Research Article

Artificial substrate experiments to investigate potential impacts of invasive quagga mussel (Dreissena rostriformis bugensis, Bivalvia: Dreissenidae) on macroinvertebrate communities in a UK river

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Abstract

Predicting potential impacts of a new invasive species remains difficult. A group of particular concern in the UK are freshwater invertebrates from the Ponto-Caspian region, including the recently established quagga mussel (Dreissena rostriformis bugensis, Bivalvia: Dreissenidae). We explored invertebrate colonisation across a series of manipulated substrate tiles with gradated densities of D. r. bugensis shells fixed to their surface (2220, 1111, 666, 222 and 0 individuals m⁻²). Across three experiments of different substrate tile deployment duration (14, 30 and 62 days), we observed significant differences in invertebrate density and richness among shell density treatments. Variation was primarily driven by low and high values on our control and highest substrate shell treatments, respectively. Within each experiment, similar taxa appeared to benefit from the physical effects of D. r. bugensis shells (e.g. Gammarus pulex, Chironomidae spp., Elmidae spp. and Hydropsyche spp.) being found with greater abundance on substrate tiles with higher D. r. bugensis shell treatments. Compared to invertebrate density, the response of taxonomic richness was weaker and only significant within our 30 and 62 day experiments of longer substrate tile deployment duration. Regardless, increased invertebrate density and richness across the highest shell treatments provided a strong indication of potential D. r. bugensis impacts on macroinvertebrates in the study river. If mussel densities were to increase to equivalent levels in other UK rivers, we could expect similar impacts to benthic fauna. While the likelihood of D. r. bugensis achieving such population densities are uncertain in such environments, our results were considered conservative because they did not account for additional facilitative impacts associated with live mussels. We add that, in the context of invasive species management, potential facilitation of native benthic fauna associated with D. r. bugensis in the UK should not be considered positively, nor necessarily sustainable over longer time periods. Further, facilitative effects could assist the establishment of other invasive invertebrates such as amphipods of Dikerogammarus spp., which were first recorded in the study river during this investigation.

Key words: Ponto-Caspian, non-native, colonisation, experiment, benthos, Dreissena spp.

Introduction

Proliferation of non-native invasive species has been documented in freshwater environments throughout the world (Lodge et al. 1998; Francis and Chadwick 2012). While researchers have had some success recording
impacts of invasive taxa on native biological communities (e.g. Gherardi and Acquistapace 2007; Stiers et al. 2011; Boltovskoy and Correa 2015); predicting impacts of a newly established species remains difficult (Williamson 1999; Roy et al. 2014). Impacts vary over time and across regions for different invasives (Strayer and Malcom 2006); potentially peaking only after considerable lag periods (Crooks 2005; Ricciardi et al. 2013).

Invasion biologists have modelled potential impacts of invasive taxa using expert knowledge and available literature (e.g. Copp et al. 2009; Roy et al. 2014) alongside statistical extrapolation of known trends (e.g. Ricciardi 2003; Kulhanek et al. 2011). Problematically, where establishments occur in a novel region, prediction of future impact is difficult in the absence of robust baseline information (Kulhanek et al. 2011). Even local records of biophysically similar invasive taxa may ignore important species-specific traits (Ricciardi 2003). This is an issue because accurate future impact scenarios are important to authorities for determining resource allocation in management (Byers et al. 2002).

A group of invasive freshwater taxa of particular concern in the UK are invertebrates of the Ponto-Caspian region of Ukraine and Russia (Gallardo and Aldridge 2013, 2015). In October 2014, a bivalve mollusc from this group, the quagga mussel (Dreissena rostriformis bugensis Andrusov, 1897) was first recorded in the United Kingdom (Aldridge et al. 2014). Prior to discovery, it was considered by experts as the most threatening potential invasive for the UK in terms of biodiversity impact (Roy et al. 2014). While subsequent study on invertebrate community structure in an invaded habitat did not suggest clear impacts to native biodiversity (Mills et al. 2017); the known range and densities of quagga mussel in the region appear to be increasing (Zoological Society of London, pers. comm., 2017) and stronger impacts of D. r. bugensis may be expected at higher densities.

