Transmission and terrestrial dispersal of non-native ectosymbionts on invasive crayfish

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Received: 2 September 2017 / Revised: 11 May 2018 / Accepted: 11 May 2018 / Published online: 19 May 2018
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Abstract  Symbionts are a fundamental component of biological systems, and their survival is highly dependent on transmission and host movement. Ectosymbionts of amphibious animals face the added challenge of having to survive dramatic environmental changes as their hosts cross ecosystem boundaries. Within freshwaters, crayfish are amongst the most widespread invasive species that readily disperse overland and are host to a wide range of ectosymbionts. Relatively little is known about the transmission of these ectosymbionts, including their ability to survive overland host migration. Here, we assessed terrestrial emigration and both inter- and intra-specific transmissions of Xironogiton victoriensis, a non-native branchiobdellidan (Annelida: Clitellata) recently found on invasive signal crayfish (Pacifastacus leniusculus) in the UK. These branchiobdellidans tolerated desiccation and did not alter host terrestrial behaviour. Transmission was rapid between natural signal and novel virile (Orconectes cf. virilis) crayfish hosts, with host interactions facilitating transmission. Thus, branchiobdellidans can disperse via amphibious host behaviour and readily infect novel hosts. These traits facilitate symbionts’ survival and provide access to additional dispersal pathways that are likely to aid transmission.

Keywords  Invasive non-native species · Branchiobdellidans · Pacifastacus leniusculus · Xironogiton victoriensis

Introduction

Dispersal is a fundamental life history trait, offering opportunities for range expansion, genetic differentiation and increased fitness. As such, both population and evolutionary dynamics are influenced by a species’ dispersal capacity (Ronce, 2007). In aquatic ecosystems, dispersal is usually limited to connected water bodies, with isolated habitats (such as ponds or lakes) only accessible via flooding events or anthropogenic activity (Hulme et al., 2008; Rahel & Olden, 2008). Amphibious behaviour, however, offers not only opportunities for foraging, reproduction and escape from unfavourable conditions, but terrestrial emigration to nearby water bodies (Sayer & Davenport, 1991). This has played an important role in the spread of invasive non-native species (Peterson et al., 2013; Marques et al., 2014; Ramalho & Anastácio,
Invasive species can detrimentally affect biodiversity, human health and industry in their introduced range (Mack et al., 2000), and determining their dispersal capacity is paramount for non-native species risk assessments (Johnson & Padilla, 1996).

Crayfish are amongst the most successful aquatic invaders, with multiple species now present in many European countries (Holdich & Pöckl, 2007; James et al., 2014; Kouba et al., 2014). Certain species have the capacity to disperse up to 1 km overland and move between isolated water bodies (Lutz & Wolters, 1999; Ramalho & Anastácio, 2014; Fialho et al., 2016), where they act as ecosystem engineers and interact with organisms on multiple trophic levels (Gherardi, 2007; Strayer, 2010; James et al., 2015a). Because of their success and impact, invasive crayfish are considered a major cause of biodiversity loss (Lodge et al., 2000; Manchester & Bullock, 2000). Non-native crayfish have further impacted their native counterparts through the introduction of co-existing symbionts (Lymbery et al., 2014). In Europe, invasion of North American crayfish has been aided by their ability to transmit crayfish plague (Aphanomyces astaci Schikora, 1906), which has caused high mortalities of susceptible native crayfish species (Holdich et al., 2014; Svoboda et al., 2016). Whilst their relationship with crayfish can vary across the symbiosis continuum (Lee et al., 2009; Brown et al., 2016) and determining their dispersal capacity is paramount for non-native species risk assessments (Johnson & Padilla, 1996).

Young, 1966; Creed et al., 2015) and broad host range (Goodnight, 1940; although host preference does occur in some species, see Brown & Creed, 2004), which has led to invading branchiobdellidan species spreading to novel hosts (Sobecka et al., 2011; Vedia et al., 2014). Considering the number of non-native crayfish introductions into Europe, reports of introduced branchiobdellidans are relatively few. Whilst this may be due to lack of reporting, branchiobdellidans could be lost during terrestrial host emigration. Hosts may also exploit their environmental tolerances to reduce infection, for example infected insects, reptiles and fish choose to inhabit different temperatures (termed “behavioural fever”), which increases their immune response and decreases pathogen survival (Vaughn et al., 1974; Müller & Schmid-Hempel, 1993; Mohammed et al., 2016). The time crayfish spend on land and frequency of amphibious behaviour could therefore be affected by branchiobdellidan infection. Alternatively, non-native branchiobdellidans may tolerate terrestrial emigration but fail to establish due to host population bottlenecks encountered during the introduction phase of invasion.

