Threatened Species Recovery Hub

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Impacts of post-fire ash and runoff sediment on the physiological tolerances of Australian freshwater aquatic fauna

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Cover image: Euastacus urospinosus. *Image: Narelle Power*

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Executive Summary

With climate warming, bushfires are predicted to become increasingly severe in south-east Australia. Post fire changes to the aquatic environment can include the loss of riparian vegetation leading to increased average aquatic temperatures and increased thermal variability, and the input of ash and sediment that can release nutrients and toxicants into the water. The ability of organisms to physiologically tolerate extensive post-fire changes likely underpins longer-term community compositions. However, we currently have little data to set environmental tolerance thresholds and inform threat mitigation or management responses. Consequently, the aims of this project were to examine how Australian freshwater fish, frogs and invertebrates are affected by acute and chronic exposure to post-fire water quality changes such as elevated temperatures, low oxygen levels and ash and sediment run-off.

This project determined the upper thermal and minimum oxygen tolerances and physiological responses to chronic ash/sediment exposures in four species of Australian freshwater fish species, three species of crayfish, and one of species of freshwater prawn. In addition, species-specific effects of both acute and chronic ash and sediment exposures on amphibian embryo, larval and metamorph survival and performance were also investigated using both laboratory tests and naturalistic mesocosms in three Australian frog species. The study species, while not at elevated bushfire risk directly, can serve as surrogates for closely related species that may be under threat by future bushfire regimes. In addition to the three taxa-specific experimental studies, a systematic review of the effects of bushfire ash and sediment exposure on the physiological tolerances of aquatic fauna was conducted.

Upper thermal tolerance and minimum aquatic oxygen tolerance thresholds differed across the fish and crustaceans consistent with their natural ecologies. Species with the lowest maximum thermal tolerances and highest minimum oxygen tolerances were those from cool, higher altitude sites, while those with broad distributions were typically more tolerant of elevated water temperatures. For the majority of fish and crustacean species tested, thermal tolerances were highly oxygen-dependent, meaning that animals could not withstand high temperatures when aquatic oxygen levels were low. For all fish and crustacean species tested, chronic exposure to conservative, sublethal concentrations of bushfire ash and sediment (200 mg/L) increased the oxygen-dependence of upper thermal tolerance limits, meaning animals were even less tolerant of high aquatic temperatures following ash and sediment exposure. In both fish and crustacean, chronic exposure to bushfire ash resulted in damage to respiratory surfaces that could impair oxygen uptake.

Amphibian embryos acutely exposed to a range of ash concentrations from 1 - 5g/L, showed evidence of increased mortality and developmental abnormalities, and increased rates of energy consumption in later stage larvae. Exposure to bushfire ash and sediment resulted in extensive eutrophication in treatment mesocosms and increased thermal and water chemistry variability. Despite these changes in water quality, there was no effect of ash or sediment exposure on survival or the size and physiological performance of tadpoles reared in these mesocosms. Indeed, tadpoles from ash and sediment treatments metamorphosed slightly earlier and at a larger size than those from control mesocosms, suggesting that increased eutrophication may have improved food availability in treatment mesocosms.

The results from this project revealed that fish and crayfish species from cooler climates or high altitude populations are at greater risk from bushfire induced changes to aquatic thermal and oxygen regimes compared to more broadly distributed and/or lowland species. Irrespective of their underlying species-specific thermal and oxygen sensitivities, chronic exposure to even very low levels of bushfire runoff can lower thermal and oxygen tolerance thresholds and damage respiratory surfaces leading to reduced scope for aerobic performance. These physiological and morphological changes could lead to reduced fitness in sensitive species and altered community composition, particularly where more resilient native or invasive species coexist. Bushfires that occur during or immediately preceding amphibian breeding events could negatively affect successful recruitment; however, low-level fuel reduction burns that occur outside of breeding seasons may benefit amphibians through the fertilisation of aquatic ecosystems, providing additional food sources for herbivorous larval stages. These data will help to guide informed and targeted management of imperilled freshwater ecosystems and species.

Introduction – including statement of the conservation challenges

Fire has played an influential role in shaping Australia's ecosystems for millennia. However, with climate warming (Fox-Hughes *et al.*, 2014, Clarke *et al.*, 2018) associated increases in evaporation, the likelihood of droughts and drier fuels, means that the risk of more intense and more frequent wildfires is expected to increase in the coming decades (Lucas *et al.*, 2007, Bradstock, 2010). The 2019/2020 'Black Summer' mega-fires along the east coast of Australia were of an unprecedented scale and intensity, with enormous fire fronts extending for hundreds of kilometres (Boer *et al.*, 2020, Wintle *et al.*, 2020). While bushfires take an undeniably heavy toll on the terrestrial environment, freshwater ecosystems in and around the burnt areas can be equally catastrophically affected. The loss of vegetative cover directly alters aquatic thermal regimes and aquatic light levels, and contributes to deposition of ash and debris into waterbodies. Vegetation destruction also reduces soil stability and can increase soil hydrophobicity, both of which exacerbate erosion with rainfall events, leading to the bulk deposition of toxic, ash-laden sediments, into freshwater catchments. Importantly, runoff impacts can affect water quality for many kilometres downstream of the fire-impact zone (Earl *et al.*, 2003, Harper *et al.*, 2019). Indeed, following the Black Summer fires, mass fish mortalities were reported 70 km downstream of fire affected areas (Silva *et al.*, 2020). Although many of the effects of bushfires on aquatic ecosystems manifest over relatively short timeframes (hours, days, weeks) (Earl, *et al.*, 2003), in some instances the effects of extreme fires can persist for years (Minshall *et al.*, 1997, Gresswell, 1999, Sheridan *et al.*, 2007).

Loss of riparian shading can have substantial and lasting impacts on aquatic temperatures. The impact of fires on water temperature can be immediate, with the severity depending on the fire's intensity, convection, and the volume of water in the burned region (Rieman *et al.*, 1997).Water temperatures increases of between 7 - 15°C have been recorded with the loss of riparian vegetation, with the effects on water temperatures lasting for up to 15 years until vegetation recovers sufficiently (Gresswell, 1999, Koontz *et al.*, 2018) (Brown *et al.*, 1970). Bushfire runoff often contains a complex milieu of nutrients (e.g., phosphorus, nitrate), ions (e.g., sodium, chloride), metals (e.g., Mg, F, Cu) and polycyclic aromatic hydrocarbons (PAHs) that can cause acute and chronic toxicity to aquatic fauna (Earl, *et al.*, 2003, Silva *et al.*, 2016, Brito *et al.*, 2017, Harper, *et al.*, 2019) and mobilise nutrients to promote the growth of aquatic plants, algae and microorganisms which strip the waterbodies of oxygen. Influxes of silt, unburned organic matter and nutrients can facilitate rapid reductions in oxygen levels in waterbodies by as much as 98% (Lyon *et al.*, 2008). While the most severe impacts of fire-associated nutrient loading on oxygen availability are generally acute, persistent eutrophication effects can remain a problem for years following some fires (Bayley *et al.*, 1992, Silva, *et al.*, 2020). The deposition of fine sediments into waterways can lead to constant increased suspended particle concentrations that can clog and damage aquatic respiratory structures, reducing oxygen uptake capacity.

