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Article type : Original Article

Invasion history shapes host transcriptomic response to a body-snatching parasite

Running title: Transcriptomics of a parasite invasion

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/MEC.16038

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ABSTRACT

By shuffling biogeographic distributions, biological invasions can both disrupt long-standing associations between hosts and parasites and establish new ones. This creates natural experiments with which to study the ecology and evolution of host-parasite interactions. In estuaries of the Gulf of Mexico, the white-fingered mud crab (*Rhithropanopeus harrisii*) is infected by a native parasitic barnacle *Loxothylacus panopaei* (Rhizocephala), which manipulates host physiology and behavior. In the 1960s, *L. panopaei* was introduced to the Chesapeake Bay and has since expanded along the southeastern Atlantic coast, while host populations in the northeast have so far been spared. We use this system to test the host's transcriptomic response to parasitic infection and investigate how this response varies with the parasite's invasion history, comparing populations representing (1) long-term sympatry between host and parasite, (2) new associations where the parasite has invaded during the last sixty years, and (3) naïve hosts without prior exposure. A comparison of parasitized and control crabs revealed a core response, with widespread downregulation of transcripts involved in immunity and molting. The transcriptional response differed between hosts from the parasite's native range and where it is absent, consistent with previous observations of increased susceptibility in populations lacking exposure to the parasite. Crabs from the parasite's introduced range, where prevalence is highest, displayed the most dissimilar response, possibly reflecting immune priming. These results provide molecular evidence for parasitic manipulation of host phenotype and the role of gene regulation in mediating host-parasite interactions.

KEYWORDS

host-parasite interactions, host adaptation, Rhizocephala, immune priming, parasitic manipulation, biological invasions, gene expression

1. INTRODUCTION

The geographic redistribution of biodiversity is one of the hallmarks of the current biological upheaval during the Anthropocene (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Pecl et al., 2017; Pereira et al., 2010). Climate change, species introductions, and changes in land use have all contributed to the ongoing shuffling of species and genes on the landscape (Chen et al.,

2011; Guo, Lenoir, & Bonebrake, 2018; Vitousek, D'Antonio, Loope, & Westbrooks, 1996). In the process, this reorganization has disrupted long-standing biotic interactions while simultaneously bringing new species into contact (Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Schweiger, Settele, Kudrna, Klotz, & Kühn, 2008). Understanding how individual species and species assemblages will respond to these changes in biogeography is a central goal for contemporary studies of ecology, evolution, and conservation (Bonebrake et al., 2018; Parmesan, 2006). By investigating the consequences of range shifts and invasions, scientists can use them as natural experiments for testing fundamental concepts of the ecology and evolution of species interactions, as well as for predicting potential responses under sustained environmental change (Moran & Alexander, 2014; Saul & Jeschke, 2015; Strauss, Lau, & Carroll, 2006).

One class of species interactions often altered by biogeographical shifts are those between hosts and their parasites (Brooks & Hoberg, 2007). In these intimate associations, the intense pressures exerted on either partner form an antagonism that can shape the evolutionary trajectory of both species. One particularly remarkable example is parasitic manipulation, where parasites exert control over their hosts by causing shifts in host physiology and behavior that improve their own reproduction and/or transmission (Moore, 2002). For example, infections by the trematode *Euhaplorchis californiensis* cause its killifish intermediate hosts to exhibit erratic swimming behaviors, making them more prone to predation by the parasite's bird definitive hosts and thus promoting completion of the parasite's life cycle (Lafferty & Morris, 1996). These behavioral changes have been linked to the targeted modification of host neurochemistry, with an infection intensity-dependent alteration of serotonin activity that is distinct from a more generalized stress response (Shaw et al., 2009; Shaw & Øverli, 2012). While we often lack such detailed information on its underlying mechanisms, behavioral manipulation of hosts is not an isolated phenomenon, and may be relatively common, having been observed across many taxa of hosts and parasites (Andersen et al., 2009; Berdoy, Webster, & Macdonald, 2000; Bethel & Holmes, 1977; Carney, 1969; Eberhard, 2000; Poulin & Latham, 2002; Thomas et al., 2002; reviewed in Lafferty & Shaw, 2013; Poulin, 2010; Thomas, Adamo, & Moore, 2005). Furthermore, examples of parasitic

manipulation can extend beyond these conspicuous behavioral syndromes and include modification of a variety of host traits including immune function, reproduction, morphology, metabolism, and development (Cézilly, Favrat, & Perrot-Minnot, 2013; Cornet, Franceschi, Bauer, Rigaud, & Moret, 2009; Thomas, Poulin, & Brodeur, 2010; Vance, 1996; Yanoviak, Kaspari, Dudley, & Poinar, 2008). The taxonomic and functional diversity of parasitic manipulation of hosts highlights the broad range and success of host "body-snatching" as an evolutionary strategy. This phenomenon represents a particularly striking outcome of host-parasite coevolution – an extreme extended phenotype (Dawkins, 1982). However, determining whether these changes in host phenotype truly represent an adaptive strategy of the parasite, rather than a side effect of infection, is challenging and demands the integration of multiple lines of inquiry to link the causal molecular mechanisms to phenotypes and eventual ecological consequences (Hughes, 2013; Thomas et al., 2005).

Given the potential for dramatic fitness costs of infection, which can include castration or death, body-snatching parasites constitute an especially potent source of selection in the evolution of their hosts. The negative consequences of parasitic infection can shape host responses across a range of temporal scales. On deep evolutionary timescales, pressure from parasites has resulted in hosts evolving complex behavioral avoidance strategies and elaborate immune systems (Buck, Weinstein, & Young, 2018; Sheldon & Verhulst, 1996). On a more constrained microevolutionary timescale, adaptation to parasitism can result in host populations that have resistance to local parasite species/genotypes, while potentially exhibiting elevated susceptibility to unfamiliar parasites to which they lack historical exposure (Eizaguirre, Lenz, Kalbe, & Milinski, 2012a; Roth, Keller, Landis, Salzburger, & Reusch, 2012). In the face of strong selection by parasites, resistance can evolve remarkably rapidly, even in just a handful of generations, provided sufficient standing genetic variation is available (Duffy & Sivars-Becker, 2007; Eizaguirre, Lenz, Kalbe, & Milinski, 2012b; Wendling & Wegner, 2015). On shorter timescales, within the lifetime of an individual, host responses to infection are often contingent on *contemporary* exposure, with immune systems providing increased resistance on subsequent contact with a pathogen. Immunological memory and other forms of adaptive phenotypic plasticity, such as learned anti-parasite behaviors, play a critical role in host fitness

(Klemme & Karvonen, 2016; Schmid-Hempel, 2009). These plastic responses themselves are often influenced by other factors in the environment (Lazzaro & Little, 2009; Martin, Hanson, Hauber, & Ghalambor, 2021), adding layers of complexity that make their study in natural settings challenging.

The application of molecular methods has greatly facilitated the study of host responses to parasitism in non-model systems (Criscione, Poulin, & Blouin, 2005). Transcriptome sequencing is of particular utility, as these responses, whether heritable or plastic, are often accompanied by changes in gene expression. These changes have been implicated in mediating a range of host-parasite interactions, including local adaptation between host and parasite (Barribeau, Sadd, Du Plessis, & Schmid-Hempel, 2014; Feis, John, Lokmer, Luttikhuizen, & Wegner, 2018; Lenz, Eizaguirre, Rotter, Kalbe, & Milinski, 2013), rapid evolution of host resistance (Bonneaud et al., 2011; Grogan et al., 2018; Lohman, Steinel, Weber, & Bolnick, 2017), and parasitic manipulation of host phenotypes, including behavior and immune function (Bankers et al., 2017; Feldmeyer et al., 2016; Geffre et al., 2017). In the context of the anthropogenic shuffling of biodiversity, transcriptomic approaches have the potential to provide unique insights into the molecular mechanisms mediating host-parasite interactions.

Biological invasions offer such an opportunity to investigate novel responses to parasitism in natural populations, as they often result in the disruption of existing host-parasite relationships and/or the establishment of new ones (Dunn, 2009; Goedknegt et al., 2016; Prenter, MacNeil, Dick, & Dunn, 2004). The loss of parasites is frequently observed in species introductions and has been implicated as a factor promoting the establishment and spread of introduced species (the enemy/parasite release hypothesis) (Keane & Crawley, 2002; Torchin, Lafferty, Dobson, McKenzie, & Kuris, 2003). In their new range, non-natives can acquire unfamiliar parasites (Blakeslee, Barnard, Matheson, & McKenzie, 2020; Criscione & Font, 2001; Gérard & Le Lannic, 2003; Torchin, Lafferty, & Kuris, 1996; Wells, O'Hara, Morand, Lessard, & Ribas, 2015; reviewed in Kelly, Paterson, Townsend, Poulin, & Tompkins, 2009); similarly, introduced species may bring with them exotic parasites that can then infect naïve hosts in the new range (parasite spillover) (Daszak, Cunningham, & Hyatt, 2000; Elsner, Jacobsen, Thieltges, & Reise, 2011; Font & Tate, 1994; Miller et al., 2018; Prenter et al., 2004). The establishment of

new associations through invasion can result in the rapid evolution of both hosts and parasites (Feis, Goedknegt, Thieltges, Buschbaum, & Wegner, 2016; Wendling & Wegner, 2015). Past studies have focused mostly on parasites infecting completely novel hosts, where each partner is encountering the other for the first time. However, when a host has a broader geographic range than its parasite, a situation can arise in which a parasite may be introduced into naïve populations of its host's range. By spilling over into a new population of a species to which it has already adapted, the parasite may be primed for success in the newly expanded range (Bushek & Allen, 1996; Tepolt et al., 2020b). Hosts within these populations, on the other hand, lack previous exposure to the parasite and are likely at an evolutionary disadvantage. This asymmetry in evolutionary history provides a natural experiment with which to examine host responses to novel parasites.

Loxothylacus panopaei (Rhizocephala) is a parasitic barnacle native to the Gulf of Mexico that infects mud crabs of the family Panopeidae (Boschma, 1955). It is a cryptic species complex, with lineages differing in patterns of host use (Kruse & Hare, 2007; Kruse, Hare, & Hines, 2012).The ER clade (hereafter simply *L. panopaei*) specifically infects the host species *Eurypanopeus depressus* and *Rhithropanopeus harrisii*, both of which have broad geographic ranges in North America, extending from the Gulf of Mexico to New England (Williams, 1984). *Loxothylacus panopaei* was introduced to the Chesapeake Bay in the mid-20th century, first observed in 1964 (Van Engel, Dillon, Zwerner, & Eldridge, 1966). Since its initial introduction, the parasite has continually spread south until meeting the putative northern edge of its native range at Cape Canaveral (Hines, Alvarez, & Reed, 1997; Kruse et al., 2012). In contrast, *L. panopaei* has not yet expanded into host populations north of the Chesapeake Bay, with the exception of one isolated introduction in Long Island Sound (Freeman, Blakeslee, & Fowler, 2013). The host crabs thus inhabit three distinct zones in relation to their parasite: where the parasite is native, where the parasite is introduced, and where the parasite is absent (Figure 1). Populations across these three regions differ in their history of exposure to the parasite, ranging from millennia to decades (<60 years) to none at all, providing an opportunity to investigate the host response to parasitism across temporal scales.

