Host developmental stage effects on parasite resistance and tolerance

Ines Klemme[1*], Paul Vincent Debes[2,3*], Craig Robert Primmer[2,4], Laura Sofia Härkönen[5,6], Jaakko Erkinaro[5], Pekka Hyvärinen[6], Anssi Karvonen[1]

*joined lead authors

[1]University of Jyvaskyla, Department of Biological and Environmental Science, P.O. Box 35, 40014 University of Jyvaskyla, Finland

[2]Organismal & Evolutionary Biology Research Program, Institute of Biotechnology, 00014 University of Helsinki, Finland

[3]Department of Aquaculture and Fish Biology, Hólar University, Sauðárkrókur, 550, Iceland

[4]Institute of Biotechnology, Helsinki Institute of Life Sciences (HiLIFE), 00014 University of Helsinki, Finland

[5]Natural Resources Institute Finland (Luke), Migratory fish and regulated rivers, Paavo Havaksen tie 3, 90570 Oulu, Finland,

[6]Natural Resources Institute Finland (Luke), Migratory fish and regulated rivers, Manamansalontie 90, 88300 Paltamo, Finland

Corresponding author: Ines Klemme, ines.klemme@jyu.fi

ORCIDs: Klemme, 0000-0001-9757-7356; Debes, 0000-0003-4491-9564; Primmer, 0000-0002-3687-8435; Härkönen, 0000-0001-5903-3999; Erkinaro, 0000-0002-7843-0364; Hyvärinen, 0000-0002-2376-3495; Karvonen, 0000-0002-2731-3344

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ABSTRACT

Hosts can defend themselves against parasites by either preventing or limiting infections (resistance), or limiting parasite-induced damage (tolerance). However, it remains underexplored how these defense types vary over host development with shifting patterns of resource allocation priorities. Here, we studied the role of developmental stage on resistance and tolerance in Atlantic salmon (*Salmo salar*). This anadromous fish has distinct life stages related to living in fresh and sea water. We experimentally exposed one-year old salmon, either at the freshwater stage or at the stage transitioning to the marine phase, to the trematode *Diplostomum pseudospathaceum*. Using 56 pedigreed families and multivariate animal models, we show that developmental transition is associated with reduced resistance, but does not affect tolerance. Furthermore, by comparing tolerance slopes (host fitness against parasite load) based on additive genetic effects among infected and unexposed control relatives, we observed that the slopes can be largely independent of the infection, that is they may not reflect tolerance. Together, our results suggest that the relative importance of different defense types may vary with host development and emphasize the importance of including control treatments for more confident interpretations of tolerance estimates.

Introduction

Hosts have two non-exclusive options to reduce negative fitness impacts of parasites: they can limit their load (resistance) and fitness loss at a given load (tolerance; Read et al. 2008; Råberg et al. 2009). In the wild, hosts show remarkable variation in these defense types both within and across populations (Schmid-Hempel 2003; Råberg et al. 2009; Sadd & Schmid-Hempel 2009; Medzhitov et al. 2012). Because resistance is expected to reduce parasite fitness, while tolerance is not (Roy & Kirchner 2000; Best et al. 2008), the relative investment to these defense types may have important epidemiological and evolutionary consequences. Identifying the sources of defense variation could therefore be critical for predicting outcomes of host-parasite interactions.

A link between defense variation against parasites and host life history has long been suggested (Sheldon & Verhulst 1996; Medley 2002; Rolff & Siva-Jothy 2003). As both resistance and tolerance consume host resources, optimal allocation to defense depends on the demands of other life functions and their relative benefits (Sheldon & Verhulst 1996; Jokela et al. 2000; Lochmiller & Deerenberg 2000; Zuk & Stoehr 2002). For example, life-stage transitions, obligatory for maturation and reproduction, often involve costly developmental processes that could temporarily become a resource investment priority. Such developmental constraint was explored in a theoretical model for insect larvae, which predicted that optimal defense allocation would be to reduce resistance, but increase tolerance (Tate & Graham 2015). Empirically, this constraint has been studied in several amphibians, where resistance across larval developmental stages either remains constant, increases or decreases, and tolerance uniformly increases (Rohr et al. 2010; Johnson et al. 2011). More generally, host age has often been linked to heterogeneity in resistance (reviewed in Ashby & Bruns 2018; Ben-Ami 2019) and tolerance (reviewed in Kutzer &

Armitage 2016). While such age-dependence may be attributable to developmental constraint, it could also relate to other factors such as acquired immunity or immunosenescence. Thus, the extent to which resistance and tolerance are shaped by host development remains underexplored.

Anadromous fish have distinct life stages related to living in fresh versus sea water. They hatch and live in fresh water until commencing a feeding migration to the sea. The endocrine-driven transition stage (smoltification) involves a number of energetically costly morphological and physiological changes that are adaptive for migration and the marine environment (Hoar 1988; Björnsson et al. 2011). In Atlantic salmon (*Salmo salar*), the age at which this transition takes place varies between 1-8 years (Marschall et al. 1998), generating developmental variation among similar-aged fish. Further, smoltification has been found to link with a reduction of several immune parameters (Muona & Soivio 1992; Melingen et al. 1995; Pettersen et al. 2003) and a down-regulation of immune genes (Johansson et al. 2016). These results are suggestive of developmental constraints on host defense; however, resistance and tolerance have not been directly compared between freshwater and transitional or migratory stages.

Here, we exposed one-year-old Atlantic salmon from 56 pedigreed families exhibiting either the resident (not yet migrating) or migrating phenotype to the trematode *Diplostomum pseudospathaceum*. This parasite commonly infects a variety of fish species in fresh and brackish water, but does not occur in sea water (Chappell 1995; Valtonen & Gibson 1997; Seppälä et al. 2011). We asked whether the host developmental stages (resident versus migrant) show differences in investment in resistance and tolerance. Resistance was quantified as inverse parasite load after controlled exposure. Tolerance is typically measured as a univariate regression slope of host post-exposure fitness against

parasite load (range tolerance; Råberg et al. 2009). This approach, however, assumes that the relationship is driven by parasite load (fig. 1 A), while host pre-exposure fitness could also affect parasite infection success or replication rate after infection (fig. 1 B).

To overcome the limitations of univariate regression methods for tolerance estimates, Graham et al. (2011) suggested the use of multivariate models (that have several responses, not to be confused with multiple regression models that are based on one response and have several regressors). Multivariate models relax causality assumptions and, additionally, allow decomposing the covariance into underlying effects of interest. Specifically, a regression slope (*b*) is defined as the ratio of the covariance (*cov*) between a regressed variable (response *y*; e.g., a fitness trait) and the regressor (predictor *x*; e.g., the parasite load) and the variance (*var*) of the regressor: $b = cov_{x,y}/var_x$. Multivariate models do not directly estimate regression slopes between the different response variables, but instead variance for each variable and covariance between them. Having estimates of covariances and variances thus allows for calculating regression slopes between the included response variables. When (co)variances are estimated at different levels that decompose the phenotypic variance into different components - such as genetic versus non-genetic or random environmental versus systematic experimental - regression slopes can be calculated at all of these levels. Under appropriate data structure, this allows for decomposing also the phenotypic regression slope, and thus for investigations of the foundational levels of the estimated phenotypic relationship. By further including uninfected control individuals, multivariate models also allow for studying the genetic covariance between fitness traits and parasite load in the presence and absence of infections. Specifically, genetic covariance can be estimated between groups of related individuals in different environments when genetic relationships connect the two groups

across environments (Falconer 1952). As a result, having genetic ties between groups of individuals in control and infection treatments may allow for assessing the role of parasites in shaping tolerance slopes with more confidence.

By employing multivariate animal models, we here provide support for developmental stage effects on resistance, but not on tolerance. Furthermore, we show that genetic tolerance slopes can also be inferred among control individuals, possibly due to an often-unconsidered interdependence of host fitness and resistance. Thus, our results not only demonstrate that host development can generate variation in defense against parasites, but also highlight important inferential considerations related to experimental design in tolerance studies.

Methods

Host-parasite system

The trematode parasite, *D. pseudospathaceum*, has a complex life cycle with three different hosts: an aquatic snail, a fish and a fish-eating bird (Karvonen 2012). Fish become infected by free-swimming larvae (cercaria) that penetrate their gills or skin, primarily when the water temperature exceeds 10°C, that is during June-August in the northern latitudes (Karvonen 2012). After penetration, the parasite actively moves within 24 hours through host tissue towards the eye and settles in the lens, where it can persist for many years. It does not multiply, but exploits its host for growth and uses it as a transmission vehicle to the final bird host. Parasitic movements and metabolic excretions cause structural damages in the lens, which lead to cataracts (Shariff et al. 1980) that increase in size with parasite load (Karvonen et al. 2004). These cataracts impair host vision and cataract size associates

negatively with host growth (Karvonen & Seppälä 2008) and positively with susceptibility to predation (Seppälä et al. 2005). Fish employ both nonspecific and specific immune responses to fend an infection with *D. pseudospathaceum* (Chappell et al. 1994). However, as the eye lens lacks blood circulation, the time window for resistance is likely restricted to the short (~1 day) period when the parasite migrates through the host. Thereafter, host defense may rely mainly on tolerance. Possible tolerance mechanisms include repair of damaged tissue, for example through eye tissue regeneration (Padros et al. 2018; Klemme et al. 2021), or behavioral and physiological adjustments that compensate for a reduced foraging success, such as increased food searching efforts or adjustments in metabolic rate.