The effects of bushfires on amphibians is particularly poorly understood. Of those existing studies, most have been conducted in North America and Europe, where population-level responses appear to be highly species-specific, focused largely on the adult life stages and vary widely according to the characteristics of the fire events (Pilliod *et al.*, 2003, Brown *et al.*, 2011, Hossack *et al.*, 2011, Hossack *et al.*, 2013, Lowe *et al.*, 2013, Noss *et al.*, 2015, Westgate *et al.*, 2018, Muñoz *et al.*, 2019). Some amphibian species display a strong aversion to oviposition sites that are surrounded by burnt vegetation, while eggs and larvae exposed to bush-fire ash and fire associated water chemistry changes displayed a variety of changes in growth, development, physiological traits, and ultimately resulting in a significant increase in the energetic cost-of-living and a decrease in overall fitness (McDonald *et al.*, 2018). Other studies have found that exposure to fire ash has no effect or even slightly beneficial impacts for aquatic amphibian life stages (Kross *et al.*, 2017). Given the highly species-specific nature of amphibian responses to post-fire aquatic ecosystems, understanding how the physiologies of native Australian frog species are impacted may provide valuable information in implementing management strategies that guard them against this increasing threat.

Ecosystem level response to extreme bush fire events largely stem from the ability (or not) of organisms to physiologically tolerate or adjust to the post-fire changes in their environments. All organisms operate within a relatively narrow range of water quality tolerances; fluctuations in water quality outside of these tolerances can be lethal or impair physiological performance and fitness. For fish, amphibians and macroinvertebrates, environmental temperature and aquatic oxygen levels are critical determinants of their physiological performance capacity and their performance capacities are optimised within a range of optimal temperature and oxygen levels that reflect their 'normal' environment. Changes in physiological performance are useful indicators of the impacts of environmental stressors on animals and so can form as biologically relevant metrics for setting allowable limits for water quality changes.

These 'tolerance thresholds' can: (i) act as triggers for management interventions such as modelling pre- and post-fire habitat suitability (Hipsey *et al.*, 2015) and monitoring species recovery trajectories, (ii) improve the structuring of water quality management guidelines (that consider species' tolerances of temperature, hypoxia and other stressors), and (iii) inform emergency actions (i.e., post-fire run-off mitigation, or population extractions/relocations (Lyon, *et al.*, 2008). However, currently we understand little about the thermal and oxygen tolerance limits of most Australian aquatic fauna in order to set appropriate tolerance limits, nor the potential for ash and sediment exposures to influence physiological tolerances and aerobic performance.

The aims of this study were to identify species-specific thermal and oxygen tolerance thresholds for a range of fish and freshwater crustacean species, and to examine how aquatic bushfire ash and sediment exposures influence the physiological function and performance of a range of fish, crustacean and amphibian species.

Methodology

Study species:

This study compared thermal and oxygen tolerance limits, and responses to ash exposure in four, small-bodied freshwater Australian fish species: mountain galaxias (*Galaxias olidus*), southern pygmy perch (*Nannoperca australis*), Agassiz's glassfish (*Ambassis agassizii*) and crimson-spotted rainbowfish (*Melanotaenia duboulayi*), and three species of freshwater crayfish: Strong spiny crayfish (*E. valentulus*), Conondale spiny crayfish (*Euastacus hystricosus*), and the tropical Redclaw crayfish (*Cherax quadricarinatus*), and one species of freshwater prawn (*Macrobrachium australiense*). The acute and chronic effects of bushfire ash on amphibian embryos and larvae were examined in Striped Marsh Frogs (*Limnodynastes peronii*), Graceful Tree Frogs (*Litoria gracilenta*) and Ornate Burrowing Frogs (*Platyplectrum ornatus*). These species have been used because they are not particularly threatened, but they have close relatives that may be threatened, in particular by bushfires, and so can serve as surrogates for the more threatened species (Table 1). Study species distribution maps are also provided in Table 1 which provide some insight into natural environmental regimes that may be experienced by animals in the wild.

Animal collection and maintenance:

Several fish species (*N. australis, M. duboulayi, A. agassizii*) and all crustacean species were collected from commercial suppliers and transported to the University of Queensland (Brisbane, Australia). *G. olidus* were wild-caught and donated to the project by the QLD Department of the Environment and Science (DES) following a trial population rescue exercise. All animals were housed in 40L tanks which formed part of 6 x 1000L recirculating aquarium systems. Water temperatures were maintained at species-specific temperatures (20 -25oC) using water chillers. Animals were fed daily with commercial fish food (bloodworms and pellets).

Eggs of six *L. peronii*, four *L. gracilenta*, and four *P. ornatus* clutches were collected after heavy rains from Brisbane and surrounding areas in SE QLD, and were transported to the University of Queensland. A subset of clutches was used for embryonic testing (detailed below). The remaining clutches were placed individually in 2 L containers filled with aged Brisbane tap water, placed in a temperature-controlled room (26°C) until tadpoles hatched and became free-swimming. On hatching, tadpoles were fed grated frozen spinach *ad libitum*.

Thermal and oxygen tolerance testing:

We conducted three tests of physiological tolerance in the fish and crustacean species. Upper thermal limits were compared using standard CT_{max} tests, minimum oxygen tolerance was measured using P_{crit} tests, and we examined the oxygen-dependence of thermal tolerance using a combination of both CT_{max} and P_{crit} test methodologies (termed PCT_{max}). All of these metrics employed a non-lethal loss of equilibrium (LOE) endpoint, where animals lose their righting reflex but recover upon return to their holding tank conditions. For CT_{max} tests, animals were introduced to a well aerated test tank at their holding tank temperature and then water temperatures were slowing increased at a rate of 0.3-0.5°C min^{-1} until animals lost equilibrium. Similarly, P_{crit} tests were conducted by placing animals in a well aerated test tank at their holding tank temperature, and then lowing the aquatic oxygen levels by bubbling nitrogen gas into the water. The aquatic oxygen level at which animals lost equilibrium was designated their P_{crit}. PCT_{max} tests were essentially CT_{max} tests conducted at different aquatic oxygen levels. Animals were placed into well oxygenated test tanks at their holding temperature and then water oxygen levels were lowered using nitrogen gas to the test level (between 5 and 50% air saturation). Once this point was reached, water temperatures were slowly increased (as described previously) until animals lost equilibrium.

Table 1. Study species habitat and distributions and potential surrogate species that may be represented by the study species. All distribution maps were sourced from Atlas of Living Australia (https://www.ala.org.au/).

