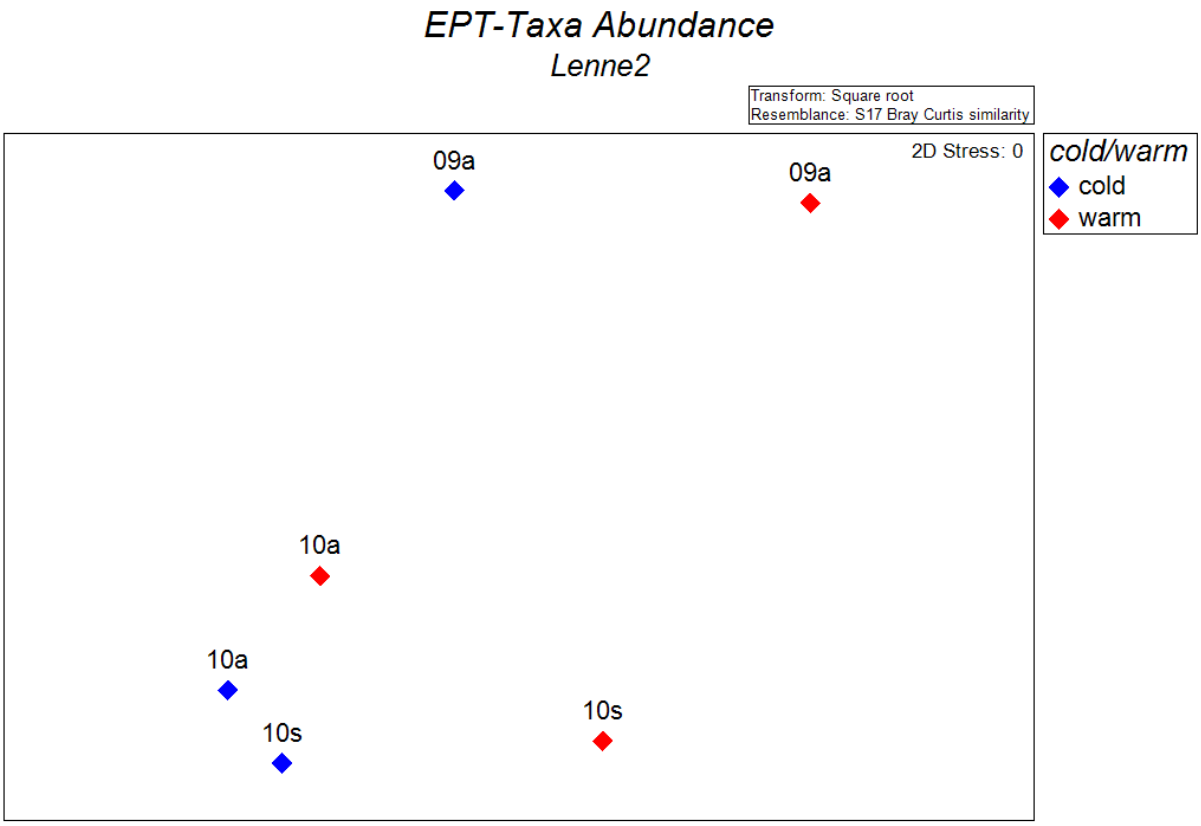

Figure 3-6: Non-metric multidimensional scaling of temperature impact on EPT-Taxa abundance at Lenne2.

Table 3-3: Results of one-way ANOSIM tests (R and p values) and SIMPER analyses on EPT-taxa abundance.

(Av. Dissimilarity = average dissimilarity between cold and warm group)

Locality	Ruhr	Lenne1	Lenne2
Global R-value	-0.074	0.111	-0.111
p-value	0.9	0.5	0.6
Av. Dissimilarity (%)	48.57	62.71	44.75

Furthermore, the SIMPER analysis identified four EPT-species (*Allogamus auricollis*, *Caenis rivulorum*, *Hydropsyche siltalai* and *Polycentropus flavomaculatus*) that were most responsible for distinctions between cold and warm groups (Table 3-4 to Table 3-6). Whereas *A. auricollis* (Trichoptera) only occurred at the Ruhr sampling sites, the three species *H. siltalai* (Trichoptera), *C. rivulorum* (Ephemeroptera) and *P. flavomaculatus* (Trichoptera) were abundant at all sampling sites. At the river Ruhr, three species (*A. auricollis*, *H. siltalai* and *P. flavomaculatus)* contributed collectively to 25.8 % of the dissimilarity between the two groups (Table 3-4). Whereas for Lenne1 and Lenne2, the two species *H. siltalai* and *C. rivulorum* were most responsible for cold and warm group distinctions (Table 3-5 and Table 3-6). Both species contributed collectively to 37.0 % (Lenne1) and 19.5 % (Lenne2) of the dissimilarity between the two groups.

Table 3-4: The outcome of a SIMPER analysis on square-root transformed data listing those EPT-species that contributed to the dissimilarity between cold and warm EPT-communities at Ruhr (cut-off set at 90 % contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Ruhr				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Allogamus auricollis	9.78	7.42	5.49	11.31
Hydropsyche siltalai	4.70	8.31	3.71	7.63
Polycentropus flavomaculatus	3.57	5.90	3.32	6.84
Lepidostoma hirtum	4.80	1.76	2.39	4.91
Anabolia nervosa	1.46	3.77	2.25	4.64
Ephemera danica	3.92	1.46	2.02	4.17
Caenis rivulorum	1.26	2.34	1.34	2.76
Goera pilosa	0.00	1.46	0.93	1.91

Table 3-5: The outcom of a SIMPER analysis on square-root transformed data listing those EPT-species that contributed to the dissimilarity between cold and warm EPT-communities at Lenne1 (cut-off set at 90 % contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Lenne1				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Hydropsyche siltalai	0.30	13.97	12.70	20.26
Caenis rivulorum	6.69	13.09	10.47	16.69
Oecetis testacea	2.79	4.66	2.69	4.36
Anabolia nervosa	0.00	2.00	1.60	2.55
Polycentropus flavomaculatus	5.22	3.60	1.51	2.40

Table 3-6: The outcome of a SIMPER analysis on square-root transformed data listing those EPT-species that contributed to the dissimilarity between cold and warm EPT-communities at Lenne2 (cut-off set at 90 % contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Lenne2				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Hydropsyche siltalai	17.59	20.07	5.30	11.84
Caenis rivulorum	5.17	3.56	3.41	7.62
Polycentropus flavomaculatus	5.65	2.51	3.17	7.09
Lepidostoma hirtum	3.33	5.31	1.60	3.58
Serratella ignita	0.00	1.76	1.07	2.40
Psychomiya pusilla	0.00	1.41	1.02	2.28
Mystacides azurea	0.00	1.46	0.87	1.93

3.3.2.2 Community Temperature Index

An overview on the community temperature index (CTI) is given in Figure 3-7. The CTI increased at the Ruhr warm sites in general. In contrast, the CTI at Lenne1 showed a general decrease at warm sites. The CTI at Lenne2 showed an increase in autumn 2009, as well as a decrease in both 2010 samplings. None of these revealed statistical significance.

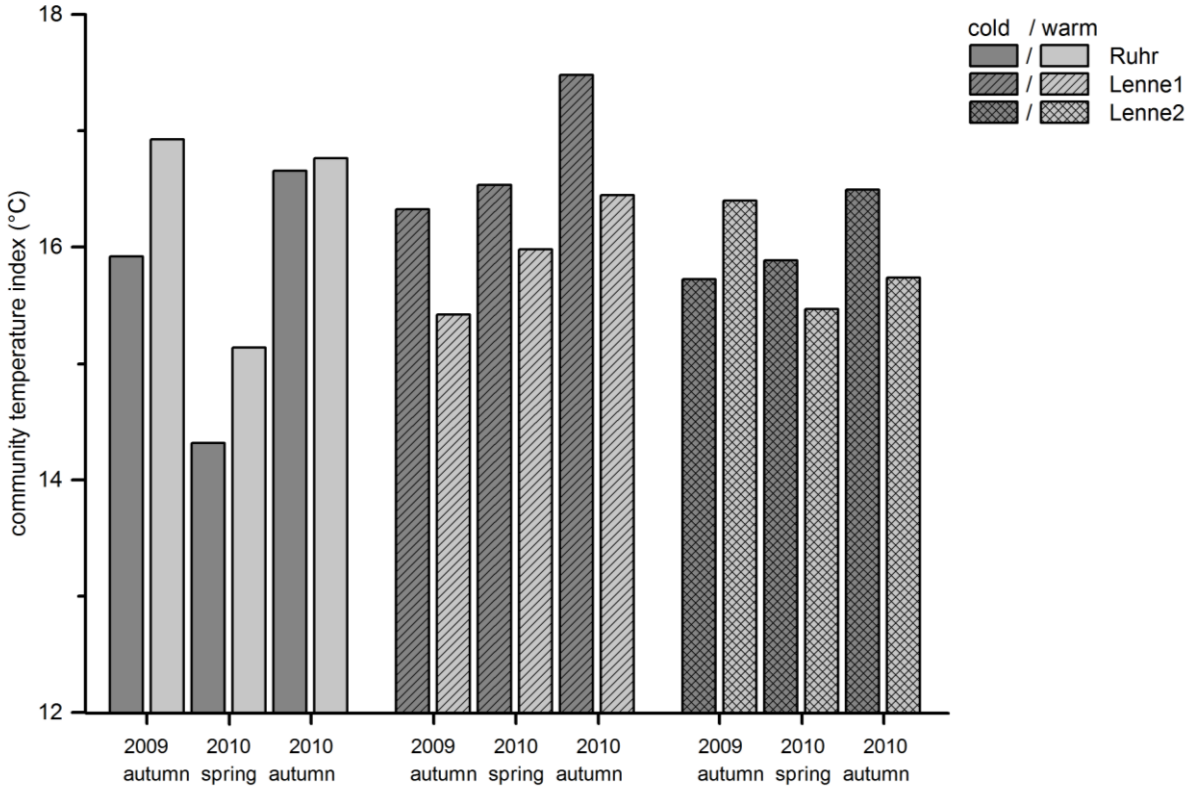

Figure 3-7: Community temperature index (CTI) in °C for macroinvertebrate communities at sampling sites.

3.4 Discussion

The results of this study indicate that differences in benthic macroinvertebrate (BMI) communities can be partly explained by water temperature changes. This finding is in accordance to the longitudinal distribution of individual species and groups of organisms (Vannote et al., 1980; Roux et al., 1992; Jacobsen et al., 1997). Greater species richness in habitats with greater thermal variability was hypothesized by Vannote et al. (1980) since more species find their specific thermal optima. Haidekker & Hering (2008) showed for mid-sized rivers that temperature explained much less of the variability in taxa distribution than in small rivers. Temperature range was even slightly higher in small rivers, which may reflect an increased share of more eurythermic and less-specialized BMI taxa.

The majority of ecological studies relate mean temperatures to the naturally occurring biota as well as degree-days (Vinson & Hawkins, 1998). Degree-days have been used in insect phenology studies (Markarian et al., 1980; Mutsunori, 1985; Watanabe et al., 1999; Haidekker & Hering, 2008). The influence of degree-days on development or distribution of BMI species have been tested in several studies and strong correlations or directional growth rates have been found (Markarian et al., 1980; Lowe & Hauer, 1999; Watanabe et al., 1999; Knispel et al., 2006). This can be easily explained by the temperature dependent development of many aquatic species.

Even though not statistically significant, the highest average dissimilarity in BMI community occur at the Lenne1 site pair. Here, also the largest difference in mean water temperature and at the same time the highest difference of DD>4 as well as DD>17 was found. A similar pattern was observed when looking at the EPT community. In absence of statistically significance, the results also show highest average dissimilarity at the Lenne1 site pair. Despite the lowest difference of both, mean water temperature and DD>4, the Ruhr site pair showed a higher average dissimilarity for BMI as well as for EPT community than at the Lenne2 site pair. This may be due to a difference in DD>17 which is twice as high for the Ruhr as for the Lenne2 site pair.

Furthermore, water temperature plays an important role concerning egg development and larval growth, especially for Ephemeroptera, Plecoptera and Trichoptera (EPT) (e.g. Humpesch, 1979; Brittain, 1983; Brittain, 1990; López-Rodríguez et al., 2009; Sand & Britain, 2009). In view of this fact, the SIMPER analysis was used to detect EPT-species that mainly contribute to differences between cold-warm site pairs. An increase in water temperature would be expected to affect such species by increasing their metabolic demand while decreasing saturation oxygen concentrations. In particular, warming in winter would more likely affect metabolism, development times or phenology of EPT (Quinn et al., 1994; Hawkins et al., 1997; Briers et al., 2004), whereas increasing summer temperatures are most likely to approach the upper limits for sensitive organisms (Quinn et al., 1994; Hawkins et al., 1997). Based on this, smaller effects on distribution and community composition would be expected by elevated winter temperature than by increasing water temperature in summer (Durance & Ormerod, 2009).

For instance, an increase of average abundance of *C. rivulorum* (Ephemeroptera) has been detected for the Lenne1 and the Ruhr warm sites, whereas the highest increase was found at the site L1w in

accordance with the highest number of DD>17 (127.4) and the largest ΔDD>4 (865.3). This fits in well with the fact that many Ephemeroptera are warm-adapted species, which develop faster at higher temperatures and often have a winter quiescence. Even though the average abundance is slightly decreasing at the L2w site with lowest DD>17 (80.3). This could be partly due to actual conditions at sampling days.

The increase of DD>17 is correlated with a rising average abundance of *H. siltalai* (Trichoptera) at all warm sites. This corresponds with the findings of Haidekker & Hering (2008) who found a positive correlation of the presence and abundance of *H. siltalai* in warmer small and medium-sized streams compared to cooler ones.

The missing of a clear pattern for the CTI results might be due to the fact that a strong variation on temperature requirements can appear within families among BMI species (Hildrew & Edington, 1979). Additionally, the large geographical ranges of most of the BMI families imply large thermal tolerance. Therefore, temperature effects will probably occur at species level rather than at family level (Bonada et al., 2007a; Bonada et al., 2007b). Likewise, identifications on family-level might conceal effects on individual species derived from species-level identification (Daufresne et al., 2004; Bêche & Resh, 2007; Durance & Ormerod, 2007). Despite a shift in BMI community composition from specialist to generalist species (e.g. Hering et al., 2009), overall metrics like abundance, diversity or richness might remain static in relation to temperature effects.

In this study, the majority of BMI were present at both cold and warm sites. This indicates that the limit of tolerance range of the majority of taxa was not exceeded by the temperature differences found in this study. Although some small changes in community composition occur, the recorded temperature increases appear unlikely to reduce taxonomic diversity. Changes in BMI communities have been noted in other studies associated with increasing water temperatures, however the magnitude of the temperature increase was often greater (e.g. Bonada et al., 2007a; Carolli et al., 2012; Worthington et al., 2015).

Unfortunately, there is still a lack of knowledge regarding the impact of changing temperature on the life cycles of BMI, as highlighted by previous studies (e.g. Haidekker & Hering, 2008; Heino et al., 2009). Most of them focus on the impact of temperature changes on egg development, larval growth rates and survival, or on hatching success. In regard to the probable impact of increasing water temperature on BMI communities in rivers, life cycle studies in controlled environments are still required. The results of the present study show that temperature changes have an impact on BMI species, especially EPT-taxa, thereby affecting also BMI communities.

The effect of water temperature on the levels of hepatic glycogen and hsp70 in brown trout (*Salmo trutta fario* L.)

4

4.1 Introduction

As a consequence of global warming, a significant increase in surface temperature is expected to occur during the present century (e.g. Webb, 1996; Mohseni et al., 2003; IPCC, 2014). Especially the effects of temperature on brown trout have received a great deal of attention (e.g. Lessard & Hayes, 2003; Jonsson & Jonsson, 2009; Almodóvar et al. 2012), particularly within the context of the predicted climate change scenarios (IPCC, 2007).

Water temperature plays a fundamental role in controlling the biology of fish species, because most fishes are poikilothermic ectotherms. Body temperature of most fishes varies as a result of changes in environmental temperature (Currie, 2011; Schulte & Columbia, 2011). Moreover, temperature within the freshwater environment may regulate their distribution, migration, survival, feeding, growth, reproduction, physiology and ecology (e.g. Pankhurst & King, 2010; Schulte & Columbia, 2011).

Cells and proteins of ectothermic fishes are vulnerable to thermal injury because body temperature fluctuates with the environment (Currie, 2011). Even small temperature variations can cause disproportionate changes in physiological processes (Pörtner & Farrell, 2008; Pörtner & Peck, 2010). Temperature as a potential physicochemical stressor acts directly at the cellular level, disturbing the cellular physiology. This leads to a disturbed homeostasis which is commonly defined as cellular stress. The cellular stress responses including those induced by environmental temperature variations determine whether an organism adapts to changed conditions, respectively withstand or suffers from physiological disturbances (Iwama et al., 1999; Wendelaar Bonga, 2011).

Biomarkers are often used tools for the detection and quantification of these various and complex stressor specific responses. A distinction is made here according to whether markers are contaminant specific (e.g. metallothioneins) or part of a general stress response (e.g. heat shock proteins) (Frank et al., 2013).

As useful biomarker for general stress, the levels of hepatic glycogen have been suggested (Vasseur & Cosso-Leguille, 2003). As glycogen is the main reserve polysaccharide of animal cells (Kilborn &

Macleod, 1920), its determination is particularly well suited to draw conclusions about the nutritional and stress state.

As a part of the organismic secondary stress responses the expression of proteins, such as heat shock proteins, especially of the 70 kDa class (hsp70), will be induced (Currie, 2011). Hsp70s act as molecular chaperones, as they protect and repair damaged proteins (Roberts et al., 2010). Thus, their induction is an integral component of the fish's response to temperature stress. In ecotoxicological research hsp70s are used as molecular biomarkers in fish to show the organismic effect of a wide range of stressors including thermal pollution (Iwama et al., 1999; Iwama et al., 2004).

This chapter aims to investigate whether temperature increase in combination with seasonality causes alterations in the physiological response of the brown trout (*Salmo trutta fario* L.). This field study also assessed the suitability of brown trout as a bioindicator for thermal pollution, particularly as this species is used commonly for experimental studies.

4.2 Materials and Methods

Detailed information on study area and sampling sites, as well as explanation on water temperature data investigations are given in chapter 2 and 3.3.1.

4.2.1 Fish Sampling and Processing

Brown trout (*Salmo trutta fario* L.) were caught by electrofishing in autumn 2009 (09a) and in spring and autumn 2010 (10s, 10a). All samples (365 fish in total, 13 to 26 individuals per locality, see Table 4-1) were taken at comparable periods of time for seasonal and annual comparison. Each cold-warm site pair was sampled on the same day. Brown trout were brought to the laboratory and kept alive in freshwater tanks until they were dissected for morphological and parasitological examination following a standardized protocol. For the analysis of biomarkers, a piece of liver of each fish (ca. 200 mg) was taken immediately after dissection using stainless steel dissecting tools, which were previously cleaned with 96 % ethanol and deionized water (Milli-Q, Q Gard 2, Millipore), to avoid contamination. Samples were rinsed with physiological solution (0.9 % NaCl), snap frozen in liquid nitrogen and later stored at -80 °C.

Total length (TL), body mass (BM), liver mass (LM) and gonad mass (GM) for each fish were determined during dissection. According to the following formulae body and organ weights were used to compute condition factor ($CF = 100 \times BM \times TL^{-3}$), hepatosomtic index ($HSI = 100 \times LM \times W^{-1}$) and gonadosomatic index ($GSI = 100 \times GM \times W^{-1}$) (Schäperclaus, 1990).

All handling procedures were conducted in compliance with the institutional guidelines for the care and use of animals.

Table 4-1: Number of S*almo trutta fario* per site and season.

	Season			
Site	**autumn 2009**	**spring 2010**	**autumn 2010**	**Sum**
Ruhr cold	25	22	23	**70**
Ruhr warm	25	13	22	**60**
Lenne1 cold	15	14	15	**44**
Lenne1 warm	24	24	26	**74**
Lenne2 cold	25	20	20	**65**
Lenne2 warm	17	15	20	**52**
Sum	**131**	**108**	**126**	

4.2.2 Biomarker

4.2.2.1 Determination of hepatic glycogen level

Hepatic glycogen level was determined using a modified method of Lo et al. (1970) and Carroll et al. (1956). For digestion of the tissue, 2 ml of 60 % KOH were added to 50 mg of liver tissue before incubating for 30 min at 90 °C in waterbath. After cooling on ice, 500 µl saturated Na_2SO_4 was added, making sure that the tissue was completely dissolved in the solution. Subsequently, 10 ml of 95 % ethanol were added to precipitate the glycogen from the alkaline digestate and samples were incubated at 80 °C for 10 min. Samples were centrifuged at 4000 rpm for 15 min, supernatant was carefully aspirated. The pellet (glycogen precipitate) was dissolved in 3 ml of deionized water (Milli-Q, Q Gard 2, Millipore), washed with 10 ml of 95 % ethanol and centrifuged again at 4000 rpm for 15 min. Ethanol washes were repeated until the precipitate was white. After drying (12 h at 50 °C) the pellet was dissolved in 5 ml of deionized water. A reagent blank and standards (0.01 up to 0.1 mg glucose/ml deionized water) were prepared. Each solution (250 µl) was mixed with 500 µl anthrone reagent (100 mg anthrone/50 ml concentrated H_2SO_4) and incubated for 10 min at 90 °C. Aliquots of 300 µl were transferred into a microwell plate and the absorbance was read at 623 nm using a microplate reader (Infinite M200, TECAN). All measurements were carried out in triplicates to minimize errors.

The calculation of glycogen (mg/g liver mass) is as follows:

$$(D_{623}/a \times 5ml) / (LM/2 \times 1000) \times 0.9$$

where D_{62}: mean of optical density of the sample and its replicates less the amount derived from the blank, a: slope of the calibration line, LM: liver mass in mg, 0.9: factor for converting glucose value to glycogen value.

