Hydrobiologia (2014) 722:61–74
DOI 10.1007/s10750-013-1675-6

PRIMARY RESEARCH PAPER

Muddied waters: suspended sediment impacts on gill structure and aerobic scope in an endangered native and an invasive freshwater crayfish

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Received: 31 May 2013 / Revised: 9 August 2013 / Accepted: 24 August 2013 / Published online: 11 September 2013

Abstract Suspended sediment (SS) loadings in freshwater habitats have increased over the past century and SS is now a significant environmental stressor. Greater tolerance to environmental stressors has been proposed as a factor in the success of aquatic invasive species. Further, parasites may interact with environmental stressors to increase host susceptibility to loss of fitness and mortality. We compared the effects of SS exposure on the gill structure and aerobic scope of the endangered white-clawed crayfish (*Austropotamobius pallipes*), and the invasive signal crayfish (*Pacifastacus leniusculus*), and assessed impacts in relation to parasite burden. SS caused gill fouling and reduction in aerobic scope in both species, though *A. pallipes* was more susceptible than invasive *P. leniusculus*. The parasite *Branchiobdella astaci*, a crayfish worm that infests the gills, interacted with the sediment to affect gill structure whereas infection with the microsporidian parasite *Thelohania contejeani* had no effect on crayfish response to SS. Juvenile *P. lenisuculus* had a higher standard metabolic rate than *A. pallipes*, which may be linked to competitive advantages such as higher growth rate and behavioural dominance. Conservation of *A. pallipes* often involves relocation of threatened populations to isolated still-waters; our findings suggest that SS levels should be assessed before relocation.

Keywords Endangered species · Water quality · Parasite · Environmental stressor · Conservation · Translocation

Handling editor: Katya E. Kovalenko

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Introduction

Freshwater fauna are proportionately more threatened by environmental change than terrestrial or marine species (Jenkins, 2003; Millennium Ecosystem Assessment, 2005); with projected extinction rates up to fivefold higher (Ricciardi & Rasmussen, 1999; Revenga et al., 2005; Xenopoulos et al., 2005). The multiple threats to freshwater systems include loss of aquatic habitats; stream fragmentation and flow regulation by dams; channelisation; pollution; and the

spread of invasive species (Poff et al., 1997; Nilsson et al., 2005; Dudgeon et al., 2006). Crayfish are a ubiquitous group, comprising up to 85% of total invertebrate biomass (Neveu, 2009), and are a functionally important component of lotic and lentic freshwater ecosystems where they feed at multiple trophic levels, modify community structure and transfer energy from primary producers to top predators (Momot, 1995; Nystrom et al., 1996; Usio & Townsend, 2002). Of the 543 species globally, 26% are classified 'vulnerable' or 'endangered', largely due to invasive species, disease and habitat degradation (IUCN, 2011).

The white-clawed crayfish (*Austropotamobius pallipes*, Lereboullet), the UK's only native crayfish species, is IUCN red-listed as 'endangered', principally due to population decline since introduction of the invasive signal crayfish (*Pacifasticus leniusculus*, Dana, 1852). *P. leniusculus* originates from North America and was initially introduced to Europe in the 1960s for the table market. Traits such as fast growth rate, high fecundity and large size made it more suitable for aquaculture than native crayfish species (Holdich & Gherardi, 1999; Gil-Sanchez & Alba-Tercedor, 2002). Where *P. leniusculus* and *A. pallipes* co-occur, the invader will typically outcompete the native within 4–5 years (Peay & Rogers, 1998), and is the main vector of *Aphanomyces astaci*, the cause of 'crayfish plague', which is fatal to *A. pallipes* (Alderman et al., 1984). Understanding the mechanisms underpinning the success of an invader is key for predicting future invasions and devising effective control measures (Davis, 2009). Greater tolerance to environmental stressors such as fluctuating temperatures and degraded water quality has been proposed as a factor in the success of aquatic invasive species (Karatayev et al., 2009; Crooks et al., 2011; Weir & Salice, 2012).

Parasites may interact with multiple environmental stressors to increase host susceptibility to loss of fitness and mortality (Lafferty & Kuris, 1999; Marcogliese & Pietrock, 2011). UK populations of *A. pallipes* are infected by a number of parasites, including the widespread microsporidian *Thelohania contejeani* which reduces function of muscle tissue and commonly infects 0–10% of crayfish in a population (Cossins & Bowler, 1974; Alderman & Polglase, 1988; Imhoff et al., 2009); and the crayfish worm *Branchiobdella astaci* which infests the gills of its host and causes pathology, most likely through the consumption of host tissue (Grabda & Wierzbicka, 1969; Vogt, 1999; Rosewarne et al., 2012).

Suspended sediment (SS) loadings in freshwater streams and lakes have increased substantially over the last century, primarily due to the intensification of agriculture (Foster et al., 2011), and SS is now a significant environmental stressor causing biodiversity loss and ecosystem change (Bilotta & Brazier, 2008; Palmer-Felgate et al., 2009; Kemp et al., 2011). The negative effects of SS on freshwater fish are well documented and include abrasion of the gills and hyperplasia, reduction in feeding rates, and increased susceptibility to disease (Martens & Servizi, 1993; Metzeling et al., 1995; Lake & Hinch, 1999). There is a mean threshold target of 25 mg l^{-1} for SS within the EU Freshwater Fish Directive (78/659/EC), and although no specific targets for SS are stated within the EU Water Framework Directive (EC 2000), mitigation for diffuse sediment pollution is considered critical for achieving 'good ecological status' by 2015 (Collins & Anthony, 2008; Defra, 2012).

