The effects of endangered freshwater pearl mussels on channel morphology and flow in a low-gradient sandy river

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Abstract

Conservation of ecosystem engineers, which modulates the surrounding habitat by causing physical state changes in biotic or abiotic materials, is important for maintaining the functional integrity of ecosystems. This study examined the effects of endangered freshwater pearl mussels (*Margaritifera laevis* and *M. togakushiensis*) on channel morphology and flow in a low-gradient sandy river. For this, we performed a field removal experiment of mussels using three treatments in twelve 10-m reaches. Mussel abundance and biomass, and three physical variables were measured before, immediately after, and two months to one year after the treatments. Mussel removal resulted in channel degradation with a 60% increase in flow depth, a 30% decrease in current velocity, and a 50% reduction in the width-to-depth ratio two months after the treatments, whereas minimal changes were measured in reaches with mussels. The results indicated that pearl mussels act as an ecosystem engineer affecting the channel morphology and flow of sandy rivers. The conservation of the pearl mussel populations is key to preserving their far-reaching benefits in ecosystem integrity including habitats for other various organisms.

Keywords: BACI, ecosystem engineer, endangered species, erosion, hydromorphology, invertebrates

Introduction

The physical environment provides a template on which a myriad of biotic processes occur in ecosystems (e.g. Poff & Ward, 1990; Huusko et al., 2007; Iwata, 2007). Some organisms are disproportionately more important in maintaining the functional integrity of ecosystems because they exert strong control over their surrounding physical environments. Such organisms are referred to as ecosystem engineers because they directly or indirectly modulate the availability of resources to other species by causing physical state changes in the biotic or abiotic environment, which have significant consequences for populations, communities, ecosystem functioning, and landscape structures (Jones, Lawron & Shachak, 1994; Jones, Lawton & Shachak, 1997). Beavers (*Castor*) are good examples of ecosystem engineers. They create dams by utilizing riparian trees, trigger habitat shifts from lotic to lentic environments, and modulate species diversity and the composition of other organisms within and around the beaver dam (Wright et al., 2002; Wright & Jones, 2004; Law et al., 2016, 2017). As beavers play such important roles, the conservation and management of their habitats are important for maintaining the functional integrity of ecosystems (Crain & Bertness, 2006; Dalbeck et al., 2014; Mccaffery & Eby, 2016). The identification of ecosystem engineers necessitates quantitative assessments of the magnitude of their effects on the physical environment (Byers et al., 2006; Crain & Bertness, 2006). Ecosystem engineer species that are rare and endangered over a relatively large spatial scale (e.g. global or regional scales) but still abundant at pristine areas also require urgent attention as future decreases in their population size could cause irreversible changes in ecosystem structure and function (Spooner & Vaughn, 2006).

As riverbed material, sediment moves frequently during high-flow events; the sediment fluxes from upstream to downstream are balanced in a dynamic equilibrium state, which determines channel morphology (Gordon et al., 2004). Sediment regimes are crucial to aquatic and riparian ecosystems (Wohl et al., 2015), whereas various types of ecosystem engineers in rivers can strongly influence the movement and flux of riverbed sediment (Corenblit et al., 2011; Statzner, 2012). These organisms can either increase or decrease riverbed erosion (stabilization or destabilization of sediment) by, e.g. foraging, bioturbation, and redd-building as well as/or act as physical substrata (e.g. mollusk shell production) (Gutiérrez et al., 2003; Sousa et al., 2009; Novais et al., 2015; Ilarri et al., 2018, 2019). However, there is a paucity of empirical research on the organisms that promote bed-sediment stabilization compared with organisms that promote bed-sediment destabilization (Statzner, 2012). More observations of potential candidate organisms in diverse environmental contexts are necessary to obtain a holistic understanding of the effects of ecosystem engineers on sediment fluxes and channel morphology.

A variety of ecosystem processes are scale-dependent, and observations at different scales may provide results that vary in the strength and direction of ecological interactions (Schindler, 1998; Beard et al., 2003). Small-scale manipulative experiments can provide a mechanistic understanding of the drivers of ecological processes, whereas generalizing or scaling up the results to those drivers operating at larger scales has been a considerable challenge in both ecology and conservation biology (e.g. Cooper et al., 1998; Tilman, 1989). For example, Kohler & Wiley (1997)'s small-scale experiment on stream invertebrate responses to manipulations of grazing caddisfly larvae (*Glossosoma nigrior* Banks, 1911) accurately predicted the direction of the algal, mobile grazer, and small-bodied filter-feeder responses, but underestimated the extent and magnitude of changes. In contrast, the results of Greathouse et al. (2006)'s experiment on benthic resources and invertebrate responses to small-scale and short-term manipulations of migratory and grazing freshwater shrimps (*Atya, Xiphocaris, Macrobrachium*) matched well those obtained at the whole catchment scale. The known effects of ecosystem engineers on riverbeds have been derived from relatively small-scale experiments or observations in the field, as well as from well-controlled laboratory environments (Johnson et al., 2009; Montgomery et al., 2011; Albertson & Daniels, 2016). This is partly because of the practical difficulty in manipulating the natural environment at large spatial scales.

Freshwater mussels (order Unionoida) are widely distributed throughout the world's freshwater habitats, occur in both lotic and lentic systems, and are among the most imperiled group of organisms (Lydeard et al., 2004; Lopes-Lima et al., 2014, 2017, 2018). Freshwater mussels are considered ecosystem engineers for several reasons. They modify the physical characteristics of the riverbed habitat through their shell production, which increases habitat heterogeneity and complexity (Bódis et al., 2014; Ilarri et al., 2018, 2019), and through bioturbation as a result of burrowing behavior, which results in sediment mixing (Gutiérrez et al., 2003; Allen & Vaughn, 2011). They can also function as stabilization agents in rivers and reduce sediment loss, possibly by forming a protective armor layer and consequently reducing the hydraulic forces of the current on substrata (e.g. Zimmerman & de Szalay, 2007). Freshwater mussels such as freshwater pearl mussels *Margaritifera* Schumacher, 1816 (family Margaritiferidae, genus *Margaritifera*, referred to as pearl mussels hereinafter) often occur in highly aggregated distribution patterns at the 1–10 m scale in the natural environment [i.e. mussel beds (see Hastie et al., 2000; Sousa et al., 2015, 2020; May & Pryor., 2016)]. Such highly gregarious spatial mussel patterns on the riverbed may not only affect sediment movements at the localized spatial scale (<1 m) but could also affect channel morphology, especially if the mussel density is high and the mussel beds extend spatially over a large portion of the entire riverbed. However, no previous studies have quantified the importance of sediment stabilization in natural field environments at a scale relevant to freshwater mussels' natural distributional patterns. This knowledge gap may result in an under- or overestimation of the role of freshwater mussels as ecosystem engineers and also prevent researchers from inferring the ecological consequences of mussel population losses.