4.2.2.2 Determination of hepatic hsp70 level

For the detection of hepatic hsp70 levels 10 fish per site and season were randomly selected. The determination of hsp70 was performed in accordance with Frank et al. (2013). In brief, tissue was homogenized and centrifuged and total protein was measured in the supernatant with a Pierce BCA

Protein Assay Kit (Thermo Scientific). Hsp70 was detected by discontinuous SDS-Page and Western Blot using monoclonal anti hsp70 antibodies (mouse anti hsp70, antibodies online) and a horseradish peroxidase labeled second antibody (goat-anti mouse, DAKO). For each sample, equivalents of 20 µg of total protein were loaded on the gel and a reference sample (fish liver homogenate) was run on all gels to allow inter-gel comparability. Band intensity was visualized by 4-chloro-1-naphthol-staining. The grey value intensity of the hsp70 bands was quantified by a densitometric image analysis (imageJ1 software) in accordance to Abramoff et al. (2004). All samples were expressed as relative hsp70 values by dividing all sample values by the value of the standard of the respective gel.

4.2.3 Statistical Treatment of Data

Results of the cold-warm site pair of each locality were tested for significant differences by the nonparametric Mann-Whitney U Test. The results were considered to be statistically significant when the p-value was less than or equal to 0.05. Levels of significance were set to $p \leq 0.0001$ (****), $0.0001 < p \leq 0.001$ (***), $0.001 < p \leq 0.01$ (**), and $0.01 < p \leq 0.05$ (*).Statistics were conducted using Statistica 12 (StatSoft 2013).

4.3 Results

4.3.1 Fish data

All morphological parameters are given in Appendix IV.1. The results of condition factors, hepatosomatic indices and gonadosomatic indices are listed in Figure 4-1 to Figure 4-3. The condition factor (CF) was significantly lower at the Ruhr warm site in autumn 2009 ($p < 0.01$) and in spring 2010 ($p < 0.05$) than at the respectively cold site. The values at the Lenne1 warm site showed a significant decrease over the whole period of investigation ($p < 0.0001$ in autumn 2009 and spring 2010; $p < 0.01$ in autumn 2010). For the cold-warm site pairs at Lenne2 no significant differences for CF were found.
The hepatosomatic index did not differ significantly between all the cold-warm site pairs.
At Ruhr and Lenne2, the gonadosomatic index (GSI) decreased for all warm sites. Whereas at Lenne1, the GSI increased in autumn 2009 and spring 2010. The only significant difference in GSI values was obtained for the cold-warm site pair at Lenne2 in autumn 2010 ($p < 0.05$).

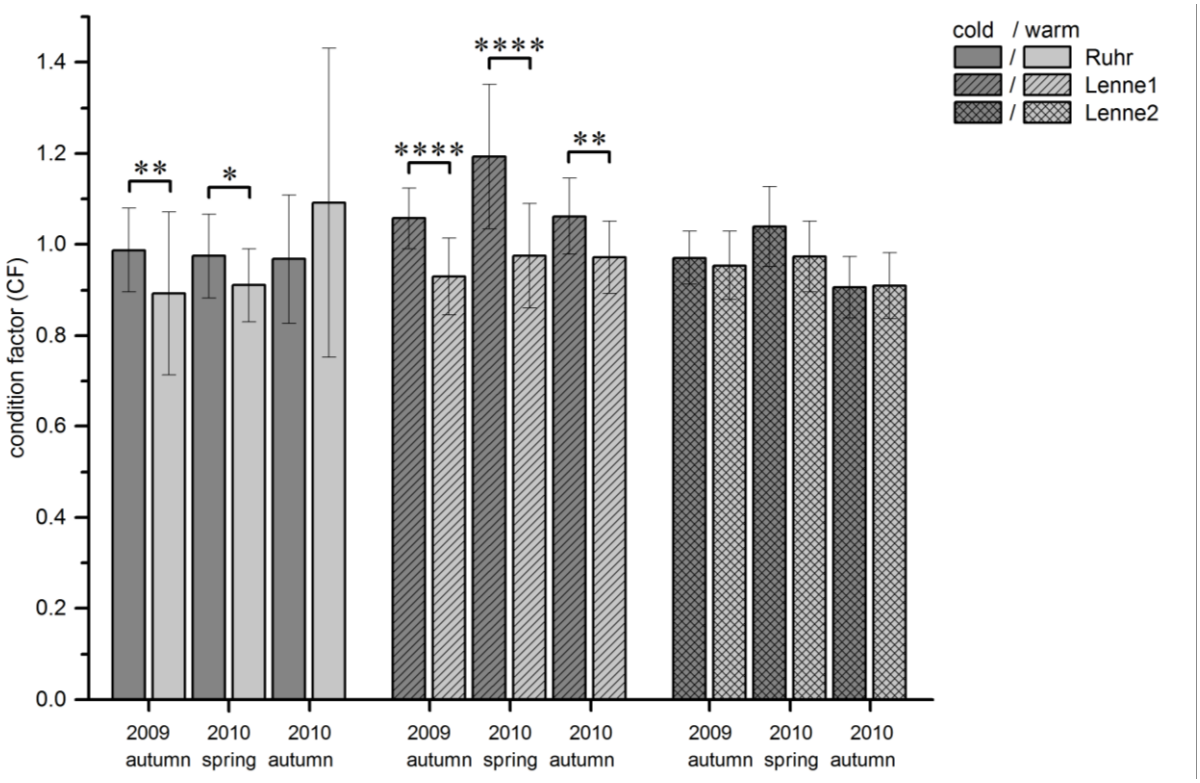

Figure 4-1: Condition factor (CF, Mean ± SD) of *Salmo trutta fario*.

n: min 13 to max 26; *: p<0.05; **: p<0.01; ****: p<0.0001, significance tested with Mann-Whitney U test.

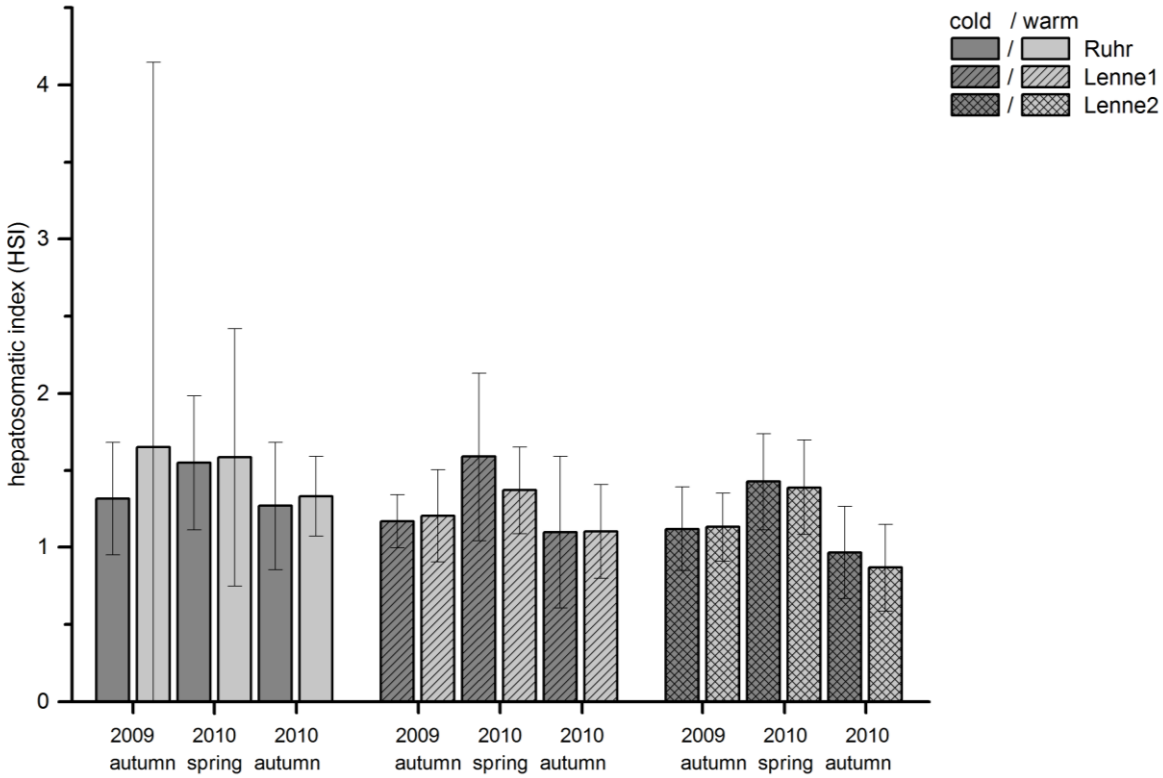

Figure 4-2: Hepatosomatic index (HSI, Mean ± SD) of *Salmo trutta fario*.

n: min 13 to max 26; significance tested with Mann-Whitney U test.

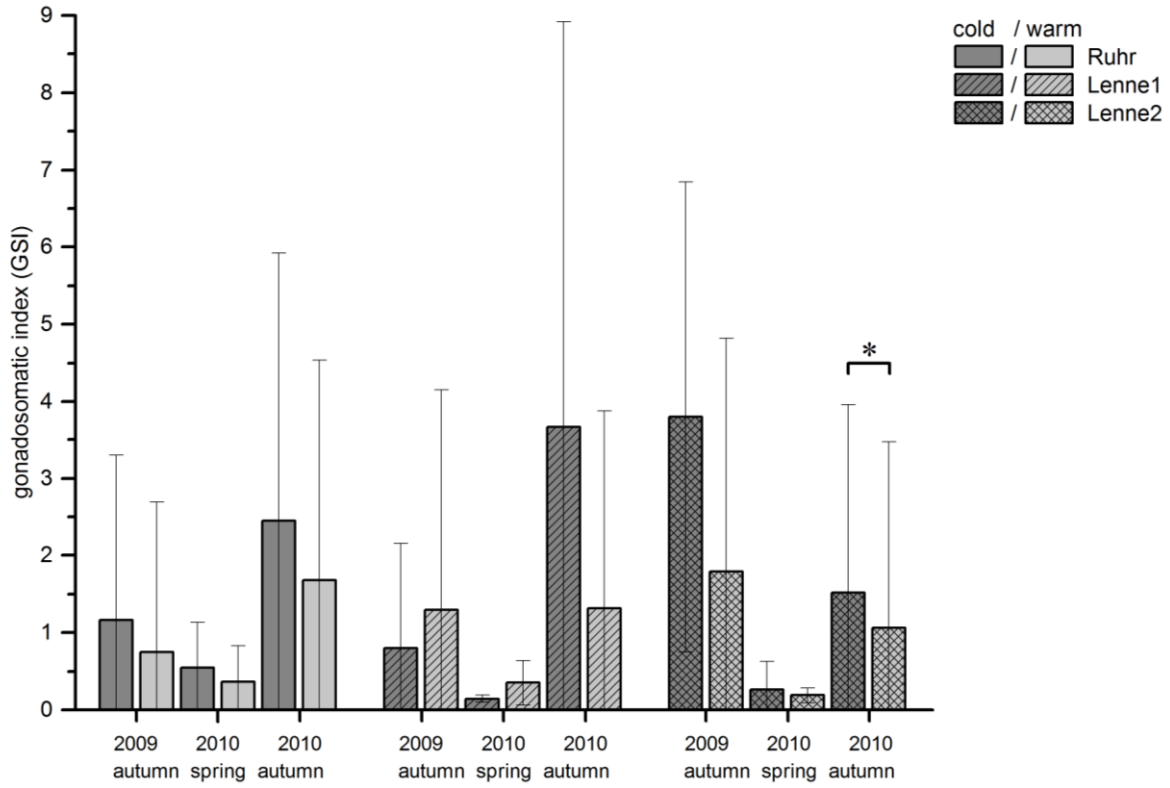

Figure 4-3: Gonadosomatic index (GSI, Mean ± SD) of *Salmo trutta fario*.

n: min 12 to max 26; *: p<0.05, significance tested with Mann-Whitney U test.

4.3.2 Biomarker

4.3.2.1 Hepatic glycogen level

The mean glycogen levels in liver tissue of brown trout are shown in Figure 4-4. Significant increases in the levels were found at the Ruhr warm site in autumn 2009 ($p < 0.05$) and at the Lenne1 warm site in autumn 2009 ($p < 0.0001$) and autumn 2010 ($p < 0.05$). Mean levels of glycogen did not differ significantly between the cold-warm site pair at Lenne2. At all locations, glycogen levels were lowest in spring 2010 at both warm and cold sites.

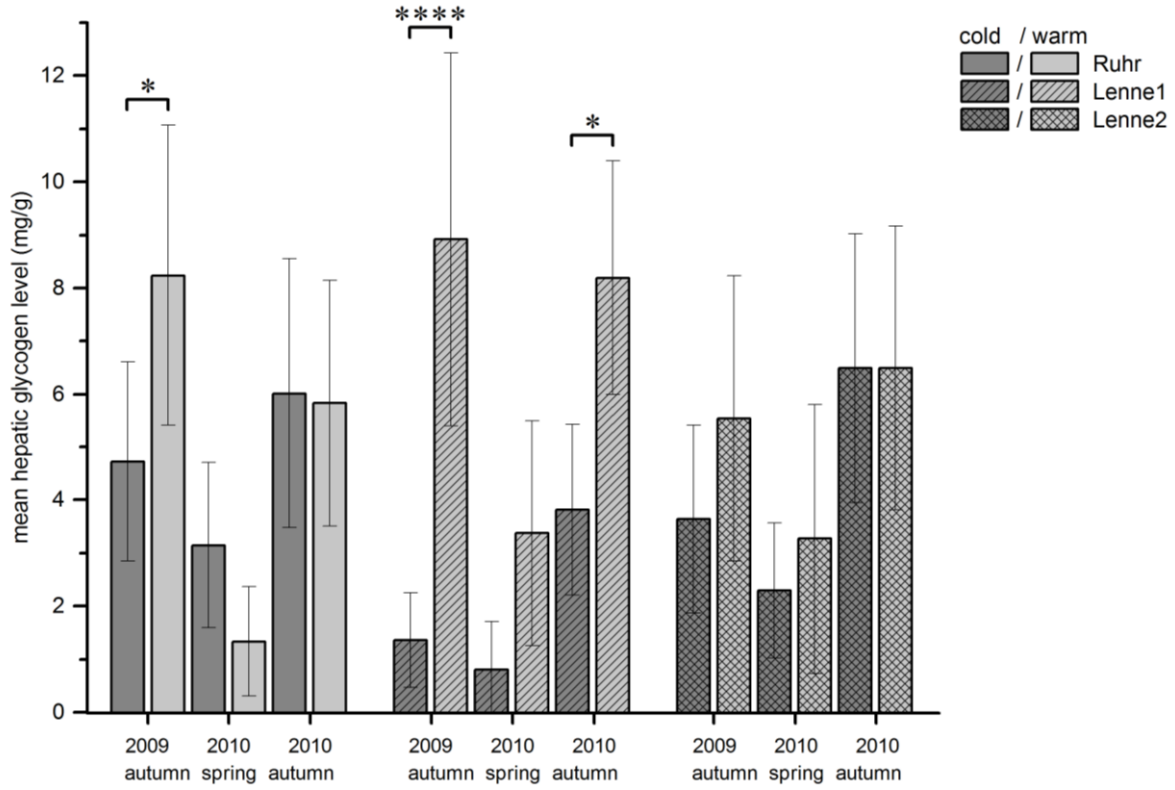

Figure 4-4: Glucogen content in liver tissue of *Salmo trutta fario*.

Mean ± 95% CI;
n: min 13 to max 26; *: p<0.05;****: p<0.0001, significance tested with Mann-Whitney U test.

4.3.2.2 Hepatic hsp70 level

Heat shock proteins were detected in liver tissues of all analyzed fish. The mean relative hsp70 levels are shown in Figure 4-5. Significantly elevated hsp70 levels were obtained for the Ruhr warm site in autumn 2009 ($p < 0.01$), whereas a significant decrease was observed in spring 2010 ($p < 0.01$). The levels of hsp70 at the Lenne1 warm site showed a significant decrease in autumn 2010 ($p < 0.0001$). At the Lenne2 warm site levels were significant lower in spring 2010 ($p < 0.0001$) and autumn 2010 ($p < 0.01$).

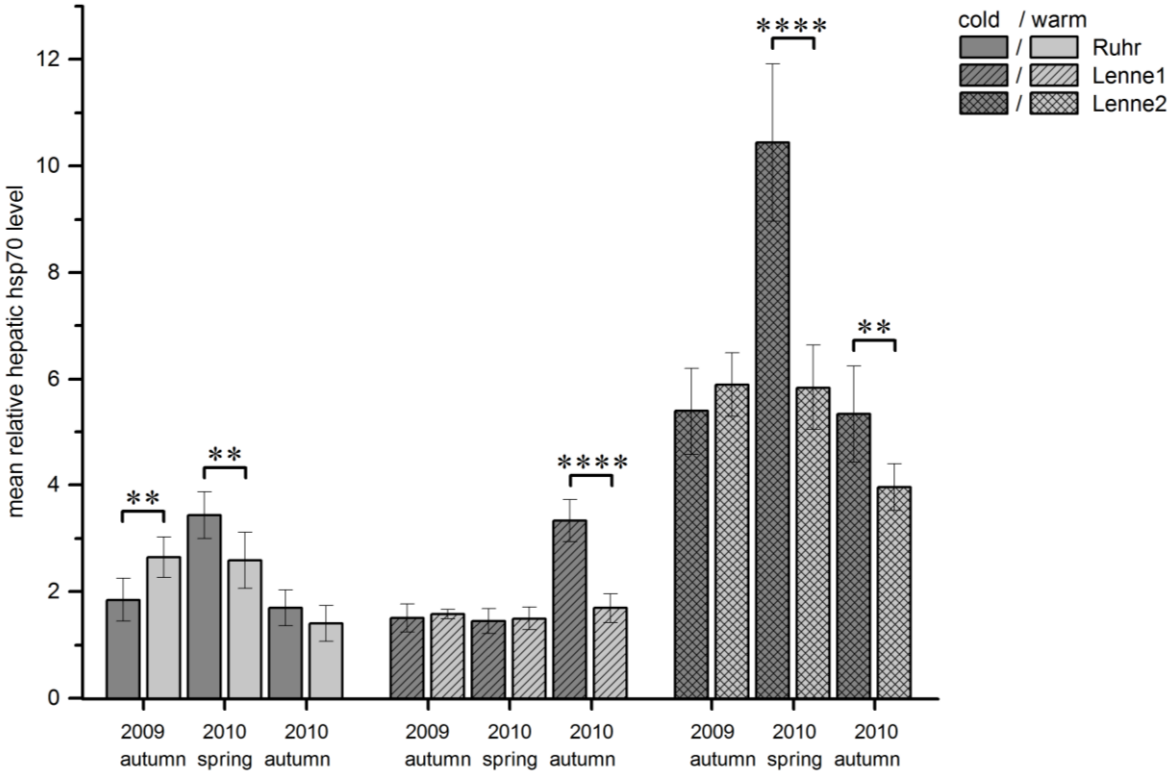

Figure 4-5: Mean relative hsp70 levels in liver tissue of *Salmo trutta fario*.

Mean ± 95% CI;
n: 10 for each site and season; **: $p<0.01$; ****: $p<0.0001$, significance tested with Mann-Whitney U test.

4.4 Discussion

In the present study, liver glycogen and hepatic hsp70 levels of brown trout were evaluated, particularly with regard to the effects of increasing water temperature and seasonal variability. Among other indicators such as hematocrit, lactate and cortisol levels, these two parameters are classically considered to be indicative of the physiological stress response in fish. When studying stress in aquatic organisms it is particularly important to constitute whether experimental procedures such as handling, sampling and other physical stressors are affecting the stress response.

Vijayan et al. (1997) demonstrated that handling stress does not adversely affect levels of hepatic hsp70 of rainbow trout. In order to exclude any further handling-related impairments in this study, all Brown trout were treated alike until examination.

For all basic physiological processes, the RGT-Regulation (Van't Hoffsch Regulation) states that all relevant processes will be enhanced two- to fourfold for a temperature rise of 10 °C. In fish, the development rate can increase to as much as five times when the temperature rises of 10 °C (Rombough, 1997). Based on the RGT-Regulation, a 0.2- to 0.4-fold increase in basic physiological processes combined with a higher use of primary energy for general metabolism was expected for the examined brown trout at warm sites (Schmidt-Nielsen, 1997; Willmer et al., 2004).

According to the condition factor temperature increase had significant effects on the condition of examined brown trout. Except of the sample in autumn 2010 at the Ruhr warm site, mean condition factors were lower at warm sites compared to respective cold sites. This is consistent with the results for *S. salar* from Murphy et al. (2006) which showed that the loss of body mass is larger in winters with warmer temperature than in cold winters.

Additionally, for both, cold and warm sites, condition factors of this study were equal or slightly higher in spring samples than in autumn samples. This corresponds with the assumption that fish often experience a compensatory growth rate in spring which could be up to three times higher than that of conspecifics tested in autumn (Larsson & Berglund, 2006).

Water temperature can alter the timing of maturation and spawning of salmonids, particularly cold winter temperatures cause delays in spawning (e.g. Henderson, 1963; Titarev, 1975; Morrison and Smith, 1986; Pankhurst et al., 1996; Pankhurst & King, 2010). In consequence, earlier spawning at warm sites could be an explanation for the decrease in gonadosomatic index (GSI) at Rw and L2w.

In general, liver size decreases with the maturation of gonads. However, hepatosomatic index (HSI) showed no significant effects in this study.

Accordingly, slightly lower hepatic glycogen levels were expected at warm sites. In this study, a general increase of hepatic glycogen levels was observed for the warm sites at Lenne1 and Lenne2; even more obvious at Lenne1 with a temperature difference of 1.92 K than at Lenne2 with a temperature difference of 0.80 K. At a temperature difference of 0.68 K between the Ruhr cold and warm site only sampling of autumn 2009 followed this trend.

The increased liver glycogen levels in brown trout at warm sites as compared to the respective cold sites indicates that a higher use of primary energy for the general metabolism was probably compensated by an increased food intake due to a general enhanced activity.

Brown trout in this study respond to normal environmental variations in their native streams by producing hsp70. The most obvious change in environmental conditions was water temperature (see chapter 3.3.1). At the cold sites at Ruhr and Lenne2, hepatic hsp70 levels followed the same trend, having highest level in spring and lowest level in autumn. For 2010 sampling periods, hepatic hsp70 levels at the warm sites at Ruhr and Lenne2 were similar to the findings at respectively cold sites, without a noticeable increase in summer 2010 compared to previous autumn samples (see Figure 4-5).