Impacts of SS on invertebrates such as zooplankton, chironomids and freshwater mussels, range from reduced feeding rates, metabolic changes, and clogged gills, to mortality (Donohue & Irvine, 2003; Bilotta & Brazier, 2008, and references therein). The effect of increased sediment loadings on crayfish has received comparably little attention, though has been implicated in declines of some species (Füreder et al., 2006; Environment Agency, 2011). Interestingly, some species of crayfish (usually the invasive ones) are considered 'ecosystem engineers' for their role as bioturbators moving substantial quantities of material, and thus are instrumental in generating SS (Statzner et al., 2003; Johnson et al., 2011).

In the absence of quantified impacts, a conservative threshold SS target of 25 mg l^{-1} (based on the target for salmon) for *A. pallipes* was suggested on the basis that sediment 'blocks gills' (Smith et al., 2003). Crayfish gills are indeed vulnerable to fouling by both particulate matter and epibionts (Holdich, 2003), which may lead to gill pathology (Bauer, 1998), although this has not previously been examined or quantified in relation to SS concentrations. Investigation of the impact of SS on *A. pallipes* is therefore important to determine acceptable ranges of SS; and also to inform conservation strategy for this endangered species which currently involves relocation of

threatened populations to isolated sites without *P. leniusculus* (Schulz et al., 2002; Kemp et al., 2003). The selection of suitable receptor sites is dependent on a thorough understanding of the habitat requirements of *A. pallipes*, including tolerance of water quality parameters such as SS (Kemp et al., 2003).

Environmental factors fundamentally influence animal activity through metabolism (Fry, 1947; Claireaux & Lefrançois, 2007). Aerobic scope denotes the maximum amount of oxygen available to an ectotherm at a particular temperature and is the difference between maximum oxygen uptake, such as that reached after exercise to exhaustion (maximum metabolic rate, MMR); and oxygen uptake at complete rest, in an unfed state (standard metabolic rate, SMR) (Brett, 1972). An individual must function within the confines of its aerobic scope, so a reduction in scope limits the energy which may be allocated to activities beyond basic survival and maintenance (e.g. growth, reproduction, foraging and predator avoidance), thereby causing loss of performance, and potentially overall fitness (Fry, 1947; Boddington, 1978; Claireaux & Lefrançois, 2007). For example, gill morphological changes induced by exposure to aluminium have been shown to reduce aerobic scope in rainbow trout *Oncorhynchus mykiss* as a consequence of reduced gill surface area (Wilson et al., 1994). SS is known to foul the gills of crayfish (Bauer, 1998), and therefore has the potential to reduce aerobic scope by modifying gill structure and limiting the area available for gas exchange.

To compare the impact of chronic exposure to SS on native *A. pallipes* and its invasive competitor *P. leniusculus*, and to investigate the influence of parasitism on the host responses to SS, we: (1) measured the effect of chronic periodic exposure to high SS on gill structure and aerobic scope of native and invasive juvenile crayfish and (2) tested whether infection by *T. contejeani* or *B. astaci* modified the effects of SS on *A. pallipes.*

Methods

We investigated the effect of the SS treatment on infected and uninfected individuals by: (1) comparing individual aerobic scope before and after the treatment; (2) measuring mortality in the different treatment groups and (3) at the end of the experiment we dissected and examined the gills for evidence of sediment accumulation and associated pathology.

Collection of animals

Juvenile *A. pallipes* (19–30 mm cephalothorax length, CL) representing ages of 2–3 years (Brewis and Bowler, 1982) were collected from Wyke Beck, UK (53°49′20.93″N, −1°28′58.73″E) using hand-search, under license from Natural England (20103521). Infection with *T. contejeani* (thelohaniasis) was determined by visual examination (Imhoff et al., 2012). Juvenile *P. leniusculus* (20–31 mm CL) were collected from the river Pant, UK (51°55′28.14″N, 0°31′16.59″E), using hand-search. Crayfish were maintained in the laboratory in de-chlorinated tap water (16°C, 16:8 light, dark regime) and fed crab pellets (Hinari) every 3 days for a minimum of 3 weeks before experiments.

Sediment exposure

Juvenile crayfish were allocated to four SS levels (0, 250, 500 and 1,000 mg l^{-1}) for 45 days. Since a large proportion of the added SS does not remain in suspension, even under moderate turbulence, this range of elevated SS levels was chosen to ensure high, but realistic levels of measurable SS in the experiment. Treatments comprised *P. leniusculus* and *A. pallipes,* with 8 individuals per treatment at each SS level, and *A. pallipes* visibly infected with *T. contejeani*, with six individuals per treatment at each SS level. The sex ratio was 1:1 in all treatments. As the presence of *B. astaci* cannot be reliably determined in live animals, it was not possible to assign crayfish to treatments based on infestation with *B. astaci*, but all *A. pallipes* were examined for *B. astaci* at the end of the exposure period. SS treatments were prepared using dried sediment re-suspended in de-chlorinated tap water. Sediment was collected from a limestone quarry in the Yorkshire Dales, UK (54°4′30.36″N, 2°2′18.29″W). The location was chosen because it is already used as an ark site for the conservation of *A. pallipes.* After collection, sediment was re-suspended in 5 l of water and the water and particles still suspended after 60 s were decanted. Sediment was allowed to settle out 7 days before subsequent air drying at room temperature. Mineralogy of the

resultant particles was determined using X-ray diffraction. To minimise potential variation in water chemistry between treatments, tap water was equilibrated during dechlorination by placing a block of limestone in the water container. Test solutions were fully replaced and the test containers rinsed with water every 3 days to both prevent the build-up of wastes, and to account for settling out of particles over time. To measure actual SS concentrations in the treatments over the 3 days settling period, random water samples (50 ml) were taken at 5, 30, 240 and 960 min, and then every 8 h thereafter from the time water was replaced, four replicates for each treatment at each time. SS concentration (mg l^{-1}) in each sample was determined by change in mass (±0.0001 g) of filter paper (0.45 µm, cellulose nitrate membrane, Whatman) during filtration.