Study Species	Habitat & distribution		Possible surrogate species
Litoria gracilenta	Breeds in temporary swamps, waterholes in spring & summer		Other ephemeral pond breeding species from family Hylidae
Limnodynastes peronii	Breeds in permanent and temporary swamps, waterholes in spring & summer		Other pond breeding species from family Limnodynastidae
Platyplectrum ornatum	Breeds in temporary swamps, waterholes in spring & summer		Other ephemeral pond breeding species from family Limnodynastidae
Nanoperca australis	Inhabit a wide range of well-vegetated slow-flowing lowland environments, including still or gently flowing streams, lakes, billabongs, drains, dams, swamps and ephemeral creeks and wetlands		Yarra Pygmy Perch (*Nannoperca obscura*) (V); Variegated Pygmy Perch (*Nannoperca variegate*) (V); Oxleyan Pygmy Perch (*Nannoperca oxleyana*) (E); Little Pygmy Perch, *Nannoperca pygmaea* (IUCN Endangered); Balston's Pygmy Perch, *Nannatherina balstoni* (V)
Ambassis agassizii	Occurs in a variety of freshwater habitats in the Murray-Darling basin		Western population *Ambassis agassizii* (NSW - E)
Galaxias olidus	Alpine and subalpine areas of south-eastern Australia. Inhabits clear pools in small, slower flowing streams, preferring areas with sandy, gravelly or rocky substrates.		Flathead Galaxias (*Galaxias rostratus*) (CE); Stocky Galaxias (*Galaxias tantangara*) (CE); Golden Galaxias (*Galaxias auratus*) (E);Swan Galaxias (*Galaxias fontanus*) (E);Barred Galaxias (*Galaxias fuscus*) (E); Clarence Galaxias (*Galaxias johnstoni*) (E); Western Trout Minnow (*Galaxias truttaceus*) (E); Black striped Dwarf Galaxias (*Galaxiella nigrostriata*) (E); Swamp Galaxias (*Galaxias parvus*) (V); Saddled Galaxias (*Galaxias tanycephalus*) (V); Eastern Dwarf Galaxias (*Galaxiella pusilla*) (V); Shannon Paragalaxias (*Paragalaxias dissimilis*) (V); Great Lake Paragalaxias (*Paragalaxias eleotroides*) (V)
Melanotaenia duboulayi	Occupies rivers, creeks, drains, ponds, dune lakes and reservoirs; slow flowing or still water; sandy coastal habitats and forested areas		Daintree Rainbowfish (*Cairnsichthys bitaeniatus*); Malanda Rainbowfish, *Melanotaenia sp.*(CE); Running river rainbowfish (*Melanotaenia sp.)*(ASFB- CE)
Euastacus valentulus	Prefer cool, clear, flowing water. Generally, inhabit upland rivers at medium to high altitudes		Mt Elliot Crayfish (*Euastacus bindal*) (CE); Fitzroy Falls Spiny Crayfish (*Euastacus dharawalus*) (CE); Glenelg Spiny Crayfish (*Euastacus bispinosus*) (E); *Euastacus balanesis (E); Euastacus brachythorax (IUCN – E);* Other mid-high altitude Euastacus sp.
Euastacus hystricosus	Prefer cool, clear, flowing water. Generally, inhabit upland rivers at medium to high altitudes		Mt Elliot Crayfish (*Euastacus bindal*) (CE); Fitzroy Falls Spiny Crayfish (*Euastacus dharawalus*) (CE); Glenelg Spiny Crayfish (*Euastacus bispinosus*) (E); *Euastacus balanesis (E); Euastacus brachythorax (IUCN – E);* Other mid-high altitude Euastacus sp.
Macrobrachium australiense	Found under a range of conditions throughout coastal and inland waters of eastern Australia		Other Australian freshwater prawns
Cherax quadricarinatus	Typically found in permanent freshwater streams, Billabongs and lakes on the north coast of the Northern Territory and northeastern Queensland		Other Cherax species, including invasive *Cherax destructor*

Effects of chronic ash and sediment exposures on fish and crustacean performance:

Fish and crustaceans were exposed to a conservative sub-lethal concentration of bushfire ash and clay sediment (kaolinite) at a rate of 200 mg of ash and sediment per litre of tank water. Bushfire ash was collected from a recent controlled burn through a eucalyptus-dominated forest and sieved to remove large (> 5 mm) particles. The amount of sediment and ash entering water systems is highly context-specific and dependent on the type of fire, vegetation characteristics, post-fire rainfall patterns and proximity to the fire event. We selected a dose rate that was likely to reflect longer-term ash and sediments inputs that may occur sometime after a fire. Similar sediments concentrations have been shown to negatively affect gill structure in juvenile reef fish (Hess *et al.*, 2015, Hess *et al.*, 2017). Fish and crustaceans were exposed to these conditions for two weeks after which time, PCT_{max} tests were repeated. To determine if ash and sediment exposure impacted oxygen uptake, transport and aerobic performance, we also measured blood oxygen carrying capacity, changes to gill structure and, for two fish species, swimming performance.

Effects of acute ash exposure on amphibian embryos and larvae:

L. gracilenta and *L. peronii* clutches were split into three treatment groups: control, low ash = 1 g L^{-1} and high ash = 3 g L^{-1}). Ash was added to replicate 2 L plastic containers half filled with filtered Brisbane tap water and then 100 eggs were added to containers and allowed to hatch. After 72 h, most of the tadpoles had hatched, and any unhatched or malformed tadpoles were counted and removed. In a separate set of trials, tadpoles reared in filtered Brisbane tap water for 8 weeks (Gosner developmental stage 26-30) were then acutely exposed to either 0, 1, 3 or 5 g L^{-1} of ash for 2 h and the effects of exposure on oxygen consumption rates (as a proxy for energy expenditure) were measured using closed system respirometry.

Effects of ash and/sediment on amphibian survival in naturalistic mesocosms:

Twenty 157 litre mesocosms were established in full sun on the roof of the Goddard building, The University of Queensland, Brisbane. These consisted of 250 L round tanks (approximately 1 m diameter x 42 cm high), with two, 40 mm mesh covered drainage holes drilled at a height of 20 cm. Mesocosms were filled with aged tap water and 5mm bird netting was secured over each mesocosm to prevent predation. Each mesocosm contained a small amount of aquarium river gravel, leaf litter and aquatic plants (*Vallisneria gigantea* and *Myriophyllum papillosum*) to provide food and structure for tadpoles. Mesocosms were divided into four treatments (control, ash-only, sediment-only, ash + sediment; 5 mesocosms per treatment). Each control mesocosm was half covered with 70% shade cloth to mimic the shading effects that come with the presence of riparian vegetation. No other treatment was covered with shade cloth. Ash (150g) was added to each ash-only and ash + sediment mesocosm, resulting in an approximate concentration of 1 g L^{-1}, while 150g of kaolinite clay and 600g of river sand was added to the ash + sediment and sediment only treatments. Water quality parameters (temperature, oxygen levels, pH) were recorded periodically through the experiment.

150 recently hatched tadpoles of *L. gracilenta, P. ornatus* and *L. peronii* were added to each mesocosm and left for 3-4 weeks. A subset of tadpoles were then removed from each mesocosm to measure growth and swimming performance characteristics. The remaining animals were left to metamorphose. Metamorphosed frogs were collected from mesocosms, weighed and tested for jumping performance to determine whether exposure to ash and/or sediment affected the physiological performance of resulting frogs.

