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Parasite infection and decreased thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change

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Summary

1. Parasites and pathogens can have an important effect on their host's thermal resistance. The impact of parasite infection on host physiological performances has traditionally been studied in controlled laboratory conditions, and much less is known about its actual effects in wild populations. Nonetheless, such knowledge is critical when assessing the effect of climate change on the future survival of the host.

2. *Tetracapsuloides bryosalmonae* is a myxozoan endoparasite causing proliferative kidney disease (PKD) in salmonids. Infection and clinical symptoms of PKD are dependent on environmental temperature and PKD has become an emerging disease of primary importance for farmed and wild salmonids in the last decades. Despite important achievements in understanding PKD pathology in recent years, there are still crucial gaps in the knowledge of the disease ecology, notably in how the parasite affects host performance in the wild.

3. We sampled juvenile (0+) brown trout (*Salmo trutta*) from the wild during early and late summer and assessed relative parasite load (DNA quantification with qPCR) and disease severity (kidney hyperplasia). We also measured haematocrit, leucocyte formula, aerobic scope and upper thermal tolerance in a field-physiology approach in order to better understand the relationships between PKD severity and host performance. By using wild-caught individuals and performing measurements directly on location, we aimed to gain insights into host physiology in a natural environment while avoiding biases caused by laboratory acclimation.

4. We found that most physiopathological symptoms in the wild were strongly correlated with kidney hyperplasia, but more weakly linked to parasite load. Disease severity was positively correlated with anaemia and abundance of circulating thrombocytes, and negatively correlated with aerobic scope and thermal tolerance.

5. Our results suggest that impaired aerobic performances and thermal tolerance in infected fish may potentially result in decreased host survival in the wild, especially in relation with predicted higher average summer temperatures and increased frequency of extreme events (summer heatwaves) in the context of global climate change.

Key-words: aerobic scope, brown trout, fish leucocyte formula, upper thermal tolerance

Introduction

Parasites and pathogens play a major role in ecosystem functioning (Dunn *et al.* 2012; Krkošek *et al.* 2013; Hatcher, Dick & Dunn 2014). In the current context of

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global climate change, host–parasite relationships are expected to be affected by multiple factors such as parasite range extension, increased virulence, modified temporal dynamics of host–parasite interactions, decreased host condition and increased frequency of disease outbreaks (Marcogliese 2008; Gallana *et al.* 2013; Paull & Johnson 2014). Notably, infections might become more detrimental for hosts under increased temperature conditions because of enhanced parasite growth (Macnab & Barber 2012), higher metabolic demands in ectothermic hosts (Muñoz *et al.* 2015) and negative impact of infections on host metabolic machinery (Seppänen 2008). While studies evaluating the effects of parasite infection on host physiology in controlled laboratory conditions are required to untangle the effects of multiple factors, it is equally critical to characterize the integrated host response in natural environments in order to understand and predict the physiological impacts of parasitism in wild populations in the context of climate change.

Proliferative kidney disease (PKD) is a widespread parasitic disease affecting salmonid fish species in North America and Europe which can result in very high mortality rates in farmed populations (e.g. Hedrick *et al.* 1984). PKD is also the suspected cause for several recent declines in wild salmonid populations (e.g. Burkhardt-Holm *et al.* 2005; Sterud *et al.* 2007) and is considered an emerging disease related to anthropogenic changes such as eutrophication and global warming (Okamura *et al.* 2011). PKD is caused by a myxozoan endoparasite (*Tetracapsuloides bryosalmonae*) proliferating in the fish kidney after infection by spores released from a bryozoan host (Anderson, Canning & Okamura 1999; Canning *et al.* 1999). Fish develop a strong immune response resulting in renal swelling, impaired excretion and anaemia. Impaired osmoregulation might be particularly challenging for anadromous fish, for example during migration to seawater (Foott, Stone & Nichols 2005), as a disrupted ionic balance would affect many physiological functions. PKD can be the primary cause of increased mortality but *T. bryosalmonae*-infected juvenile fish may also become more sensitive to secondary infections and harsh environmental conditions (Hedrick, MacConnell & de Kinkelin 1993).

The distribution of *T. bryosalmonae* is highly heterogenous and its prevalence can be very high in certain wild salmonid populations (Okamura *et al.* 2011; Skovgaard & Buchmann 2012; Dash & Vasemägi 2014). Its geographic range is expected to expand in the future in relation with increased temperature (Tops, Hartikainen & Okamura 2009; Okamura *et al.* 2011), raising further concern about its effect on wild salmonid populations (Borsuk *et al.* 2006). Increased temperature can affect PKD host–parasite interactions both directly and indirectly (Clifton-Hadley, Richards & Bucke 1986; Bettge *et al.* 2009a,b). First, increased temperature results in an increased number of spores released to the water from the bryozoan host (Tops, Lockwood & Okamura 2006). Secondly, parasite proliferation in the salmonid host after infection might be stimulated by increased temperature (Gay, Okamura & de Kinkelin 2001; Bettge *et al.* 2009a,b). Thirdly, the increased temperature exacerbates the fish immune response to PKD and results in more severe lesions in the renal tissues (excretory and interstitial haematopoietic tissues) due to the inflammatory response (Bettge *et al.* 2009b). Finally, fish metabolic demands will increase with temperature, which can be problematic if the fish physiological functions (e.g. oxygen transport in the blood) are simultaneously impaired by the PKD symptoms.