Crayfish were weighed (wet mass, g) numbered on the carapace using non-toxic correction fluid and transferred to aerated experimental containers with two individuals per container. The set-up comprised upturned funnels (300 mm diam., 1 l vol.), and central platform on which the crayfish were housed. Air flow was provided via an air stone secured in the base of each container; the flow of bubbles also served to maintain the sediment in suspension. The platform was composed of aluminium insect screen (mesh size 1.5 mm) which whilst solid enough to support the crayfish, permitted water flow throughout the experimental chamber. To reduce stress and aggressive interactions, crayfish were provided refugia in the form of PVC pipe sections (5 cm × 3 cm diam.), one per animal (Fig. 1). Crayfish were fed every 2 days with Hinari crab pellets, two pellets per crayfish. Any uneaten food was removed after approximately 3 h to prevent fouling of the water.

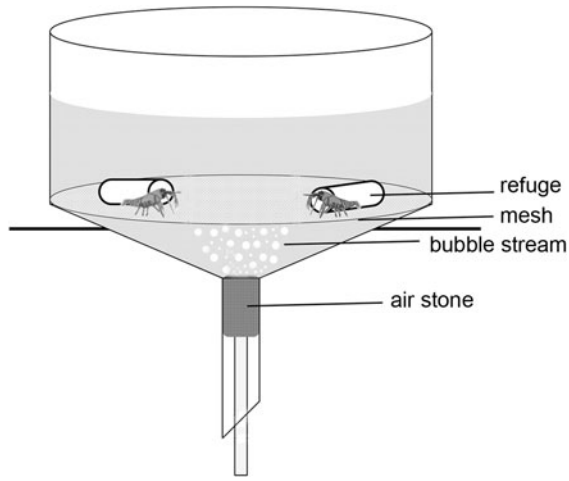

Fig. 1 An experimental chamber

Gill examination

To investigate the impact of SS on the gills, and to screen for *B. astaci* infection, at the end of the experiment all crayfish were euthanised by freezing and the branchiostegites removed to enable in situ examination of the podobranchs. Individuals that did not survive to day 45 were examined as close as possible to the time of death (<10 h). For infected individuals, *B. astaci* burden was recorded as the number of cocoons (egg and embryo stage) attached to the gill filaments (see Rosewarne et al., 2012). The presence of sediment particles was visually assessed for each of the 12 podobranchs as percentage of total area affected. A photograph was taken of each side of the animal under a dissecting microscope, and a grid superimposed over the photo (Adobe Photoshop CS6). The number of grid squares where sediment particles were visible in >50% of the square was counted, relative to the total number of squares containing gill tissue, and was used to calculate % total gill area affected. The area of melanised tissue was assessed using the same method.

Respirometry

Oxygen consumption rates (MO_2) were measured using intermittent flow respirometry. This method overcomes the problem of metabolites building up over long measurement periods, without the difficulties of achieving steady state required by flow-through respirometry (Steffensen, 1989). Single channel Loligo Autoresp respirometry equipment and software (LoligoSystems, ApS, Tjele, Denmark) were used. The glass respirometer chamber (4.5 cm inner diameter, 10 cm length) and mixing pump were submerged in 50 l dechlorinated tap water, aerated using air stones and maintained at 16 ± 0.1°C by means of a cooling coil and temperature regulation unit. Water in the chamber was re-circulated through tubing connected at each end. Total respirometric volume was 0.25 l. Oxygen partial pressure of the circulating water was recorded each second using a fibre optic oxygen

sensor dipping probe (Fibox 3, Presens, Regensburg, Germany) mounted into the tubing via a Y-connector. Water in the respirometer was fully replaced in the flush period; ensuring oxygen levels did not fall below 20 kPa. The coefficient of determination (R^2) associated with each MO_2 measurement was >0.9 (Behrens & Steffensen, 2007). Blank runs with no animal present were made each week to determine background respiration levels.

Standard metabolic rate was measured in a subset of individuals of *P. leniusculus* ($n = 6$), *A. pallipes* infected with *T. contejeani* ($n = 6$) and uninfected *A. pallipes* ($n = 6$) (1:1 males to females) prior to the start of the experiment. Crayfish were starved for 24 h before being transferred to the respirometer and MO_2 measured at least every 13 min for 24 h, 3 pm to 3 pm the following day. A refuge in the form of a section of PVC pipe (5 cm length, 2 cm diam.) was placed in the respirometer to minimise stress and activity in the crayfish. The respirometer and tank were loosely covered with a lid to limit visual disturbance to the crayfish, but gaps around the edge permitted entrance of light to denote photoperiod. Animals that were suspected of being immediately pre-moult, or had moulted in last 3 days were excluded from measurements because ecdysis increases oxygen consumption rate in crayfish (Rice & Armitage, 1974).

