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Supplementary material

Feral horses (*Equus caballus*) increase suspended sediment in subalpine streams

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Supplementary text S1

A random subset of the long-term turbidity data was created because there were unequal numbers of data points among sites and seasons which violates one of the main assumptions of parametric statistics (Underwood 1997). Prior to the major analyses, a stratified random sampling procedure (stratified by site and season) was applied to the full data set to produce five independent replicate new data sets with ~125 data points within each cell of the site x season matrix (from the 17 000 to 20 000 available within each cell). A one-factor ANOVA was used to test for differences in overall mean turbidity among the five replicate data sets (d.f. 4, 9410), and two one-way ANOVAs (Factors: data sub-set 1–5 (random factor; d.f. = 4) and site (fixed; d.f. = 3; interaction d.f. = 12; MSE d.f. = 9395); and data sub-set 1–5 (random factor; d.f. = 4) and season (fixed; d.f. = 3; interaction d.f. = 12; MSE d.f. = 9395) were used to test for differences in site and season means among data sets. The mean overall turbidity for the five sub-sets of data did not differ significantly among sets (One way ANOVA, $P = 0.97$), and there were no significant interactions between data source (sub-set) and site or season ($P = 0.77$, 0.91 respectively), indicating that there were no significant differences among the random means from sub-sets by site or season. On this basis all subsequent analyses relate to the first random sub-set generated, with the assumption that it is generalisable to other randomly sub-sampled data sets.

Supplementary text S2
Spatial extent of turbidity

Methods

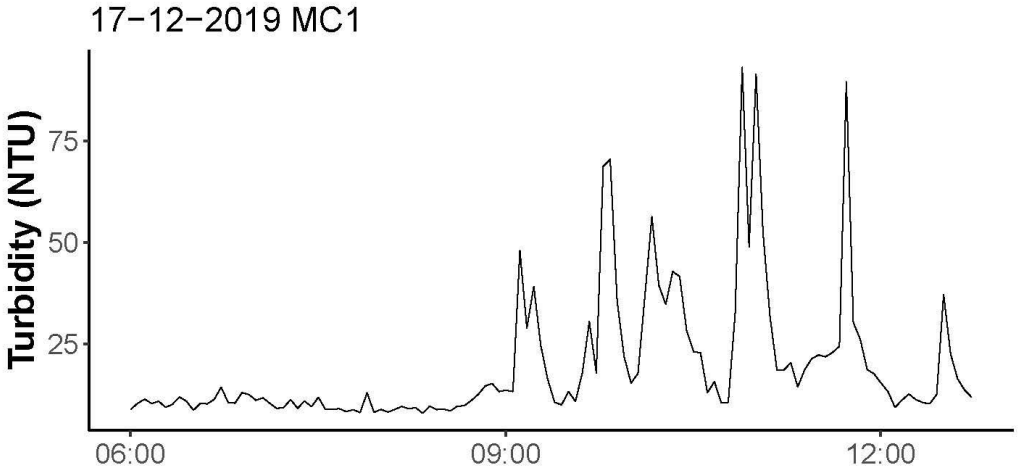
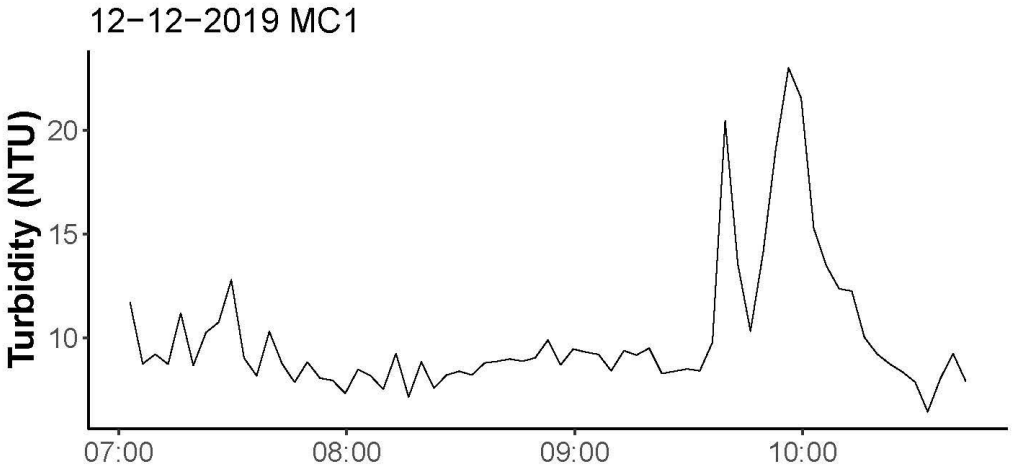
Spatial extent of turbidity was determined by an experimental simulation of instream animal activity at MR1, a stream that is regularly affected by horses (which meant that there were fine sediments on the stream bed), but at the time of this experiment (June 2020) was not affected. A 150-m stretch of stream (~3 m wide and 300–600 mm deep) was marked and turbidity loggers were placed on the stream bed at 2, 20, 70 and 150 m downstream. A researcher entered the water at 0 m and for two minutes walked backwards and forwards across the stream to simulate stream bed disturbance from horses crossing and in-water activities. Cross sectional average stream velocity at each distance downstream was measured at the end of the experiment using a handheld flow meter (Global Water Instruments FP311, USA).