This study aimed to quantify the role of pearl mussels as ecosystem engineers. Testing directly whether the mussels act as a stabilization agent of the riverbed is practically difficult in the natural environment especially at large spatial scales. To this end, an indirect approach was adopted based on the assumption that species removal provokes erosion when they act as a stabilization agent of a riverbed. Removal experiments have generally been used for testing the role of ecosystem engineers in natural systems (Wilby et al., 2001; March et al., 2002; Ranvestel et al., 2004). The present study used a removal experiment in which endangered pearl mussel species, *Margaritifera laevis* (Haas, 1910) and *M. togakushiensis* Kondo & Kobayashi, 2005, were removed from 10-m reaches of a low-gradient sandy river. Their engineering effects were assessed based on channel morphology (cross-sectional width-to-depth ratio) and associated physical environment characteristics (flow depth and current velocity). The hypothesis tested was that the removal of pearl mussels affects erosion and sedimentation on the riverbed. More specifically, it was predicted that the removal of pearl mussels from 10-m reaches would result in sediment destabilization, erosion, and lowering of a riverbed, which would cause an increase in the flow depth and a decrease in the current velocity, thereby resulting in channel degradation.

Materials and Methods

Study site and target species

The study was conducted from August 2015 to August 2016 in a lowland segment (approximately 2.0 km long) of a tributary of the Bekanbeushi River, located in eastern Hokkaido, northern Japan (Bekanbeushi River: 43°09′N, 144°50′E; Figs. 1a and 1b). The exact location and name of the tributary will remain undisclosed to protect the habitat of endangered species. The study tributary is characterized by low and stable discharge during early summer. The highest and more variable discharge occur in late summer to autumn because of the heavy rains by such as typhoon. Besides, higher mean discharge than other seasons occurs in early spring during snowmelt runoff. Water temperatures ranged from -0.2 to 16.9 ℃ and stream water was nearly saturated with dissolved oxygen. Other detailed descriptions of the study tributary are available in Miura et al. (2017). The study segment had a low-gradient (mean slope value of 0.1%) meandering channel and pool-glide sequences. The substratum was homogenous and mostly composed of sand (0.5–2.0 mm) without any coarse gravel materials (the maximum particle size was approximately 4.0 mm), regardless of the variations in flow depth, current velocity, and sediment depth. The channel had an incised cross-section with steep riverbanks fringed by slightly raised natural levees (Fig. 1c). The height from the water surface to the bank top was approximately 1 m on both sides during base-flow conditions. Large woody debris was sparsely distributed throughout the channel, on average, 1.6% of the surface area of the study segment was covered by large woody debris. In the fish community, *Pungitius* spp. were the most abundant, and *Salvelinus leucomaenis leucomaenis* (Pallas, 1814) and *Gymnogobius urotaenia* Hilgendorf, 1879 were among the secondary-dominant fish species (Ishiyama et al., 2020). In addition, *Oncorhynchous masou masou* (Brevoort, 1856), *Lethenteron* sp., *Cottus nozawae* Snyder, 1911, and *Platichthys stellatus* (Pallas, 1788) were occasionally found.

Two pearl mussel species, *Margaritifera laevis* and *M. togakushiensis,* are found in Japan. The Red List of Japan has classified these two pearl mussel species as endangered (Ministry of the Environment of Japan, 2018). These two species co-occurred in the study segment (Fig. 1d) with a relative abundance ratio of approximately 9:1 (Miura, unpublished data) and an average pearl mussel density of 175 individuals/m^2. They have relatively long shells (<150 mm and <100 mm, respectively), no shell surface sculptures, and similar morphologies (Kondo & Kobayashi, 2005; Miura et al., 2019a; Miura et al., 2019b). The accurate species identification of the two pearl mussel species needs checking the inner-shell morphology, DNA-based identification, or measurement of shell morphological features (Kondo & Kobayashi, 2005; Miura et al., 2019b). The former two criteria need to sacrifice or slightly injure mussel individuals if the known protocols for the two species are applied (Kondo & Kobayashi, 2005; Miura et al., 2019b). Besides, the shell morphological criterion is time-consuming and not practical when a large number of individuals needs to be handled (see below). For these reasons, we didn't distinguish the two species in the present study. No mussels from the study tributary were sacrificed, and when mussels were transplanted, the distance was kept at a minimum level to reduce physiological stresses on them without conflict against legislation on animal experimentation of Hokkaido University (Hokkaido University, 2007).

<u>Experimental treatments</u>

The before-after-control-impact (BACI) design was adapted to experimentally assess the effects of the removal of mussels on the physical environment. The experimental design, which has prior information on the potential impact involving the comparison of impact areas with control areas, is referred to as BACI design (Smith, 2014). This design is a powerful tool in ecological studies where the responses to the treatment, which cannot be accounted for by the observations in the control environment, are attributable to the impact (Smith, 2014; Smokorowski & Randall, 2017).

Four 65–170-m study sections (referred to as S1, S2, S3, and S4) with a wetted channel width of 4–6 m were selected in a downstream to upstream direction, and separated by at least 500 m from each other (Fig. 1b). A total of twelve 10-m reaches, three for each section, were established and assigned for three treatments, i.e. "Removal", "Removal-Release", and "Control", with four replicates each, namely one of each treatment in each section. The former two types of treatments were established for experimental manipulations and the latter was not subject to any treatment for comparisons with manipulated reaches. All mussels were removed from reaches classified as either "Removal" or "Removal-Release", and those removed from the latter were released in the same reaches. The "Removal-Release" was set to assess the influence of mussel removal without being confounded by experimental artifacts associated with the excavation procedure during the manipulation. "Removal" and "Removal-Release" were established in glides within each study section (a total of eight reaches), such that all reaches had as much similar physical conditions as possible (Table 1, Fig. S1). However, the mean wetted channel width of "Removal-Release" was 42.88 ±24.33 cm (mean ±SD) (range: 11.17–63.08 cm) greater than that of "Removal" (P<0.05, Table1). To reduce the potential manipulation effects in the immediate upstream on downstream reach, each reach within the same section was longitudinally spaced apart from upstream reaches by 36.25 ±10.31 m (range: 25–50 m). Furthermore, the positional assignments of "Removal" and "Removal-Release" were alternated in the longitudinal direction in order to prevent systematic errors in the experimental treatment in association with an upstream-downstream order. In addition, one 10-m reach within each section was designated as "Control". As additional sites similar to these experimental reaches were unavailable, the "Control" reach had greater flow depth before the manipulations compared with those of the other two experimental manipulations (Table 1). "Control" reaches were established 40.00 ±15.14 m (range: 5–70 m) apart from the treatment reaches. The close distance (5 m) of some sites was not considered to be a problem because of the absence of manipulation in the "Control" reaches. There was no woody debris >10 cm in diameter in any of the experimental 10-m reaches, indicating that large woody debris was not an important control agent of channel morphology. The physical conditions immediately before the manipulative treatment are shown in Table 1.