Findings

Upper thermal tolerance and minimum aquatic oxygen tolerance thresholds differed across the fish and crustacean species consistent with their natural ecologies (Figures 1 and 2). Species with the lowest maximum thermal tolerances and highest minimum oxygen tolerances were those from cool, higher altitude sites, while those with broad distributions were typically more tolerant of elevated water temperatures. For the majority of fish and crustacean species tested, thermal tolerances were highly oxygen-dependent, meaning that animals could not withstand high temperatures when aquatic oxygen levels were low.

For all fish and crustacean species tested, chronic exposure to conservative, sublethal concentrations of bushfire ash and sediment (0.2 g/L) increased the oxygen-dependence of upper thermal tolerance limits, meaning animals were even less tolerant of high aquatic temperatures following ash and sediment exposure (Figure 3). Exposure to ash and sediment decreased swimming performance by ~20% in *A. agassizii*, but not in *N. australis* (Figure 4A). Ash exposure resulted in an increase in blood oxygen carrying capacity in *N. australis* that may underpin the lack of effect of ash exposure on aerobic swimming performance. There was no change in oxygen carrying capacity in either *G. olidus*, *A. agassizzi* or in redclaw crayfish (*C. quadricarinatus*) (Figure 4b). In both fish and crustaceans, chronic exposure to bushfire ash resulted in damage to respiratory surfaces that could impair oxygen uptake.

Amphibian embryos acutely exposed to a range of ash concentrations from 1 - 5g/L, showed evidence of increased mortality and developmental abnormalities, and increased rates of energy consumption in later stage larvae (Figure 5A, B). Addition of bushfire ash and sediment resulted in extensive eutrophication in treatment mesocosms, and increased thermal and water chemistry variability. Despite these changes in water quality, there were no effects of ash or sediment exposure on survival, or the size and physiological performance of tadpoles reared in these mesocosms. Indeed, tadpoles from ash and sediment treatments metamorphosed slightly earlier and at a larger size than those from control mesocosms, suggesting that increased eutrophication may have improved food availability in treatment mesocosms (Figures 6, 7).

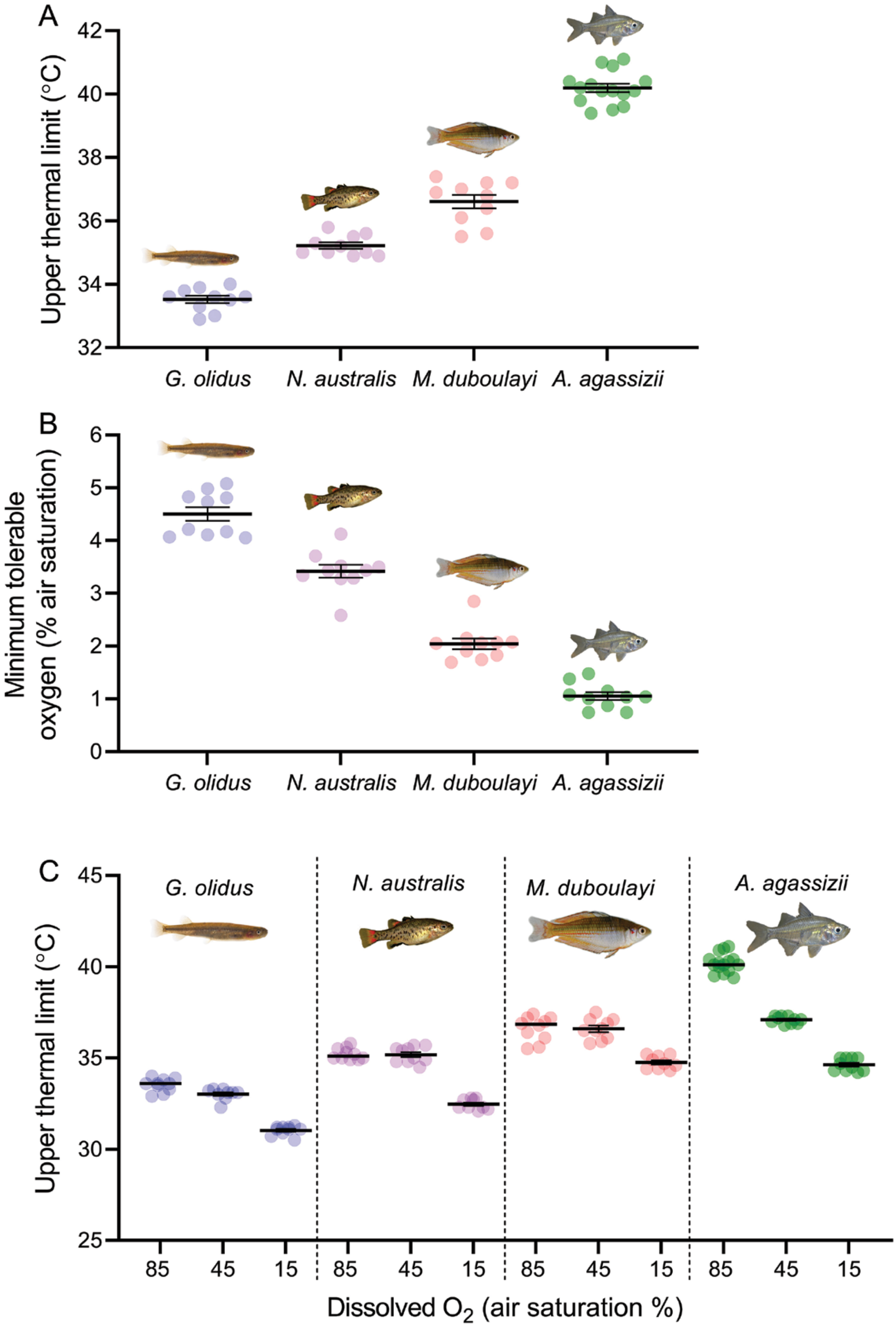

Figure 1. (A) Upper maximum thermal tolerance limits, (B) minimum tolerance oxygen concentration and (C) the oxygen dependence of thermal tolerance in four Australian freshwater fish species.