MMR was initially determined in all crayfish at commencement of the study, and again on day 45. Prior to measurement, each crayfish was placed in a shallow tray of dechlorinated water (40 cm × 25 cm, 4 cm water depth) and exhausted using a two-stage protocol. First, the crayfish was induced to tail flip by gently touching the telson with a blunt pencil. This escape behaviour is common among crayfish whereby the abdomen is rapidly contracted propelling the animal backwards. The tail musculature has a limited capacity for aerobic contraction and on depletion of arginine phosphate reserves in the tail the animal will adopt stationary threat posture (England & Baldwin, 1983). Once tail flipping ceased, the second stage entailed repeatedly turning the crayfish onto its back until it could no longer right itself. At this point the crayfish was immediately transferred to the respirometer.

Both MMR and SMR represent measurements that cannot be self-regulated (Priede, 1985), and are therefore repeatable within individuals (Norin & Malte, 2011).

Data analysis

The survival of crayfish across the four sediment treatments was analysed using cox proportional hazard survival models (Therneau & Lumley, 2011) in R v 2.14.0 (R Development Core Team, 2011). In the first instance time to death was modelled as a function of SS concentration, species and crayfish mass on day 1. As only *A. pallipes* were parasitised in this study, a second analysis was undertaken. Time to death for *A. pallipes* only was modelled as a function of SS concentration, infection with *T. contejeani*, infection with *B. astaci*, and first-order interactions between the two parasites, and between each parasite and SS concentration. The assumption of constant hazard in all models was tested using function cox.zph.

All MO_2 measurements were corrected for background respiration (i.e. oxygen consumed by bacteria) prior to analysis. Using raw MO_2 data, SMR can be derived in a number of ways. In this study, SMR was calculated for each crayfish using two different methods. First, a frequency distribution was fit to the set of MO_2 values collected during the entire 24 h period, typically revealing a bi-modal normal curve. The distribution reflects a short 'adaptation phase' when oxygen consumption is high reflecting stress in response to handling, then a longer period of lower, settled values. The peak of the second curve of lower values was taken to be SMR (see Svendsen et al., 2012). Curve-fitting was carried out in Tablecurve2D v.5.01 (Systat Software Inc., California). In the second method, values in the 'adaptation phase' were disregarded and lowest 10% of values in the following settled period, excluding outliers, were averaged to arrive at SMR (see Herrmann & Enders, 2000).

To enable comparison of MO_2 values between crayfish of different mass, SMR and MMR were corrected to a body mass of 5 g using the equation $MO_2\ (5\ g) = MO_{2(BM)} x (BM/5)^{(1-A)}$, where $MO_{2(BM)}$ is oxygen consumption of animal with body mass BM and A is the mass exponent describing the relationship between metabolic rate and oxygen consumption (Steffensen et al., 1994). A value of 0.71 for the mass exponent determined for crayfish species *Orconectes rusticus* using field metabolic rate in 137 individuals of different populations (McFeeters et al., 2011) was used within this study.

Prior to all analyses, data were tested for normality using Shapiro–Wilk test. Generalised linear models

(GLMs) with quasibinomial error distributions were used to first, test for a species effect on the level of sediment accumulated in crayfish gills at day 45, and second to investigate predictors of sediment at day 45 in *A. pallipes* only. Variables included in the latter maximal model were SS concentration, infection with *T. contejeani*, burden of *B. astaci* and first order interaction terms. GLMs with quasibinomial error distributions were also used to investigate predictors of melanisation levels in *P. leniusculus* and *A. pallipes*. Variables included in the maximal model for *P. leniusculus* were: sediment accumulation in gills at time of death, sediment concentration, and days in experiment. For *A. pallipes*, variables were sediment accumulation in gills at time of death, sediment concentration, days in experiment, and *B. astaci* burden. Non-significant terms were stepwise deleted from the maximal model and model fit assessed by examination of residual plots and tests for normality of residuals.

Independent sample t-tests were used to test differences in mean SMR and aerobic scope between groups. General linear models (LM) were used to determine significant predictors of aerobic scope on day 1. Aerobic scope (mg O_2 kg^{-1} h^{-1}) on day 1 was modelled as a function of species, sex, start mass, number of tail contractions before exhaustion, and time to exhaust. Linear models were also used to explore change in aerobic scope between days 1 and 45 for the same individuals. In the maximal model, change in aerobic scope (mg O_2 kg^{-1} h^{-1}) was modelled as function of sediment treatment, gill area affected by sediment on day 45 (%), gill area affected by melanisation on day 45 (%), species, difference in number of tail flips during exhaustion protocol between days 1 and 45, and difference in time to exhaustion between days 1 and 45. Non-significant variables were excluded stepwise. All models were carried out in R v 2.14.0 (R Development Core Team, 2011).

Results

SS treatments

Composition of the sediment was 53.7% calcite, 43.6% magnesium calcite and 2.7% quartz silica. Actual SS concentrations, time-averaged for the 45-day exposure period were 2.5, 42, 65 and 133 mg l^{-1}, for control, 250, 500 and 1,000 mg treatments, respectively.

Survival

Suspended sediment concentration was not a significant predictor of crayfish survival, for either species, through the 45-day period ($z = 0.94$, $n = 92$, $P = 0.35$) (Fig. 2.). Species was the only significant predictor of survival (z = 1.92, $n = 92$, $P = 0.05$) with higher survival for *P. leniusculus* than *A. pallipes* across all treatments (Fig. 2.). Adult *B. astaci* or cocoons were found in 18 *A. pallipes* individuals (32%), with a maximum of six adult worms and 80 cocoons per individual. The number of individuals parasitised with *B. astaci* did not vary significantly between the four SS levels ($X^2 = 5$, $P = 0.17$, 3 d.f.). Survival of *A. pallipes* was not affected by infection with *T. contejeani*, burden of *B. astaci* (no. cocoons), or the interaction between them ($z = -0.81$, $n = 57$, $P = 0.42$; $z = -0.33$, $n = 57$, $P = 0.74$; and $z = 1.29$, $n = 57$, $P = 0.64$, respectively).

