Increased activity and growth rate in the non-dispersive aquatic larval stage of a damselfly at an expanding range edge

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SUMMARY

1. While evolutionary changes in adult traits during range expansion have been recorded in many species, similar changes in the non-dispersive larval stage have only rarely been documented. Increased activity in the non-dispersive larval stage is an important ecologically relevant trait in aquatic communities that may be expected to evolve in the edge populations (i) as a result of the combination of spatial sorting in dispersal-related adult activity and a coupling between adult and larval behaviour and (ii) to meet higher energy demands to allow higher growth rates and a higher investment in costly dispersal-related traits.

2. We specifically address whether activity is higher in the larval non-dispersive aquatic stage at an expanding range front by comparing larvae of replicated core and edge populations of the damselfly Coenagrion scitulum in three common garden experiments where larvae were reared from the egg stage.

3. As expected, activity in the non-dispersive larval stage was consistently higher in the edge populations. Although changes in larval activity probably have consequences for ecological interactions, the higher activity was not associated with increased predation rates by dragonfly larvae, potentially because of associated compensatory changes in other antipredator mechanisms.

4. We documented one of the few cases of a positive coupling of activity in the larval and adult stages. Yet, contrary to larval activity, adult activity did not differ between core and edge populations. This indicates that the higher larval activity we documented is not shaped by a coupling with adult activity. Instead, our results are consistent with the hypothesis that a higher energy need in edge populations shaped the higher larval activity. Edge larvae showed a higher growth rate which is expected to evolve at the initial low population densities in newly founded edge populations. Moreover, higher growth rate showed the expected positive covariation with larval activity.

5. Increases in activity in the non-dispersive stage in edge populations at an expansion front should be included in the ongoing debate whether evolutionary changes at invasion fronts are driven by adaptive versus non-adaptive evolution. Moreover, they may have the potential to affect ecological interactions at expanding range fronts.

Keywords: behavioural coupling, global change, life-history evolution, Odonata, range expansion

Introduction

Many species ranges are currently shifting polewards triggered by climate change (Hickling et al., 2006; Thomas, 2010; Chen et al., 2011). The majority of these species have a complex life cycle with a non-dispersive larval stage where growth occurs and a dispersive adult stage where reproduction occurs (Moran, 1994; Stoks &
Coenagrion scitulum (Rambur, 1842) is a small Mediterranean damselfly preferring small ponds (Dijkstra, 2006). The historical northern range limit is situated in northern France and since the 1990s a north-eastward range expansion occurred (Wasscher & Goudsmits, 2010). The expansion front was situated in the north-west of the Netherlands and in Western Germany in 2010. We studied two core populations [Rosnay (C1) and Merlimont (C2), France] within the historical core distribution of the species, and four edge populations [Webenheim, Germany (E1), Zülpich, Germany (E2), Hoofdplaat, the Netherlands (E3) and Cadzand, the Netherlands (E4) situated at the expansion front and founded <4 years before sampling (Fig. 1). Characteristics of the breeding pond (pond size, predator regime and larval temperature regime) of each population are given in Appendix S1.

