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Experimental increases in detritus boost abundances of small-bodied fish in a sand-affected stream

Running head: Fish responses to detritus in a sand-affected stream

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Abstract

1. Restoration projects often rely on the assumption that a local intervention will restore diminished populations, without fully understanding the constraints that limit the target species in the first place. In rivers, one common restoration technique is to place large structures, like wood and boulders, on the bed, with the assumption that fish will subsequently arrive and use them. Nonetheless, providing large habitat structure may not overcome demographic or resource constraints on fish populations, and thus may not aid recovery. We aimed to test if resource constraints (food and cover) are limiting local densities of fishes in a degraded stream by experimentally alleviating these constraints. If the abundance of one or more species is constrained by resource availability, then local numbers of these species should increase following an increase in resources.
2. To test our prediction, we increased the availability of food and microhabitat complexity (cover) at sites in Hughes Creek, a degraded stream in south-east Australia that has extensive accumulations of sand and limited in-stream structure. At treatment sites, we hammered pairs of wooden stakes (25 cm apart) into the stream bed so that the end of stakes protruded just above the water surface at moderate flows. Stakes effectively trapped passing sticks and leaves, which increased local detrital densities and, subsequently, invertebrate densities, hence providing food and cover for fish. Over the course of a year, we compared the changes in fish abundances at treatment sites and unmanipulated control sites.
3. Fish responded quickly to enhanced retention of detritus, with assemblage differences observed between treatment and control sites. We caught more river blackfish (*Gadopsis marmoratus*), southern pygmy perch (*Nannoperca australis*), Macquarie perch (*Macquaria australasica*) and mountain galaxias (*Galaxias olidus*) at treatment sites on some occasions, indicating that these species may be subject to resource constraints in the stream. The magnitude of observed positive fish responses was influenced by the life-stage of individuals and local stream conditions. Importantly, treatment effects varied through time and were no longer observed after a large flood affected the study stream.
4. Our results show that resource constraints limit local species abundances and demonstrate a novel method of overcoming these constraints in a small, degraded stream. This is a necessary first step. Future work is needed to examine whether increases in abundance are due to the provided resource increasing growth rates, survivorship or reproduction. This work also highlights the importance of understanding species' life history, the broader landscape setting and the disturbance regime when undertaking site scale restoration.

INTRODUCTION

River channels present clear gradients in physical variables (e.g. in substrate size, water temperature, channel width, etc.), and these gradients have been a strong focus for understanding the distribution and abundance of plants and animals along channels (e.g. Power et al., 1988; Statzner & Higler, 1986). However, densities of organisms are also affected by access to essential resources, such as food and living space. For example, in-stream algae and allochthonous detritus are food resources for multiple riverine taxa, and these resources can also show gradients along channels, with implications for the abundance and distribution of taxa (e.g. Rosi-Marshall & Wallace, 2002). Thus, it is necessary to look at both physical conditions and resources to understand constraints on population numbers.

Knowledge of the interacting roles of environmental and resource constraints is critical to guide actions to repair or rehabilitate environments. Many rivers and streams worldwide have been degraded by anthropogenic disturbances (Dudgeon et al., 2006), and significant funds are expended globally on restoration actions (Bernhardt et al., 2005; Brooks & Lake, 2007). The focus for in-stream restoration is often on repairing the physical environment by returning habitat structure (e.g. wood and rocks) or complexity (e.g. bends, riffles) to the channel (Hale, Mac Nally, Blumstein, & Swearer, 2019), but many projects fail to deliver improvements in species diversity or abundances of target organisms (Palmer, Hondula, & Koch, 2014; Roni, Hanson, & Beechie, 2008). There are multiple, prospective reasons for these failures (Bond & Lake, 2003b; Hale, Blumstein, Mac Nally, & Swearer, 2020) but one problem could be that the stated goals of many projects are to “improve habitat” by increasing physical structure in the environment or by altering flows (Palmer et al., 2014). This approach assumes that physical conditions primarily determine species abundances, and the implicit hypothesis is that an increase in the amount of habitat structure leads automatically to greater population abundances (the 'Field of Dreams Hypothesis': Palmer, Ambrose, & Poff, 1997). However, predictions of population numbers are unlikely to be successful without including the effects of essential resources (Lancaster & Downes, 2010).

A major reason why rehabilitation focuses on returning structures to streams is that some fish species are associated with in-stream structure (e.g. rocks, wood, macrophytes), which can enhance both survival and reproduction (Crook & Robertson, 1999). Moreover, the removal of wood from streams is associated with declines in fish numbers (Tonkin et al., 2020). Although fish are known to require large woody debris for cover, some fish, particularly small-bodied species, may depend more upon the complex arrangement of spaces provided by branches (Howson et al. 2012). This small-scale habitat complexity can be created by benthic packs of leaves, twigs, bark and small sticks (coarse particulate organic matter, hereafter detritus) that typically accumulate in areas of low flow and around wood and boulders and provide essential resources of food (such as macroinvertebrates) and shelter (Dala-Corte et al., 2016). A hypothesis for declines in small-bodied fish populations is that the loss of in-stream structure has led to low levels of detritus, and thus reduced essential resources for some species.

While loss of detritus may have deleterious impacts on streams, the removal of woody debris – either directly or because of clearance of riparian vegetation – causes a loss of channel retentiveness (the capacity of channels to trap and hold floating detritus: Bovill, Downes, & Lake, 2020; Webster, Covich, Tank, & Crockett, 1994). Clearance of native vegetation can also trigger erosion that deposits tonnes of sediment into streams, which smothers the bed (Davis & Finlayson, 2000). Shifting sediments rapidly bury retentive structures, leading to reduced channel retention capacity and consequent very low densities of benthic detritus and diminished macroinvertebrate diversity and densities (Downes, Lancaster, Glaister, & Bovill, 2017). A recent experiment demonstrated that improving the retentiveness of a sand-affected stream (Hughes Creek in south-eastern Australia; location of this study) boosted densities of benthic detritus across the stream bed by up to 42x, and this doubled the species richness and density of macroinvertebrates (Lancaster & Downes, 2017). Theoretically, these effects should have boosted food and cover for fish, but, to our knowledge, there has been limited work examining resource constraints caused by a lack of benthic detritus on stream fishes, especially in a restoration context.

Here, we conducted two experiments in which densities of detritus were manipulated along Hughes Creek. Over > 12 months, we tested whether abundances of several key fish species increased following local enhancement of detritus, hypothesising that, if fishes are subject to resource constraints, then abundance of those species will increase at sites where detritus was increased. The two experiments were located in different parts of the creek that had been subject to different intensities of human impact, hence we also measured pertinent environmental variables and explored their variability along the creek and relations to fish abundances. Monitoring our experiments over a longer time frame also allowed us to explore how experimental effects changed over time, particularly in relation to the environmental context.