The establishment of invasive Dreissenia spp. has been widely associated with shifts in the structure of pre-existing freshwater communities. In particular, invasions have been linked with increased benthic invertebrate density (Ricciardi 2003; Yakovleva and Yakovlev 2011; Ward and Ricciardi 2007). The structural complexity of Dreissenia spp. mussel beds provide predator refugia (González and Downing 1999; Ward and Ricciardi 2007), protection from wave action (Ricciardi et al. 1997) and increased habitable surface area (Stewart et al. 1998) to facilitate invertebrate taxa. Further, grazing herbivorous species may benefit from biofilm development on mussel shells (Kobak et al. 2013) and Dreissenia spp. pseudofoeces excretion can provide an exploitable food source for detritivores (Izvekova and Lvova-Katchanova 1972; Gergs and Rothhaupt 2008). While antagonistic biofouling of Unionid mussels and feeding competition with other filterers may cause deleterious impacts to certain taxa (Schloesser et al. 1998; Sousa et al. 2011), species of omnivorous Amphipoda and grazing Gastropoda
have been shown to benefit significantly from invasions, alongside overall increases to invertebrate taxonomic richness (MacIsaac 1996; Ricciardi et al. 1997; Bially and MacIsaac 2000). Facilitative impacts of *Dreissena* spp. may also favour varieties of other invasive species; particularly from the Ponto-Caspian region, increasing risk of “Invasional Meltdown” processes (Gallardo and Aldridge 2015; *sensu* Simberloff and Von Holle 1999).

Given such issues, the first objective of this study was to simulate and measure potential impacts of *D. r. bugensis* in UK rivers at densities higher than currently recorded. Invasive *Dreissena* spp. have shown varied population trends over time (Haynes et al. 1999; Strayer and Malcom 2006). In the Great Lakes Region, for example, peak mussel densities were achieved after 10 years since first record in Lake Michigan (Fahnenstiel et al. 2010), 6 years in Lake Erie (Karatayev et al. 2014) and 1 year in Lake Huron (Nalepa et al. 2003). In the UK, the zebra mussel (*Dreissena polymorpha*; Pallas, 1771) recently and unexpectedly increased its range and density, over a century after first national record and for uncertain reasons (Aldridge et al. 2004). If at any point, *D. r. bugensis* populations were to similarly expand in UK rivers, we might expect greater impacts than currently found. By simulating such an incidence here, we improve knowledge of this highly concerning invasive species in the absence of sufficient baseline data for UK rivers.

Using an experimental approach, we aimed to observe invertebrate colonisation across a series of manipulated substrate tiles treated with different densities of *D. r. bugensis* specimens; including some higher than currently recorded in the UK. Due to biosecurity considerations, we could not use live quagga mussels and so simulated *D. r. bugensis* individuals through the use of dead shell analogues. It was expected that substrate tiles with higher shell treatment densities would present increased invertebrate density and richness following deployment in a UK river. A second objective was to evaluate our novel methodology as a quantitative approach to determine potential impacts of invasive *D. r. bugensis* in the UK.

**Materials and methods**

**Study area**

Manipulated substrate tile experiments were conducted in the Wraysbury River (Aldridge et al. 2014; Mills et al. 2017), a shallow tributary of the river Thames (< 0.5 m depth), short in length (c. 8.7 km) and situated near Staines-upon-Thames (western London; Figure 1a). Catchment geology is Devensian gravels and the river had a predominantly sandy gravel/pebble substrate with laminar, glide flow conditions throughout. Surrounding land uses include semi-natural moorland, disused canals, sparse suburban housing and a section of the London orbital motorway (M25). Seasonal records collected by the UK Environment Agency between January 2015 and
April 2017 gave mean nutrient concentrations for the Wraysbury River as total oxidised nitrogen 10.7 N mg L\(^{-1}\), and orthophosphate 2.7 mg L\(^{-1}\) with stream alkalinity as 223 mg L\(^{-1}\) as CaCO\(_3\) (Environment Agency, pers. comm. 2018). The reach in which we deployed our substrate tiles was 20 m long (Lat 51.451842; Long –0.520814). It was chosen for homogeneous stream width (5 m), depth (0.3–0.4 m), flow velocity (0.2–0.3 m s\(^{-1}\)) and substrate typology, which was sandy pebble-gravel dominated.

**Experimental design**

Our substrate tiles were designed to identical specification before gradated treatments of *D. r. bugensis* shells were added to their surface. A series of coarse pebbles (40–60 mm on *a*-axis) were firstly collected by hand from Wraysbury River, washed and oven dried in the laboratory (6 hrs; 400 °C). 20 pebbles were then randomly selected and glued with silicon aquarium sealer onto a 150 × 150 mm patio tile base. The structural arrangement of clasts on each tile covered all of its surface and clast edges overlapped, leaving minor interstices between each pebble. Twenty-five substrate tiles were constructed for each of three experiments.
For substrate tile shell treatments, adult *D. r. bugensis* specimens (24–30 mm shell length) were collected from a known site on the Wraysbury River (Lat 51.455889; Long −0.518917) before removal of all inner-shell animal tissue through boiling and extraction using forceps. For each specimen, shell valves were glued back together with aquarium sealant in analogue appearance to a live animal. Analogue mussels (each containing two shell valves) were then glued to the surface of our substrate tiles at numbers of 50, 25, 15, 5 or 0 per substrate, or 2220, 1111, 666, 222 and 0 *D. r. bugensis* individuals m$^{-2}$ of tile, respectively (Figure 1b). For comparative purposes, mean density of *D. r. bugensis* recorded in the Wraysbury River, c.10 m upstream of the study reach, during the same period of time was ~200 individuals m$^{-2}$ (Mills 2017, unpublished), roughly equivalent to our lowest 5 shell substrate treatment.