Fader et al. (1994) have shown for a series of fish including brown trout that even under unpolluted conditions, seasonal variability resulted in higher hepatic hsp70 levels in late spring and summer. They also pointed out that this seasonality was temperature independent. Triebskorn (2012) indicated that biomarkers show different basis level according to seasons and basis levels of hepatic hsp70 in brown trout are much lower in autumn and winter than in spring and summer.

The observed changes in the hepatic glycogen levels as well as in the hepatic hsp70 levels are indications of the ability of the fish to respond to increased energy demands as part of an increase in water

temperature without losing their regulatory capability. In light of the temperature differences found in this study, one should bear in mind that the limit of tolerance range might not have been reached yet. Besides this, parasites are normally considered as stressors to their host´s and induced stress response measured by induced levels of HSP70 is to be expected (Basu et al., 2002). Additionally, parasites interfere with the physiology of their hosts and may therefore modify the host´s protection mechanisms (Sures, 2008a; Sures, 2008b). In addition, there´s also an additional stress on the nutritional status and therefore its energy reserves by the parasite´s metabolism (Bush et al., 2002). Therefore, parasites should be included as additional stressors when effects of thermal pollution are monitored under field conditions, as will be examined under chapter 5 and 6.

The impact of water temperature on the parasite communities in brown trout (*Salmo trutta fario* L.)

5

5.1 Introduction

Numerous studies described how knowledge on ecosystem function and integrity can be extended when taking a closer look on the relation between parasitism and ecological conditions in a given environment (Hudson et al., 2006; Lafferty et al., 2008). In aquatic ecosystems, parasites are frequently used as bioindicators for various aspects of host biology (Sures, 2008b). The species richness, as well as the abundance of fish parasites can even correlate with the "health" of aquatic ecosystem due to the close relationship between parasites, host biology and environmental conditions (Marcogliese et al., 2005). Life cycles of parasites are often complex, compromising definitive hosts as well as several intermediate hosts. For example, parasitic helminths often undertake substantial growth in aquatic invertebrates and fish intermediate hosts before achieving sexual maturation in definitive hosts (Mueller, 1965). This clearly demonstrates that parasite survival depends on the occurrence of all hosts in a stable community structure (Marcogliese & Cone, 1997).

Due to the complexity and the manifoldness of their life cycles, parasites are suitable bioindicators for changes in environmental conditions. Compared to their hosts, parasites are even more sensitive to certain environmental stressors, including water temperature (Landsberg et al., 1998). Such changes in environmental conditions, which affect their hosts directly or indirectly, lead to significant differences in the diversity and composition of fish parasite communities (MacKenzie et al., 1995). Depending on the type of stressor and parasite taxon, there are different ways of interactions between parasites and environmental stressors. Besides neutral effects, stressors could exhibit positive effect on parasites, which might benefit from weakened host and its increased vulnerability. On the other hand, stressors could cause negative impacts for parasites by increasing host´s mortality rate or reducing the number of intermediate hosts (Möller, 1987; Khan & Thulin, 1991; Lafferty, 1997; MacKenzie et al., 1995; Valtonen et al., 2003).

Water temperature, as major abiotic factor plays a fundamental role in controlling the biology of fish. As most fishes are poikilothermic ectotherms, their body temperature depends on seasonal variations in water temperature (Currie, 2011; Schulte & Columbia, 2011), which then again strongly influences physiological and immunological processes in fish (Martinez et al., 1994).

Therefore in poikilothermic hosts, parasites are also expected to be immediately and severely affected by alterations in temperature (Deutsch et al., 2008; Thomas & Blanford, 2003). As a consequence, variations in parasite susceptibility reflect the impact of temperature alterations on physiological and immunological processes in intermediate and definitive hosts, and on free living stages of parasitic life cycles (Blanar et al., 2009). For these reasons, parasites rank among the most sensitive bioindicators.
Recently, many studies on parasites of freshwater fish have been carried out (e.g. Kennedy et al., 1986; Sures et al., 1999; Knopf et al., 2007) with some focusing specifically on brown trout (Kennedy, 1978; Molloy et al., 1995; Kennedy & Hartvigsen, 2000; Dezfuli et al., 2001; Byrne et al., 2002; Quilichini et al., 2007). Furthermore, the effects of temperature on brown trout have gained attention (e.g. Lessard & Hayes, 2003; Ficke et al., 2007; Jonsson & Jonsson, 2009; Almodóvar et al., 2012), particularly within the context of the predicted climate change scenarios (IPCC, 2007). However, only a few studies addressed the use of trout parasites as bioindicators for thermal pollution (e.g. Hernandez et al., 2007; Quilichini et al., 2010).
The aim of this study was to investigate the influence of temperature alterations and seasonality on parasite communities of brown trout (*Salmo trutta fario* L). Furthermore the suitability of fish parasites as a bioindicator for thermal pollution was evaluated, as the occurrence and abundance of intermediate and paratenic hosts could be influenced by temperature regime and seasonality and thus indirectly affect the diversity and composition of parasite communities.

5.2 Materials and Methods

Detailed information on study area and sampling sites, as well as water temperature data are given in chapter 2.
Fish data used for parasite community structure analyses refer to chapter 4.3.1.

5.2.1 Parasite Sampling and Data Processing

Brown trout (see chapter 4.2.1) were dissected for parasite recovery following a standardized protocol. In brief, skin, scales, fins, gills, eyes, the digestive tract, liver, gall bladder, spleen, kidney, swim bladder, gonads muscle tissue and visceral cavity were examined for presence of parasites using a stereomicroscope (magnification x8 to x50). Individuals of each parasite species were identified and counted.
Prevalence (P, %), mean intensity (MI), relative abundance (pi, %), mean abundance (MA) and aggregation index (AI) of the respective parasite species were determined according to Bush et al. (1997).
The ecological indices of richness, diversity and equitability (Shannon and Weaver 1949; Pielou, 1978) for the description of the parasite communities (Kennedy, 1993) such as Brillouin index (HB), Shannon index (H),Shannon evenness (EH), Simpson's index (1-D) and Berger-Parker index (d) were calculated according to Magurran (1988) and Sures et al. (1999).

A comparative assessment of the impact of water temperature on parasite community structure dynamics was carried out using non-metric multi-dimensional scaling (NMDS) to ordinate cold and warm sampling sites using parasites abundance. The similarity in species composition between sampling sites was evaluated using the Bray-Curtis coefficient (Bray & Curtis, 1957) on square root transformed species abundance data. Contributions to similarity by abundant species were reduced by square root transformation, thereby increasing the importance of rare or uncommon species in the dataset (Clarke, 1993). NMDS was performed using 50 random starting configurations of sample points, while for cold vs. warm comparison a two-dimensional solution is presented.

In order to detect the parasite species mainly contributing to differences ("key discriminating" species), a SIMPER analysis was applied (Clarke, 1993; Clarke & Warwick, 2001). In addition, an analysis of similarities (ANOSIM) with a one-way layout for each locality (Ruhr, Lenne1 and Lenne2) was performed to test for differences between cold-warm sampling site groups. All analyses were performed using the software PRIMER (Version 6.1.13, PRIMER-E Ltd, 2009).

Nonparametric methods were used for further statistical analysis, because parasite abundances were highly skewed and contained many zero-counts. Differences in abundances between cold-warm site pairs were tested for significance using the Mann-Whitney U Test. Spearman's rank correlation coefficient was used to investigate associations between Brillouin index (HB) and physiological variables as the calculated condition factor (CF), hepatosomatic index (HSI) and gonadosomatic index (GSI) (see chapter 4.3.1).

All results were considered to be statistically significant when the p-value was less than or equal to 0.05. Levels of significance were set to $p \leq 0.0001$ (∗∗∗∗), $0.0001 < p \leq 0.001$ (∗∗∗), $0.001 < p \leq 0.01$ (∗∗), and $0.01 < p \leq 0.05$ (∗). Statistics were conducted using Statistica 12 (StatSoft 2013).

5.3 Results

5.3.1 Parasite Fauna

A total of 12 metazoan parasite species were recorded, including six salmon-specific specialists and six generalist species (see Table 5-1). Most of the parasite species were detected in the intestine followed by the swim bladder and inside the eyes.

Two species of the genus *Crepidostomum* were found, *C. farionis* (Müller, 1784) and *C. metoecus* (Braun, 1900). A clear distinction between these two species based on morphological characteristics could only be made using adult individuals. Due to the high number of juvenile individuals the findings have been pooled under the genus *Crepidostomum*.

Table 5-1: Parasite fauna of examined S*almo trutta fario*.

Infected Organ	Class	Parasite species	Specialist	Generalist
Eye	Trematoda	*Diplostomum* sp. (Nordmann, 1832)		X
		Tylodelphis clavata (Nordmann, 1832)		X
Swim bladder	Nematoda	*Cystidicola farionis* Fischer, 1798	X	
Intestine	Trematoda	*Crepidostomum* sp.	X	
	Cestoda	*Cyatocephalus truncatus* (Pallas, 1781)	X	
	Nematoda	*Cucullanus truttae* Fabricius, 1794		X
		Cystidicoloides ephemeridarum (Linstow, 1872)	X	
		Pseudocapillaria salvelini (Polyansky, 1952)	X	
		Raphidascaris acus (Bloch, 1779)		X
	Acanthocephala	*Echinorhynchus truttae* (Schrank, 1788)	X	
		Neoechinorhynchus rutili (Müller, 1780)		X
		Pomphorhynchus laevis (Zoega in Müller, 1776)		X

5.3.2 Metapopulation

The complete data on metapopulations are given in Appendix V.1.

Two genera of digenean trematodes (*Diplostomum* (metacercariae) and *Crepidostomum*) and two species of nematodes (*C. ephemeridarum*, *R. acus*) were recovered at all sampling sites during each sampling.

Metacercariae of the digenean trematode *T. clavata* were only found at the Ruhr cold site in autumn 2009 and were found at the Lenne1 sampling sites (except in spring and autumn 2010 at the cold site). At Lenne2, only one fish was infected with *T. clavata* (warm site autumn 2010).

The parasite species *C. farionis* (Nematoda) did not occur at both Ruhr sampling sites, was found at the Lenne1 sampling sites apart from one missing (warm site autumn 2009) and occurred at both Lenne2 sampling sites during each sampling.

The nematode species *C. truttae* was found at the cold Ruhr site in autumn 2010 only, merely occurred at Lenne1 warm site and was not detected at both Lenne2 sampling sites.

There were no significant differences in abundance of *T. clavata*, *C. farionis*, *C. truttae* and *P. laevis* between all cold-warm site pairs during the sampling period.

Three generalist species (*Diplostomum* sp., *C. truncatus* and *R. acus*) and one salmon-specific species (*P. salvelini*) showed an increase in abundance at the warm sampling sites, even with statistical significance in some cases as shown in Figure 5-1 to Figure 5-4.

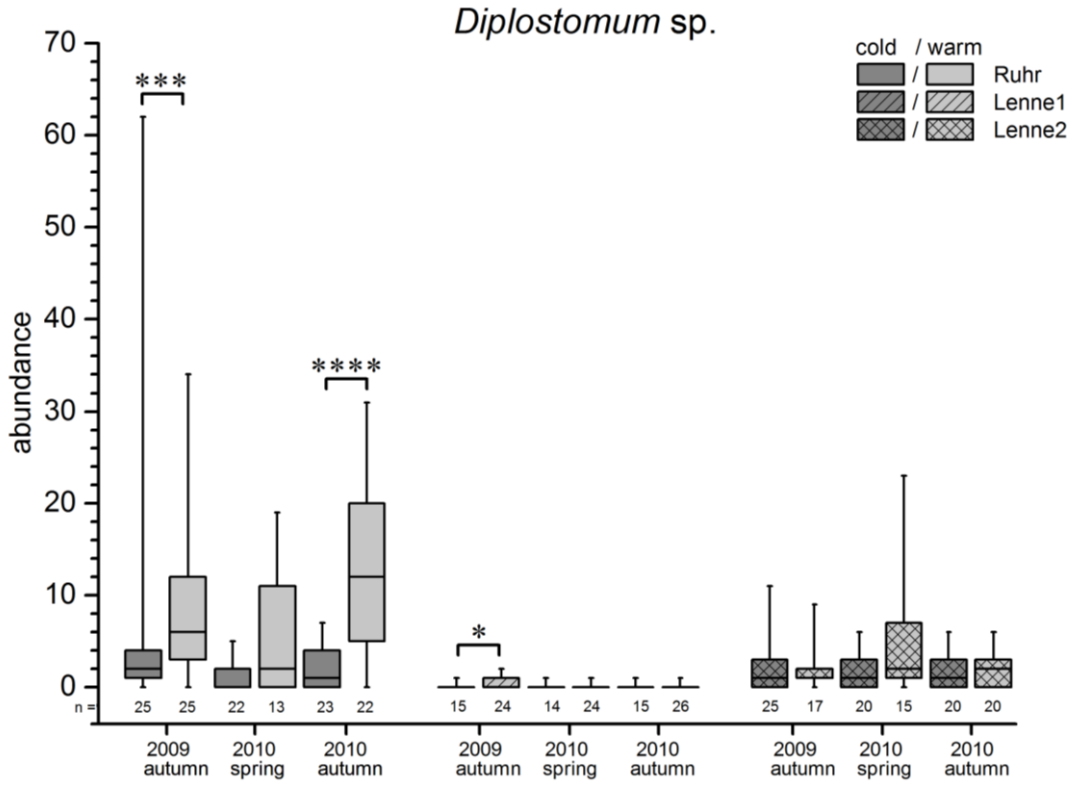

Figure 5-1: Differences in abundance of *Diplostomum* sp.

(median box: 25-75%, whisker: min/max; n: number of brown trout; *: p<0.05; ***: p<0.001;****: p<0.0001, significance tested with Mann-Whitney U test)

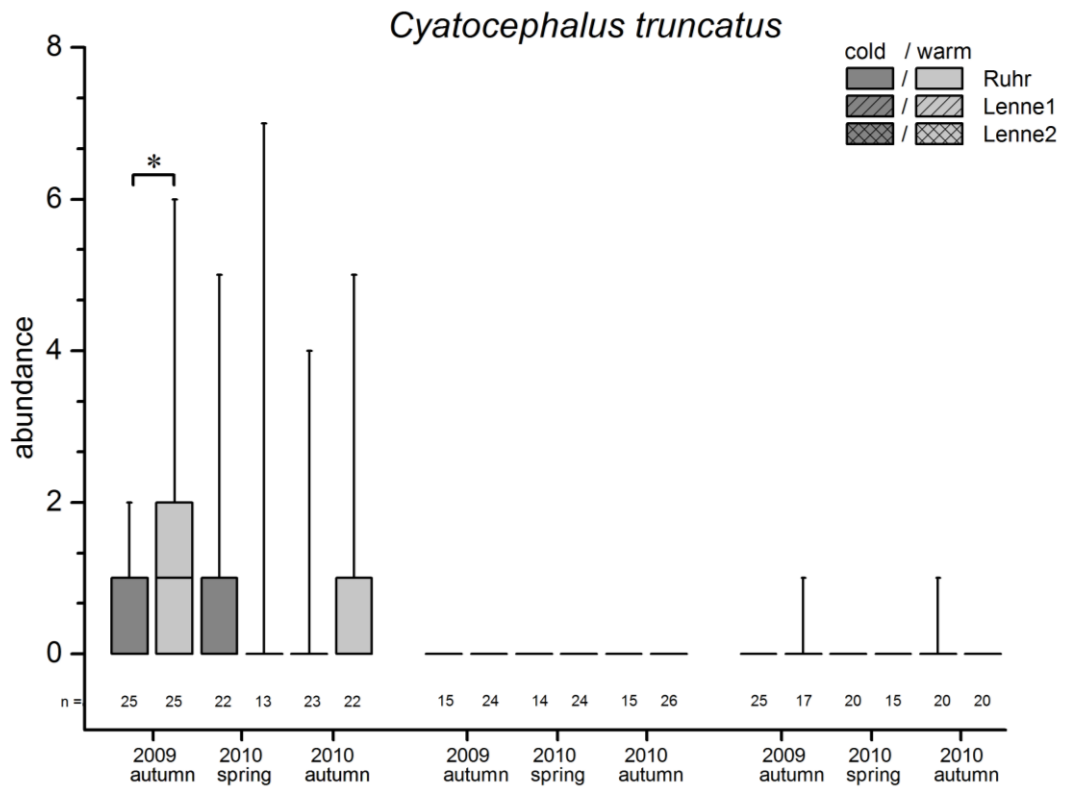

Figure 5-2: Differences in abundance of *Cyatocephalus truncatus.*

(median box: 25-75%, whisker: min/max; n: number of brown trout; *: $p<0.05$, significance tested with Mann-Whitney U test.)

The cestode *C. truncatus* was found at both Ruhr sampling sites over the whole sampling period. *C. truncatus* was not found at both Lenne1 sampling sites and was only detected twice at the Lenne2 sampling sites (warm site autumn 2009; cold site autumn 2010).

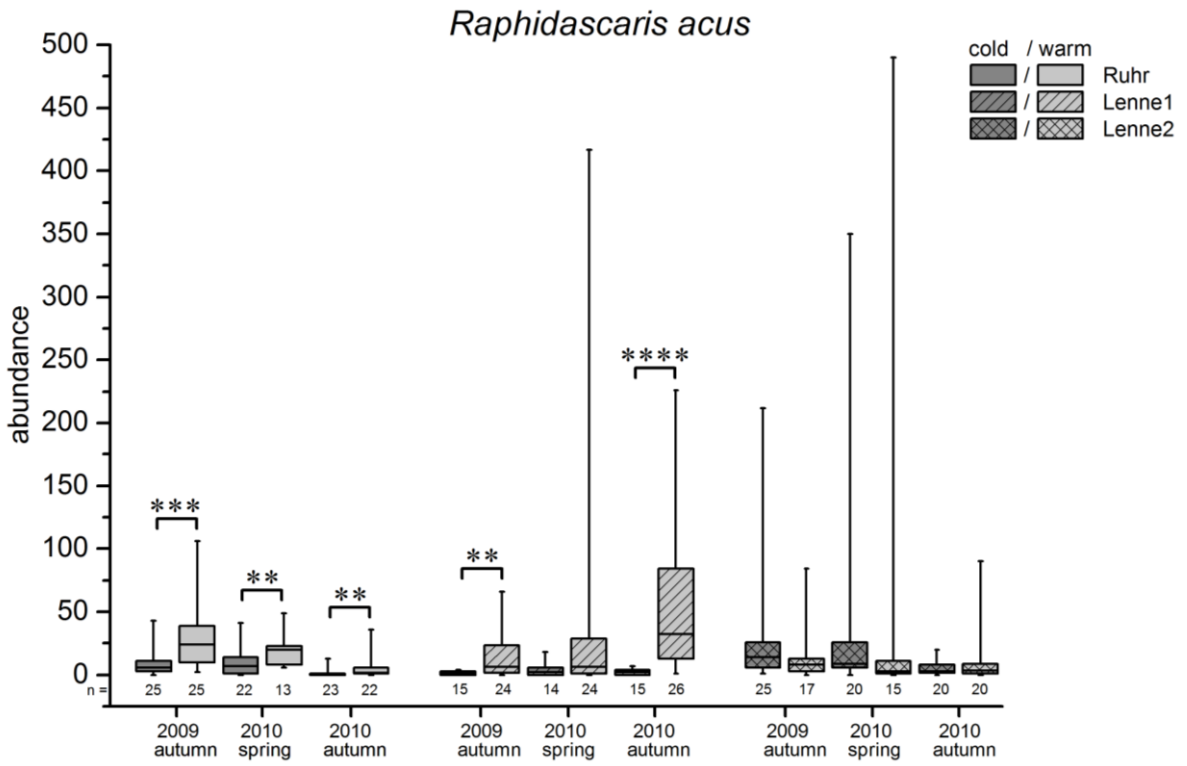

Figure 5-3: Differences in abundance of *Raphidascaris acus.*

(median box: 25-75%, whisker: min/max; n: number of brown trout; **: p<0.01; ***: p<0.001;****: p<0.0001, significance tested with Mann-Whitney U test.)

P. salvelini (Nematoda) was found during the whole sampling period at both Lenne1 and at both Lenne2 sampling sites as well as at Ruhr sites (autumn 2009 and spring 2010, cold site; autumn 2010, warm site).

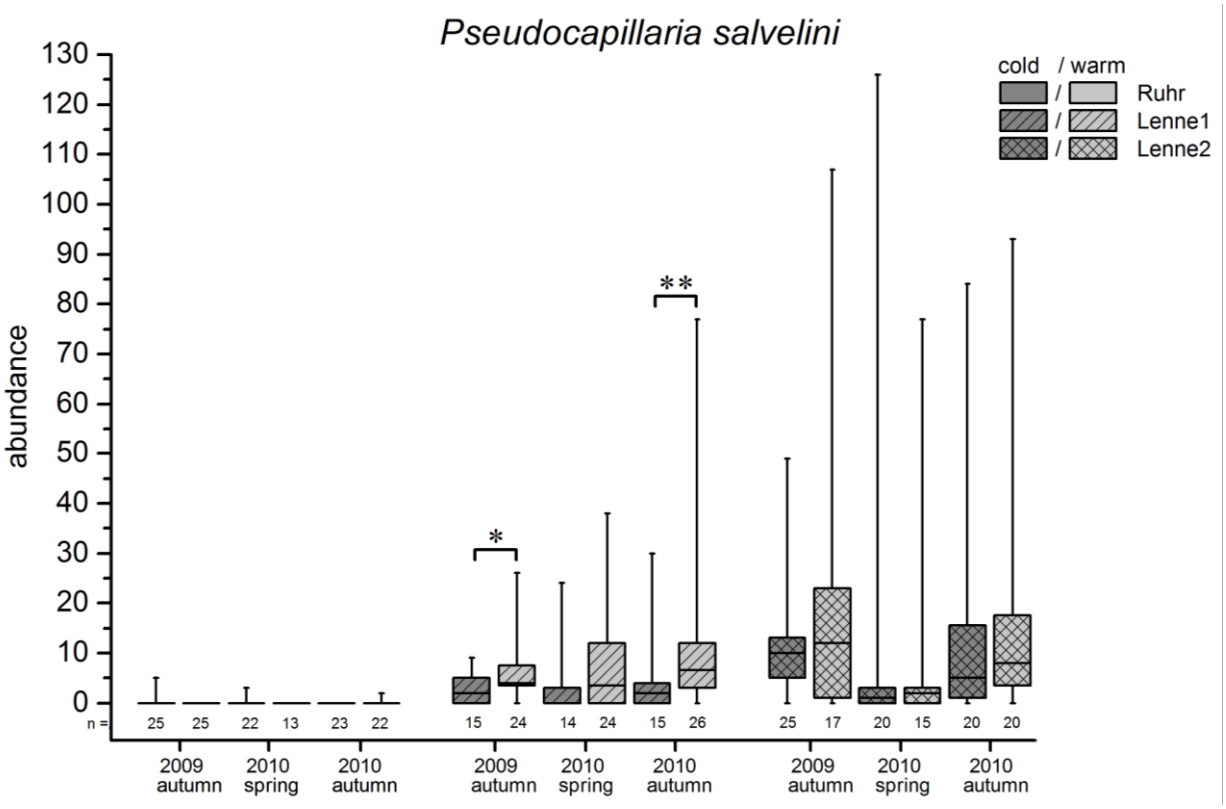

Figure 5-4: Differences in abundance of *Pseudocapillaria salvelini*.