The manipulative removal treatments of mussels were conducted in "Removal" and

“Removal-Release” reaches from August 22–26, 2015. One to three investigators gently collected all visible mussels by hand to minimize the disturbance to the riverbed sediment. Relatively large organic materials such as twigs and leaves with >10-cm long axes exposed from the riverbed were also carefully collected and removed. The mussels collected from the “Removal” reaches were transplanted to similar habitats in nearby 5-m or 10-m reaches from “Removal” reaches in each section (Fig. S1). The mussels collected from the “Removal-Release” reaches were stored in 2-mm mesh net bags located in the river water between the experimental reaches, and were gently released back to their home reaches within 24 h of collection. It was not feasible to place them back to their original individual positions; instead, they were placed evenly across the reaches by hand. All experimental manipulations were undertaken in the upstream direction.

<u>Mussel abundance, biomass, and physical variable measurements</u>

The present study set a total of five measurement time periods; on August 22–24 (immediately before the treatment; “before” hereinafter), August 27–28 (immediately after the treatment; “short” hereinafter), October 22–23 (two months after the mussel treatment; “medium” hereinafter), November 24–26 (three months after the treatment; “long_1” hereinafter), 2015, and August 4–6, 2016 (approximately one year after the treatments; “long_2” hereinafter) to measure the mussel abundance and biomass, and three physical variables (i.e. flow depth, current velocity, and wetted channel width) along with the BACI design.

The abundance of pearl mussels was quantified in each reach on “before”, “short”, and “long_1” time periods. No mussel samples were collected from the “Control” reaches during the “short” time period, in order to minimize the disturbances on the mussels. In addition, no mussel surveys were conducted in the “medium” and “long_2” time periods because the water was too turbid to perform mussel surveys accurately.

Mussels were collected using quadrat-based sampling (area 0.063 m^2). Each reach was divided into 25 same-sized compartments, five of which were chosen, starting from the compartment on either of the furthest sides (randomly chosen) of the downstream row located diagonally to the upstream row in each reach. One quadrat was established at the center points of each of these five compartments.

In each selected quadrat, the riverbed was excavated by hand or foot to a depth of 10 cm, followed by the collection of pearl mussels. This process was undertaken consistently by the one trained investigator (N. Watanabe) in order to maintain similar sampling efforts. The sediments excavated during quadrat sampling were not sieved because the preliminary intensive survey that entailed excavation and the sieving of the bed materials, showed that pearl mussels had a shell length of >50 mm (Fig. S2), and the majority of individuals were considered large enough to be caught by hand. The collected mussels were counted and photographed digitally on a white tray with a ruler and a level gauge. Mussels were then released to the quadrat points they originated in immediately after being photographed.

The bed sediment stabilization functions of riverine organisms can be changed not only by their abundance, but also by their biomass (Albertson & Allen, 2015). Therefore, the dry mass of each pearl mussel individual was estimated using an empirical relationship between shell length and dry biomass (Online Resource 2), and the shell length (mm) of each individual was estimated using Image J software (Schneider et al., 2012).

Flow depth, current velocity, and wetted channel width were quantified in five time periods; three times along with mussel abundance measurements during the “before,” “short,” and “long_1” time periods, and once during the “medium” time period. In addition, flow depth and wetted channel width were measured on “long_2” time period. The wetted channel width was not measured during the “short” time period because visual observations revealed that it did not change within a very short period (within five days) from the manipulation. This assumption from visual observations was supported by the results in which there was no change of wetted channel width during the experiment (see Results section).

All the physical variable measurements were carried out immediately before the collection of mussel samples when these were conducted within the same time period. The flow depth and current velocity, averaged over 6 s at 40% flow depth from the streambed, were measured at the central point of the quadrat using a meter stick and a propeller-based flow meter (model CR-11; Cosmo-Riken Inc., Tokyo, Japan). The wetted channel width was measured at three transects each 2.5 m from the upstream end of each reach using a 50-m tape measure. As the quadrats were not set up for physical measurements, the flow depth during the “long_2” time period was measured on each transect at 10-cm intervals from one channel bank to the other.

The width (W)-to-depth (D) ratio (W/D) was calculated as an index of the cross-sectional shapes of stream reach (Gordon et al., 2004). The mean wetted channel width was calculated for each reach during the “before,” “medium,” “long_1,” and “long_2” time periods. For each reach, the mean flow depth of the quadrats was calculated during the “before,” “medium,” and “long_1” time periods, while that of the transects was calculated during the “long_2” time period.

The sediment volume lost by removal of pearl mussels was estimated and converted to sediment volume retained by per-kilogram biomass of pearl mussels. The sediment volume was calculated using channel length (i.e. 10 m), average wetted channel width, mussel biomass, and the changes in flow depth (thus bed surface elevation) across all the “Removal” reach against “Control” treatment level using the following formula: Sediment volume (m^3) = [channel length (m) × average wetted channel width (m) × flow depth (m)].

<u>Statistical analyses</u>

Generalized linear mixed models (GLMMs) were constructed to examine the physical variable differences among the three treatments (i.e. “Removal,” “Removal-Release,” and “Control”) and measurement time periods. The measurements obtained from each quadrat (flow depth, and current velocity), transect (wetted channel width), or reach (W/D) were used as response variables, with time period, treatment level, and their interaction used as explanatory variables, and section identity as a random effect. The full and reduced models without the interaction term were compared to test the effect of the interaction of time period and treatment level. When the interaction was significant, the models were compared further with the treatment level as an explanatory variable and the null model. That is, the model in which the effects of the intercept were considered but not the fixed effects, on each time period; models that included each of all combinations of three treatment as groups, as well as a null model, were developed and compared with each other. When the interaction was not significant, the models were compared further with the treatment level as an explanatory variable and the null model throughout the experimental period. In this case, the time period was included as a random effect together with the section identity.