Offspring from females collected in populations C1, C2, E2 and E4 were used in the first experiment, while offspring from females collected in populations C1, C2, E1 and E3 were used in the second and third experiments. Paired females were caught during June–July (2010: experiment 1, 2011: experiment 2, 2012: experiment 3) and allowed to lay eggs in oviposition chambers with wet filter paper. Eggs were transported to Belgium, where eggs and larvae were kept at a constant temperature (experiment 1: 20 °C, experiment 2 and 3: 22 °C) and a 14-h light–10-h dark photoperiod throughout the experiments. Larvae were placed individually in 100-mL plastic rearing cups filled with aged tap water and fed ad libitum with brine shrimp nauplii 6 days a week.
We set up three independent common garden experiments where animals were reared from the egg stage to test for a higher activity in edge larvae compared with core larvae. Each experiment additionally tested an extra hypothesis. In the first experiment, we also tested for activity-mediated higher larval growth rate in edge larvae by scoring growth rate and activity during the growth period. Larval activity of 125 individuals (C1: 34, C2: 31, E2: 30, E4: 30) originating from 12 different females (C1: 2, C2: 4, E2: 4, E4: 2) was scored when larvae were 50 days old. Due to some mortality, growth rate was scored on the 113 individuals that survived until the penultimate larval stage. Larva needed on average 155.9/2.4 days (mean /SE) to reach the penultimate larval stage. In the second experiment, we also tested for an activity-mediated higher vulnerability to predation in edge larvae. Therefore, we quantified the activity of each larva in the absence and in the presence of cues from larval dragonfly predators and subsequently monitored survival time in the presence of these predators. As predators, we used large Anax imperator dragonfly larvae, important visual predators of coenagrionid damselfly larvae (Stoks, De Block & McPeek, 2005b), that occur in each of the four study populations (Appendix S1). All response variables were scored on forty larvae per population (total of 160 larvae) when they were 100 days old. We used offspring of all available mothers per population (C1: 39, C2: 24, E1: 25, E3: 25), resulting in 1–2 larvae scored per mother. In the third experiment, we additionally tested for a positive covariation of individual activity between the larval and the adult stages. Larval activity of 93 individuals (C1: 34, C2: 27, E1: 7, E3: 25) obtained from 49 different females (C1: 21, C2: 9, E1: 4, E3: 15) was scored. The number of scored adults (n = 77) was lower than the number of scored larvae due to the exclusion of animals that did not emerge successfully.

Experiment 1: Activity and growth rates

We scored larval activity when the larvae were 50 days old and closely followed the protocols by Stoks (1998) and Johansson et al. (2001). To equalise hunger levels, damselfly larvae were deprived of food 24 h before the start of the activity trials. Each larva was placed separately in a container (17 × 10.5 × 11.5 cm, filled to a height of 2 cm with aged tap water) with a grid (0.5 × 0.5 cm squares) at the bottom. After a 10-min acclimation period, the position of each larva was recorded every 15 min during 3 h. From these positions, we calculated the activity score: the number of position changes in a larva ranging from 0 (no position changes) to 12. A position change was defined as the displacement of the centre of the head to another square of the grid. To correct for potential effects of larval size on activity, we measured the head width of each damselfly larva using a binocular linked to an image analyser. Larval head width is an often-used proxy for larval size in Odonata larvae (e.g. Brodin & Johansson, 2004; Mikolajewski et al., 2005). Afterwards, larvae were placed back in their rearing cups and further reared until the penultimate larval stage, and larval head width was measured for the second time when larvae entered the penultimate larval stage. Growth rate was calculated as \( \ln(\text{head width in the penultimate larval stage})/\text{time between egg hatching and entering the penultimate larval stage.} \)

Experiment 2: Activity and vulnerability to predation

We scored larval activity when larvae were 100 days old using the method described in experiment 1. In this experiment, we recorded the position change in each larva every 10 min during 2 h. We chose a shorter time interval between subsequent recordings of positions compared with experiment 1 as larvae were older, hence larger and more active. Each larva was tested twice on the same day, once at 9 am and once at 12 am. Half of the larvae per population were tested first in the absence of predator cues, the other half with predator cues present. Predator cues were applied by adding 5 mL of...
medium in which an *A. imperator* dragonfly larva was kept for 2 weeks. Damselfly larvae are expected to reduce activity in response to the presence of chemical dragonfly predator cues to reduce detection by the predator (Brodin, Mikolajewski & Johansson, 2006; Mortensen & Richardson, 2008). The dragonfly larvae were fed chironomid larvae throughout the experiment. To correct for potential effects of larval size on activity, we measured the head width of each damselfly larva.