(median box: 25-75%, whisker: min/max; n: number of brown trout; *: $p<0.05$; **: $p<0.01$, significance tested with Mann-Whitney U test.)

The two salmon-specific species *Crepidostomum* sp. and *C. ephemeridarum* showed a decrease in abundance at the warm sampling sites, in some cases significantly as shown in Figure 5-5 and Figure 5-6.

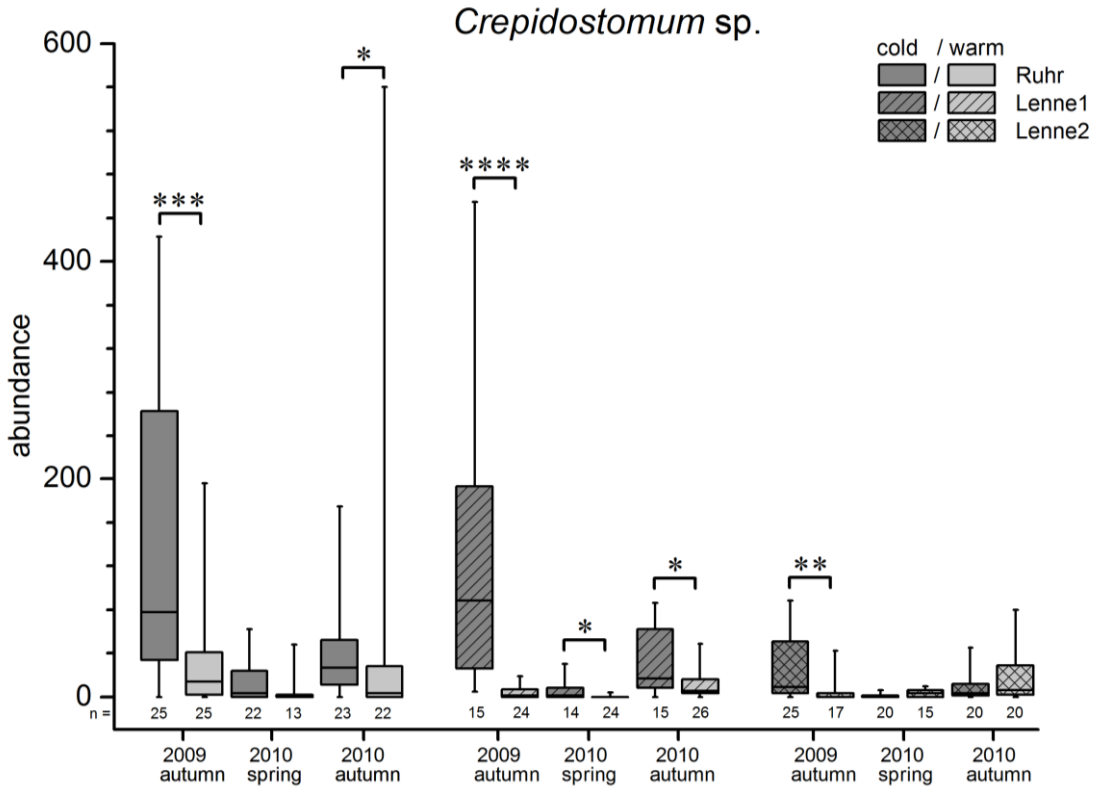

Figure 5-5: Differences in abundance of *Crepidostomum* sp.

(median box: 25-75%, whisker: min/max; n: number of brown trout; *: p<0.05; **: p<0.01; ***: p<0.001;****: p<0.0001, significance tested with Mann-Whitney U test.)

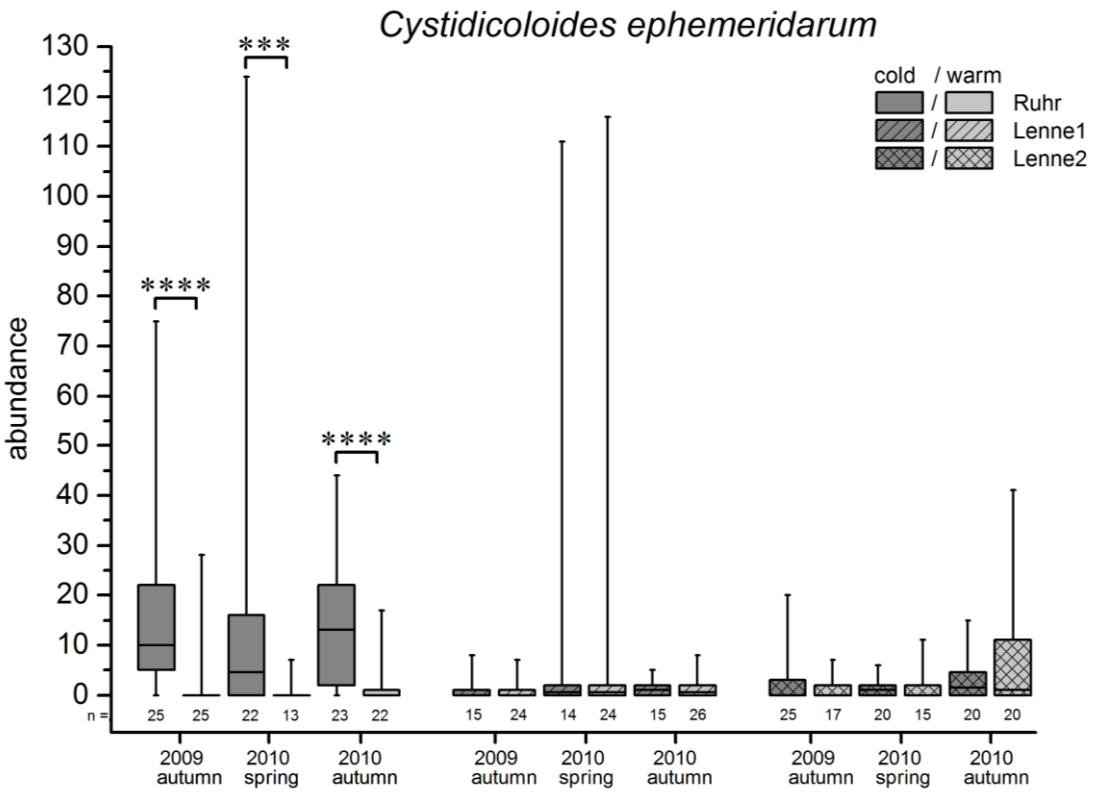

Figure 5-6: Differences in abundance of *Cystidicoloides ephemeridarum*.

(median box: 25-75%, whisker: min/max; n: number of brown trout; ***: p<0.001;****: p<0.0001, significance tested with Mann-Whitney U test.)

The abundance of the salmon specific acanthocephalan *E. truttae* as well as the abundance of *N. rutili* (generalist) indicated a general increase at the warm sites. However, the analyses showed only for samplings at the Ruhr a significant increase in abundance from cold to warm (see Figure 5-7 and Figure 5-8).

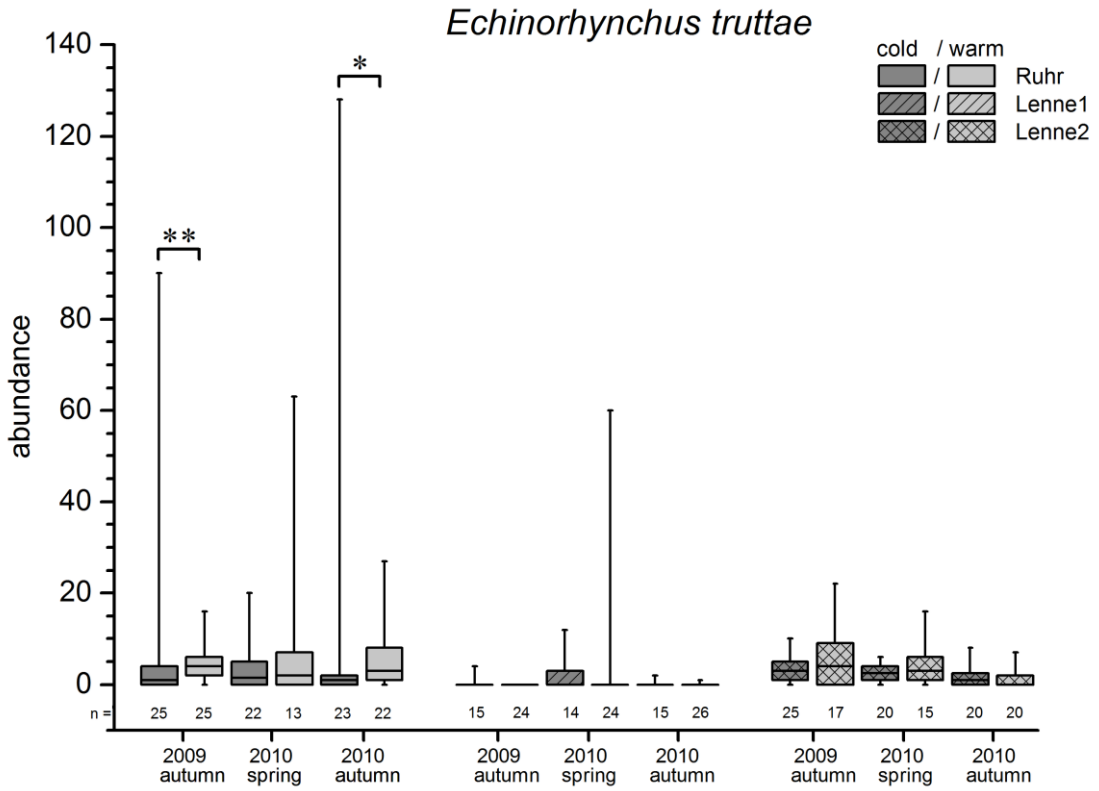

Figure 5-7: Differences in abundance of *Echinorhynchus truttae.*

(median box: 25-75%, whisker: min/max; n: number of brown trout; *: $p<0.05$; **: $p<0.01$, significance tested with Mann-Whitney U test.)

The parasite species *E. truttae* was found at all the Ruhr, Lenne1 and Lenne2 sampling sites during each sampling with one exception at the Lenne1 warm site in autumn 2009.

N. rutili also occurred at all the cold and warm sampling sites (Ruhr, Lenne1 and Lenne2) with two missings in autumn 2010 at the Lenne1 and the Lenne2 cold sites.

The acanthocephalan *P. laevis* was not detected at both Ruhr, as well as both Lenne1 sampling sites and only occurred at the Lenne2 cold site in autumn 2010.

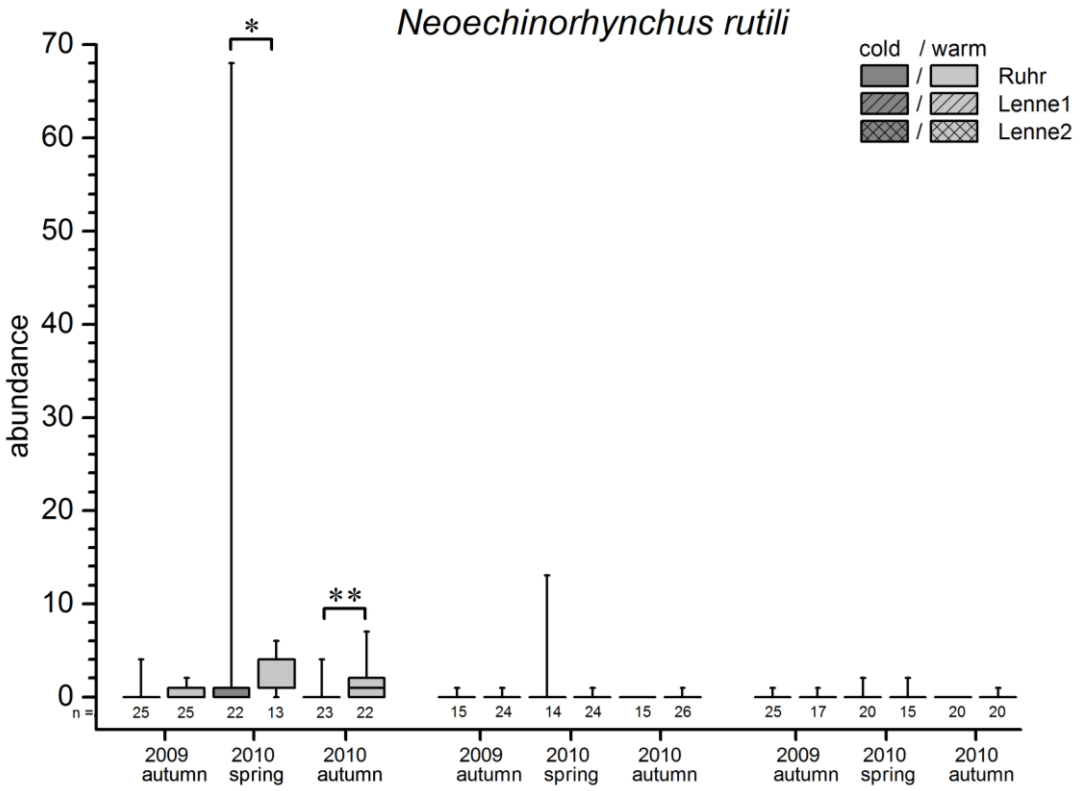

Figure 5-8: Differences in abundance of *Neoechinorhynchus rutili.*

(median box: 25-75%, whisker: min/max; n: number of brown trout; *: $p<0.05$; **: $p<0.01$, significance tested with Mann-Whitney U test.)

5.3.3 Component Community

Diversity characteristics of the component communities are presented in Table 5-2. Species richness varies between seven and ten taxa over all sampling sites, following no clear pattern.

Higher parasite diversity (Shannon index and Simpson's index) was recorded in autumn at the warm sites at Ruhr and Lenne1, whereas in spring 2010 parasite diversity was lower than at the cold sites at Ruhr and Lenne1. At the Lenne2 warm site, only 2009 autumn samples showed the same pattern. Samples in 2010 followed an opposite trend.

As expected, the values of Berger-Parker dominance index for parasites at the Ruhr and the Lenne1 warm sites followed a contrary pattern, being lowest in autumn samples and reaching the highest values for spring samples. At the Lenne2 warm site, values of Berger-Parker dominance index were lower than the respective cold site at all sampling periods.

Similar dominance structure of parasite communities was observed at the Ruhr and the Lenne1 sites with *Crepidostomum* sp. (Trematoda) being the dominant species in autumn samples and the nematode species *R. acus* being dominant in spring samples. Species dominance at the Lenne2 sites broke ranks by being dominated by *R. acus* in autumn 2009 and spring 2010 and being dominated by the nematode species *P. salvelini* in autumn 2010.

Table 5-2: Comparison of the diversity characteristics of the component communities of parasites at sampling sites.

(n: number of brown trout; n_{taxa}: number of parasite taxa; Ind.: number of parasite individuals; H: Shannon index; EH: Shannon evenness; 1-D: Simpson´s index; d: Berger-Parker index; *C. ephemeridarum*: *Cystidicoloides ephemeridarum*; *P. salvelini*: *Pseudocapillaria salvelini; R. acus: Raphidascaris acus*)

Site and Season	n	n_{taxa}	Ind.	H	EH	1-D	d	Dominant species
Rc_09a	25	9	4322	0.82	0.37	0.37	0.79	*Crepidostomum* sp.
Rw_09a	25	7	1948	1.36	0.70	0.68	0.39	*R. acus*
Rc_10s	22	8	1104	1.58	0.76	0.75	0.38	*C. ephemeridarum*
Rw_10s	13	7	588	1.45	0.74	0.69	0.49	*R. acus*
Rc_10a	23	8	1481	1.10	0.53	0.55	0.63	*Crepidostomum* sp.
Rw_10a	22	8	1495	1.28	0.61	0.61	0.58	*Crepidostomum* sp.
L1c_09a	15	9	2021	0.29	0.13	0.10	0.95	*Crepidostomum sp.*
L1w_09a	24	8	654	1.30	0.62	0.64	0.54	*R. acus*
L1c_10s	14	8	450	1.50	0.72	0.70	0.50	*C. ephemeridarum*
L1w_10s	24	10	1433	1.15	0.50	0.55	0.65	*R. acus*
L1c_10a	15	7	563	0.85	0.44	0.39	0.77	*Crepidostomum sp.*
L1w_10a	26	10	2162	0.95	0.41	0.49	0.69	*R. acus*
L2c_09a	25	10	2057	1.48	0.64	0.72	0.38	*R. acus*
L2w_09a	17	9	911	1.57	0.69	0.73	0.36	*R. acus*
L2c_10s	20	8	1232	1.13	0.54	0.52	0.67	*R. acus*
L2w_10s	15	8	936	1.34	0.65	0.60	0.61	*R. acus*
L2c_10a	20	9	700	1.67	0.76	0.77	0.37	*P. salvelini*
L2w_10a	20	9	1115	1.57	0.72	0.75	0.32	*Crepidostomum sp.*

The ordination through NMDS revealed a clear separation of Ruhr and Lenne1 samples according to temperature exposure (Figure 5-9 and Figure 5-10). However, for the Lenne2 samples such clear separation was not observed (Figure 5-11).

The ANOSIM test based on Bray-Curtis similarity confirmed such separation, showing no significant effects of temperature on parasite component communities. The SIMPER analysis showed that the average dissimilarity between cold and warm groups was highest at Lenne1 (51.1 %), followed by Ruhr (32.2 %) and Lenne2 (20.6 %) (Table 5-3).

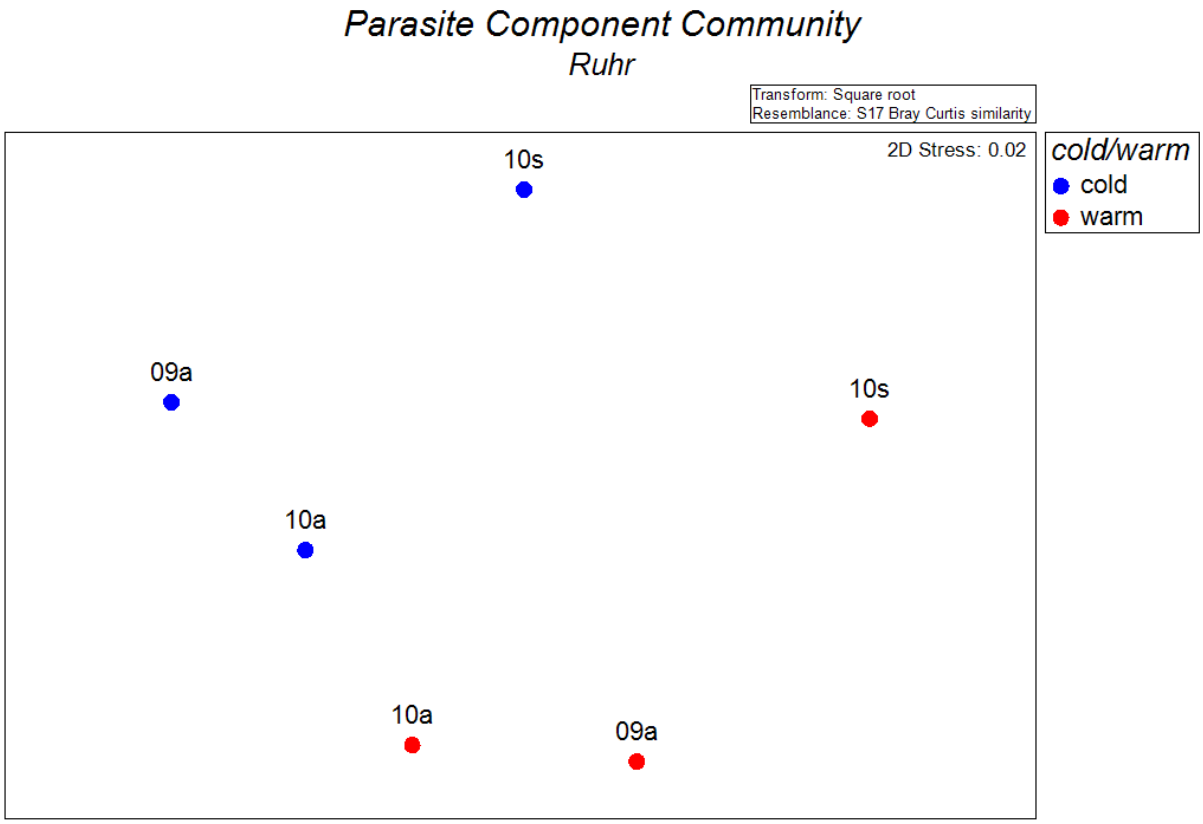

Figure 5-9: Non-metric multidimensional scaling of temperature impact on parasite component communities at Ruhr.

