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Population-level variation of digestive physiology costs of mounting an immune response in damselflies

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Abstract. 1. Trade-offs are often predicted to occur between energetically costly activities, such as somatic growth and eliciting immune responses to parasites. Although parasitism frequently reduces growth via lowered consumption, it remains unclear if the energetic demands of generating immune responses also affect the digestive physiological processes necessary for growth. Moreover, as local environmental conditions affect energetic investment towards growth and immune responses, the extent of any digestive–immune response trade-offs may vary among populations and not be fixed at the species-level.

2. To test these ideas, melanisation – a general innate immune response – was first induced in damselfly larvae (*Enallagma vesperum*) from two populations. The study then quantified growth and consumption rates, assimilation and production efficiencies, and daily metabolic rates to determine if digestive–immune response trade-offs were present and, if so, whether they differed between populations.

3. There was no evidence of any trade-offs between immune responses and digestive physiology components in either population. However, the results did show that populations differentially allocated energy towards different digestive physiology components after an immune response was elicited: one population increased their relative consumption and daily metabolic rates, while the other population had lower assimilation efficiencies and consumption rates.

4. Although researchers lack a mechanistic understanding of the observed population-level differences, these results suggest that accounting for population-level variation in digestive physiology and immune responses is critical to inferences about how immunological defences to parasitism may affect the ability for organisms to both acquire and utilise resources.

Key words. *Enallagma*, growth, immune response, metabolism, parasitism, trade-off.

Introduction

The acquisition and utilisation of resources via digestive physiological processes are foundational tasks for all organisms. Yet optimal energy allocation towards particular tasks is often unattainable due to the competing demands of energetically costly activities, such as somatic growth versus mounting immune responses to combat pathogens (Lima & Dill, 1990; Werner & Anholt, 1993; Freitak *et al.*, 2003). In order to circumvent potential trade-offs and prioritise specific energetically costly activities, organisms may undergo temporary behavioural changes such as reduced food consumption (illness-induced anorexia; Hart, 1988; Dunn *et al.*, 1994). For instance, reduced food consumption may allow organisms to devote energy stores to mounting an immune response (Weers & Ryan, 2006; Adamo, 2008; Adamo *et al.*, 2010), increase tolerance towards an infection (Ayres & Schneider, 2009), or be less exposed to predators while recovering from an infection or illness (Hart, 1988). While this change in feeding behaviour may increase survival rates of infected individuals (Murray & Murray, 1979; Ayres & Schneider, 2009; Adamo *et al.*, 2010), it may also have negligible effects on survival rates (Donegan & Lighthard, 1989) or even increase mortality rates if individuals are exposed to pathogens (Furlong & Groden, 2003). Although changes in

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food consumption when organisms are infected appears common, it remains unclear how activating immune responses may affect key digestive physiological processes (e.g. consumption, assimilation, production), and whether trade-offs between these physiological processes and immune responses exist.

If energetic trade-offs between digestive physiology and immune function do exist (digestive–immune trade-off), their magnitude may be influenced by local environmental conditions and ecological interactions that affect resource acquisition and utilisation. For instance, somatic growth frequently varies among populations because of negative density-dependence due to resource competition (McPeek, 1990, 1998; Stoks *et al.*, 2005; Siepielski *et al.*, 2011), interference competition (McPeek & Crowley, 1987; McPeek, 2004), or digestive physiological stress responses to crowding (McPeek *et al.*, 2001). Similarly, parasite infection rates also vary among populations because of differences in local ecological conditions (e.g. resource levels, parasite loads, predator densities). As a result, populations tend to allocate energy differentially towards disease resistance and immune responses at the expense of growth (density-dependent prophylaxis; Wilson & Reeson, 1998; Wilson *et al.*, 2002). While these studies highlight differences in somatic growth and immune function at the population level, most studies have examined digestive–immune trade-offs in single populations (Dunn *et al.*, 1994; Adamo *et al.*, 2010). Thus, addressing whether digestive physiology–immune trade-offs exist and vary between populations will increase our understanding of the influence of local environmental and ecological conditions on investments towards both digestive physiology and immune function.