Non-native branchiobdellidans [Xironogiton victoriensis (Gelder & Hall 1990)] were recently found on invasive signal crayfish [Pacifastacus leniusculus (Dana, 1852)] in the UK (James et al., 2015b). Compared to other freshwater annelids, X. victoriensis possess a relatively short reproduction time (cocoons hatch 10–27 days after laying) and can quickly colonise a host following transmission (Govedich et al., 2009; James et al., 2017). Like other Xironogiton spp., X. victoriensis are predominantly located on the crayfish chela (Gelder et al., 2009; James et al., 2015b). This location may facilitate transmission as crayfish often lock chela during aggressive interactions, but it could also promote desiccation during terrestrial host movements. Although branchiobdellidans are generally considered commensals, X. victoriensis reduces crayfish aggression and foraging efficiency, which could alter signal crayfish invasion success (James et al., 2015c). Here, we tested whether these aquatic ectosymbionts can tolerate amphibious host behaviour, which in turn could affect symbiont dispersal with potential consequences for host invasion dynamics. Specific aims were to (i) verify whether X. victoriensis can survive terrestrial host emigration, and if so; (ii) determine if terrestrial crayfish behaviour is affected by X. victoriensis; (iii)
examine transmission of *X. victoriensis* from the environment and between intra-specific and inter-specific crayfish pairs, and finally; (iv) assess how host interactions facilitate transmission.

**Materials and methods**

**Animal origin and maintenance**

Crayfish were captured throughout March–October in 2012–2016 via baited traps and manual searching. Animals were maintained in the lab for 2–4 months prior to the experiments described below. Signal crayfish infected with *Xironogiton victoriensis* were caught at the Gavenny River (Abergavenny, Wales, Grid Reference: SO308164), whilst uninfected, branchiobdellidan naïve signal crayfish were obtained from Dderw Farm Pond (Powys, Wales, Grid Reference: SO138375). Uninfected virile crayfish [*Orconectes virilis* (Hagen, 1870)] were collected from the River Lee (London, England, Grid Reference: TL370028). Although *X. victoriensis* have never been reported on virile crayfish, signal and virile crayfish co-exist in the UK (James et al., 2015d).

All crayfish were caught under licence (NT/CW065-C-652/5706/01) and held at Cardiff University (W C ILFA 002), where infected and uninfected crayfish were maintained separately at a density of 20 individuals per m² in tanks of dechlorinated water (14 ± 1°C). The tanks contained a pea gravel substrate (2 cm) and refugia (plant pots and plastic tubes) with no access to a terrestrial area. Crayfish were fed daily with bloodworm (*Tubifex* spp.) and frozen peas with 50% water changes performed weekly to maintain water quality. All experiments were conducted under these laboratory conditions in windowless rooms to prevent external lighting. Experiments 1 (Dispersal) and 4 (Intra-specific group transmission) were performed under a 12-h-light:12-h-dark photoperiod to provide equal periods of time for diurnal and nocturnal crayfish activity during behavioural observations. A 16-h-light:8-h-dark photoperiod was used during Experiments 2 (Environmental transmission) and 3 (Intra- and inter-specific dyad transmission).

Only healthy branchiobdellidan naïve crayfish in the intermoult stage were used in experiments, with all crayfish measured (carapace length, accurate to 0.1 mm) and sexed. Experiments 1 (Dispersal) and 4 (Intra-specific group transmission) were conducted using 189 L aquaria (0.5 m² area) stocked with groups of four crayfish (density within natural UK signal crayfish populations which can range from 2 to 34 crayfish per m²: Guan & Wiles, 1996; Bubb et al., 2004). These experiments were also recorded using 24-h infrared CCTV cameras (Sentient Pro HDA DVR 8 Channel CCTV, Maplin) with individual crayfish numbered using non-toxic yellow paint on the dorsal carapace (a region *X. victoriensis* does not inhabit; James et al., 2015b) for visual identification. In accordance with the Wildlife and Countryside Act 1981, signal and virile crayfish were humanely destroyed by freezing at −20°C upon termination of experiments as they could not be returned to the wild due to their invasive status.