Most restoration projects are assessed using indicators of changes in population size or community composition (Hale et al., 2019). Showing that populations of target taxa have increased following restoration is important but only a first step; it does not tell us if increases were due to changes in vital rates or simply because restoration has led to a redistribution of individuals within the system. Further and more detailed experiments are needed to test the mechanisms of these changes. We therefore discuss our results as an initial exploration of the importance of resource contraints on stream fishes, and highlight some potential options for future mechanistic experiments.

METHODS

Study area

We undertook fieldwork in Hughes Creek in Victoria, Australia (Fig. 1). Hughes Creek is spring-fed and generally has year-round surface flow, but discharge varies seasonally in relation to rainfall patterns. Peak rainfall is typically between June and August (Erskine, 2016). The creek rises on the Strathbogie Ranges and flows off the Highlands Plateau through an area with relatively intact vegetation (riparian width > 200 m, fragmentation ~ 10%: Downes et al., 2017). Our sites were located in three contiguous zones, the upper, middle and gorge zones. Zones were determined based on geographic location and the characteristics of the reaches in which they occurred, with reaches as defined by Erskine (2016), who described the entire length of Hughes Creek in detail (Fig. 1). The upper zone (Reaches 6 and 7) is moderately sandy, with some deeper pools and in-stream structure. Below this, the creek flows through the middle zone (Reach 5), which is an area widely cleared of vegetation for agriculture, although a discontinuous strip of trees remains along the banks of the creek (riparian vegetation width ~ 50 m, fragmentation ~ 80%: Downes et al., 2017). This zone is characterised by large aggradations of sand and limited pool development. The creek then flows through the gorge zone (upper section of Reach 4) for approximately 2.5 km, with some pools and riffles present. The riparian vegetation is mostly intact through the gorge zone, but the surrounding land has been largely cleared. Immediately downstream of the gorge zone (within Reach 4), the creek flows through an area of cleared vegetation followed by a second gorge with intact vegetation (average riparian width ~ 100 m, 20% fragmentation: Bovill et al., unpublished data). The creek then emerges onto a flat floodplain that has again been largely cleared of vegetation for agriculture (average riparian width ~ 30 m, fragmentation ~45%; Bovill et al., unpublished data), whereafter it joins the Goulburn River.

The original morphology of Hughes Creek was characterised by a series of pools, runs, riffles and bars (Erskine, 2016). Land use change through the 1900s associated with vegetation clearance and a large flood in 1916 resulted in erosion that deposited substantial amounts of granitic sand into the creek, which largely covered the stream bed all the way to the town of Avenel (Fig. 1). A further large flood in 2010 re-mobilised this sand, which buried riffles and in-filled pools in Reach 4 and also deposited a lot of sand on the bed, channel margins, bars and benches of Reach 5 (Erskine, 2016).

Study design

We tested our hypothesis by manipulating the amount of plant detritus retained on the stream bed in two experiments. First, we used some of the original, and on-going, experimental sites of Lancaster & Downes (2017), who increased benthic detrital retention in 40 m-long, straight channel lengths of Hughes Creek during 2013 by hammering multiple pairs of wooden stakes (120 cm long, 0.13 – 0.19

pairs of stake.m^{-2}, spread throughout each site) into the stream bed, approximately 25 cm apart. The ends of stakes protruded just above the surface of the water at moderate flows, trapping passing detritus. These stakes were left in place to maintain experimental treatments, with control sites left unmanipulated. We re-used eight of their sites that spanned the upper and middle zones (four manipulation sites, four controls: “Experiment 1”, Fig. 1). While these sites had well-established experimental effects of almost four years duration, no data on fish abundances were collected prior to the experiment being started. Moreover, the sites were deliberately restricted to straight lengths of channel (to minimise losses of stakes from erosion or deposition of sediments). Thus, we established eight new sites in the gorge zone (four manipulation and four controls: “Experiment 2”, Fig. 1) in the same manner as Experiment 1. Sites were selected randomly from places where sand was across the bed but, unlike Lancaster & Downes (2017), we did not exclude places where the channel contained bends given the latter may benefit fish by providing undercut bank habitat. Sites were randomly assigned to treatment and established following the same protocols of Lancaster & Downes (2017).

Invertebrate and detritus measurements from concurrent studies

Because Experiment 1 sites were from an on-going experiment, we were unable to sample detritus in this study. Instead we relied on samples that were collected during February 2018 (using methods which leave most detritus intact at sites) as part of the aforementioned long-term experiment (Lancaster & Downes, 2021). Detritus (bark, leaves, twigs, small sticks) was collected using 15 randomly placed Surber samples at each site and dried and weighed as per Lancaster & Downes (2017). While these samples were collected prior to the end of fish sampling (Fig. 2), we are confident treatment differences were maintained because wood stakes retain detritus throughout the year (Lancaster & Downes, 2017). Our intention was to also sample detritus and invertebrates from Experiment 2, but a large flood (Fig. 2) unexpectedly removed many of the stakes in early December 2017. Because Lancaster & Downes (2021) demonstrates that stakes reliably retain detritus over a long length of Hughes Ck (across three reaches), we think that study provides sufficient evidence for a treatment effect in Experiment 2. However, for completeness, we also report as yet unpublished data collected in late November 2017 for a different project. This second project had an identical set of new experimental sites that were installed within and downstream of the second gorge in Reach 4, approximately 6 km downstream of Experiment 2 (Bovill et al. unpublished data). It had three control and three manipulation sites within the agricultural area and a reference site within the second gorge. Detritus and invertebrate densities for this second project were sampled prior to treatment and again in late November 2017 before the aforementioned flood, and as per the protocols in Lancaster & Downes (2017).

Field measurements of site characteristics

The physical environment of sites was characterised by measuring environmental variables typically used to quantify fish habitat (Bond & Lake, 2003a) on 15 and 22 March 2017 (Experiment 1) and 14 May 2018 (Experiment 2). Mean daily discharge reflected typical base flows on both of these dates (Fig. 2). At 20 randomly selected points in the active channel of each site, we measured the water depth (cm) and the mid-water flow velocity ($m.s^{-1}$) using a Valeport Flow Meter (Model 002, 50 mm diameter impellor; Valeport Ltd, Totnes, Devon, United Kingdom) to calculate average water depth and velocity. We assessed each site's structural complexity by measuring the total area of backwater (m^2), the total length of undercut bank (m), the length of bank that had emergent macrophytes or trailing terrestrial vegetation in the water (m), and the total area of aquatic macrophytes in the channel (m^2). In-stream macrophytes and bank macrophytes were distinguished as they potentially represent different microhabitats; bank-restricted macrophytes may be interacting with undercut banks and backwaters in areas of low flow, while instream macrophytes were often in areas of higher flow.