Results

Simulated animal activity created a turbidity front that moved downstream. The peak turbidity value decreased with distance downstream but was clearly detectable 150 m from source (Fig. S1). A logarithmic function was fitted to the smoothed peak turbidity and distance downstream ($y = -19.78\ln(x) + 117.92$; $r^2 = 0.97$) and a value of 10 NTU (which is the approximate value of background turbidity in horse affected areas) was extrapolated to occur ~230 m downstream. Based on the time taken for the turbidity smoothed peak to move downstream the rate of travel was 0.36 m s^{-1}, average water velocity for the reach (mean of velocity at each turbidity measurement point) was measured at 0.4 m s^{-1}, so the turbidity peak travels at approximately the speed of the water.

Table S2. Turbidity patterns from selected types of horse interaction

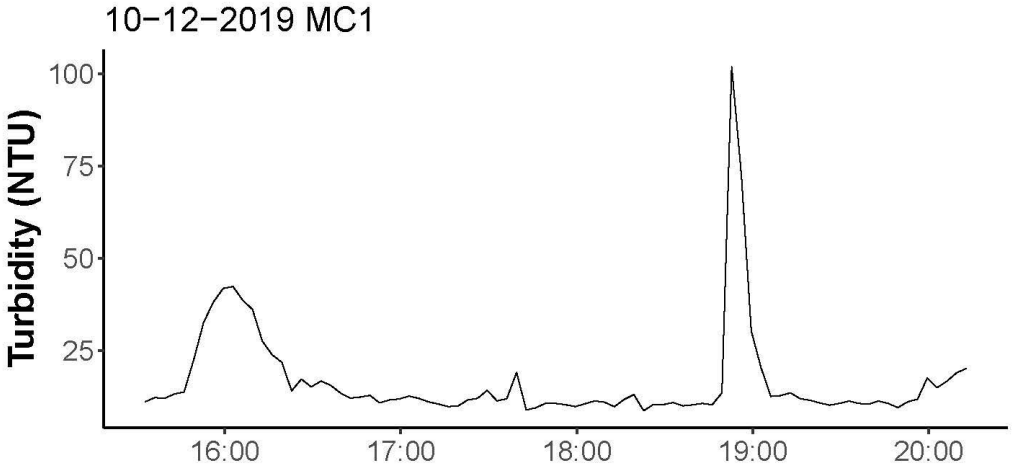
Note that background turbidity ranges between 10 and 20 NTU

Interaction	Turbidity Profile
MC 1 17 December 2019 Large group of horses walking through water and walking/standing in water. Group remains on banks for over 3 h with multiple crossings. Interactions were noted at 0932, 1020, 1217 hours. Multiple peaks occur on logger, some reaching maximum value. Turbidity peaked prior to first capture by motion sensed camera, presumably due to interactions outside camera range.	
MC 1 12 December 2019 Single horse enters stream margins to drink with small disturbance, walks away without crossing. Interaction was detected by camera at 0949 hours. Small increase in turbidity is registered on logger, clears within 20 min.	