Individual *X. victoriensis* (identified according to James et al., 2015b) were carefully dislodged from stock crayfish after encouraging them to move using blunt forceps and placed onto a petri dish (experiment 1—dispersal), ceramic tile (experiment 2—environmental transmission) or new, branchiobdellidan naïve crayfish (experiments 3—Intra- and inter-specific dyad transmission and 4—intra-specific group transmission). Removal from the original host did not cause any visible damage to the worms, and they readily re-attached to the petri dish, tile or new host. Previous in vitro experiments show that worms removed using this method survive and successfully reproduce long-term (James et al., 2017). Only adult (> 3.0 mm), *X. victoriensis* that were visually healthy were used in experiments, and any *X. victoriensis* that detached 30 min following transfer were replaced. All other crayfish used in transmission experiments were sham infected to ensure they experienced the same handling procedure and period of time out of water. Crayfish were immediately used in experiments following artificial infection to ensure the same starting number of worms across experiments as host grooming can rapidly reduce worm numbers following infection. In addition, an acclimation period could have resulted in worm reproduction and/or crayfish moulting (James et al., 2017).

**Experiment 1: dispersal**

To investigate the maximum survival time out of water, *X. victoriensis* were removed from their host.
and subjected to periods of dehydration at 15°C (59% RH) and 23°C (41% RH). Five individual *X. victoriensis* were added to a petri dish containing 10 ml water (*n* = 10 petri dishes per temperature treatment). After ensuring all worms were alive, active and firmly attached to the bottom of the petri dish, the water was gently poured from the petri dishes leaving the worms in situ, with excess water removed using an absorbent paper wick. Each petri dish was refilled with dechlorinated water following a set period of dehydration (1, 2, 4, 8, 15, 20, 25, 30, 40 or 50 min; times selected following preliminary trials). The worms were then left in water for 24 h at 15 or 23°C to allow them to rehydrate and reanimate, and then the number of worms alive in each petri dish was counted. In this study, we did not attempt to monitor *X. victoriensis* desiccation survival on the host as crayfish had a tendency to groom some of the branchiobdellidans from their chelae immediately after artificial infection (also reported previously by Farrell et al., 2013; Skelton et al., 2014).

The capacity of *X. victoriensis* to exploit new hosts in recipient water bodies following terrestrial host emigration was assessed by placing infected signal crayfish (*n* = 4) into an aquarium containing ramps (29 × 43 cm) that provided access to a terrestrial bridge (250 × 20.5 cm). The bridge was connected to another aquarium containing uninfected signal crayfish (*n* = 4) (Fig. 1). The ramps and bridge were lined with affixed pea gravel substrate to aid crayfish movement. Crayfish were left to acclimatise for 1 day with an opaque divider on the bridge preventing movement or visual exchange between the two aquaria. Following removal of the divider, individuals were screened for branchiobdellidans every morning (09:00 h) until a crayfish in the uninfected population became infected (maximum of 7 days).

To determine whether *X. victoriensis* infection influenced how often signal crayfish left the water and the length of time spent on land (per exit and total time), infected (*n* = 4) and uninfected (*n* = 4) crayfish were placed into aquaria and given access to a 125 × 20.5 cm section of land. Crayfish were filmed for 24 h with individual terrestrial activity determined from analysis of video footage (*n* = 10 replicates, 80 crayfish).

**Experiment 2: environmental transmission**

To investigate indirect transmission of *X. victoriensis* from the environment to crayfish, 30 individuals of *X. victoriensis* attached to a ceramic tile (5 × 5 cm²) were placed in a 15-l aquarium with one uninfected signal crayfish (*n* = 20). The number of *X. victoriensis* on the crayfish was recorded after 1, 2, 4, 6, 8, 10, 12, 18 and 24 h.

**Experiment 3: intra- and inter-specific dyad transmissions**

To assess both intra- and inter-specific transmissions of *X. victoriensis*, artificially infected ‘donor’ signal crayfish (30 worms per individual) were placed in a 15-l aquarium at 14:00 h with either a ‘recipient’ signal (n = 20) or virile crayfish (n = 20) sex and size matched to within 10% carapace length (mm) of the donor. The number of *X. victoriensis* worms on the recipient crayfish was recorded after 1, 2, 4, 6, 8, 10, 12, 18 and 24 h. The experiment was repeated for intra-specific virile crayfish pairs (*n* = 20).