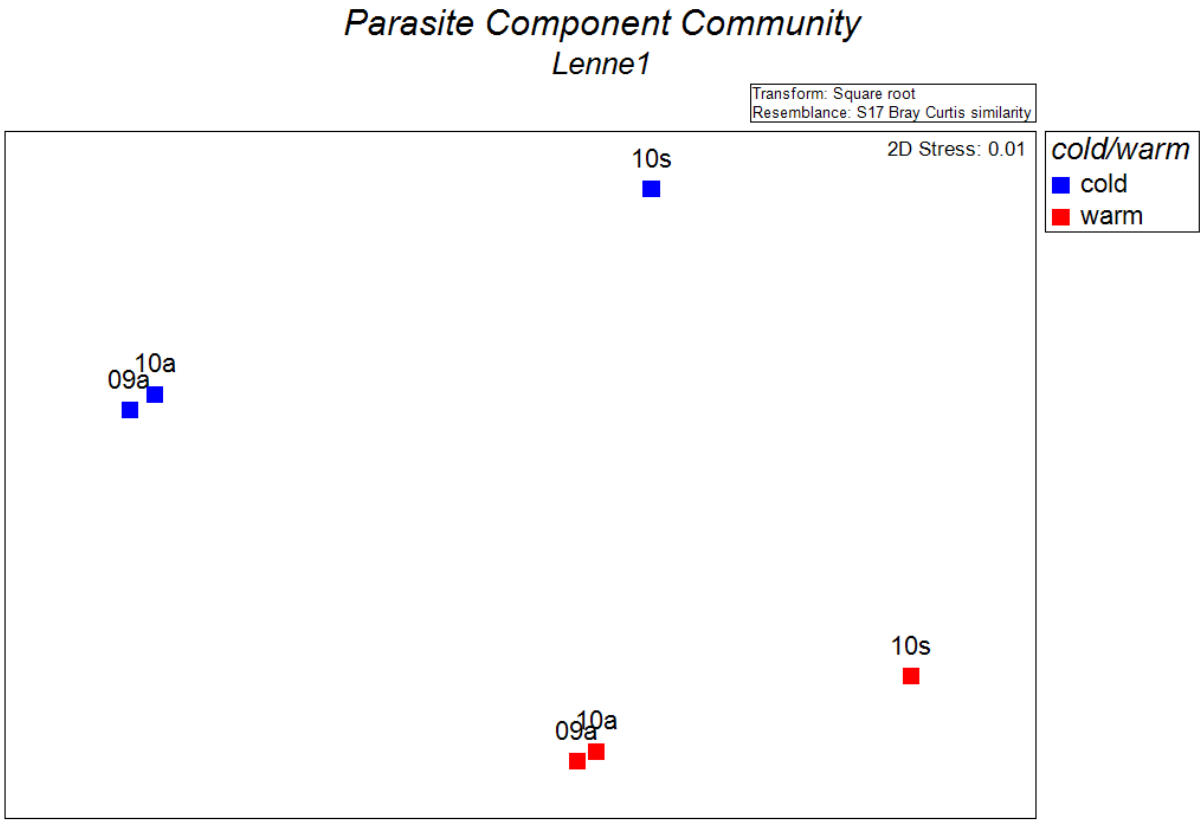

Figure 5-10: Non-metric multidimensional scaling of temperature impact on parasite component communities at Lenne1.

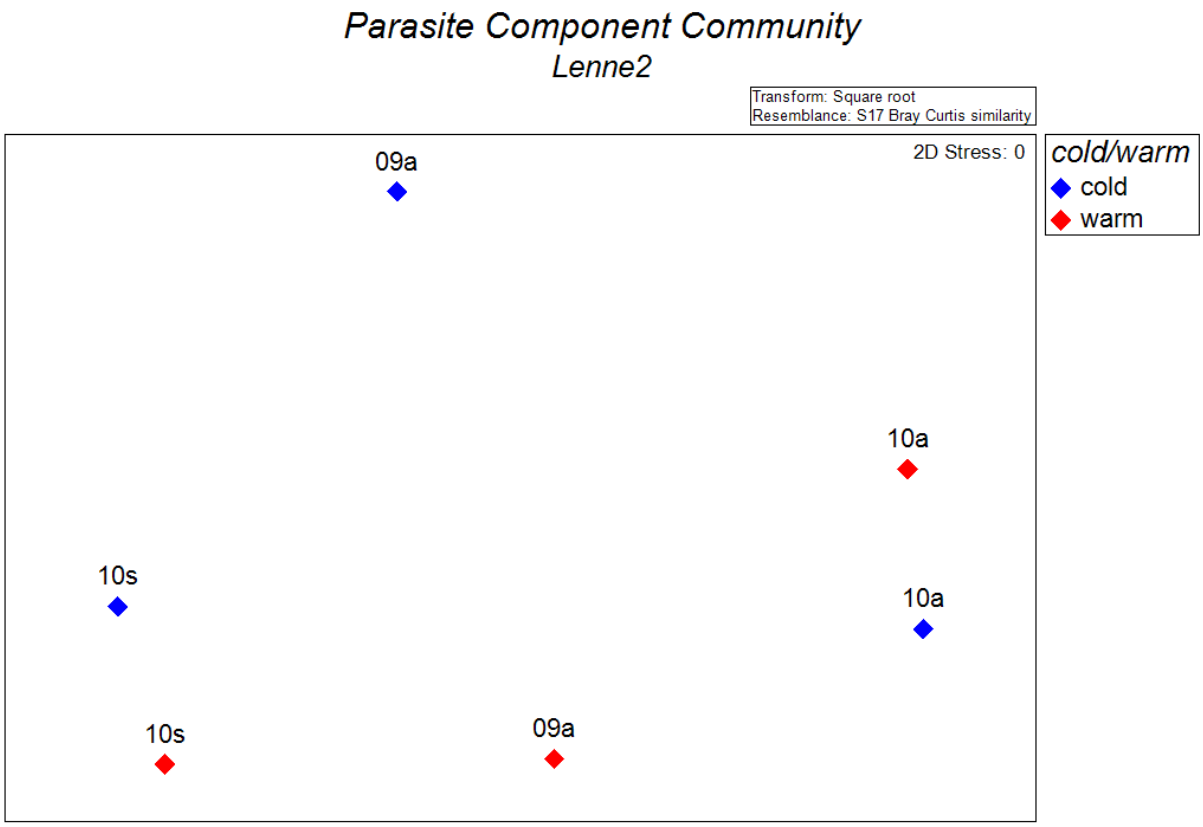

Figure 5-11: Non-metric multidimensional scaling of temperature impact on parasite component communities at Lenne2.

Table 5-3: Outcome from one-way ANOSIM tests (R and p values) and SIMPER analyses on parasite component communities.

(Av. Dissimilarity = average dissimilarity between cold and warm group)

Locality	Ruhr	Lenne1	Lenne2
Global R-value	0.593	0.852	-0.222
p-value	0.1	0.1	0.8
Av. Dissimilarity (%)	32.19	51.10	20.56

Further, the SIMPER analysis identified five species that were most responsible for distinctions between cold and warm groups at the Ruhr (Table 5-4). The two species, *Crepidostomum* sp. and *C. ephemeridarum*, contribute collectively to 58.4 % of the dissimilarity between the two groups. Three additional discriminating species were identified (*R. acus*, *Diplostomum* sp. and *N. rutili*).

At the Lenne1 sites, seven species were most responsible for cold and warm group distinctions (Table 5-5). At the Lenne1 sites the two species, *R. acus* and *Crepidostomum* sp., contribute collectively to 57.9 % of the dissimilarity between the two groups. Furthermore five additional species (*P. salvelini*, *C. ephemeridarum*, *E. truttae*, *C. truttae* and *N. rutili*) were identified as being most responsible for cold and warm distinctions (Table 5-5).

At the Lenne2 sites, the SIMPER analysis identified also seven species that were most responsible for distinctions between cold and warm groups (Table 5-6). The two species, *R. acus* and *Crepidostomum* sp., contribute collectively to 55.3 % of the dissimilarity between the two groups. The following five additional discriminating species were identified: *P. salvelini*, *C. ephemeridarum*, *C. farionis*, *E. truttae* and *Diplostomum* sp.

Table 5-4: The outcome of a SIMPER analysis on square-root transformed data listing those species that contributed most to the dissimilarity between cold and warm component communities at Ruhr (cut-off set at 90% contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Ruhr				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Crepidostomum sp	35.12	21.48	10.52	32.68
Cystidicoloides ephemeridarum	19.10	5.07	8.29	25.75
Raphidascaris acus	11.37	18.38	5.08	15.77
Diplostomum sp.	7.67	13.54	3.74	11.62
Neoechinorhynchus rutili	5.11	4.99	1.55	4.81

Table 5-5: The outcome of a SIMPER analysis on square-root transformed data listing those species that contributed most to the dissimilarity between cold and warm component communities at Lenne1 (cut-off set at 90% contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Lenne1				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Raphidascaris acus	6.10	29.24	17.12	33.56
Crepidostomum sp	24.18	9.28	12.41	24.33
Pseudocapillaria salvelini	6.73	14.79	5.99	11.74
Cystidicoloides ephemeridarum	7.92	8.19	3.67	7.20
Echinorhynchus truttae	3.46	3.89	3.37	6.61
Cucullanus truttae	0.00	3.32	2.64	5.17
Neoechinorhynchus rutili	2.03	1.47	1.47	2.89

Table 5-6: The outcome of a SIMPER analysis on square-root transformed data listing those species that contributed most to the dissimilarity between cold and warm component communities at Lenne2 (cut-off set at 90% contribution to total similarity).
(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Lenne2				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Raphidascaris acus	22.23	18.44	5.93	28.82
Crepidostomum sp	14.71	11.87	5.45	26.48
Pseudocapillaria salvelini	15.97	15.61	2.24	10.92
Cystidicoloides ephemeridarum	7.37	7.37	1.92	9.32
Cystidicola farionis	7.29	5.09	1.54	7.49
Echinorhynchus truttae	7.12	7.68	1.25	6.09
Diplostomum sp.	6.43	6.75	0.65	3.18

5.3.4 Infracommunity

Prevalences (P, %) of coexisting parasite species of brown trout are presented in Figure 5-12 to Figure 5-14. Brown trout at the Ruhr cold site were infected with up to eight parasite species, 70 % of all fish were infected with three to five parasite species. At the Ruhr warm site the number of coexisting parasite taxa ranged between one and seven, more than 58 % of brown trout were infected with five or six parasite species simultaneously (see Figure 5-12).

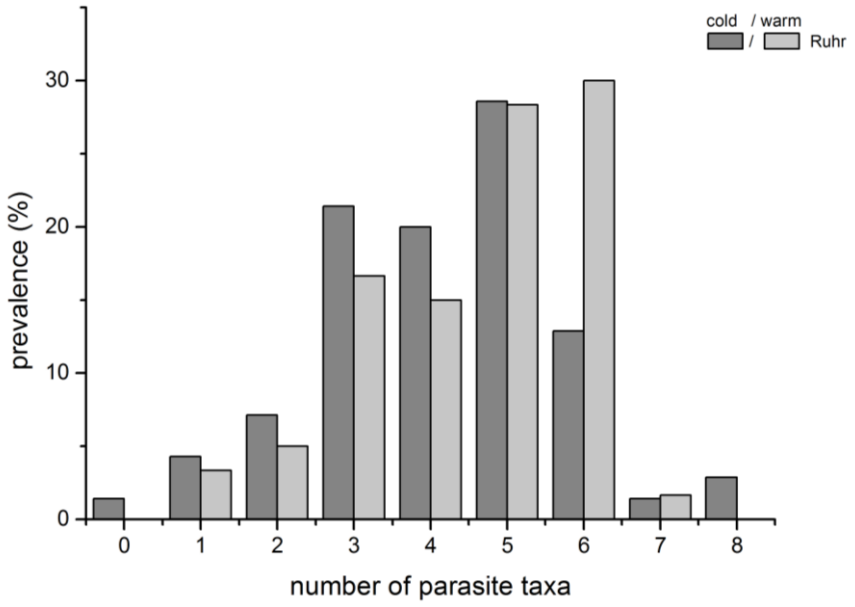

Figure 5-12: Prevalence of coexisting parasite taxa of *Salmo trutta fario* at Ruhr.

The number of coexisting parasite species ranged between zero to seven for the Lenne1 cold site as well as for the Lenne1 warm site.

At both sites more than 50 % of brown trout are infected with three or four parasite species (see Figure 5-13).

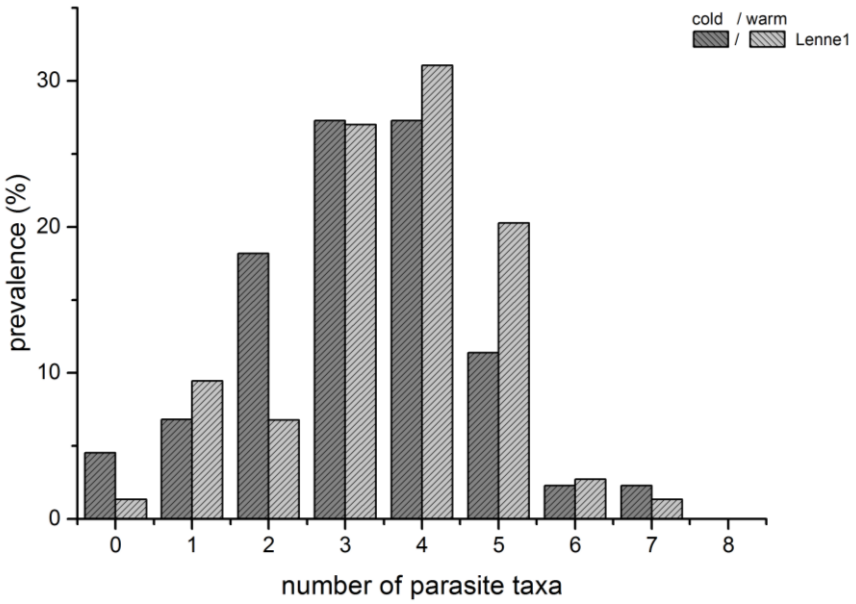

Figure 5-13: Prevalence of coexisting parasite taxa of *Salmo trutta fario* at Lenne1.

At the Lenne2 cold site brown trout were infected with two to seven parasite species, 55 % of all fish were infected with either five or six parasite species simultaneously. At the Lenne2 warm site, the number of coexisting parasite taxa ranged between two and eight, more than 60 % of all brown trout were infected with four or five parasite species (see Figure 5-14).

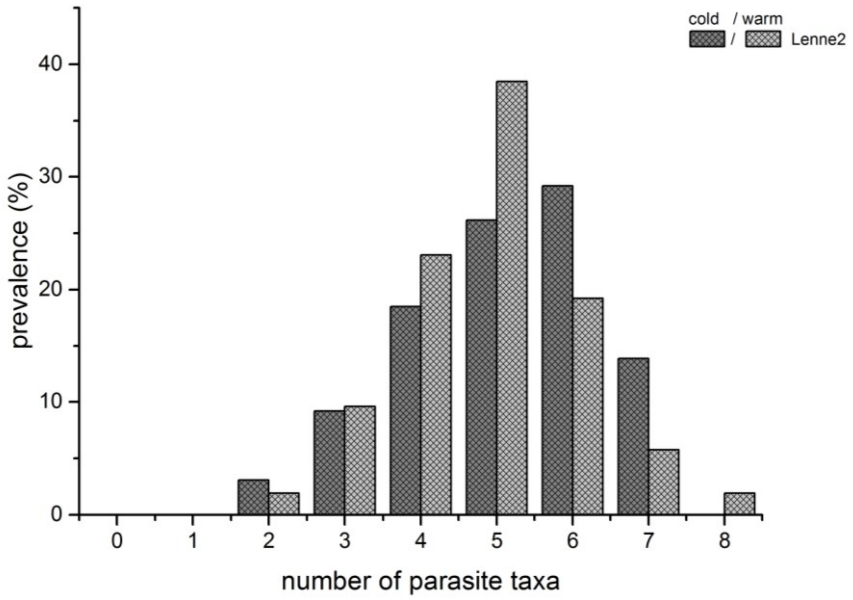

Figure 5-14: Prevalence of coexisting parasite taxa of *Salmo trutta fario* at Lenne2.

Summarized data on the parasite infracommunity structures are given in appendix VI.2.

Mean Brillouin-index (HB) showed a significant increase in autumn samples at the Ruhr warm site (09a: $p < 0.01$; 10a: $p < 0.05$), with a slightly decrease in spring 2010; likewise for the Lenne1 warm site, but only significant in autumn 2009 ($p < 0.001$) (Figure 5-15). Mean HB at the Lenne2 warm site showed an inverse trend with a decrease in autumn 2009 and 2010 and an increase in spring 2010 (see Figure 5-15).

Positive correlations were obtained between the Brillouin-index and hepatosomatic index for the Ruhr cold ($r = 0.267$; $p < 0.05$) and warm ($r = 0.321$; $p < 0.05$) samplings, but not for any sampling at Lenne1 and Lenne2.

Brillouin-index also showed negative correlations with gonadosomatic index for the Lenne1 cold site ($r = -0.326$) and the Lenne2 warm site ($r = -0.346$) samplings, both significant at $p < 0.05$.

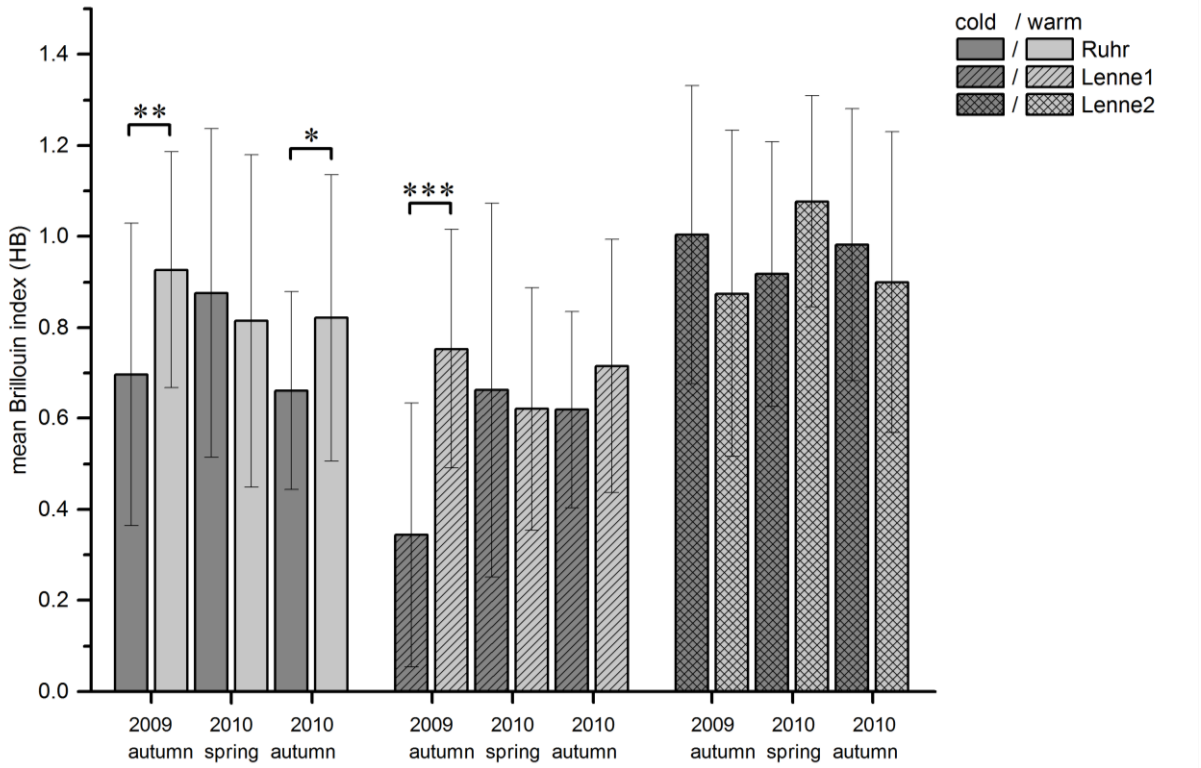

Figure 5-15: Brillouin index (mean ± SD) of *Salmo trutta fario*.

n: min 13 to max 26; *: p<0.05; **: p<0.01; ***: p<0.001, significance tested with Mann-Whitney U test.

The ordination through NMDS revealed a clear separation of the Ruhr and the Lenne1 samples according to temperature exposure (Figure 5-16 and Figure 5-17). However, no clear separation of the Lenne2 samples was revealed (Figure 5-18).

The ANOSIM test based on Bray-Curtis similarity confirmed the latter results, whereas no significant effects of temperature on parasite infracommunities were observed. The SIMPER analysis showed that the average dissimilarity between cold and warm groups was highest at Lenne1 (67.7 %), followed by Ruhr (61.7 %) and Lenne2 (52.2 %) (Table 5-7).

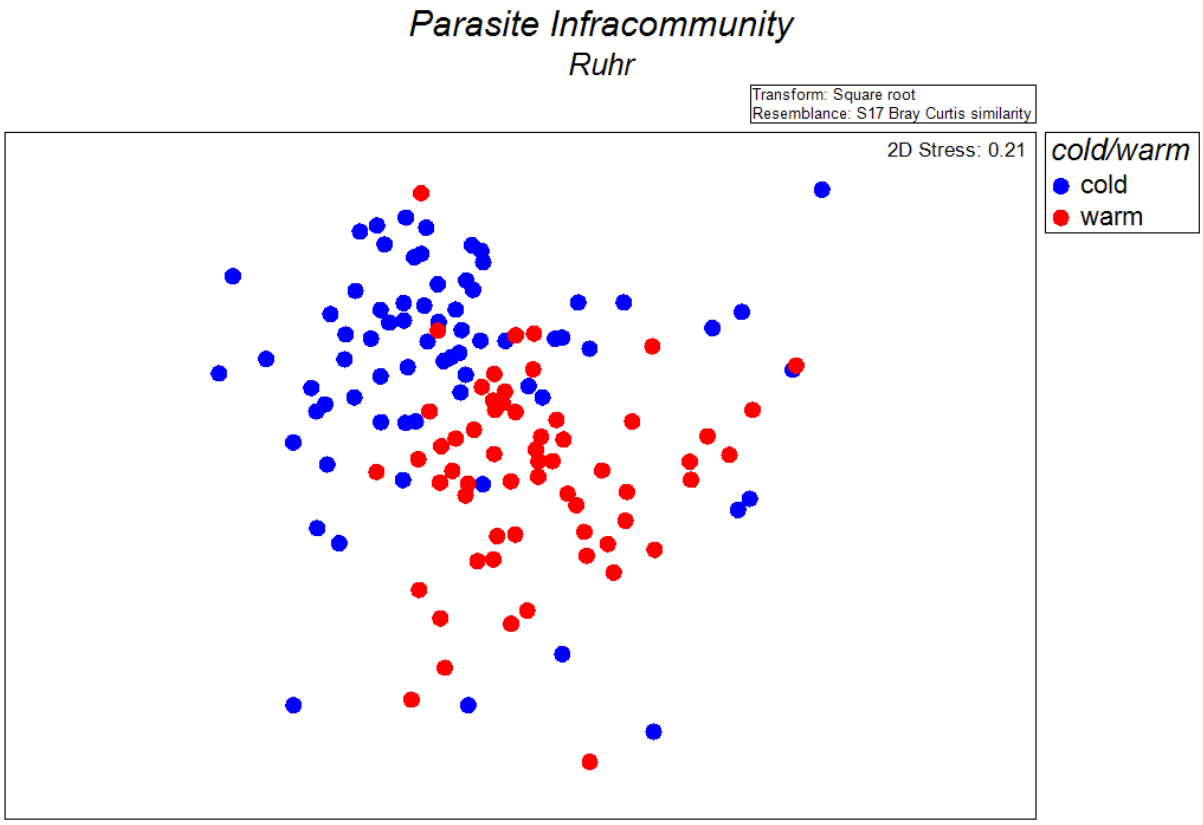

Figure 5-16: Non-metric multidimensional scaling of temperature impact on parasite infracommunities at Ruhr.