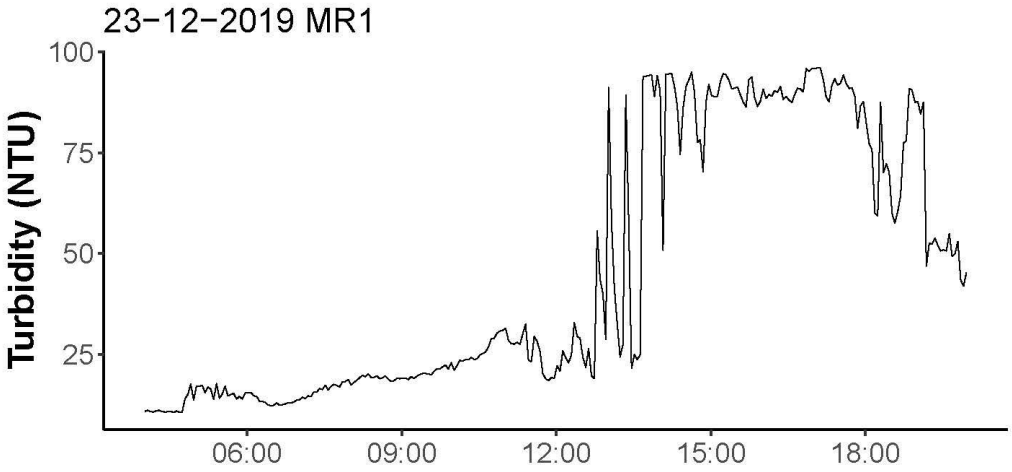
Interaction

Turbidity Profile

MC 1 10 December 2019
Multiple horses, single crossing as a group were noted in camera records at 1849 hours. Turbidity showed a brief spike to maximum then declined to base in ~20 min.



MR1 23 December 2019
Extended interaction involving multiple horses and visits over at least 7 hours. Interactions were noted at 1140, 1154, 1228, 1552, 1632, 1834, 1949 hours. Multiple horses were observed standing in the stream to graze and to kick at submerged sediments.
Turbidity was initially elevated small amount prior to detection by the motion sensed camera, but soon reached maximum and remained there for 5 h before it began to decline.



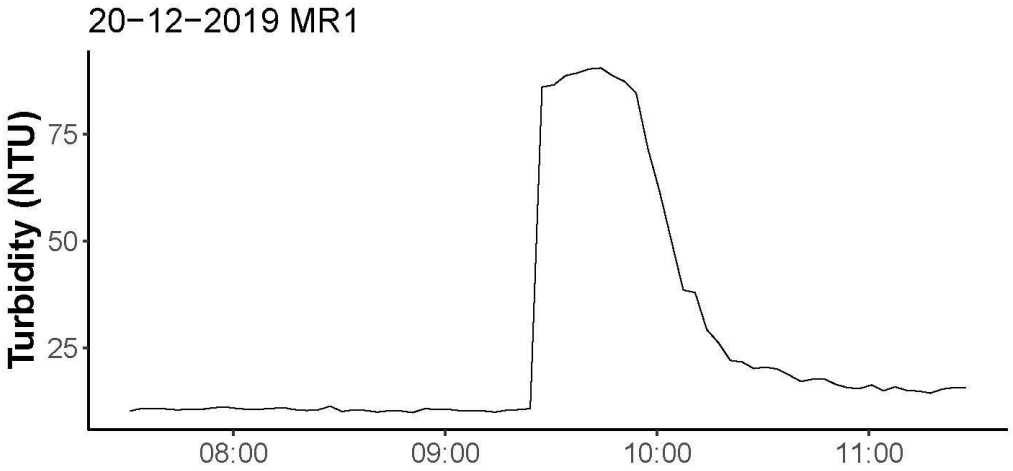
Interaction

MR 1 20 December 2019

Moderate length interaction involving multiple horses standing and walking in the stream. The camera date stamp shows interactions at 0912 hours and the logger detected turbidity at 0927 hours.

Turbidity again appears on logger slightly later than interaction time. Turbidity reaches maximum very quickly, and remains there for 25 min, after which it drops back towards background levels after ~ 1 h.

Turbidity Profile



MR1 18 December 2019

Extended interaction involving multiple horses in the water. Horses recorded in water from 1743 to 1802 hours, subsequent interactions at recorded at 1810, 2046 and 2055 hours. In the interaction at 1743 hours, a horse was observed actively digging at the stream bed.

The turbidity record starts a few minutes after horse interaction, reflecting that logger is downstream of camera. Turbidity rapidly reaches the logger maximum and the continued interaction, suspended fine material and low flow rate mean that that level of turbidity is maintained at maximum for 2 h, before it begins to decline. It then climbs to very high for a further 900 min following a second round of interactions.