![Fig. 1 Terrestrial dispersal of *Xironogiton victoriensis* experimental design. To determine how quickly *X. victoriensis* could transmit to separate host populations following overland host movement, crayfish were given free movement between tanks across a bridge](/images/association/138/fig1.png)
Insufficient virile crayfish were available to investigate virile-to-signal crayfish transmission.

Experiment 4: intra-specific group transmission

Xironogiton victoriensis transmission within a host population was assessed using groups of signal crayfish \( n = 10 \) replicates, 40 crayfish. Crayfish \( n = 4 \) were placed into an aquarium with a single uniform shelter large enough for all the crayfish \((29 \times 43 \text{ cm}^2)\) and left to acclimatise for 3 days. After acclimatisation, an individual crayfish \((44–48 \text{ mm carapace length})\) was experimentally infected with 154 \( X. \) victoriensis worms to match natural intensities (James et al., 2015b). This created a starting group of one infected donor crayfish and three uninfected recipient crayfish. Crayfish behaviour was then observed for 1 week. As branchiobdellidan transmission is linked to host contact (Young, 1966), all interactions between individual crayfish (aggressive and shelter sharing behaviours) were analysed via video recording. Intensity of \( X. \) victoriensis infection was quantified after 1 week for each individual. No branchiobdellidan reproduction could have occurred during the experiment as \( X. \) victoriensis cocoons take at least 10 days to hatch (under the same laboratory conditions used for these experiments: James et al., 2017).

Statistical analyses

Models were refined through stepwise deletion of insignificant terms and/or AIC comparisons with visual examination of model plots to check standardised residuals for normal distribution and homogeneity of variance (Crawley, 2007). Generalised Linear Mixed Models (GLMMs) were implemented using the glmmADMB package (Skaug et al., 2016) to include trial number as a random factor accounting for group effects. In all tests, the level of significance was taken as \( P < 0.05 \).

To assess whether temperature and \((\log)\) time significantly affected the proportion of worms that survived dehydration, we used a General Linear Model (GLM). For terrestrial emigration, a GLMM with a binomial error distribution and logit link function was used to compare successful transmission (yes/no) to the number of host crossings (total number normalised using log transformation). To determine the influence of \( X. \) victoriensis intensity on the tendency of crayfish to leave the water, we also used a GLMM with a binomial error distribution and logit link function. Another GLMM with a gaussian error distribution and identity link function was then used to examine whether the duration of time crayfish spent on land \((\text{square root transformed}, n = 29\) as crayfish that did not leave the water were discounted) was affected by \( X. \) victoriensis intensity.

Transmission of \( X. \) victoriensis to uninfected hosts was analysed using two separate GLMs; The first model investigated the effect of transmission pathway \((\text{environment–signal, signal–signal, virile–virile, signal–virile})\) and crayfish size \((\text{mean pair carapace length})\) on the speed of transmission \((\log \text{ time to first worm transfer})\). The second model determined the effect of transmission pathway and crayfish size on the \((\log x + 1)\) maximum number of worms transferred to each crayfish. Significant differences between transmission pathways were examined with a Tukey’s Honest Significant Difference means comparison.

To determine if time spent interacting, sharing shelter, host size \((\text{carapace length})\), chelae size or sex influenced transmission of \( X. \) victoriensis in a group, a GLMM was used with a negative binomial error distribution and log link function. A GLM was also performed to examine whether time crayfish spent interacting with conspecifics was influenced by crayfish size or sex.

All statistical analyses were conducted in R statistical software v3.3.1 (R Core Team, 2016).