We determined vulnerability to predation following an adapted protocol of Janssens and Stoks (2012). One hour after the last activity trial, individual larvae were placed in a container (10 × 10 × 6 cm, filled with 3 cm of aged tap water) and allowed to acclimate for 10 min. Then, one penultimate instar larval *A. imperator* predator was introduced in the container and faced to the corner opposite to the *C. scitulum* larva. The containers were observed continuously, and a trial ended when the *C. scitulum* larva was caught by the *A. imperator* larva. Survival times longer than 120 min were considered right-censored. We used 25 predator individuals in the experiment. Predators were not fed 24 h before predation trials, and when used several times, the interval between trials was at least 1 day. To be able to correct for predator size on prey survival time, we measured the predator’s head width.

**Experiment 3: Activity across metamorphosis**

In experiment 3, we first scored larval activity levels in the final larval stage in the absence of predator cues, using the method described in experiment 2. Afterwards, the sex of the larvae was determined. Wet mass was determined by first blotting the larvae dry on an absorbent tissue, then weighting the larvae on a balance that had an accuracy of 0.01 mg (McPeek, Grace & Richardson, 2001). Larvae were placed back in their rearing cup and further reared until adult emergence. In the final larval stage, animals were fed three chironomid larvae in addition to their regular diet to meet the higher food requirements. Adult activity was scored 24 h after emergence with an analogue method as used to score larval activity. Each adult was placed separately in a plastic aquarium (32 × 18 × 16 cm) containing a diagonally placed wooden willow branch for perching. Grids (2 × 2 cm) on the bottom and side walls of the aquarium allowed three-dimensional position recording. Animals were acclimatized in their aquarium for 4 h before activity trials began. The position of each adult was recorded every 15 min during 3 h. Adult activity scores were calculated as the number of position changes during the 3 h and ranged from 0 (no position changes) to 12. A position change was defined as the displacement of the head to another square of the grid. Short flights were often observed during the activity test, despite the relative small aquarium size. Short flights, as observed in the experiment, are probably ecologically relevant as a higher number of short flights per unit of time were associated with higher mass gain in immature individuals of *Lestes sponsa* under seminatural conditions in a large outdoor insectary (Stoks, 2001a). However, we admit that scoring adult behavioural data in an aquarium is artificial compared with field observations.

**Statistics**

In the first experiment, the effect of population status (core versus edge) on log-transformed larval activity and growth rate was tested using AN(C)OVAs. The larval head width was added as covariate when testing for larval activity. Population nested in population status and female nested in population were added as random factors. To evaluate the positive covariation pattern between larval activity and larval growth rate, we added larval activity as covariate to the ANCOVA testing for growth rate and tested its effect one-sided.

In the second experiment, we tested for the effect of population status, predator cues and larval head width on larval activity using a repeated-measures ANCOVA with the log-transformed activity level of the same larva in the absence and in the presence of predator cues as repeats (hence with predation risk as within-class variable). Population nested in population status and testing order (first in the absence or first in the presence of predator cues) were added as random factors. Note that as larvae were tested at day 100, they were too small to be sexed. We tested the effects of population status on survival time using a Cox regression in proc PHREG of SAS v9.4 (SAS Institute, Cary, NC, U.S.A.). Population was nested in population status. As continuous covariates, we included larval head width and predator head width. Subsequently, we tested the effect of activity on survival time by adding larval activity (in the presence of predator cues) to the Cox regression.

In the third experiment, the effects of population status and sex on larval and adult activity were tested using ANCOVAs with log-transformed activity level. Population nested in population status was added as random factor and individual mass as a covariate. To specifically evaluate the presence of a positive covariation pattern between adult and larval activity levels, we added log-transformed larval activity and larval body
mass as covariates to the ANCOVA of adult activity. The one-tailed \( P \)-value is reported for the covariation between adult and larval activities given the \textit{a priori} unidirectional prediction (Rice & Gaines, 1994) of a positive covariation (Brodin, 2009).