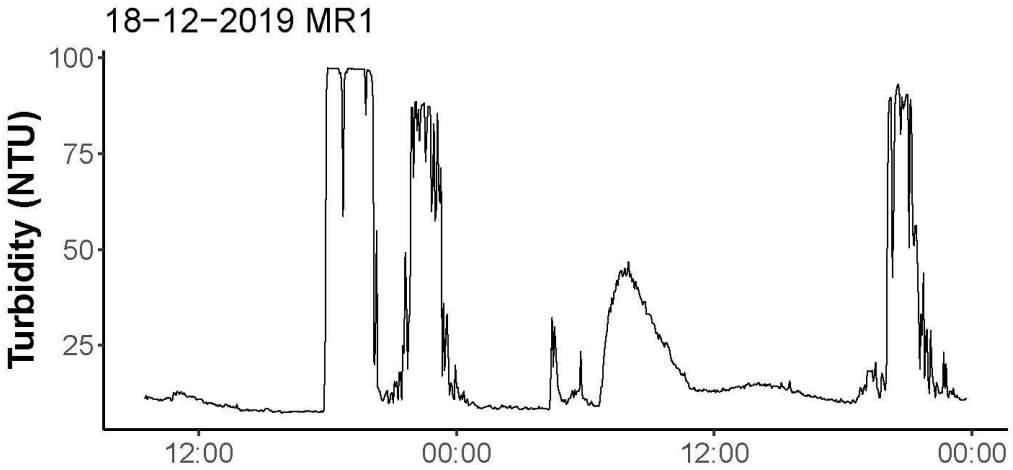


Table S3. Riparian assessments

A) Damage was assessed using 9 indices. Those indices were combined to derive 3 summary sub-indices. Data are presented by sub-index and as a summed score in the Raw Score (B) where high scores are in best condition; The penetrometer measures resistance to insertion, so a high value (max. 6) is more compacted than a low value (min 0). Each unit on the penetrometer scale is ~50 g mm^{-2}. (C) Data are recalculated as a normalised disturbance index (low disturbance indices are in best condition)

A) Components of Sub-Indices within Robertson Score

Sub Index	Robertson Score Indices
animal presence	pugging damage, number of animal paths, level of impact of animal paths
vegetation	grazing disturbance, projected foliage cover, proportion of cover that is native
bank/channel	stream bank stability, longitudinal profile, sediment level on stream bed

B) Raw Score

Site	Animal Presence	Channel Morphology	Vegetation	Summed Scores	Mean Soil Compaction (n = 20) (penetrometer scale units)
BH1	12	12	14	38	1.43
ER1	12	10	14	36	1.65
MR3	9	7	7	23	2.9
MR1	6	5	8	19	3.77
MC2	6	5	8	19	3.95
MC1	4	3	8	15	4.76

C) Normalised score

Site	Animal Presence	Channel Morphology	Vegetation	Summed Scores
BH1	0.00	0.00	0.00	0.00
ER1	0.00	0.22	0.00	0.09
MR3	0.33	0.56	0.64	0.65
MR1	0.67	0.78	0.55	0.83
MC2	0.67	0.78	0.55	0.83
MC1	0.89	1.00	0.55	1.00

Table S4. Analysis and interpretation of differences in frequency distributions of turbidity in a random sub sample of the turbidity data

Interpretation is a visual assessment of observed distribution in relation to expected distribution under hypothesis of no difference in distribution among sites. Differences were determined using Fishers Exact Test. Ns, no significant differences among distributions. ***, significant differences among distributions $P < 0.001$. Sites with obvious horse activity: MC, Mosquito Creek; MR, Murrumbidgee River. Sites with minimal to none horse activity; ER Eucumbene River; BH, Bullocks Head Creek

Differences among seasons at a site		
Site	Test Result	Interpretation: skew to the right indicates greater than expected turbidity; skew to the left indicates lesser than expected turbidity
BH	ns	
ER	ns	
MC	***	Summer skewed to the right; autumn and winter skewed to the left
MR	***	Summer skewed to the right; spring, autumn and winter skewed to the left
Differences among sites within a season		
		Interpretation: skew to the right indicates greater than expected turbidity; skew to the left indicates lesser than expected turbidity
Autumn	***	MC, MR skewed to the right; BH skewed to the left
Spring	***	MC, MR skewed to the right; BH skewed to the left
Summer	***	MC, MR skewed to the right; BH, ER skewed to the left
Winter	***	MC skewed to the right