As the majority of earlier immunological and physiological works on PKD have been performed in laboratory conditions using farmed rainbow trout (*Oncorhynchus mykiss*) as a model species, there is an urgent need for detailed characterization of the physiological effects of PKD on wild salmonid populations in their natural habitat, focusing on native potentially co-adapted host–parasite complexes (Okamura *et al.* 2011). This study aimed at characterizing the effect of *T. bryosalmonae* infection on physiological performance of brown trout (*Salmo trutta*) individuals directly collected from the wild to test whether PKD can have a negative impact on fish metabolic capacities and thermal resistance. Aerobic scope (AS) and upper thermal tolerance (UTT), which integrate the effects of infection at the level of the whole organism, were measured in a field-physiology approach (Costa & Sinervo 2004). Aerobic scope, the difference between maximum metabolic rate and standard metabolic rate, represents the aerobic metabolic power available once essential maintenance functions are provided for (e.g. ventilation and circulation at rest, protein turnover). This available metabolic power has to be partitioned through trade-offs between non-maintenance functions (e.g. growth, reproduction and locomotion, Guderley & Pörtner 2010). Organism performances such as resistance to disease (Castro *et al.* 2013) or migratory behaviour (Dalziel, Ou & Schulte 2012) depend on aerobic scope, and an impaired aerobic scope can therefore have deleterious effects on individual fitness. Upper thermal tolerance (UTT) is a proxy for the capacity of fish to withstand stressful temperature conditions in the wild. Additionally, circulating leucocyte abundances were measured to gain insights into brown trout immune response to PKD infection. Those measurements were tested for correlations with parasite load and severity of the disease symptoms (kidney hyperplasia and anaemia). The knowledge of the impact of infection on host physiological performances is critical to understand how both increased temperatures and resulting *T. bryosalmonae* range expansion might influence wild salmonid populations in the near future (Rohr *et al.* 2011, 2013).

Materials and methods

SAMPLING AND PRE-EXPERIMENTAL HANDLING

Juvenile (age 0+) anadromous brown trout (*Salmo trutta* L.) were caught by electrofishing in two locations in Estonia (Mustoja and Vainupea rivers) during 1 week at the end of July and 1 week at

the end of September 2011 (Table 1 and Table S1 in Supporting Information). Based on our earlier studies, both sampled river stretches are PKD-positive, while *T. bryosalmonae* has not been detected in the upstream areas above dams in River Mustoja (Dash & Vasemägi 2014). In July, a small number of individuals from both rivers were sampled ($n_{Mustoja} = 13$, $n_{Vainupea} = 11$). As clinical signs of PKD infection are more marked after the warm summer period, a larger number of fish were screened in September. However, since the density of 0+ trout was very low in River Mustoja in September we mainly focused on analysis of juveniles from River Vainupea for this month ($n_{Mustoja} = 3$, $n_{Vainupea} = 77$). Based on 10-year monitoring data, the density of juvenile fish in Mustoja was the lowest in 2011 (Kesler, Taal & Svirgsden 2015). After capture, fish were kept to fast in keepnets in the river before carrying out the performance experiments. Experiments were conducted in a field laboratory adjacent to the rivers to avoid long transportation steps to the laboratory. UTT experiments and blood smears for leucocyte counts were performed only in the September experiment. Given the low throughput of respirometry experiments compared to UTT, only 21 of the fish underwent respirometry in September. All experiments were performed according to the animal experimentation permit No 53 issued by the Estonian Ministry of Agriculture (issued on 17.11.2010, valid from 17.11.2010 until 31.12.2014).

METABOLIC RATE MEASUREMENTS

After fasting at least 24 h in keepnets in the river, standard metabolic rate (SMR) measurements were started in the evening and performed overnight in a flow-through respirometry system. Swimming trials for the maximum metabolic rates (MMRs) were performed during the next day with the same individuals. Aerobic scope (AS) was calculated as the difference between mean MMR and mean SMR for each individual. Water temperature in the respirometry measurement tanks was the same in July and in September (20 ± 1 °C). This was close to the river water temperature in July, but the river temperatures were lower in September (13–14·5 °C). To minimize thermal shock in September, individuals were acclimated to respirometry temperature over a temperature gradient before SMR experiments in a thermostated tank filled with river water and kept at this temperature during all subsequent experiments. See Appendix S1 in Supporting Information for details about the respirometry methods.

UPPER THERMAL TOLERANCE (UTT) TRIALS

Estimation of the critical thermal maximum is considered a good proxy to determine and compare upper thermal tolerance in ectotherms, even though care must be taken when comparing results from studies using different methodologies (Lutterschmidt & Hutchison 1997; Terblanche *et al.* 2007; Ribeiro, Camacho & Navas 2012). We used the loss of righting response (LRR) as an endpoint to determine upper thermal tolerance using a temperature ramp in a thermostated tank filled with river water. See Appendix S1 for the details of UTT trials.

Table 1. Sampling information. Coordinates are for the beginning of the sampling stretches

River	Date (1 week per month)	Coordinates	*N* (resp)
Mustoja	End of July 2011	59·582N, 26·177E	13 (13)
Vainupea	End of July 2011	59·567N, 26·256E	11 (11)
Mustoja	End of September 2011	59·582N, 26·177E	3 (2)
Vainupea	End of September 2011	59·567N, 26·256E	77 (19)

N, number of sampled individuals; (resp), number of sampled individuals used for respirometry experiments.

TISSUE SAMPLING AND ANALYSIS

After recovery from the experiments, fish were killed by an overdose of buffered MS222 (tricaine methanesulfonate) and body mass and fork length (FL, from snout to the end of the middle caudal fin) were measured. Condition factor can potentially reveal differences in food intake and was calculated as $\frac{\text{body mass (g)}}{(\text{fork length (mm)})^3} \times 10^6$ (Mills & Eloranta 1985). The tail was severed after carefully blotting each individual on paper towel, and blood was collected into sodium-heparinized glass capillaries (internal diameter of 0·5–0·6 mm, Marienfeld). When enough blood could be collected, several capillaries (up to three) were used and haematocrit values were averaged. In September, blood drops were also used for microscopic examination and determination of leucocyte formula. Capillaries were centrifuged at 12 250 *g* in a microcentrifuge for 5 min to assess haematocrit and leucocrit. After centrifugation, the length of the blood fractions corresponding to packed red blood cells and to the buffy coat (containing the white blood cells) were measured with a ruler and divided by the total length of the centrifuged sample to estimate haematocrit and leucocrit, respectively. We only measured leucocrit in September as the fish were larger and more blood could be collected to determine more reliably the buffy coat fraction size. Leucocyte formula was also determined for those fish (see Appendix S1 for details about the methods).