Significance of random factors was verified with likelihood ratio tests (Littell \textit{et al.}, 1996). Random factors were removed from the final analysis when not significant. All tests were performed in SAS v9.4.

Results

Experiment 1: Activity and growth rates

Larval activity was c. 31\% higher in edge than in core populations (Table 1a, Fig. 2a), and head width had no effect on the activity level (Table 1a). Larval growth rate was higher in edge populations (Table 1b–c, Fig. 2b) and covaried positively with larval activity during the growth period (Table 1c, Fig. 2c).

Experiment 2: Activity and vulnerability to predation

Larvae of edge populations made c. 70\% more position changes than larvae of core populations (Table 2a, Fig. 3a). The presence of predator cues did not affect activity level (Table 2a). Neither head width nor any of the pairwise interactions between the independent variables were significant (Table 2a).

Survival time in the presence of the predator was on average marginally non-significantly shorter in edge populations (\( P = 0.052 \), Table 2b). This pattern was, however, driven by only one of the edge populations (E2), while the other edge population (E1) had the longest survival time (population nested in population status: \( P = 0.023 \), Table 2b, Fig. 3b). Larger larvae had higher mortality rates compared with smaller larvae (estimate of the slope for larval head width: 1.03 ± 0.45, Table 2b). Predator head width and larval activity level did not influence survival time, nor were there pairwise interactions between the independent variables (Table 2b).

Experiment 3: Activity across metamorphosis

Although not significant (\( P = 0.14 \), Table 3a), larval activity was c. 13\% higher in edge than in core populations (Fig. 4a). Larval activity level did not depend upon sex or larval body mass. None of the pairwise interactions between the independent variables were significant (Table 3a).

Table 1 ANCOVAs testing for (a) the effects of population status (core versus edge) and larval head width on larval activity; (b) the effect of population status on larval growth rate; and (c) testing the covariation between larval growth rate and larval activity of \textit{Coenagrion scitulum} in experiment 1. A one-tailed \( P \)-value is reported for the covariation between larval activity and growth rate given the \textit{a priori} unidirectional prediction.

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<th></th>
<th>d.f.</th>
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<tbody>
<tr>
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Fig. 2 Mean (with 1 SE) activity level (a), growth rate (b) and their positive covariation pattern (c) in two core (grey) and two edge populations (white) of \textit{Coenagrion scitulum} in experiment 1. C1 and C2 are core populations, E2 and E4 are edge populations.

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Core and edge populations did not differ in adult activity (Table 3b, Fig. 4b), and adult activity did not differ between females and males (Table 3b). Adult body mass had no effect on adult activity and none of the pairwise interactions between the independent variables were significant. Larval activity covaried positively with adult activity (Table 3c, Fig. 4c).

Activity pattern across experiments
Combining the $P$-values of the three independent experiments testing the same hypothesis of a higher larval activity in edge larvae compared with core larvae (see Sokal & Rohlf, 1995, p. 794) resulted in a combined $P$-value of 0.0034 ($\chi^2 = 19.51$, d.f. = 6).

Discussion
As expected, we found a consistent higher activity in larvae from the edge populations at the expanding range front compared with larvae from the core populations. Given logistic constraints, we could only sample eggs from two core and two edge populations per experiment, yet across the three experiments, we used in total two core and four edge populations. The repeatable pattern here presented across populations, over larval ontogeny and across years suggests that the documented pattern of higher activity levels in edge populations is widespread in this species. The generality of the higher larval activity at the expansion front in this species is further supported by a recent mesocosm experiment showing a higher larval foraging activity in one edge population compared with two core populations and where one of the core populations and the edge population were not included in current experiment (L. Therry, unpubl. data). Moreover, core and edge populations did not consistently differ in the presence of important predators ($A$. imperator and fish), abundance of submerged vegetation or larval temperature regime (Table S1, Appendix S1). Temperature and
predator regime are key factors shaping activity and growth in Coenagrion damselfly larvae (Stoks & Johansson, 2000; Brodin & Johansson, 2004; Van Doorslaer & Stoks, 2005; Nilsson-Ortman et al., 2012) and the absence of consistent differences in these factors between core and edge populations suggests that chance local influences unlikely were driving the observed differences in activity and growth rate.