Infection by *L. panopaei* likely represents a strong selective force in the evolution of its crab hosts. Rhizocephalans are large, conspicuous parasites that exert dramatic fitness effects on their hosts (Høeg, 1995). After colonization by the infective larval stage, rhizocephalans grow an extensive internal "root" system throughout the host; at later stages of the infection, the parasite's reproductive organ (externa) emerges through the abdominal wall, mimicking the female crab's egg mass (Høeg, 1995). Rhizocephalan infections represent compelling cases of adaptive parasite manipulation, as they often result in the castration of both sexes, expression of female traits in males (feminization), disruption of the molt cycle, and alteration of feeding and habitat choice (Belgrad & Griffen, 2015; Høeg, 1995; Mouritsen & Jensen, 2006; O'Shaughnessy, Harding, & Burge, 2014; Takahashi, Iwashige, & Matsuura, 1997; Toscano, Newsome, & Griffen, 2014; Waser et al., 2016). In addition to the utility of *L. panopaei*'s introduction history for studying how the host response to infection varies with its history of parasitism, this system is ideal for investigating the molecular basis of parasitic manipulation of host phenotype.

Here we test the transcriptomic response to *L. panopaei* infection of *R. harrisii* hosts from populations with different histories of exposure to the parasite. We previously conducted experimental infections in this system, and found differences in susceptibility among the geographic source populations (Tepolt et al., 2020b). Naïve crabs from sites with no history of parasitism were more susceptible to infection than those from sites where the parasite is native, providing evidence of the potential for host adaptation to parasitism in the native range (Tepolt et al., 2020b). Surprisingly, there was no difference in susceptibility between host crabs from regions where the parasite is introduced and where it is native. However, because we used adult crabs from natural populations, it is unclear whether this similarity in susceptibility reflects rapid adaptation of host resistance in the introduced range or a plastic response arising from previous exposure to the parasite. In this study, we take advantage of tissue samples collected during these susceptibility experiments to explore differences in the transcriptomic response to parasitism by these hosts across the species' range. Using mRNA sequencing of these samples, here we compare infected and uninfected hosts to investigate the molecular basis of potential parasitic manipulation. We further compare host populations from regions

where the parasite is native, introduced, and absent to investigate whether and how differences in gene expression may correspond with the observed differences in susceptibility. We hypothesize that rhizocephalan infection is accompanied by widespread, coordinated changes in host gene expression corresponding to putatively manipulated host phenotypes. Furthermore, we hypothesize that the transcriptomic responses exhibited within crab populations will reflect the observed differences in susceptibility and vary according to their history of exposure to the parasite. Such patterns could be reflective of host adaptation to parasitism, though we acknowledge that this possibility cannot be disentangled from plastic responses driven by other ecological factors. By examining a host's transcriptomic response to rhizocephalan infection, and how this may vary depending on a host population's history of exposure, this work contributes to a growing understanding of the molecular mechanisms of parasitic manipulation and host-parasite interactions more broadly.

2. MATERIALS AND METHODS

2.1 Collections and experimental infections

All collections and experiments were carried out during the work described in Tepolt et al. (2020b). Briefly, uninfected *R. harrisii* adults were collected from nine estuaries along the Gulf and Atlantic coasts, three from each of three regions differing in their degree of historical exposure to *L. panopaei*: the parasite's native range [Terrebonne Bay, LA (LA); Apalachicola, FL (AP); St. Lucie River, FL (FP)], the parasite's introduced range [Pellicer Creek, FL (ML); Ashley River, SC (SC); Chesapeake Bay, MD (MD)], and outside of the parasite's current range [Mullica River, NJ (NJ); Moonakis River, MA (MA); Squamscott River, NH (NH)] (Figure 1). Crabs were kept individually in 50 ml of 15 PSU artificial seawater at 20°C and a 12-hr:12-hr light:dark cycle. Control crabs were never exposed to the parasite in the laboratory, while "treatment" crabs were exposed to infective parasite larvae within 24 hours of molting, when they are most susceptible to infection (Alvarez, Hines, & Reaka-Kudla, 1995). Naturally infected crabs from MD with mature parasite externae were used as the source of parasite larvae for all experimental exposures. Crabs were maintained in the laboratory through an additional molting cycle, after which their infection status was determined by visual inspection for parasite externae. Tissue samples from 82 crabs, 51 control and 31 parasitized (Table 1, Table

S1), two "reference" parasite externae from LA and MD, were collected and stored in RNAlater at -80 °C.

2.2 RNA extraction, library preparation, and sequencing

Sequence data from these specimens was generated previously, as described in Tepolt et al. (2020a). As the largest and most accessible neural organ, the thoracic ganglion was chosen for sequencing due to its likely functional importance to the parasite's impact on host behavior. RNA extractions were performed using Trizol with 1-bromo-3-chloropropane, and cDNA libraries were generated using Illumina TruSeq Stranded mRNA Library Prep Kits. Libraries were sequenced across seven lanes of an Illumina HiSeq 2000 in 50 bp single-end reads at the University of Utah High Throughput Genomics Core Facility. Further methodological details, including the results of a population genomic investigation of the host crab, can be found in Tepolt et al. (2020a).

2.3 Sequence processing, *de novo* transcriptome assembly, and functional annotation

With the exception of sequence read trimming and assembly of the parasite transcriptome, code for all steps of the bioinformatic pipeline, downstream analyses, and plotting are given in a publicly accessible GitHub repository (http://www.github.com/tepoltlab/RhithroLoxo_DE/). Please see the included README.md file for a more detailed description of the steps briefly outlined here. The pipeline was constructed and executed using the workflow engine Snakemake (Köster & Rahmann, 2012), and all computation was performed on the Poseidon high-performance computing cluster at Woods Hole Oceanographic Institution.

QC and trimming of raw reads were performed with Trim Galore! v0.4.0 (Krueger, 2015), a Perl wrapper for Cutadapt (Martin, 2011) and FastQC (Andrews, 2010). Read ends were trimmed of Illumina adapters and bases with Phred quality scores of less than 20; reads shorter than 20 bp after trimming were discarded. One sample, MA_C_3, displayed low quality scores and was removed from subsequent analyses. To identify potential contamination from reads originating from the parasite, sequences were aligned to a *L. panopaei* transcriptome that had been previously assembled from sequencing of two parasite externae, one each from the parasite's native (LA) and invasive (MD) ranges. The parasite transcriptome was assembled as

per Tepolt et al. (2020a) and reads from host libraries were screened against it using Magic-BLAST (Boratyn, Thierry-Mieg, Thierry-Mieg, Busby, & Madden, 2019); reads aligning across their entire length with ≥98% identity were removed using bbtools (https://sourceforge.net/projects/bbmap).

De novo assembly of the *R. harrisii* transcriptome was performed using Trinity v2.8.5 (Grabherr et al., 2011; Haas et al., 2013). To minimize inclusion of any residual parasite-derived reads in the assembly, only cleaned reads from the uninfected individuals were used. A range of parameter values was used for assembly, and the optimal transcriptome was selected based on the N50 and ExN50 quality metrics generated by utility scripts within Trinity. Only the longest isoform per 'gene' was retained and contigs less than 200 bp were discarded. Contigs were then queried against the NCBI nt database with blastn using BLAST+ v2.7.1 (Camacho et al., 2009) to screen for potential contaminant taxa including fungi, bacteria, platyhelminthes, and nematoda. Contigs with alignments to contaminant taxa references sequences with e-values of 1^{-10} or lower were removed.

Functional annotation was performed using the software EnTAP v0.9.0-beta (Hart et al., 2020), which uses DIAMOND v0.9.9 (Buchfink, Xie, & Huson, 2015) to perform similarity searches against user-specified reference sequence databases and retrieve gene family assignments and associated gene ontology (GO) information using the eggNOG v4.5 database (Huerta-Cepas et al., 2016). Six-frame translated contigs were queried against the following protein databases: UniProt's Swiss-Prot, TrEMBL, and UniRef90, and NBCI's nr and RefSeq (NCBI Resource Coordinators, 2017; UniProt Constortium, 2018). In addition to those identified as contamination in the nucleotide BLAST search, transcripts matching contaminant reference sequences in protein space were removed from the finalized transcriptome prior to analyses.

2.4 Transcript quantification, differential expression analysis, and co-expression network analysis

Transcript quantification was performed using the pseudo-aligner Salmon v0.14.2 (Patro, Duggal, Love, Irizarry, & Kingsford, 2017) from within Trinity. Differential expression analysis was performed in R v3.6.1 (R Core Team, 2013) using the package DESeq2 v1.26.0 (Love, Huber, & Anders, 2014). Prior to the analysis, initial screening and filtering of latent

contaminant transcripts from *L. panopaei* and other parasites was carried out and four expression outliers (MA_C_1, MA_C_2, MA_C_4, and AP_C_1) were identified by a principal components analysis and removed, as such samples can impede estimation of dispersion and model fitting. These four outlier samples likely harbored intense field-derived fungal infections, as transcripts driving this outlier pattern were homologous to sequences from fungal genera (*Microsporidium*, *Hepatospora, Enterospora*, etc.). We also performed expression-based filtering, removing transcripts with normalized counts of less than 10 in over 90% of samples. After these steps, we conducted three separate sets of analyses to test the host's general response to infection, and how this response differed depending on host sex and history of parasitism (native, introduced, and absent).

2.4.1 General response to infection

First, using the trimmed dataset of 77 individuals (original 82 less one failed library and four outliers), we tested for differentially expressed transcripts between parasitized and control crabs, irrespective of their site of origin. We used a likelihood ratio test to evaluate a generalized linear model of site and infection status as coefficients against a null model with site alone to account for site-specific differences.

2.4.2 Sex-specific response to infection

Second, using this same dataset, we tested for differences in the magnitude of response to infection between males and females as a means of investigating signatures of host feminization. We used Wald tests to identify where expressed transcripts differed significantly in each of the two sexes. Because the sexes differed in their number of control and parasitized crabs, and thus power to resolve regulatory differences, we randomly down-sampled each sex to 21 control and 14 parasitized crabs, their respective minima in either sex. This randomization procedure was repeated across 1000 iterations, and we used a Welch's t-test to examine the difference in the mean number of differentially expressed genes (upregulated, downregulated, and overall) between males and females in their response to infection. We estimated effect sizes using Cohen's *d*.