Damselflies (Odonata: Zygoptera) are an ideal study system to examine whether digestive–immune trade-offs differ between populations. First, damselfly growth rates and physiological components vary between populations (McPeek, 1990, 1998; McPeek *et al.*, 2001; Siepielski *et al.*, 2016; Bried & Siepielski, 2019). Second, late-instar damselfly larvae are frequently attacked by ectoparasitic water mites (*Arrenurus* spp.; Smith, 1988; Corbet, 1999). Third, their resulting immune response, encapsulating an attacking ectoparasite's feeding tube via melanin, is well established (Ratcliffe *et al.*, 1985; Gillespie *et al.*, 1997; Siva-Jothy, 2000). In addition, the ability to generate this response should be condition-dependent because it requires an energetic investment that should vary with resource availability.

Here we test the predictions that: (i) a digestive–immune trade-off exists, such that mounting an immune response would decrease relative consumption rate, assimilation efficiency, and production efficiency; and (ii) energetic trade-offs between immune responses and digestive physiology would differ at the population level. To test these predictions, we induced immune responses in damselfly larvae from two different populations. We then conducted short-term growth trials in a controlled environment and quantified digestive physiology responses to determine whether digestive–immune trade-offs were present and if they varied between populations.

Materials and methods

Sample collection

We conducted immune assays and growth trials of *Enallagma vesperum* (Calvert, 1919) during December 2018. Sixty larvae were collected with a D-frame dipnet from each of two lakes in northwest Arkansas, U.S.A., Lake Fayetteville (henceforth population 1; lake surface area = 0.68 km^2; 36.1328, −94.1392, WGS84) and Lake Wilson (henceforth population 2; lake surface area = 0.11 km^2; 36.001, −94.1353, WGS84). Repeated sampling has shown that these populations have vastly different densities (population 1, *c*. 94/m^2; population 2, *c*. 6/m^2; A.M. Siepielski, unpublished; Ousterhout *et al.*, 2019). Both Lake Fayetteville and Lake Wilson contain predatory fish at densities of *c*. 9.7 m^{-2} and 3.1 m^{-2}, respectively (A.M. Siepielski, unpublished; Ousterhout *et al.*, 2019). Water quality assessments conducted in 2016 suggest that Lake Fayetteville is eutrophic and Lake Wilson is mesotrophic (NWQMC, 2017). Gene flow between these two populations of *E. vesperum* was unlikely to occur, as the inhabited lakes were geographically isolated (*c*. 14.7 km apart) and *Enallagma* species tend to disperse very short distances (< 1 km; McPeek, 1989). We used natural variation in larvae size at both lakes, and most individuals were probably between instars 7 and 10 given the sampling time.

All larvae were then housed in an environmental chamber maintained at 18 °C and LD 12:12 h. Each individual was placed in a 20-ml scintillation vial with aged, dechlorinated water and a 2-mm wooden dowel that served as a perch. The sides of each vial were covered in opaque tape to prevent exchange of visual cues between conspecifics, which may reduce their growth rates via stress responses (McPeek, 1998, 2004; McPeek *et al.*, 2001). Larvae were fed *Daphnia pulex* for 1 week *ad libitum* to allow for acclimation to the environmental chamber and feeding regime and to allow them to moult before the experiments began. Individuals were then starved for 24 h to standardise their hunger levels. The following day, we dried each larva by gently blotting them on absorbent tissue and measured their initial wet mass to the nearest 0.001 mg on a microbalance (XP6; Mettler Toledo, Columbus, Ohio). The initial mass of larvae from population 1 ($n = 60$, $\bar{x} = 10.90$ mg, SE = 0.51) and population 2 ($n = 60$, $\bar{x} = 10.83$ mg, SE = 0.51) were not significantly different from each another ($t_{106} = 0.10$, $P > 0.92$).

Immune responses

The generalised innate immune response in invertebrates recognises and encapsulates non-self tissue with melanin, which isolates infections and parasites from the host's tissue (Ratcliffe *et al.*, 1985). In turn, dermal cells darkened via melanisation may be used as a proxy to indicate the strength of an invertebrate immune response (González-Santoyo & Córdoba-Aguilar, 2012). We therefore used melanisation levels as a measure of the innate immune response to ectoparasitism, as done in many other studies with damselflies (González-Santoyo & Córdoba-Aguilar, 2012; Iloven & Suhonen, 2016).