Instead, larval activity probably evolved in response to the process of range expansion. A genetic study based on microsatellite markers indicated that a major mechanism of range expansion in this species is through a stepping-stone colonisation pattern at the expansion front (J. Swaegers, unpubl. data). Yet, long-distance dispersal also plays a role as suggested by new populations at the expansion front at a large distance from the nearest populations (e.g. Weihrauch et al., 2011). The key processes of spatial sorting in dispersal ability and a shift towards higher r-selection (Phillips, Brown & Shine, 2010) are expected to take place under both a stepping-stone and a long-distance colonisation pattern. The study species shows meta-population dynamics with frequent local extinctions and recolonisations (Male-Malherbe, 2010). If we by chance would have selected recently recolonised core populations, these core populations may then also have had more active larvae compared with the other core populations (McCauley, Brodin & Hammond, 2010), making the finding of even higher larval activity in the edge populations conservative. The documented pattern in larval activity probably reflects genetic differences as larvae were reared from the egg stage under identical laboratory conditions, although we cannot exclude the possibility of a contribution of maternal effects (see also McCauley et al., 2010).

### Table 3
ANCOVAs testing for the effects of population status (core versus edge), sex and body mass on (a) larval activity and (b) adult activity and (c) testing the covariation between adult and larval activity of Coenagrion scitulum in experiment 3. A one-tailed $P$-value is reported for the covariation between larval and adult activities given the *a priori* unidirectional prediction.

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**Fig. 4** Mean (with 1 SE) activity levels in the larval stage (a) and in the adult stage (b), and their positive covariation pattern (c) in two core (grey) and two edge populations (white) of Coenagrion scitulum in experiment 3. C1 and C2 are core populations, E2 and E3 are edge populations.
minor role in shaping life-history traits associated with activity (Strobbe & Stoks, 2004; Shama et al., 2011). Whatever the nature of these activity differences, this is the first demonstration that behaviour in the larval nondispersive stage can differ predictably between edge and core populations in a species with an expanding range margin.

In contrast with previous studies in damselfly larvae (e.g. Stoks et al., 2005a), including those using Coenagrion prey and dragonfly predators (Brodin & Johansson, 2004), larvae and populations with a higher larval activity did not suffer a higher predation rate nor did larvae reduce activity in response to predator cues (for another example, see Wohlfahrt et al., 2006). The predator used co-occurs with C. scitulum in all studied populations, and antipredator responses are especially expected in response to cues of familiar predators (Wohlfahrt et al., 2006). While antipredator responses might have been stronger in response to chemical cues from predators fed conspecific prey, this pattern is not generally observed in damselfly larvae (see e.g. Stoks, 2001b). It is unlikely that the small predation arenas artificially caused the absence of a link between activity level and predation rates. Indeed, in a study on a related damselfly species using similar-sized predation arenas and the same species of dragonfly predator, it was found that larvae with a higher activity had shorter survival times (Janssens & Stoks, 2012). Potentially, C. scitulum larvae in edge populations have a higher escape swimming speed that compensates for their higher activity, hence assumed higher detection chance by predators, but this remains to be tested. Such trait compensation (sensu Dewitt, Sih & Hucko, 1999), where more active larvae possess higher swimming speeds thereby making them less vulnerable to dragonfly predation, has been found in another coenagrionid damselfly where it was also associated with the absence of a decrease in activity level in the presence of predator cues (Janssens & Stoks, 2012). Another reason for the lack of behavioural antipredator response may be that predator and prey differ in vertical distribution or microhabitat use within the same pond (Johansson, 2000), which may reduce encounter rates of the two species. The higher activity in edge populations is likely to shape interactions with other natural enemies such as fish predators (against which the suggested compensation in escape swimming may not be effective) and other odonate larvae (e.g. Suhling, 2001; Witt, Forkner & Kraus, 2013), and competitors (Beckerman et al., 2010).