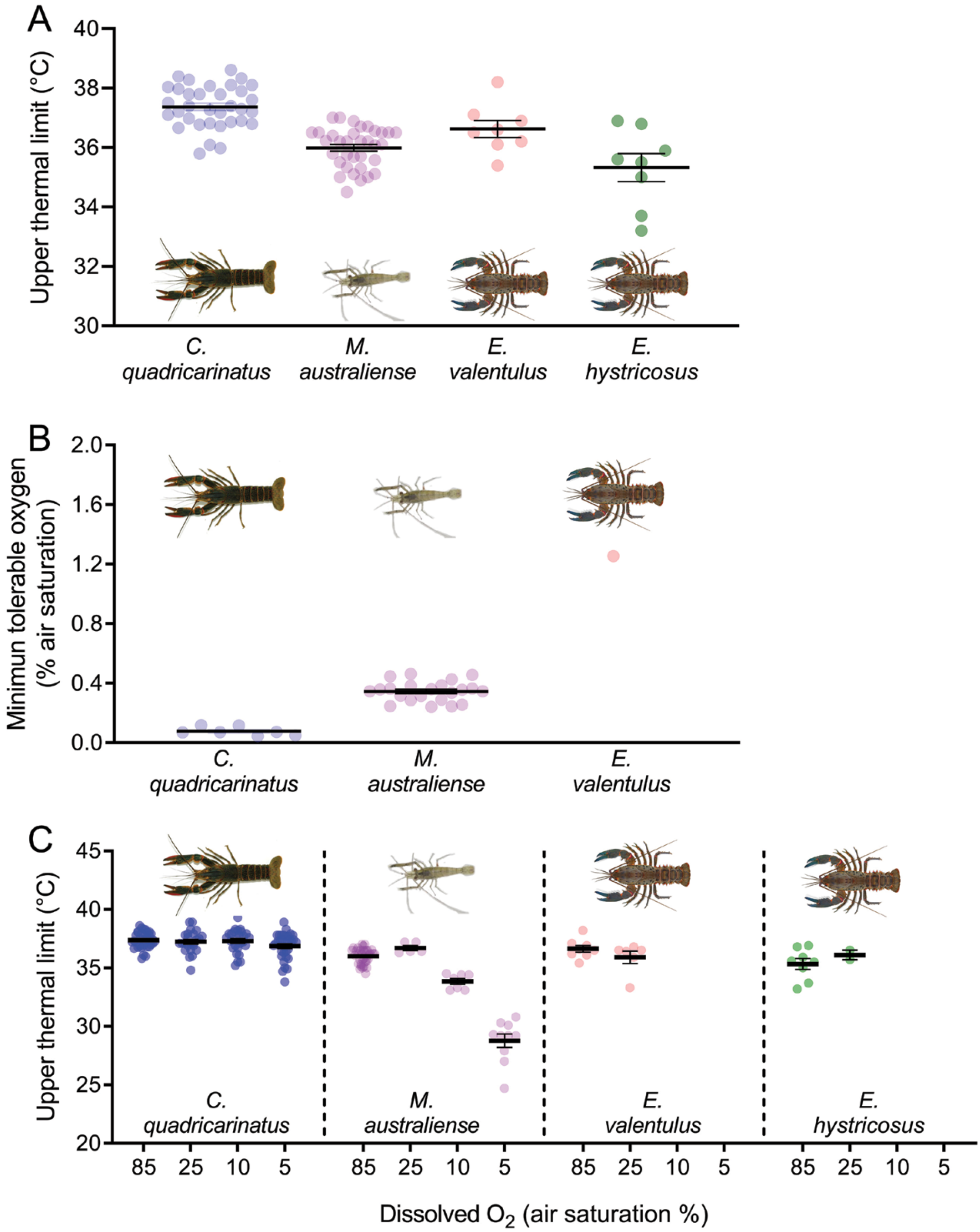

Figure 2. (A) Upper maximum thermal tolerance limits, (B) minimum tolerance oxygen concentration and (C) the oxygen dependence of thermal tolerance in four Australian freshwater crustacean.

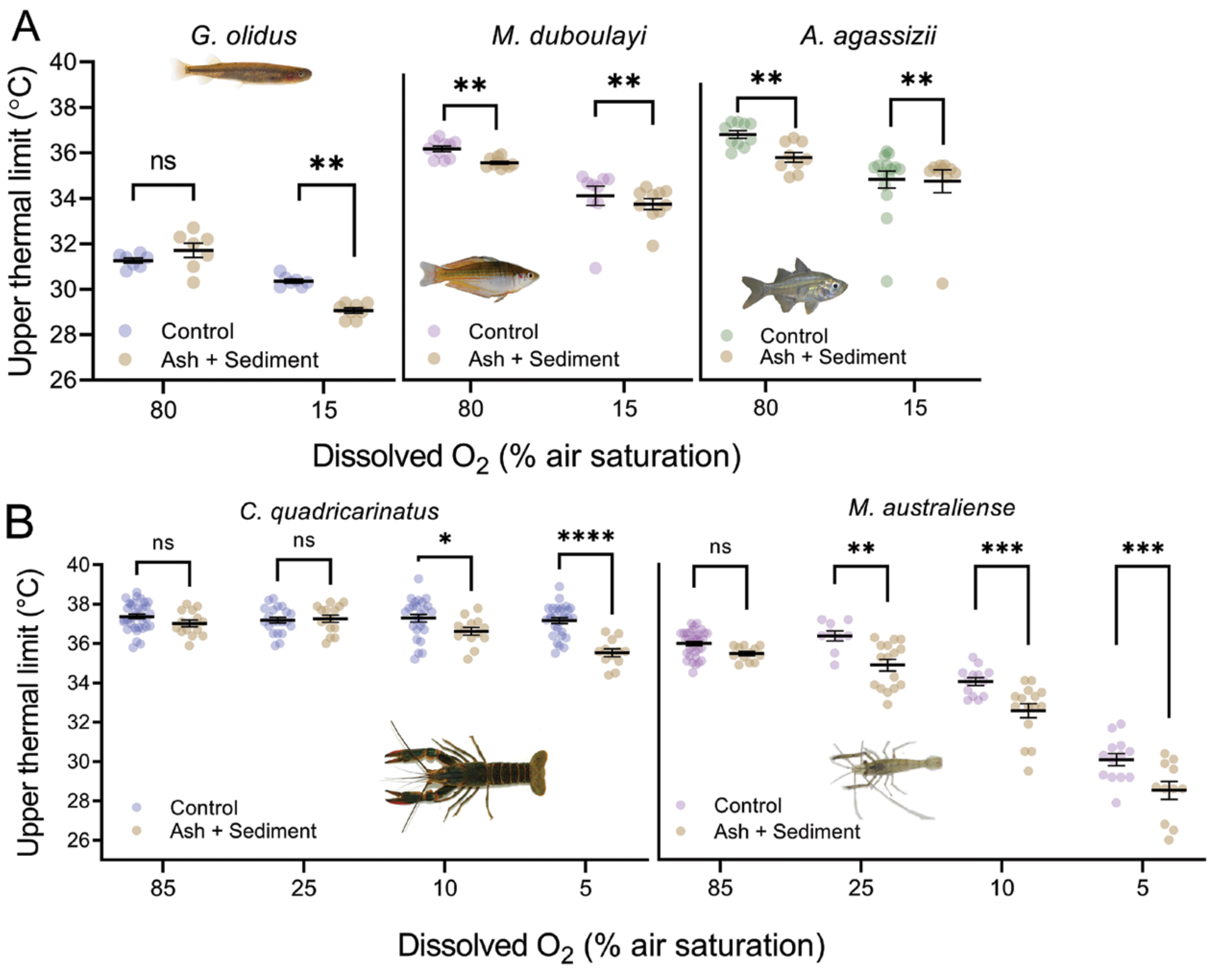

Figure 3. *The effect of 2-weeks exposure to bushfire ash and sediment (0.2 mg/L) on the oxygen-dependence of thermal tolerance in freshwater fish (A) and freshwater crustacean (B). Lines represent means ± s.e. In all species, exposure to ash and sediment increased the oxygen dependence of thermal tolerance, meaning that animals had a lower tolerance of high temperatures when water oxygen levels were low.*