In order to obtain quantitative estimates of kidney hyperplasia, a cross section was produced by cutting the body between the front tip and the end of the dorsal fin. This section was then photographed with a digital camera for measurement of the kidney-to-body thickness ratio (K/B ratio, Fig. 1a). This ratio provides a quantitative estimate of the PKD-induced hyperplasia of the kidney rather than relying on a more subjective scoring system used earlier (e.g. Bettge *et al.* 2009a). Body sections were then stored in 95% ethanol for relative quantification of *T. bryosalmonae* DNA in the kidney tissue using qPCR.

QUANTIFICATION OF RELATIVE PARASITE LOAD BY QPCR

To assess individual relative parasite load, the parasite DNA was quantified relative to the host DNA in kidney samples from the body sections used to quantify kidney hyperplasia. DNA was extracted from kidney tissue based on a salt extraction method (Aljanabi & Martinez 1997). The parasite DNA was quantified using *T. bryosalmonae*-specific primers (Grabner & El-Matbouli 2009) that amplify 166-bp-long 18S rDNA sequence. To allow quantification of the parasite relative to the host DNA, the putative salmon prefoldin subunit 6 gene (EST_ssal_eve_10615, GenBank: EG831853.1) was used as an endogenous control based on the following primers (F: GGCTGGATTCAGTGCTTTTC, R: CAAGGTCTGACCAGCAGATG; Vasemägi *et al.* 2010). The comparative C_T ($\Delta\Delta C_T$) method was used to quantify the real-time PCR measurements using a reference calibrator sample. For each sample, relative parasite load compared to the calibrator sample was calculated as $2.\exp(-\Delta\Delta C_T)$. In order to keep as much quantitative information as possible for correlation analyses, no minimum C_T cut-off was applied.

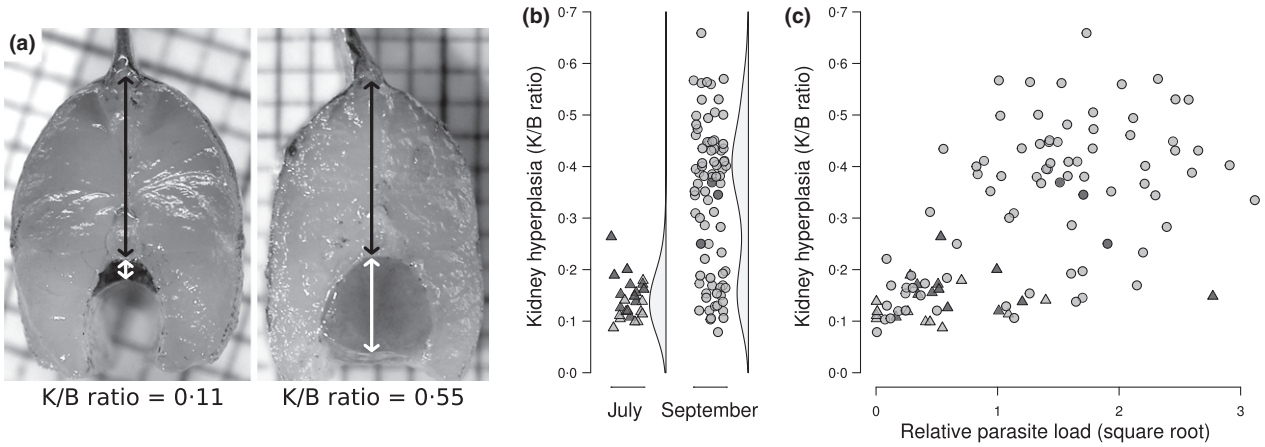

Fig. 1. Distribution of kidney hyperplasia and relation with parasite load. (a) Left, body section of a fish with normal kidney; right, body section of a fish with swollen kidney (hyperplasia). Black arrows, measured body thickness; white arrows, measured kidney thickness. K/B ratios are calculated as the ratio between the lengths of the white arrows and of the black arrows. (b) Temporal variation in distributions of K/B ratio (raw values, no FL-standardization). Light grey, Vainupea individuals; dark grey, Mustoja individuals. The corresponding density distributions are plotted vertically on the side of each month data points. Spreading of the data points along the horizontal axis is for visual convenience only (c) Relation between parasite load and kidney hyperplasia (raw values, no FL-standardization). Relative parasite load is estimated by qPCR. Triangles, July sampling; circles, September sampling; light grey, Vainupea individuals; dark grey, Mustoja individuals. Spearman's $\rho = 0{\cdot}63$, $P < 0{\cdot}001$. The regression line is fitted using the Theil-Sen estimator.

STATISTICAL ANALYSES

All statistical analyses were performed with the R software (version 3.2, R Core Team 2015). To avoid issues with potentially nonlinear relationships and heteroscedasticity of residuals, and since our primary interest was to detect monotonous relationships between PKD symptoms and traits rather than to estimate linear predictors, associations between variables (after fork-length standardization if needed) were estimated and tested for significance using Spearman's ρ.

Prior to correlation analyses, the effect of FL, origin, month and all their interactions on trait values was tested by stepwise linear regression with backward elimination in order to perform trait standardization to mean FL when appropriate (see Appendices S1 and S2 for details; metabolic rates were standardized by body mass, and no standardization by fork length was performed for these traits). FL had an effect on relative parasite abundance, haematocrit, leucocrit and UTT performance, and there was a significant interaction between FL and origin for relative parasite abundance; FL-standardization was performed accordingly (see Appendix S2). For kidney hyperplasia, the model including FL, month and origin showed strong heteroscedasticity of the residuals and linear regression was thus performed separately for each month. Subsequent correlation analyses involving relative parasite abundance were performed separately for each river of origin, and those involving kidney hyperplasia were performed separately for each month.