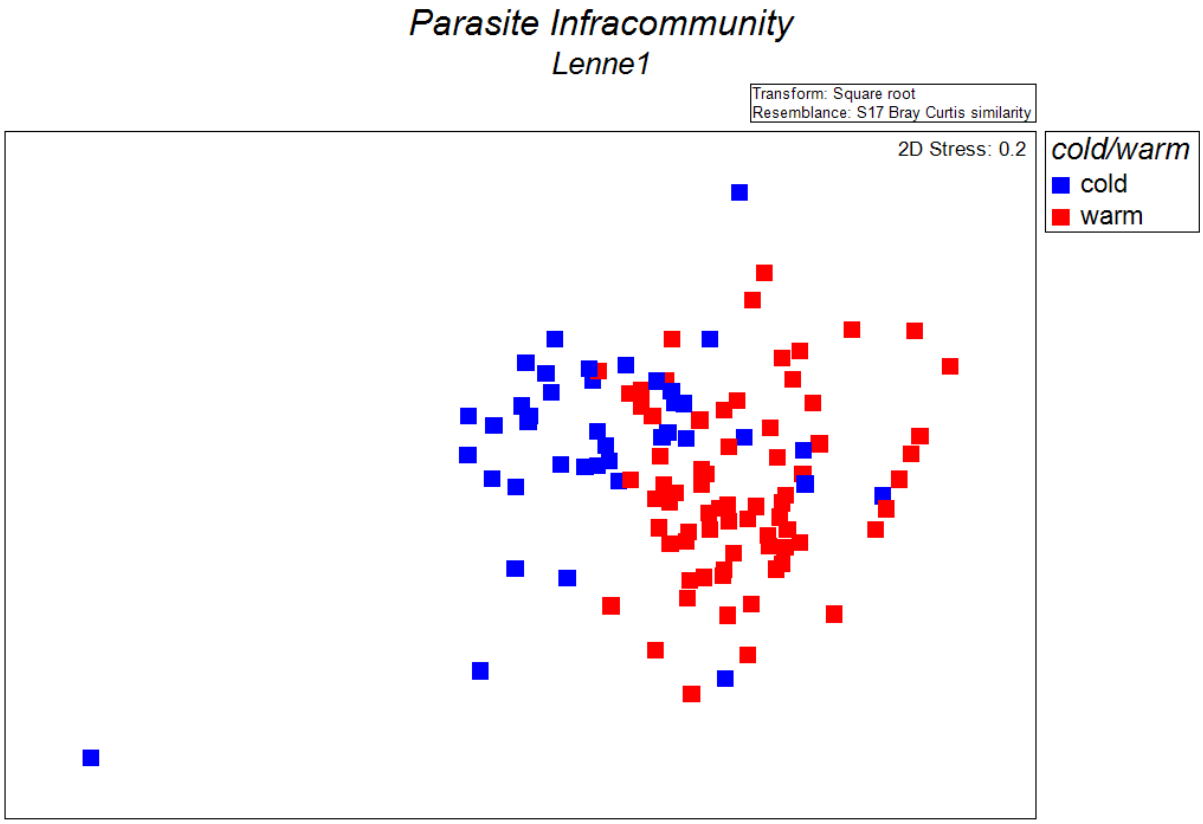

Figure 5-17: Non-metric multidimensional scaling of temperature impact on parasite infracommunities at Lenne1.

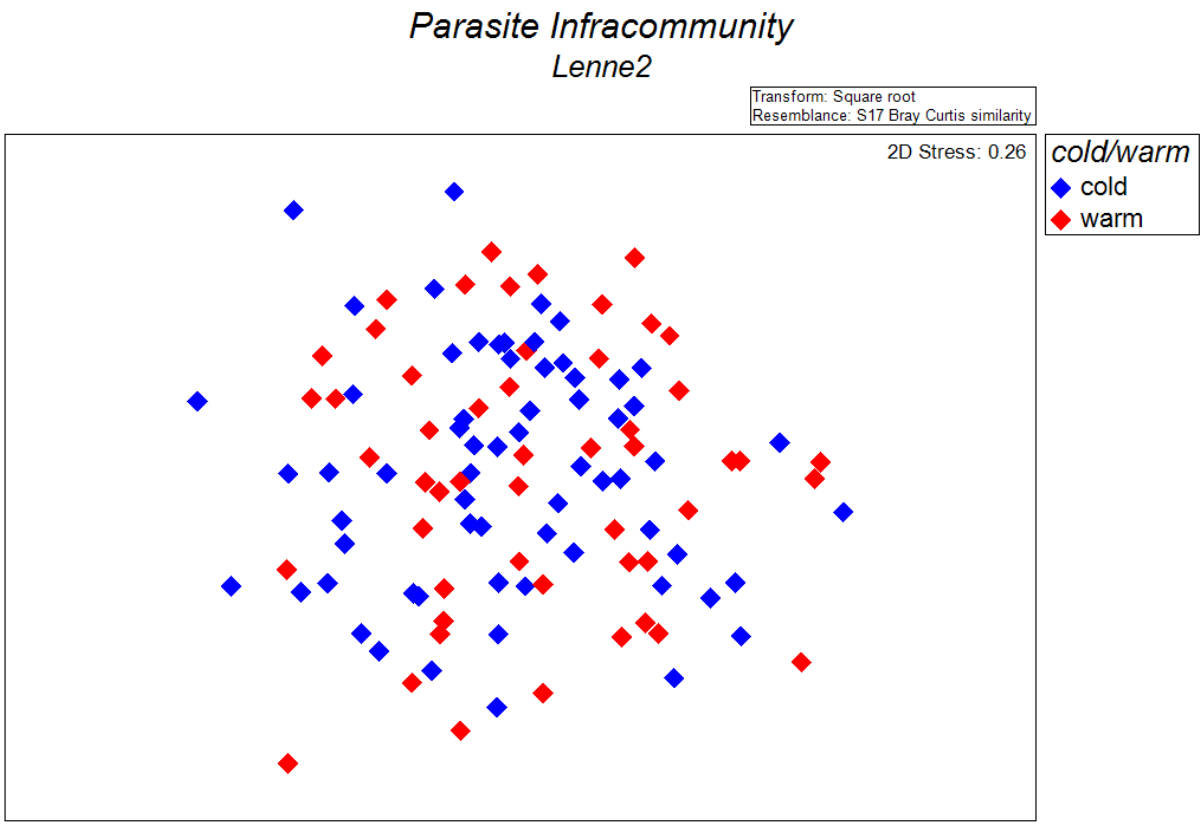

Figure 5-18: Non-metric multidimensional scaling of temperature impact on parasite infracommunities at Lenne2.

Table 5-7: Outcome from one-way ANOSIM tests (R and p values) and SIMPER analyses on parasite infracommunities. (Av. Dissimilarity = average dissimilarity between cold and warm group)

Locality	Ruhr	Lenne1	Lenne2
Global R-value	0.289	0.308	0.021
p-value	0.001	0.001	0.109
Av. Dissimilarity (%)	61.73	67.74	52.20

Further, the SIMPER analysis identified six species that were most responsible for distinctions between cold and warm groups at Ruhr (Table 5-8). Three species, *Crepidostomum* sp., *C. ephemeridarum* and *R. acus* contribute collectively to 64.2 % of the dissimilarity between the two groups. Three additional discriminating species were identified (*Diplostomum* sp., *E. truttae* and *N. rutili*).

For Lenne1, six species were most responsible for cold and warm group distinctions (Table 5-9). The two species, *Crepidostomum* sp. and *R. acus*, contribute collectively to 59.0 % of the dissimilarity between the two groups. *P. salvelini*, *C. ephemeridarum*, *E. truttae* and *Diplostomum* sp. were identified as the four additional discriminating species.

For Lenne2, three species, *R. acus*, *P. salvelini* and *Crepidostomum* sp., contribute collectively to 60.6 % of the dissimilarity between the two groups. Four additional discriminating species were identified (*C. ephemeridarum*, *E. truttae*, *C. farionis* and *Diplostomum* sp.).

Table 5-8: The outcome of a SIMPER analysis on square-root transformed data listing those species that contributed most to the dissimilarity between cold and warm infracommunities at Ruhr (cut-off set at 90 % contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Ruhr				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Crepidostomum sp	6.20	3.35	18.45	29.89
Cystidicoloides ephemeridarum	3.19	0.51	10.59	17.15
Raphidascaris acus	1.91	3.64	10.57	17.13
Diplostomum sp.	1.13	2.64	8.51	13.78
Echinorhynchus truttae	1.41	2.04	7.08	11.46
Neoechinorhynchus rutili	0.47	0.81	3.42	5.55

Table 5-9: The outcome of a SIMPER analysis on square-root transformed data listing those species that contributed most to the dissimilarity between cold and warm infracommunities at Lenne1 (cut-off set at 90 % contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Lenne1				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Crepidostomum sp	5.57	1.45	22.22	33.10
Raphidascaris acus	1.29	4.64	17.35	25.85
Pseudocapillaria salvelini	1.24	2.40	10.02	14.93
Cystidicoloides ephemeridarum	1.21	0.97	7.32	10.90
Echinorhynchus truttae	0.43	0.25	2.58	3.85
Diplostomum sp.	0.07	0.33	2.05	3.05

Table 5-10: The outcome of a SIMPER analysis on square-root transformed data listing those species that contributed most to the dissimilarity between cold and warm infracommunities at Lenne2 (cut-off set at 90 % contribution to total similarity).

(Av. Abd. = average abundance; Av. Dissim. = average contribution to overall dissimilarity between samples)

Lenne2				
Species	**Av. Abd.**		**Av. Dissim.**	**Contribution**
	cold	**warm**		**%**
Raphidascaris acus	3.74	2.93	11.19	21.43
Pseudocapillaria salvelini	2.56	2.91	10.25	19.63
Crepidostomum sp	2.46	2.05	10.20	19.54
Cystidicoloides ephemeridarum	1.11	1.21	5.46	10.46
Echinorhynchus truttae	1.26	1.37	5.23	10.02
Cystidicola farionis	1.19	0.85	4.57	8.75
Diplostomum sp.	1.06	1.31	4.42	8.46

5.4 Discussion

The parasitological examination of 365 brown trout revealed a total of 12 metazoan parasite species, including five species of Nematoda, three Trematoda species, three species of Acanthocephala and one species of Cestoda. All parasite species were identified to species level except the trematodes of the genus *Diplostomum* and *Crepidostomum*.

The results demonstrate that an increase in water temperature caused both qualitative and quantitative changes in composition of parasite communities. This is indicated by significant, temperature dependent changes in abundances of the most parasite species, as well as by the alterations in diversity parameter on community level. According to these results, two opposing trends were observed:

1) a temperature-dependent increase of infection indices as well as parameters of species richness and diversity parameters which concerns both, specific parasites of salmonids such as *E. truttae*, *P. salvelini* as well as rather generalist parasites such as *Diplostomum* sp., *R. acus*, *C. truncatus* and *N. rutili*.

And 2) a temperature-dependent decrease of infection indices as well as parameters of species richness and diversity parameters which only concerns the two salmon-specific species *Crepidostomum* sp. and *C. ephemeridarum*.

For various aquatic parasites seasonal differences in their transmission were reported, whereat climate conditions played a relevant role. For example, seasonal fluctuations in the temperature of the habitat can have consequences for the oscillations in prevalence of acanthocephalans (Kennedy, 1985). The acanthocephalan *E. truttae* occurs mainly in various species of salmonids, while cystacanths of this parasite were recorded from naturally infected amphipods (Awachie, 1965; Awachie, 1966; Moravec 2004). Kennedy (2006) reported that prevalence of cystacanths was slightly higher in summer but lower

during winter. Furthermore, low temperatures affect the feeding behaviour and the reproduction activity of the amphipods, which also may decrease the transmission efficiency of the acanthocephalans. Coupled with a reduced fish activity and a corresponding altered feeding behaviour at lower temperatures, temperature-related alterations are affecting transmission processes (Kennedy, 1985). In addition, the development of *E. truttae* in *Gammarus pulex* (intermediate host) and in *S. trutta* (final host) is delayed at low water temperatures (Awachie, 1966). As the establishment of *E. truttae* in the intestine of *S. trutta* is temperature dependent (Awachie, 1972), lower intensities of *E. truttae* in autumn samples at cold sites could be explained by a depressed host rejection reaction at low temperatures, along with an elimination of a large number of worms at higher summer temperature in relation to an increased activity of the immune system (Moravec, 2004). The permanently increased temperature at the warm sites could overstress the immune system of brown trout, which would explain the increased abundance of *E. truttae* despite higher temperature.

Concerning the increased abundance of the generalist acanthocephalan *N. rutili* at higher temperature, higher abundances of thermophile intermediated host species invading the warm sites could be an additional explanation. The main definitive hosts of *N. rutili* are cyprinds and various species of Ostracoda are required as intermediate host. Additionally, *Sialis lutaria* (Megaloptera) is considered as intermediate host (Moravec & Scholz, 1994), as well as *Erpobdella octoculata* (Hirudinea) (Lassiere & Crompton, 1988). The latter species showed a twofold higher average abundance at the Ruhr warm site, where *N. rutili* was most abundant.

The generalist nematode *R. acus* occurs mainly in piscivorous fish species belonging to various families, including salmonids (Dorucu et al., 1995; Moravec, 2004). Larvae (L2) of *R. acus* appear in numerous aquatic invertebrates (Koubková et al., 2004; Moravec, 2004). At an average temperature of 22 °C, laboratory tests showed that the free-living period of hatched *R. acus* larvae in the water is reduced to 1-2 days, in contrast to 3-10 days at 7 °C. Also, the development of L1- to L2-larvae took place within three days at 22 °C (Moravec 2004). Therefore, faster development at higher temperature would increase the risk of host´s infection. The results of this study showed an almost fivefold higher average abundance at the Lenne1 warm site.

The final hosts of the nematode species *P. salvelini* are fishes of the orders Salmoniformes and Scorpaeniformes. The complete life cycle of this species is not known and seasonal cycle of maturation has not been studied in detail so far. In experimental studies, Lomakin & Trofimenko (1982) succeeded in infecting oligochaetes with eggs of *P. salvelini*. Birtwell & Arthur (1980) recorded a 96-h LC50 at 34° C for the freshwater oligochaete *Tubifex tubifex* (Müller, 1774). Furthermore, Chapman et al. (1982) showed that *Tubifex tubifex* is tolerant to high temperatures. In this study, higher abundances of Oligochaets occurred at the Ruhr and the Lenne2 warm sites. In addition, the abundance of *P. salvelini* also increased at warm sites with highest abundances in autumn samples. Campbell (1974) detected *P. salvelini* in trout all the year round without seasonal changes in its prevalence. The intensity of this

parasite reached its maximum in June and at the beginning of winter (Campbell, 1974; Alvarez-Pellitero, 1979). Both authors point out that maturation of larvae acquired in the autumn-winter period proceed more slowly than those originating from spring.

In the present study, two species of the genus *Crepidostomum* were found, *C. farionis* (Müller, 1784) and *C. metoecus* (Braun, 1900). First intermediate hosts are, among others, molluscs of the genus *Sphaerium* and *Pisidium*. *Gammarus pulex* (Amphipoda) is the second intermediate host for both.

Higher abundances for *Pisidium* sp. and *Ancylus fluviatilis* occurred at the Ruhr and the Lenne2 cold sites. In addition, the abundance of *Crepidostomum* sp. also increased at cold sites with highest abundances in autumn samples, regardless of a twofold higher appearance of *Gammarus pulex* at the Ruhr warm site and the absence at both Lenne1 sampling sites. This shows the pronounced impact of temperature increase at the first intermediate host level. This goes along with the vulnerability to rising temperature of *Pisidium amnicum* and *P. supinum* as shown by Domisch et al. (2011). Thomas (1958), Awachie (1968) and Moravec (1982) found an annual rhythm in the appearance of *C. metoecus* in trout. A minimum in summer is caused by the death of older trematodes as well as by a smaller number of benthic macroinvertebrate species which might act as intermediate hosts. Related to this and to the feeding behaviour of trout, new infections mainly occur in spring and autumn (Moravec, 1982). In this study, highest abundances of *Crepidostomum* sp. were found in autumn samples.

The genus *Diplostomum* contains several species. Their identification only on morphological characteristics is considered problematic and often lead to failures in species identification (Moravec, 2004; Georgieva et al., 2013). Species within the genus *Diplostomum* show complex life cycles involving freshwater lymnaeid snails and fish as intermediate hosts. Piscivorous birds serve as final hosts. Metacercarie of the digenean trematode *Diplostomum* sp. were found in the eyes of brown trout. As no significant differences in lymnaeid snail abundance were found in the present study, up to sixfold higher abundances of metacercariae occur at the warmer sections. These findings could be partly explained by the cercarial shedding. In lymnaeid hosts, cercarial shedding is strongly temperature dependent. Up to 60,000 cercariae per snail per day shed at 20 °C, in contrast to only up to 10,000 cercariae per snail per day at 10 °C (Lyholt & Buchmann, 1996; Karvonen et al. 2004). Additionally, infective cercariae at a temperature of 7 °C were four to five times less infective than at 15 °C (Lyholt & Buchmann, 1996). Chubb (1979) showed that seasonal occurrence is primarly determined by temperature. However, the findings of the present study revealed no specific seasonal pattern.

The cestode *C. truncatus* is a common parasite of freshwater fish, mainly salmonids and a total of 18 amphipod species have been recorded as intermediate hosts of this parasite so far (Protasova & Roytman, 1995; Dezfuli et al., 2000). The maximum number of infective procercoids in gammarids occurs at the beginning of spring (Moravec, 2004).

In the intermediate host, the parasite is infective to fish after remaining in the amphipod´s hemocoel for about 10 weeks (Okaka, 1984). Okaka (2000) also revealed that young specimens of *Gammarus pulex*

harboured the mature procercoids for over one year and a half. Thus the normal heteroxenous life cycle could be supplemented by a monoxenous cycle under particular ecological conditions (Moravec 2004). In addition, Franceschi et al. (2007) recently showed that the larval cestode is capable of affecting the behaviour of *G. pulex*, enhancing transmission of the parasite to the final host. The flexibility in transmission processes could be one reason for a constant or positive effect of increasing temperature on abundance of *C. truncatus*.

The final hosts of the nematode species *C. ephemeridarum* are salmonids, in Europe mainly brown trout. Different species of mayflies (Ephemeroptera) are utilized as intermediate hosts, whereas small fishes (for example *Cottus gobio*) may serve as paratenic hosts. The complete larval development in the intermediate host lasts 24-38 days at a water temperature of 13-15° C. The presence of larvae of *C. ephemeridarum* does not avoid metamorphosis completion of mayfly nymphs, as shown for *Ephemera danica*. At higher water temperature, both larval development and maturation of mayfly nymphs proceed faster. As being a preferred prey for trout, higher infection occurs also by feeding on infected winged imagoes and not only by mayfly nymphs (Moravec, 2004). This is expanding the transmission potential on brown trout. Water temperature also influences the maturation of larvae in the final host, as has been demonstrated experimentally. Moravec (1971) and De & Moravec (1979) showed that the fourth moult of nematode larvae took 28-32 days post infection at 7-16° C, but only 12-20 days p.i. at 18° C. This pattern of seasonal maturation differs considerably in different localities in depending on local ecological conditions, particularly the temperature regime and seasonal changes in the presence of intermediate hosts (Moravec, 2004). In the present study, lower abundance of *Ephemera danica* at the Ruhr warm site, could be the reason for the significant decrease in abundance of *C. ephemeridarum* compared to the colder reference site. The effects of water temperature on developing and transmission processes of *C. ephemeridarum* could be diminished by the larger occurrences of intermediate hosts.

The development of all detected parasites is connected with benthic macroinvertebrates. The infection with these species occurs both by the active attack of the host (for example cercariae) and via feeding on BMI. The difference in infracommunity composition and structure suggest that parasite communities in *S. trutta fario* reflect the communities of free living animals in the Ruhr and the Lenne rivers depending on water temperature. This may be related to differential occurrence and abundance of intermediate hosts of the key discriminating species, namely a decline of *Pisidium* sp., *Ancylus fluviatilis* and *E. danica* abundance and an increase of *S. lutaria*, *E. octoculata* and Oligochaeta Gen. sp abundance at the site with higher water temperature.

Likewise, a mean annual temperature difference of 0.7 K (Ruhr) and 1.9 K (Lenne1) caused a two- to threefold increase of parasite component community in autumn.

The higher intensity of specific parasites found in brown trout from the warmer sites in the present study is likely due to accelerated life cycles, which is, however, well above the value anticipated by the RGT-

Regulation. Also indirect effects such as increased densities of intermediate and definitive host may contribute to higher parasite intensities. Besides this, the increase of unspecific parasites can be explained by higher abundances of thermophile species preferring the warm sites.

The present study provides evidence that metazoan fish parasites will benefit from water temperature increase following climate change and associated shifts of host communities. The results revealed substantial differences in composition and structure of parasite communities in brown trout depending on temperature suggesting that those may have reflected an effect of temperature increase on communities of free living animals acting as intermediate hosts for parasites. This could be related to differential occurrence and abundance of the intermediate hosts of a few species which were identified as key discriminating species in chapter 4. The detection of different parasite species in fish is more efficient than in a single macroinvertebrate individual, because of enrichment processes. Additionally, the community composition of parasites in fish reflect the whole intermediate host range. Thus parasite communities reveal patterns that are not detectable at the community level of the benthic macroinvertebrate system.