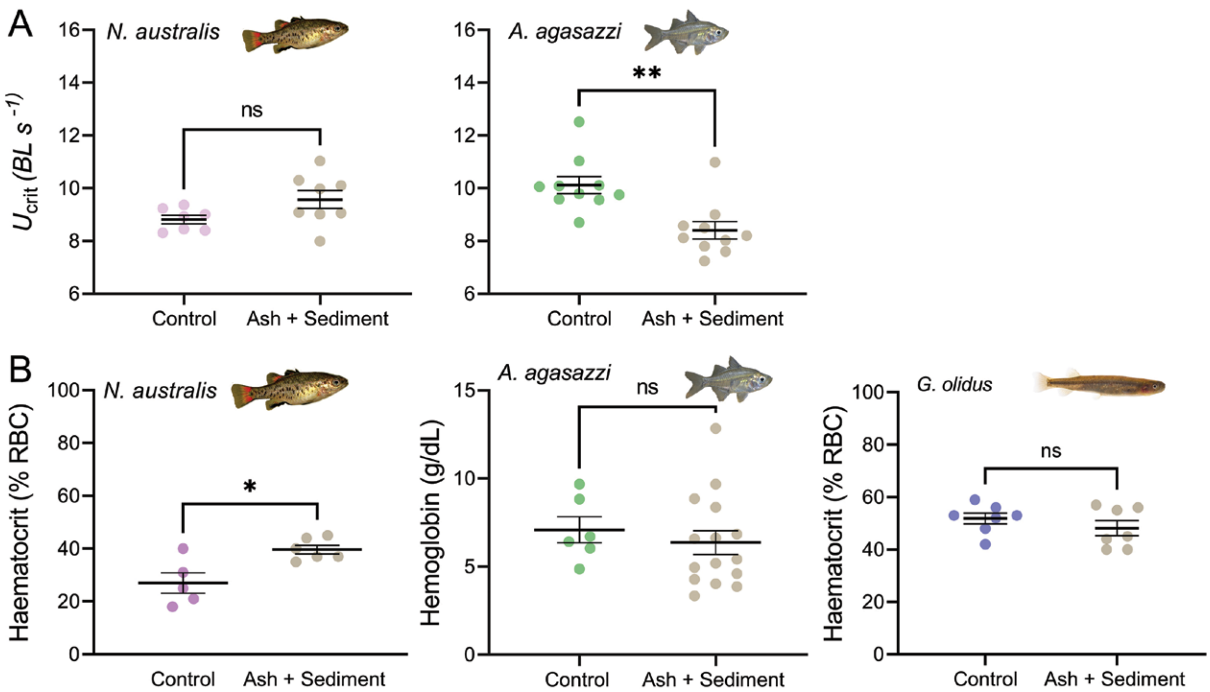

Figure 4. *The effects of chronic ash exposure on (A) the aerobic swimming performance and (B) blood oxygen carrying capacity (as haematocrit or haemoglobin concentration) in freshwater fish.*

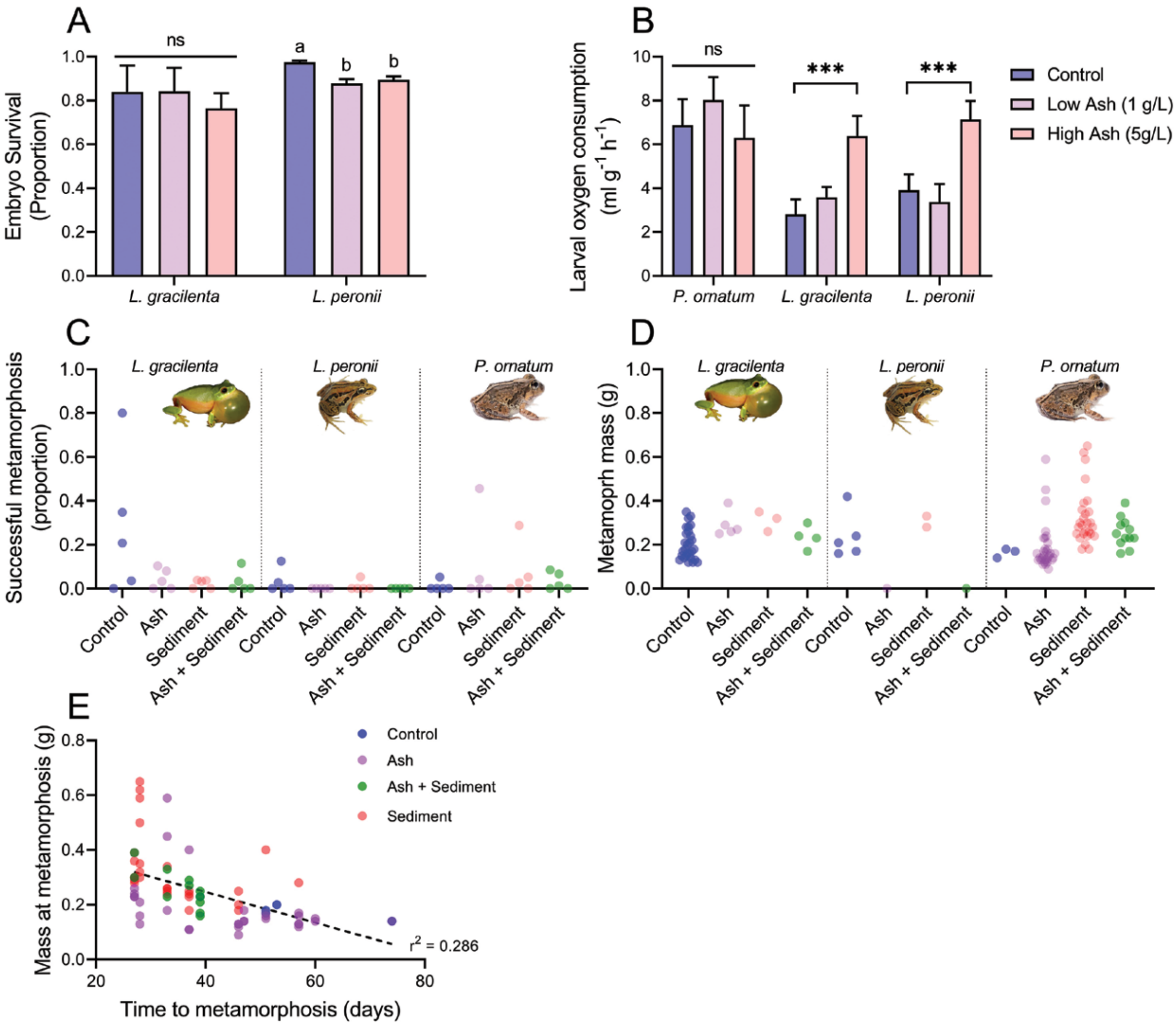

Figure 5. A-B: The effects of acute ash and/or sediment exposure on (A) embryonic survival and hatching success; (B) larval energy expenditure measured as rate of oxygen consumption. Acute ash exposure reduced embryonic survival in L. peronii *but not* L. gracilenta*, while it increase larval energy expenditure in* L. gracilenta *and* L. peronii *larvae, but not* P. ornatum*. C-E: There was no effect of chronic ash and/or sediment exposure in naturalistic mesocosms on (C) larval survival to metamorphosis and (D) mass at metamorphosis. There was a slight trend towards animals from treatment mesocosms metamorphosing earlier and at a larger size than control animals.*

Figure 6. Comparison of mesocosm algal growth between a control (left) treatment and a sediment (right) treatment after 5 days of exposure.