Two mechanisms can lead to increased larval activity in populations at an expanding range edge. Firstly, a higher activity in edge populations can be due to the combination of (i) the process whereby more active adults are more abundant in the edge populations resulting from spatial sorting in dispersal ability (Shine et al., 2011) and/or in situ evolution of higher dispersal ability in edge populations (Hill et al., 2011) and (ii) a positive covariation of behaviour traits between life stages (Moran, 1994; Watkins, 2001; Gyuris, Fero & Barta, 2012). Secondly, a higher larval activity can evolve to meet a higher energy demand in edge larvae which are expected if growth rate is higher in edge populations (Phillips, 2009) and/or if the investment in costly dispersal-related traits is higher in edge populations (Hill et al., 2011). In support of the first mechanism, we did find a behavioural coupling between life stages. Yet, this was not associated with a higher activity level in adults from edge populations, leading us to reject the first mechanism as a driver of higher larval activity in the edge populations of the study species. Instead, our results are consistent with the hypothesis that a higher energy need in edge populations shaped the higher larval activity.

We indeed found a higher growth rate in edge populations compared with core populations, and this higher growth rate was associated with a higher activity. A positive covariation between activity and growth is well documented (e.g. Sundt-Hansen et al., 2009), including in Coenagrion damselflies (Brodin & Johansson, 2004). Patterns in growth rates observed in the laboratory and under natural conditions in field enclosures are very similar in damselfly larvae (e.g. McPeek et al., 2001; Stoks et al., 2005a), indicating the relevance of the measured growth rates for the situation in nature. The process of range expansion can select for a higher growth rate to achieve a greater number of generations per year, which is favoured at the initially low population densities in the newly founded edge populations (Phillips, 2009; Burton, Phillips & Travis, 2010). We hypothesise that a similar process acts at the expanding range edge of C. scitulum to maintain a univoltine life cycle also at higher latitudes. Alternatively, one may argue that the observed behavioural differentiation between edge and core populations is actually a side effect of geographic patterns in temperature regimes with cooler temperatures at the range edge causing higher compensatory growth and associated activity levels (cf. Chiba, Arnott & Conover, 2007). This explanation, however, seems unlikely as ponds used in the study were chosen to show a latitudinal overlap between the core and edge populations (Fig. 1). Furthermore, calculated site-specific degree days available for larval growth show that larval

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growth seasons are actually longer in three of the four edge populations compared with the most northern core population C2 (see Appendix S1).

In addition to the need to generate a higher larval growth rate, increased investment in flight-related traits may also explain the higher larval activity. We indeed demonstrated in another common garden experiment a higher investment in flight muscle mass in freshly emerged adults (Therry et al., 2014). The investment in this energetically costly flight-related trait took place during the larval stage, as individuals were not fed in the adult stage. Such higher investment in flight-related traits has been observed in edge populations of several poleward-moving insect species (Hill et al., 2011) and can be explained by natural selection and spatial sorting on dispersal-related traits during range advance (Travis & Dytham, 2002; Shine et al., 2011).