Animal origin

We used parental fish from two different Atlantic salmon broodstocks maintained by the Natural Resources Institute Finland (LUKE). Their home rivers, Oulu and Tornio, drain into the Bothnian Bay of the Baltic Sea. We used 24 2×2 factorial matings, each including one female and male from both Oulu and Tornio, to produce 96 families. Thus, each family belonged to one of four crosses: Oulu, Tornio or their respective hybrids (female/male reciprocal).

We fertilized the eggs in October 2017 and incubated them under standard conditions. In March 2018, we transferred 4000 eggs each to two replicate tanks (3.14 m^2) per cross. Hatching occurred in May 2018, and the fish were maintained in these tanks until February 2019, when we combined the cross-specific duplicates. Following combination, we randomly selected 800 fish from each cross for injection of HDX PIT-tags (12×2 mm, Oregon RFID) under mild anesthesia (40 mg l^{-1} benzocaine). Because the families had been mixed during rearing, we took a fin tissue sample to assign family and determine genotypic sex

(see Aykanat et al. 2016 for details on genotyping; see Debes et al. 2021 for details on assignment protocol). Subsequently, we maintained the fish in two round tanks (15 m^2), both containing 1600 individuals from all four crosses in equal proportions. Throughout the rearing period, the fish were fed with commercial fish food and the light-cycle and water temperature corresponded to natural conditions. The study was carried out with permission from the Finnish Regional State Administrative Agency (license no. ESAVI/5184/04.10.07/2017) and complied with the animal care legislation of Finland.

Experimental setup

The experiment was conducted at Kainuu Fisheries Research Station (www.kfrs.fi) of LUKE, a flow-through experimental facility supplied with water from a nearby lake at natural temperatures. The setup consisted of two main parts. At first, we assessed the developmental stage (resident or migrant) of each fish and subsequently, measured their resistance and tolerance to infection with *D. pseudospathaceum*. We assigned the fish to 16 experimental replicate groups of 120 individuals each, with equal proportions of the four population crosses. Experimental animals in the 16 replicates originated from a total of 56 of the initial 96 families, each represented by 16-80 individuals, and each family was distributed randomly across 10-16 replicates (according to family size).

Following assignment, we transferred the replicate groups to 16 ring-shaped, semi-natural streams (fig. 2 A, B). The streams had a width of 1.5 m, a central perimeter of 26.15 m and an average water depth of 30 cm with an induced current of 40 l s^{-1} (0.09 m s^{-1}). The stream bed consisted of coarse gravel (ø 30–80 mm) and larger boulders for shelter. During the experiment, the fish relied on natural food, consisting of benthic and drifting invertebrates. After transfer, we initially placed the fish for 12-36 hours (variation due to a

randomized pre-transfer-measurement order) into large flow-through boxes (80 x 60 x 45 cm) within the streams for acclimation, and then released them simultaneously to the streams. After one week of further acclimation, we started to collect high-precision movement data using a PIT based radio frequency identification (RFID) system for developmental stage assessment (see below). Each stream was equipped with four PIT antennas (fig. 2 B) spaced at equal intervals and signal detections from by passing PIT-tagged fish were automatically recorded with a frequency of nine times per second. Because simultaneous recording was limited to 32 antennas (8 streams), we switched the surveillance between two sets of eight streams every third day.

Assessment of developmental stage

To assess salmon developmental stage, that is resident or migrant, we used a combination of morphological and activity indices. Following the migration period in the outdoor streams, we returned all fish temporarily to the laboratory (July 2019) and measured their length and mass under mild anesthesia (40 mg l^{-1} benzocaine). We also examined them for external migration indices, specifically skin coloration (fig. 2 C). At the transition stage, salmon skin changes from dark to silver for improved camouflage in open water (Björnsson et al. 2011). We categorized each individual as either 'silver' (fully silver) or 'not silver' (no silver color or partly silver).

Because individuals at an intermediate stage (partly silver) are sometimes observed to migrate (Vainikka et al. 2012), we additionally collected individual movement data during Jun 01-30. This period corresponds with the natural migration time of Atlantic salmon in the northern Baltic Sea area (Jutila et al. 2005; Otero et al. 2014). To infer downstream movement activity indicating seaward migration, we obtained individual counts of antenna

downstream bypasses. We filtered signal detections using the PIT Data software package (http://pitdata.net/) and calculated seaward migration activity as completed downstream rounds per hour, averaged across the period (15 days per individual). An average activity-threshold value of five rounds per hour was set for migrants according to the activity shown by 95% of the 'silver' individuals (fig. S1). We classified all 'silver' individuals as migrants (N = 201). Additionally, we also classified ´not silver´ individuals with an average activity above the threshold as migrants (N = 299). All individuals without phenotypic change and with an average activity below the threshold were classified as residents (N = 1129).

Parasite exposure and host defense

Following the assessment of morphological traits in July 2019, we conducted the parasite exposures. Prior to infection, we examined the eyes of 25 fish using slit-lamp microscopy and confirmed the absence of any earlier infections with *D. pseudospathaceum*. Infective larval stages (cercariae) for the exposures originated from 18 naturally infected *Lymnaea stagnalis* snails that we had collected from the wild 1-2 weeks before the exposures. Before the exposures, we placed the snails in individual containers with lake water for 3 hours, after which we combined the cercariae-containing solutions and determined average cercarial density using ten 1 ml aliquots. We assigned each of the 16 streams to either a parasite-exposed or a sham-exposed (control) treatment. All fish were then parasite-exposed (infected; N = 821, N = 8 streams) or sham-exposed (control; N = 793, N = 8 streams), that is we placed them individually in 1 l of lake water (16.4-16.7°C) with either 300 or zero cercariae, respectively, for 30 minutes. Immediately after the exposures, we returned the fish to their original outdoor stream. For logistic reasons, we measured and exposed two streams per day and eight streams in each of two consecutive weeks.

Ten weeks post-exposure (September 2018), we returned all surviving fish to the laboratory and measured their length and mass (742 infected and 752 control fish). We euthanized all parasite-exposed individuals using an overdose of benzocaine and studied both eyes for cataracts using slit-lamp microscopy (Karvonen et al. 2004). We scored the overall impact of the cataracts (fig. 2 D) on host vision by recording their coverage and thickness as 0-100% in increments of 10%. Subsequently, we dissected the eye lenses, measured their diameter and counted the number of parasites using a microscope. All parasite-exposed individuals that survived until the end of the experiment harbored infections in at least one eye.

We quantified inverse resistance as parasite load, that is the total number of successfully established parasites in the left and right eye of each host. We defined tolerance as the reaction norm between three fitness-related traits and parasite load. As one trait, we used the magnitude of lens tissue damage, which measured the host's ability to prevent or repair it (see above). We corrected the relative measure of percental cataract coverage for lens volume (based on the measured lens diameter) to determine the absolute quantity of affected tissue, that is total damage per parasite (cataract volume; Klemme et al. 2020). The other two fitness-related host traits were growth in length and change in body condition (Fulton's condition factor *K*; K = mass [g] / length^3 [cm] * 100), which quantified host feeding success despite infection effects on their vision (see above). Although the fitness consequences of growth rate vary generally among species and environments (Metcalfe & Monaghan 2003), maintaining higher growth rates and energy reserves during an infection with *D. pseudospathaceum* may be expected to increase survival probability until reproduction.