Five replicates of each shell density were used for each of three experiments (substrate tile $n = 25 \times 3$) with the 0 shell treatment acting as control. Care was taken to ensure *D. r. bugensis* shells were glued to the substrate tiles by their posterior keel and orientated in a randomised direction; simulating field observations of live mussels in the Wraysbury River. For higher treatment densities (e.g. 50, 25 shells), complex interstices were formed between shell individuals and the substrate tile appearance strongly resembled that of a natural mussel bed or druse. With the lower shell treatments (15, 5 shells), coverage on the substrate tiles was sparser and care was taken to ensure that glued individuals were approximately equidistant (Figure 1b).

Substrate tiles were deployed at the study site for the first experiment between 30th June–30th July 2017 (30 days), second experiment between 30th July–30th September 2017 (62 days) and third experiment between 30th September–15th October 2017 (14 days). The deployment periods of our experiments were staggered due to limited space and availability of homogenous transects within the Wraysbury River. In conducting tests of different length, we hoped to evaluate the effects of substrate deployment duration on the density and taxonomic richness of colonising invertebrates. Across other environments, artificial substrates have appeared to achieve stabilised invertebrate communities within 30–60 days (Roby et al. 1978; Meier et al. 1979; Boothroyd and Dickie 1989) or occasionally, shorter periods (e.g. 19 days; Wise and Molles 1979 and 14 days; Figueroa et al. 2006). We thought comparison of experiments across similar time frames would help guide future study using our approach.

In each case, 25 substrate tiles were constructed, labelled and transported to the study reach for deployment on the first day of the test duration. Here they were placed in the stream 1 m from the wetted bank and 1 m equidistant, in randomised order (Figure 1c). Particularly coarse pebbles and cobbles had to be occasionally removed from the stream bed.
immediately underneath some substrate tiles; ensuring flattened elevation and increased stability in situ. In no cases was stream flow velocity sufficient to dislodge or transport any substrate tile during deployment.

At the start of each experiment, stream flow and various physicochemical parameters were measured with samples at 0.6 depth above the deployment location of each substrate tile. Parameters included stream pH, dissolved oxygen (DO; mg L$^{-1}$), conductivity (μs cm$^{-1}$), temperature (°C) and depth (cm). Aside from depth, all were recorded using a HACH™ HQ30d multi-probe and HI9811-5N pH/EC/TDS/°C portable meter. Stream flow measurements were also taken using a Valeport electromagnetic flow meter (model 801) using a 30 second-average velocity function. These measurements were conducted to assess variability in stream conditions among substrate deployment locations both within and across experiments.

On the last day of each experiment’s deployment period (after 14, 30 and 62 days, respectively), colonised invertebrate communities were sampled from the substrate tiles in-stream. In all cases, a surber sampler net (mesh size 250 μm$^{-1}$) was lowered by hand to the stream bed to envelop the surface of the deployed substrate tile. The substrate tile was then carefully loosened from the bed and lifted from the stream inside the covering net. The contained substrate tile and netting were thoroughly washed in a basin on the river bank and inspected carefully for attached invertebrates. Mineral and biological material washed and picked from each substrate tile was collected in a 180 μm$^{-1}$ mesh field-sieve before preservation in a labelled 50 ml polyethene vial using Industrial Methylated Spirit (90%).

In the laboratory, all invertebrates per sample were enumerated and identified under a high power ocular microscope. Identification was made to species level except for *Simulium* spp., Oligochaeta, and the family Chironomidae (identified to tribe). Individuals of Limnephilidae and Hydropsychidae were also grouped at family level due to morphological ambiguity at their smallest size-ranges.