We placed 20 individuals from each lake into three treatments: no parasitism (control), piercing of the exoskeleton with no

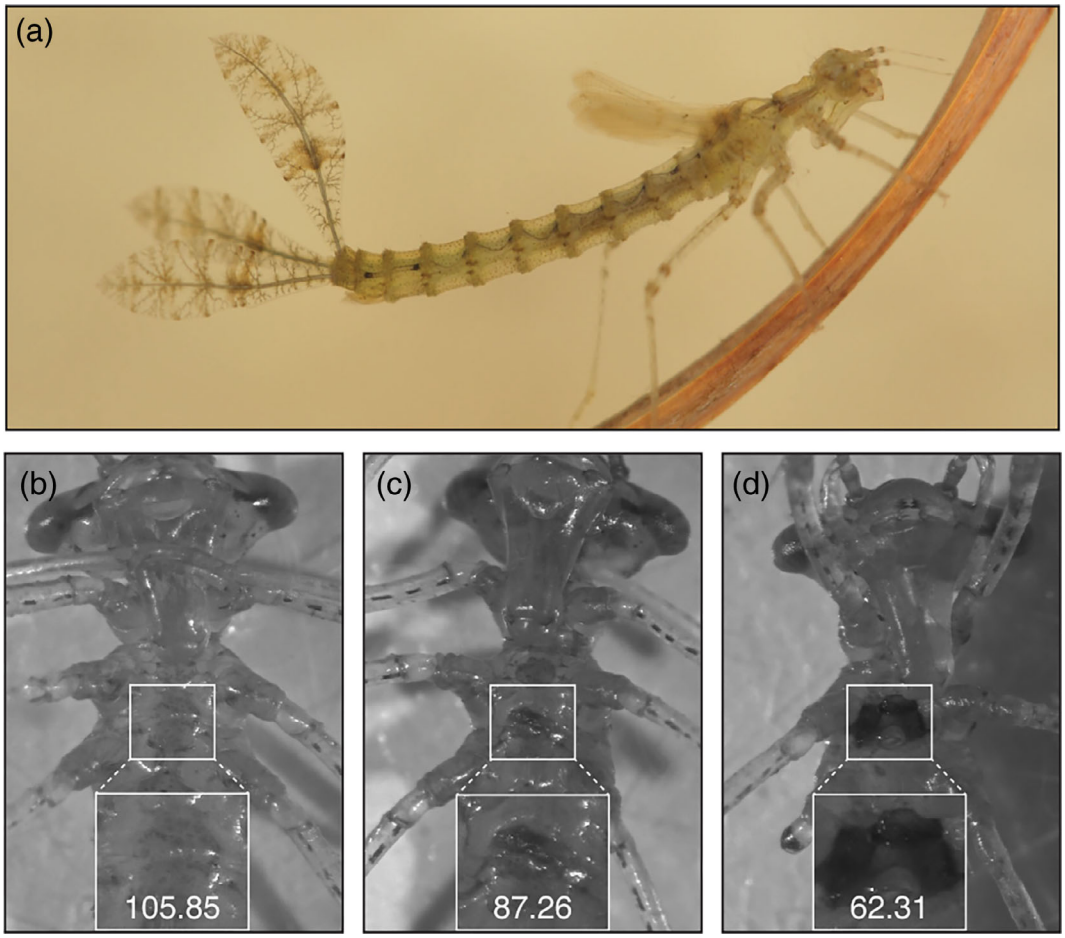

Fig. 1. (a–d) Photograph of the study organism, *Enallagma vesperum* (a) and examples of the three treatments: control (b), puncture (c), and implant (d). Insets show puncture/implant location, as well as mean grey values for each individual. Note that a stronger immune response means a lower grey value [cf. (b), (d)]. The photograph in (a) was kindly provided by Stephen R. Krotzer. [Colour figure can be viewed at wileyonlinelibrary.com].

implant (henceforth 'puncture'), and piercing of the exoskeleton and insertion of a monofilament implant (henceforth 'implant'; Fig. 1). As piercing the exoskeleton of numerous insect taxa induces an immune response (Wigby *et al.*, 2008; Ardia *et al.*, 2012), comparisons between the control and the puncture treatment isolate any injury effects, whereas comparisons between the puncture and implant treatment represent the possible effects of mounting an immune response on digestive physiology components.