Statistics

Proportions of residents and migrants, survival rates

To collect summary statistics for the proportion of individuals at the migrant developmental stage, we fitted a univariate generalized animal model with probit-link function using Bayesian Markov chain Monte Carlo (MCMC) simulations implemented in MCMCglmm v. 2.32 (Hadfield 2010). We modelled the developmental-stage phenotypes (resident, migrant) as a function of infection treatment, cross, sex and the cross-by-sex interaction, and estimated the variance associated with stream and animal effects (equation 1; explained below). The residual variance was fixed to 1 because it cannot be estimated in threshold models. For the variances, we specified univariate χ_1^2 priors as recommended by de Villemereuil et al. (2013). We ran the model with four chains for 322,500 iterations each and sampled every 250 iterations. We then determined i) sampling convergence as indicated by a scale reduction factor around 1 per chain (Brooks & Gelman 1998), ii) the number of samples to discard ("burn-in") as when consistently reaching a scale reduction factor < 1.1 across chains (Brooks & Gelman 1998), and iii) the thinning per chain that resulted in autocorrelations at lag 2 < 0.1. In addition, we checked for sufficient mixing via MCMC per chain by visual examination of trace plots. These criteria resulted in combined posteriors across chains based on 3560 iterations. The model for the proportion of residents and migrants, recorded as individual binary vector (*Y*) for resident (coded as 0) or migrant (coded as 1) followed:

$$Y = \mu + \beta_1 Treatment + \beta_2 Cross + \beta_3 Sex + \beta_4 CrossSex + animal + stream + \varepsilon rror \quad (1)$$

where μ is a constant, $\beta_1 Treatment$ the infection treatment effect (infected or control), $\beta_1 Cross$ the cross effects (Oulu, Tornio or their hybrids), $\beta_2 Sex$ the sex effect (female or male), and $\beta_3 CrossSex$ the corresponding interaction effect. The terms animal, stream, and

εrror refer to genetic effects (breeding values, estimating additive genetic variance; V_A), random common environmental effects (stream identifications, estimating common environmental variance; V_C), and residual effects (combining random environmental effects and measurement error, estimating residual variance; V_R). As characteristic of traditional animal models, we estimated breeding values and the associated genetic variance via the pedigree-based inverse-relationship matrix (Henderson 1973). We report model predictions based on back-transformed posterior estimates obtained using the package QGglmm (de Villemereuil et al. 2016).

We also tested whether the two developmental stages differed in survival in the presence and absence of infection. A differential survival rate can lead to biased results if data are not missing at random. Therefore, we fitted a univariate generalized animal model with probit-link function that followed the methodology as outlined above for the proportion of migrants, but we retained 2380 iterations. As another difference, the model equation for the response of the binary record vector (Y) for dying (coded as 0) or surviving (coded as 1) followed:

$$Y = \mu + \beta_1 Treatment + \beta_2 Development + \beta_3 Cross + \beta_4 Sex + \beta_5 TreatmentDevelopment + \beta_6 CrossSex + \beta_7 TreatmentCross + \beta_8 TreatmentSex + \beta_9 TreatmentCrossSex + stream + animal + \varepsilon rror \quad (2)$$

Resistance: parasite load and pre-exposure host traits

We fitted a multivariate animal model to estimate inverse resistance (parasite load) and study how it relates to additional pre-exposure parameters of interest (for an overview of all multivariate models see table S1). Specifically, we assessed whether residents and migrants vary for resistance and whether resistance covaries with body length and condition prior to

parasite exposure, while controlling for the effects of sex and cross. In this model, we included three traits as response variables (*Y*): the log of parasite load (LN of parasite load + 1), the log of pre-exposure body length (LN of body length in mm) and pre-exposure body condition (Fulton´s *K*). The model followed the equation per response:

$Y = \mu + \beta_1 Development + \beta_2 Sex + \beta_3 Cross + DevelopmentAnimal + DevelopmentStream + DevelopmentError$ (3),

where μ is a constant, $\beta_1 Development$ the developmental stage effect (resident or migrant), $\beta_2 Sex$ the sex effect (female or male), and $\beta_3 Cross$ the cross effect (Oulu, Tornio or their hybrids). The random terms were as in (1) but conditionally fit for each of the two developmental stages. Covariance matrices for all random terms (including residuals) were specified as 6 × 6 matrices, referring to the interaction of three traits and two developmental stages. This specification allowed estimating variances for each trait separately per developmental stage, covariances between developmental stages for each trait, and covariances between traits per developmental stage (see also table S2). Based on these developmental stage-specific trait variances and their covariances, we were able to construct developmental stage-specific regression slopes between traits at each random level (genetic slopes based on 'animal', common environmental slopes based on 'stream', and random environmental slopes based on 'residual'). These slopes are assumed to represent a causal relationship of pre-exposure traits (length, condition) on infection susceptibility as represented by parasite load.

Tolerance: parasite load and post-exposure host traits

Models on tolerance, that is the relationship between parasite load and host post-exposure fitness-related traits, were similar to equation (3). We fitted two multivariate models on

parasite load and altogether three fitness-related host traits: one model that included the two responses of parasite load and cataract volume (tolerance model 1) and one model that included the three responses of parasite load, growth in length (Δ Length, the log of the size difference), and change in body condition (Δ Condition, the difference in Fulton's condition index) during 10 weeks following parasite exposure (tolerance model 2).

For tolerance model 1, we modelled the response of cataract volume with the additional mean-centered covariate of lens volume (standardizing estimates to the average lens volume of 21.54 mm^3), because cataract volume induced by a given number of parasites appeared to depend on lens volume (saturation effect, fig. S2). However, the latter relationship changed with the number of parasites (fig. S2) and we therefore fitted the additional continuous interaction term of lens volume with parasite load. Furthermore, we modelled cataract volume by its square root transformation, which related (conditional on the parasite-load-specific lens volume adjustment) linearly to the log of parasite load, as non-linearity in a tolerance relationship can lead to spurious estimates (Tiffin & Inouye 2000).

In both tolerance models, covariance matrices for all random terms (including residuals) were specified, similar to the resistance models, as 4 × 4 (tolerance model 1) or 6 × 6 (tolerance model 2) matrices, referring to the interaction of trait and developmental stage (see also tables S3 and S4). As for resistance, we were able to construct developmental stage-specific regression slopes between traits at each random level (genetic slopes based on animal, common environmental slopes based on stream, and random environmental slopes based on residual). However, to represent tolerance, these slopes are assumed to represent causal relationships of parasite load on host fitness-related traits (cataract volume, post exposure change of length or condition).

Tolerance: including control individuals

For the change of fitness-related traits (length, condition) between pre- and post-parasite exposure, we had also collected data for unexposed control individuals, originating from the same full- and half-sib families as the infected individuals. This allowed us to test a) whether the parasite indeed affected the fitness-related host traits and b) for differences in tolerance slopes based on genetic effects of control versus infected individuals to support or weaken the causality assumption for tolerance slopes.

To test for a treatment effect on average expression per developmental stage of each fitness-related trait (a), we specified two models with only one response trait each: one for growth (Δ Length) and one for condition change (Δ Condition). These two models were similar to (3) but also included a fixed infection treatment term (infected, control) and treatment interactions with all fixed and random terms. The covariances for these models were thus based on 4 × 4 matrices (two treatments by two developmental stages).

To test for differences in tolerance slopes based on genetic effects of control versus infected individuals per developmental stage (b), we first respecified the data per fitness-related trait as a different trait in each treatment (e.g., a trait pair for growth as 'growth control' and 'growth infected'). This made it easier to restrain the covariances (see below). We then fitted a multivariate model per fitness-related trait pair with parasite load as a third response (trait). To reduce the dimensions of the covariance matrices, we fitted each model conditional for each developmental stage (see also tables S5 and S6). However, to make the model specification meaningful for the covariances, we restrained the covariances to zero for effects that were not observed in either the same group of streams or individuals. For genetic effects, on the other hand, covariances based on unobserved effects could be estimated, and were thus not restrained, due to reasons explained above.

General settings

We fit all non-generalized models under REML in ASReml-R v. 3.0 (Butler et al. 2009) and present effects as predicted from the models, which we back-transformed to the observed scale (if applicable). Effects are reported as means with either 95% credible interval across posteriors (for generalized models fitted via MCMC) or as approximate 95% confidence interval (mean ± 2*standard error) for models fitted with REML. We evaluated fixed REML model terms using *F*-tests and conducted associated contrasts using *t*-tests with denominator degrees of freedom approximated according to Kenward and Roger (1997). Based on multivariate models, we extracted regression coefficients (*b*; including tolerance slopes) at all random levels (genetic, common environmental, residual) based on the estimated (co)variances between traits *x* and *y* as: $b = cov_{x,y}/var_x$. We defined heritability (h^2) as the conventional ratio of V_A to the total phenotypic variance (V_P) $h^2 = V_A/V_P$, and defined V_P as the sum of all variance components: $V_P = V_A + V_C + V_R$.

Results

Proportions of residents and migrants, survival rates

Using a generalized animal model and averaging across crosses and sexes, we estimated that the proportion of migrants among all surviving individuals was 0.28 in both infected (95% credible interval, 95% CI: 0.20-0.36) and control individuals (95% CI: 0.20-0.37). Heritability for the probability to be a migrant on the observed scale was h^2 = 0.143 and significantly different from zero (defined as not including zero in its 95% CI: 0.066-0.237). Thus, the probability to be a resident or migrant differed among genotypes, which justifies

to statistically account for individual genotypes conditional for developmental stage in the subsequent analyses.