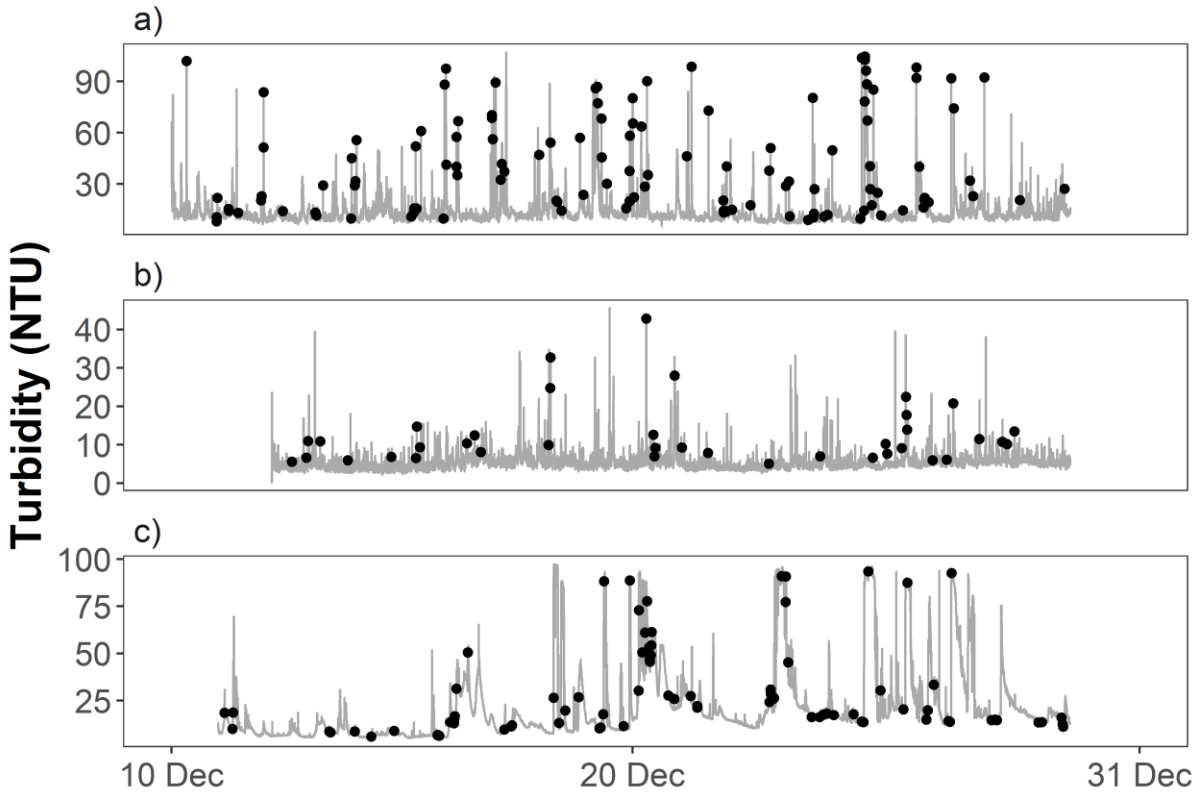


Figure S1. Turbidity (grey line) and instances when horses were photographed interacting with the stream (black circles) at site (a) MC1, (b) MC2, (c) MR1.