For both the puncture and implant treatments, we used a 0.5-μl syringe (7000; Hamilton Company, Reno, Nevada) to penetrate the second abdominal pleura on the dorsal side of the sternal–tergal margin, which has been shown to generate a melanisation response in damselfly larvae (Iloven & Suhonen, 2016; Fig. 1). For this procedure, individuals were chilled on ice for *c*. 15 s to slow their movements, after which the syringe was inserted to similar depths to avoid confounding effects caused by differential abdominal and gut damage to individuals. For the implant treatment, we inserted one 1-mm-long nylon monofilament through the penetration point and into each body cavity. The syringe, monofilaments, tweezers, and Petri dishes were sterilised with 90% ethanol and dried before use. Larvae were then returned to separate scintillation vials in the environmental chamber for 24 h. The next day, the point of penetration for each individual was photographed at 10.0× magnification with a high-definition microscope camera (MC170 HD; Leica, Buffalo Grove, Illinois; Fig. 1). All photographs were sequentially taken via the same microscope and under the same lighting conditions. An unused monofilament was placed next to each individual to standardise grey values and melanisation levels. Images were saved in colour and converted to greyscale in PHOTOSHOP (Adobe, San Jose, California) to preserve both colour types. We used IMAGEJ v.2.0 to calculate the mean grey value of each individual to use an index for the amount of melanisation present. Grey values of 0 and 255 represent black and white, respectively, such that lower mean grey values imply increased melanisation levels and a stronger immune response was induced.

Digestive physiology

Once treatments were applied, short-term growth trials were then conducted over a 4-day period (McPeek, 2004). On the

first day, 35 *D. pulex* were offered to each individual to estimate their relative consumption rate. For the next 3 days, individuals were fed the quantity of *D. pulex* eaten the previous day and at least two additional prey items to account for daily variation in consumption rate. Similar growth rate estimates of damselfly larvae have been obtained from field and laboratory experiments using this method (McPeek, 1998). Each morning, we recorded the number of uneaten *D. pulex* and collected faeces from each vial, which were then stored in separate 5-ml microcentrifuge tubes. After 4 days of regimented feeding, we removed all *D. pulex* from the vials and waited 24 h to allow damselfly digestive processes to complete (Johnson *et al.*, 1975). The following day, we collected the final faecal samples and obtained the final wet mass of each individual by blotting each dry on absorbent tissue and measuring their mass to the nearest 0.001 mg on a microbalance. Faecal pellets were dried at 60°C for at least 24 h, after which they were weighed to the nearest 0.001 mg on a microbalance. *Daphnia pulex* were reared in tanks housed within a greenhouse. All *D. pulex* were size-standardised by sequentially passing them through mesh sieves with perforations between 0.84 and 1.00 mm.

We quantified five aspects of digestive physiology (relative growth rate, relative consumption rate, assimilation efficiency, production efficiency, and average daily metabolic rate) based on equations in Culler *et al.* (2014). Relative growth rates represent daily changes of an individual's dry mass with respect to body size, and were calculated as:

$$[(\text{Final dry mass} - \text{initial dry mass})/(4\,\text{days} \times W_e^m)]$$

where W_e was the exponential mean dry mass (Gordon, 1968; Ayres & Scriber, 1994) and m was an allometric scaling factor that related to body size ($m = -0.33$; Niven & Scharlemann, 2005). We calculated W_e by obtaining the wet masses of 55 damselfly larvae, that were not used in the immune assays and growth trials, on a microbalance and then drying these individuals at 60°C for at least 24 h to obtain their dry masses on a microbalance. We then performed a linear regression to determine the ratio between dry and wet masses ($r^2 = 0.94$).

Relative consumption rates describe the amount of food consumed relative to the amount of food available for consumption on a daily basis, and were calculated as:

$$[(\text{Total dry mass of } \textit{Daphnia} \text{ given}) - \text{total dry mass of all uneaten } \textit{Daphnia})/(4\,\text{days} \times W_e^m)]$$

Assimilation efficiencies represent the proportion of food that was consumed and not excreted, and were calculated as:

$$[(\text{Total dry mass of } \textit{Daphnia} \text{ consumed} - \text{total dry mass of faecal pellets})/\text{total dry mass of } \textit{Daphnia} \text{ consumed}]$$

Production efficiencies detail gains in biomass relative to the amount of food that was consumed and not excreted, and were calculated as:

$$[(\text{Final dry mass} - \text{initial dry mass})/(\text{total dry mass of } \textit{Daphnia} \text{ consumed} - \text{total dry mass of fecal pellets})]$$

Lastly, average daily metabolic rates describe the amount of consumed food that was available for cellular growth and maintenance but not directly converted into biomass (Gordon, 1968; Ayres & Scriber, 1994), and were calculated as (Culler *et al.*, 2014):

$$[(\text{Relative consumption rate} \times \text{assimilation efficiency}) - \text{relative growth rate}]$$

Statistical analysis

We used simple correlation analyses to examine if trade-offs between digestive physiology and immune function existed in either population. If immune–digestive trade-offs were present, increased melanisation should correlate with reductions in digestive physiology components.