Sediment accumulation in gills

Sediment particles were evident within the podobranchs of all crayfish exposed to the medium and highest SS concentrations and in 92% and 56% of *A. pallipes* and *P. leniusculus* exposed to the low concentration, respectively. *Austropotamobius pallipes* accumulated particles significantly more readily

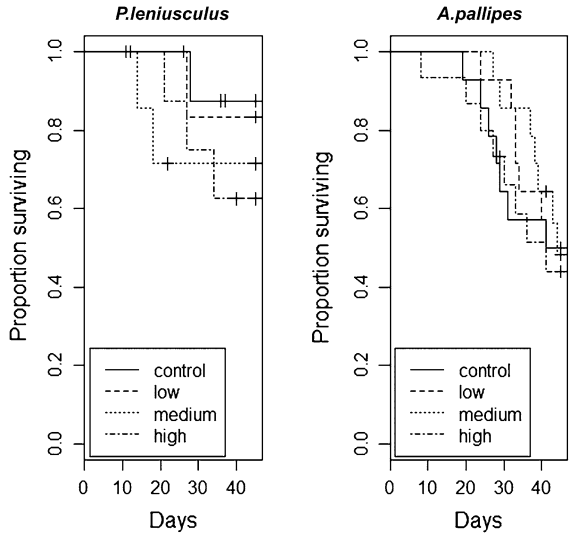

Fig. 2 Stepped survival plots for crayfish *Pacifastacus leniusculus* and *Austropotamobius pallipes* during the 45-day exposure to four SS levels (control, low, medium and high)

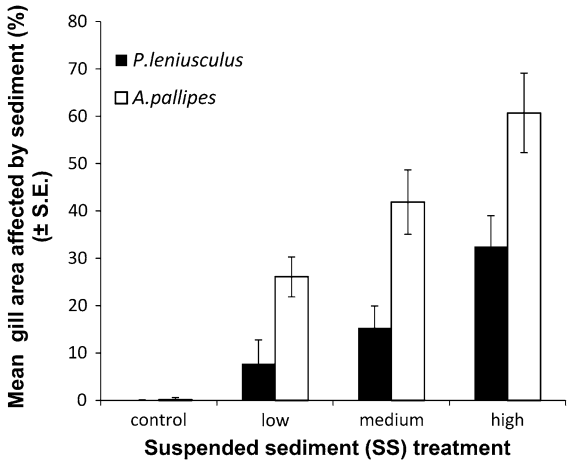

Fig. 3 Mean gill area (%) (±SE) of crayfish *Pacifastacus leniusculus* and *Austropotamobius pallipes* visibly affected by sediment accumulation after 45 days exposure to four SS levels (control, low, medium and high)

than *P. leniusculus* over the 45-day period (23% residual deviance, $P < 0.01$, 1 and 43 d.f.) (Fig. 3). Sediment accumulation in *A. pallipes* was not affected by the *B. astaci* burden, or infection with *T. contejeani*; hence SS concentration was the only significant predictor of sediment accumulation in the gills (51% residual deviance, $P < 0.01$, 3 and 20 d.f.). In some animals, despite heavy sediment accumulation elsewhere in the gills, there was a clear band at the posterior edge of the podobranchs, near the joints of the thoracic appendages (Fig. 4).

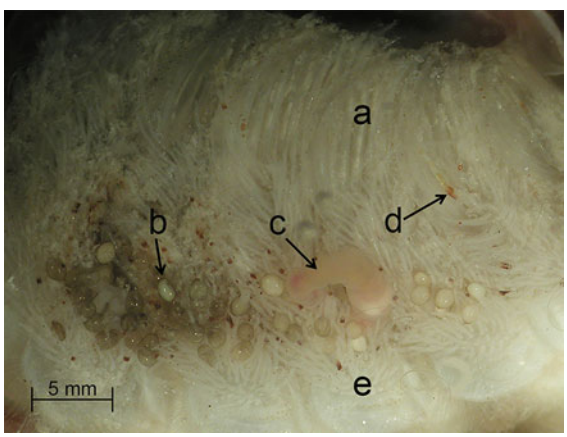

Fig. 4 Podobranchs of crayfish *Austropotamobius pallipes* after exposure to SS (1,000 mg l^{-1}) for 45 days showing heavy sediment accumulation (**a**), cocoons of *Branchiobdella astaci* (**b**), *Branchiobdella astaci* (**c**), gill filament with melanisation spot (**d**) and base of podobranchs free from sediment (**e**)

Melanisation as a percentage of total gill area ranged from 0 to 18% in *P. leniusculus* and 0 to 22% in *A. pallipes*. For *P. leniusculus*, melanisation did not significantly reflect SS concentration, or accumulated sediment level in the gills (7% residual deviance, 23 d.f., and <1% residual deviance, 22 d.f., respectively). In *A. pallipes*, burden of *B. astaci* was a strong predictor of melanisation levels, irrespective of time spent in the experiment (27% residual deviance, $P < 0.01$, 1 and 48 d.f.), and there was a significant interaction between *B. astaci* burden and accumulated sediment in the gills, explaining 10% of residual deviance, 1 and 46 d.f., $P < 0.01$). This result indicated a potential additive effect between *B. astaci* and SS to cause melanisation of gill tissue.