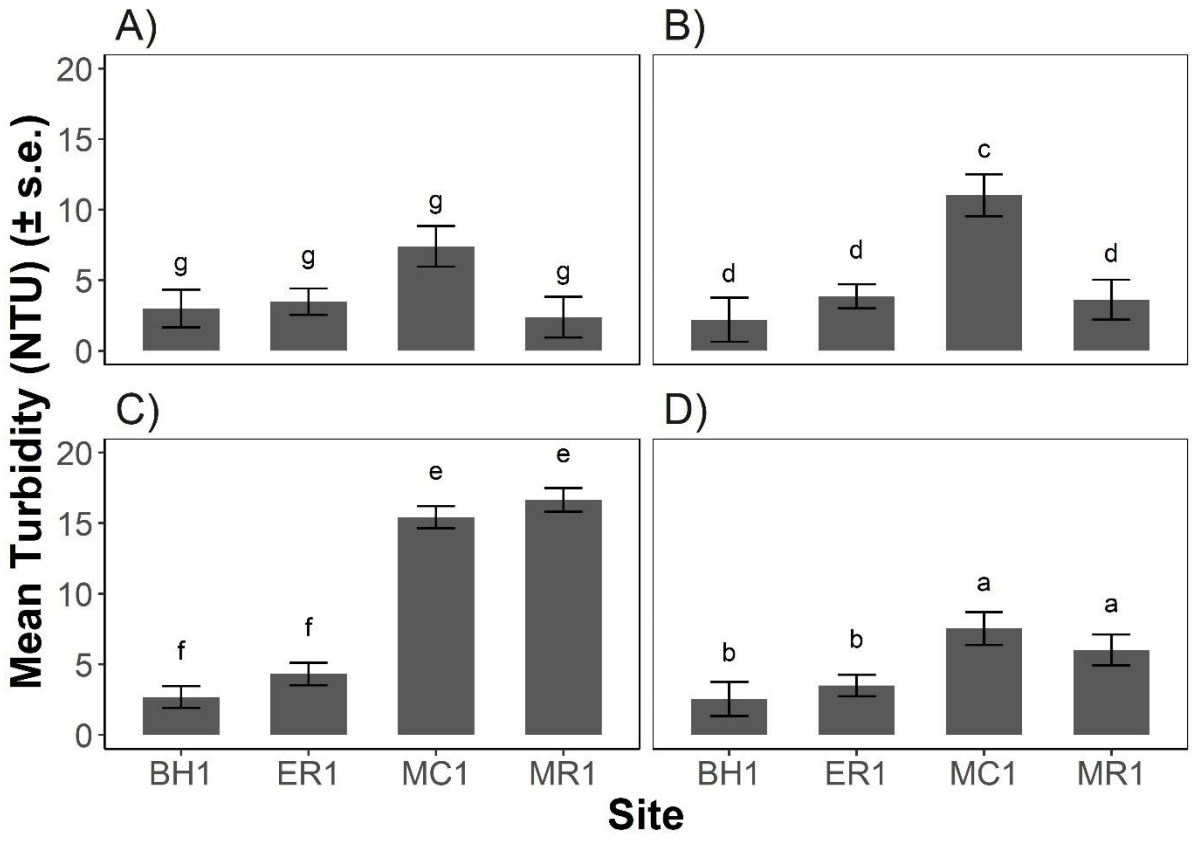
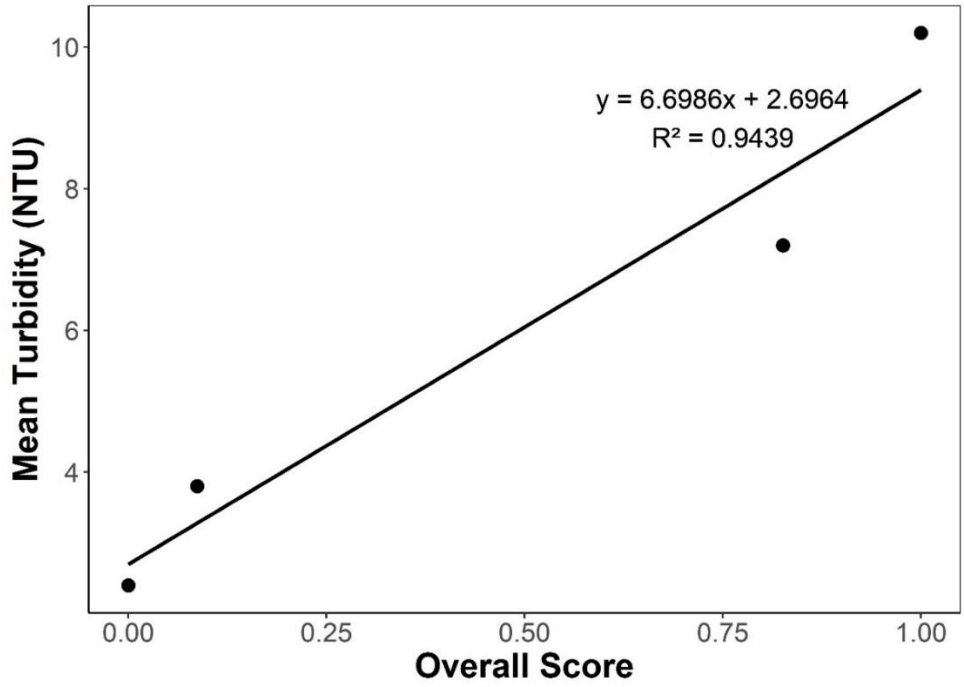


Figure S2. Result of TK post-hoc significance tests among sites within a season. Letters above bars show which means are significantly different from each other (TK *post-hoc* test, p=0.05). Y axis indicates turbidity (NTU). A, winter; B, spring; C, summer; D, autumn.