To determine if there were any differences in digestive physiology attributes of uninjured individuals (e.g. control treatments) between populations, we used a two-sample *t*-test (assuming equal variance) to compare the means of each digestive physiology measure. We then used general linear models with a Gaussian error distribution and an identity link function to compare the effects of treatments (control, puncture, and implant) and lake (i.e. different population) on digestive physiology. Each model included terms for treatment, lake, and their interaction, with each digestive physiology component as the response. Interaction terms were removed if not statistically significant ($P < 0.05$). Pairwise comparisons among treatments were made with Tukey's honestly significant difference (HSD).

Data accessibility

Data are available from the Dryad Digital Repository (Tye *et al.*, 2019).

Results

Collectively, we found no evidence of trade-offs between digestive physiology and immune responses in either population (Fig. 2). All correlation coefficients were extremely low and variable in sign among digestive physiology components and immune responses, as well as between populations (all $r < 0.4$, mean $r = -0.026$). The only statistically significant correlation was between assimilation efficiency and the extent of melanisation in population 2 (Fig. 2), but this was a positive and relatively weak association ($r = 0.33$).

While mounting a stronger immune response did not reduce any of the digestive physiology components that we measured, the puncture and implant treatments did induce an immune response as measured by the extent of melanisation ($F_{2,104} = 19.009$, $P < 0.0001$; Fig. 3a). Melanisation levels were 7% higher in the implant treatments ($n = 37$, $\bar{x} = 79.983$, SE = 1.687) than in the control treatments ($n = 36$, $\bar{x} = 92.760$, SE = 1.179; Tukey's HSD, $P < 0.0001$). Moreover, there were no statistically significant differences between melanisation levels of control ($n = 36$, $\bar{x} = 92.760$, SE = 1.787) and puncture

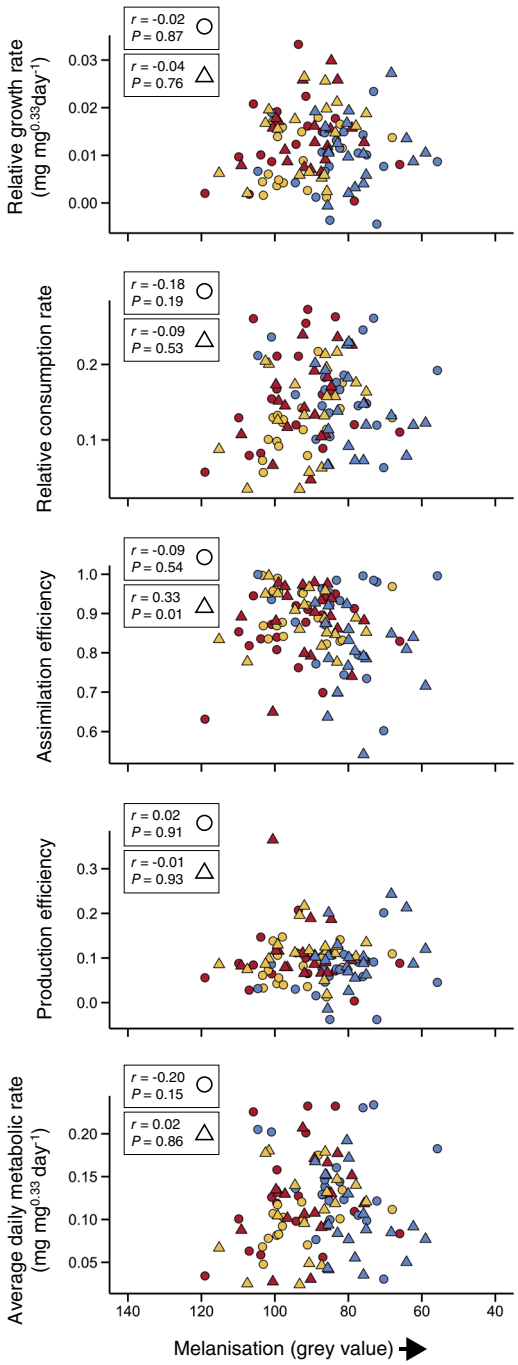

Fig. 2. Associations between digestive physiology and extent of melanisation of *Enallagma vesperum* from two populations: Lake Fayetteville (population 1; circles, $n = 53$) and Lake Wilson (population 2; triangles, $n = 55$). Colours correspond to treatments: red, individuals exposed to no immune challenge (control); yellow, exoskeleton punctured with a syringe (puncture); blue, monofilament inserted into body cavity (implant). Note that lower mean grey values indicate higher melanisation levels and stronger immune responses. Neither population showed any signs of trade-offs between digestive physiology components and immune responses. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. General linear models summarising the effects of immune challenge treatments and lake (i.e. different population) for digestive physiology measurements in *Enallagma vesperum*. Significant values are in bold.