In the particular case of metabolic rates (SMR, MMR and AS), splitting the data according to origin and month would result in small data sets ($n = 13$, 11, 2 and 19) with low power for Spearman's ρ test within each data set. Hence, since we were interested in testing if there was an overall consistent monotonous relationship between metabolic rates and other variables within each group but not in the differences in the strength of the relationship between groups, we decided to produce normalized ranks for the samples within each group (i.e. rank within the group divided by the size of the group) and pooled those normalized ranks together to perform a single Spearman's ρ test using all 45 samples together. Simulations were performed to check that this approach did not result in artefactually low *P*-values for our data (see Appendix S1 for details).

Results

TEMPORAL DYNAMICS OF *T. BRYOSALMONAE* INFECTION AND PKD-INDUCED KIDNEY SWOLLENNESS

Brown trout kidneys exhibited a wide range of hyperplasia levels, from approximately normal to extremely swollen (Fig. 1a). The distribution of non-standardized K/B ratios differed between early and late summer (Fig. 1b, two-sample Kolmogorov–Smirnov test, $n = 24$ vs. 80, $D = 0{\cdot}67$, $P < 0{\cdot}001$). In July, the majority of juveniles showed no or very mild kidney hyperplasia (Fig. 1a left). In September, K/B ratios varied over a larger range: some individuals showed low to moderate kidney hyperplasia as in July but most individuals showed severe kidney hyperplasia (Fig. 1b right).

Parasite DNA was detected in all individuals by conventional PCR on kidney samples (data not shown). There was a strong positive correlation between *T. bryosalmonae* DNA abundance in kidney tissue (relative parasite load estimated by qPCR) and kidney hyperplasia (K/B ratio) ($n = 104$, $\rho = 0{\cdot}63$, $P < 0{\cdot}001$ using non-FL-standardized values for this comparison) (Fig. 1c). Interestingly, whereas low quantities of parasite DNA were associated with no or moderate swollenness, medium to high quantities of parasite DNA were associated with a large range of kidney swollenness, ranging from normal to extremely swollen kidneys.

CORRELATION BETWEEN PKD SYMPTOMS AND CONDITION FACTOR

Condition factor globally increased with the severity of kidney hyperplasia (Fig. S1). Because kidney hyperplasia was standardized for FL separately within each month,

Spearman's ρ was calculated separately for July and September (no significant interaction was found between kidney hyperplasia and origin within each month): the relation between condition factor and kidney hyperplasia was stronger in July, but significant in both cases (July, $n = 24$, $\rho = 0{\cdot}45$, $P = 0{\cdot}03$; September, $n = 80$, $\rho = 0{\cdot}33$, $P = 0{\cdot}003$).

RELATIONSHIPS BETWEEN INFECTION AND HAEMATOLOGICAL INDICES

Haematocrit decreased with increasing severity of kidney hyperplasia (Fig. 2; significant interaction between K/B ratio and origin in July, $P = 0{\cdot}004$; not tested in September since $n = 3$ for Mustoja fish; correlation between haematocrit and K/B ratio, in July for Mustoja: $n = 13$, $\rho = -0{\cdot}71$, $P = 0{\cdot}008$; in July for Vainupea: $n = 10$, $\rho = -0{\cdot}82$, $P = 0{\cdot}007$; in September for Vainupea: $n = 76$, $\rho = -0{\cdot}32$, $P = 0{\cdot}005$). Haematocrit was also negatively correlated with relative parasite load (significant interaction between relative parasite load and month for Vainupea fish, $P = 0{\cdot}03$; not tested for Mustoja fish since $n = 3$ for Mustoja fish in September; correlation between haematocrit and relative parasite load in July for Mustoja: $n = 13$, $\rho = -0{\cdot}29$, $P = 0{\cdot}33$; in July for Vainupea: $n = 10$, $\rho = -0{\cdot}39$, $P = 0{\cdot}26$; in September for Vainupea: $n = 76$, $\rho = -0{\cdot}44$, $P < 0{\cdot}001$).

In September, leucocyte abundance increased with increasing kidney hyperplasia ($n = 65$, Spearman's $\rho = 0{\cdot}44$, $P < 0{\cdot}001$; Fig. S2). The abundance of circulating thrombocytes increased with increasing kidney hyperplasia ($n = 59$, $\rho = 0{\cdot}31$, $P = 0{\cdot}02$) but no such association was detected for granulocytes ($n = 59$, $\rho = 0{\cdot}22$, $P = 0{\cdot}10$), lymphocytes ($n = 59$, $\rho = 0{\cdot}08$, $P = 0{\cdot}53$) or monocytes ($n = 59$, $\rho = 0{\cdot}004$, $P = 0{\cdot}98$) (Fig. 2). Overall leucocyte abundance and cell-type-specific leucocyte abundances were not correlated with relative parasite load (all Spearman's ρ P-values > 0·05).

CORRELATION BETWEEN INFECTION INDICES AND HOST PHYSIOLOGICAL PERFORMANCES

Metabolic rate measurements were highly repeatable, with intraclass correlation coefficients (ICC) of 0·98 for both standard and maximum metabolic rates.

When analysing the relationship between metabolic rates and PKD symptoms (haematocrit, kidney hyperplasia and relative parasite load), there were significant interactions of each symptom with origin and/or month for at least one of SMR, MMR or AS. We thus used pooled ranks normalized within sampling group for all metabolic rates, which showed that MMR and AS, but not SMR, were positively correlated with haematocrit ($n = 44$; MMR, $\rho = 0{\cdot}46$, $P = 0{\cdot}002$; AS, $\rho = 0{\cdot}38$, $P = 0{\cdot}01$; SMR, $\rho = 0{\cdot}13$, $P = 0{\cdot}38$) and negatively correlated with kidney hyperplasia ($n = 45$; MMR, $\rho = -0{\cdot}40$, $P = 0{\cdot}007$; AS, $\rho = -0{\cdot}44$, $P = 0{\cdot}002$; SMR, $\rho = 0{\cdot}08$, $P = 0{\cdot}58$) (Fig. 3). MMR and AS were also negatively correlated with relative parasite load ($n = 45$; MMR, $\rho = -0{\cdot}36$, $P = 0{\cdot}02$; AS, $\rho = -0{\cdot}33$, $P = 0{\cdot}03$) but SMR was not ($n = 45$; SMR, $\rho = -0{\cdot}16$, $P = 0{\cdot}30$).