Wood loading was assessed by counting and measuring all submerged, in-stream woody structure at each site using the methods of Webb & Erskine (2003). This included individually distinguishable pieces of wood and small and large debris dams (accumulations of multiple pieces of wood and detritus). The lengths and diameters of pieces of wood were measured and multiplied to calculate an area of wood in the stream. The wetted area of debris dams was calculated by estimating the percent area of a 1 x 1 m quadrat that each dam covered. All wood in a randomly selected 10 m length was assessed, and then multiplied by four to estimate the wood loading in m^2 for the 40 m site (as per Downes et al., 2017). At experimental sites, wood loading surveys did not include any debris that was clearly trapped by pairs of stakes, because the aim was to assess the broader stream characteristics independent of the experimental effect.

Fish sampling

Experiment 1 sites were surveyed for fish on seven separate occasions from late November 2016 to mid-April 2018, and Experiment 2 sites were surveyed prior to treatment in April 2017 and then on five occasions from June 2017 to mid-April 2018 after treatments were established and simultaneously with Experiment 1 (Fig. 2). We used a backpack electrofisher (Smith-Root Model LR-20B, Smith-Root Inc., Vancouver, WA, U.S.A.) to sample adult and juvenile fish. We followed the electrofishing protocol of Bond & Lake (2003a), in which electrofisher effort was related to the habitat complexity within each site, which was exhaustively sampled and therefore ensures comparable sampling efficacy between sites. Some fish were observed within the bounds of sites while fishing but escaped before they could be caught by the net operator. If the species could be identified, they were noted as present and the number observed was added to the total abundance for that site. Fish that could not be identified were not added to counts for that site, but this was a very rare occurrence. Mosquito fish (*Gambusia holbrooki*) were often observed in large schools too numerous to count and were therefore excluded from all analyses. Additionally, only three individuals each of flathead gudgeon (*Philypnodon grandiceps*) and carp gudgeon (*Hypseleotris spp.*), a small number of tench (*Tinca tinca*) and a single goldfish (*Carassius auratus*) were captured, and these species will not be discussed further nor were they used in any analyses. Finally, the common yabby *Cherax destructor* was also caught. Numbers were recorded but given electrofishing under-samples small individuals and there can be a strong size difference between males and females (Lake & Sokol, 1986), we do not include them in specific tests for experimental effects.

We measured the standard length of each fish to the nearest millimetre. All animals were returned to the stream after measurement, apart from the non-native mosquito fish and carp (*Cyprinus carpio*), which were humanely killed as required by our fisheries permit and according to methods approved by The University of Melbourne Animal Ethics Committee (#1614041.1). River blackfish (*Gadopsis marmoratus*) were classified as either juvenile (< 123 mm) or adults (> 123 mm) as per Howson et al. (2012) because their study showed juvenile and adult blackfish have different resource requirements. Other species were not caught in sufficient numbers to permit a similar analysis.

Data analysis

The environmental characteristics of sites were $\log_{10}$-transformed where necessary to reduce skew (all variables except average water depth, average water velocity, average stream width), normalised and then converted to a resemblance matrix using Euclidean distance. PERMANOVA was used to test for differences in environmental characteristics between sites in different Zones (fixed factor: upper, middle, gorge) crossed with Treatment (fixed: control, manipulation). Significance was determined using 999 permutations (Anderson, Gorley, & Clarke, 2008). Differences between manipulation and control sites were not expected (given sites were allocated to treatment randomly), and so we also used a one-way model comparing environmental characteristics of different zones. Differences detected by PERMANOVA were illustrated with a Principal Components Analysis (PCA) ordination conducted on the transformed and normalised data and solved in two dimensions (Clarke & Gorley, 2006).

To analyse the species assemblage as a whole, data on species abundances for surveys 2-7 (which were common to both experiments) were $\log_{10}(x+1)$ transformed and then converted to a resemblance matrix using the Bray-Curtis index, which was analysed using PERMANOVA. The model contained Treatment (fixed: control, manipulation) crossed with Zone (fixed: upper, middle, gorge) and with Sites nested within the interaction term (i.e. Treatment x Zone). Time (fixed: 6 levels) was a repeated measure that was crossed with both Treatment and Zone. A response by species to increased detritus is revealed by significant Treatment effects, which can also involve interactions with Zone or Time. Differences between zones, times or treatments were visualised using non-metric multi-dimensional scaling (nMDS) applied to the resemblance matrix and solved in two dimensions. For clarity, we plotted the centroids of each Treatment x Zone x Survey combination (Clarke & Gorley, 2006).

We tested experimental effects on species-specific abundance using separate models for Experiment 1 and 2 because they were started at different times. All abundance data, for both experiments, were $\log_{10} + 1$ transformed to satisfy assumptions of homogeneity of variance. We did not convert fish numbers to densities because the area of sites can be unrelated to abundance and hence the calculation of densities can produce misleading values (Downes, 2010). Experiment 1 used the same model structure as that used to analyse the fish assemblage data, given above. Because of environmental differences among zones and high temporal variability in conditions such as discharge (which affects detritus levels), we expected the effects of treatment to vary with zone and survey. We used planned contrasts (i.e. manipulation vs control) to assess treatment effects in different zones or times, however we had no *a priori* expectation about which comparisons (i.e. which surveys or zones) should show treatment differences. To control Type I errors, we used the following procedure. For each interaction, we determined the number of contrasts where treatments were significant at $\alpha = 0.05$. We then used a Bonferroni procedure to adjust alpha by dividing 0.05 by the number of these tests. Thus, if we detected three significant contrasts at 0.05, we adjusted alpha to 0.0167 and used this value to determine which of these tests, if any, were deemed to be statistically significant. Bonferroni corrections are overly conservative but provide increased confidence in cases where multiple tests are required (Quinn & Keough, 2002).

The model for Experiment 2 had a single fixed factor of Treatment (control, manipulation) crossed with the repeated measure of time. Treatment effects are revealed by a significant Treatment x Time term, which tests whether numbers of fish changed from before to after the start of the experiment at manipulation sites relative to controls. We used planned contrasts to test whether manipulation and controls differed before the start of the experiment, as described above.

RESULTS

Experimental effects on detritus and invertebrate densities – evidence from concurrent studies

Evidence from concurrent studies showed that increased retention by the wood stakes boosted detritus and increased macroinvertebrate densities at manipulation sites. At sites in Experiment 1, detritus densities were significantly greater at manipulation than control sites in both the upper (means ± SE: 274 ± 136 vs 132 ± 6 g.m^{-2}, respectively) and middle (287 ± 40 vs 134 ± 87 g.m^{-2}, respectively) zones, and invertebrate densities at manipulation sites were twice those of controls; these effects were also apparent at two sites downstream (Lancaster & Downes, 2021) that were not included in our samples. Comparable results were found in the separate experiment downstream of ours after 11 months of manipulation for both detritus (350 ± 50 g/m^2 vs 51 ± 29 g/m^2) and invertebrate (11756 ± 495 individuals/m^2 vs 5292 ± 697 individuals/m^2) densities (Bovill et al. unpublished data).