Measure	*F*	*P*
Relative growth rate		
Treatment	2.136	0.123
Lake	3.304	0.071
Relative consumption rate		
Treatment	1.864	0.16
Lake	0.6559	0.418
Assimilation efficiency		
Treatment	1.859	0.161
Lake	2.095	0.15
Treatment × lake	**5.08**	**0.007**
Production efficiency		
Treatment	1.357	0.262
Lake	**10.046**	**0.002**
Average daily metabolic rate		
Treatment	**3.397**	**0.0372**
Lake	0.138	0.71
Treatment × lake	**3.175**	**0.0459**

treatments ($n = 37$, $\bar{x} = 92.355$, SE = 1.666; Tukey's HSD, $P = 0.981$). There was, however, a lake effect on melanisation levels ($F_{1, 104} = 3.665$, $P = 0.058$), which indicated that the melanisation response was *c*. 2% higher in population 2 than in population 1 (Fig. 3a).

We found no differences in relative growth rate ($t_{34} = -0.599$, $P = 0.552$), relative consumption rate ($t_{34} = 0.538$, $P = 0.593$), assimilation efficiency ($t_{34} = -1.568$, $P = 0.126$), production efficiency ($t_{34} = -1.34$, $P = 0.188$), and average daily metabolic rate ($t_{34} = 0.344$, $P = 0.739$) between control treatments from each population (Fig. 3). However, population-level differences in some digestive physiology components were apparent across treatments, even though there were no detectable effects of treatments within populations. Individuals from population 1 tended to have lower relative growth rates than individuals from the population 2 across treatments (Table 1; Fig. 3b), even though there were no significant differences in relative consumption rates across lakes or treatments, or their interactions (Table 1; Fig. 3c).

For assimilation efficiencies, there was a treatment × lake interaction; assimilation efficiencies varied with treatment in population 2 ($F_{2,52} = 6.28$, $P = 0.003$; Table 1), but not in population 1 ($F_{2,52} = 1.855$, $P = 0.167$; Fig. 3d). Assimilation efficiencies in population 2 were 10.4% higher in the control treatment ($n = 19$, $\bar{x} = 0.891$, SE = 0.021; Tukey's HSD, $P = 0.007$; Fig. 3d) and 9.7% higher in the puncture treatment ($n = 18$, $\bar{x} = 0.884$, SE = 0.064; Tukey's HSD, $P = 0.012$) than in the implant treatment ($n = 18$, $\bar{x} = 0.798$, SE = 0.101). Assimilation efficiencies in population 1 were 5.4% lower in the control treatment ($n = 17$, $\bar{x} = 0.845$, SE = 0.021; Tukey's HSD, $P = 0.369$; Fig. 3d) and 1.3% higher in the puncture treatment ($n = 17$, $\bar{x} = 0.903$, SE = 0.061; Tukey's HSD, $P = 0.843$) than in the implant treatment ($n = 19$, $\bar{x} = 0.891$, SE = 0.112). Production efficiencies of population 1 were 30.3% lower than those