Standard metabolic rate

Estimates of SMR generated using the curve-fitting method (Svendsen et al., 2012), and using 10% of lowest MO_2 values (Herrmann & Enders, 2000), differed by 10–15%. Curve-fitting estimates were always higher and are the only values used hereafter. For juveniles, mean size-corrected (5 g) estimates of SMR were significantly higher in *P. leniusculus* (86.2 ± 3 mg O_2 kg^{-1} h^{-1}, ±SE) than *A. pallipes* (infected and uninfected combined) (70.2 ± 3 mg O_2 kg^{-1} h^{-1}, ±SE) ($t = 3.35, P = 0.005, 14$ d.f.). Within *A. pallipes,* mean SMR was not affected by infection with *T. contejeani* ($t = 1.09$, $P = 0.30$, 8 d.f). It was determined during subsequent dissection that *A. pallipes* infested with *B. astaci* were not present in this subset of animals for which SMR was measured.

Aerobic scope

Individual aerobic scope was determined for juveniles for which SMR had been previously determined. Mean aerobic scope did not differ significantly between *A. pallipes* individuals with and without thelohaniasis ($t = 0.47$, $P = 0.65$, 8 d.f), or between *P. leniusculus* (251.5 ± 18 mg O_2 kg^{-1} h^{-1}, ±SE) and *A. pallipes* (infected and uninfected combined) (206.6 ± 15 mg O_2 kg^{-1} h^{-1}, ±SE) ($t = 1.80$, $P = 0.09$, 14 d.f.). Factorial scope, the ratio of MMR to SMR, ranged 3.3–5.7 among all subjects, with mean value 3.77 ± 0.16 (±SE). For all individuals, MMR was at least 2.8-fold higher than the highest MO_2 values

recorded during routine phase in SMR measurements as the result of spontaneous activity.

On days 1 and 45 of the SS exposure experiment aerobic scope was determined for all juvenile crayfish (>3 g) using individual MMR and, in the absence of estimates of SMR for each individual, mean SMR for the species. Mean scope on day 1 was 221.67 ± 14 mg O_2 kg^{-1} h^{-1} (±SE) for *P. leniusculus* and 223.4 ± 12 mg O_2 kg^{-1} h^{-1} (±SE) for *A. pallipes* (infected and uninfected combined), with no significant difference between the species ($t = 0.09$, $P = 0.93$, 41 d.f.). Although mean scope on day 1 was lower for crayfish infected with *T. contejeani* than those without, the difference was not significant ($t = 1.9$, $P = 0.29$, 19 d.f., equal variances not assumed). Burden of *B. astaci* (no. cocoons) did not affect aerobic scope on day 1.

During the exhaustion protocol *P. leniusculus* on average performed more tail contractions before exhaustion than *A. pallipes* with means 57.2 ± 3 and 48.0 ± 3 (±SE), respectively, though the difference was not significant ($t = 1.72, P < 0.09, 36$ d.f.). There was no significant difference in the mean number of tail flips performed by *A. pallipes* with and without thelohaniasis (49.1 ± 4.5 and 46.0 ± 4.2 (±SE) flips, respectively, $t = 0.469, P = 0.65, 17$ d.f.). Number of tail flips and time to exhaustion were positively correlated ($b = 0.2$, $r^2 = 0.47$, $n = 36$). In the best fitting LM describing variation in scope at day 1, time to exhaust was the only significant predictor of scope ($b = -0.61$, $r^2 = 0.17$, $P = 0.01$, $n = 38$) (Fig. 5).

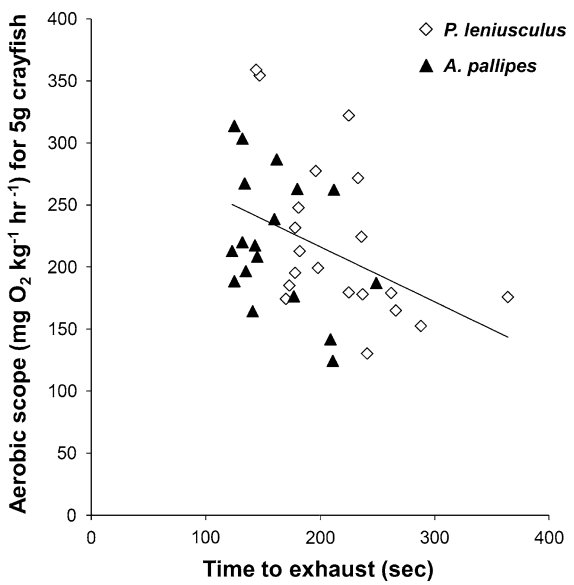

Fig. 5 Aerobic scope (mg O_2 kg^{-1} $^{h-1}$) for crayfish *P. leniusculus* and *A. pallipes* corrected to 5 g individual, as a function of time to exhaust (s) ($b = -0.61$, $r^2 = 0.17$, $P = 0.01$, $n = 38$)

Aerobic scope after SS exposure

For all individuals besides two, aerobic scope on day 45 was lower than on day 1 across all treatments. The minimum adequate model describing 40% of variation in change in aerobic scope over the exposure period identified three significant predictors. Reduction in scope was greater in *A. pallipes* than *P. leniusculus* ($b = 98.17$, $t = 2.62$, $P = 0.02$), and was positively related to both sediment concentration ($b = 0.15$, $t = 2.90$, $P < 0.01$), and level of accumulated sediment in the gills on day 45 ($b = -2.79$, $t = -2.85$, $P = 0.01$) ($r^2 = 0.4$, $n = 23$). Scope was reduced on average 17 and 28% more in the two highest SS concentrations, relative to control. Melanisation level in the gills, change in time taken to reach exhaustion, and change in number of tail flips performed before exhaustion were not significant predictors of change in scope. All interaction terms were non-significant.