Environmental characteristics of sites

There were no differences in the environmental characteristics of sites in different treatments but there were strong differences among zones (Table 1a). PERMANOVA analysis comparing only zones showed the upper zone was different to both other zones (pairwise tests, both with $P < 0.05$), which did not differ from each other. The PCA ordination showed the upper zone was typically narrower, deeper and had slower water velocities compared to sites in other zones (Fig. 3a). The gorge and middle sites spanned a wide range of environmental conditions and were aligned with variation in areas of backwater and both bank and mid-stream macrophytes (Fig. 3a). Wood loading and the lengths of undercut banks were only weakly associated with differences between zones. Although the environmental characteristics of the gorge zone were assessed after the flood while the other zones were assessed before, it is unlikely that this critically impacted our results. The differences between the upper zone and both other zones were in characteristics relatively unaffected by floods of the scale observed. Macrophyte cover in the gorge zone may have been reduced by the flood, although values were similar between the middle and gorge zones (mean ± SE of bank macrophytes: 33 ± 13 m and 49 ± 11 m, respectively; mean ± SE of in-stream macrophytes: 7 ± 5 m^2 and 4 ± 3 m^2, respectively).

Faunal responses to experimentally increased detritus

Six native and five introduced species of fish were captured during the study (including the uncommon species noted above). The most common native fish were river blackfish and southern pygmy perch (*Nannoperca australis*), with substantially lower numbers of mountain galaxias (*Galaxias olidus*) and Macquarie perch (*Macquaria australasica*). The most common introduced species were redfin perch (*Perca fluviatilis)* and carp (*Cyprus carpio*).

The assemblage of species at sites varied strongly between zones (Table 1b), which separated along Axis 1 of the nMDS plot (Fig. 3b). The middle zone differed from both the upper and gorge zones (PERMANOVA pairwise tests, both with $P < 0.05$), whereas differences between the latter two were marginally non-significant ($P = 0.059$). Faunal composition also varied between surveys (Table 1b), which separate along Axis 2 and show marked effects due to the flood (Fig. 2b). Experimental effects were present in some surveys (Table 1b), however, because Experiments 1 and 2 started at different times we explore these experimental effects, as well as zonal differences, by looking at individual taxa.

In Experiment 1, southern pygmy perch, mountain galaxias and adult and juvenile river blackfish were relatively abundant, while other species of fish were very rare (redfin perch) or absent (Macquarie perch). There were strong differences in abundances between zones. River blackfish were most abundant in the upper zone (Table 2, Fig. 4a,b), while southern pygmy perch and mountain galaxias were restricted to the middle zone (Table 2, Fig. 4c,d). Southern pygmy perch and mountain galaxias responded to increased detrital densities during some surveys (significant interactions involving Treatments: Table 2). *A priori* contrasts revealed that southern pygmy perch attained higher numbers at manipulation sites in the middle zone on two occasions (Fig. 4c). Mountain galaxias were frequently found in higher numbers at manipulation sites than controls, but differences were statistically significant on only one occasion (Fig. 4d). In contrast, neither juvenile nor adult river blackfish responded to manipulation of detrital levels (non-significant terms involving Treatment and contrast, Table 2). Numbers of both pygmy perch and juvenile river blackfish varied strongly over time (Table 2) and both declined noticeably after the flood (Fig. 4a,c).

Six species of fish were sufficiently common for analysis in Experiment 2 (Table 3). In each case, an *a priori* contrast within the Treatment x Time term showed that, prior to treatment, abundance did not significantly differ at control and manipulation sites (Fig. 5a-d). Macquarie perch attained higher abundances in manipulation sites compared to controls on one occasion (Fig. 5a) and juvenile river blackfish on two occasions (Fig. 5c), which contrasts with juvenile river blackfish responses in Experiment 1. Redfin perch also responded to the treatment but was more common at control sites (Table 3, Fig. 5b). Three species of fish did not respond to treatment: adult river blackfish, carp, and, in contrast to Experiment 1, southern pygmy perch (Table 3). The latter species was, however, uncommon in the gorge (only 10 individuals were collected across Experiment 2). Mountain galaxias was not caught in the gorge zone and so is not considered in the Experiment 2 analyses. Both the exotic fish species and juvenile river blackfish varied strongly over time (Table 3) and, similar to Experiment 1, abundances of juvenile blackfish declined sharply after the flood (Fig. 5c).

DISCUSSION

Using field-based experiments, we show that increased detrital densities can significantly increase the abundance of native fish, but that these effects are modulated by the environmental context. When analysed individually, we found that all four, common native species of fish attained higher abundances at manipulation sites at least once during our experiments. In the experiment where we recorded pre-treatment abundances, the response was also relatively rapid, with the highest abundances of responding species recorded after only two months of treatment application. Our results support the hypothesis that boosting benthic detritus, in the form of leaves, sticks, small twigs and bark, relieved some resource constraints that otherwise limit local fish numbers in degraded areas of Hughes Creek.

Fish numbers may increase with higher levels of detritus because the latter can boost food supplies. In both experiments, macroinvertebrate densities increased substantially with increases in detritus. All of our responding fish species feed on benthic invertebrates either directly from the bed or via the drift (Cadwallader & Eden, 1979; Closs, 1994; Humphries, 1995; Rees, Shackleton, Watson, Dwyer, & Stoffels, 2020). Most previous studies that have added wood as a restoration technique failed to increase macroinvertebrate densities as well (Palmer et al., 2014; Roni et al., 2008), possibly because the sizes, shapes or positions of logs and branches did not lead to increases in detritus at restored sites (Entrekin, Tank, Rosi-Marshall, Hoellein, & Lamberti, 2009). This lack of detritus, and therefore dearth of macroinvertebrates, may explain why adding wood can have equivocal effects on fish abundances (Langford, Landford, & Hawkins, 2012). A second benefit for fish may be that detritus packs provide areas of cover. Scour holes develop around stakes with detritus packs on sandy beds in Hughes Ck (Lancaster & Downes, 2017) creating areas of slower-flowing, deeper water associated with structural cover and small interstitial spaces. Such areas are used preferentially by some fish (Bond and Lake, 2003a), including those observed responding to treatments in this study. Southern pygmy perch are found commonly in backwaters and beds of macrophytes (Humphries, 1995), and juvenile river blackfish have been observed using small woody debris (Howson et al., 2012). All the experimental effects on fish occurred in zones inundated with sand and with poor background densities of detritus. Thus, increases in food and cover resources are likely causal mechanisms for increases in fish numbers.