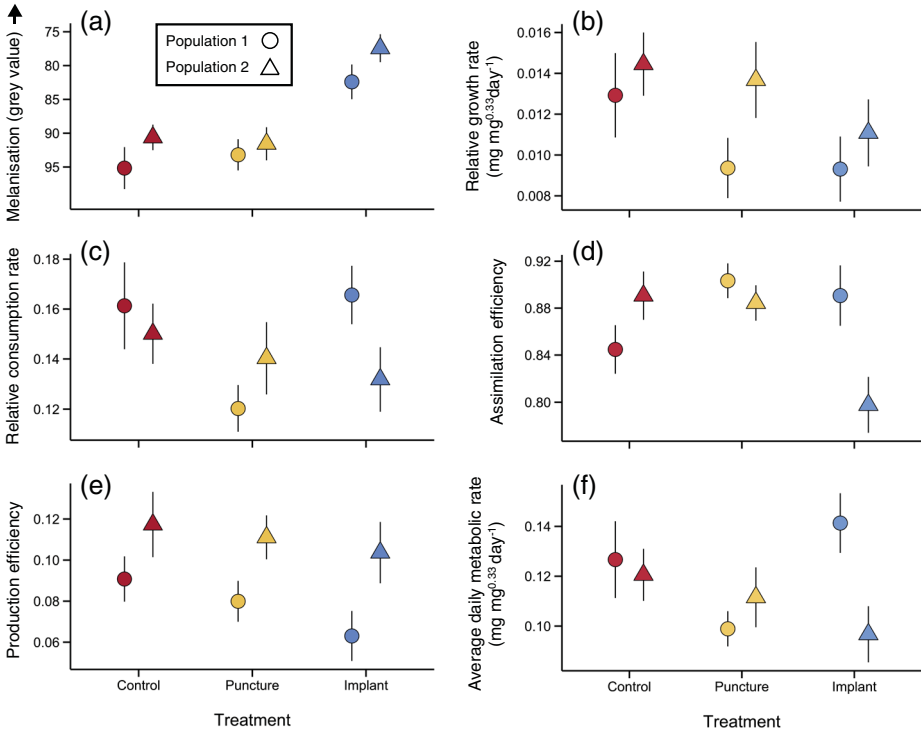

Fig. 3. (a–f) Extent of melanisation (a) and digestive physiology measurements (b–f) of *Enallagma vesperum* from two populations: Lake Fayetteville (population 1; circles) and Lake Wilson (population 2; triangles). Colours correspond to treatments: red, control; yellow, puncture; and blue, implant. Symbols and error bars represent mean values and ± SEM of each measurement, respectively. Note that lower mean grey values indicate higher melanisation levels and stronger immune responses. Several individuals died when exposed to each treatment (control, puncture, implant), such that there were 17, 19, and 17 individuals from population 1, and 19, 18, and 18 individuals from population 2, respectively. [Colour figure can be viewed at wileyonlinelibrary.com].

of population 2 across treatments, but they did not vary by treatment (Fig. 3e).

Average daily metabolic rates did, however, vary among treatments (Fig. 3f), and there was a significant treatment × lake effect, indicating that treatment effects were population-specific (Table 1). There were no treatment effects for average daily metabolic rate in population 2 ($F_{2,52} = 1.152$, $P = 0.323$), but there was a treatment effect in the population 1 ($F_{2,50} = 3.24$, $P = 0.047$; Fig. 2). This treatment effect was due to metabolic rates of population 1 being 29.8% higher in the implant treatment than in the puncture treatment (Tukey HSD, $P = 0.039$; Fig. 3f), indicating that mounting an immune response elevated metabolic rates. There were no differences in average daily metabolic rates between the control and the puncture treatments (Tukey's HSD, $P = 0.253$) or between the control and implant treatments (Tukey's HSD, $P = 0.661$) in population 1.

Discussion

Trade-offs between somatic growth, digestive physiology, and defence mechanisms are predicted to exist in many taxa (Lima & Dill, 1990). Yet we found no support for the prediction of a digestive–immune trade-off in either damselfly population. This lack of support was surprising given that we successfully induced immune responses, our estimated values of digestive physiology components corroborate studies with similar methodologies (McPeek *et al.*, 2001; McPeek, 2004; Culler *et al.*, 2014), and we found no intrinsic differences in digestive physiology components between control treatments of each population. Instead, population-specific differences in digestive components only became apparent when immune responses were induced, which supported our predictions that immune responses decrease some digestive physiology processes and that these decreases vary between populations.

Our estimates of digestive physiology components should reflect high condition responses because the larvae were physically and visually isolated, provided food *ad libitum*, and reared in water without predator kairomones. While this approach isolated the physiological costs of immune responses from the negative effects of parasitism that could occur in the wild, the resulting high condition responses may also have prevented us from observing any digestive–immune trade-offs if they were condition-dependent. As damselfly populations typically do not achieve optimum levels of resource acquisition and somatic growth due to exploitative and interference competition (McPeek & Crowley, 1987; Anholt, 1997; McPeek, 1998), feeding individuals food *ad libitum* may have prevented the emergence of digestive–immune trade-offs. Even so, our data show that digestive physiological components and immune responses are not fixed at the species level and vary greatly both between and within populations.