Covariation patterns across metamorphosis between larval and adult traits are receiving increased attention as they have the potential to generate carry-over effects of environmental conditions encountered in one stage towards the other stage and thereby to couple aquatic and terrestrial ecosystems (Knight et al., 2005; McCauley et al., 2010; Stoks & Cordoba-Aguilar, 2012). Few studies, however, demonstrated the coupling between behavioural traits across life stages (but see Brodin, 2009; Gyuris et al., 2012). In line with our results, activity was positively coupled between the larval and adult stages in the damselfly Lestes congener (Brodin, 2009). Yet, such behavioural coupling across metamorphosis is not general. In a related study, Brodin et al. (2013) showed more exploratory and bold behaviour in Rana temporaria tadpoles and froglets in isolated island populations compared with mainland populations, which was not associated with a behavioural coupling between these life stages. The emerging picture suggests the complexities of the trait patterns across metamorphosis and indicates that similar trait patterns between treatment groups may occur across metamorphosis despite the absence of a trait coupling across metamorphosis (Brodin et al., 2013), and that dissimilar trait patterns between treatment groups may occur across metamorphosis despite trait coupling across metamorphosis (current study). This urges caution when, for example, assuming that a behavioural correlation across life stages automatically translates behavioural patterns in the larval stage into the same behavioural patterns in the adult stage.

Our results add to the recent insight that besides the widely documented changes in the dispersive adult stage (Hill et al., 2011), traits in the non-dispersive larval stage may also evolve during range expansions as shown before for larval growth rate in the cane toad (Phillips, 2009). We extend this insight by demonstrating for the first time differentiation in a behavioural trait in the larval stage at a moving range front. Noteworthy, McCauley et al. (2010) documented similar changes in activity level of larval dragonflies at the much smaller scale of colonisation of new patches within a local metapopulation. They reported higher larval activity in the progeny of dragonflies that dispersed further (>400 m) compared with those that dispersed for shorter distances. We hypothesise that the same underlying mechanism, selection for a higher activity in dispersive phenotypes to meet the energetic demand of a higher growth rate and a higher investment in dispersal-related morphology, may generate both the here documented large-scale geographic patterns at range expansion fronts and the small-scale geographic patterns as documented in the study by McCauley et al. (2010).

The key finding of a higher activity in the non-dispersive larval stage in edge populations of a poleward-moving damselfly has two important broad implications that deserve further attention. First, there is ongoing debate whether evolutionary trait changes at moving range fronts are driven by adaptive versus non-adaptive evolution (Shine et al., 2011). Our results indicate that besides traits in the adult dispersive stage, behavioural traits in the larval non-dispersive stage may also differentiate between edge and core populations, hence should also be considered when studying the adaptive nature of phenotypic differentiation at expanding range edges. Second, increased activity levels may affect ecological interactions and eventually modify community dynamics (Beckerman et al., 2010). Larval activity measured under laboratory conditions has been shown to be under survival selection in damselflies using a field enclosure experiment (Strobbe et al., 2011), indicating that the measured activity in the laboratory probably has fitness consequences under natural conditions. Although activity did not determine predation risk by dragonfly larvae in current study, it is expected that the higher larval activity of edge individuals will influence other ecological interactions. We may expect a higher vulnerability of high active larvae to more efficient predators such as fish predators (Stoks, McPeek & Mitchell, 2003). Further, more active and fast-growing animals often have a higher competitive ability (Grill & Juliano, 1996; Laurila, 2000; Beckerman et al., 2010), which may change competitive outcomes with other species at the new habitats they colonise at the range edge. Finally, damselfly larvae can structure zooplankton communities (Burks, Jeppesen & Lodge, 2001), and the higher feeding
rates of active larvae are expected to enhance the impact on their prey communities (Hunt & Swift, 2010). This creates the hypothesis that the rapid evolution of higher activity levels at the moving range edge as observed in current study may feedback to ecology, hence generate eco-evolutionary dynamics (Matthews et al., 2011; Hanski, 2012).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Characteristics of the study populations.
Table S1. Location, coordinates, the reconstructed number of site-specific larval degree days and the predator regime [presence (+) or absence (−) of Anax imperator and fish predators] for the set of two core (C1 and C2) and four edge (E1, E2, E3 and E4) populations of Coenagrion scitulum.

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