Observed fish responses to our experimental treatment varied through time. The large flood in early December 2017 abruptly terminated Experiment 2 and was associated with sharp declines in fish numbers in all zones, likely explaining why experimental effects on fish were absent during the later surveys. However, there was also species-specific variation in responses to the experimental treatment through earlier parts of 2017. For example, southern pygmy perch attained higher numbers at manipulation sites on two dates but not during an intervening survey, and both Macquarie perch and mountain galaxias responded clearly on only a single date. Because we used a conservative procedure to determine statistical significance, we do not think these outcomes are chance (i.e. Type I errors). Some of this variability may be due to fish movements along stream channels that occurs only at particular times. For example, mountain galaxias disperse upstream during winter (Dexter, Bond, Hale, & Reich, 2014), which is when we detected a significant response by that species. Likewise, Macquarie perch may occasionally disperse away from home pools for several hundred metres, sometimes in response to discharge fluctuations (Koster, Dawson, Morrongiello, & Crook, 2013). These movements would then mean their occurrence may not be strongly correlated to local habitat or resource conditions.

Fluctuations in treatment effects may also be caused by variability in detritus densities over time. Densities of detritus depend on both the supply of debris from the surrounding terrestrial environment and the sizes of discharges that move it within the channel. Peak leaf and bark fall in south-eastern Australia occurs in late summer (Reid, Lake, Quinn, & Reich, 2008), hence detritus densities are highest in Hughes Creek in autumn prior to the onset of higher discharges during winter (Downes et al., 2017). If discharges are low over autumn, control sites can accumulate relatively high amounts of detritus despite their lack of retentiveness. Winter floods will then remove this detritus, but if winter floods are few or small in size, detritus densities can remain relatively similar at manipulation and control sites and this results in only small differences in invertebrate densities (Lancaster & Downes, 2021). Because autumn and winter floods during 2017 were relatively small (for contrast, the winter of 2016 was a wet year), it is feasible that treatment differences between manipulation and control sites were small over the autumn to mid-winter period of 2017, and this may explain why numbers of southern pygmy perch at controls gradually increased and reached parity with manipulation sites before subsequently dropping again by the end of winter.

Some fish did not increase in number with greater amounts of detritus. Adult river blackfish did not respond to treatment in either the upper or gorge zone. Howson et al. (2012) also found that adult river blackfish did not respond to addition of small woody debris. Adult river blackfish are typically found in deeper pools and around large woody debris and undercut banks (Khan, Khan, & Wilson, 2004; Koehn, O'Connor, & Jackson, 1994) and it is thus unlikely that they would use small detritus packs in shallow water for cover. Adult river blackfish may benefit from more food because of enhanced invertebrate densities, but they are often stationary during the day, and move around at night, when they may be found in open water (Khan et al., 2004; Koster & Crook, 2008). River blackfish's diel movement behaviour obviates detection of any treatment effects through simple abundance measures during the day if they are moving into treatment areas at night to feed. In contrast, juvenile river blackfish did not respond to treatments in the upper zone, whereas they did in the gorge zone. Detritus levels were increased experimentally by a relatively small amount in the upper zone (2x increase) compared to the gorge zone (9x increase). Background detritus densities in the upper zone are consistently higher than other zones (Lancaster & Downes, 2021) and may be sufficient to provide food and cover for fish. Another interesting outcome is that redfin perch attained higher numbers at controls rather than manipulation sites. Redfin perch were relatively uncommon and the effect was only marginally significant, however this species becomes an aggressive piscivore when individuals reach sufficient size (Morgan, Hambleton, Gill, & Beatty, 2002), and the species has been recognised as a possible threat to the recovery of the nationally endangered Macquarie perch (Department of the Environment and Energy, 2018). Given redfin perch co-occur with Macquarie perch in the gorge, reduced numbers of redfin perch with increased detritus is a result worthy of further investigation to determine its cause.

Most studies of rivers degraded by sediment deposition or de-snagging have tested whether the increases in physical complexity gained by adding large rocks or wood (large branches, logs) can enhance local numbers of fish. Much of this research has been conducted in North America and collectively has not revealed any consistent effects on fish numbers, except for some salmonids (Foote, Biron, & Grant, 2020). Alternatively, an Australian study recorded higher abundances of juvenile river blackfish and flathead gudgeon after experimental additions of small woody debris (ten branches, 0.01 – 0.1 m stem diameter), which provided essential cover for small-bodied fish and were also possibly used as spawning sites (Howson et al., 2012). Woody debris placed for cover can sometimes accumulate benthic detritus (Bond & Lake, 2003a; Howson et al., 2012), which may also have benefited fish, but this has not been the focus of experiments. To our knowledge, this is the first study to directly test the effects on fish of boosting benthic accumulations of leaves, twigs, small sticks and bark.

We have presented evidence that increased retention of benthic detritus boosted local numbers of native fish. Of the four species that responded positively to increased detritus, one is threatened in the state of Victoria (southern pygmy perch: Whiterod et al., 2019) and one is threatened in Victoria and endangered nationally (Macquarie perch: Department of the Environment and Energy, 2018). The most likely explanation for increases in fish numbers in our experiment is that these species are otherwise constrained by shortages of essential resources associated with detritus, such as food and cover. Our study spanned some 30 km of river length comprising morphologically distinct reaches that had different fish assemblages, with fish responding in those reaches with the greatest accumulations of sand. This outcome increases confidence in the results and reflects the importance of collecting data over scales of multiple kilometres (Fausch, Torgersen, Baxter, & Li, 2002).

Our results indicate the potential importance of resource constraints on stream fishes and how these can be alleviated. However, like almost all comparable studies we have not yet demonstrated that fish accrued the fitness benefits (e.g. greater reproduction or reduced mortality) that are needed for increases in population sizes. It is therefore possible that the responses we observed were due to fish being attracted from nearby locations to those where detritus increased. This ‘attraction versus production’ hypothesis has been discussed in relation to the use of artificial reefs in marine systems (Bohnsack, Ecklund, & Szmant, 1997) but is relevant to all restoration actions. A recent detailed assessment of the available literature for riverine fishes (mostly salmonids) found that abundance increases at improved sites were sometimes due to enhanced survival but in other cases occurred through attraction (Roni, 2019). Significantly, < 5% of over 600 papers specifically addressed this question (Roni, 2019), so it remains greatly understudied. Clearly, an important next step for our system will be to undertake tests to determine if increased detritus results in improved demographic responses (e.g. higher growth rates or recruitment), coupled with experiments examining colonisation rates to focal habitats that do or do not have neighbouring sites which may redirect colonists (Stier & Osenberg, 2010). Collectively, this information would allow a definitive test of the importance of resource constraints on fish populations, and how such constraints can be addressed.