**Data analysis**

Within each experiment, a series of one-way ANOVAs were performed to assess variability of physicochemical parameters across substrate tiles, per shell treatment category. Similar testing was then conducted to assess variability of physicochemical parameters among all substrate tiles, between experiments. In all analyses, when data for physicochemical measurements did not meet assumptions of normality, even after transformation, ANOVA on ranks was used. Where significant variation was found for physicochemical parameters both within or across experiments; post hoc pairwise comparisons were undertaken using a Tukey test.
For each substrate tile and corresponding shell treatment, post-deployment invertebrate density (individuals m$^{-2}$) and taxon richness was calculated. Graphical summaries of invertebrate richness and the contribution of different taxonomic orders to mean total density per treatment were made, including: Amphipoda, Coleoptera, Diptera, Trichoptera, Ephemeroptera, Gastropoda and Bivalvia. Following this, we conducted two-way ANOVAs on mean invertebrate density and richness using shell substrate treatment and experiment duration category as factors. This was undertaken to assess the impact of shell treatments across all experiments, ensuring no interactions between experiment duration and shell density effects. For invertebrate density, data were natural-log transformed to meet parametric assumptions prior to analysis. Where significant differences were found across levels of shell substrate treatment or experiment length; post hoc multiple comparison procedures were undertaken using the Holm-Sidak method. To test for variation in invertebrate density and richness within experiments; we also conducted one-way ANOVAs between shell treatments per experiment. Where there was significant variation between shell treatments, post hoc pairwise comparisons were undertaken using a Tukey test. All calculations were made using Sigmaplot 13.0 (Systat Software, Chicago, Illinois, USA).

Ordinations of community structure were performed with statistical software package PRIMER-E Ltd. 2009 (Clarke 1993; Clarke and Warwick 2001; Clarke and Gorley 2006). Across all experiments, Non-Metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarities was used to assess community composition per shell treatment based on mean invertebrate density (individuals m$^{-2}$) per taxon. Prior to statistical analysis, all data were Log(X+1) transformed to moderate for the effects of rare or highly abundant taxa (Clarke and Green 1988; Legendre and Gallagher 2001) and all taxa accounting for less than 0.5% of total mean density per experiment were excluded to reduce distortion of assemblage differences. NMDS is a widely used approach for displaying invertebrate community structure data (e.g. Barquin and Death 2004; Wikström and Kautsky 2007; Herbst et al. 2012).

**Results**

Physicochemical measurements suggested strong homogeneity of conditions above deployed substrates at the start of each experiment. Parameters, including stream dissolved oxygen (mg L$^{-1}$), pH, conductivity (μs cm$^{-1}$), temperature (°C), flow rate (m s$^{-1}$) and depth (cm) presented a small range of mean values with very low standard error (Table 1). Within experiments, one-way ANOVAs between shell substrate treatments failed to detect significant differences for any parameter. However, different ranges for parameter values between experiments suggested some variation...
Table 1. Summary of physicochemical measurements sampled above deployed substrate tiles, including: stream dissolved oxygen (mg L\(^{-1}\)), pH, conductivity (\(\mu\)S cm\(^{-1}\)), temperature (°C), flow (m S\(^{-1}\)) and depth (cm). Table shows the range, mean and standard error of each parameter for all measurements per experiment. Also shown: results of one-way ANOVA for parameter values between substrate *Dreissena rostriformis bugensis* shell treatments per experiment.

| Parameter measured          | Range all samples | Mean all samples | SE   | ANOVA (between substrate treatments) |
|-----------------------------|-------------------|------------------|------|--------------------------------------|
| **Dissolved oxygen mg L\(^{-1}\)** | 9.4 – 9.8         | 9.6              | 0.02 | F(4, 20) = 2.9                       | 0.878 |
| **pH**                      | 8.02 – 8.05       | 8.0              | 0.002| H = 1.2(4)                           | 0.884 |
| **Conductivity µS cm\(^{-1}\)** | 810 – 811         | 810              | 0.07 | H = 2.2(4)                           | 0.702 |
| **Temp °C**                 | 17.32 – 17.35     | 17.35            | 0.002| H = 2.8(4)                           | 0.59  |
| **Flow m S\(^{-1}\)**      | 0.2 – 0.3         | 0.25             | 0.003| F(4, 20) = 0.1                       | 0.99  |
| **Stream depth cm**         | 28 – 35           | 31.3             | 0.36 | F(4, 20) = 1.5                       | 0.23  |

| Parameter measured          | Range all samples | Mean all samples | SE   | ANOVA (between substrate treatments) |
|-----------------------------|-------------------|------------------|------|--------------------------------------|
| **Dissolved oxygen mg L\(^{-1}\)** | 8.5 – 9.0         | 8.6              | 0.02 | F(4, 20) = 0.6                       | 0.65  |
| **pH**                      | 8.02 – 8.03       | 8.0              | 0.001| F(4, 20) = 1.0                       | 0.431 |
| **Conductivity µS cm\(^{-1}\)** | 891 – 892         | 891              | 0.07 | H = 5.8(4)                           | 0.213 |
| **Temp °C**                 | 17.09 – 17.13     | 17.10            | 0.003| F(4, 20) = 7.4                       | 0.578 |
| **Flow m S\(^{-1}\)**      | 0.2 – 0.3         | 0.27             | 0.01 | H = 6.1(4)                           | 0.962 |
| **Stream depth cm**         | 27 – 31           | 29.4             | 0.46 | F(4, 20) = 2.9                       | 0.884 |