The *manipulation effect* of the mussel removal manipulation on the two physical variables (flow depth and current velocity that had changed with time, see Results) was quantified and the loss of the *engineering effect* on the two physical variables in “Removal” was estimated. The mussel removal manipulation inevitably involved the physical disturbance of the riverbed during excavations, thus could lead to changes in the physical environment, thereby blurring the presence or absence of any mussel *engineering effects*. The “Removal-Release” data were used to examine the extent of the excavation effect. First, the mean value of each physical variable for each reach during each time period was obtained. Secondly, the mean change rates of each physical variable of “Control” on each time period relative to the “before” time period were obtained. Thirdly, these rates were applied to values from the “before” time period in “Removal” and “Removal-Release” to obtain the predicted values of the two physical variables for each time period. Finally, the observed to predicted value ratio of “Removal” and “Removal-Release” were calculated as the *manipulation effect* of each treatment (“Removal” and “Removal-Release”). A value of one in “Removal-Release” indicated that physical disturbances during excavations did not cause any changes in the physical environments. If this was proven true, values <1 (or >1) in “Removal” indicated that the changes in “Removal” were fully attributable to the *engineering effects* of mussels. Furthermore, the predicted values for “Removal” were obtained for each time period using the change rates of the physical variables in “Removal-Release.” The *engineering effect* loss was estimated as the proportional changes (percentage) in the observed relative to the predicted values of “Removal.” GLMMs were constructed with the *manipulation effect* or *engineering effect* losses as response variables, with days since the treatment (3, 60, and 90 days) as explanatory factors, and reach as a random factor.

In the GLMMs, a Gaussian distribution was used for all cases with an identity function. For models with more than one explanatory variable, multiple models were compared based on the Akaike information criterion (AIC) (Akaike, 1974), which is an estimator of out-of-sample prediction error, thus also of the relative statistical model quality for a given set of data. We considered models with delta AIC of less than two to be meaningful representations of the relationships between the explanatory and response variables (Burnham & Anderson, 2002). The model significance was tested using likelihood-ratio tests. All statistical analyses were conducted using R 3.0.3 (R Development Core Team, 2015) with a significance level α set at P=0.05. The models were fitted and compared using the glmmADMB package. The variables were ln(x)- or ln(x + 1)-transformed when necessary, to improve normality before analysis.

Results

A total of 74,200 mussel individuals were removed from the eight reaches by the manipulative treatments. In the “Removal” and “Removal-Release” reaches, the number of removed pearl mussel individuals was 6,968 ±2,100 individuals (range: 4,143–9,195 individuals) and 8,779 ±5,369 (range: 1,312–14,061 individuals), respectively. Mussel abundance and biomass were largely decreased only in “Removal” reaches after manipulative treatments whereas those two levels were very similar to the levels of the other two reaches in the “before” time period (Fig. S3, Table S1). The decreased mussel abundance and biomass in “Removal” persisted until the end of the experiment as intended (Fig. S3).

The flow depth, current velocity, and W/D changed with time, and the extent of the changes varied among the treatment levels (treatment level × time period interaction, $P<0.05$ for all; Table 2, Figs. 2 and 3). During the “before” time period, the current velocity did not differ among the treatment levels ($P>0.05$, Table 1). On the contrary, the flow depth and W/D in “Removal” and “Removal-Release” were on average 6.35 and 6.20 cm smaller, and 4.43 and 4.51 larger, respectively, than those in “Control” ($P<0.05$; Figs. 2 and 3). The differences or similarities of all three variables among the treatments changed significantly; this was true for current velocity and flow depth during the “short” time period, and W/D during the “medium” time period. These delayed responses to the manipulative treatment were observed largely in “Removal,” whereas the relative differences in all three variables between “Removal-Release” and “Control” remained similar throughout the experiment. During the “medium” and “long_1” time periods, the flow depth and current velocity in “Removal” increased on average by 27.2 ±8.67 cm (range: 14.4–38.2 cm) and decreased on average by 7.49 ±3.18 cm/s (range: 3.37–11.29 cm/s), respectively (both $P<0.05$, respectively; Fig. 2). In addition, the W/D in “Removal” decreased on average by 8.60 ±3.66 (range: 5.00–13.65) during the “medium” time period ($P<0.05$, Fig. 3). Unlike the other physical variables, the wetted channel width did not change during the experimental period (treatment level × time period interaction, $P>0.05$; Table 2), indicating that the temporal changes in W/D were caused by alterations in flow depth.

The manipulation effect was not significant in "Removal-Release," and this was reflected in the absence of the effects of time (Figs. 4a1 and b1; both $P>0.05$ for flow depth and current velocity, respectively). The *manipulation effect* was observed in "Removal," and the extent of the effects progressively increased over time with both the flow depth and current velocity values deviating from one in different directions (Figs. 4a2 and b2); the effects of time on flow depth and current velocity were both significant (both $P<0.05$). The *engineering effect* loss followed the temporal trajectory of the *manipulation effect* and resulted in a 60% increase in flow depth and a 30% decrease in current velocity (Figs. 4a3 and b3); the effects of time on flow depth and current velocity were both statistically significant ($P<0.05$). The total sediment volume lost by removal of pearl mussels and sediment volume retained by per-kilogram biomass of pearl mussels were estimated at 12.87 m^3 and 4.7×10^{-2} m^3, respectively.

Discussion

Previous studies have demonstrated sediment stabilization by organisms, such as freshwater mussels, in artificial streams and mesocosms (Zimmerman & de Szalay, 2007; Albertson et al., 2014). The findings of these studies are inherently constrained by the over-simplified characteristics of physical environments in terms of sediment characteristics, flow depth, current velocity, and their temporal changes during high flow events (e.g. Nowell & Jumars, 1987). In addition, how important these sediment-mediating traits of organisms are in ecosystem properties that extend beyond local erosion or sediment deposition has remained untested. The results of our upscaled field experiment, which allowed us to examine the effects of mussels without such constraints and at the river reach scale, clearly demonstrated that the removal of mussels had an effect on channel morphology as well as the physical environment in a sandy river. The temporally delayed responses of the channel to the removal of mussels resulted in a slower and deeper flow environment (i.e. increased flow depth and decreased current velocity), and these physical changes in the channel were preserved for at least three months after mussel removal. Furthermore, the changes in channel morphology (i.e. reduced W/D) were sustained for almost one year. These responses are in agreement with the initial hypothesis and supported our predictions.