Discussion

Understanding a species' habitat requirements and range of environmental tolerances is important for conservation planning, particularly if conservation is to be facilitated through relocation, as is the case for the endangered white-clawed crayfish (IUCN, 1995; Kemp et al., 2003; Armstrong & Seddon, 2008). This study demonstrated a reduction in aerobic scope of crayfish after medium-term (45-day) exposure to SS, a common environmental stressor in freshwater systems, and that native *A. pallipes* crayfish was more susceptible to the SS than invasive *P. leniusculus*. We also found that juveniles of the invasive crayfish had a higher SMR than the native.

The highest time-averaged SS concentration in this study (133 mg l^{-1}) exceeded the highest mean concentration that *A. pallipes* has been found to persist at in the wild (34 mg l^{-1}) (Trouilhe et al., 2007) and exceeded the current recommended levels of 25 mg l^{-1}. Survival in the study did not reflect SS treatment for either species, however, there was mortality in all treatments

and the control, particularly for *A. pallipes*, inferring that a factor other than SS was responsible for the lower overall survivorship in this study than would be expected for crayfish maintained in aquarium facilities (Sáez-Royuela et al., 2002; Gonzalez et al., 2009). One explanation for this may be that the bubble stream within the experimental chambers impaired successful moulting as several crayfish were found dead during their moult, which for the juvenile life-stage used in this study, occurs 4–6 times per year (Pratten, 1980). Ecdysis is naturally associated with heightened risk of mortality in crayfish, both through the physical difficulties of leaving the hardened cuticle and increased risk of cannibalism in the wild (Pratten, 1980, Olsson & Nystrom, 2009). Further investigation, ideally over a longer time period than the 45 days used in this study, is therefore required to elucidate on the implications of SS for crayfish survival.

Crayfish exposed to SS showed signs of sediment accumulation, or fouling, in their gills. Highly fouled individuals showed common patterns with clear bands towards the base of the podobranchs and highest sediment load towards the tips of podobranchs, which likely reflects higher branchial flow velocities near water intake points at the bases of the thoracic joints. Crayfish posses several mechanisms to reduce fouling of the gill filaments by particulates and epibionts. Most important are the setae on the epipods, setobranchs, scaphogthathites and inner surface of the branchiostegites, which are entwined around the gill filaments and furnished with scale setules that scrape over the gills (Bauer, 1998; Batang & Suzuki, 2000). 'Limb rocking' behaviour has also been observed in *Procambarus clarkii* (Bauer, 1998) and *Cherax quadricarinatus* (Batang & Suzuki, 2000), whereby crayfish moved limbs apparently to jostle the setae to aid cleaning. We were unable to make observations of limb rocking in this study. Periodic reversal of direction of branchial flow is another mechanism used to clear debris on the gills of decapods (Arudpragasam & Naylor, 1966). However, cleaning mechanisms were apparently unable to prevent widespread sediment accumulation in the gills under all SS concentrations. The generally lower accumulation in *P. leniusculus* compared to *A. pallipes* may reflect more efficient gill cleaning, though there is to date no comparison of cleaning mechanisms.

SS alone did not cause melanisation of gill tissue; however there was an additive effect between SS accumulation and burden with *B. astaci.* As a mainly gill-dwelling parasite, the impacts of *B. astaci* would be expected to be most apparent in the gills. Adult worms reside mainly in the branchial chamber and the cocoons are deposited among, and attached to, the gill filaments. The presence of melanised gill tissue in *A. pallipes* strongly reflected burden with *B. astaci*, as has been shown in previous studies (Quaglio et al., 2006; Rosewarne et al., 2012), most likely the result of consumption of gill tissue by the adult worms. Melanisation is a generic immune response among Crustacea and results from release and activation of the enzyme phenoloxidase, induced by the presence of microbial products or tissue damage by mechanical wounding, and also possibly due to enzymes released from pathogens (Cerenius & Söderhäll, 2004; Cerenius et al., 2008 and references therein). The increased presence of melanisation observed in parasitised individuals exposed to high SS concentrations may result from sediment particles causing mechanical injury to gill tissues which were already affected by the action of the parasite (Cerenius & Söderhäll, 2004).

Crayfish that accumulated high levels of sediment within their gills showed greater decline in aerobic scope compared to unaffected crayfish. Sediment particles coating the filaments presumably reduced the gill surface area effective for gas exchange, and thus limited the maximum oxygen uptake capacity of individuals with heavy fouling. Scope reduction did not reflect the amount of melanised gill tissue in the present study; however, there was a general decline in scope among all individuals, even those unaffected by sediment, which may reflect sub-optimal conditions in the experimental set-up. A similar study, in which crayfish were exposed to lead, showed impairment of oxygen uptake in *P. clarkii* due to gill damage (Torreblanca et al., 1989). Oxygen uptake capacity in decapods is dependent on several factors including ventilation rate, gill area, diffusion distance, and haemolymph capitance (Harrison & Humes, 1992). Inefficient or damaged gills may induce functional hypoxia to which crayfish are able, up to a point, to compensate for using a variety of measures such as increased ventilation rate, increased diffusive conductance of the gill (Wheatly & Taylor, 1981), and also bradycardia in some cases (Reiber & McMahon, 1998). However, the impacts of gill fouling were evident when crayfish were under increased oxygen demands, as is the case during intense activity.