Despite having the same proportions of migrants in both treatments at the end of the experiment, survival rate during 10 weeks post-exposure was, averaged across crosses and sexes, slightly lower for infected (0.95; 95% CI: 0.92-0.98) than control residents (0.97; 95% CI: 0.95-0.99), but the contrast of 0.02 was non-significant (95% CI: -0.01-0.05). The survival rates of migrants were lower than of residents and lower for infected (0.77; 95% CI: 0.69-0.86) than control individuals (0.86; 95% CI: 0.79-0.92), whereby the contrast of 0.08 was marginally significant (95% CI: 0.00-0.18). These estimates may (despite their statistical non-significance) indicate a mortality-biased sample of migrants at the end of the study in the infection relative to the control treatment, but largely unbiased samples of residents. However, when we visually evaluated whether non-surviving individuals (that could not be phenotyped for post-infection traits) originated preliminarily from families whose surviving relatives showed relatively low resistance or high eye-tissue damage, we concluded this was not the case (figs. S3, S4). The conclusions were supported by the relatively low, and what may be considered as non-significant, heritability estimate for survival on the observed scale of h^2 = 0.043 (95% CI: 0.000-0.103). Altogether, these results may indicate that a possible survival bias on the following study results may have been relatively minor in respect to genotypes.

Resistance: parasite load and pre-exposure host traits

We fitted one multivariate model for the three responses of parasite load (inverse resistance), pre-exposure host body length and condition to assess whether resistance varied between residents and migrants and whether pre-exposure size or condition affected

resistance. We detected evidence for developmental-stage effects on resistance (table 1, fig. 3 A, B). Specifically, residents had an average parasite load of 12.4, which was significantly lower (0.75 times, 95% CI: 0.66-0.85) than the parasite load of 16.6 estimated for migrants. Residents were also significantly shorter than migrants (119.9 mm versus 138.6 mm) and had a significantly higher body condition (0.77 versus 0.65).

Parasite load showed a negative phenotypic relationship with pre-exposure host body length, but this was significantly different from zero only in residents (fig. 3 A). A 1% longer body was associated with a 1.21% (0.53–1.89%) lower parasite load in residents and a 0.56% (-1.05–2.14%) lower parasite load in migrants. For residents, significant common environmental and residual effects, and non-significant genetic effects, contributed to the negative phenotypic relationship (fig. 3 C, E, G). Migrants, on the other hand, showed positive relationships for all slope components, except for the genetic effect, and all components were non-significant (fig. 3 C, E, G). Overall, a higher pre-exposure growth based on common and random environmental effects, rather than genetic growth potential, appeared to decrease parasite load in residents, but not in migrants (see also table S7 for correlations between traits at each level). Nevertheless, all traits showed significant and moderate heritability estimates in both developmental stages (table S8).

Parasite load exhibited a non-significant and weakly positive phenotypic relationship with pre-exposure host body condition for residents and a significant negative phenotypic relationship for migrants (fig. 3 B). Back-transformed, increasing body condition by one unit was associated with a 32.5% (-53.6–278.4%) higher parasite load in residents and 96.2% (60–99%) lower parasite load in migrants. For residents, the phenotypic relationship was underlaid by non-significant positive genetic and common environmental effects and a negative residual effect (fig. 3 D, F, H). For migrants, it was underlaid by negative genetic

and residual effects and a positive common environmental effect, but estimates for the phenotypic slope components were non-significant, which is in contrast to their summed effects (the significant phenotypic slope). Overall, a presumed better nutritional state prior to parasite exposure in migrants, which resulted from primarily genetic and residual effects, appeared to decrease parasite load.

Tolerance: parasite load and post-exposure host traits

We fitted two multivariate models for infected individuals to assess the tolerance relationships between parasite load and three fitness-related host traits (cataract volume, growth in length and change in body condition) 10 weeks post exposure, and whether these tolerances differed between residents and migrants. The model results indicated that residents had, on average, significantly smaller cataracts, higher growth and a higher condition reduction than migrants (table S9). Furthermore, we estimated strong phenotypic relationships between parasite load and each host trait. Higher load was significantly associated with larger cataracts, more strongly reduced growth in both developmental stages and with lower condition change in residents but not migrants (fig. 4). However, for condition change, similar phenotypic slopes were estimated in migrants and residents, despite the different significance levels.

Adjusted to a common lens volume, the phenotypic tolerance slope for cataract volume of migrants was somewhat steeper than that of residents (fig. 4; first column). Back-transforming phenotypic slope estimates, an increase of parasite load by a factor of 2.72 (the base of the natural logarithm), associated with increasing the square root of the cataract volume by 2.06 $mm^{3/2}$ (1.85–2.30 $mm^{3/2}$) for residents and by 2.16 $mm^{3/2}$ (1.87–2.49 $mm^{3/2}$) for migrants. However, 95% confidence intervals of slope differences between

developmental stages encompassed zero (Δb_P = -0.046, -0.154–0.062; Δb_R = -0.094, -0.252–0.064), which renders tolerance variation between residents and migrants statistically non-significant. Genetic, common environmental and residual effects contributed to the phenotypic tolerance slopes. It is worth noting the significant and very high partial correlations between parasite load and cataract volume at all investigated levels (table S10) and the moderate heritability for cataract volume that was significant (defined as estimate minus two times the standard error not including zero) in residents but not in migrants (table S11).

Back-transformed model estimates suggested that a 1% increase in parasite load was associated with a phenotypic growth-reduction of 0.33% (0.24–0.43%) in residents and 0.46% (0.27–0.66%) in migrants. The phenotypic relationships were underlaid by negative and relatively similar estimates for both residents and migrants at all levels, albeit most estimates reached significance only for residents and there was a somewhat steeper slope for migrants based on residual effects (fig. 4; second column). However, the confidence intervals for the migrant to resident ratios of the phenotypic and the residual growth reduction percentage estimates (that is, the test whether the back-transformed slopes differed between developmental stages) both were non-significant as they included one (Δb_P = 0.88, 0.70–1.10; Δb_R = 0.80, 0.53–1.20), providing again little evidence for tolerance variation between developmental stages.

The effect of 1% increase in parasite load associated with a significant phenotypic body condition (K) decrease of 0.00020 (0.00005–0.000035) in residents and a non-significant decrease of 0.00018 (-0.00015 to 0.00051) in migrants, that is having five times more parasites reduced K during the 10 weeks post-exposure by 0.10 and 0.09, respectively. The negative phenotypic relationship was underlaid by negative and relatively similar

estimates for both residents and migrants, based on non-significant genetic and significant residual effects (fig. 4; third column). Based on non-significant common environmental effects (which may reflect differences among streams), the estimated relationship was positive and stronger for migrants.

Tolerance: including control individuals

Using separate models for post-exposure growth and condition change, which also included control treatment data, we tested for assumptions of tolerance relationships. First, we modeled fitness change data for both infection treatments and both developmental stages. These fitness-trait-specific models enabled testing whether the infection treatment indeed affected growth or condition change and whether the infection treatment affected developmental stages differently. We did not detect a significant infection treatment term for growth but for condition change, and no significant infection-treatment-by-developmental-stages interaction term for either growth or condition change (table S12). Nonetheless, estimates for each trait per developmental stage were lower in the infection than the control treatment (fig. 5 A, B). Based on the actual developmental-stage-specific treatment contrasts, we did not detect any significant treatment effect for either developmental stages on growth (residents: $t_{13.3} = 1.13$, $P = 0.279$; migrants: $t_{13.3} = 1.52$, $P = 0.151$; fig. 5 A), or on condition change of migrants, but on condition change of residents (residents: $t_{15.2} = 4.04$, $P = 0.001$; migrants: $t_{15.2} = 1.29$, $P = 0.215$; fig. 5 B).

In addition to testing for differences in means between infection treatments, we evaluated how the association between genetic effects for each fitness-related trait and parasite load (that is, presumed tolerance) differed between infection treatments. Such a comparison was possible, because we were able to estimate treatment-specific breeding

values of individuals (such as for the parents, which were not phenotyped) based on the phenotypes of their relatives in each infection treatment. These analyses showed that tolerance estimates based on genetic effects for growth and condition change may have largely been independent of the infection. Specifically, we detected high, positive and significant genetic correlations (defined as estimate ± 2 standard errors not including zero) in residents based on breeding values between infected and control treatments for growth and condition change (fig. 5 C, D). Although the corresponding genetic correlation for growth in migrants was non-significant, it was still high and positive (fig. 5 C). However, the correlation for condition change in migrants was non-significant and even negative (fig. 5 D). The high positive genetic correlations indicate that breeding value rankings are very similar in the presence and absence of a parasite infection, whereas a genetically based tolerance relationship may assume that ranking of growth or condition change is determined by tolerance to the infection. Secondly, we estimated tolerance-like slopes between breeding values for growth or condition change (estimated based on phenotypic data from control individuals) and parasite load (estimated based on phenotypic data from infected individuals) that were surprisingly similar to the corresponding slopes based on only infected individuals, again with an exception for condition change of migrants (fig. 4 E, F versus fig. 5 E, F). However, only one of these estimates - the slope between condition change and parasite load of residents - was statistically significant.