2.4.3 Range-specific response to infection

Third, we tested for differences in the response to infection according to range. This analysis used a reduced dataset of 58 individuals across six populations, including two populations from each of the three ranges. We excluded FP because although it has historically been considered as part of *L. panopaei*'s native range, this was based on a single historical record that (1) predates recognition of the cryptic species complex and (2) may have occurred in a different panopeid species misidentified as an *E. depressus* or *R. harrisii*. Because MD was used as the source of *L. panopaei* larvae in the experimental infections, we excluded this site to avoid potentially confounding signatures of strong, estuary-level adaptation of the parasite to local hosts. And after the removal of the aforementioned sample outliers, no crabs remained from MA. We first investigated differences in the magnitude of transcriptional response to infection, using Wald tests to identify transcripts that were differentially expressed *within* each range separately (native, introduced, or absent) while controlling for site-specific effects. As in the case of sex-specific differences, each range differs in their number of control and infected crabs. We therefore repeated the same randomization procedure, down-sampling to five control crabs and two infected crabs, their respective minima across the sites, but repeating this across 5000 iterations. Significance testing of differences in the magnitude of response among ranges was performed using an ANOVA, followed by Tukey's honestly significant difference (HSD) post-hoc tests for pairwise comparisons. We again used Cohen's *d* to estimate effect size. We then tested for differences *among* ranges in their response to infection by using a likelihood ratio test to evaluate a full model including range, infection status, and their interaction as coefficients against a null model of range and infection status alone. Further sub-comparisons were made using Wald tests to reveal transcripts displaying a significant range x infection status interaction *between pairs* of ranges. Significance testing for all differential expression analyses was performed with adjusted p-values using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

2.4.4 Co-expression analysis

We also used weighted gene co-expression network analysis (WGCNA) to characterize transcriptome-wide expression patterns and relate them to infection status and host sex (Langfelder & Horvath, 2008). Rather than test each transcript individually, this approach

clusters transcripts exhibiting similar expression patterns across all samples, agnostic to experimental conditions. The overall expression signatures (eigengene) of the resulting co-expression modules are then tested for significant correlations with sample characteristics, here, infection status and sex.

2.5 Functional enrichment analysis

To investigate enrichment of functions across the various differential expression and co-expression analyses described above, we used the R package GO_MWU (Wright, Aglyamova, Meyer, & Matz, 2015). This approach uses Mann-Whitney U tests to determine which GO terms are significantly overrepresented among the up- or downregulated transcripts, or within WGCNA co-expression modules. We included only GO terms for transcripts mapping to arthropod clusters of orthologous genes (COGs) with e-values of 1^{-50} or less, except when analyzing WGCNA modules, for which we used GO terms for transcripts mapping to COGs with e-values of 1^{-10} regardless of taxonomy. Mann-Whitney U tests were performed on $-\log_{10}$p-values from the differential expression analyses and on the transcript module membership scores (kMEs) of select WGCNA modules. Significance testing was performed using Benjamini-Hochberg adjusted p-values for differential expression results and a permutation-based procedure for inclusion of GO terms in WGCNA modules.

3. RESULTS

3.1 Transcriptome assembly and annotation

Sequencing yielded 1.19 Gb of cleaned sequence data from 50 control and 31 parasitized crabs, of which 0.82 Gb from control crabs were used for *de novo* transcriptome assembly. After the removal of 3,179 putative contaminant contigs, the final transcriptome assembly consisted of a total of ~87 Mbp, containing 146,332 contigs with an average length of 577 bp, a total N50 of 774 bp, and an ExN50 of 1,119 bp for those transcripts which constituted 90% of total expression. Of these, 19,217 contigs (13.1%) had an alignment to reference sequences and 24,483 (16.7%) were assigned to gene families, with 21,550 (14.7%) having at least one GO term. After further contaminant screening and expression-based filtering, 60,488 transcripts were retained in the first differential expression dataset (general response and sex-

specific response) and 59,579 were retained in the second dataset, which encompassed six sites (range-specific response).

3.2 Transcriptional response to infection

The overall response to infection, regardless of range, was investigated by comparing the expression of 46 control and 31 parasitized individuals. A likelihood ratio test identified 852 upregulated and 743 downregulated transcripts ($p_{adj} < 0.05$) in the parasitized group, with 71 and 65 significant transcripts having absolute $\log_2$-fold changes (LFCs) > 2, respectively (Figure 2). Of these 1,595 transcripts, 269 (16.9%) mapped to decapod references (Figure S1). A Mann-Whitney U test for overrepresentation of GO terms from high-confidence, arthropod-specific gene family assignments among differentially expressed transcripts indicated enrichment of molecular functions including chitin binding ($p_{adj}= 1.22E^{-5}$), RNA binding ($p_{adj} = 1.13E^{-6}$), and molecular transducer activity ($p_{adj} = 4.90E^{-5}$); biological processes such as cell surface receptor signaling pathway ($p_{adj} = 5.70E^{-5}$), carbohydrate metabolic process ($p_{adj} = 1.28E^{-6}$), and RNA processing ($p_{adj} = 2.88E^{-5}$); and cellular localizations including extracellular region ($p_{adj} = 1.66E^{-11}$), ribonucleoprotein complex ($p_{adj} = 5.39E^{-9}$) and actin cytoskeleton ($p_{adj} = 2.17E^{-5}$) (Figure 2a, Table S2).

WGCNA identified 16 co-expression modules, two of which were negatively associated with infection (Figure S2). One infection-associated module ($r = -0.41$, $p = 2E^{-4}$, 180 transcripts) was enriched for GO terms associated with nervous system processes, for example, regulation of glutamatergic synaptic transmission ($p_{adj} = 0.033$) and positive regulation of neuron projection development ($p_{adj} = 0.065$, Table S3). The second ($r = -0.34$, $p = 0.002$, 175 transcripts) was enriched for GO terms associated with cytoskeletal functions, for example, cytoskeleton organization ($p_{adj} = 0$), actin filament-based process ($p_{adj} = 0$), and microfilament motor activity ($p_{adj} = 0$), among others.

3.3 Sex-specific response to infection

Male and female crabs differed in the magnitude of their transcriptional response to *L. panopaei* infection. Across 1,000 random iterations to account for differences in sample size, females averaged 120 upregulated and 206 downregulated transcripts in response to infection, whereas males exhibited a significantly higher number of differentially expressed transcripts,

with an average of 663 upregulated and 537 downregulated (Figure 3). In addition to those modules discussed above, WGCNA recovered an additional module containing 467 transcripts that exhibited associations consistent with feminization of male hosts (Figure S3). This sex-associated module exhibited lower expression in uninfected males relative to uninfected females ($r = -0.34$, $p = 2E^{-3}$), but was more highly expressed in infected males relative to uninfected males ($r = 0.47$, $p = 1E^{-5}$). GO term enrichment of this module revealed an overrepresentation of a diverse set of functions, but none with obvious links to host feminization. These included GO terms such as response to interleukin-1 ($p_{adj} = 0$), cell surface receptor signaling pathway ($p_{adj} = 0.0125$) and antioxidant activity ($p_{adj} = 0$) (Table S3)

3.4 Range-specific responses to infection

Crabs from locations where the parasite is native, introduced, or absent differed in the number of transcripts differentially expressed according to infection status. Across 5,000 iterations, there were an average of 128 upregulated and 173 downregulated transcripts in the native range, 805 and 407 in the introduced range, and 1,074 and 410 where the parasite is absent (Figure 4a). A likelihood ratio test revealed 4,449 transcripts that displayed a significant interaction between range and infection status, of which 547 (12.3%) aligned to decapod reference sequences (Figure S4). Interestingly, most significant interactions involved the introduced range, where transcripts tended to exhibit the opposite response relative to the native range and/or where the parasite is absent (Figure 4b,c). This set of transcripts was enriched for molecular functions including RNA binding ($p_{adj} = 3.85E^{-3}$), oxidoreductase activity ($p_{adj} = 0.0194$), and structural constituent of ribosome ($p_{adj} = 1.89E^{-13}$); biological processes such as mitotic cell cycle process ($p_{adj} = 8.08E^{-5}$), ATP synthesis coupled electron transport ($p_{adj} = 3.52E^{-4}$), and cellular response to stress ($p_{adj} = 0.03$); and cellular localizations including cytosolic ribosome ($p_{adj} = 1.27E^{-10}$), transcription repressor complex ($p_{adj} = 0.0331$) and mitochondrial protein complex ($p_{adj} = 2.32E^{-7}$) (Figure S5, Table S4).

In the analysis of transcripts displaying a significant range x infection status interaction *between pairs* of ranges, there was considerably less overlap between pairs including the introduced range than between the absent and native range (Figure S6). Functional enrichment of transcripts exhibiting an interaction when comparing the native and introduced ranges

revealed the overrepresentation of the GO terms "immune response" (p_{adj} = 0.0187), "neurotransmitter receptor activity" (p_{adj} = 1.63E^{-4}), and "chitinase activity" (p_{adj} = 0.0267) (Table S5). Despite the greater overlap in the response of transcripts between the native range and absent range, functional enrichment analysis recovered hundreds more divergent GO terms in this comparison than for the introduced-native range pair (899 v. 106 <0.1 FDR, Table S6). Similar results were found for the absent-introduced range pair (598 GO terms <0.1 FDR, Table S7), perhaps resulting from the greater and more balanced sample size in the absent range.

4 DISCUSSION

The redistribution of biodiversity through species invasions has disrupted existing host-parasite relationships and established new ones, creating opportunities to study novel host-parasite interactions in the wild (Goedknegt et al., 2016). Building upon our previous work demonstrating differences in *R. harrisii*'s susceptibility to *L. panopaei* across a mosaic of historical parasite exposure (Tepolt et al., 2020b), here we use transcriptomic analyses to investigate the molecular mechanisms underlying the host response. We demonstrate that rhizocephalan infection has a widespread effect on patterns of host gene regulation, with changes in expression including patterns that may reflect the modification of key biological processes implicated in parasitic manipulation, including host immunity and molting. Male crabs exhibited a stronger response than females, which may be indicative of sex-specific changes associated with host feminization. On a regional level, hosts differed in their response to infection depending on their degree of historical exposure to the parasite, a pattern consistent with previously observed differences in susceptibility in the laboratory and prevalence in the field. By exploring *R. harrisii*'s gene regulatory response to *L. panopaei* infection, we contribute to a growing understanding of the molecular basis of parasitic manipulation and host-parasite interactions more broadly.

4.1 General response to infection: signatures of parasitic manipulation?

Individuals from all parts of *R. harrisii*'s range, regardless of historical exposure to the parasite, displayed a core response to *L. panopaei* infection. Perhaps the most striking component of this pattern is the widespread downregulation of transcripts involved in

immunity. Crustaceans, like all invertebrates, canonically lack an acquired (adaptive) immune system, instead relying on non-specific innate immunity (Cerenius, Jiravanichpaisal, Liu, & Söderhäll, 2010; Iwanaga & Bok, 2005; but see Melillo, Marino, Italiani, & Boraschi, 2018). Central processes in crustacean innate immunity include pattern recognition, prophenoloxidase (proPO) activation, encapsulation, and clotting, among others (Vazquez et al., 2009). Pattern recognition is dominated by proteins that bind carbohydrates and glycoproteins on the cell surface of pathogens, with lectins playing a central role (Drickamer & Taylor, 1993; Marques & Barracco, 2000). In this experiment, infected crabs exhibited a reduction in GO terms associated with carbohydrate binding, with several transcripts encoding for lectins orthologous to hemocytin, ficolin 2, and C-type lectins significantly downregulated during infection (Figure 2). We also found evidence for the disruption of the clotting response, with a significant reduction in transcripts for hemolymph clottable protein and heme binding protein 1 (Theopold, Schmidt, Söderhäll, & Dushay, 2004). Additionally, potential disruption of the proPO activation system, the invertebrate analog to the complement system (Vazquez et al., 2009), may be reflected in the reduction of the peptidase and oxidoreductase GO categories in parasitized individuals.