| Parameter measured          | Range all samples | Mean all samples | SE   | ANOVA (between substrate treatments) |
|-----------------------------|-------------------|------------------|------|--------------------------------------|
| **Dissolved oxygen mg L\(^{-1}\)** | 8.7 – 9.3         | 8.9              | 0.03 | F(4, 20) = 2.4                       | 0.085 |
| **pH**                      | 7.95 – 7.98       | 8.0              | 0.002| F(4, 20) = 0.7                       | 0.616 |
| **Conductivity µS cm\(^{-1}\)** | 871 – 873         | 810              | 0.07 | H = 0.2(4)                           | 0.993 |
| **Temp °C**                 | 18.13 – 18.15     | 18.14            | 0.001| F(4, 20) = 0.5                       | 0.722 |
| **Flow m S\(^{-1}\)**      | 0.2 – 0.4         | 0.35             | 0.02 | F(4, 20) = 0.4                       | 0.838 |
| **Stream depth cm**         | 27 – 34           | 32.2             | 0.47 | F(4, 20) = 2.4                       | 0.083 |

in physicochemical conditions (Table 1). According to one-way ANOVAs on ranks, significant variation in mean stream dissolved oxygen (mg L\(^{-1}\)), pH, conductivity (\(\mu\)S cm\(^{-1}\)), temperature (°C) and depth (cm) above deployed substrate tiles was found between experiments (Supplementary material Table S1). This was expected due to seasonal environmental variation, which will be discussed further in the context of our results.

A total of 4826 invertebrate individuals were identified on substrate tiles across all three experiments, including 44 taxa. For each case, the three most abundant groups found were amphipod shrimps of Gammaridae, riffle beetles of Elmidae and non-biting midges of Chironomidae. The net spinning caddis family Hydropsychidae was the fourth most abundant group for both 30 day and 62 day experiments, but it was Oligochaeta for the 14 day experiment (Table S2). While nearly all recorded invertebrates were native, several invasive species were found at low density across experiments, including *Crangonyx pseudogracilis* (Bousfield, 1958), *Dikerogammarus haemobaphes* (Eichwald, 1841), *Potamopyrgus antipodarum* (Gray, 1843) and *D. r. bugensis* (Table S2).

Mean invertebrate density was highest for the 50 shell treatment and lowest on the control in all three experiments (Figure 2). With two-way ANOVA, significant differences in mean invertebrate density (\(Ln\)-transformed) were found among substrate shell treatments. In post hoc Holm-Sidak tests, the 50 shell treatments presented significantly higher invertebrate density compared to all others. In addition, the moderate 15 and 25 shell substrate treatments showed significantly higher density compared
Figure 2. Proportional contribution of different taxa groups to total mean invertebrate density (individuals m$^{-2}$) across substrate tile shell treatments ($n$ substrate tiles = 5 per treatment) for all experiment duration categories. Error bars denote standard error. Symbols denote significant differences between substrate treatment categories after allowing for effects of experiment duration category according to two-way ANOVA ($p$ = < 0.001).

Within experiments, one-way ANOVA presented significant differences in invertebrate density between treatments for all tests. According to post-hoc Tukey’s procedures, the 50 shell treatment had significantly higher mean density than all others in the 30 and 62 day experiments. For the 14 day test, the 50 shell treatment was only significantly higher than the control (Table S4).

Taxa groups contributing to total invertebrate density appeared consistent between experiments and across shell treatments, but for some exceptions. In the 30 day experiment, Amphipoda showed a higher percent contribution to total density for the 50 shell treatment (41%) compared to others (23–28%). Similarly for the 62 day experiment, Trichoptera (50 shell treatment; 15%, others 8–11%) and Diptera (50 shell treatment; 16%, others 5–11%) were more dominant contributors to the 50 shell treatment. Within the 14 day experiment, proportional contributions of taxa groups appeared more homogenous throughout treatments. Notably in this case, total density appeared consistently lower across treatments than for the 30 and 62 day tests (Figure 2).

With two-way ANOVA, significant differences in mean total invertebrate density (Ln-transformed) were found according to experiment duration category, after allowing for differences in substrate shell treatment.
In Holm-Sidak tests, the 14 day experiment presented strongly significant, lower invertebrate densities compared to both others (Table S3). The 30 day experiment also presented higher densities compared to the 62 day experiment, but at weaker significance.