Results

Experiment 1: terrestrial emigration

\( Xironogiton victoriensis \) tolerated desiccation and survived significantly better at \(15°C (40–50 \text{ min})\) than at \(23°C (> 15 \text{ min}) \) \( F_{1,37} = 11.52, P < 0.001 \). Terrestrial emigration frequency of signal crayfish between two connected water bodies significantly affected whether \( X. \) victoriensis transmission to the uninfected host population was successful \( (\chi^2 = 12.22, \text{df} = 1, P < 0.001) \). Terrestrial emigration of an infected crayfish to the uninfected
population resulted in successful transmission in 70% of trials after a maximum of 7 days, with the fastest transmission occurring after 1 day. The majority of crayfish that left their aquarium proceeded to cross over to the adjacent aquarium (90%, \( n = 182 \) instances of leaving the water with 163 full crossings) with the shortest crossing taking 2 min and the longest 14 min. The longest period of time spent on the bridge was 30 min; however, that individual did not enter the new aquarium and instead returned to its original aquarium. During the terrestrial behaviour trials, the tendency of signal crayfish to leave the water and duration of time spent on land was not significantly affected by \( X. victoriensis \) intensity. The total time individuals spent on land (calculated from multiple outings) ranged from 2 to 112 min with 36% of crayfish leaving the water (\( n = 80 \)). The longest single period of time spent out of water by an individual crayfish was 58 and 41 min for uninfected and \( X. victoriensis \) infected crayfish, respectively.

Experiments 2 and 3: environmental, intra- and inter-specific dyad transmission

Table 1 summarises the results of \( X. victoriensis \) transmission from the environment to signal crayfish and between host pairs. Transmission within 24 h was 100% successful from the environment to signal crayfish, 95% for both intra-specific signal–signal and virile–virile, and 70% for interspecific signal–virile pairs. Success was significantly lower for interspecific signal–virile pairs compared to environment, signal–signal and virile–virile trials (\( P < 0.05 \)). The time until first worm transfer and maximum number of worms transferred was significantly dependent upon transmission route (\( F_{3,59} = 3.67, P = 0.020; F_{3,76} = 28.59, P < 0.001 \)), with the fastest first worm transfer occurring between virile conspecifics and highest total number of worms transferred from the environment to signal crayfish. Crayfish size had no effect on either the speed of transmission or the number of \( X. victoriensis \) transferred.

Experiment 4: intra-specific group transmission

Following one week of co-habituating with infected crayfish, \( X. victoriensis \) intensity on recipient crayfish significantly increased with time spent sheltering (\( \chi^2 = 17.10, \ df = 1, \ P < 0.001 \)) and interacting (\( \chi^2 = 5.35, \ df = 1, \ P = 0.020 \)) (Fig. 2). In addition, male crayfish became infected with significantly more worms than female crayfish (\( \chi^2 = 5.55, \ df = 1, \ P = 0.018 \)). Males also spent more time interacting with conspecifics (1130 min for males versus 653 min for females); however, this was not a significant difference. Crayfish size did not significantly affect \( X. victoriensis \) intensity or time spent interacting with conspecifics. By the end of the experimental period (1 week), all recipient crayfish had branchiobdellidan cocoons and 0–41 \( X. victoriensis \) worms.

**Discussion**

Symbioses are implicit in biological systems (Brooks, 2012); however, pathways of symbiont dispersal are often unknown. This study shows that branchiobdellids possess several life-history traits which aid dispersal, establishment and spread. *Xironogiton*
victoriensis survived 40–50-min desiccation and infection did not alter terrestrial crayfish behaviour, as such, amphibious host movement provides an additional emigration opportunity. Furthermore, X. victoriensis readily infected both signal and virile crayfish with host interactions facilitating transmission.

To establish, ectosymbionts of amphibious invaders have to survive exposure to different environments when their hosts cross ecosystem boundaries. Ectosymbionts typically tolerate desiccation far less than their hosts; however, some species are capable of surviving overland migration. Ectoparasitic gyrodaetlyids are prone to desiccation but can survive on killifish (Rivulus hartii Boulenger, 1890), which migrate terrestrially (Sayer & Davenport, 1991; Cable et al., 2013), whilst aquatic leeches can be transported overland via crocodilians and waterfowl (Davies et al., 1982; Leslie et al., 2011). X. victoriensis is evidently capable of surviving translocation and dispersal due to the number of successful introductions and established populations across Europe (currently confirmed in seven countries; see James et al., 2015b).