In many systems, there can be distinct chronosequences following restoration, which can influence when responses are likely to occur. For instance, different bird guilds will likely recolonize replanted sites based on when the habitat resources they require are returned – ground and shrub cover in the short term, then arboreal vegetation, and ultimately tree hollows and fallen timber after longer than a century (Vesk, Mac Nally, Thomson, & Horrocks, 2008). In sand-affected streams, a similar long-term view is required. Replanting of riparian trees is often needed, but it can take decades for logs and branches to be recruited to the stream bed. In the short term and at some sites, particularly those with low detritus densities, it may be beneficial to use wooden stakes to increase detritus. The technique is cheap, easy to deploy and hardwood stakes can last for at least 5 years, as long as they are placed in areas not subject to high erosion or deposition of sediment during floods (Lancaster & Downes, 2021). Our results suggest there is potential to 'kick start' a longer-term recovery process by producing increases in fish numbers in the short-term. Obviously, managers will need to weigh up any potential disadvantages such as impacts on public amenity or recreation. Either way, we hope the results reported here may encourage other researchers to test whether detritus acts as a constraint on fish numbers and explore whether boosting stream retentiveness can have long-term benefits for fish populations.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare there are no conflicts of interest.

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Table 1 Summary of PERMANOVA analyses of (a) differences among zones and treatments as assessed across eight environmental variables and (b) differences among zones, treatments, and surveys in the composition of the fauna present at sites. SS, sum of squares; df, degrees of freedom; MS, mean square; F, value of pseudo-F, P, probability. Significant P-values (P < 0.05) are denoted with an asterisk.

Source	**df**	**MS**	***F***	***P***
(a) Environmental variables				
Treatment	1	4.22	0.57	0.78
Zone	2	13.96	1.89	0.03*
Treatment x Zone	2	6.48	0.88	0.58
Error	10	7.40		
(b) Species assemblage				
Treatment (T)	1	541.5	0.25	0.872
Zone (Z)	2	17507	8.00	0.002*
T x Z	2	1220	0.56	0.772
$\text{Site}_{\text{(nested within T x Z)}}$	10	2187		
Survey (S)	5	2356	4.31	0.001*
S x Z	10	864	1.58	0.057
S x T	5	1008	1.85	0.033*
S x Z x T	10	717	1.319	0.176
Error	50			

Table 2 Repeated measures analyses of variance of fauna in experiment 1 in different treatments (Manipulation *vs* Control) and zones (Upper *vs* Middle) over time (seven separate surveys) for southern pygmy perch, mountain galaxias, and juvenile and adult river blackfish. df, degrees of freedom; MS, mean square; F, value of pseudo-F, P, probability. Significant P-values are in denoted with an asterisk.

Source	df	MS	*F*	*P*	MS	*F*	*P*
		juvenile river blackfish			*adult river blackfish*		
T	1	0.209	0.366	0.578	0.167	1.298	0.318
Z	1	7.942	13.905	0.020*	1.488	11.54	0.027*
T x Z	1	0.062	0.108	0.758	0.167	1.298	0.318
Site $_{\text{within(T x Z)}}$	4	0.571			0.129		
S	6	0.469	22.128	<0.001*	0.078	1.833	0.135
S x T	6	0.026	1.213	0.334	0.034	0.802	0.578
S x Z	6	0.337	15.91	<0.001*	0046	1.077	0.404
S x T x Z	6	0.032	1.522	0.214	0.023	0.537	0.774
Residual error	24	0.021			0.043		
		southern pygmy perch			*mountain galaxias*		
Treatment (T)	1	0.064	10.511	0.032*	0.106	0.689	0.453
Zone (Z)	1	2.378	390.29	<0.001*	0.284	1.853	0.245
T x Z	1	0.064	10.511	0.032*	0.164	1.072	0.359
Site $_{\text{within(T x Z)}}$	4	0.006			0.153		
Survey (S)	6	0.231	6.573	<0.001*	0.019	1.124	0.378
S x T	6	0.094	2.684	0.039*	0.052	3.081	0.022*
S x Z	6	0.231	6.573	<0.001*	0.027	1.586	0.194
S x T x Z	6	0.094	2.684	0.039*	0.035	2.057	0.097
Residual error	24	0.035			0.017		

Table 3 Repeated measures analyses of variance of the abundances of fish in experiment 2 in different treatments (Manipulation *vs* Control) over time (six separate surveys) for pygmy perch, Macquarie perch, juvenile and adult river blackfish, carp and redfin perch. Symbols and abbreviations are as for Table 2.

Source	**df**	**MS**	***F***	***P***			***P***
		southern pygmy perch			*Macquarie perch*		
Treatment (T)	1	0.008	0.579	0.476	0.006	0.282	0.615
Sites$_{(\text{within T})}$	6	0.013			0.022		
Survey (S)	5	0.019	0.966	0.454	0.061	2.338	0.066
S x T	5	0.012	0.588	0.709	0.055	2.135	0.088
Error	30	0.020			0.026		
		juvenile river blackfish			*adult river blackfish*		
T	1	0.4	1.63	0.249	0.024	0.145	0.716
Sites$_{(\text{within T})}$	6	0.245			0.167		
S	5	0.318	6.225	<0.001*	0.068	1.012	0.428
S x T	5	0.174	3.412	0.015*	0.132	1.977	0.111
Error	30	0.051			0.067		
		carp			*redfin perch*		
T	1	0	0.005	0.945	0.118	6.142	0.048*
Sites$_{(\text{within T})}$	6	0.01			0.019		
S	5	0.078	5.375	0.001*	0.064	3.414	0.015*
S x T	5	0.025	1.724	0.16	0.029	1.552	0.204
Error	30	0.015			0.019		

Fig. 1. Map of Hughes Creek in south-eastern Australia, with dots indicating study sites (filled: manipulation; open: controls). Sites in the middle and upper stream zones were established by

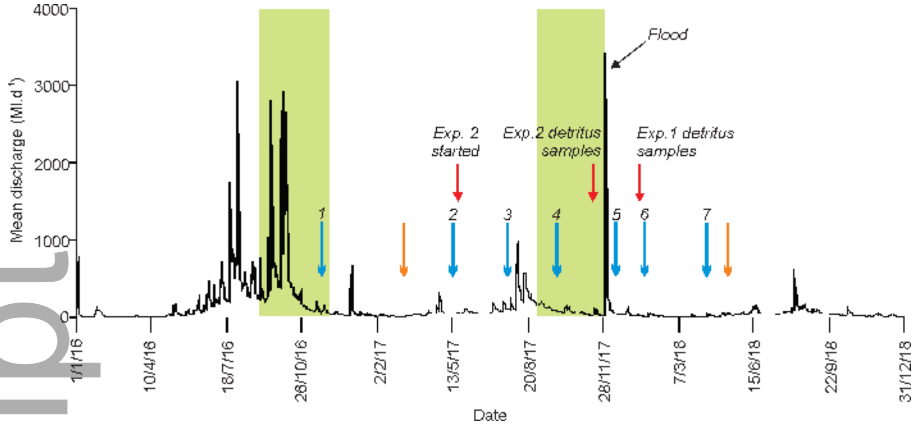
Lancaster and Downes (2017) in summer 2013 (their sites 1-4, 7-10). Sites in the gorge zone were established in this study in May 2017. Reaches throughout our study area are as defined by Erskine (2016) and are shown because they reflect different environmental conditions (see *Study Area*). Zones are defined in this study based on geographical groupings and broad environmental similarities of the conditions of the reaches. For reference, we show Erskine's reaches that bracket our study area: Reach 8 (the most upstream of Erskine's study) and Reach 3. Reaches 2 and 1 are further downstream and are not illustrated because the relevant length of Hughes Ck would become too small to show any detail.