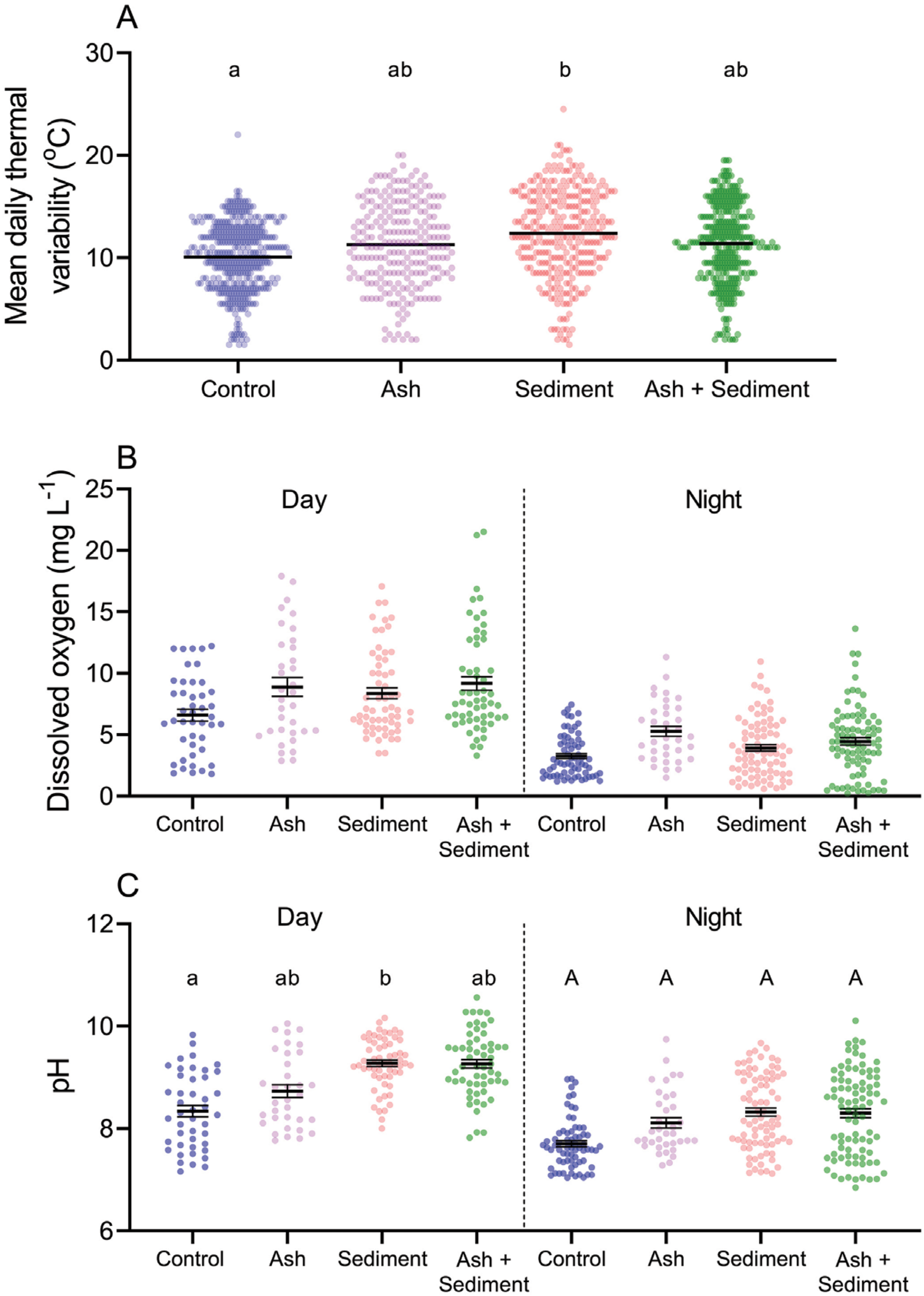

Figure 7. The effects of ash and/or sediment on water quality parameters in 157 L naturalistic mesocosms exposed to full summertime sun. (A) Mean daily thermal variability was slightly higher in the sediment-only treatment mesocosms compared with the control mesocosms; (B) There was no effect of ash and/or sediment treatments on aquatic oxygen levels, however there was a strong diurnal effect; and (C) aquatic pH was higher in the sediment only treatment mesocosms compared with the controls. Again there was a strong diurnal effect on aquatic pH.

Discussion

The results from this study revealed that fish (e.g. Galaxids) and crayfish species (e.g. Euastacus species) from cooler climates or high altitude populations are likely to be at greater risk from bushfire-induced changes to aquatic thermal and aquatic oxygen regimes compared to more broadly distributed and/or lowland species. This is because both their species-specific thermal and aquatic oxygen tolerance limits are lower than those of species with low-land or more tropical distributions. These finding are consistent with existing literature on the physiological tolerances of ectothermic animals with temperate or tropical distributions. All animals were held at similar acclimation temperatures preceding testing, meaning that differences in thermal tolerances are the result of inherent species differences and not the result of differences in acclimation temperatures.

Irrespective of their underlying species-specific thermal and aquatic oxygen sensitivities, chronic exposure to even very low levels of bushfire runoff can lower thermal and oxygen tolerance thresholds and damage respiratory surfaces leading to reduced scope for aerobic performance. Our data are also consistent with studies conducted in other parts of the world showing that relatively acute changes in water quality following a bushfire may be better tolerated by aquatic fauna, but effects may accumulate with sustained sub-lethal exposures. In both fish and crustacean, just two weeks of exposure to low-dose ash and sediment solutions reduced thermal tolerances. With the loss of riparian vegetation along burned water courses, the negative effects of the resulting increase in mean water temperatures and increased thermal variability on aquatic ectotherms is likely to be compounded by the presence of ash and sediment in the water. These data indicate that long-term assessments are necessary gain insight into the lasting impacts of bushfire associated changes in water quality on aquatic fauna.