The pronounced reduction in immune-associated functions in parasitized individuals is consistent with active immunomodulation by *L. panopaei*. Evasion of the host's immune response is a common feature of infection across parasitic taxa, either through passive detection avoidance, for example, by infecting minimally surveilled tissues, or actively by directly interfering in immune pathways (Maizels & Yazdanbakhsh, 2003; Sacks & Sher, 2002; Schmid-Hempel, 2008, 2009). While passive immune evasion may occur simultaneously, rhizocephalans, with their conspicuous externae and internally ramifying "root" systems, likely have little recourse for escaping detection and thus would be expected to rely principally upon active mechanisms (Viney & Cable, 2011). To date, there have been no comprehensive studies of a host's immune response to rhizocephalan infection. However, suppression of host immunity has been observed in other parasitic arthropods, including parasitoid wasps and ticks (Schmidt, Theopold, & Strand, 2001; Wikel, 1999). Additionally, other parasites of crustaceans have been shown to interfere with host immune systems (Cornet et al., 2009; Helluy & Thomas, 2010).

In addition to host immunity, we found several differentially expressed transcripts encoding for proteins involved in ecdysis (Figure 2). Transcripts for cryptocyanin, hemocyanin, and a chitinase, all of which have been implicated in the crustacean molt cycle (Rocha et al., 2012; Terwilliger, 2012), were significantly downregulated in *L. panopaei*-infected crabs. By contrast, other studies have found elevated levels of hemocyanin in the hemolymph of rhizocephalan-infected crabs (Manwell & Baker, 1963; Shirley, Shirley, & Meyers, 1986). This could be due to differences in expression among tissues, as the source of mRNA for the present study was the thoracic ganglion. Furthermore, hemocyanin and cryptocyanin are part of the larger hemocyanin gene family, of which there are many constituent genes, paralogs, and isoforms with diverse functions (Burmester, 2002). In fact, prophenoloxidase, of the aforementioned proPO system, is itself a member of the hemocyanin family. This, along with evidence for the potential involvement of hemocyanin and cryptocyanin in immunity (Lei et al., 2008; Liu, Su, Wang, & Wang, 2006), makes it difficult to disentangle the effects of homologous transcripts potentially involved in both immunity and molting. Nonetheless, the observed response may be reflective of inhibition of molting by the parasite, in what has been termed parasitic anecdysis (O'Brien & Van Wyk, 1984).

Rhizocephalan infection is intimately associated with the molt cycle (Høeg, 1995). In general, hosts that have recently molted are susceptible to penetration by cyprid larvae, with emergence of the parasite externa typically occurring after the following molt, beyond which ecdysis is often inhibited (Alvarez et al., 1995; O'Brien & Skinner, 1990). This can be considered a putatively adaptive manipulation, as host ecdysis would divert resources from parasite growth and reproduction, result in greater exposure to predation, and may cause severe physical damage to parasite tissues. While the underlying mechanisms are unclear, parasitic anecdysis likely relies on modulation of the host neuroendocrine system (Høeg, 1995). Molting inhibition in rhizocephalan-infected crabs has been associated with the regression of the host Y-organ (Chassard-Bouchaud & Hubert, 1976), a neuroendocrine gland which mediates the ecdysis cycle (Chang & Mykles, 2011). Correspondingly, we observed the upregulation of many transcripts involved in neuronal and signaling processes, such as neuroparsins and cell-surface receptors, and the enrichment of molecular function GO categories including neurotransmitter

receptor activity and G-protein coupled receptor activity. Our WGCNA analysis also recovered a differentially expressed coexpression module that was enriched for terms associated with neuronal processes, further highlighting the potential for host neuromodulation by the parasite (Figure S2, Table S3). However, rhizocephalan infection results in the modification of numerous aspects of behavior beyond molting (Mouritsen & Jensen, 2006; Toscano et al., 2014), and it is unclear how the changes in expression observed here may influence individual behavioral processes.

Another hallmark of host manipulation by rhizocephalans is the feminization of male hosts, which includes changes in behavior (Brockerhoff, McLay, & Rybakov, 2010; Høeg, 1995; Kristensen et al., 2012). We found that males exhibited a greater transcriptional response to infection compared to females, which may reflect differences in how *L. panopaei* manipulates its host depending on its sex. Furthermore, we found one WGCNA module that exhibited patterns of expression consistent with male feminization, albeit rather slight (Figure 3). The annotations of its constituent transcripts and the enrichment of GO terms did not appear to be dominated by any one biological function, obscuring its specific role in host feminization. Nonetheless, the coordinated change in expression of this set of genes points towards its involvement in this process, and this analysis reveals key candidates for further investigation.

4.2 Variation in susceptibility, immune priming, and host adaptation

Crabs from populations differing in their historical exposure to *L. panopaei* were expected to differ in their transcriptional response to infection. Specifically, we hypothesized that crabs from the parasite's introduced range would mount a response most similar to that of crabs from the native range, consistent with previous results demonstrating similar susceptibility (Tepolt et al., 2020b) and possibly reflecting of recent parallel adaptation to parasite pressure. In contrast, we expected that crabs from locations without the parasite, which were more prone to infection, would exhibit a contrasting reaction. Contrary to expectations, the vast majority of transcripts that displayed a significant interaction involved range pairs that included the introduced range. In other words, it was crabs from the introduced range of the parasite that displayed the most dissimilar transcriptional response.

While we cannot conclusively ascribe this seemingly paradoxical result to a specific mechanism, we provide two likely explanations.

First, this may result from differences in contemporary parasite pressure among the populations. We previously found that naïve *R. harrisii* populations that lacked historical exposure to *L. panopaei* were more prone to infection than those from the parasite's native range, possibly due to evolution of host resistance over millennia of sympatry (Tepolt et al., 2020b). Interestingly, there was no difference in host susceptibility between the introduced and native ranges, which at first glance might suggest rapid adaptation to parasitism in just dozens of generations. However, *L. panopaei* prevalence is substantially higher in its introduced range than in its native range (Tepolt et al., 2020b). As the crabs used in experimental infections were collected as adults from the wild, hosts from different populations also differ in their degree of *contemporary* exposure to the parasite. Crabs from the introduced range, where average prevalence was 25.9%, were much more likely to have already avoided or terminated infection than those from the native range, where prevalence averaged just 1.2% (Tepolt et al., 2020b). The dramatically higher prevalence in the introduced range likely resulted in the collection of crabs that were biased towards those with increased resistance, as variability in parasite susceptibility is commonly observed within populations, potentially mediated by innate genetic and/or plastic mechanisms (Ebert, Zschokke-Rohringer, & Carius, 1998; Henter & Via, 1995). The idiosyncratic transcriptional response of crabs from the introduced range may thus reflect constitutive differences in gene expression among ranges and/or an elevated immune response potentiated by previous exposure.

Innate immunity, in contrast to the adaptive immune system exclusive to vertebrates, is classically perceived as lacking memory; that is, exposure to a pathogen should not elicit an improved response upon subsequent contact (Zinkernagel et al., 1996). Instead, pattern recognition in innate immunity is more general, relying on germline-encoded receptors that bind broadly to evolutionarily-conserved pathogen antigens (Janeway & Medzhitov, 2002). However, a number of studies from the field of ecological immunity have established a potentiating effect of previous exposure within innate immunity, which has been termed trained innate immunity or immune priming (Kurtz, 2005; Moret & Schmid-Hempel, 2001;

Moret & Siva-Jothy, 2003; Netea et al., 2016; Rodrigues, Brayner, Alves, Dixit, & Barillas-Mury, 2010). Host immune priming in *L. panopaei*'s introduced range may help explain the divergent expression profile observed. In the parasite's introduced range, where prevalence is high, the response of many host transcripts to infection was opposite of that observed in both the native range and where the parasite is absent. Many of the most highly significant transcripts displaying a range x infection status interaction are involved in immunity, and mostly increased in abundance in the introduced range while decreasing in the others. Functional enrichment analysis in a comparison of range x infection status interaction transcripts between the native and introduced range revealed overrepresentation of the GO term "immune response", further supporting the potential for immune priming. While immune priming in rhizocephalan host-parasite systems has not been established, it has been observed in crustacean diseases such as vibriosis and white spot syndrome (Chang, Kumar, Ng, & Wang, 2018).

Alternatively, the differences in the expression patterns seen in the introduced range may indeed reflect rapid adaptation to parasitism. Our expectation was rooted in the assumption that putative evolution of host resistance in the parasite's introduced range may render the host's transcriptional response more similar to that seen in the native range. However, a growing body of evidence has indicated that parallel phenotypic evolution may be mediated by *non-parallel* molecular evolutionary trajectories (Bolnick, Barrett, Oke, Rennison, & Stuart, 2018; Elmer et al., 2014; Therkildsen et al., 2019; Wang et al., 2020). Thus, the unexpected host response in the parasite's introduced range may be reflective of the evolution of a novel reaction to rhizocephalan parasitism that is distinct from that in the native range. Independent evolutionary trajectories are particularly likely in *R. harrisii*; population structure in this species is very high, with each estuary in this study having a distinct population genomic signature (Tepolt et al., 2020a). This strong structure, as well as the species' larval behavior (Forward, 2009), suggests very limited larval dispersal among estuaries. Given this, rapid evolution would very likely act on the standing genetic variation present in each individual estuary rather than drawing from the variation present in a larger metapopulation.

Conversely, the heightened reaction in the introduced range may instead reflect a *lack* of evolved host resistance – a transcriptomic uproar indicative of an uncoordinated and

overzealous immune response that may be maladaptive and/or be in response to secondary damage by the parasite. Studies of emerging wildlife diseases, such as chytrid fungus in amphibians and white-nose syndrome in bats, have shown that susceptible host lineages often launch more robust transcriptomic responses than their tolerant counterparts, with specific dysregulation of transcripts with immune functions (Davy et al., 2017; Eskew et al., 2018; Grogan et al., 2018; Savage, Gratwicke, Hope, Bronikowski, & Fleischer, 2020). However, if this were the case in the present study, we would expect to observe a similar response by the crabs from where the parasite is absent. This observation, coupled with the results of the analysis including all crabs, irrespective of origin, demonstrating the overall downregulation of transcripts involved in immunity, renders the possibility of immune priming and/or non-parallel adaptation more likely.

An additional source of complexity is the potential for this pattern to be driven by evolution of the parasite. All parasites were sourced from naturally infected crabs in Maryland, and it is possible that the idiosyncratic response in the introduced range reflects strong adaptation of the parasite to local hosts. We excluded MD crabs from the analysis to avoid potentially confounding signals from tight, estuary-level adaptation of parasite to host, but cannot rule out local adaptation of the parasite across the introduced range as a potential driver of this pattern. Further study is required to determine how these processes, immune priming or rapid evolution of the host and/or parasite, directly influence susceptibility in the introduced range.

5 CONCLUSIONS

Range shifts caused by rapidly changing environmental conditions and continued anthropogenic transport of exotic species are redistributing global biodiversity, blurring biogeographical lines and bringing novel combinations of species and genes into contact. Understanding how species will respond to these novel encounters, spanning recent and distant timescales, is of critical importance to predicting and managing impacts to native ecosystems. Our study investigated the gene regulatory response of *R. harrisii* to infection by *L. panopaei*, and how it differed among populations with different histories of parasitism. Our transcriptomic results demonstrated signatures consistent with parasitic manipulation and

previous observations of differences in susceptibility and parasite prevalence among regions. (Tepolt et al., 2020b). Despite the constraints of limited annotation of the *R. harrisii de novo* transcriptome, we were able to observe meaningful changes in the regulation of key transcripts involved in innate immunity and the molting cycle, two host processes with direct impacts on parasite viability. While follow-up experiments should be conducted to demonstrate causality, the data presented here provide insights into the molecular basis of an extreme extended phenotype – parasitic manipulation.