Mean invertebrate richness was highest for the 50 shell treatment in the 30 and 62 day experiments and for the 15 shell treatment in the 14 day experiment (Figure 3). According to two-way ANOVA, significant differences in richness were found between substrate shell treatments, after allowing for experiment duration category. In Holm-Sidak tests, control treatments (0 shells) presented significantly lower invertebrate density compared to all others except for the 5 shell treatment. The latter was also significantly lower than the 50 shell treatment. For this test we again found no significant interactions between experiment duration and shell density effects (Table S3).

Within experiments, one-way ANOVA also presented significant variability of richness between treatments; though only in the 30 and 62 day experiments. Tukey’s tests showed that in the 30 day test, richness was significantly higher for the 50 shell treatment compared to the 5 shell treatment. For the 62 day test, the 50 shell treatment was significantly higher than the control (Table S4).

In general, mean richness appeared lower across treatments in the 14 day experiment when compared to equivalents in the 30 and 62 day tests (Figure 3).
With two-way ANOVA, significant differences in richness were found according to experiment duration category, after allowing for differences in substrate shell treatment. In Holm-Sidak tests, the 14 day experiment presented significantly lower invertebrate richness compared to both others (Table S3) but there was no significant difference between the 30 and 62 day tests.

In the NMDS plot (stress 0.05), mean community composition across experiments (driven by taxa contributions to total invertebrate density) appeared most segregated by experiment rather than shell treatment (Figure 4). For the 62 day experiment, the three highest shell treatments were closely grouped together and more distanced in the plot from their respective lower treatments. Similarly, the highest two shell treatments in the 30 day experiment were closely grouped and separated from their respective lower shell treatments. In contrast, the 14 day experiment presented closer association between the lower three treatments, while the remaining higher treatments appeared more isolated on the plot.

**Discussion**

The response of invertebrates to substrate tile treatments complimented facilitative associations of invasive *Dreissena* spp. (e.g. Stewart and Haynes 1994; Kuhns and Berg 1999; Ward and Ricciardi 2007; Ozersky et al. 2011). For example, the largest mean increase of invertebrate density was consistently found between the control and highest substrate shell categories (14 day: 119%, 30 day: 124%, 62 day: 148%); with these treatments driving significant variation both within and across experiments. Given homogenous physicochemical conditions for substrate tiles *in situ*; we
could attribute this variation to differences in *D. r. bugensis* shell densities. Such effects were expected because invertebrates have shown positive responses to the physical structure of shells (Botts et al. 1996; Horvath et al. 1999).

Dominant taxa in our study, including the riffle beetle *Elmis aenea* (Müller, 1806), caddisfly *Hydropsyche* spp., and shrimp *Gammarus pulex* (Linnaeus, 1758) were consistently found at increased densities on higher substrate shell treatments and feasibly benefitted from effects provided. For *E. aenea*, the taxon’s size permits flow refuge by exploitation of small interstices (Peris et al. 2015) characteristic of *Dreissena* spp. mussel beds. With *Hydropsyche* spp., shells may provide protrusive bed features on which nets and tubular refuges are constructed for suspension feeding (Edington 1968). For *G. pulex*, shell structures may provide predator refugia, as suggested during *ex-situ* laboratory experiments on *Dreissena* spp. (Reed et al. 2004; Kobak et al. 2014). In all cases, taxa could benefit from increased habitable surface area, as associated with natural *Dreissena* spp. beds (Ricciardi et al. 1997; Stewart et al. 1998; Ward and Ricciardi 2007).

Such physical traits have also explained the positive response of invertebrate richness to invading *Dreissena* spp. (Griffiths 1993; Stewart and Haynes 1994; Ricciardi 2003), which was similarly correlated with higher substrate shell treatments in our study. Across experiments, significant variation between treatments was driven by low and high richness values for the control and highest shell categories, respectively. This was similar to trends for invertebrate density, however the response of richness appeared reduced. In particular, significant differences within experiments were only found between treatments for the 30 and 62 day tests; alongside weaker *p* values than for density. Viewing too, comparatively lower richness values throughout the 14 day experiment, this test appeared subject to different colonisation effects than the other experiments.

One possibility was that methodologically, 14 days was an insufficient duration for colonising invertebrates to achieve taxonomic richness representative of shell treatment effects. Artificial substrates are typically saturated by invertebrates to stabilised richness within 30–60 days (Roby et al. 1978; Meier et al. 1979; Boothroyd and Dickie 1989) despite variation across sampling methodologies and geographical regions of deployment (Boothroyd and Dickie 1989). While some studies suggest shorter durations are sufficient for representative assemblages to appear (e.g. 19 days; Wise and Molles 1979 and 14 days; Figueroa et al. 2006); we found that significant variation in richness between experiments, after allowing for effects of substrate shell treatment, was driven only by lower values for the 14 day experiment. This suggested temporal factors were of importance to our methodology.