Across all four taxa, species differed in their relative sensitivity to ash and sediment-induced physiological disruptions. These findings indicate that post-fire conditions may result in selection for species with more resilient physiologies, which in turn may lead to significant changes in community composition. This may be a particularly important consideration for managing the post-fire risk of invasion by noxious or highly invasive species such as carp or *Gambusia* that have extreme tolerances to a broad range of environmental variables (Lennox *et al.*, 2015). It is important to note that many of species examined in this study are common and widespread with relatively broad tolerances to a range of water conditions (excluding *Euastacus* sp. and *Galaxias olidus*); consequently they are likely to be more resilient against fire-induced changes than rare and or threatened species with more restricted distributions and a narrower range of water quality tolerances. Despite this, we were able to detect detrimental physiological changes in response to ash and sediment exposures in a number of typically robust species. While many of these changes were subtle, it is possible that the magnitude of effects could be substantially higher in other species, or life stages.

Bushfires may differentially affect different life stages of animals within aquatic environments. Our study suggests that bushfires that occur during or immediately preceding amphibian breeding events could negatively affect successful recruitment through the detrimental effects of ash exposure on embryonic and larval survival. The metabolic costs of smaller, juvenile animals are disproportionally greater than larger adult life stages because of the allometric scaling of metabolism. This means that changes to the aquatic environment that increase metabolic costs such as increases in water temperature, may differentially affect juvenile life stages. A recent study of Murray cod covering a 3-fold range of body sizes show that thermal and hypoxia tolerances are reduced in both very small and very large animals (McPhee, unpublished finding), meaning that these life stages may be disproportionately affected by post-fire thermal and oxygen disturbances. Despite this though, our data also suggest that the timing and magnitude of fire events is important in determining whether fires will have a detrimental impact on amphibians. Indeed, low-level fuel reduction burns that occur outside of breeding seasons may benefit amphibians through the fertilisation of aquatic ecosystems, providing additional food sources for largely herbivorous larval stages.

We did not examine the effects of ash and sediment exposures on animal behaviours that may allow animals to either moderate their exposure to deleterious post-fire conditions or exacerbate the negative impacts on physiological fitness. Bushfire ash has been shown to significantly alter various behaviours of fish (Gonino *et al.*, 2019). Fish exposed to high ash loads showed drastic reduction in activity levels, spending 56% of their time resting compared control (unexposed) fish, which spent just 31% of their time resting. Bushfire ash also decreases fish boldness and shoal cohesion. These behavioural alterations are likely to have fitness consequences as reduced exploratory behaviours that would prevent fish from escaping poor water conditions, and decreased shoaling behaviours may increase the risk of detecting and evading predators (Ward *et al.*, 2011, Goldenberg *et al.*, 2014). Contrarily, some behaviours changes may be adaptive, namely boldness, preventing fish from adopting risky behaviours and increase their survivorship in adverse environments (Biro *et al.*, 2009).

Application of research (to-date and anticipated)

This incidence of fire in Australia is expected to increase substantially in the coming years, and with this the need for further research in into the impacts of fire on both terrestrial and aquatic ecosystems. Post-fire impacts can be devastating for aquatic fauna. The multifactorial nature of these impacts supports the need for informed, species-specific, and adaptive management in fire-affected systems, particularly for systems containing threatened or vulnerable aquatic species. Although the study species in this project were largely chosen as surrogates for related threatened species, our data suggest that those with existing restricted, alpine or temperate distributions are likely to be disproportionately affected by post-fire changes to aquatic environments.

Our study provides a suite of thermal and oxygen tolerance limits for several freshwater fish and crustacean species that may serve as management intervention triggers. However, given the endpoint of our metrics are close to lethal limits, thermal and oxygen tolerance limits should be lowered by several degrees to ensure that interventions can occur in a timely manner well before lethal limits are approached. Thermal and oxygen tolerances are likely to be phylogenetically constrained (Hoffmann, 2010) and therefore similar between closely related species occupying similar environments. Importantly, our study shows that for some fish and crustacean species, interactions between thermal and oxygen stresses can lower overall tolerance thresholds. This is likely to be the case for other competing stressors such as exotic competitors and predators, and other water quality changes (Lintermans, 2000, Raadik, 2014, Todd *et al.*, 2017) and needs to be considered in management actions.

Our data also suggest that while acute bulk input of ash and sediment into aquatic systems following fires can cause severe decreases in water quality, the magnitude of impact on individuals in these systems is likely to be highly species- and life history stage specific. Early life stages are likely to be particularly sensitive to acute changes in water quality. However, chronic exposure to ash and sediment could have contrasting impacts across taxa, with some deriving benefit while other experience a decline in physiological fitness. These contrasting effects underline the importance of a species- and life-stage-specific understanding of the impacts of post-fire changes to water quality.

Physiological tools can be used to increase our understanding of the factors driving post-fire changes to aquatic ecosystems and predicting the impacts of increased fire frequency and severity. The utility of experimental physiology means that the environmental boundaries within which animals operate can be determined from the level of a single stressor to more ecologically realistic multi-stressor studies. A management strategy that incorporates the use of physiological limits will provide a more holistic picture of the consequences of fire aquatic ecosystems as a whole.

Impact of the research (to-date and potential in future)

This research has recently been used by DAWE staff to inform EPBC assessments for a potentially fire-impacted freshwater lamprey, and other native freshwater fish.

Future research priorities

Key future research areas should prioritise further investigation into the relationship between aquatic oxygen levels and elevated temperatures for a greater range of aquatic fauna, and the compounding nature of ash and sediment exposure on physiological tolerance limits. Future studies should explore impacts across the range of life stages to understand how fire-related effects change with ontogeny. Additionally, the stress of severe acute and/or protracted exposure to post fire water quality changes may compromise immune function and this has implications for the management of existing and emerging diseases on aquatic communities. Finally, an examination of the toxicity of fire retardants on native aquatic fauna is urgently required.

Data sets

All data will be made available through The University of Queensland's eSpace repository (to follow).

Recommendations

- Post-fire changes to aquatic systems can result in complex changes in water quality that may differentially affect species persistence. Further research into the nature, magnitude and continuance of these effects and their implications for animal physiological fitness is required.
- Species are likely to be differentially affected by post-fire changes to water quality - species from cooler climates or high altitude populations are at greater risk compared to more broadly distributed and/or lowland species. Preventative management of at-risk ecological communities is required to limit the impact of future fire events on these environments.
- Chronic exposure to even very low levels of bushfire runoff can lower thermal and oxygen tolerance limits. These complex interactions need to be considered when developing management intervention triggers/thresholds.
- Bushfires that occur during or immediately preceding amphibian breeding events could negatively affect successful recruitment; however, low-level fuel reduction burns that occur outside of breeding seasons may benefit amphibians through the fertilisation of aquatic ecosystems, providing additional food sources for herbivorous larval stages

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Ethics statement:

All studies were conducted with the approval of The University of Queensland Animal Ethics Committee (SBS/352/20), the QLD Department of the Environment and Science (Scientific Purposes Permit WA0017092) and the Queensland Department of Fisheries (General Fisheries Permit 208676).

Graceful tree frog. Image: Coen Hird

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Further information:

http://www.nespthreatenedspecies.edu.au

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