Fig. 2 Hydrograph for Hughes Creek showing the mean daily discharge (megalitres per day) from 1 January 2016 to 31 December 2018; green blocks indicate Spring. Orange arrows indicate date of site surveys; blue arrows indicate first day of each of 7 electrofishing survey for the experiments (**1**: 21-22 November 2016; **2**: 16-18 May 2017; **3**: 26-27 July and 1 August 2017; **4**: 28-29 September; **5**: 11 and 15 December 2017; **6**: 18 and 27 February 2018 and **7**: 16-17 April 2018); red arrows indicate the date on which stakes were added to manipulation sites for experiment 2 and the dates of detritus samples for each experiment. On 2 December 2017, a large discharge event attained a daily flow that was an estimated 1 in 500 year event (flow exceeded on only 0.2% of days during the last 45 years, 1975-2020). While the flood is clearly large, the estimate of its frequency should be treated with caution given the shortness of the discharge record and likely recent changes in precipitation due to climate change. Data downloaded from the Victorian Dept of Environment, Water, Land & Planning (http://data.water.vic.gov.au, Victorian Government monitoring site no. 405228, Hughes Creek at Tarcombe Road).

Fig. 3 (a) Eighteen sites from three zones (upper: red, middle: orange, gorge: blue) plotted with respect to principal components that represent eight environmental variables, which are illutrated as vectors (solid, black lines). Direction and length of each vector indicates correlation with axes (maximum value of 1 is represented by the circle). **(b)** nMDS plot of the assemblage of species in different zones (upper, middle, gorge) and treatments (open: control; closed: manipulation) over time. Each point is the centroid of replicates. The black symbols indicate the first survey (survey 2) and the line connects the centroids in the order in which they were collected (up to survey 7). P: post-flood sample.

Fig. 4. The mean abundance per site (± SE) of (a) juvenile and (b) adult river blackfish in both the upper and middle zone and (c) southern pygmy perch and (d) mountain galaxias in the middle zone, and at experiment 1 sites on seven sample dates. A site was a 40m length of stream sampled with proportional electrofisher effort based on habitat complexity, ensuring all habitat was equally sampled. Illustrated are differences between Manipulation (Manip) and control (Cont) sites or between zones (Middle or Upper) over time (see Table 2). * indicates manipulation and control sites (pygmy perch, mountain galaxias) or zones (juvenile river blackfish) differed as revealed by *a priori* contrasts conducted within the relevant analysis of variance term (see Table 2) and using a value for alpha adjusted for number of tests (see text for explanation). P-value of each test is provided next to the asterisk. The dotted line indicates when the large flood (Fig. 2) occurred.

Fig. 5. The mean abundance per site (± SE) of (a) Macquarie perch, (b) redfin perch, and (c) juvenile and (d) adult river blackfish at experiment 2 sites (manipulation vs control) on six sample dates within the gorge zone. A site was a 40m length of stream sampled with proportional electrofisher effort based on habitat complexity, ensuring all habitat was equally sampled. The first sample was taken immediately before stakes were added to manipulation sites, and a planned contrast used to test specifically whether manipulation and control sites differed prior to treatment, which was non-significant (ns) for all species. * indicates manipulation and control sites (Macquarie perch, mountain galaxias) differed as revealed by *a priori* contrasts conducted within the relevant analysis of variance term (see Table 3) and using a value for alpha adjusted for number of tests (see text for explanation). P-value of each test is provided next to the asterisk. The dotted line indicates when the large flood (Fig. 2) occurred.



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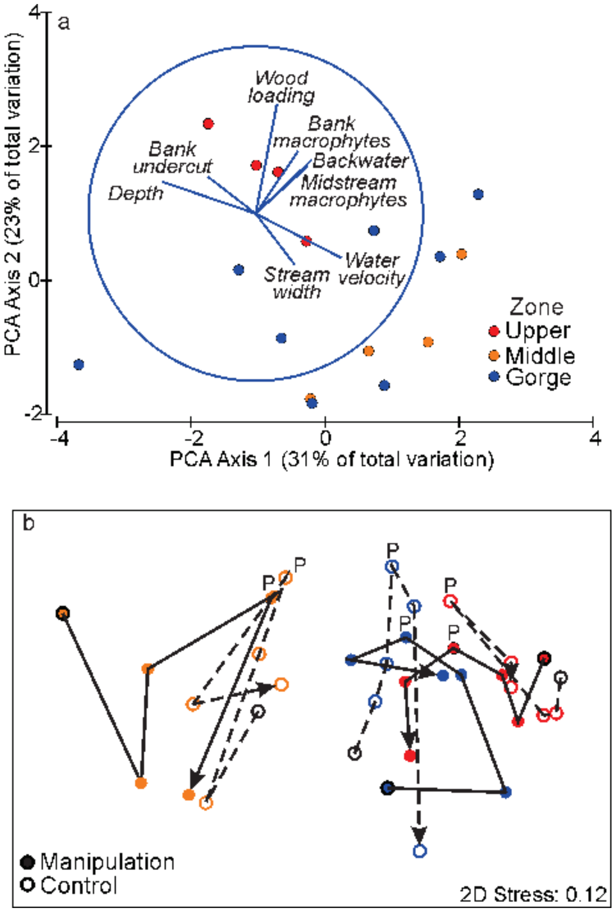