A



B

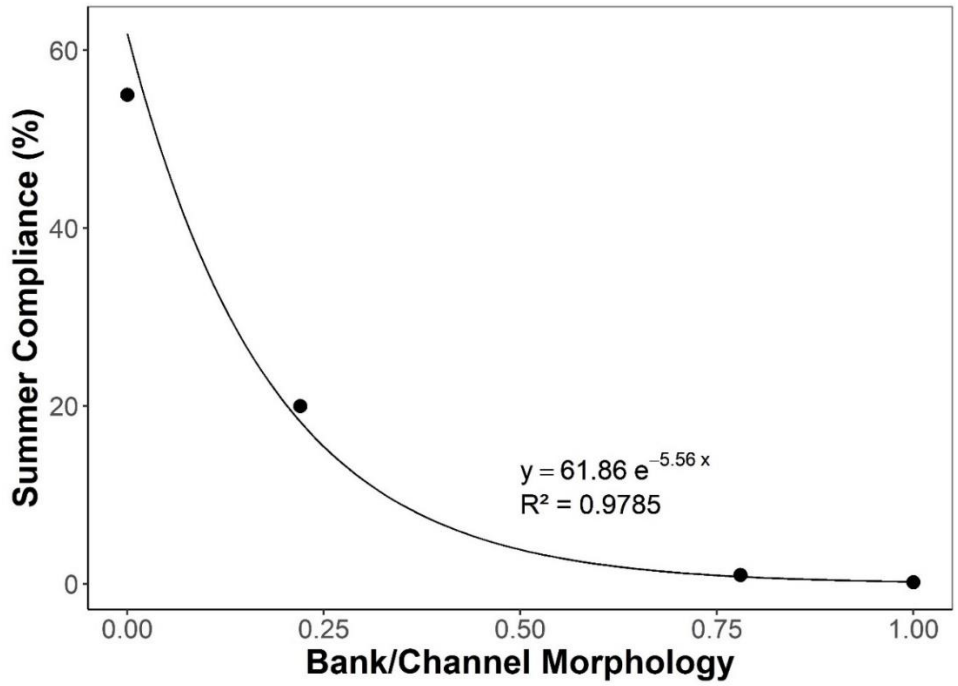


Figure S3. Relationship between (A) Mean turbidity and Overall Score, and (B) Summer compliance with guideline values with and Bank/Channel Morphology sub-score (details of scoring in Table 5)