The differences in the host's transcriptional response to infection between the native range and where the parasite is absent may reflect long-standing host adaptation to parasitism in the native range. The unexpected pattern in the introduced range, however, could be suggestive of alternative processes (e.g., immune priming, non-parallel evolution, local adaptation of parasite to hosts) occurring in place of rapid parallel adaptation of *R. harrisii* to a novel parasite. Continued empirical investigations, ideally with crabs reared for multiple generations in the laboratory and parasites from different source populations, will help to disentangle the potential contributions of heritable and plastic mechanisms. By examining *R. harrisii*'s transcriptomic response to *L. panopaei* infection across a mosaic of historical exposure, this study adds to a growing understanding of the foundational role of gene regulation in both host-parasite interactions and novel biotic encounters facilitated by human introductions.

ACKNOWLEGMENTS

This project was supported by the Smithsonian Institute for Biodiversity Genomics, via an award for project sequencing and a fellowship to C.K.T. We are particularly grateful to our extraordinary interns, Darrin Rummel and Connor Hinton. Special thanks for significant help in the field to Michael Greene, Rebecca Bernard, Scott Hausmann, Sherry Reed, Jesse Schmidt, and Paul Stacey. We thank Cory Berger, Otto Cordero, Ann Tarrant, and Stefan Sievert for helpful discussions and comments on an earlier version of this manuscript. We also thank all the other people and organizations who helped support this project, including Tuck Hines, Monaca Noble, Alison Cawood, Lee Weigt, GeeGee Lang, Inken Kruse, LUMCON,

Pointe-aux-Chenes WMA, Sportsman's Lodge, Bill Eaton, Tommy Ward, Smithsonian Marine Station at Fort Pierce, Riverwatch Marina, Harbour Ridge Yacht Club, Nikki Dix, Faver-Dykes State Park, Princess Place Preserve, George O'Dell, CSX, Andrew Oaks, Graham Williams, Rivers Edge Marina, Campbell's Boat Yard, scores of Chesapeake-area citizen scientists, Roland Hagan, Allen's Dock, Stockton College, Frans Rowaan, Rick York, Brent Tepolt, and Gary Tepolt. We particularly want to acknowledge the wonderful National Estuarine Research Reserve network, especially the reserves in which we sampled: Apalachicola Bay, Guana Tolomato Matanzas, Jacques Cousteau, Waquoit Bay, and Great Bay. The United States Environmental Protection Agency and its Office of Research and Development did not provide any funding support for the data collection and analysis described here. Though this manuscript has been subjected to US EPA administrative review and approved for publication, its content does not necessarily reflect official Agency policy. The authors declare that they have no conflicts of interest regarding this publication.

REFERENCES

Alvarez, F., Hines, A. H., & Reaka-Kudla, M. L. (1995). The effects of parasitism by the barnacle *Loxothylacus panopaei* (Gissler) (Cirripedia: Rhizocephala) on growth and survival of the host crab *Rhithropanopeus harrisii* (Gould) (Brachyura: Xanthidae). *Journal of Experimental Marine Biology and Ecology*, *192*, 221–232. https://doi.org/10.1016/0022-0981(95)00068-3

Andersen, S. B., Gerritsma, S., Yusah, K. M., Mayntz, D., Hywel-Jones, N. L., Billen, J., … Hughes, D. P. (2009). The life of a dead ant: the expression of an adaptive extended phenotype. *The American Naturalist*, *174*, 424–433. https://doi.org/10.1086/603640

Andrews, S. (2010). FastQC: A Quality Control Tool for High Throughput Sequence Data. Retrieved from http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Bankers, L., Fields, P., McElroy, K. E., Boore, J. L., Logsdon, J. M., & Neiman, M. (2017). Genomic evidence for population-specific responses to co-evolving parasites in a New Zealand freshwater snail. *Molecular Ecology*, *26*, 3663–3675. https://doi.org/10.1111/mec.14146

Barribeau, S. M., Sadd, B. M., Du Plessis, L., & Schmid-Hempel, P. (2014). Gene expression differences underlying genotype-by-genotype specificity in a host-parasite system.

693 *Proceedings of the National Academy of Sciences of the United States of America*, *111*,
694 3496–3501. https://doi.org/10.1073/pnas.1318628111
695 Belgrad, B. A., & Griffen, B. D. (2015). Rhizocephalan infection modifies host food consumption
696 by reducing host activity levels. *Journal of Experimental Marine Biology and Ecology*, *466*,
697 70–75. https://doi.org/10.1016/j.jembe.2015.02.011
698 Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and
699 powerful approach to multiple testing. *Journal of the Royal Statistical Society B*, *57*, 289–
700 300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
701 Berdoy, M., Webster, J. P., & Macdonald, D. W. (2000). Fatal attraction in rats infected with
702 *Toxoplasma gondii*. *Proceedings of the Royal Society of London. Series B: Biological*
703 *Sciences*, *267*, 1591–1594. https://doi.org/10.1098/rspb.2000.1182
704 Bethel, W. M., & Holmes, J. C. (1977). Increased vulnerability of amphipods to predation owing
705 to altered behavior induced by larval acanthocephalans. *Canadian Journal of Zoology*, *55*,
706 110–115. https://doi.org/10.1139/z77-013
707 Blakeslee, A. M. H., Barnard, R. B., Matheson, K., & McKenzie, C. H. (2020). Host-switching
708 among crabs: Species introduction results in a new target host for native parasites. *Marine*
709 *Ecology Progress Series*, *636*, 91–106. https://doi.org/10.3354/meps13214
710 Bolnick, D. I., Barrett, R. D. H., Oke, K. B., Rennison, D. J., & Stuart, Y. E. (2018). (Non)parallel
711 evolution. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 303–330.
712 https://doi.org/10.1146/annurev-ecolsys-110617-062240
713 Bonebrake, T. C., Brown, C. J., Bell, J. D., Blanchard, J. L., Chauvenet, A., Champion, C., … Pecl, G.
714 T. (2018). Managing consequences of climate-driven species redistribution requires
715 integration of ecology, conservation and social science. *Biological Reviews*, *93*, 284–305.
716 https://doi.org/10.1111/brv.12344
717 Bonneaud, C., Balenger, S. L., Russell, A. F., Zhang, J., Hill, G. E., & Edwards, S. V. (2011). Rapid
718 evolution of disease resistance is accompanied by functional changes in gene expression in
719 a wild bird. *Proceedings of the National Academy of Sciences of the United States of*
720 *America*, *108*, 7866–7871. https://doi.org/10.1073/pnas.1018580108
721 Boratyn, G. M., Thierry-Mieg, J., Thierry-Mieg, D., Busby, B., & Madden, T. L. (2019). Magic-

722 BLAST, an accurate RNA-seq aligner for long and short reads. *BMC Bioinformatics*, *20*, 405.
723 https://doi.org/10.1186/s12859-019-2996-x
724 Boschma, H. (1955). The described species of the family Sacculinidae. *Zoologische*
725 *Verhandelingen*, *27*, 1–76.
726 Brockerhoff, A. M., McLay, C. L., & Rybakov, A. V. (2010). Occurrence of *Heterosaccus*
727 (Cirripedia: Rhizocephala) in the New Zealand Crab *Metacarcinus novaezelandiae*
728 (Decapoda: Cancridae) and distribution of other Rhizocephala in the South Pacific. *Journal*
729 *of Crustacean Biology*, *30*, 377–383. https://doi.org/10.1651/09-3270.1
730 Brooks, D. R., & Hoberg, E. P. (2007). How will global climate change affect parasite-host
731 assemblages? *Trends in Parasitology*, *23*, 571–574.
732 https://doi.org/10.1016/j.pt.2007.08.016
733 Buchfink, B., Xie, C., & Huson, D. H. (2015). Fast and sensitive protein alignment using
734 DIAMOND. *Nature Methods*, *12*, 59–60. https://doi.org/10.1038/nmeth.3176
735 Buck, J. C., Weinstein, S. B., & Young, H. S. (2018). Ecological and evolutionary consequences of
736 parasite avoidance. *Trends in Ecology & Evolution*, *33*, 619–632.
737 https://doi.org/j.tree.2018.05.001
738 Burmester, T. (2002). Origin and evolution of arthropod hemocyanins and related proteins.
739 *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental*
740 *Physiology*, *172*, 95–107. https://doi.org/10.1007/s00360-001-0247-7
741 Bushek, D., & Allen, S. K. (1996). Host-parasite interactions among broadly distributed
742 populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus*
743 *marinus*. *Marine Ecology Progress Series*, *139*, 127–141.
744 https://doi.org/10.3354/meps139127
745 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L.
746 (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, *10*, 421.
747 https://doi.org/10.1186/1471-2105-10-421
748 Carney, W. P. (1969). Behavioral and morphological changes in carpenter ants harboring
749 dicrocoeliid metacercariae. *The American Midland Naturalist*, *82*, 605–611.
750 https://doi.org/10.2307/2423801

751 Cerenius, L., Jiravanichpaisal, P., Liu, H., & Söderhäll, I. (2010). Crustacean Immunity. In K.
752 Söderhäll (Ed.), *Invertebrate Immunity* (pp. 239–259). Boston, MA: Springer.
753 Cézilly, F., Favrat, A., & Perrot-Minnot, M. J. (2013). Multidimensionality in parasite-induced
754 phenotypic alterations: Ultimate versus proximate aspects. *Journal of Experimental*
755 *Biology*, *216*, 27–35. https://doi.org/10.1242/jeb.074005
756 Chang, E. S., & Mykles, D. L. (2011). Regulation of crustacean molting: A review and our
757 perspectives. *General and Comparative Endocrinology*, *172*, 323–330.
758 https://doi.org/10.1016/j.ygcen.2011.04.003
759 Chang, Y. H., Kumar, R., Ng, T. H., & Wang, H. C. (2018). What vaccination studies tell us about
760 immunological memory within the innate immune system of cultured shrimp and crayfish.
761 *Developmental and Comparative Immunology*, *80*, 53–66.
762 https://doi.org/10.1016/j.dci.2017.03.003
763 Chassard-Bouchaud, C., & Hubert, M. (1976). On the fine structure of the regressing ecdysial
764 glands of *Carcinus maenas* L. (Crustacea Decapoda) parasitized by *Sacculina carcini*
765 Thompson. *Cell Tissue Research*, *167*, 351–361. https://doi.org/10.1007/BF00219147
766 Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of
767 species associated with high levels of climate warming. *Science*, *333*, 1024–1026.
768 https://doi.org/10.1126/science.1206432
769 Cornet, S., Franceschi, N., Bauer, A., Rigaud, T., & Moret, Y. (2009). Immune depression induced
770 by acanthocephalan parasites in their intermediate crustacean host: Consequences for the
771 risk of super-infection and links with host behavioural manipulation. *International Journal*
772 *for Parasitology*, *39*, 221–229. https://doi.org/10.1016/j.ijpara.2008.06.007
773 Criscione, C. D., & Font, W. F. (2001). The guest playing host: Colonization of the introduced
774 mediterranean gecko, *Hemidactylus turcicus*, by helminth parasites in Southeastern
775 Louisiana. *Journal of Parasitology*, *87*, 1273–1278. https://doi.org/10.1645/0022-
776 3395(2001)087[1273:TGPHCO]2.0.CO;2
777 Criscione, C. D., Poulin, R., & Blouin, M. S. (2005). Molecular ecology of parasites: Elucidating
778 ecological and microevolutionary processes. *Molecular Ecology*, *14*, 2247–2257.
779 https://doi.org/10.1111/j.1365-294X.2005.02587.x