Based on the fish from Vainupea caught in September, PKD infection severity and individual upper thermal tolerance (UTT) were negatively correlated: parasite load showed a marginally significant negative correlation with upper thermal tolerance ($n = 76$, $\rho = -0{\cdot}21$, $P = 0{\cdot}06$), but there was a stronger negative correlation between UTT and kidney hyperplasia ($n = 76$, $\rho = -0{\cdot}45$, $P < 0{\cdot}001$) (Fig. 4). UTT was positively correlated with haematocrit ($n = 75$, $\rho = 0{\cdot}27$, $P = 0{\cdot}02$) and positively but non-significantly correlated with aerobic scope ($n = 19$, $\rho = 0{\cdot}35$, $P = 0{\cdot}14$).

Discussion

Predicting the effects of parasites on host species in the wild in the context of global change is challenging, as it requires a thorough understanding of host–parasite interactions from ecological to molecular levels under changing conditions. Our results demonstrate the dramatic correlation between PKD symptoms and decreased host performances in the wild, which is especially worrying given the already known effects of increased temperature on this host–parasite system.

PKD AND THE REDUCTION OF HOST PHYSIOLOGICAL PERFORMANCES

Reduced aerobic scope

Previous works in adult sockeye salmon (*Oncorhynchus nerka*) infected by another myxosporean kidney parasite and monitored under laboratory conditions showed no effect of infection on metabolic rates and swimming performance, but an effect on recovery from exercise (Wagner *et al.* 2005). Similarly, no effect of PKD on time to exhaustion in a swimming challenge in juvenile Chinook salmon was observed (Foott, Stone & Nichols 2005). In contrast, our results show a negative correlation between PKD severity and MMR but not with SMR in wild, juvenile brown trout. Low AS resulting from reduced MMR can have a direct and immediate effect on juvenile survival in 0+ brown trout in the wild by limiting foraging, competition with other individuals and predator avoidance (Auer *et al.* 2015; Killen *et al.* 2015). This is especially relevant during summer heat waves when water temperature increases, oxygen solubility decreases and oxygen demand is higher. Low AS in juveniles could potentially have lasting effects carried over to later life stages in the surviving individuals such as changes in timing of smoltification or reduced survival in marine environment.