All these facts underline the demand to involve parasites in future aquatic ecosystem research. In order to assess the usefulness of parasite communities in biological water quality assessments and to gain further information on parasite biology.

Effects of elevated water temperature on aquatic organisms: a meta-analysis

6

6.1 Introduction

Since water temperature has a direct influences on all forms of aquatic life, aquatic organisms are particularly affected by thermal changes. Effects of water temperature on aquatic organisms, especially poikilothermic ectotherms, are profound (e.g. Ward & Stanford, 1982; Daufresne et al., 2004; Caissie, 2006; Ficke et al., 2007). Thermal stress and coupled changes in water temperature poses new challenges to watercourses which will enforce in the course of climate change. In this context, a progressive loss of cold water habitats in the upper reaches of rivers in the temperate zone will affect cold-adapted species by the loss of their retreat areas.

Although there has been an increasing interest in biological consequences of global warming in the recent decades, there is still a lack of knowledge of potential changes in the parasite fauna of animal populations, especially those in aquatic ecosystems.

Aquatic parasites respond directly to increasing water temperature but also indirectly to changes in other abiotic parameters, such as the distribution and abundance of their hosts. Additionally, increasing water temperatures can promote the transmission of parasites and raise their local abundance (Marcogliese, 2001; Poulin, 2006).

Due to the complexity of host-parasite systems, potential temperature-related changes in freshwater community composition are manifold and diverse. This illustrates the difficulty in making precise predictions on the effects of temperature-related changes for aquatic ecosystems.

Therefore, the response ratio (Osenberg et al., 1997) was used to compare benthic macroinvertebrate, brown trout and parasite communities between warm and cold sampling sites. The response ratio is often used as a measure of the effect magnitude in experimental ecological research. By using this metric a standardized effect size was calculated. This allowed a comparison of the different units used in the previous chapters. For quantifying the simple two-group experimental design in this study, a modification of the response ratio was applied. In this chapter, experimental data from chapter 4, 5 and 6 were compiled and a meta-analysis was performed in order to evaluate the sensitivity of benthic macroinvertebrates (BMI), brown trout (*Salmo trutta fario* L.) and parasites to increasing water temperature.

6.2 Materials and Methods

Concerning study area, sampling sites and water temperature, the overall sampling design is described in detail in chapter 2.

The data on benthic macroinvertebrates, fish and parasites used for effect size analyses refer to chapters 3.3, 4.3 and 5.3.

6.3 Data Processing

Meta-analysis depends upon estimating effect size for each independent experiment (Rosenberg et al., 2000). Based on the results (see chapter 3.3, 4.3 and 5.3.) for benthic macroinvertebrates (community temperature index), fish (condition factor, hepatosomatic index, gonadosomatic index, hepatic glycogen and hsp70 level) and parasites (Brillouin-Index, mean abundance), the response ratio (Osenberg et al., 1997) was calculated to quantify the effects of a temperature increase on the mentioned parameters.

Therefore, a modified version of the response ratio was used to quantify differences between warm (treatment) and cold (control) groups, as parasite mean abundances contained many zero-values:

$$\Delta r = ln\left(\frac{(Xw+1)}{(Xc+1)}\right)$$

where Xw: parameter at warm site (treatment group), Xc: parameter at cold site (control group).

Thereby, values > 0 denote an increase for respective parameters (e.g. diversity, mean abundance) at warm sites, and negative values a decrease. The response ratio is dimensionless since *Xw* is divided by *Xc*.

6.4 Results

6.4.1 Response ratio based on different parameters

Results of the meta-analysis of elevated temperature on different parameters are presented in Figure 6-1. A comparison of the response ratios revealed differences in the direction of the effect, as well as differences in the size of the effect.

A comparison of the response ratios showed that the community temperature index (CTI) was slightly increasing at the Ruhr, whereas at both Lenne site pairs the CTI was slightly decreasing with the exception of Lenne2 in autumn 2009. The response ratio for CTI ranged between 0.06 (R_09a) and -0.06 (L1_10a).

Response ratios for condition factor (CF) ranged between 0.06 (R_10a) and -0.10 (L1_10s). An increase was only given for CF at the Ruhr sites in autumn 2010, whereas a decrease was given for all others, except for Lenne2 in autumn 2010 (Δr: 0.00).

The obtained effects on the hepatosomatic index (HSI) ranged between 0.13 (R_09a) and -0.09 (L1_10s). At the Ruhr site, the HSI was increasing over all three sampling periods. The Lenne1 and the Lenne2 site showed an increase only for autumn 2009 samples.

Response ratios for the gonadosomatic index (GSI) ranged between 0.24 (L1_09a) and -0.70 (L1_10a) and a decrease for all Ruhr and Lenne2 samplings was obtained. At Lenne1, the only increase for GSI occurred, as well as the highest decrease (Δr: -0.70).
For hepatic glycogen, response ratios ranged between 1.43 (L1_09a) and -0.58 (R_10s). At Lenne1 and Lenne2 hepatic glycogen is increasing throughout, with only one exception for Lenne2 in autumn 2010 (Δr: 0.00).
Obtained effects on hsp70 levels ranged between 0.25 (R_09a) and -0.51 (L2_10s). Samples in 2010 showed a decrease for all sampling sites, except for spring sampling at Lenne1 (Δr: 0.02).
The response ratios for Brillouin-Index (HB) ranged between 0.27 (L1_09a) and -0.07 (L2_09a). An increase for the HB was given for both autumn samples at Ruhr and Lenne1, whereas a decrease occurred in spring 2010 (Δr Ruhr: -0.04; Δr Lenne1: -0.02). Conversely, response ratios at Lenne2 decreased for both autumn samples and an increase was only given for HB in spring 2010 (Δr: 0.08).

Results of the meta-analysis of temperature increase on the abundance of different parasite taxa are presented in Figure 6-2. Digeneans of genus *Diplostomum* sp., showed generally an increase within a range of 1.53 to 0.08, except for both autumn samples at Lenne2 (Δr 09a: -0.04; Δr 10a: -0.02).
In contrast, *Crepidostomum* sp. showed mostly a decrease within a range of -0.03 to -3.28, with only one exception for autumn 2009 sample at Lenne2 (Δr 09a: 1.46).
Within a range of 2.90 to 0.08, the obtained effects on *R. acus* showed a generally increase, with only one decrease in autumn 2010 sample at Lenne2.
The response ratio for *P. salvelini* was negative at Ruhr in spring 2009 (Δr: -0.18) and autumn 2010 (Δr: -0.17) and at Lenne2 in both autumn samples (Δr 09a: -0.38; Δr 10a: -0.30). The remaining samples showed an increase within a range of 1.13 to 0.09.
Conclusions from meta-analysis for *C. ephemeridarum* showed generally a decrease within a range of -0.04 to -2.46. Positive response ratios were obtained for autumn 2009 samples at Lenne1 and Lenne2.
Response ratios for *C. truncatus* showed mostly no or only small increases in the range 0.47 - 0.00, with one exception in autumn 2010 (Δr: -0.05) at Lenne2.
Meta-analytic results for both acantocephalan species *N. rutili* and *E. truttae* presented a mixed picture, with an increase (*N. rutili*: 0.59 to 0.06, *E. truttae*: 0.67 to 0.00), as well as a decrease (*N. rutili*: -0.97 to -0.02, *E. truttae*: -0.48 to -0.14).

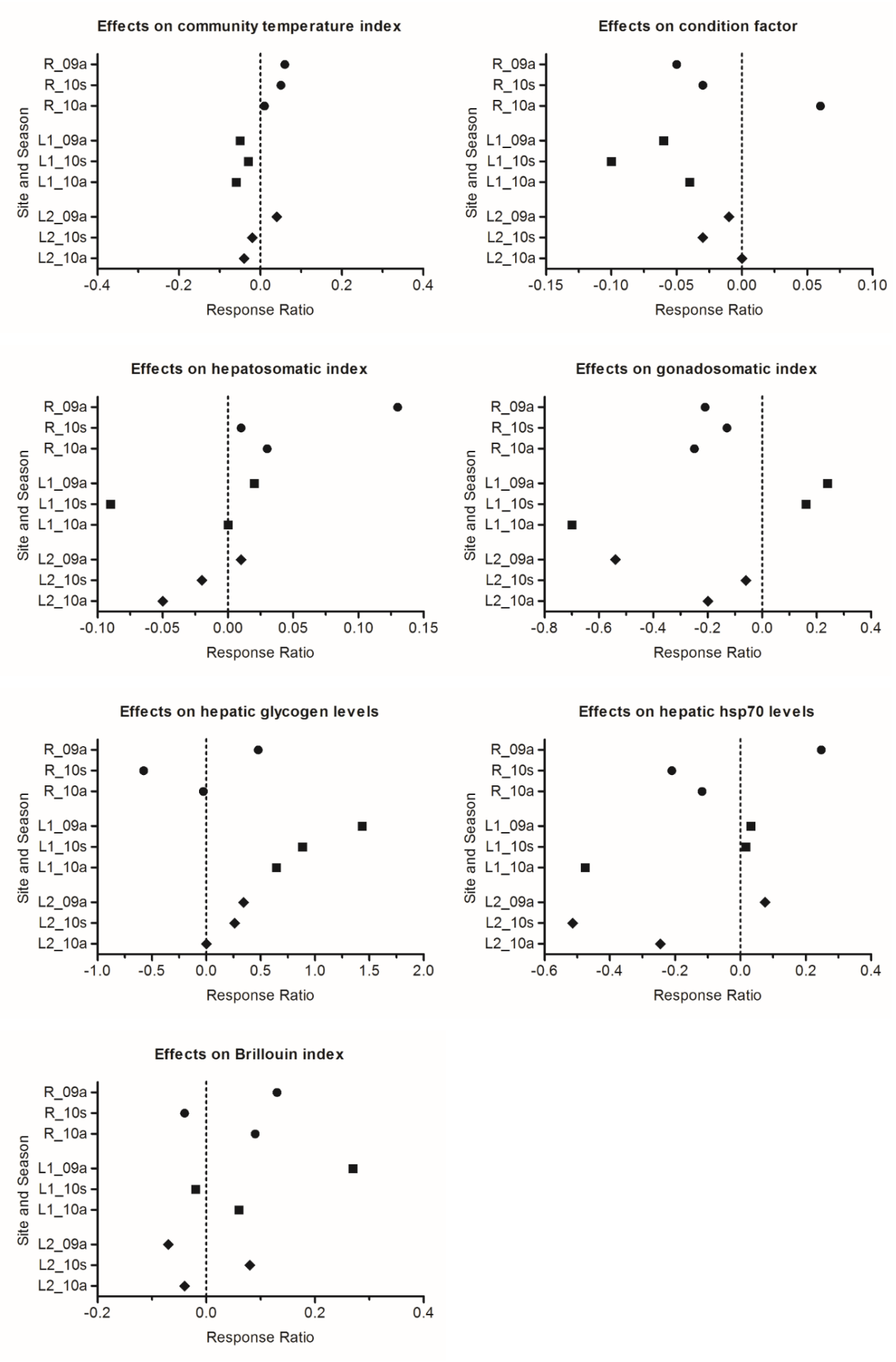

Figure 6-1: Comparison of modified response ratios based on different parameters at river Ruhr (R) and Lenne (L1 and L2) in 2009 (09) and 2010 (10) in autumn (a) and spring (s).

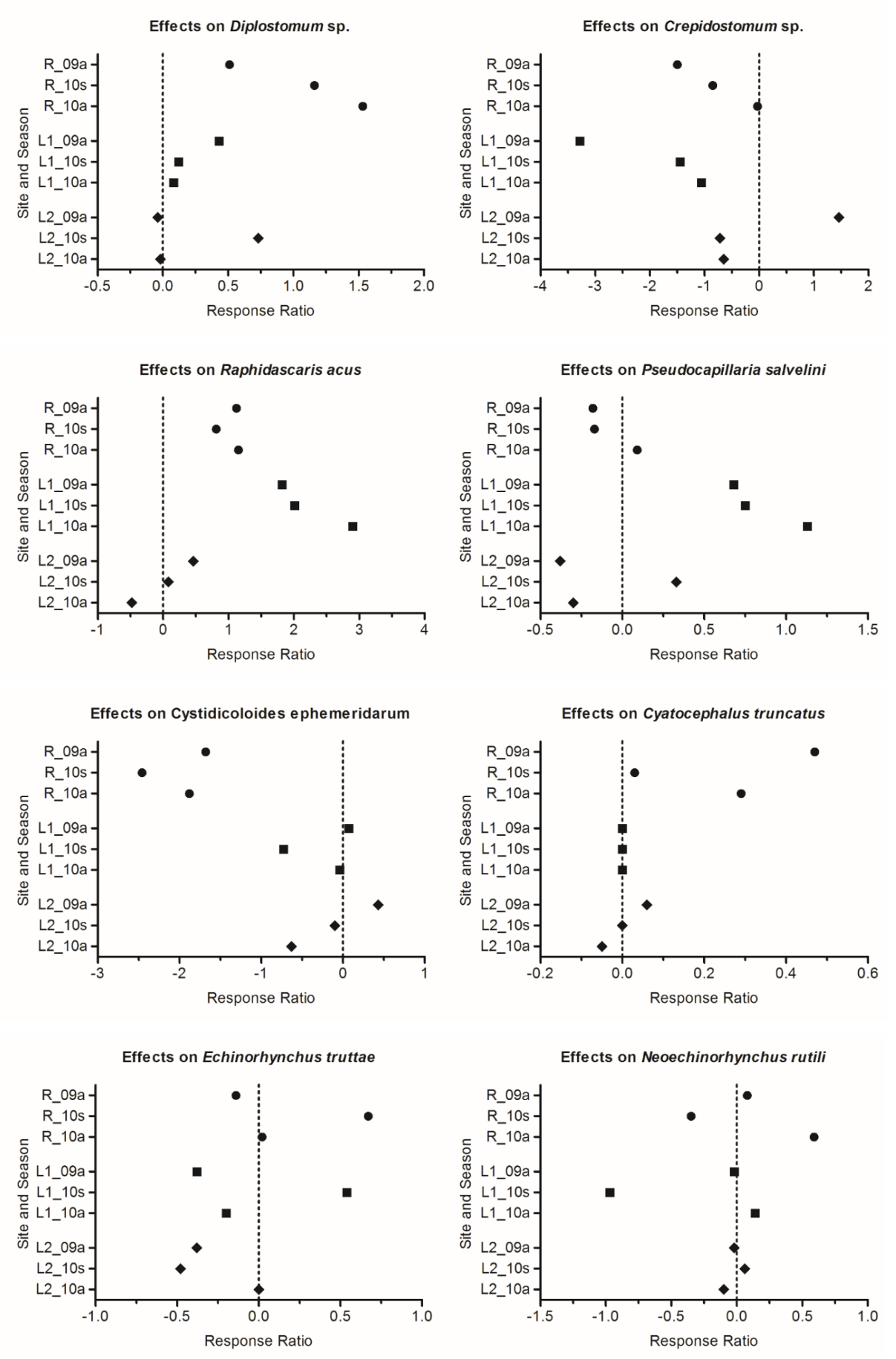

Figure 6-2: Comparison of modified response ratios based on different parasite taxa at river Ruhr (R) and Lenne (L1 and L2) in 2009 (09) and 2010 (10) in autumn (a) and spring (s).

6.4.2 Response Ratio grouped by site and season

The grouping by site and season generally show that minor effects appear on BMI community level (CTI) and on brown trout individuals (CF, HSI and GSI), whereas the greatest dynamic is likely to occur in metapopulations of parasites. Here, a general increase (e. g. *Diplostomum* sp. and *R. acus*), as well as a general decrease (e. g. *C. ephemeridarum*) was detected. The different expressions of the response ratio can be related to seasonal dynamics. An overview on the potential effects arising from elevated water temperature is given in Figure 6-3 to Figure 6-5.

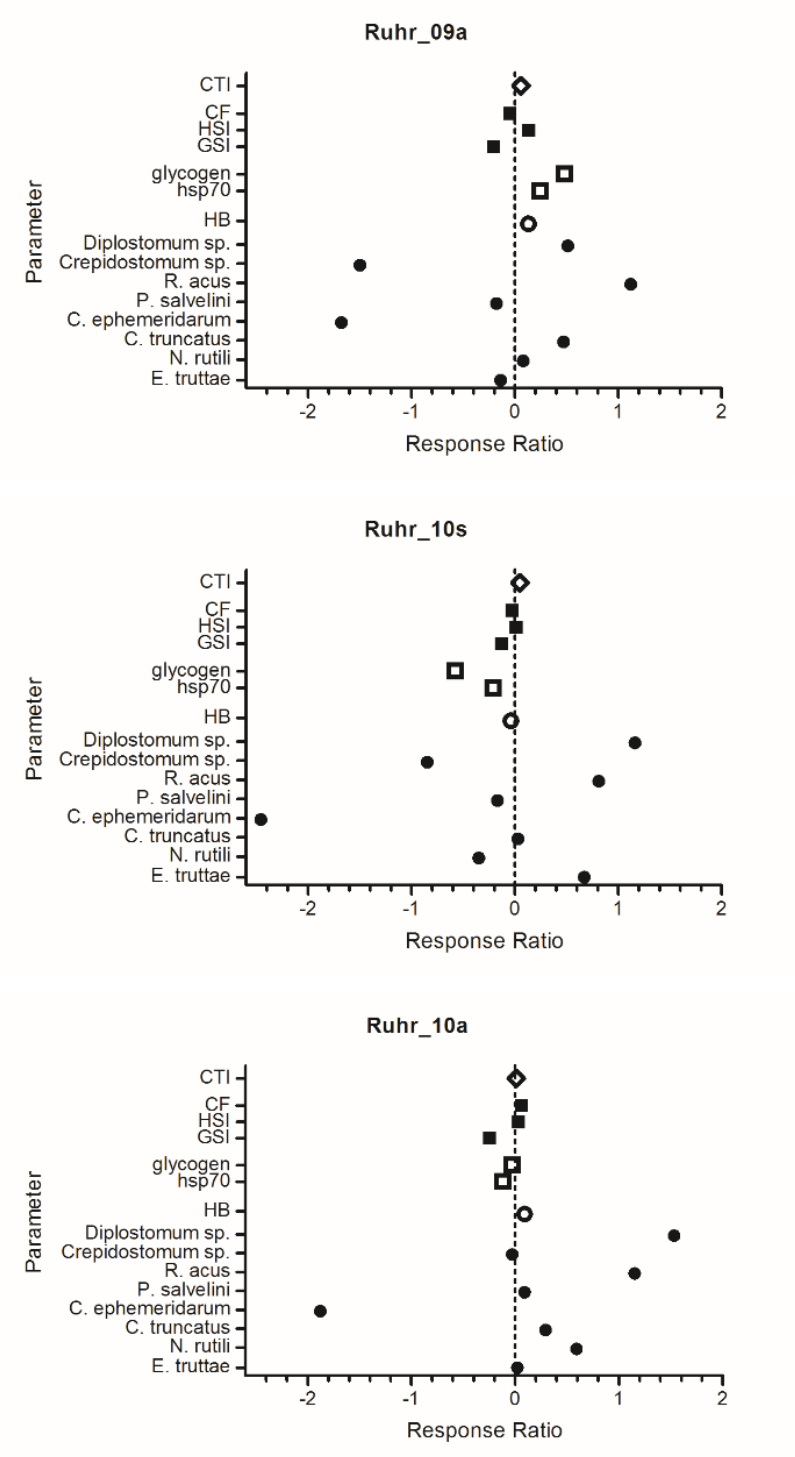

Figure 6-3: Comparison of modified response ratios at river Ruhr. (2009 (09) and 2010 (10) in autumn (a) and spring (s))

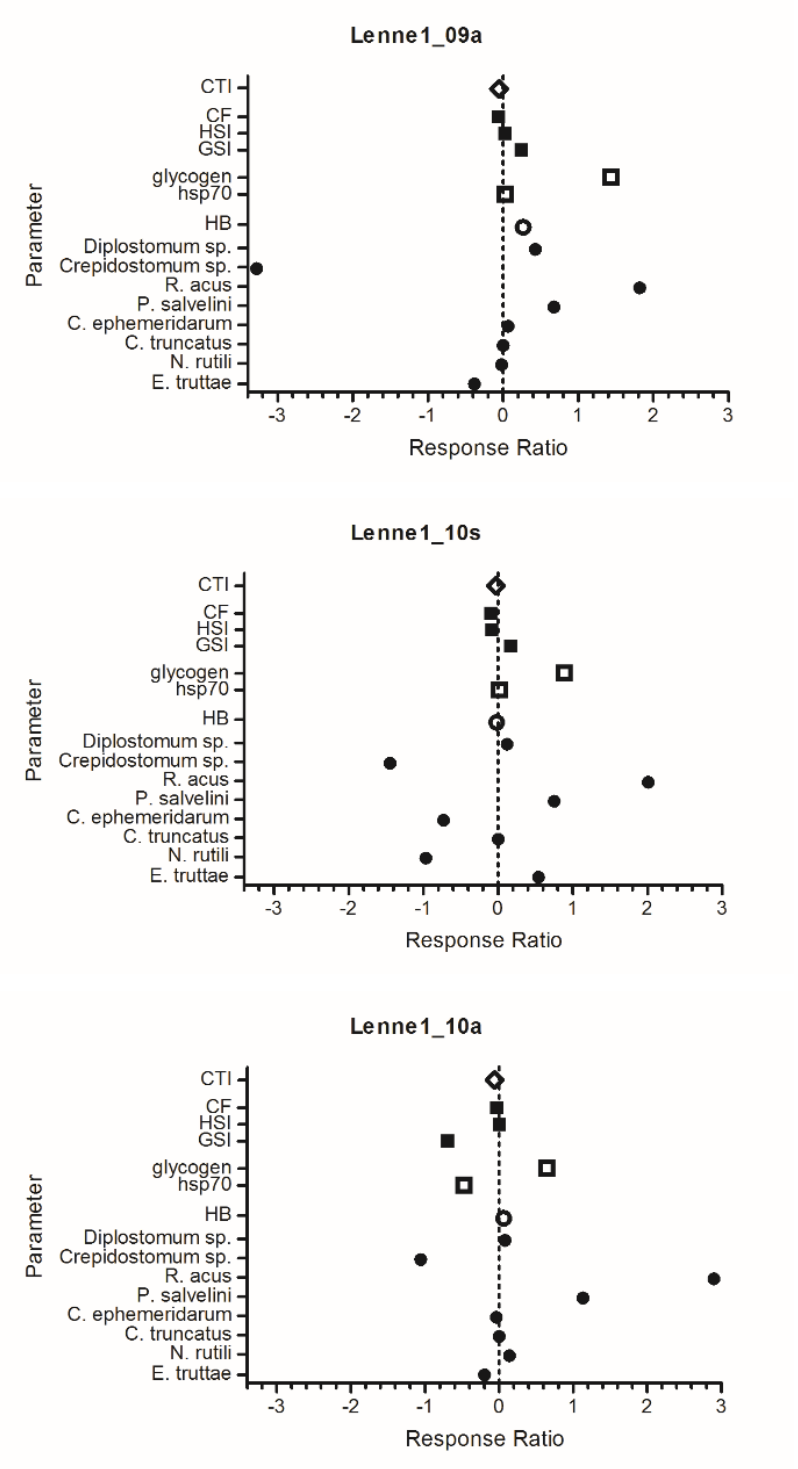

Figure 6-4: Comparison of modified response ratios at river Lenne1. (2009 (09) and 2010 (10) in autumn (a) and spring (s))