Our lack of support for a digestive–immune trade-off may have also been due to the considerable intraspecific variation in digestive physiological components and immune responses that we observed. For example, intraspecific variation of resting metabolic rates is common (Burton *et al.*, 2011) and may be influenced by local environmental conditions (Le Lann *et al.*, 2010). In addition, digestive morphology may vary within populations due to the availability and nutrient composition of prey items (Sassi *et al.*, 2007), as well as the intensity of competitive interactions within an environment (Relyea & Auld, 2004). McPeek *et al.* (2001) suggested that damselfly species inhabiting food-limited environments may have larger foreguts to consume more prey when or if they become available. Thus, population-level variation in digestive morphology (e.g. larger foregut) may also have influenced our estimates of digestive physiology components, particularly relative consumption and average daily metabolic rates. Lastly, our implantation methods may have caused differential gut and abdominal damage, which may generate changes in host microbiome environment that affect digestive physiology components and immune function (Perrin & Sibly, 1993; Wong *et al.*, 2014).

The strength of an immune response (i.e. melanisation level) may also greatly vary within insect populations (Smilanich *et al.*, 2009). For instance, the abundance of phenoloxidase, an enyzme that helps to facilitate the recognition of non-self tissue (Brey & Hultmark, 1997) and melanin production (Shiao *et al.*, 2001), may increase when individuals are exposed to predators (Joop & Rolff, 2004). Predator exposure may be especially applicable to digestive–immune trade-offs in damselfly populations, as conspecifics are perceived as predators and their presence can generate a strong stress response that affects growth rates (McPeek, 1998, 2004; McPeek *et al.*, 2001). Thus, as the population densities of damselflies we used varied greatly (A.M. Siepielski, unpublished; Ousterhout *et al.*, 2019), latent effects of natural population densities may have affected our estimated melanisation levels. Lastly, single and repeated implantations of nylon microfilaments in invertebrates may result in different melanisation responses and survival rates (Krams *et al.*, 2011). Thus, even though we generated an immune response, our single implantation method may not have accurately represented the numerous ectoparasitic attacks that damselfly larvae probably experience in the wild. While these factors may help to explain the high levels of intraspecific variation in both digestive physiology and immune responses we observed, what maintains such variation is an intriguing question.

Notably, both populations experienced incremental declines in relative growth rate and production efficiency across treatments, suggesting that both populations allocated fewer resources to somatic growth with increasing levels of activated immunity. This implies that some trade-offs between growth physiology and immune responses may be present but they are only manifested by considering population-level mean responses, not differences at the individual level – a result that further underscores the need for population-level comparisons. However, incongruent trends between other digestive physiology components and the magnitude of immune response at the population-level might reflect the effects of local environmental conditions or ecological interactions.

Differences in population density are one possible ecological condition that could account for some of these patterns, because populations 1 and 2 have high and low densities of *E. vesperum*, respectively (A. M. Siepielski, unpublished; Ousterhout *et al.*, 2019). Thus, our observation of slightly higher melanisation values across treatments of population 1 (Fig. 3a) may be indicative of density-dependent prophylaxis, which has been documented for high-density populations of beetles, locusts, and moths (Wilson & Reeson, 1998; Barnes & Siva-Jothy, 2000; Wilson *et al.*, 2001, 2002). Comparatively, population 2 experienced a sharp decline in assimilation efficiency under the implant treatment. Lowered assimilation efficiencies correlate with higher net growth efficiencies in aquatic consumers (Welch, 1968), which suggests that a greater proportion of energy from ingested food was allocated towards somatic growth when an immune response was induced in this population. However, we cannot attribute any of the observed differences in digestive physiology and/or immune responses to population density alone, because with only two populations it is not possible to disentangle the possible effects of density from the many other factors that could vary between lakes.

Although we did not detect consistent individual-level trade-offs between digestive physiology components and immune responses, our observations do suggest disproportional prioritisation of energy allocation towards growth, development, and immune function between damselfly populations. Moreover, they provide insights into the multiple life-history strategies that may be employed by a single species. While we did not identify the environmental conditions or ecological interactions responsible for the observed population-level differences in digestive physiology and immune responses, future studies on digestive–immune trade-offs should develop such a mechanistic understanding. Doing so may help to determine spatial shifts in investment in immune responses and digestive physiology, as well as providing insights into how organisms engage in the numerous energetically costly activities that arise within complex communities.

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Author contributions

AMS conceived the ideas; SPT and BKB collected the data; AMS and SPT analysed the data; SPT and AMS led the writing of the manuscript.

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