780 Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife -
781 Threats to biodiversity and human health. *Science*, *287*, 443–449.
782 https://doi.org/10.1126/science.287.5452.443
783 Davy, C. M., Donaldson, M. E., Willis, C. K. R., Saville, B. J., McGuire, L. P., Mayberry, H., … Kyle,
784 C. J. (2017). The other white-nose syndrome transcriptome: Tolerant and susceptible hosts
785 respond differently to the pathogen *Pseudogymnoascus destructans*. *Ecology and*
786 *Evolution*, *7*, 7161–7170. https://doi.org/10.1002/ece3.3234
787 Dawkins, R. (1982). *The Extended Phenotype* (Vol. 8). Oxford University Press Oxford.
788 Drickamer, K., & Taylor, M. E. (1993). Biology of animal lectins. *Annual Review of Cell Biology*, *9*,
789 237–264. https://doi.org/10.1146/annurev.cb.09.110193.001321
790 Duffy, M. A., & Sivars-Becker, L. (2007). Rapid evolution and ecological host-parasite dynamics.
791 *Ecology Letters*, *10*, 44–53. https://doi.org/10.1111/j.1461-0248.2006.00995.x
792 Dunn, A. M. (2009). Parasites and biological invasions. *Advances in Parasitology*, *68*, 161–184.
793 https://doi.org/10.1016/S0065-308X(08)00607-6
794 Eberhard, W. G. (2000). Spider manipulation by a wasp larva. *Nature*, *406*, 255–256.
795 Ebert, D., Zschokke-Rohringer, C. D., & Carius, H. J. (1998). Within- and between-population
796 variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*.
797 *Proceedings of the Royal Society B: Biological Sciences*, *265*, 2127–2134.
798 https://doi.org/10.1098/rspb.1998.0549
799 Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012a). Divergent selection on locally
800 adapted major histocompatibility complex immune genes experimentally proven in the
801 field. *Ecology Letters*, *15*, 723–731. https://doi.org/10.1111/j.1461-0248.2012.01791.x
802 Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012b). Rapid and adaptive evolution of
803 MHC genes under parasite selection in experimental vertebrate populations. *Nature*
804 *Communications*, *3*, 1–6. https://doi.org/10.1038/ncomms1632
805 Elmer, K. R., Fan, S., Kusche, H., Spreitzer, M. L., Kautt, A. F., Franchini, P., & Meyer, A. (2014).
806 Parallel evolution of Nicaraguan crater lake cichlid fishes via non-parallel routes. *Nature*
807 *Communications*, *5*, 5168. https://doi.org/10.1038/ncomms6168
808 Elsner, N. O., Jacobsen, S., Thieltges, D. W., & Reise, K. (2011). Alien parasitic copepods in

mussels and oysters of the Wadden Sea. *Helgoland Marine Research*, *65*, 299–307. https://doi.org/10.1007/s10152-010-0223-2

Eskew, E. A., Shock, B. C., Ladouceur, E. E. B., Keel, K., Miller, M. R., Foley, J. E., & Todd, B. D. (2018). Gene expression differs in susceptible and resistant amphibians exposed to *Batrachochytrium dendrobatidis*. *Royal Society Open Science*, *5*. https://doi.org/10.1098/rsos.170910

Feis, M. E., Goedknegt, M. A., Thieltges, D. W., Buschbaum, C., & Wegner, K. M. (2016). Biological invasions and host–parasite coevolution: different coevolutionary trajectories along separate parasite invasion fronts. *Zoology*, *119*, 366–374. https://doi.org/10.1016/j.zool.2016.05.012

Feis, M. E., John, U., Lokmer, A., Luttikhuizen, P. C., & Wegner, K. M. (2018). Dual transcriptomics reveals co-evolutionary mechanisms of intestinal parasite infections in blue mussels *Mytilus edulis*. *Molecular Ecology*, 1505–1519. https://doi.org/10.1111/mec.14541

Feldmeyer, B., Mazur, J., Beros, S., Lerp, H., Binder, H., & Foitzik, S. (2016). Gene expression patterns underlying parasite-induced alterations in host behaviour and life history. *Molecular Ecology*, *25*, 648–660. https://doi.org/10.1111/mec.13498

Font, W. F., & Tate, D. C. (1994). Helminth parasites of native Hawaiian freshwater fishes: an example of extreme ecological isolation. *The Journal of Parasitology*, *80*, 682–688. https://doi.org/10.2307/3283246

Forward, R. B. (2009). Larval biology of the crab *Rhithropanopeus harrisii* (Gould): A synthesis. *Biological Bulletin*, *216*, 243–256. https://doi.org/10.2307/25548158

Freeman, A. S., Blakeslee, A. M. H., & Fowler, A. E. (2013). Northward expansion of the rhizocephalan *Loxothylacus panopaei* (Gissler, 1884) in the northwest Atlantic. *Aquatic Invasions*, *8*, 347–353. https://doi.org/10.3391/ai.2013.8.3.11

Geffre, A. C., Liu, R., Manfredini, F., Beani, L., Kathirithamby, J., Grozinger, C. M., & Toth, A. L. (2017). Transcriptomics of an extended phenotype: Parasite manipulation of wasp social behaviour shifts expression of caste-related genes. *Proceedings of the Royal Society B: Biological Sciences*, *284*. https://doi.org/10.1098/rspb.2017.0029

838 Gérard, C., & Le Lannic, J. (2003). Establishment of a new host-parasite association between the
839 introduced invasive species *Potamopyrgus antipodarum* (Smith)(Gastropoda) and
840 *Sanguinicola* sp. Plehn (Trematoda) in Europe. *Journal of Zoology*, *261*, 213–216.
841 https://doi.org/10.1017/S0952836903004084
842 Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for
843 community interactions under climate change. *Trends in Ecology and Evolution*, *25*, 325–
844 331. https://doi.org/10.1016/j.tree.2010.03.002
845 Goedknegt, M. A., Feis, M. E., Wegner, K. M., Luttikhuizen, P. C., Buschbaum, C., Camphuysen,
846 K. (. C. J.)., … Thieltges, D. W. (2016). Parasites and marine invasions: Ecological and
847 evolutionary perspectives. *Journal of Sea Research*, Vol. 113, pp. 11–27.
848 https://doi.org/10.1016/j.seares.2015.12.003
849 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Regev, A.
850 (2011). Full-length transcriptome assembly from RNA-Seq data without a reference
851 genome. *Nature Biotechnology*, *29*, 644–652. https://doi.org/10.1038/nbt.1883
852 Grogan, L. F., Cashins, S. D., Skerratt, L. F., Berger, L., McFadden, M. S., Harlow, P., … Mulvenna,
853 J. (2018). Evolution of resistance to chytridiomycosis is associated with a robust early
854 immune response. *Molecular Ecology*, *27*, 919–934. https://doi.org/10.1111/mec.14493
855 Guo, F., Lenoir, J., & Bonebrake, T. C. (2018). Land-use change interacts with climate to
856 determine elevational species redistribution. *Nature Communications*, *9*, 1–7.
857 https://doi.org/10.1038/s41467-018-03786-9
858 Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., … Regev, A.
859 (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity
860 platform for reference generation and analysis. *Nature Protocols*, *8*, 1494–1512.
861 https://doi.org/10.1038/nprot.2013.084
862 Hart, A. J., Ginzburg, S., Xu, M. (Sam), Fisher, C. R., Rahmatpour, N., Mitton, J. B., … Wegrzyn, J.
863 L. (2020). EnTAP: Bringing Faster and Smarter Functional Annotation to Non-Model
864 Eukaryotic Transcriptomes. *Molecular Ecology Resources*, *20*, 591–604.
865 https://doi.org/10.1111/1755-0998.13106
866 Helluy, S., & Thomas, F. (2010). Parasitic manipulation and neuroinflammation: Evidence from

the system *Microphallus papillorobustus* (Trematoda) - *Gammarus* (Crustacea). *Parasites and Vectors*, *3*, 1–11. https://doi.org/10.1186/1756-3305-3-38

Henter, H. J., & Via, S. (1995). The potential for coevolution in a host-parasitoid system. I. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution*, *49*, 427–438. https://doi.org/10.1111/j.1558-5646.1995.tb02275.x

Hines, A. H., Alvarez, F., & Reed, S. A. (1997). Introduced and native populations of a marine parasitic castrator: Variation in prevalence of the rhizocephalan *Loxothylacus panopaei* in xanthid crabs. *Bulletin of Marine Science*, *61*, 197–214.

Høeg, J. T. (1995). The biology and life cycle of the Rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom*, *75*, 517–550. https://doi.org/10.1017/S0025315400038996

Huerta-Cepas, J., Szklarczyk, D., Forslund, K., Cook, H., Heller, D., Walter, M. C., … Bork, P. (2016). EGGNOG 4.5: A hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Research*, *44*, D286–D293. https://doi.org/10.1093/nar/gkv1248

Hughes, D. (2013). Pathways to understanding the extended phenotype of parasites in their hosts. *Journal of Experimental Biology*, *216*, 142–147. https://doi.org/10.1242/jeb.077461

Iwanaga, S., & Bok, L. L. (2005). Recent advances in the innate immunity of invertebrate animals. *Journal of Biochemistry and Molecular Biology*, *38*, 128–150. https://doi.org/10.5483/bmbrep.2005.38.2.128

Janeway, C. A., & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, *20*, 197–216. https://doi.org/10.1146/annurev.immunol.20.083001.084359

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, *17*, 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Kelly, D. W., Paterson, R. A., Townsend, C. R., Poulin, R., & Tompkins, D. M. (2009). Parasite spillback: A neglected concept in invasion ecology? *Ecology*, *90*, 2047–2056. https://doi.org/10.1890/08-1085.1

Klemme, I., & Karvonen, A. (2016). Learned parasite avoidance is driven by host personality and

resistance to infection in a fish-trematode interaction. *Proceedings of the Royal Society B: Biological Sciences*, *283*. https://doi.org/10.1098/rspb.2016.1148

Köster, J., & Rahmann, S. (2012). Snakemake—a scalable bioinformatics workflow engine. *Bioinformatics*, *28*, 2520–2522. https://doi.org/10.1093/bioinformatics/bts480

Kristensen, T., Nielsen, A. I., Jørgensen, A. I., Mouritsen, K. N., Glenner, H., Christensen, J. T., … Høeg, J. T. (2012). The selective advantage of host feminization: A case study of the green crab *Carcinus maenas* and the parasitic barnacle *Sacculina carcini*. *Marine Biology*, *159*, 2015–2023. https://doi.org/10.1007/s00227-012-1988-4

Krueger, F. (2015). Trim Galore!: A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files.