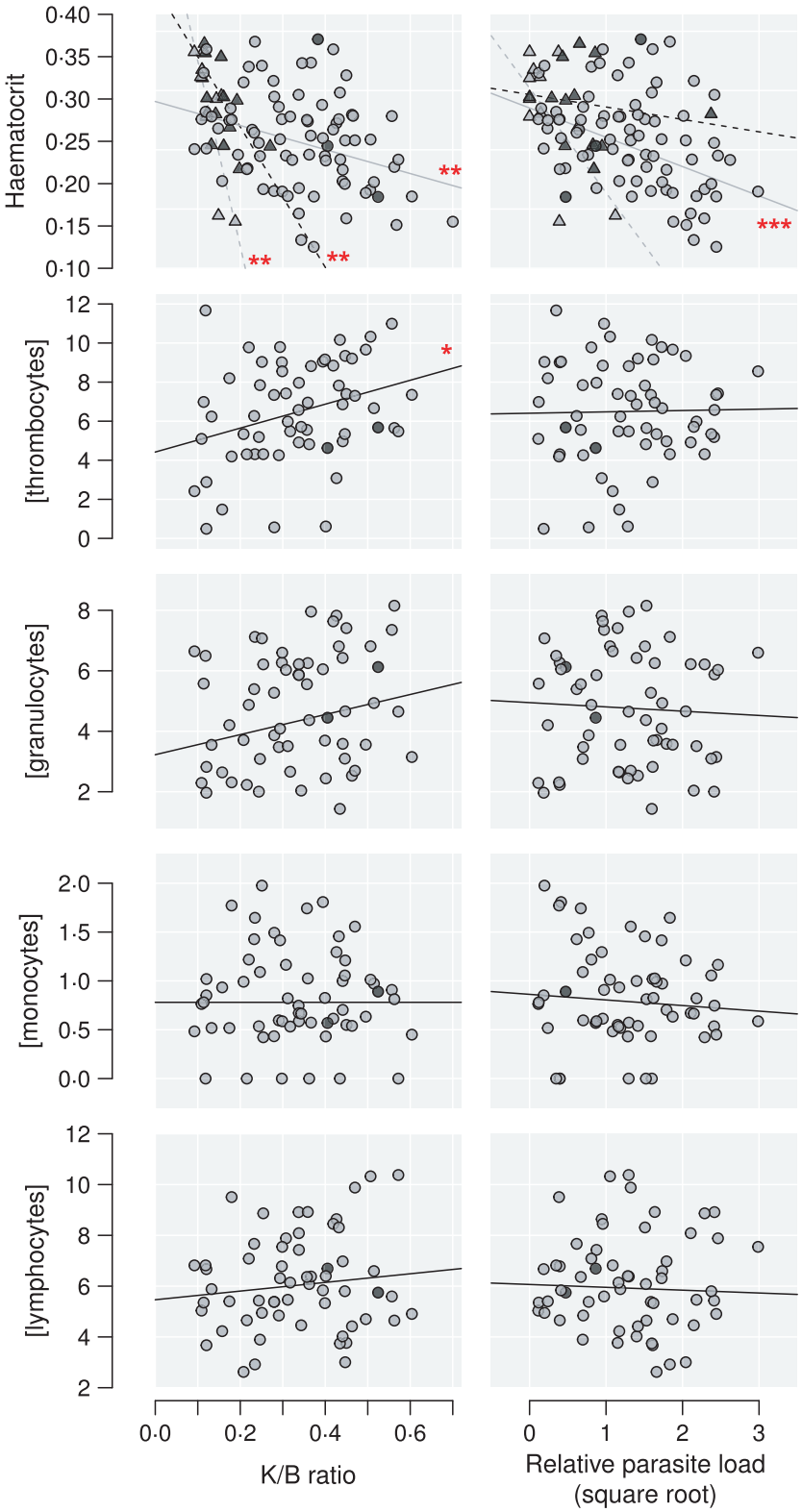

Fig. 2. Effect of PKD on blood cell abundances. Concentration of white blood cell types are estimated by (n_{cells} per 10 000 red blood cells) × haematocrit (values are then square root transformed). Relative parasite load is estimated by qPCR, and kidney hyperplasia is measured by the K/B ratio. Haematocrit, relative parasite load and kidney hyperplasia were standardized by fork length. Triangles, July sampling; circles, September sampling; light grey, Vainupea individuals; dark grey, Mustoja individuals. Regression lines are drawn using Theil-Sen estimators, using: black solid line, all fish from September; black dashed line, Mustoja fish from July; grey dashed line, Vainupea fish from July; grey solid line, Vainupea fish from September. Red asterisks denote significance levels for corresponding Spearman's ρ: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

Lowered upper thermal tolerance

PKD severity was negatively correlated with the upper thermal tolerance of infected fish (Fig. 4). While UTT trials using fast temperature ramps might not perfectly reflect resistance to global warming (Nguyen *et al.* 2011), they do measure general organism performance and capacity to cope with demanding conditions. Moderately increased temperatures are a challenge in themselves for aerobic metazoans, even though temperate species are thought to be less close to their thermal limit compared to tropical species (Stillman 2003; Tewksbury, Huey & Deutsch 2008).

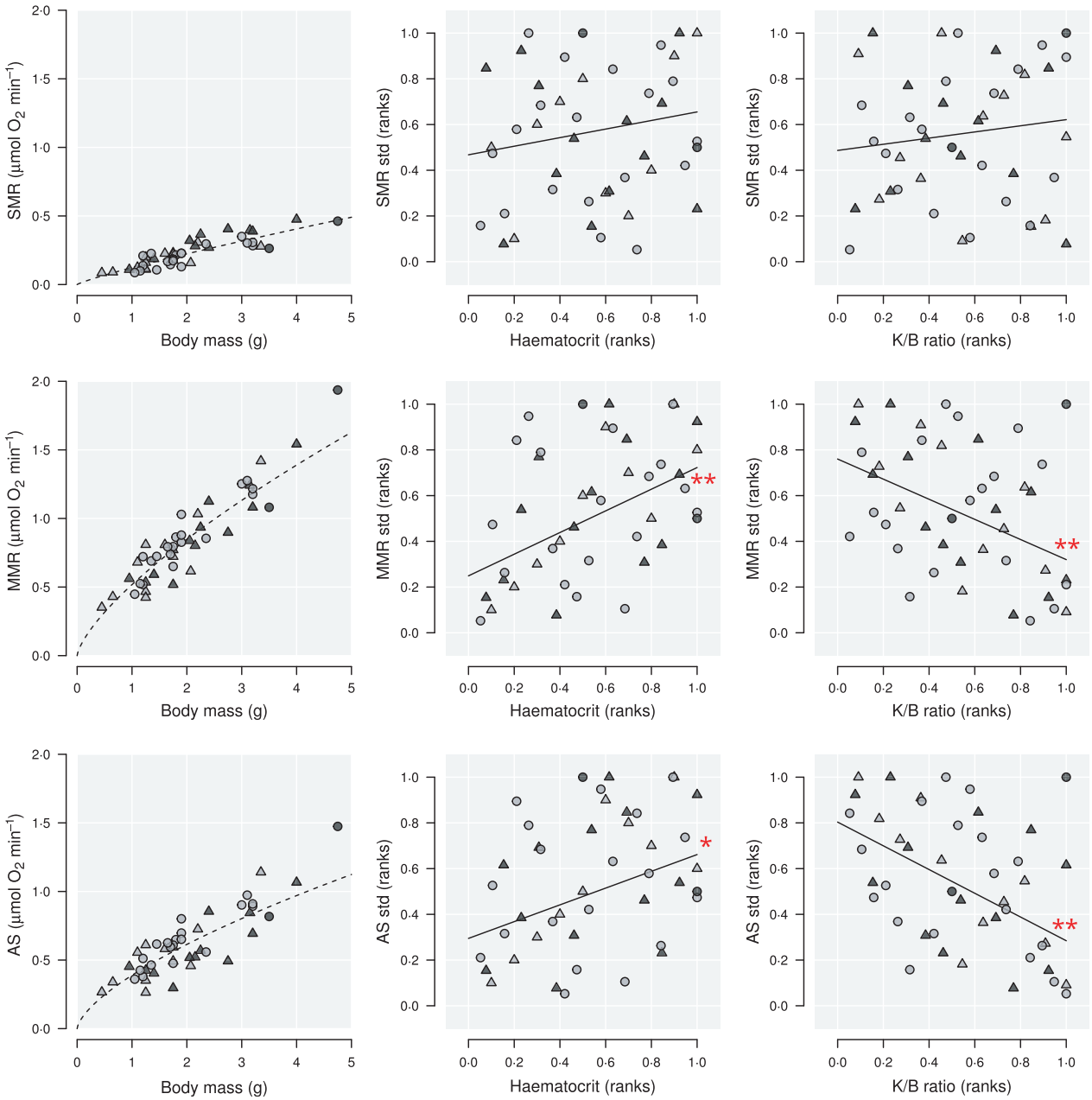

Fig. 3. Effect of PKD on aerobic performances. Leftmost panels, relation between body mass and metabolic rates and aerobic scope. Central panels, relation between haematocrit and standardized mass-specific metabolic rates and aerobic scope (ranked values within sampling group). Rightmost panels, relation between kidney hyperplasia and standardized mass-specific metabolic rates and aerobic scope (ranked values within sampling group). Haematocrit and kidney hyperplasia were standardized by fork length. Triangles, July sampling; circles, September sampling; light grey, Vainupea individuals; dark grey, Mustoja individuals. The regression lines are based on the Theil-Sen estimator. Red asterisks denote significance levels for corresponding Spearman's ρ: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

Salmonids are, however, a special case of temperate species, since they are dependent on cooler freshwater and might in this respect be more sensitive to temperature increase than other temperate freshwater species.