The experimental design of the present study was successful in detecting the *engineering effects* of mussels by benefitting from two robust design strategies. First, the BACI design was implemented. Although “Control” reaches had slightly smaller wetted channel widths and larger flow depth (both $P<0.05$, Table 1), their physical environments remained relatively unmodified throughout the experiment, whereas substantial changes were observed in “Removal” reaches. Secondly, manipulation control in “Removal-Release” made it possible to exclude the artifact of mussel removals that could erroneously inflate the estimate of their *engineering effect*. In ecological studies that involve the experimental intervention in natural systems, there have been concerns about the potential bias in the outcome (Peterson & Black, 1994). In this study, the removal of mussels inevitably involved sediment disturbances, which can be directly associated with the physical environment responses. This manipulation effect was almost non-existent in “Removal-Release,” thereby indicating that physical disturbances associated with the removal of mussels generated negligible changes in physical environments throughout the experiment. In contrast, the manipulation effect was significant in “Removal,” thereby demonstrating that the observed physical environment changes in this treatment can be fully attributed to the independent effects of the removal of mussels and the loss of their *engineering effects*. The responses in “Removal-Release” followed the trajectory of the changes in “Control” and this further provided support that temporally progressive changes in “Removal” were also caused by the mussel removal treatment.

The *engineering effect* loss of the mussels was observed as an increase in flow depth and channel incision three months into the experiment. The decrease in current velocity was likely the physical consequence of the increased cross-sectional area through which the same flow rate in river water passed. The significantly reduced W/D in “Removal” that was observed since the “medium” time period was largely associated with a disproportionately greater increase in flow depth compared to “Removal-Release” and “Control.” The constantly small W/D in “Control” was caused by the relatively smaller wetted channel width and larger flow depth. The W/D differences among the treatments persisted until August 2016, based on the fact that there were no treatment-related changes in the wetted channel width of any of the reaches throughout the study period. Therefore, although flow depth, current velocity, or mussel measurements were not obtained one year after the manipulation at a resolution comparable to the time periods within three months of the manipulation, it was inferred that the *engineering effect* loss of the mussels persisted for at least one year. It is obvious that sediment was eroded from the channel, leading to the degradation of the riverbed. Interestingly, the mussel removal effects were not only continuous but also amplified over time. Delayed responses of channel form or riverbed sediment properties to external forces or physical structures are well known (e.g. Konrad, 2009; Nilsson et al., 2005). In natural rivers, riverbed elevation is maintained through sediment supplies from upstream, which balances the loss downstream in a dynamic equilibrium state (Gordon et al., 2004). Thus, it is probable that the increase in flow rates from a total of five major rainfall events and associated increases in hydraulic forces between the sampling time periods (Fig. S4) induced disproportionately greater loss than the upstream sediment supply and gradually lowered the riverbed.

The experiments indicated that pearl mussels retained the sandy bed-sediment by their *engineering effects* of pearl mussels suggesting their role as sediment stabilization agent. There are two possible mechanisms by which pearl mussels may have exhibited such an *engineering effect*. Firstly, mussels acted as an armoring layer (resistant to higher shear stress with a higher threshold required to initiate movements) on the riverbed, preventing the dislodgement of sandy particles (Zimmerman & de Szalay, 2007). Secondly, mussels reduced the shear velocity at the boundary layer on the riverbed surface with their shell protruding into the water column. The *engineering effect* of mussels is probably a function of the proportion of the riverbed surface occupied by mussel shells. Furthermore, the clear response of the channel is related to the relatively small riverbed materials, which are responsive to changes in hydraulic environments (Grant, 2012). Thus, relatively smaller *engineering effects* of pearl mussels are expected to occur in systems with larger substrate particles and/or lower mussel density.

Our results should be viewed with some cautions because of uncertainty and limitations.

First, there were no data on the changes in substrate size during the experiment. The substrate size has a close relation to bed-sediment erosion (Gordon et al., 2004). Even with the relatively homogenous size composition of sandy particles in the study segment, there may have occurred changes in the size composition due to the manipulative removal of mussels. Future studies should assess these possible changes. Second, there were no replicates at the river scale owing to the unavailability of other river sites where pearl mussels were abundant, and the river size accommodated manipulation. Third, the physical environment monitoring did not extend over several years over which more (or less) overall changes in physical environments might have occurred. Lastly, the estimated engineering effects can not be directly interpreted as a possible consequence of species extinction in the future. This is because there might have been an artifact of how mussels were removed in the experiment. The conditions through which all mussels disappeared from the channel were mimicked. In reality, the spent shell would remain for some time, possibly leaving partial engineering effects on ecosystems (Vaughn et al., 2008). The position of shells in the river bed would be different and the effects can also change in accordance in such a situation. Additionally, a gradual decrease in abundance is likely to lead to a more gradual acceleration of erosion, which would not necessarily cause the exact same responses in the physical environment. Despite these limitations, this study design clearly highlights the potential significance of the engineering roles of pearl mussels at an unprecedented spatial scale.

Overall results of this study demonstrated that endangered freshwater pearl mussels served as integral components of channel morphology in the study river. The influence of biota on the physical processes that shape the landscape has been debated in recent decades (Dietrich

& Perron, 2006; Corenblit et al., 2011; Jones, 2012). The results of this study demonstrate that the presence of pearl mussels not only directly affected the riverbed sediment dynamics, but also indirectly affected the flow environment by controlling the channel morphology in a low-gradient sandy river at a scale of 10-m reaches. The empirical data obtained at this spatial scale has important implications for the habitat management of this taxon. Freshwater mussels commonly form habitat patches consisting of highly aggregated individuals at a scale of several tens of meters (e.g. Strayer, 1999), and population dynamics such as the cessation of reproduction and extirpation of this taxon also take place at similar or larger spatial scales. The abundance of freshwater mussels has declined across their global geographical distribution range owing to various human impacts such as dam construction, water pollution, land-use change, and overexploitation (Haag, 2012; Haag & Williams, 2014; Ferreira-Rodríguez et al., 2019). The flow environment forms a crucial habitat for various organisms and facilitates material cycling in river ecosystems (Poff et al., 1997), where sediment characteristics strongly affect benthic processes (Wohl et al., 2015). Considering the findings of this study as an empirical projection of the consequences of the loss of pearl mussels in quality sandy river habitats, it is possible to foresee substantial changes in the channel morphologies that can potentially lead to chain reactions affecting the habitats of diverse aquatic organisms and the ecosystem functioning supported by them.