Kruse, I., & Hare, M. P. (2007). Genetic diversity and expanding nonindigenous range of the rhizocephalan *Loxothylacus panopaei* parasitizing mud crabs in the western North Atlantic. *Journal of Parasitology*, *93*, 575–582. https://doi.org/10.1645/ge-888r.1

Kruse, I., Hare, M. P., & Hines, A. H. (2012). Genetic relationships of the marine invasive crab parasite *Loxothylacus panopaei*: An analysis of DNA sequence variation, host specificity, and distributional range. *Biological Invasions*, *14*, 701–715. https://doi.org/10.1007/s10530-011-0111-y

Kurtz, J. (2005). Specific memory within innate immune systems. *Trends in Immunology*, *26*, 186–192. https://doi.org/10.1016/j.it.2005.02.001

Lafferty, K. D., & Shaw, J. C. (2013). Comparing mechanisms of host manipulation across host and parasite taxa. *Journal of Experimental Biology*, *216*, 56–66. https://doi.org/10.1242/jeb.073668

Lafferty, K., & Morris, K. (1996). Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology*, *77*, 1390–1397. https://doi.org/10.2307/2265536

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*, 559. https://doi.org/10.1186/1471-2105-9-559

Lazzaro, B. P., & Little, T. J. (2009). Immunity in a variable world. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*, 15–26. https://doi.org/10.1098/rstb.2008.0141

925 Lei, K., Li, F., Zhang, M., Yang, H., Luo, T., & Xu, X. (2008). Difference between hemocyanin
926 subunits from shrimp *Penaeus japonicus* in anti-WSSV defense. *Developmental &*
927 *Comparative Immunology*, *32*, 808–813. https://doi.org/10.1016/j.dci.2007.11.010
928 Lenz, T. L., Eizaguirre, C., Rotter, B., Kalbe, M., & Milinski, M. (2013). Exploring local
929 immunological adaptation of two stickleback ecotypes by experimental infection and
930 transcriptome-wide digital gene expression analysis. *Molecular Ecology*, *22*, 774–786.
931 https://doi.org/10.1111/j.1365-294X.2012.05756.x
932 Liu, W., Su, J., Wang, G., & Wang, S. (2006). Proteomic approach for acute-phase proteins of
933 hemolymph and muscles in Scylla serrata challenged by a pathogenic bacterium. *Frontiers*
934 *of Biology in China*, *1*, 254–258. https://doi.org/10.1007/s11515-006-0031-x
935 Lohman, B. K., Steinel, N. C., Weber, J. N., & Bolnick, D. I. (2017). Gene expression contributes
936 to the recent evolution of host resistance in a model host parasite system. *Frontiers in*
937 *Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01071
938 Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion
939 for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550. https://doi.org/10.1186/s13059-
940 014-0550-8
941 Maizels, R. M., & Yazdanbakhsh, M. (2003). Immune regulation by helminth parasites: Cellular
942 and molecular mechanisms. *Nature Reviews Immunology*, *3*, 733–744.
943 https://doi.org/10.1038/nri1183
944 Manwell, C., & Baker, C. M. A. (1963). Starch gel electrophoresis of sera from some marine
945 arthropods: Studies on the heterogeneity of hemocyanin and on a “ceruloplasmin-like
946 protein.” *Comparative Biochemistry and Physiology*, *8*, 193–208.
947 https://doi.org/10.1016/0010-406X(63)90124-5
948 Marques, M. R. F., & Barracco, M. A. (2000). Lectins, as non-self-recognition factors, in
949 crustaceans. *Aquaculture*, *191*, 23–44. https://doi.org/10.1016/S0044-8486(00)00417-8
950 Martin, L. B., Hanson, H. E., Hauber, M. E., & Ghalambor, C. K. (2021). Genes, environments,
951 and phenotypic plasticity in immunology. *Trends in Immunology*, *42*, 198–208.
952 https://doi.org/10.1016/j.it.2021.01.002
953 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing

reads. *EMBnet. Journal*, *17*, 10–12. https://doi.org/10.14806/ej.17.1.200

Melillo, D., Marino, R., Italiani, P., & Boraschi, D. (2018). Innate immune memory in invertebrate metazoans: a critical appraisal. *Frontiers in Immunology*, *9*, 1915. https://doi.org/10.3389/fimmu.2018.01915

Miller, M. A., Kinsella, J. M., Snow, R. W., Hayes, M. M., Falk, B. G., Reed, R. N., … Romagosa, C. M. (2018). Parasite spillover: indirect effects of invasive Burmese pythons. *Ecology and Evolution*, *8*, 830–840.

Moore, J. (2002). *Parasites and the Behavior of Animals*. Oxford University Press on Demand.

Moran, E. V, & Alexander, J. M. (2014). Evolutionary responses to global change: lessons from invasive species. *Ecology Letters*, *17*, 637–649. https://doi.org/10.1111/ele.12262

Moret, Y., & Schmid-Hempel, P. (2001). Immune defence in bumble-bee offspring. *Nature*, *414*, 506. https://doi.org/10.1038/35107138

Moret, Y., & Siva-Jothy, M. T. (2003). Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 2475–2480. https://doi.org/10.1098/rspb.2003.2511

Mouritsen, K. N., & Jensen, T. (2006). The effect of *Sacculina carcini* infections on the fouling, burying behaviour and condition of the shore crab, *Carcinus maenas. Marine Biology Research*, *2*, 270–275. https://doi.org/10.1080/17451000600874752

NCBI Resource Coordinators. (2017). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *46*, D8–D13. https://doi.org/10.1093/nar/gkx1095

Netea, M. G., Joosten, L. A. B., Latz, E., Mills, K. H. G., Natoli, G., Stunnenberg, H. G., … Xavier, R. J. (2016). Trained immunity: A program of innate immune memory in health and disease. *Science*, *352*, 427. https://doi.org/10.1126/science.aaf1098

O'Brien, J. J., & Skinner, D. M. (1990). Overriding of the molt-inducing stimulus of multiple limb autotomy in the mud crab *Rhithropanopeus harrisii* by parasitization with a rhizocephalan. *Journal of Crustacean Biology*, *10*, 440–445. https://doi.org/10.2307/1548333

O'Brien, J., & Van Wyk, P. (1984). Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. In A. Wenner (Ed.),

Crustacean Issues 3: Factors in Adult Growth (pp. 191–218). Rotterdam, The Netherlands: Balkema, AA.

O'Shaughnessy, K. A., Harding, J. M., & Burge, E. J. (2014). Ecological effects of the invasive parasite *Loxothylacus panopaei* on the flatback mud crab *Eurypanopeus depressus* with implications for estuarine communities. *Bulletin of Marine Science*, *90*, 611–621. https://doi.org/10.5343/bms.2013.1060

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, *14*, 417. https://doi.org/10.1038/nmeth.4197

Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I. C., … Williams, S. E. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*, *355*. https://doi.org/10.1126/science.aai9214

Pereira, H. M., Leadley, P. W., Proença, V., Alkemade, R., Scharlemann, J. P. W., Fernandez-Manjarrés, J. F., … Walpole, M. (2010). Scenarios for global biodiversity in the 21st century. *Science*, *330*, 1496–1501. https://doi.org/10.1126/science.1196624

Poulin, R. (2010). Parasite manipulation of host behavior: An update and frequently asked questions. *Advances in the Study of Behavior*, *41*, 151–186. https://doi.org/10.1016/S0065-3454(10)41005-0

Poulin, R., & Latham, A. D. M. (2002). Parasitism and the burrowing depth of the beach hopper *Talorchestia quoyana* (Amphipoda: Talitridae). *Animal Behaviour*, *63*, 269–275. https://doi.org/10.1006/anbe.2001.1938

Prenter, J., MacNeil, C., Dick, J. T. A., & Dunn, A. M. (2004). Roles of parasites in animal invasions. *Trends in Ecology and Evolution*, *19*, 385–390. https://doi.org/10.1016/j.tree.2004.05.002

R Core Team. (2013). *R: A Language and Environment for Statistical Computing*. Retrieved from http://www.r-project.org/

Rocha, J., Garcia-Carreño, F. L., Muhlia-Almazán, A., Peregrino-Uriarte, A. B., Yépiz-Plascencia, G., & Córdova-Murueta, J. H. (2012). Cuticular chitin synthase and chitinase mRNA of whiteleg shrimp *Litopenaeus vannamei* during the molting cycle. *Aquaculture*, *330–333*, 111–115. https://doi.org/10.1016/j.aquaculture.2011.12.024

Rodrigues, J., Brayner, F. A., Alves, L. C., Dixit, R., & Barillas-Mury, C. (2010). Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science*, *329*, 1353–1355. https://doi.org/10.1126/science.1190689

Roth, O., Keller, I., Landis, S. H., Salzburger, W., & Reusch, T. B. H. (2012). Hosts are ahead in a marine host-parasite coevolutionary arms race: Innate immune system adaptation in pipefish *Syngnathus typhle* against *Vibrio* phylotypes. *Evolution*, *66*, 2528–2539. https://doi.org/10.1111/j.1558-5646.2012.01614.x

Sacks, D., & Sher, A. (2002). Evasion of innate immunity by parasitic protozoa. *Nature Immunology*, *3*, 1041–1047. https://doi.org/10.1038/ni1102-1041

Saul, W. C., & Jeschke, J. M. (2015). Eco-evolutionary experience in novel species interactions. *Ecology Letters*, *18*, 236–245. https://doi.org/10.1111/ele.12408

Savage, A. E., Gratwicke, B., Hope, K., Bronikowski, E., & Fleischer, R. C. (2020). Sustained immune activation is associated with susceptibility to the amphibian chytrid fungus. *Molecular Ecology*, *29*, 2889–2903. https://doi.org/10.1111/mec.15533

Schmid-Hempel, P. (2008). Parasite immune evasion: a momentous molecular war. *Trends in Ecology and Evolution*, *23*, 318–326. https://doi.org/10.1016/j.tree.2008.02.011

Schmid-Hempel, P. (2009). Immune defence, parasite evasion strategies and their relevance for "macroscopic phenomena" such as virulence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*, 85–98. https://doi.org/10.1098/rstb.2008.0157

Schmidt, O., Theopold, U., & Strand, M. (2001). Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. *BioEssays*, *23*, 344–351. https://doi.org/10.1002/bies.1049

Schweiger, O., Settele, J., Kudrna, O., Klotz, S., & Kühn, I. (2008). Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, *89*, 3472–3479. https://doi.org/10.1890/07-1748.1