Our results can be interpreted in the framework of the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis stating that aerobic scope is the main factor determining thermal tolerance in aerobic ectotherms. The OCLTT hypothesis predicts that aerobic scope increases with temperature until it reaches a peak (optimum temperature for aerobic scope) but then decreases at higher temperature because SMR would increase faster than MMR as the rate of oxygen delivery by the cardiorespiratory system becomes limiting (Pörtner 2001; Pörtner & Knust 2007; Farrell *et al.* 2009). This theory is currently being challenged and might not always explain observations satisfactorily (Gräns *et al.* 2014; Norin, Malte & Clark 2014). It has been proposed that aerobic scope is only one physiological function among others that can become limiting at high temperature (Clark, Sandblom

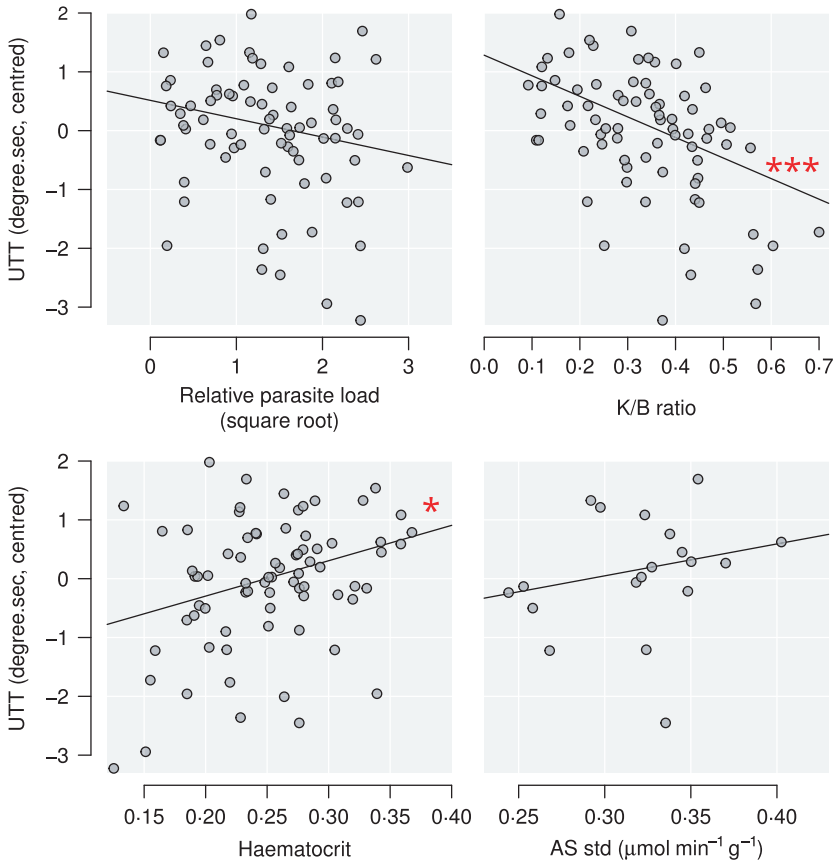

Fig. 4. Effect of PKD on thermal tolerance. Upper thermal tolerance estimates (UTT) are centred across trials. UTT, haematocrit and kidney hyperplasia values are standardized by fork length. AS std, standardized mass-specific AS. The regression lines are based on the Theil-Sen estimator. All fish from September sampling; light grey, Vainupea individuals; dark grey, Mustoja individuals. Red asterisks denote significance levels for corresponding Spearman's ρ: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

and Jutfelt 2013; but see also Gräns *et al*. 2014; Pörtner 2014; Jutfelt *et al*. 2014; Bozinovic & Pörtner 2015). However, the OCLTT hypothesis provides a reasonable framework to interpret our results, as we observe a correlation between reduced haematocrit (and hence admittedly aerobic scope) and reduced UTT.

CLINICAL SYMPTOMS ASSOCIATED WITH PKD IN THE WILD

Kidney hyperplasia and anaemia

Similarly to earlier studies (e.g. Hedrick, MacConnell & de Kinkelin 1993), the most striking PKD symptoms in wild 0+ brown trout were kidney hyperplasia and mild to severe anaemia. Kidney hyperplasia and lesions are due to the proliferation of lymphocytes and macrophages when *T. bryosalmonae* multiplies in the interstitial tissue (Clifton-Hadley, Bucke & Richards 1987; Hedrick, MacConnell & de Kinkelin 1993; Chilmonczyk, Monge & de Kinkelin 2002). While renal swelling and anaemia were relatively mild in July, we observed very drastic signs of PKD in September. This is consistent with the seasonality of PKD outbreaks (Wahli *et al*. 2002; Sterud *et al*. 2007; Dash & Vasemägi 2014) and previous laboratory experiments demonstrating the existence of a time-lag and a minimal temperature for the onset of PKD symptoms in infected fish (Bettge *et al*. 2009a,b).

The head kidney produces erythrocytes in teleost fishes (Fänge 1994) whereas the excretory tissue is involved in osmoregulation and urine production (Safford Black 1957) and is mostly found in the back kidney (e.g. Pacific salmon, Groot, Margolis & Clarke 1995). Haemoglobin genes are down-regulated in infected brown trout kidney (Kumar, Abd-Elfattah & El-Matbouli 2014), which suggests suppressed erythropoiesis. Spleen, which is involved in red blood cell destruction and iron recycling to the haematopoiesis sites (Soldatov 2005), is also swollen as a consequence of PKD (Hedrick, MacConnell & de Kinkelin 1993). It has therefore been proposed that PKD-induced anaemia is the result of both impaired production and problematic red blood cell destruction (Hedrick, MacConnell & de Kinkelin 1993).