Conclusion

This study provided the first evidence that endangered freshwater pearl mussels can be an ecosystem engineer stabilizing sediments at a 10-m reach scale in the natural river. The manipulative removal experiment revealed that removal of mussels resulted in channel degradation with a 60% increase in flow depth, a 30% decrease in current velocity, and a 50% reduction in the W/D ratio two months after treatments in the reaches where mussels were removed. The states of increased flow depth and decreased current velocity were preserved at least for three months and a reduced W/D ratio was also observed almost one year after treatments. The results of the study suggested that pearl mussels can affect channel morphology and physical environments on a larger spatio-temporal scale than previously revealed by small-scale experiments. Importantly, different levels of engineering roles of mussels may exist, and thus the effects of removal and projected changes in the face of population decrease may greatly differ in other rivers. Channel responses to mussel removal likely depend both on mussel abundance and dominant riverbed substrate particle size. For example, in rivers having coarser riverbed gravels and steeper channel gradient, channel change after removals may occur over a longer time scale or the level of change may be smaller even with the abundance being similarly high to our study system. The magnitude of hydrological and sediment interventions by mussels could be reduced in such systems. These predicted variable engineering roles of mussels in relation to hydrogeomorphic contexts of rivers may warrant an evaluation in future studies. Overall, the findings of this study inform the river managers and stakeholders of the importance of habitat management and sustaining the pearl mussel populations at the river-reach scale as the keys to their far-reaching benefits in ecosystem integrity at least in low-gradient sandy rivers.

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Data availability

Data are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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Table 1 Summary of physical variables [mean (±SD)] in reaches for each treatment ("Removal–Release," "Removal," and "Control") on each time period ("before," "short," "medium," "long_1," and "long_2") or throughout the experimental period ("Overall the experimental period") from 2015 to 2016, and the results based on GLMMs with the likelihood ratio tests comparing with null models (a) (i.e. only with intercept). Bold type indicates statistical significance α at P=0.05. " † " indicates the result of the testing effect of the main factor throughout the experimental period on the basis of GLMMs with the likelihood ratio tests comparing with null models (a) (i.e. only with intercept). "-" means unmeasured variable (see "Methods"). Different letters of superscripts indicate a statistical difference among treatment levels on each time period (P<0.05, multiple comparisons based on generalized linear mixed models with delta AIC<2, and the likelihood-ratio tests).

Variable	Removal-Release	Removal	Control	*P*
"before"				
Flow depth (cm)	33.05(±11.28)^{b}	32.90(±10.46)^{b}	39.25(±12.12)^{a}	**<0.05**
Current velocity (cm/s)	34.65(±8.21)	34.46(±7.30)	36.50(±9.66)	0.39
W/D	17.45(±3.81)^{a}	17.37(±4.35)^{a}	12.94(±3.56)^{b}	**<0.05**
"short"				
Flow depth (cm)	33.55(±9.68)^{b}	33.25(±9.50)^{b}	38.85(±10.20)^{a}	**<0.05**
Current velocity (cm/s)	30.42(±5.37)	29.25(±5.69)	31.84(±9.75)	0.64
W/D	-	-	-	-
"medium"				
Flow depth (cm)	37.30(±12.30)^{c}	58.00(±18.27)^{a}	47.00(±10.62)^{b}	**<0.05**
Current velocity (cm/s)	33.14(±7.33)^{a}	26.03(±6.05)^{b}	35.03(±7.89)^{a}	**<0.05**
W/D	15.60(±4.28)^{a}	8.77(±1.89)^{b}	10.46(±1.83)^{b}	**<0.05**
"long_1"				
Flow depth (cm)	34.25(±13.37)^{c}	62.20(±17.47)^{a}	43.75(±10.36)^{b}	**<0.05**
Current velocity (cm/s)	36.57(±5.76)^{a}	27.91(±6.32)^{b}	35.68(±9.09)^{a}	**<0.05**
W/D	16.63(±3.37)^{a}	8.75(±2.30)^{c}	10.95(±0.85)^{b}	**<0.05**
"long_2"				
W/D	13.80(±3.94)^{a}	9.02(±3.33)^{a}	9.88(±2.06)^{b}	**<0.05**
"Overall the experimental period"				
Wetted channel width (m)	5.57(±0.62)^{a}	5.14(±0.57)^{b}	4.81(±0.58)^{c}	**<0.05†**

“W/D”: width-to-depth ratio

Table 2 Summary of GLMMs with the likelihood ratio tests comparing with reduced models (i.e. no interaction model) to test interactions between treatment level ("TL") and time period ("TP") on four physical variables.

Variable	Model	AIC	p
Flow depth (cm)	TL+TP+(TL×TP)	120.0	**<0.05**
	TL+TP	146.8	
Current velocity (cm/s)	TL+TP+(TL×TP)	33.1	**<0.05**
	TL+TP	34.2	
W/D	TL+TP+(TL×TP)	-11.7	**<0.05**
	TL+TP	-2.3	
Wetted channel width (m)	TL+TP+(TL×TP)	241.7	0.49
	TL+TP	235.1	