The overall fitness consequences of reduced scope reflect limitations of energy available for different activities. An individual's maximum scope may only be required at times of extreme oxygen demand such as during rapid bursts of activity to escape from predators, or to compete with compatriots during challenges (Brown et al., 2004). However, if reduced enough it will affect all aspects of the individual's performance as less energy can be devoted to feeding, growth and reproduction (Fry, 1947; Nilsson et al., 2009). In salmonids it has been suggested that at least 60–75% of the aerobic scope is required to avoid daily metabolic constraints and increased risk of mortality (Priede, 1977; Priede, 1985; Evans, 2007). It may be inferred from the lower susceptibility of *P. leniusculus* to SS that this invader would suffer less reduction in fitness and is therefore likely to be more successful than native *A. pallipes* in anthropogenically impacted environments such as those with high SS loadings. Although spread of the crayfish plague pathogen *A. astaci* is the predominant means by which introduced *P. leniusculus* replaces *A. pallipes*, these findings support the hypothesis that tolerance to environmental stressors facilitates the spread of invasive species such as *P. leniusculus* in their introduced range (Crooks et al., 2011).

We found no clear evidence that parasites exacerbated the negative impact of SS on crayfish fitness or mortality, as may be expected when parasites increase the host's susceptibility to a stressor, resulting in disproportionate increases in mortality or loss of fitness (Lafferty & Kuris, 1999). Parasites may interact with environmental stressors in a variety ways, including the converse relationship whereby a stressor reduces the immunological capabilities of hosts making them more susceptible to parasitism, as shown for bivalve molluscs (McDowell et al., 1999). Recognition of the cumulative effects of multiple stressors on organism health, and ecosystems more generally, is increasing (Marcogliese & Pietrock, 2011), though this topic remains largely unaddressed for crayfish.

The mean factorial scopes in this study (3.3–5.7) corresponded to the range quoted for the majority of crustaceans, i.e. 3–5 (Adamczewska & Morris, 1994). Values were lower than the only known previous study of crayfish aerobic scope that recorded a factorial mean scope of 12.4 (at 15°C) for adult *P. leniusculus* (Rutledge & Pritchard, 1981; Wheatly & Taylor, 1981), which is comparable to aerobic scopes reported for the most active of salmonid fish, for example, 12.5 for sockeye salmon (*Oncorhynchus nerka*) (Brett, 1964), and 7–8.5 for pink salmon (*Oncorhynchus gorbuscha*) (Clark et al., 2011). Crayfish, in contrast to fish, have an open circulatory system with generally less efficient gill arrangement and lower oxygen carrying capacity (Rutledge & Pritchard, 1981). Crayfish exhaustion protocols differed between the current and previous study; Rutledge & Pritchard (1981) employed a forced swimming method and measured oxygen consumption concurrently for longer periods, however, they found that consumption rate peaked within the first 5 min of forced activity.

Although crayfish in this study accumulated sediment in time-averaged concentrations at 42 mg l^{-1} and above, it is not clear whether this fouling would persist between moults. Moulting has been hypothesised as a means to escape gill fouling in crayfish (Bauer, 1998), so the impacts on aerobic scope may only persist until the next moult; however, in habitats where SS inputs are chronic and prolonged, lowered scope could be an almost constant state.

Estimates of SMR indicated that juvenile *P. leniusculus* has higher basal energy requirements than *A. pallipes*. SMR denotes the minimum energy requirements for life, excluding all non-essential activity and the specific dynamic action of digestion, and can be indicative of several life history traits and behaviours (Sih, 2004). Although indicative of higher energy costs, higher SMR is commonly associated with faster growth rates and dominance (Metcalfe et al., 1995; Biro & Stamps, 2010; Burton et al., 2011). For example, Brown et al. (2003) found resting metabolic rate a key determinant of outcomes of aggressive interactions between prawns. Higher SMR in *P. leniusculus* compared to *A. pallipes* is indeed consistent with known differences in life history traits between the species such as higher growth rates in *P. leniusculus* (Guan & Wiles, 1999), higher predatory functional response (Haddaway et al., 2012), and dominance during interspecific interactions (Holdich et al., 1995). Such life-history traits are also frequently cited explanations for the invasive capabilities of *P. leniusculus*, and other invasive crayfish (Gherardi et al., 2002; Gherardi & Daniels, 2004; Pintor & Sih, 2009).

The current study suggests that although crayfish are reasonably tolerant of periodic chronic exposure to SS, the structure and function of gills is impaired at

high SS concentrations. Water quality decline with respect to sediment loadings is likely to further exacerbate the effects of the invasive species on the decline of the white-clawed crayfish, which tends to occur in less turbid waters (Trouilhe et al., 2003). It is acknowledged that limitations of the laboratory set-up employed hinder direct inference to natural conditions; nevertheless, our findings provide a valuable first step towards understanding the impact of this common environmental stressor on crayfish. When formulating appropriate relocation strategies for white-clawed crayfish, findings suggest that SS concentration within potential receptor sites should be considered before selection. We recommend that SS concentrations should not exceed 65 mg l^{-1} to minimise negative impacts on gill function, and potentially, population fitness.

Acknowledgements This work was funded by NERC case-studentship with project partners Lafarge Tarmac to PJR, and supported by a grant (SFRH/BPD/89473/2012) from the Foundation for Science and Technology (FCT) in Portugal to JCS. We thank two anonymous referees for helpful suggestions of improvements to the original manuscript.

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