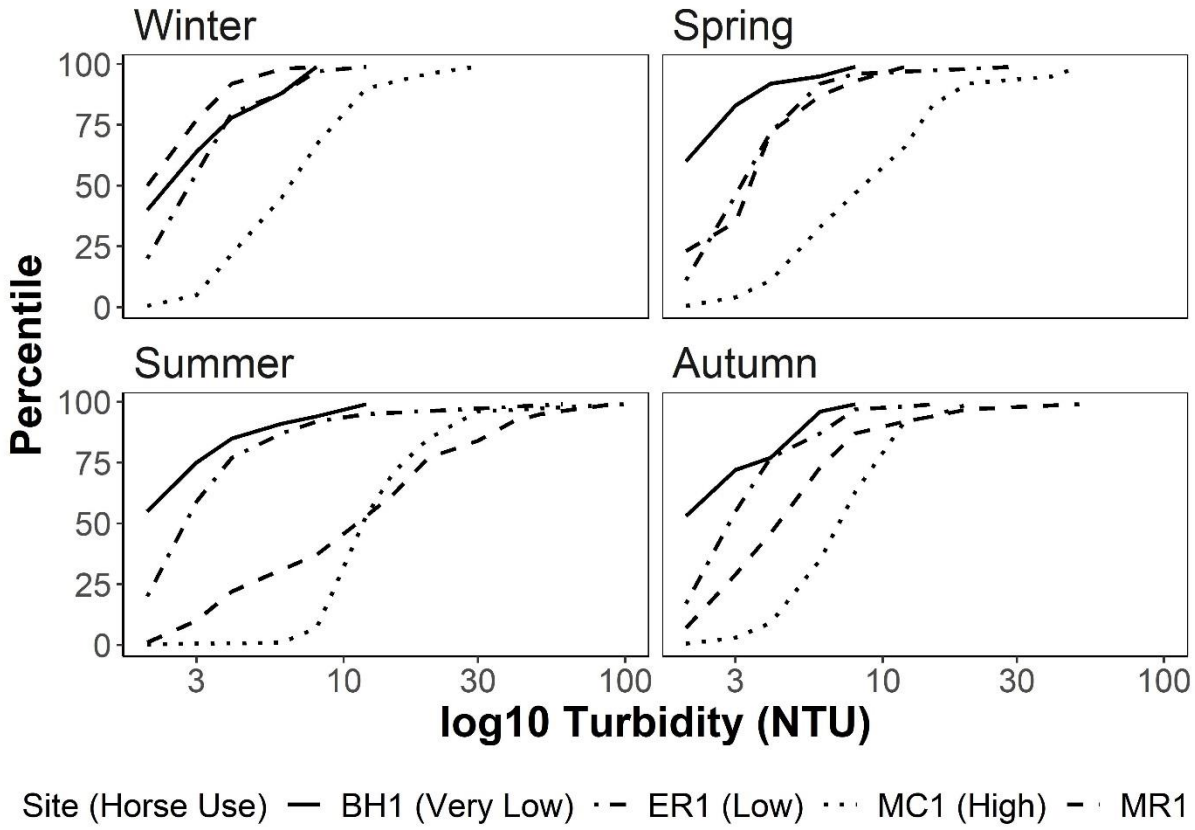


Figure S4. Frequency distributions of turbidity data from sites BH1 (solid line), ER1 (dot-dash line), MC1 (dotted line) and MR1 (dashed line) in (A) Winter, B) (Spring, (C) Summer and (D) Autumn. The y axis is the percentile value for each turbidity reading (i.e. the percentage of the dataset less than a nominated *x*-axis value). Therefore, the higher that the starting point of a site's curve is on the y axis, the greater compliance with Guideline Value of 2 NTU.

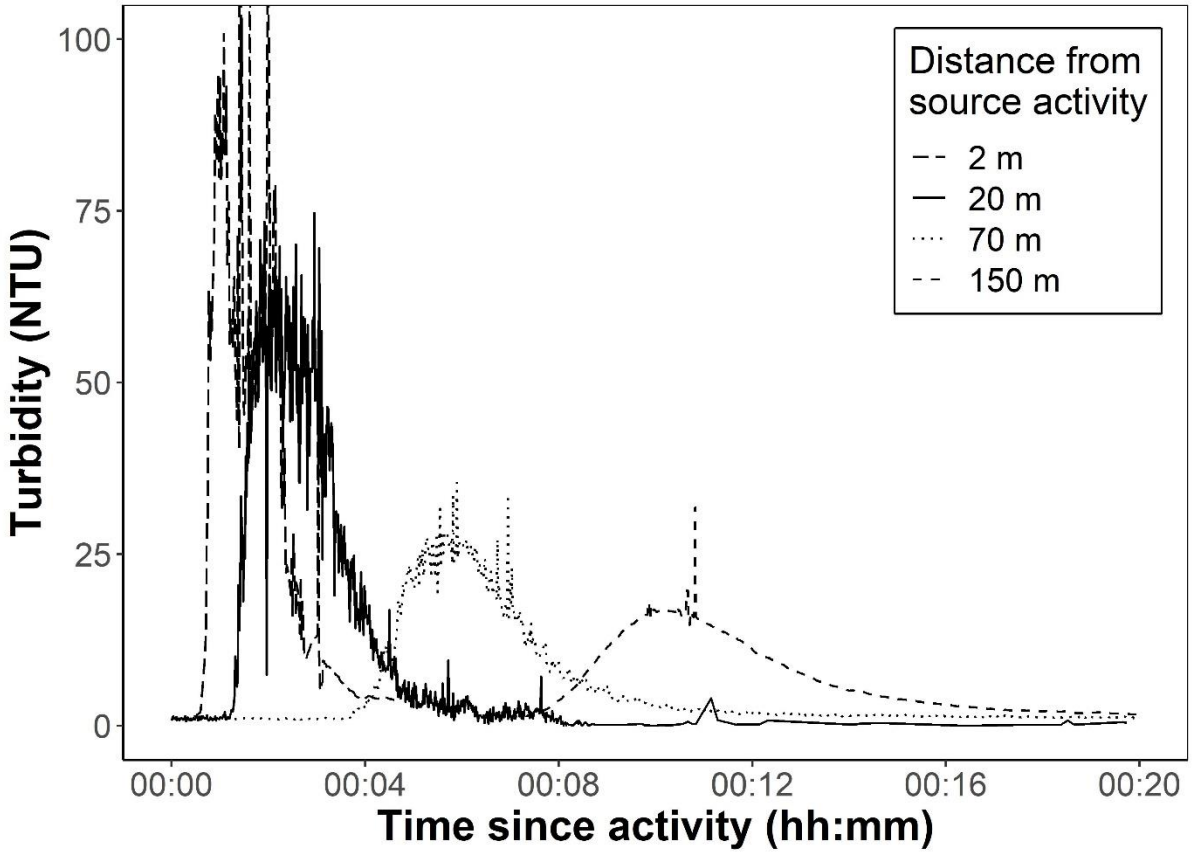


Figure S5. Turbidity measured downstream of simulated in-stream disturbance. The *y*-axis has been truncated, values of 580, 172 and 122 NTU were recorded at 2 m.