"W/D": width-to-depth ratio

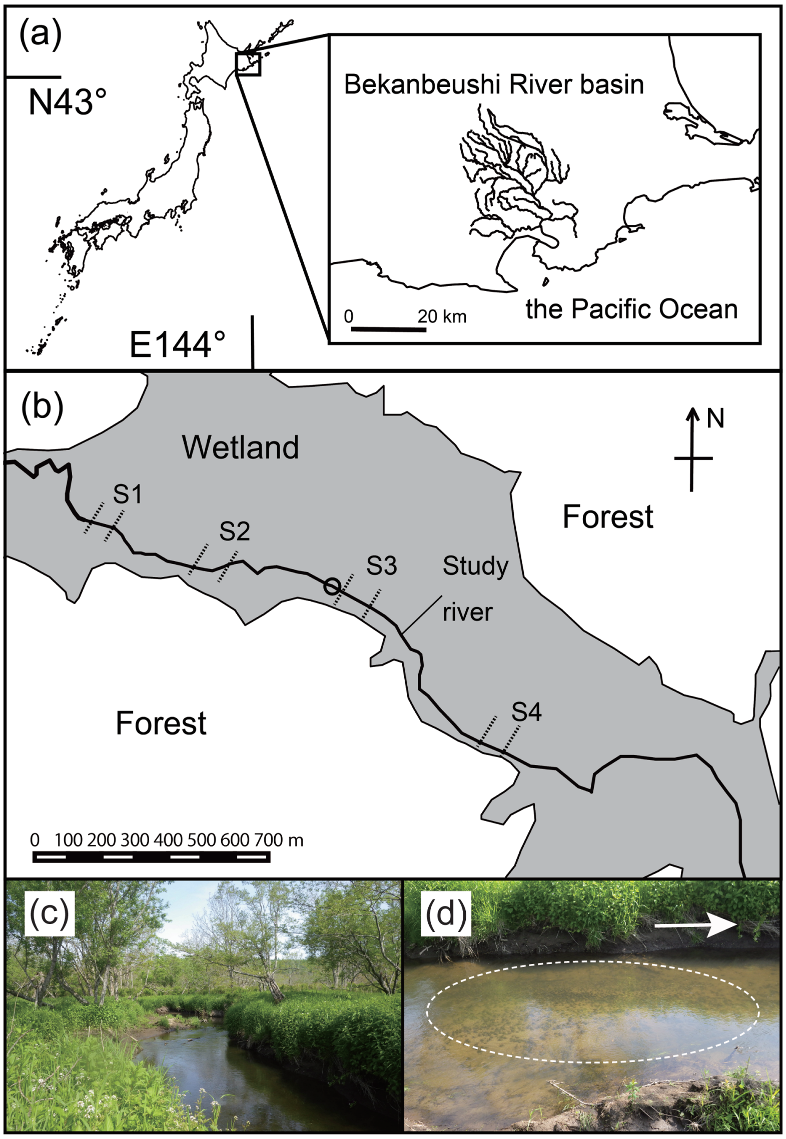

Fig. 1 Geographical location of the Bekanbeushi River basin (a), landscape structures around the study river (b), the photo of the study segment (c), and an example of the pearl mussel bed (enclosed by a dashed line) (d). The gray and white areas as well as the thick line in (b) denote a wetland, forest, and the study river, respectively. Four pairs of broken lines across the river and an open circle in (b) indicate the boundaries of each study section (S1, S2, S3, and S4) and the water level monitoring point, respectively. An arrow in (d) indicates the water flow direction.

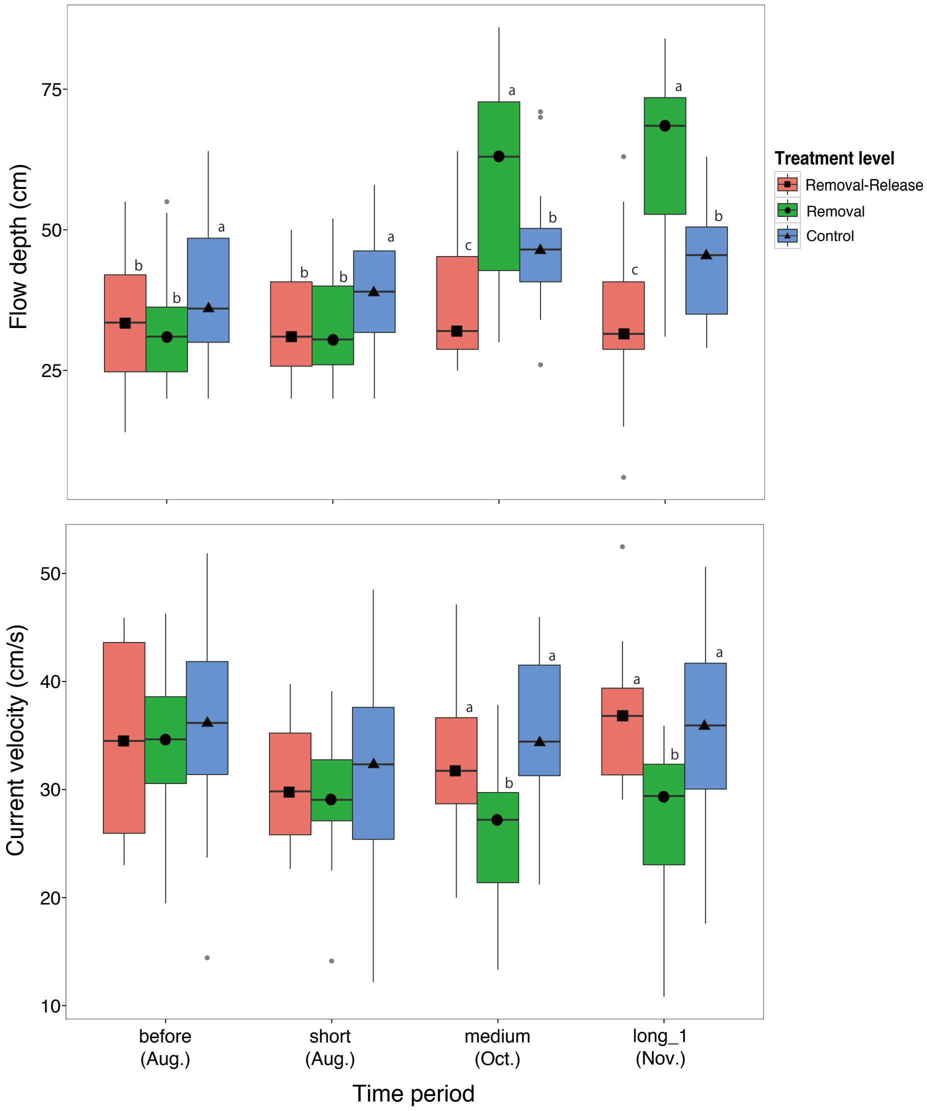

Fig. 2 Boxplots showing flow depth (top) and current velocity (bottom) in reaches for each treatment (“Removal–Release,” “Removal,” and “Control”) on each time period (“before,” “short,” “medium,” and “long_1”) in 2015. The lower ends, top ends, and central thick lines of boxes represent 25% and 75% quartile ranges, and medians, respectively. Error bars indicate a maximum value within 1.5 times of the box height, with outliers as gray circles. Different letters above the boxes indicate a statistical difference among treatment levels on each time period ($p<0.05$, multiple comparisons based on generalized linear mixed models with delta AIC<2, and the likelihood-ratio tests).