Changes in circulating leucocyte types

While several recent studies have characterized the immune response in the kidney tissues during PKD (Gorgoglione *et al*. 2013; Kumar, Abd-Elfattah & El-Matbouli 2014), few studies have focused on the response of circulating leucocytes during infection and PKD development. Chilmonczyk, Monge & de Kinkelin (2002) examined the abundance of lymphocytes and granulocytes in PKD-infected rainbow trout and found that the blood cell populations underwent virtually no change during the course of infection, with about five times more lymphocytes than

granulocytes. However, they found that the pronephros exhibited an increase in the lymphocyte population compared to before infection when it had almost equal amount of both cell types. Foott, Stone & Nichols (2005) found no marked change in white blood cell profiles during the development of PKD in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). In contrast to what was observed for rainbow trout, we found that lymphocytes and granulocytes were similarly abundant in the blood of infected brown trout (raw counts, see Data accessibility section). We also found that thrombocytes increased in abundance with increasing severity of PKD symptoms (K/B ratio), independently from relative parasite load. The thrombocyte response might be related to a reaction against haemorrhage (one of PKD clinical signs). Alternatively, this response could be targeted against secondary infections, or reflects a general systemic response to immune challenge of the organism.

Our results and the work from Chilmonczyk, Monge & de Kinkelin (2002) show that the immune response mediated by circulating leucocytes during PKD infection differs between brown trout and rainbow trout. The immune response to PKD also differs between brown trout and rainbow trout at the kidney level (Kumar, Abd-Elfattah & El-Matbouli 2015). This variability demonstrates the importance of using native host–parasite combinations to characterize species- and tissue-specific immune responses (Okamura *et al.* 2011).

Variation in resistance and sensitivity to T. bryosalmonae infection or/and recovery from PKD

We identified large variation both in resistance (parasite load) and sensitivity (kidney hyperplasia and haematocrit for a given parasite load) to *T. bryosalmonae* infection among studied fish. The very loose coupling between actual parasite load (measured by qPCR) and PKD symptoms suggests that individuals in the wild differ in their sensitivity to *T. bryosalmonae* infection: while individuals with low parasite load always had normal kidneys, fish with medium to high parasite load exhibited kidney hyperplasia ranging from weak to extreme (Fig. 1a,c). Similarly, the majority of clinical symptoms observed in our study (anaemia, circulating leucocyte response, physiological performances) were more strongly correlated with kidney hyperplasia than with parasite load. Since kidney hyperplasia is linked to the intensity of the immune response (Hedrick, MacConnell & de Kinkelin 1993), it might be that tolerant individuals avoid hyperplasia of the kidney interstitial tissue with a milder immune response. Alternatively, some individuals may recover from the symptomatic stage of the disease faster than others and the observed discrepancies between parasite load and symptoms could be due to differences in recovery status, infection date or potential secondary infections by pathogens other than *T. bryosalmonae*. Both hypotheses are not mutually exclusive, and monitoring of the temporal dynamics of parasite load and PKD symptoms in the wild would be needed to distinguish between the two.

CONSEQUENCES OF PKD ON BROWN TROUT PERFORMANCE IN THE CONTEXT OF CLIMATE CHANGE

Earlier ecological and physiological studies have shown that the salmonid fish/*T. bryosalmonae* host–parasite system is extremely sensitive to temperature increase because higher temperature has multiple effects that all negatively impact the host at different levels (production of parasite spores by the bryozoan host, infection rates of the fish by *T. bryosalmonae* and activation of the fish immune system triggering the emergence of PKD symptoms). Here, we reported negative correlations between PKD symptoms and both aerobic scope and thermal tolerance observed in the wild, but controlled experiments are necessary to formally test the causal relationships between the disease and the lowered physiological performances. However, several lines of evidence strongly support a limitation of aerobic performances due to PKD infection mediated through decreased oxygen transportation capacity of the circulatory system. In particular, (i) the correlation between kidney hyperplasia and anaemia (impaired haematopoiesis in the pronephros of the hyperplasic kidney), (ii) significant relationships between haematocrit and active metabolic rate (MMR) but not baseline rate (SMR) and (iii) the absence of correlation between PKD severity and SMR support the functional link between PKD and host performance.

Taken together, our results suggest that *T. bryosalmonae* could have substantial physiological costs for the host, and such costs may result in substantial mortalities among juvenile brown trout. Earlier studies have documented declines of juvenile brown trout and juvenile salmon related to increased summer temperature and reduced discharge (Jonsson & Jonsson 2009; Almodóvar *et al.* 2012), but climate-driven changes may have severe consequences on host populations also via the emergence of parasitic disease(s) (Marcogliese 2008; Rohr *et al.* 2013). Due to the existence of a temperature threshold for the onset of PKD symptoms, this host–parasite system is likely to be susceptible both to an increase in average summer temperature but also to an increase in temperature variability and in the frequency of extreme events, as is expected in future scenarios (Schär *et al.* 2004; Rohr *et al.* 2013).

The brown trout/*T. bryosalmonae* host–parasite system illustrates the importance of a more integrated view to study both the physiological and ecological mechanisms that mediate climate-change impacts on host–parasite interactions (Gallana *et al.* 2013). Studies that aim to predict the effects of global change in natural ecosystems should not solely rely on laboratory experiments that quantify the effect of temperature on host performance and combine it with predictions on the expansion of the parasite range: the real impact of climate change is likely to be more than the superimposition of independent

predictions because of the interactions between parasite and host performance in a changing environment.

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Competing interests

The authors declare no competing interests.

Data accessibility

The data set related to this work is publicly available on Dryad Digital Repository http://dx.doi.org/10.5061/dryad.591d4 (Bruneaux *et al*. 2016).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Relation between kidney hyperplasia and condition factor.
Fig. S2. Relation between kidney hyperplasia and leukocrit.
Fig. S3. Correlogram for main variables.
Fig. S4. Test for absence of significance inflation when pooling normalised ranks in a Spearman's ρ test.
Table S1. Sampling locations complete coordinates.
Appendix S1. Methods.
Appendix S2. Standardisation for body size effect.
Appendix S3. Legends to supplementary figures.