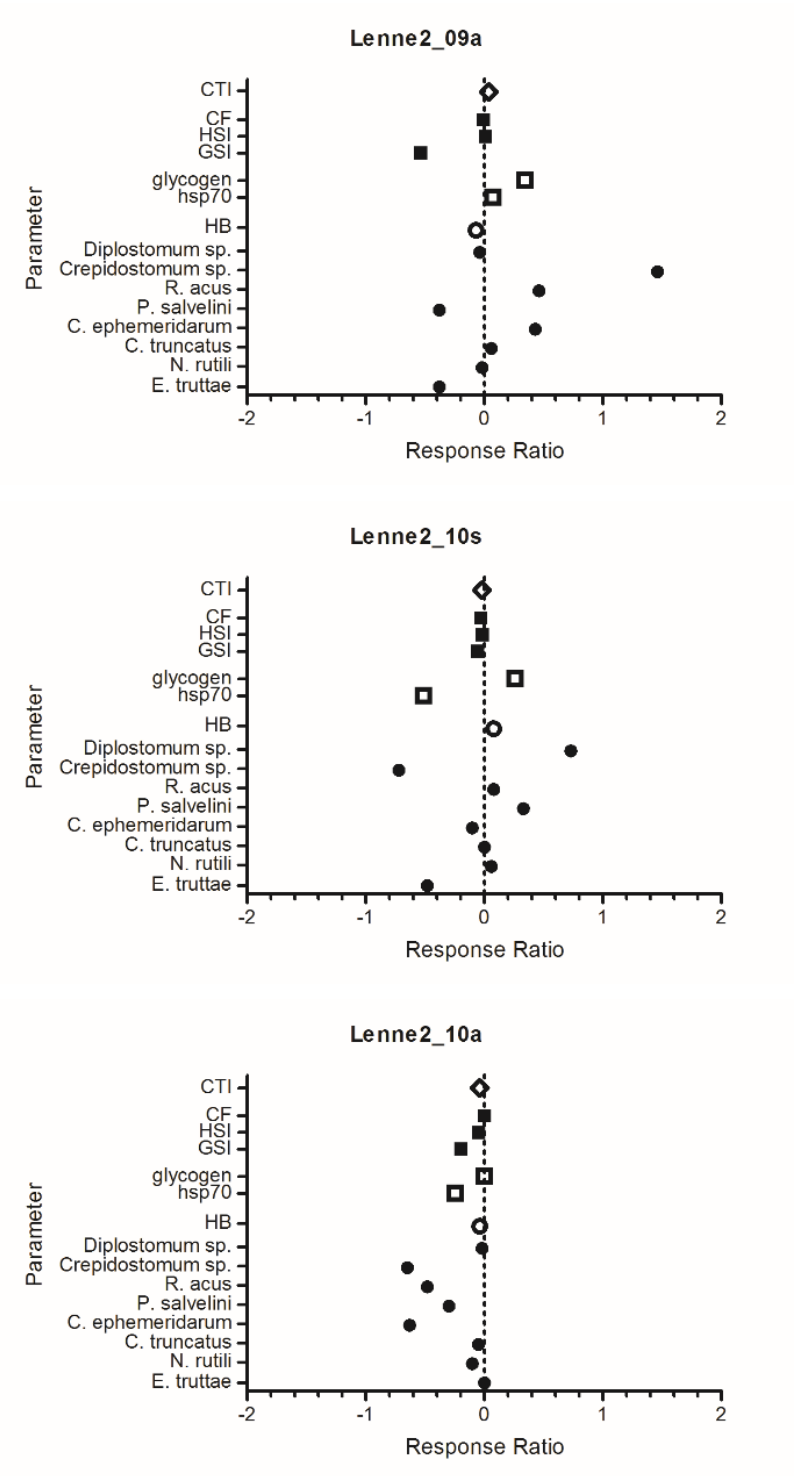

Figure 6-5: Comparison of modified response ratios at river Lenne2. (2009 (09) and 2010 (10) in autumn (a) and spring (s))

6.5 Discussion

This meta-analysis estimated the impact level of elevated water temperature on aquatic organisms and in particular on parasites of brown trout at two temperate zone rivers.

Meta-analysis has been used by various authors for different purposes in ecological research. For example, with a view on BMI, meta-analysis was applied to quantify BMI responses to in-stream habitat restoration (Miller et al., 2010) or to quantify impacts of alien species invasions to BMI communities (Ward & Ricciardi, 2007).

In the study of Kamiya et al. (2014), meta-analysis has been used for testing the relationship between parasite richness across animal, plant and fungal hosts. According to Bush et al. (1997) and Blanar et al. (2009), parasite mean abundance (number of parasites divided by the total number of host individuals examined) was judged to be a reliable, standardized indicator of parasitism because it integrates information on both prevalence (percent of hosts infected) and intensity (number of parasites per infected host).

Generally, meta-analysis permits the statistical analysis and comparison of a set of different experimental data. Here, the modified response ratio was used to compare BMI, brown trout and parasite communities between warm and cold sampling sites. The modified response ratio gives an instant and reliable indication of whether the magnitude and direction of an effect differs among different categories of the separate studies.

The results of the meta-analysis conducted herein confirmed the findings from the previous chapters. No major changes were found for BMI and only small effects occurred for recorded parameters of brown trout. Concerning parasites, effects on infracommunity level are barely recognizable, whereas the greatest impacts are likely to occur in metapopulations of parasites.

The majority of effect sizes for the salmon-unspecific parasites such as *Diplostomum* sp., *R. acus*, *C. truncatus* and *N. rutili* were positive, indicating that increasing water temperature typically has positive impacts on the parasite taxa considered.

On the other hand, increasing water temperature has negative impacts on the two salmon-specific species *Crepidostomum* sp. and *C. ephemeridarum*, as the majority of effect sizes were typically negative.

Most recent climate projections indicate an average warming by 0.8 to 1.7°°C by 2050 across the German federal state North Rhine-Westphalia. Air temperature affects water temperature via several mechanisms that include solar radiation and direct sensible heat transfer, thus a similar development is expected for water temperature.

As the annual mean water temperature differences of this study range within the expected scope (0.7 to 1.9 K), the results show that changes in temperature due to global climate change can already affect the transmission of parasites.

On the one hand, changes in water temperature directly affect the parasite species. On the other hand, as parasite survival depends on the presence of all required hosts in a stable community structure (Marcogliese & Cone, 1997), consequences of temperature increase for an individual parasite species allow also conclusions on intermediate and definitive hosts.

Finally, the potential impact of increasing water temperature suggests further studies on long-term effects on biota. More research is needed to understand how temperature-related changes in freshwater community composition, affect life cycles of aquatic parasites.

Summaries

7

7.1 Summary and Conclusions

The present study evaluated and compared the response of benthic macroinvertebrate, fish and parasite communities to changes in water temperature across two mid-sized mountain rivers in the south-eastern part of the German federal state North Rhine-Westphalia (NRW). In 2009 and 2010, the two rivers Ruhr and Lenne have been investigated at comparable river sections up- and downstream of thermal pollution by cold hypolimnetic water from reservoirs as well as by cooling water released from a power station.
The main focus was to extend the knowledge about the use of metazoan fish parasites as bioindicators for changes in freshwater biodiversity in relation to water temperature. The research focus was aimed at the faunistical and ecological aspects of parasite communities of Brown trout (*S. trutta fario*) in relation to temperature variations.
At 6 river sections (3 cold-warm site pairs) benthic macroinvertebrate, fish and parasite communities were examined in autumn 2009 and in spring and autumn 2010. Additionally, thermal characteristics were monitored over one and a half year. The annual mean water temperature differences of this study ranged within 0.7 K (Ruhr) to 1.9 K (Lenne1). The analysis of degree-days confirmed the separation of sampling sites by temperature.
Looking at **the influence of water temperature on benthic macroinvertebrate communities (chapter 3)**, BMI communities showed highest average dissimilarity at Lenne1 (ΔWT: 1.9 K). A similar but more differentiated pattern was observed when looking at the EPT community. An increase of average abundance of *H. siltalai* (Trichoptera) has been detected at all warm sites as well as rising average abundance of *C. rivulorum* (Ephemeroptera) for the Lenne1 and the Ruhr warm sites.
As the majority of BMI were present at both cold and warm sites, the limit of tolerance range of the majority of taxa was not exceeded by the temperature differences found in the present study. The results indicated that temperature changes have an impact on BMI species, in particular EPT-taxa, and as a consequence BMI communities are affected.
The results of **chapter 4 – the effect of water temperature on the levels of hepatic glycogen and hsp70 in brown trout (*Salmo trutta fario* L.)** – showed a general increase for hepatic glycogen levels at warmer sections and a clear seasonal pattern with higher concentrations in autumn.

Besides, increasing temperature had effects on the condition of examined brown trout. Mean condition factors were lower at warm sites compared to respective cold sites. Additionally, condition factors were equal or slightly higher in spring samples than in autumn samples. Gonadosomatic indices showed a general decrease at warmer sections, while hepatosomatic indices showed no clear pattern.

Hepatic hsp70 levels showed no clear pattern at warm sites, while the concentrations in the liver tissues remained more or less similar in spring and autumn. At Ruhr and Lenne2 cold sites, hepatic hsp70 levels had their maximum in spring. The results obtained indicated the ability of brown trout to respond to increased energy demands as part of an increase in water temperature without losing their regulatory capability. In light of the temperature differences found in this study, one should bear in mind that the limit of tolerance range might not have been reached yet.

The impact of water temperature on the parasite communities in brown trout (*Salmo trutta fario* L.) is the content matter of **chapter 5**. Between autumn 2009 and autumn 2010, twelve metazoan parasite species including five nematodes, three acanthocephalans, three trematodes and one cestode were found in 365 *S. trutta fario* examined from six sites.

The results revealed significant, temperature dependent changes in abundances for most of the parasite species found. According to these results, two opposing trends were observed:

- an increase in calculated infection indices (prevalence, abundance and mean intensity) which concerns both, specific parasites of salmonids (*E. truttae* and *P. salvelini*) as well as rather generalist parasites (*Diplostomum* sp., *R. acus*, *C. truncatus* and *N. rutili*)
- a decrease in calculated infection indices (prevalence, abundance and mean intensity) which only concerns the two salmon-specific species *Crepidostomum* sp. and *C. ephemeridarum*.

The results also showed that changes in water temperature cause both qualitative and quantitative changes in parasite communities of brown trout.

Since the difference in parasite infracommunity composition and structure reflect the difference of the community composition of free living animals, the study shows an effect of water temperature on free living animals such as BMI in both rivers Ruhr and Lenne. This is related to differential occurrence and abundance of intermediate hosts of a few species which were identified as key discriminating species in chapter 3.

Likewise, a mean annual temperature difference of 0.7 K (Ruhr) and 1.9 K (Lenne1) caused a two- to fourfold increase of diversity parasite component community in autumn.

The present study provides evidence that metazoan fish parasites will benefit from water temperature increase following climate change and associated shifts of host communities.

Finally, the present study delivered a detailed parasite monitoring on Brown trout in the river Ruhr and Lenne.

The results of the meta-analysis in **chapter 6 – Effects of elevated water temperature on aquatic organisms: a meta-analysis –** confirmed the findings off the previous chapters. The quantitative analysis based on a modified response ratio provided information on both the typical magnitude and

direction of effect. The effects on infracommunity level are barely recognizable, whereas the greatest impacts are likely to occur in metapopulations of parasites.

The results of the present study revealed the parasite´s susceptibility to increasing water temperature and showed that changes in temperature due to global climate change can already affect the transmission of parasites. The findings provide evidence that the parasite community patterns of brown trout may differ essentially in relation to changes in water temperature.

All these facts highlight the usefulness of parasite communities for aquatic monitoring and research and therefore should be considered in future studies. However there is also need for further research regarding autecological characteristics of parasite species.

In many rivers it is still impossible to monitor and quantify the status of biodiversity due to a dearth of species occurrence data at relevant scales and compatible formats and a lack of knowledge on parasite life cycles and its linked intermediate hosts.

For these reasons, considering parasites, will improve the validity of biological water quality assessments. Future research as well as success of tackling climate change can profit from increasing the awareness of aquatic parasites and their hosts as a functional unity. Therefore, available data on freshwater parasites and their hosts groups should be collected as a basis for analyses of distribution patterns in different river types. Additionally, aquatic parasite communities and freshwater organism groups should be monitored over longer time spans. The improvement and conservation measures on biodiversity of freshwater ecosystems should also broaden the focus on aquatic parasite communities. Finally, a standardized assessment method should be developed to quantify temperature effects on aquatic parasite communities including the identification of indicator species specific for different fish types. More research is needed to understand how temperature-related changes in freshwater community compositions, affect life cycles of aquatic parasites.

7.2 Zusammenfassung und Schlussfolgerungen

Die Artenvielfalt natürlicher Ökosysteme ist durch zunehmende menschliche Eingriffe in den letzten Jahrhunderten stark bedroht. Bereits geringe Veränderungen ökologischer Bedingungen in Ökosystemen können eine Reihe vielfältiger Wirkungsketten initiieren. Deshalb ist ein fundiertes Wissen über die genetische Vielfalt, die ökologischen Funktionen und Interaktionen der unterschiedlichen Lebensräume eine wichtige Voraussetzung für eine effiziente Planung von Erhaltungs- und Förderungsmaßnahmen zum Schutz der Biodiversität.

Die Wassertemperatur ist einer der wesentlichen Faktoren, welcher sich maßgeblich auf die Ausbildung, Zusammensetzung und Verteilung der Lebensgemeinschaften in Fließgewässern auswirkt. Darüber hinaus stellen thermische Belastungen und damit einhergehende Änderungen der Wassertemperatur Fließgewässer vor Herausforderungen, welche sich im Zuge der Auswirkungen des Klimawandels noch verstärken werden. In den Fließgewässern der gemäßigten Breiten wird dies zu einer fortschreitenden Potamalisierung rithraler Abschnitte führen. Davon sind besonders kaltstenotherme Arten betroffen, da ihnen Ausweich- und Rückzugsmöglichkeiten nur eingeschränkt zur Verfügung stehen.

Obwohl in Süßwasserökosystemen rund 10 % aller weltweit bekannten Arten vorkommen, werden insbesondere in Fließgewässern Parasiten in ökosystemaren Betrachtungen kaum berücksichtigt.

In der vorliegenden Arbeit wurden im Freiland die Auswirkungen veränderter Wassertemperaturen auf Parasitengemeinschaften der Bachforelle an unterschiedlichen Standorten untersucht. Dazu wurden an zwei Mittelgebirgsflüssen in Nordrhein-Westfalen, der Ruhr und der Lenne, an vergleichbaren Abschnitten jeweils ober- und unterhalb von thermischen Belastungen Makrozoobenthos-gemeinschaften, Bachforellen (*Salmo trutta fario* L.) sowie deren Parasitengemeinschaften über einen Zeitraum von zwei Jahren betrachtet. Die mittleren jährlichen Differenzen der Wassertemperatur betrugen über diesen Zeitraum 0,7 K (Ruhr), 0,8 K (Lenne2) und 1,9 K (Lenne1).

Sowohl bei der allgemeinen Betrachtung der Makrozoobenthosgemeinschaften, als auch bei der genaueren Betrachtung der EPT-Taxa (Ephemeroptera, Plecoptera und Trichoptera) traten die größten Unterschiede am Probestellenpaar Lenne1, mit der größten Differenz in der mittleren Jahreswassertemperatur (1,9 K) auf. An allen drei Probestellen mit erhöhter Wassertemperatur konnte ein Anstieg der mittleren Abundanz von *H. siltalai* (Trichoptera) sowie für *C. rivulorum* (Ephemeroptera) an den warmen Abschnitten der Probestellenpaare Ruhr und Lenne1 festgestellt werden.

Die dieser Studie zu Grunde liegenden Temperaturunterschiede wirkten sich auf unterschiedliche Art auf die Gesamtkondition der Bachforellen aus. An den warmen Abschnitten lagen im Mittel niedrigere Konditionsfaktoren vor. Dabei wurden im saisonalen Vergleich jeweils in den Frühjahrsproben leicht erhöhte Konditionsfaktoren festgestellt.

Auch der Gonadosomatische Index war bei den Bachforellen an den warmen Abschnitten im Mittel niedriger. Die Ergebnisse für den Hepatosomatische Index wiesen hingegen keine klaren Muster auf.

Bei den Biomarkeruntersuchungen konnte für alle warmen Abschnitte ein genereller Anstieg des Glykogengehalts im Lebergewebe der Bachforellen gemessen werden. Die Ergebnisse zeigen ein ausgeprägtes saisonales Muster mit höheren Glykogengehalten in den Herbstproben.

Die HSP70-Gehalte im Lebergewebe der Bachforellen an den warmen Abschnitten wiesen dagegen kein deutliches Muster auf. Die Konzentrationen waren sowohl in den Frühjahrs- als auch den Herbstproben ähnlich hoch. An den kalten Abschnitten der Ruhr und Lenne2 traten in den Frühjahrsproben die höchsten HSP70-Gehalte auf.

Bei der parasitologischen Untersuchung der insgesamt 365 Bachforellen (Anzahl pro Abschnitt = 13-26) wurden insgesamt 12 Endoparasitenarten gefunden. Dabei handelte es sich um die Nematodenarten *C. ephemeridarum*, *C. farionis*, *C. truttae*, *P. salvelini* und *R. acus*, die Acantocephalenarten *E. truttae*, *N. rutili* und *P. laevis*, die beiden Gattungen der digenen Trematoden *Crepidostomum* sp., *Diplostomum* sp. und die Trematodenart *T. clavata* sowie den Cestoden *C. truncatus*.

An den warmen Abschnitten wurden sowohl für spezifische Parasiten der Bachforelle (*E. truttae* und *P. salvelini*), als auch für die Generalisten *C. truncatus*, *Diplostomum* sp., *N. rutili* und *R. acus* signifikante Anstiege in den jeweiligen Abundanzen, Prävalenzen und mittleren Intensitäten an den warmen Abschnitten beobachtet. Im Gegensatz dazu wurde ein signifikanter Rückgang dieser

Parameter für die beiden forellenspezifischen Parasiten *C. ephemeridarum* und *Crepidostomum* sp. an den warmen Abschnitten festgestellt.
Die Ergebnisse dieser Studie zeigen, dass eine Veränderung der Wassertemperatur sowohl qualitative als auch quantitative Veränderungen der Parasitengemeinschaften der Bachforelle bewirkt. Dies konnte u. a. in einem zwei- bis vierfachen Anstieg der Diversität in den Komponentengemeinschaften der warmen Abschnitte an Ruhr und Lenne1, als auch in signifikanten Veränderungen der Abundanzen und Intensitäten bei einem Großteil der gefundenen Fischparasitenarten dokumentiert werden.
Die zusammenfassende Metaanalyse, über die Auswirkungen erhöhter Wassertemperatur auf Makrozoobenthosgemeinschaften, Bachforellen sowie deren Parasitengemeinschaften, gibt einen Überblick über die jeweilige Effektgröße als auch über die jeweilige Effektrichtung. Die Auswertung zeigt das Auftreten geringer Effekte auf der Ebene der Parasiteninfragemeinschaften. Die größten Auswirkungen wurden auf der Ebene der jeweiligen Metapopulationen der Parasiten nachgewiesen.

Zusammenfassend konnte mit dieser Arbeit gezeigt werden, dass eine Erhöhung der Wassertemperatur im Zuge des Klimawandels die Parasitentransmission beeinflusst. Dabei treten die beobachteten Unterschiede in den Parasitengemeinschaften der Bachforelle in Relation zum Ausmaß der Temperaturveränderungen auf.
Dies unterstreicht die Forderung, aquatische Parasiten in zukünftige ökosystemare Betrachtungen mit einzubeziehen. Darüber hinaus besteht weiterer Forschungsbedarf zur Autökologie von Parasiten im Zusammenspiel mit abiotischen und biotischen Umweltfaktoren.
Insbesondere in Fließgewässern wird das Monitoring der Artenvielfalt aus Mangel an entsprechenden Daten zum Vorkommen von Parasitenarten, sowie durch nur unvollständige Kenntnis der jeweiligen Lebenszyklen und der involvierten Zwischenwirte erschwert.
Die Ergebnisse dieser Studie legen nahe, die Auswirkungen einer erhöhten Wassertemperatur auf das Vorkommen und die Transmission von aquatischen Parasiten weiter zu untersuchen. Insbesondere langfristige Veränderungen sowie die unterschiedlichen Auswirkungen in verschiedenen Flussgebietstypen sollten dabei im Fokus künftiger Forschungsprojekte liegen.
Aus diesem Grund wäre es auch für zukünftige gewässerökologische Untersuchungen nur von Vorteil, die Parasiten aquatischer Organismen stärker in Monitoringuntersuchungen mit einzubeziehen, um erfolgreiche Anpassungsmaßnahmen an den Klimawandel entwickeln und umsetzen zu können.

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Appendix

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Lebenslauf Daniel Rainer Dangel

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