1041 Shaw, J. C., Korzan, W. J., Carpenter, R. E., Kuris, A. M., Lafferty, K. D., Summers, C. H., & Øverli.
1042 (2009). Parasite manipulation of brain monoamines in California killifish (*Fundulus*
1043 *parvipinnis*) by the trematode *Euhaplorchis californiensis*. *Proceedings of the Royal Society*
1044 *B: Biological Sciences*, *276*, 1137–1146. https://doi.org/10.1098/rspb.2008.1597
1045 Shaw, J. C., & Øverli, O. (2012). Brain-encysting trematodes and altered monoamine activity in
1046 naturally infected killifish *Fundulus parvipinnis*. *Journal of Fish Biology*, *81*, 2213–2222.
1047 https://doi.org/10.1111/j.1095-8649.2012.03439.x
1048 Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and
1049 trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, *11*, 317–321.
1050 https://doi.org/10.1016/0169-5347(96)10039-2
1051 Shirley, S. M., Shirley, T. C., & Meyers, T. R. (1986). Hemolymph responses of Alaskan king crabs
1052 to rhizocephalan parasitism. *Canadian Journal of Zoology*, *64*, 1774–1781.
1053 https://doi.org/10.1139/z86-267
1054 Strauss, S. Y., Lau, J. A., & Carroll, S. P. (2006). Evolutionary responses of natives to introduced
1055 species: What do introductions tell us about natural communities? *Ecology Letters*, *9*, 357–
1056 374. https://doi.org/10.1111/j.1461-0248.2005.00874.x
1057 Takahashi, T., Iwashige, A., & Matsuura, S. (1997). Behavioral manipulation of the shore crab,
1058 *Hemigrapsus sanguineus* by the rhizocephalan barnacle, *Sacculina polygenea*. *Crustacean*
1059 *Research*, Vol. 26, pp. 153–161. https://doi.org/10.18353/crustacea.26.0_153
1060 Tepolt, C. K., Blakeslee, A. M. H., Fowler, A. E., Darling, J. A., Torchin, M. E., Miller, A. W., & Ruiz,
1061 G. M. (2020a). Strong genetic structure in a widespread estuarine crab: A test of potential
1062 versus realized dispersal. *Journal of Biogeography*, 1–11.
1063 https://doi.org/10.1111/jbi.13919
1064 Tepolt, C. K., Darling, J. A., Blakeslee, A. M. H., Fowler, A. E., Torchin, M. E., Miller, A. W., & Ruiz,
1065 G. M. (2020b). Recent introductions reveal differential susceptibility to parasitism across
1066 an evolutionary mosaic. *Evolutionary Applications*, 1–14.
1067 https://doi.org/10.1111/eva.12865
1068 Terwilliger, N. B. (2012). Gene expression profile, protein production, and functions of
1069 cryptocyanin during the crustacean molt cycle. *Invertebrate Reproduction and*

Development, *56*, 229–235. https://doi.org/10.1080/07924259.2011.595972

Theopold, U., Schmidt, O., Söderhäll, K., & Dushay, M. S. (2004). Coagulation in arthropods: defence, wound closure and healing. *Trends in Immunology*, *25*, 289–294. https://doi.org/10.1016/j.it.2004.03.004

Therkildsen, N. O., Wilder, A. P., Conover, D. O., Munch, S. B., Baumann, H., & Palumbi, S. R. (2019). Contrasting genomic shifts underlie parallel phenotypic evolution in response to fishing. *Science*, *365*, 487–490. https://doi.org/10.1126/science.aaw7271

Thomas, F., Adamo, S., & Moore, J. (2005). Parasitic manipulation: where are we and where should we go? *Behavioural Processes*, *68*, 185–199. https://doi.org/10.1016/j.beproc.2004.06.010

Thomas, F., Poulin, R., & Brodeur, J. (2010). Host manipulation by parasites: A multidimensional phenomenon. *Oikos*, *119*, 1217–1223. https://doi.org/10.1111/j.1600-0706.2009.18077.x

Thomas, F., Schmidt-Rhaesa, A., Martin, G., Manu, C., Durand, P., & Renaud, F. (2002). Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? *Journal of Evolutionary Biology*, *15*, 356–361. https://doi.org/10.1046/j.1420-9101.2002.00410.x

Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J., & Kuris, A. M. (2003). Introduced species and their missing parasites. *Nature*, *421*, 628–630. https://doi.org/10.1038/nature01346

Torchin, M. E., Lafferty, K. D., & Kuris, A. M. (1996). Infestation of an introduced host, the European green crab, *Carcinus maenas*, by a symbiotic nemertean egg predator, *Carcinonemertes epialti*. *The Journal of Parasitology*, 449–453. https://doi.org/10.2307/3284084

Toscano, B. J., Newsome, B., & Griffen, B. D. (2014). Parasite modification of predator functional response. *Oecologia*, *175*, 345–352. https://doi.org/10.1007/s00442-014-2905-y

UniProt Constortium. (2018). UniProt: the universal protein knowledgebase. *Nucleic Acids Research*, *46*, 2699. https://doi.org/10.1093/nar/gky092

Van Engel, W. A., Dillon, W. A., Zwerner, D., & Eldridge, D. (1966). *Loxothylacus panopaei*

(Cirripedia, Sacculinidae) an introduced parasite on a xanthid crab in Chesapeake Bay, U.S.A. *Crustaceana*, *10*, 110–112. https://doi.org/10.1163/156854066x00135

Vance, S. A. (1996). Morphological and behavioural sex reversal in mermithid-infected mayflies. *Proceedings of the Royal Society B: Biological Sciences*, *263*, 907–912. https://doi.org/10.1098/rspb.1996.0134

Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A., & Zenteno, E. (2009). Immunity mechanisms in crustaceans. *Innate Immunity*, Vol. 15, pp. 179–188. SAGE Publications. https://doi.org/10.1177/1753425909102876

Viney, M., & Cable, J. (2011). Macroparasite life histories. *Current Biology*, *21*, R767–R774. https://doi.org/10.1016/j.cub.2011.07.023

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Scientist*, *84*, 468– 478.

Wang, Y., Zhao, Y., Wang, Y., Li, Z., Guo, B., & Merilä, J. (2020). Population transcriptomics reveals weak parallel genetic basis in repeated marine and freshwater divergence in nine-spined sticklebacks. *Molecular Ecology*, *29*, 1642–1656. https://doi.org/10.1111/mec.15435

Waser, A. M., Goedknegt, M. A., Dekker, R., McSweeney, N., Witte, J. I. J., Van Der Meer, J., & Thieltges, D. W. (2016). Tidal elevation and parasitism: Patterns of infection by the rhizocephalan parasite *Sacculina carcini* in shore crabs *Carcinus maenas*. *Marine Ecology Progress Series*, *545*, 215–225. https://doi.org/10.3354/meps11594

Wells, K., O'Hara, R. B., Morand, S., Lessard, J. P., & Ribas, A. (2015). The importance of parasite geography and spillover effects for global patterns of host-parasite associations in two invasive species. *Diversity and Distributions*, *21*, 477–486. https://doi.org/10.1111/ddi.12297

Wendling, C. C., & Wegner, K. M. (2015). Adaptation to enemy shifts: Rapid resistance evolution to local *Vibrio* spp. in invasive pacific oysters. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20142244. https://doi.org/10.1098/rspb.2014.2244

Wikel, S. K. (1999). Modulation of the host immune system by ectoparasitic arthropods: Blood-feeding and tissue-dwelling arthropods manipulate host defenses to their advantage.

Bioscience, *49*, 311–320. https://doi.org/10.2307/1313614

Williams, A. B. (1984). Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida. In *Smithsonian Institution Press*. https://doi.org/10.2307/1352125

Wright, R. M., Aglyamova, G. V, Meyer, E., & Matz, M. V. (2015). Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. *BMC Genomics*, *16*, 371. https://doi.org/10.1186/s12864-015-1540-2

Yanoviak, S. P., Kaspari, M., Dudley, R., & Poinar, G. (2008). Parasite-induced fruit mimicry in a tropical canopy ant. *American Naturalist*, *171*, 536–544. https://doi.org/10.1086/528968

Zinkernagel, R. M., Bachmann, M. F., Kündig, T. M., Oehen, S., Pirchet, H., & Hengartner, H. (1996). On immunological memory. *Annual Review of Immunology*, *14*, 333–367. https://doi.org/10.1146/annurev.immunol.14.1.333

DATA ACCESSIBILITY STATEMENT

R. harrisii sequence reads are available in NCBI's Sequence Read Archive (SRA) under BioProject ID PRJNA633282. Individual BioSample IDs and SRA accession numbers are provided in Table S1. *L. panopaei* sequence reads are included within BioProject ID PRJNA739649 (SRR 14872363-4). The host transcriptome, parasite transcriptome, gene expression matrix, and sample metadata files can be found at https://doi.org/10.5061/dryad.rxwdbrv8m.

AUTHOR CONTRIBUTIONS

C.K.T., G.M.R., A.E.F., A.M.H.B., J.A.D., M.E.T., and A.W.M. conceived and designed this study. C.K.T. conducted the experimental work. Z.J.C.T. analyzed the data. Z.J.C.T. drafted the manuscript with assistance from C.K.T., and all authors contributed to editing it.

TABLES

		Control			**Parasitized**		
Range	**Site**	Female	Male	**Total**	Female	Male	**Total**
Native	LA	*3*	*3*	6	*0*	*2*	2

	AP[†]	2	4	6	*3*	*1*	4
	FP	*3*	*3*	6	*3*	*3*	6
Introduced	ML	*3*	*3*	6	*1*	*1*	2
	SC	*3*	*3*	6	*1*	*2*	3
	MD	*1*	*4*	5	*1*	*1*	2
Absent	NJ	*3*	*3*	6	*5*	*1*	6
	MA[‡]	*0*	*4*	4	*0*	*0*	0
	NH	*3*	*3*	6	*3*	*3*	6
Total		***21***	***30***	**51**	***17***	***14***	**31**

FIGURE LEGENDS

TABLE 1 Summary of *R. harrisii* individuals included in infection experiment. [†]One AP control female removed as expression outlier. [‡]Four MA controls were removed, including three as expression outliers and one due to sequencing failure.

FIGURE 1 Map of *L. panopaei* invasion history and survey data from Tepolt et al. (2020b). Pie charts represent parasite prevalence in *R. harrisii* at each site in summer 2015. *At AP, no parasitized *R. harrisii* were found, but presence of ER clade *L. panopae*i was confirmed by infections in co-occurring *E. depressus* hosts.

FIGURE 2 (a) Hierarchical clustering of Molecular Function GO terms significantly enriched among upregulated (red) and downregulated (blue) transcripts between infected and control crabs, as determined by a Mann-Whitney U test. (b) Curated subset of highly significant transcripts involved in innate immunity, ecdysis, and neurotransmission, corresponding to GO term categories shown in (a). Error bars represent 95% confidence intervals. Note $\log_{10}$ y-axis scale.

FIGURE 3 Number of differentially expressed transcripts in response to *L. panopaei* infection by host sex, across 1000 iterations of random downsampling. *** = $p << 0.001$, numbers indicate Cohen's *d* effect size.

FIGURE 4 (a) Number of differentially expressed transcripts in response to *L. panopaei* infection according to parasite status in the host range, across 5000 iterations of random downsampling.

*** = p << 0.001, numbers indicate Cohen's *d* effect size. Some datapoints for the absent range not shown due to truncation of y-axis limit for display purposes. (b) Most significant transcript in likelihood ratio test of range:infection status interaction. Error bars represent 95% confidence intervals. Significant within-range t-test result indicated by *** ($p < 0.001$). (c) Composite response of the 848 transcripts exhibiting a significant range:infection status interaction with $p < 0.01$. Each line represents a single transcript, with y-axis indicating the mean expression level within each group relative to the overall mean, in units of standard deviation.

SUPPORTING INFORMATION

See attached xlsx for sample metadata and functional enrichment results. Supplementary figures are included in attached doc. All code for analyses and plotting can be accessed at http://www.github.com/tepoltlab/RhithroLoxo_DE/.