Altogether, although the infection treatment lowered average growth and condition change relative to a control treatment (fig. 5 A, B), the observed pattern of genetic correlations and genetic slope similarities between infected and non-infected individuals suggests that genotypes with low resistance also show less growth and a stronger condition reduction than genotypes with high resistance in the absence of an infection. In other

words, a genetic relationship that adheres to the definition of tolerance may exist between breeding values for infection susceptibility and genetic growth potential or condition change even when the organism is not infected (fig. 5 E, F).

Discussion

Two key results emerge from our study linking host development with two types of parasite defense. First, Atlantic salmon show developmental stage-specific investment in resistance to parasites, but not in tolerance. Specifically, individuals transitioning to the seawater stage (migrants) were less resistant to a trematode infection than their similar-aged siblings in the freshwater stage (residents), suggesting that host development can generate variation in defense against parasites. Second, tolerance estimates may be uncertain due to an interdependency of its two components: parasite load and host fitness. As a result, variation in fitness traits causing covariation in resistance may be misinterpreted as tolerance relationship.

Developmental stage effects

The observed lower resistance in migrants, relative to residents, is consistent with the idea of developmental constraints on host defense. The habitat shift from fresh to sea water requires significant physiological, biochemical, morphological and behavioral changes, the magnitude of which has been compared to that of metamorphosis (Björnsson et al. 2011). The developmental transition is thought to initiate several months before the habitat shift (Thorpe et al. 1998; Debes et al. 2020) and culminates in a seaward migration. Our results may thus indicate that this time- and energy-demanding process monopolizes resources over those required for resistance to parasites.

Alternatively, or in addition, a reduction in resistance could be adaptive for migrants. From an eco-immunological perspective, hosts should employ costly defense mechanisms in relation to parasite exposure risk (Lee 2006). Many organisms face varying exposure risks during development, as life stages often differ in habitat use, resources or behavior (Tate & Graham 2015). Salmonid fishes switch from being territorial to schooling during transition to the migratory stage (Björnsson et al. 2011), which could dilute individual exposure risk to free-living parasite stages (Poulin & FitzGerald 1989) and thus favor a downregulation of immune responses. However, at the same time migrants become more active, which is expected to increase parasite exposure (Barber & Dingemanse 2010), especially during migration across habitats (Poulin & de Angeli Dutra 2021).

Further, Atlantic salmon migrants spatially escape infection risk with *D. pseudospathaceum* after migrating to the sea as this parasite is restricted to fresh and brackish water. In our experiment, migrants were in fresh water at the time of exposure and thus, not expected to have downregulated resistance (unless this process is coupled with developmental transition). However, individuals migrating at a younger age may generally show lower resistance than later migrating conspecifics due to a lower lifetime exposure risk (Miller et al. 2007). If so, the observed pattern of reduced resistance and unaltered tolerance in migrants versus same-aged residents would seem plausible. Specifically, while resistance mechanisms are often parasite-specific, tolerance mechanisms, such as tissue repair, are typically unspecific to the cause of tissue damage (Medzhitov et al. 2012), making a downregulation of tolerance less advantageous. However, a higher overall susceptibility of individuals migrating at a younger age seems unlikely for this system. It would lead to increased infection rates before transitioning to the migrant stage, and thus to larger eye cataracts (Karvonen et al. 2004), which reduce growth (Karvonen & Seppälä 2008) and

therefore, could delay the host development that depends on growth (Metcalfe & Thorpe 1990).

Comparable tolerance across developmental stages could also be explained by relatively low costs of tolerance compared to those of resistance, allowing effective host defenses even under limited resource availability. While immunological resistance is generally associated with large energetic and nutritional costs (Lochmiller & Deerenberg 2000), the magnitude of costs arising from tolerance are often not known. Independent of the mechanisms, the inferred change of resistance but not tolerance with a developmental-stage transition, together with the expected opposing effects of both defense mechanisms on parasite fitness (Roy & Kirchner 2000; Best et al. 2008), may cause variation in epidemiological and evolutionary outcomes of parasitism in varying host life-stage structures. This is especially relevant for salmonid species (such as salmons, trouts and charrs), which show a particularly large variation in the age at seaward migration, both within and among populations (Metcalfe & Thorpe 1990; Marschall et al. 1998). Although our study included only one-year-old salmon, we assume that the observed developmental effects are consistent across later migrating age classes, as the developmental changes involved in the transition to migrants remain the same. Moreover, previously observed downregulation of immune parameters during the transition stage were found to be comparable among one- and two-year-old Atlantic salmon (Muona & Soivio 1992).

Pitfalls in tolerance estimations

The lack of developmental stage effects on some of the tolerance traits studied here may also be explained by large uncertainty in their estimates. We detected negative associations (tolerance slopes) between parasite load and both host growth and body condition change

post-exposure . However, parasite load itself associated phenotypically with host size (in residents) and host body condition (in migrants) before exposure. These associations were largely driven by random and common environmental effects for size and, although with larger uncertainty, by genetic and random environmental effects for condition. Thus, a higher realized size (residents) or condition (migrants) based on random environmental effects before exposure appeared to decrease parasite load. If these environmental effects on size and body condition persisted after exposure, a relationship with parasite load could be mistaken for tolerance. In other words, host growth or condition change post-exposure may be independent of parasite load, but appear to be driven by it, because pre-infection size and condition affected resistance.

Although we were not able to infer causality in the observed tolerance slopes directly, we conducted additional statistical tests examining the slopes aided by genetic effects (breeding values) and estimates based on unexposed control individuals. Specifically, we tested whether genetic covariance between the post-exposure fitness traits and parasite load differed between infected and control relatives. If driven by the infection, we expected the relationship to be absent in control individuals. This was not the case for growth in either residents or migrants, suggesting that genotypes with low resistance also show lower growth under similar environmental conditions but in the absence of an infection (although the relationships did not reach statistical significance). Thus, the estimated similar relationships based on infected individuals are unlikely to fully reflect tolerance. A similar result was also found for resident body condition change. For migrants, on the other hand, the relationship was strongly negative for infected individuals, but only weakly so for control individuals, which may provide more confident support for a tolerance relationship in infected individuals. As this was detected for only one of four investigated relationships,

estimated tolerance slopes may have to be interpreted with caution. Specifically, the similar genetic relationships of uninfected and infected individuals, even if some were estimated as being non-significant, may still contribute to the value and statistical significance of the phenotypic relationships (and thus the phenotypic tolerance slopes). These findings may therefore question the validity of tolerance slopes that are based on relationships with unknown causation and do not critically assess the relationship via uninfected controls. Thus, this finding emphasizes the importance of including control individuals in tolerance studies using a split-family design, not only for evolutionary inferences (Graham et al. 2011), but also to more reliably support causation in the observed relationships.

Uncertainty surrounding causation in tolerance slopes does not, however, apply to analyses that use the magnitude of parasite-induced tissue damage as host fitness trait, as tissue damage only arises post-exposure. Thus, our tolerance slopes based on eye cataract volume likely reflect tolerance. However, these slopes were underlaid by both genetic and environmental effects. While environmental effects on this type of tolerance slopes do not reduce their certainty, effect presence indicates that conclusions on evolutionary responses of host tolerance mechanisms need to be made with caution if based on phenotypic relationships (Graham et al. 2011).

Another challenge for obtaining reliable tolerance estimates is survivor bias. In studies with mortalities, like ours, the least tolerant individuals may be lost before being measured. If so, survival differences between host types can eradicate existing tolerance differences. Here, for migrants, but not residents, survival was lower in the infection than the control treatment. It is possible that the energy-demanding process of developmental transition contributed to the increased mortality of migrants, which was further amplified by the higher infection rates. However, we determined that mortality among migrants was largely

independent of genotypes. Nevertheless, we were unable to determine whether non-survivors may have had the largest cataracts relative to parasite load, that is low tolerance. Inclusion of such data could have led to a steeper migrant tolerance slope, possibly resulting in tolerance differences between residents and migrants. A solution in future experiments would be to more vigorously monitor experimental individuals to be able to sample them immediately following decease.