Aside from substrate deployment length; the timing of each test, though seasonally proximate, was also different (particularly comparing the 30 and
14 day experiment). This could mean invertebrate life histories, such as the pupation or emergence of adult insects, prevented important taxa groups from achieving comparable assemblages across tests. For example, Trichoptera found in the longer two experiments, but not in the 14 day test, included Goera pilosa (Fabricus, 1775), Brachycentrus subnubilis (Curtis, 1834) and Limnephilus lunatus (Curtis, 1834). Given the diversity of life history patterns shown by such taxa (Meier et al. 1979; Jannot et al. 2008), their colonisation could have been restricted at this time compared to others. Generally, seasonal shifts in stream primary production or autochthonous inputs would also be expected in temperate streams (e.g. Roby et al. 1978; Hawkins and Sedell 1981); causing variation in food availability to impact invertebrate distribution and life history patterns (Vannote et al. 1980; Jannot et al. 2008). In our study, distinct community compositions within experiments, identified in the NDMS plot, could have been due to such factors; although they remain difficult to isolate from effects of experiment duration.

In evaluating our methodology, the issues of substrate deployment time and seasonal period should be more carefully standardised in future experiments. Chiefly, substrate deployment of 30 days or longer, during consistent seasonal periods could be used for achieving more comparable results between experiments. Where experiments of different duration are conducted, simultaneous start times could also be employed; given sufficient homogenous space for deployment. However, we argue our current study still provides a useful benchmark for potential impacts of D. r. bugensis in Wraysbury River. A significant, positive response of invertebrate density and richness was clearly shown across experiments, despite differently timed deployment periods. At particular population levels (between 1110 and 2220 individuals m$^{-2}$), we may conclude D. r. bugensis would significantly impact benthic community structure in this system. Considering the likelihood D. r. bugensis would reach such densities, we may comment whether such impacts would feasibly occur in future.

In this respect, there remains uncertainty regarding potential invasiveness of D. r. bugensis in environments like the Wraysbury River. Initial establishment of the species at this site was considered surprising (Aldridge et al. 2014) and the majority of high density, invasive populations have been found in deep, lentic environments of North America. For example, 16,000 individuals m$^{-2}$ (Lake Michigan; Nalepa et al. 2009), 75,000 individuals m$^{-2}$ (Lake Huron; Nalepa et al. 1995) and 342,000 individuals m$^{-2}$ (Lake Erie; Howell et al. 1996). In more illuminated, shallow systems, Dreissena spp. are vulnerable to visual predation by waterfowl and fish (Karatayev et al. 1997; Petrie and Knapton 1999; Haynes et al. 1999), while early-stage larval veligers exhibit higher mortality with increased exposure to ultraviolet radiation (Thaw et al.
2014) and flow turbulence (Horvath and Lamberti 1999; Rehmann et al. 2003). In Wraysbury River, such limitations could prevent *D. r. bugensis* populations increasing from the modest densities currently recorded (maximum: 198 individuals m$^{-2}$; Mills 2017, *unpublished*). We suggest clear impacts of *D. r. bugensis* on cohabiting invertebrate communities would be unlikely to occur in current conditions.

However, the fact we used analogue *D. r. bugensis* rather than live mussels should be noted. While comparative studies of invertebrate communities on live and dead *Dreissena* spp. suggest invertebrates primarily respond to the physical structure of shells (Botts et al. 1996; Horvath et al. 1999); specific effects of live *D. r. bugensis* may provide further benefits for some taxa. In particular, *D. r. bugensis* suspension feeding of phytoplankton and subsequent pseudofaces excretion has been shown to concentrate phytic biomass, nutrients and minerals on the bed (Izvekova and Lvova-Katchnanova 1972; Stewart and Haynes 1994). Such materials may be consumed by invertebrates (e.g. MacIsaac 1996; Pace et al. 1998) or encourage the development of biofilm and submersed macrophytes (Arnott and Vanni 1996; Stoeckmann and Garton 1997); providing additional food sources and greater habitat heterogeneity. In particular, the facilitation of Chironomidae by *Dreissena* spp. has been associated with consumption of pseudofaeces (Griffiths 1993; Botts et al. 1996); another prominent group found in our study. We would expect even stronger facilitation of such taxa when colonising equivalent populations of live mussels.