In the present study, X. victoriensis survived terrestrial host movement and did not alter this behaviour, whilst also tolerating 40–50-min desiccation off the host. The terrestrial walking speed of signal crayfish is unknown, but adult male red swamp crayfish (Procambarus clarkii Girard, 1852), another invasive North American species, walk on average 58 m h⁻¹ (Ramalho & Anastácio, 2014). At this rate, we estimate X. victoriensis could survive terrestrial migration up to 43.5 m, although this does not take into account variable environmental conditions (Marques et al, 2014; Ramalho & Anastácio, 2014; Yoder et al., 2016). It is also possible that branchiobdellidans could tolerate longer periods of desiccation in vivo by retreating into host crevices or gill chambers, but for X. victoriensis this is unlikely given that this species is a chelae specialist (Gelder & Hall, 1990; James et al., 2015b). Regardless, it is likely that even based on our conservative estimates, branchiobdellidans would be able to survive natural crayfish overland dispersal, which can exceed 20 m (Puky, 2014; Ramalho & Anastácio, 2014). Cocoons present another opportunity for dispersal: oligochaete cocoons, for example, can have a higher desiccation tolerance than juvenile and adult worms (Holmstrup, 2001; Govedich et al., 2009). Therefore, even where juvenile/adult worms desiccate, cocoons may survive terrestrial dispersal and proceed to hatch and establish a viable population.

Following establishment, dispersal of non-native branchiobdellidans is likely promoted by their low host-specificity and the co-existence of multiple North American crayfish species (Kouba et al., 2014). In the present study, X. victoriensis transmitted readily...
between signal and virile crayfish, which co-exist in the UK (James et al., 2015d). Intra-specific transmission occurred faster between novel virile crayfish hosts, compared to signal crayfish, potentially due to the higher aggression of this species and thus more frequent interactions (James et al., 2015d). The maximum number of worms transmitted, however, was not dependent upon the recipient host species. This supports previous reports indicating that whilst some branchiobdellidan species exhibit host preferences (Brown & Creed, 2004), most crayfish species are acceptable hosts (alongside certain crabs and shrimps; Gelder & Messick, 2006; Govedich et al., 2009; Niwa et al., 2014). X. victoriensis has been reported on invasive red swamp crayfish populations in Spain, likely due to cohabitation with infected signal crayfish (Vedia et al., 2014). Red swamp and virile crayfish are present in the UK together with five other non-native and one native crayfish species (James et al., 2014). In addition, other suitable crustacean hosts are present, for example, invasive Chinese mitten crabs (Eriocheir sinensis H. Milne Edwards, 1853), which X. victoriensis could exploit (Sobecka et al., 2011). Access to multiple hosts could, however, prompt a ‘dilution effect’ whereby infection on signal crayfish (i.e. the natural host of X. victoriensis) is reduced due to the increasing diversity of suitable hosts (Keesing et al., 2006).

As branchiobdellidan transmission is dependent on host contact (Manus, 1960; Young, 1966), crayfish behaviours that increase contact will evidently promote symbiont spread. Both host shelter sharing and, in particular, aggressive interactions (involving interlocking chelae, the primary niche of X. victoriensis; James et al., 2015b) positively increased X. victoriensis transmission in the current study. We also found male signal crayfish were infected with significantly more X. victoriensis than females. Whilst grooming behaviours can play a major role in the control of crayfish ectosymbionts (Farrell et al., 2014; Skelton et al., 2014), it is likely that this sex difference in branchiobdellidan intensity was due to a disparity in male/female interactions. Male crayfish are typically more aggressive (Ranta & Lindström, 1993) and, although not statistically significant, spent almost twice the time interacting compared to females.

Overall, we demonstrate that X. victoriensis can survive terrestrial host emigration, and this may facilitate the movement of these symbionts between isolated water bodies. Given that X. victoriensis is capable of reducing signal crayfish aggression and foraging efficiency, these results have potential implications for the invasion dynamics of these highly successful invasive species. The spread of these branchiobdellidians in the UK could be further promoted by their propensity to transmit to novel non-native crayfish hosts, as demonstrated in the current study. As crayfish are keystone species and ecosystem engineers that interact with organisms on multiple trophic levels, the presence of these behaviour-altering symbionts may have ecosystem-wide consequences.

Acknowledgements We thank Amanda Chapman for laboratory assistance. The Cardiff University Morgan E Williams Helminthology Scholarship, the Natural Environment Research Council (studentship NE/L002434/1) (RH) and the Coleg Cymraeg Cenedlaethol (JRT) funded the project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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