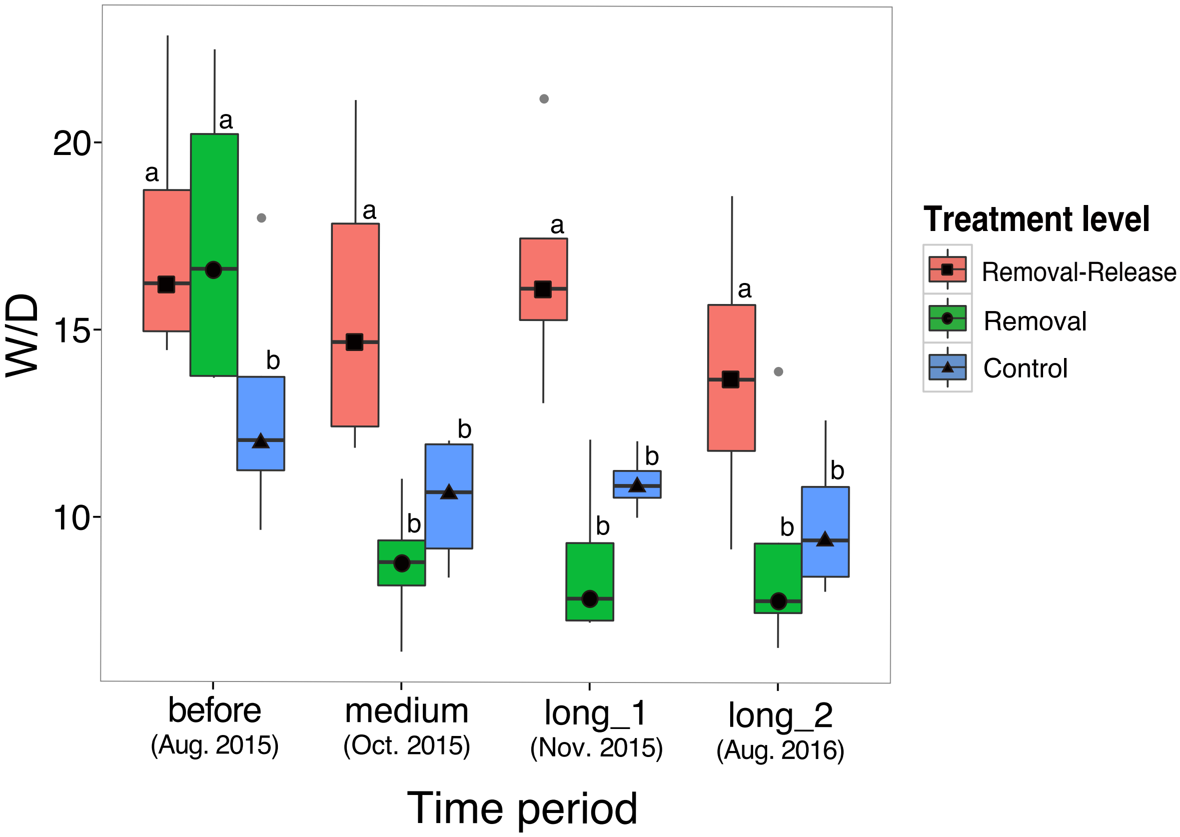

Fig. 3 Boxplots showing the width-to-depth ratio (W/D) of each treatment level ("Removal-Release," "Removal," and "Control") during each time period ("before," "medium," "long_1," and "long_2") from 2015 to 2016. See Figure 3 for boxplot explanations and letters above the boxes.

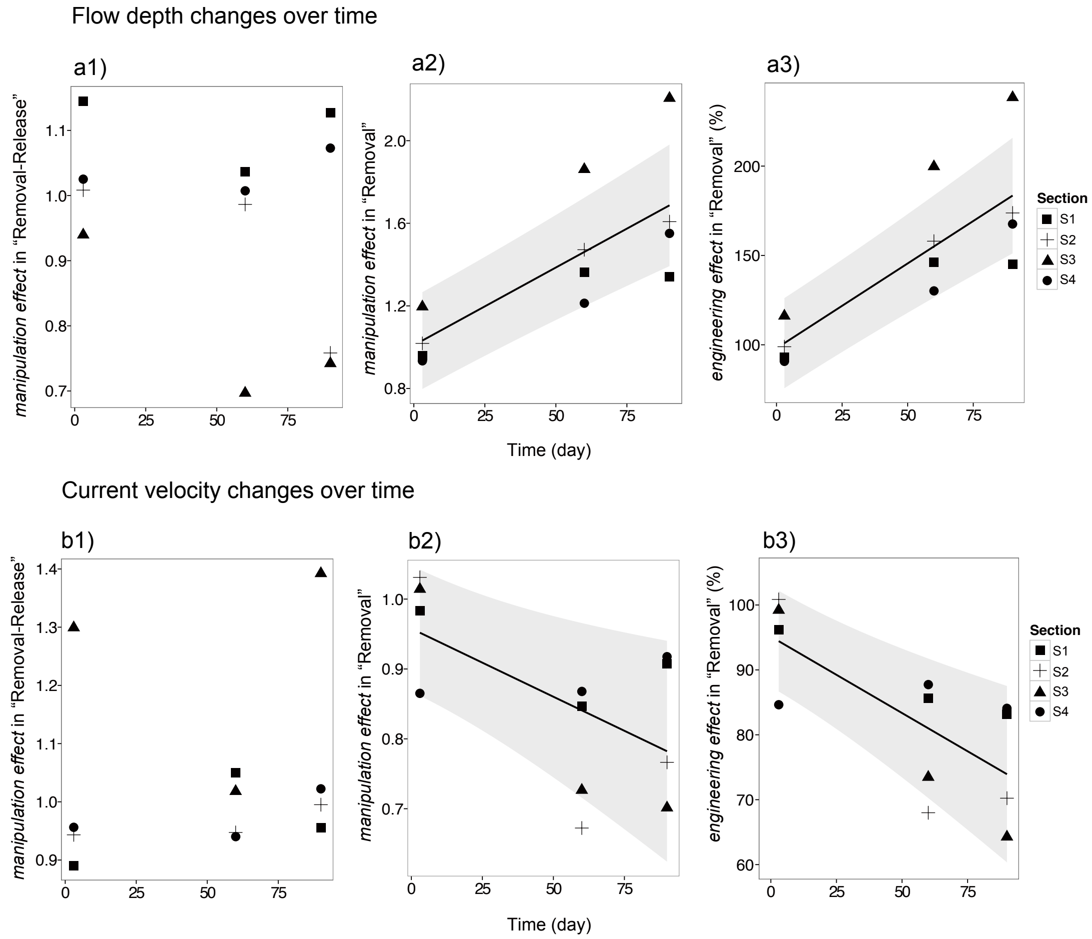

Fig. 4 Biplots showing the relationship between the extent of the differences in the physical environments (flow depth and current velocity) in the "Removal-Release" and "Removal" treatments compared to those in the "Control" treatment and the time elapsed since the manipulative treatment of mussels commenced (*manipulation effect*: a1–2 and b1–2, *engineering effect* loss: a3 and b3). The fitted lines were derived from generalized linear mixed models. Gray shaded areas are the 95% confidence intervals of the means in models.