Conclusion

Host defense shows tremendous variation in the wild (Schmid-Hempel 2003; Råberg et al. 2009; Sadd & Schmid-Hempel 2009; Medzhitov et al. 2012), but the processes that create and maintain this variation are often not clear. We show that resistance, but not tolerance, can vary across ontology and suggest that costly developmental processes associated with life-stage transition may drive this variation. This information is important for predictions of disease dynamics. At the same time, the results emphasize the importance of controlling for developmental stages in studies on host defense, for example when comparing populations that differ in life stage structure. We encourage more studies exploring the role of host development on resistance and tolerance, and strongly recommend study designs that employ control treatments for infection, to produce more reliable tolerance estimates.

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Statement of authorship

All authors were involved in conceptualization, experimental design and data collection, PVD and IK conducted data analysis and data visualization, IK and PVD wrote the first draft of the manuscript, which all authors reviewed and edited.

Data and code availability

The data set used in this study is available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.02v6wwq4d; Klemme et al. 2022).

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Table 1: *F*-test results from a multivariate model on host resistance

Trait	Term	df	ddf	*F*	*P*	Effect	Estimate (± SE)
Parasite load	Intercept						2.5749 ± 0.1370
	Dev. stage	1	508.3	21.0	< 0.001	Migrant	0.2916 ± 0.0636
	Sex	1	248.7	25.3	< 0.001	Male	-0.1983 ± 0.0395
	Cross	3	956.6	0.2	0.877	OUL×TOR	0.0467 ± 0.0817
						TOR×OUL	0.0620 ± 0.0868
						TOR×TOR	0.0667 ± 0.1024
Length	Intercept						4.7743 ± 0.0203
	Dev. stage	1	2206.0	67.6	< 0.001	Migrant	0.1524 ± 0.0185
	Sex	1	181.2	0.0	0.864	Male	0.0013 ± 0.0074
	Cross	3	410.8	0.8	0.494	OUL×TOR	-0.0109 ± 0.0147
						TOR×OUL	0.0133 ± 0.0147
						TOR×TOR	0.0151 ± 0.0191
Condition	Intercept						0.7623 ± 0.0085
	Dev. Stage	1	2206.0	159.6	< 0.001	Migrant	-0.1133 ± 0.0090
	Sex	1	248.6	15.9	< 0.001	Male	0.0181 ± 0.0045
	Cross	3	459.5	5.9	0.001	OUL×TOR	0.0117 ± 0.0070
						TOR×OUL	-0.0208 ± 0.0069
						TOR×TOR	-0.0051 ± 0.0081

Note. The responses in the model were parasite load, host pre-exposure body length, and host pre-exposure condition. Model coefficients on the modeled scale (parasite load: natural log of [count + 1], length: natural log of length (mm), condition: Fulton's K) are also reported. Some selected contrasts on the measured scale are reported in the text.

Figure legends

Figure 1: A common challenge in tolerance estimation: reversing cause and effect. Tolerance is typically measured as the regression slope of host fitness against parasite load for a group of individuals of a specified host type (e.g. genotypes). If the slopes of two host types differ (A), that is the host types differ in the rate of fitness change as parasite load increases, they are assumed to differ in tolerance. However, this concept assumes that host post-exposure fitness is causally related to parasite load, while host fitness before parasite exposure could also determine subsequent parasite infection success or within-host reproduction, and thus the measured parasite load after infection (B). For example, pre-exposure host fitness may associate positively with immunocompetence, which then limits parasite infection success or replication. If host types differ in the strength of the relationship between parasite load and pre-exposure fitness (for example, because they react differently to the experimental environment) and if fitness remains consistent from pre- to post-exposure, host types only appear to differ in tolerance (underlying data for A and B are identical).

Figure 2: Timeline of the experiment starting in May 2019 with the release of 120 one-year old Atlantic salmon to each of 16 semi-natural streams (A). A PIT-based radio frequency identification system with four antennas (B) per stream allowed us to record fish movement at the time of seaward migration in June. In July, we re-captured the fish, measured them for size, studied morphological indices of phenotypic change (silvering, C) and based on this, together with movement data, classified them as either residents (lower individual) or migrants (upper individual). At the same time, we exposed all fish from half of the streams to the eye parasite *Diplostomum pseudospathaceum*. Subsequently, we returned the fish to

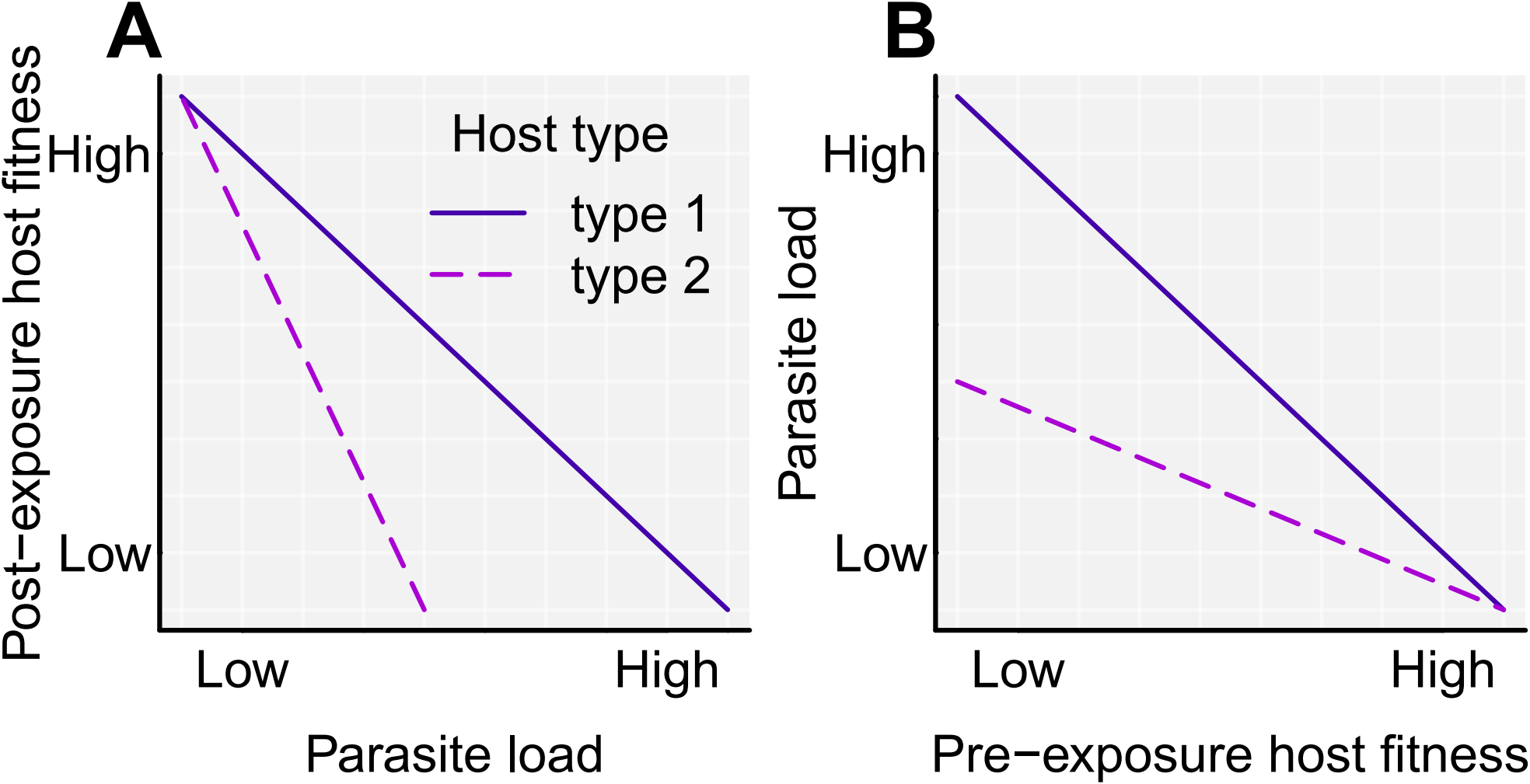
the streams until September, when we measured them again for size and studied parasite-exposed individuals for parasite load and the magnitude of parasite-induced tissue damage (cataracts) in the eye lenses (D). Photo credit: Ines Klemme.

Figure 3: Relationship between parasite load (inverse resistance) and pre-exposure host body length (left column) or body condition (Fulton's *K*, right column) in juvenile Atlantic salmon infected with the eye parasite *Diplostomum pseudospathaceum*. The multivariate model allowed us to decompose the phenotypic relationships (A, B) into genetic (breeding value) effects (C, D), common environmental (stream) effects (E, F) and residual effects (G, H). Slopes characterize the model-predicted relationships back-transformed to the observed scale, whereas estimates with standard error refer directly to the modelled scale. Points show individual data records (A, B) or model-predicted random effects (C-H).