In describing our findings in the context of invasive species management, we hope not to overstate the potentially positive, facilitative impacts of *D. r. bugensis* on cohabiting invertebrate communities. Over annual time periods, invasive *Dreissena* spp. have been associated with periodic desaturation of dissolved oxygen in North American rivers due to population respiration demand (Effler and Siegfried 1994; Effler et al. 1996; Caraco et al. 2000). In poorly aerated systems, resulting anoxia may degrade, rather than facilitate faunal diversity and richness (Effler et al. 1996), including vertebrate groups such as fish. In addition, research in other invaded environments has shown initial benthic responses to *Dreissena* spp. weaken after several years (*sensu* Strayer and Malcom 2006; Karatayev et al. 2015); possibly driven by developing predatory regulation of invertebrates by fish (Karatayev et al. 1997; Haynes et al. 1999). In Wraysbury River and other UK environments, such effects could be driven by widespread benthivorous species like bullhead *Cottus gobio* (Linnaeus, 1758) and gudgeon *Gobio gobio* (Linnaeus, 1758). Outwardly positive effects of *D. r. bugensis* for benthic fauna in UK rivers may be similarly unsustainable and decline over time.

Further, facilitative impacts of *D. r. bugensis* may allow other invasive invertebrates, including several Ponto-Caspian taxa, to benefit from *D. r. bugensis* proliferation in the UK (Gallardo and Aldridge 2013, 2015). For
example, invasive, predatory amphipods of *Dikerogammarus* spp. have been shown to present an affinity to *Dreissena* spp. shells (Kobak and Żytkowicz 2007) and like other taxa, benefit from increased habitat complexity provided by mussel beds (Gallardo and Aldridge 2013). Interestingly, in both our 30 and 62 day experiments, invasive shrimp *Dikerogammarus haemobaphes* (Eichwald, 1841) was explicitly identified on our higher shell treatments. While found at low abundance, this was our first record of a Ponto-Caspian shrimp for the Wraysbury River after approximately three years of recent study. While the possibility *D. r. bugensis* may facilitate other Ponto-Caspians in Wraysbury River can only be highlighted and not conclusively demonstrated here; additional research might examine such interactions further and progress understanding on the implications of *D. r. bugensis* establishment in UK rivers.

**Conclusions**

In three colonisation experiments with manipulated substrate tiles, significantly higher invertebrate density was found with increasing *D. r. bugensis* shell treatment. Prominent taxa recorded at greater density with higher shell treatments included amphipod *G. pulex*, riffle beetle Elmidae, net spinning caddis *Hydropsyche* spp. and dipteran Chironomidae. Increasing shell treatments may have provided more habitable surface area, predator and flow refugia for invertebrates alongside facilitation of feeding strategies for certain taxa.

Positive responses of invertebrate taxonomic richness were also found with higher *D. r. bugensis* shell treatment; though weaker than for invertebrate density. Within experiments, significantly increased richness with higher shell treatments occurred only for the longer, 30 and 62 day experiments. Our shortest, 14 day experiment presented comparably homogenous richness values between shell treatments.

Given evidence of similar physicochemical conditions between experiments, less clear variation in richness and density across treatments for the 14 day test could have been driven by insufficient substrate deployment duration for stabilised invertebrate communities to develop. Further, experiments were not performed concurrently and seasonal change may have impacted invertebrate responses. In evaluating our methodology for future study; substrate deployment of 30 days or longer, during consistent seasonal periods was recommended.

Despite differences in experiment duration and timing, we observed consistent, significantly increased invertebrate density and richness across the highest substrate shell treatments, equivalent to 2220 *D. r. bugensis* individuals m⁻². If similar mussel densities developed in the Wraysbury River, we would expect comparable impacts on benthic fauna to occur. The feasibility of such populations occurring in this site appears low, however our results may be conservative, failing to account for additional impacts of live mussels.
In the context of invasive species management, potential facilitation of benthic fauna by *D. r. bugensis* in Wraysbury River was not considered particularly positively, nor sustainable. In other invaded regions, *Dreissena* spp. have been associated with periodic stream anoxia and other feedbacks which may degrade aquatic communities. Further, facilitative effects on the benthos may assist the establishment of other invasive invertebrates such as amphipods of *Dikerogammarus* spp., first recorded in the Wraysbury River during this study.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** Results of 1-way ANOVA assessing variability of physicochemical parameters above substrate tiles, between experiments, measured at the start of the deployment period.

**Table S2.** Mean density of invertebrates per taxa (individuals m⁻²) sampled on substrate tiles according to shell treatment category and experiment.

**Table S3.** Results of two-way ANOVA for mean invertebrate density (individuals m⁻²) and taxonomic richness using substrate tile shell treatment and experiment duration category as factors.

**Table S4.** Mean invertebrate density (individuals m⁻²) and taxonomic richness for *Dreissena rostriformis bugensis* shell treatments per substrate tile exposure period (± SE).

This material is available as part of online article from:

http://www.aquaticinvasions.net/2019/Supplements/AI_2019_Mills_etal_SupplementaryMaterial.xlsx

Mills et al. (2019), *Aquatic Invasions* 14(2): 365–383, https://doi.org/10.3391/ai.2019.14.2.13