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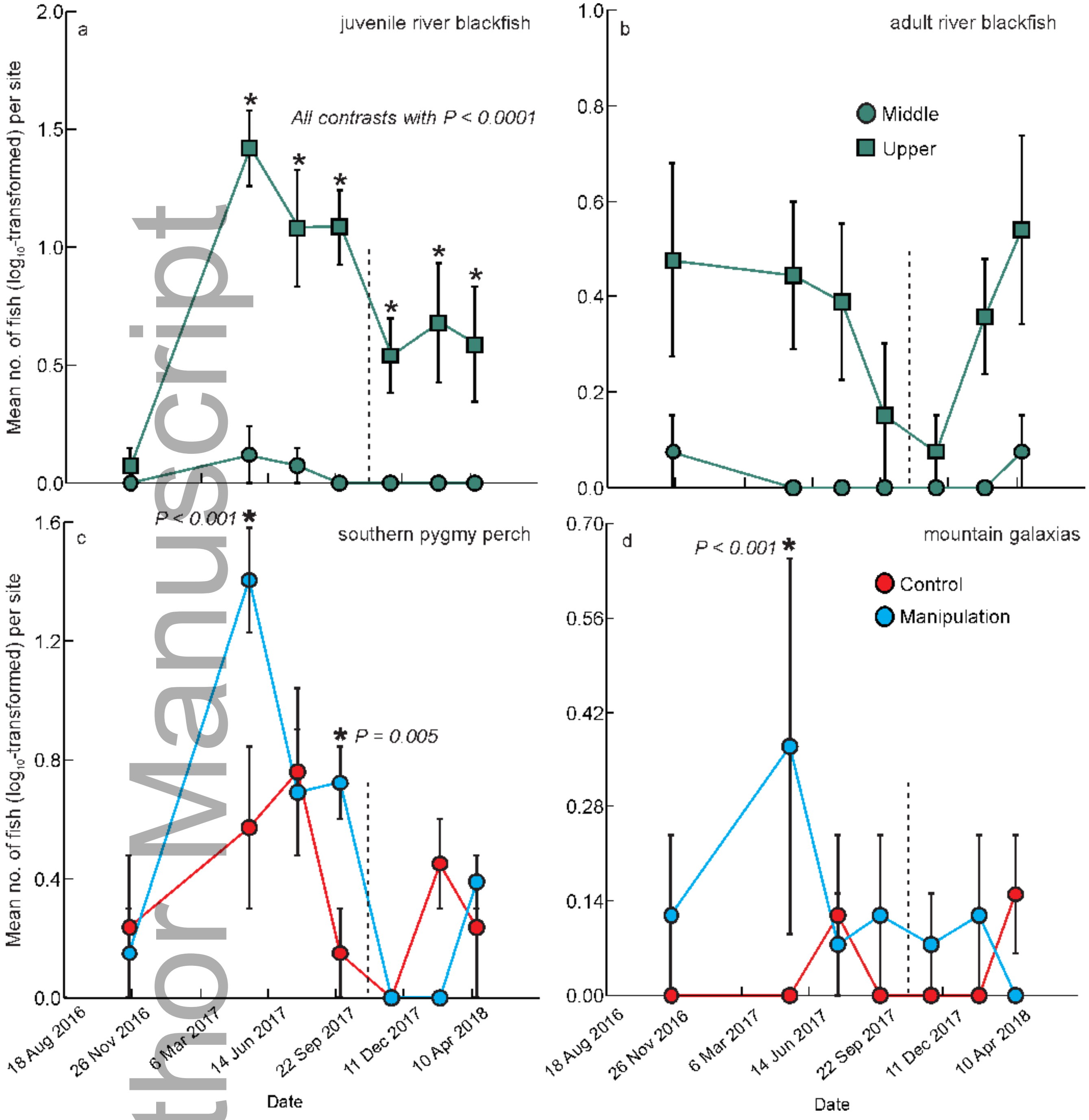

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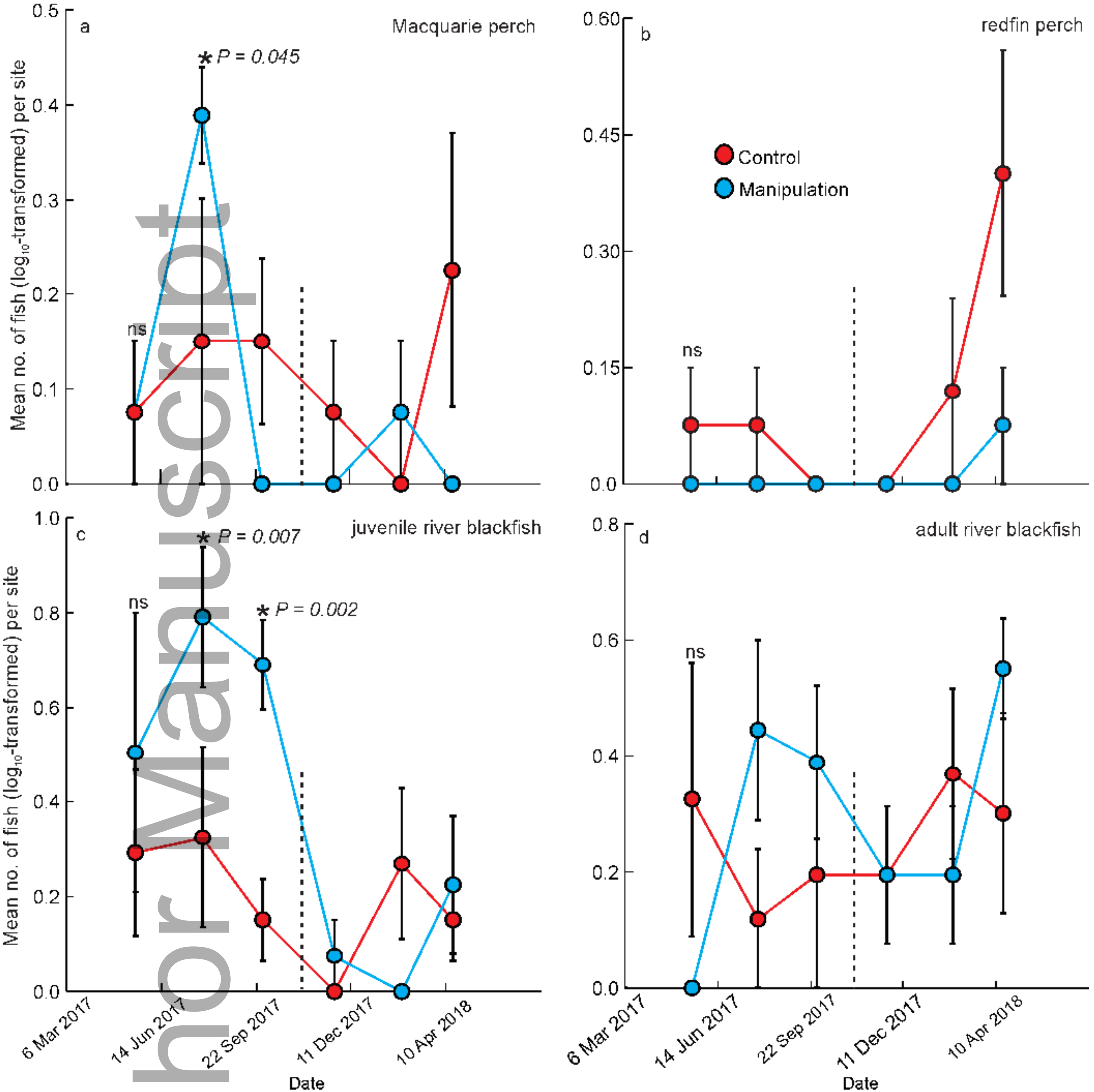

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