Figure 4: Relationship between parasite load and either parasite-induced cataract volume (left column), post-exposure host growth (middle column) or post-exposure change in body condition (Fulton's *K*, right panel) in juvenile Atlantic salmon infected with the eye parasite *Diplostomum pseudospathaceum*. The phenotypic relationships (A-C) were decomposed into genetic effects (estimated breeding values; EBV; D-F), common environmental effects (streams; G-I) and residuals (J-L). Slopes characterize the estimated tolerance relationships at the measured scale, whereas estimates with standard error refer to the modelled scale. Points show individual data records (A-C) or model-predicted random effects (D-L).

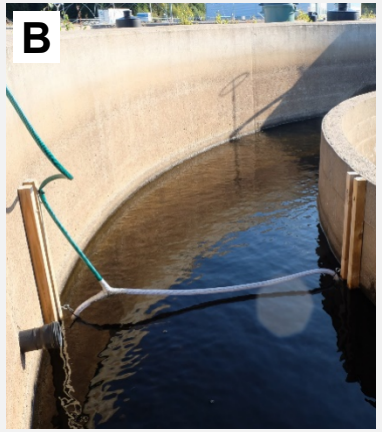
Figure 5: Comparison of parameter estimates (back-transformed to the measured scale) in presence (infected) and absence (control) of an infection with the eye parasite *Diplostomum*

pseudospathaceum for two developmental stages (resident, migrant) of juvenile Atlantic salmon. Contrasts of mean effects (A, B) are reported in the main text. The genetic correlations based on parental estimated breeding values (EBV) were estimated for the same traits but between infection treatments (C, D). The slopes based on EBV between either growth (E) or condition change (F) of control individuals and parasite load of infected individuals reflect the presumed predictive relationship reported in panels E and F in Figure 3, respectively, but are here based on uninfected individuals for EBVs for growth or condition change. These slopes may wrongly be interpreted as predictive tolerance slopes.


A
B
Post−exposure host fitness
High
Low
Low
High
Parasite load
Host type
type 1
type 2
Parasite load
High
Low
Low
High
Pre−exposure host fitness

transfer to seminatural streams

21-23 MAY



recording of movement data

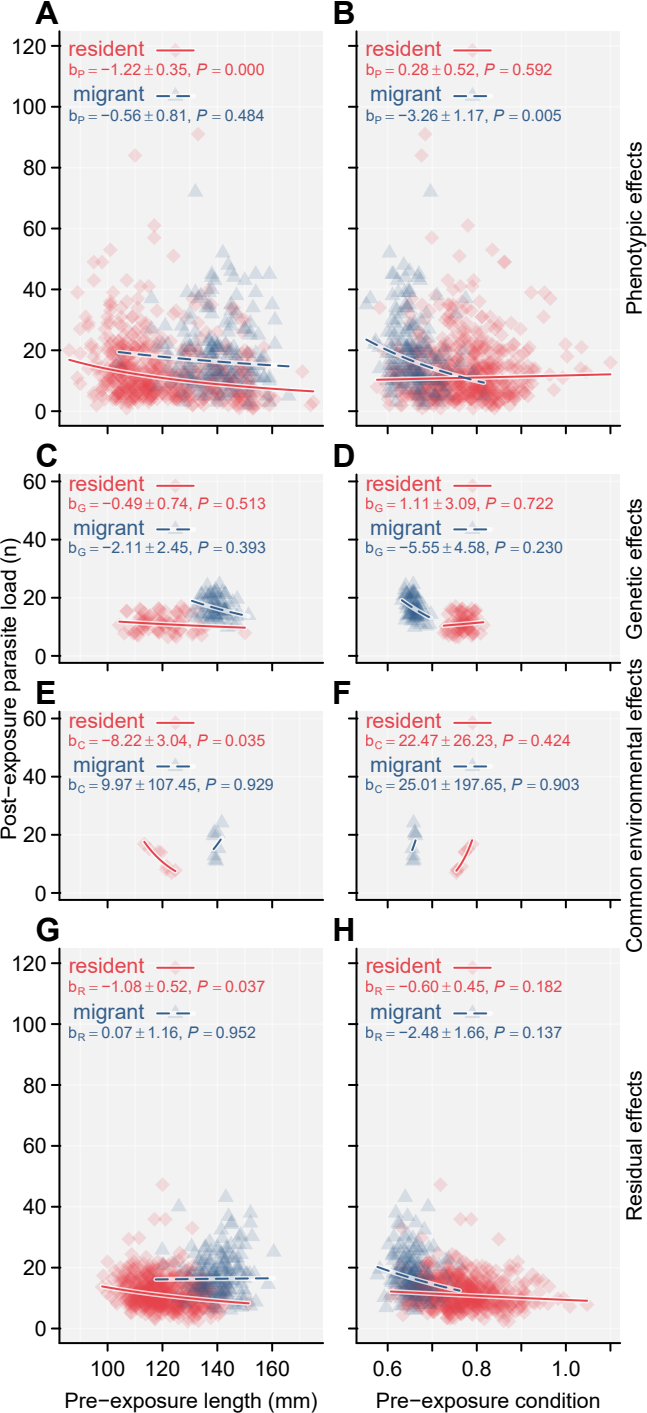
1-30 JUN

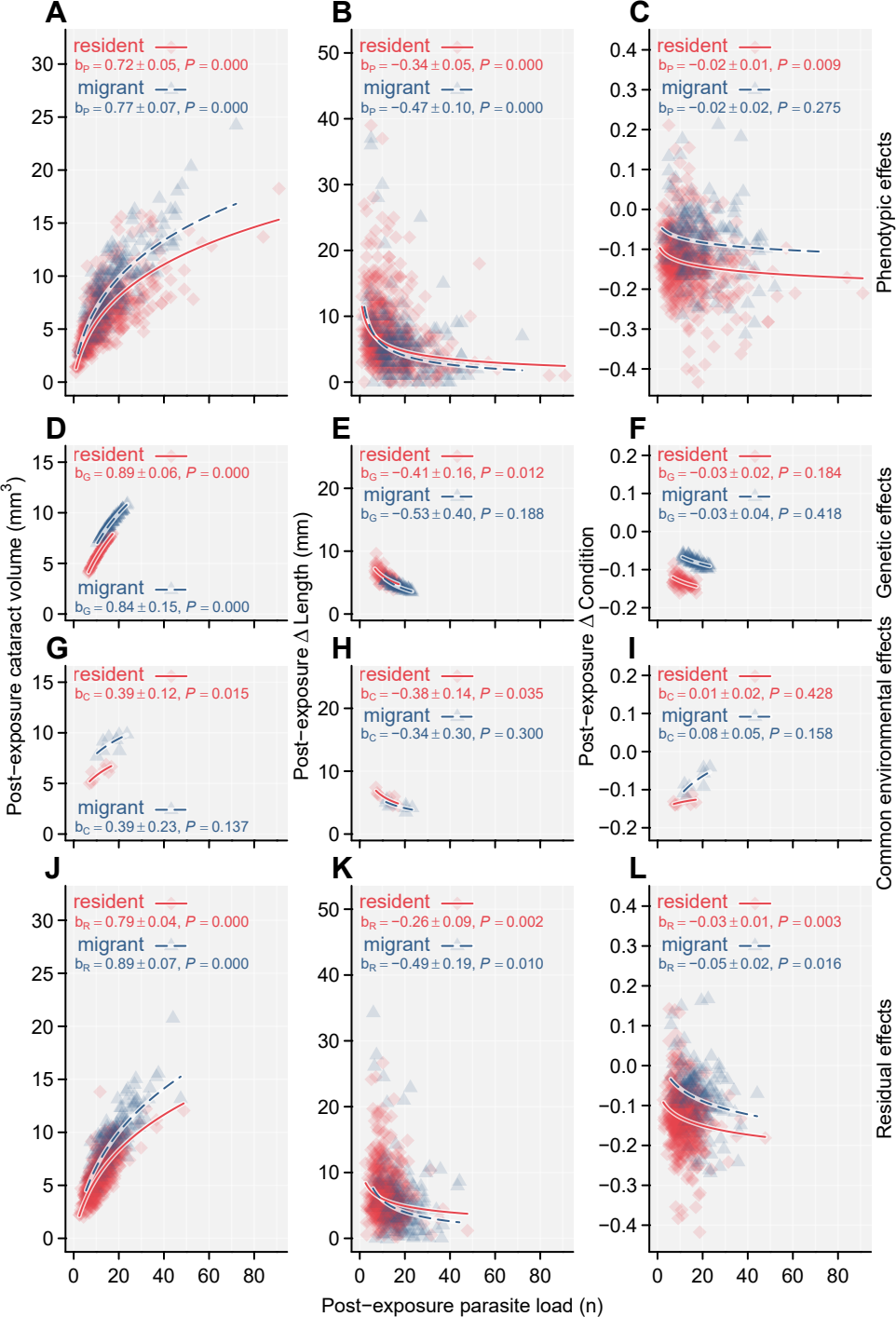
developmental stage assessment, parasite exposure

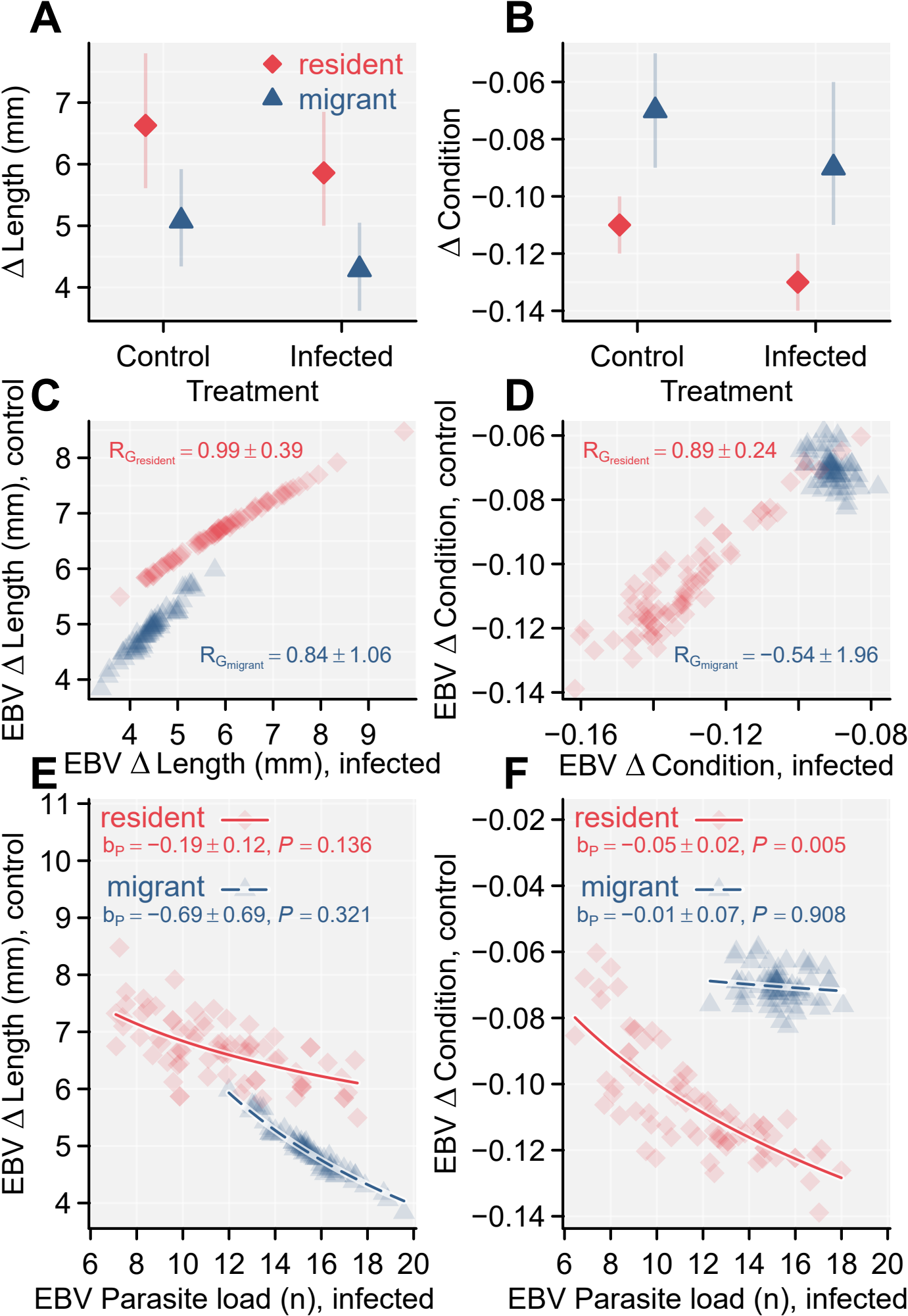
02-12 JUL

resistance, tolerance

11-20 SEP


A
resident
$b_P = -1.22 \pm 0.35$, $P = 0.000$
migrant
$b_P = -0.56 \pm 0.81$, $P = 0.484$
B
resident
$b_P = 0.28 \pm 0.52$, $P = 0.592$
migrant
$b_P = -3.26 \pm 1.17$, $P = 0.005$
Phenotypic effects
C
resident
$b_G = -0.49 \pm 0.74$, $P = 0.513$
migrant
$b_G = -2.11 \pm 2.45$, $P = 0.393$
D
resident
$b_G = 1.11 \pm 3.09$, $P = 0.722$
migrant
$b_G = -5.55 \pm 4.58$, $P = 0.230$
Genetic effects
Post−exposure parasite load (n)
E
resident
$b_C = -8.22 \pm 3.04$, $P = 0.035$
migrant
$b_C = 9.97 \pm 107.45$, $P = 0.929$
F
resident
$b_C = 22.47 \pm 26.23$, $P = 0.424$
migrant
$b_C = 25.01 \pm 197.65$, $P = 0.903$
Common environmental effects
G
resident
$b_R = -1.08 \pm 0.52$, $P = 0.037$
migrant
$b_R = 0.07 \pm 1.16$, $P = 0.952$
H
resident
$b_R = -0.60 \pm 0.45$, $P = 0.182$
migrant
$b_R = -2.48 \pm 1.66$, $P = 0.137$
Residual effects
Pre−exposure length (mm)
Pre−exposure condition


A
resident
$b_P = 0.72 \pm 0.05$, $P = 0.000$
migrant
$b_P = 0.77 \pm 0.07$, $P = 0.000$
B
resident
$b_P = -0.34 \pm 0.05$, $P = 0.000$
migrant
$b_P = -0.47 \pm 0.10$, $P = 0.000$
C
resident
$b_P = -0.02 \pm 0.01$, $P = 0.009$
migrant
$b_P = -0.02 \pm 0.02$, $P = 0.275$
Phenotypic effects
D
resident
$b_G = 0.89 \pm 0.06$, $P = 0.000$
migrant
$b_G = 0.84 \pm 0.15$, $P = 0.000$
E
resident
$b_G = -0.41 \pm 0.16$, $P = 0.012$
migrant
$b_G = -0.53 \pm 0.40$, $P = 0.188$
F
resident
$b_G = -0.03 \pm 0.02$, $P = 0.184$
migrant
$b_G = -0.03 \pm 0.04$, $P = 0.418$
Genetic effects
G
resident
$b_C = 0.39 \pm 0.12$, $P = 0.015$
migrant
$b_C = 0.39 \pm 0.23$, $P = 0.137$
H
resident
$b_C = -0.38 \pm 0.14$, $P = 0.035$
migrant
$b_C = -0.34 \pm 0.30$, $P = 0.300$
I
resident
$b_C = 0.01 \pm 0.02$, $P = 0.428$
migrant
$b_C = 0.08 \pm 0.05$, $P = 0.158$
Common environmental effects
J
resident
$b_R = 0.79 \pm 0.04$, $P = 0.000$
migrant
$b_R = 0.89 \pm 0.07$, $P = 0.000$
K
resident
$b_R = -0.26 \pm 0.09$, $P = 0.002$
migrant
$b_R = -0.49 \pm 0.19$, $P = 0.010$
L
resident
$b_R = -0.03 \pm 0.01$, $P = 0.003$
migrant
$b_R = -0.05 \pm 0.02$, $P = 0.016$
Residual effects
Post−exposure cataract volume (mm^3)
Post−exposure Δ Length (mm)
Post−exposure Δ Condition
Post−exposure parasite load (n)


A
resident
migrant
Δ Length (mm)
7
6
5
4
Control
Infected
Treatment
B
Δ Condition
−0.06
−0.08
−0.10
−0.12
−0.14
Control
Infected
Treatment
C
$R_{G_{resident}} = 0.99 \pm 0.39$
$R_{G_{migrant}} = 0.84 \pm 1.06$
EBV Δ Length (mm), control
EBV Δ Length (mm), infected
D
$R_{G_{resident}} = 0.89 \pm 0.24$
$R_{G_{migrant}} = -0.54 \pm 1.96$
EBV Δ Condition, control
EBV Δ Condition, infected
E
resident
$b_P = -0.19 \pm 0.12$, $P = 0.136$
migrant
$b_P = -0.69 \pm 0.69$, $P = 0.321$
EBV Δ Length (mm), control
EBV Parasite load (n), infected
F
resident
$b_P = -0.05 \pm 0.02$, $P = 0.005$
migrant
$b_P = -0.01 \pm 0.07$, $P = 0.908$
EBV Δ Condition, control
EBV Parasite load (n), infected