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METHODS AND RESOURCES

Temperature-dependent spatial and seasonal distribution patterns of the invasive zebra mussel in an artificial lake assessed using environmental DNA

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Abstract

Environmental DNA (eDNA) combined with quantitative PCR analysis is a fast and accurate alternative to more costly and laborious physical methods to detect the highly invasive zebra mussel (*Dreissena polymorpha*). We have developed, in collaboration with the managing authorities of an artificial reservoir, a cost-effective eDNA qPCR-High resolution melt (HRM) assay for zebra mussel which we used for a pilot monitoring of spatial-temporal fluctuations in density across the reservoir. Zebra mussel eDNA densities varied significantly across sampling locations and months, being lower in the winter (when zebra mussel growth is slower) and the highest in April (about a month ahead of the reproductive peak). Temperature was a significant predictor of eDNA concentration. We hypothesise that extreme temperatures might have triggered early reproduction, highlighting the need to plan regular monitoring exercises considering environmental variation, particularly in years with extreme variations. Establishing fast, accurate and affordable methods for regular zebra mussel monitoring is particularly relevant in relation to climate change and may allow prediction of reproductive peaks or distribution shifts. The collaboration with the managing authorities is essential for the regular monitoring of aquatic invasive species such as the zebra mussel.

Keywords Invasive species · eDNA-barcoding · QPCR · Freshwater

Introduction

Artificial lakes and reservoirs favour the colonisation and establishment of invasive species mainly because they are more recent, more disturbed and have higher human accessibility than natural lakes (Frehse et al. 2021; Sultan and Pei 2023). Reservoirs are also one of the leading causes of aquatic fauna homogenisation, mainly driven by the combined effects of introduced species and local extinctions of native species (Britton et al. 2023). Moreover, the impact is not limited to impoundments created by large hydropower dams or extensive artificial lakes, even small dams' reservoirs alter the benthic communities, facilitating the colonisation of non-native species (Linares et al. 2019). Reservoirs tend to accumulate different invasive species, and their interactions often facilitate each other's invasions or even act synergistically (making their combined impacts larger than those of each separate invasion) (Preston et al. 2012; Ricciardi 2003), potentially leading to invasion meltdowns (Braga et al. 2018).

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The Ponto-Caspian region is an area from where a particularly high number of freshwater species have invaded European and North American rivers, lakes and reservoirs (Gallardo and Aldridge 2015; Ricciardi and MacIsaac 2000), due to their general tolerance to high salinities (Krijgsman et al. 2019). The zebra mussel (*Dreissena polymorpha*) is one of the most common Ponto-Caspian invaders causing widespread ecologic and economic loss in Europe and North America (Connelly et al. 2007; Minchin et al. 2002) by competing directly for food and space with native species (Karatayev et al. 2015; Haubrock et al. 2024) and can drive local populations to extinction (Zwarych et al. 2025). Zebra mussels can also facilitate the establishment of other invasive species, for example, they can engineer dense mats both on natural and artificial surfaces (Lovell et al. 2006) which can be used as refugium by killer shrimp (Rolla et al. 2019). Navigation is a common vehicle of introduction and dispersal for many aquatic invaders, including the zebra mussel (Robertson et al. 2020; Rodríguez-Rey, Consuegra, Börger, & Garcia de Leaniz, 2021), making artificial lakes used for sailing activities hot spots for aquatic invaders.

Environmental DNA (eDNA) the DNA of all organisms present in environmental samples (Pawlowski et al. 2020), including DNA released by organisms through their blood, urine, skin, mucus or faeces, is commonly used to detect aquatic species which are rare, threatened or difficult to sample by traditional means (Biggs et al. 2015; Robinson et al. 2019). eDNA analysis has been recommended as an alternative or complement to more costly and laborious physical methods to detect zebra mussel, particularly in lentic habitats (Amberg et al. 2019) and for that purpose a number of assays have been developed (Amberg et al. 2019; Blackman et al. 2020; Feist and Lance 2021; Sepulveda et al. 2020a). In combination with quantitative PCR (qPCR), eDNA analysis has proved to be a reliable method for detecting targeting endangered and invasive species (Díaz-Ferguson et al. 2014; Carlsson et al. 2017), using specific probes (Gingera et al. 2017) or high-resolution melt (HRM) curve analysis (Minett et al. 2020; Robinson et al. 2018). The analysis of eDNA from water samples is commonly used to identify spatial and temporal changes in species distribution patterns (Handley et al. 2018; Sigsgaard et al. 2017; Yamamoto et al. 2016), spawning migrations (Maruyama et al. 2018), to assess habitat connectivity for individual species (Yamanaka and Minamoto 2016) and community changes (Muha et al. 2021). Yet, some reticence remains to their application for monitoring and management purposes and it has been suggested that involvement of the management authorities in the design of the sampling strategy may be key to overcome it (Feist and Lance 2021; Sepulveda et al. 2020b).

Cardiff Bay is a 200 ha. artificial freshwater lake (depth = 4–14 m) located in Wales in the mouths of the rivers Taff and Ely, limited by a barrage. It was built between 1994 and 2001 as part of a regeneration project of the old docklands area (Alix 2010) and is used for recreational sailing activities. Zebra mussel was introduced in the Bay by visiting vessels around 2004 for the first time (Alix et al. 2016), representing the first confirmed sighting of the species in Wales. Zebra mussels are actively controlled in Cardiff Bay by annual removals (Alix 2010) but, while its presence has not been identified in the upper reaches of the rivers Taff and Ely, the Bay poses an important risk for invasion and dispersal. Previous analyses using physical sampling indicated significant spatial variation in mussels' growth and settlement rates across the Bay which, coupled with multiple cohorts, could be helping the persistence of the species in the lake (Rolla, Consuegra, Hall, & Garcia de Leaniz, 2020). In collaboration with the managing authority of the lake (Cardiff Harbour Authority) we developed an eDNA-qPCR based assay to detect zebra mussel in the lake and assessed its ability to reflect changes in abundance through laboratory trials and temporal sampling on key locations across the lake.

Materials and methods

Study site, water collection and DNA extraction

We collected water samples from 5 different locations across Cardiff Bay (51.4539° N, 3.1694° W): Channel Dry Dock (A), Inner Harbour (B), Sand Wharf- Lower Taff (C), Lower Ely (D) and Cardiff barrage (E); 4 of them (B-E) also sampled in Rolla et al. 2020 (Fig. 1). The sampling points were chosen for the variable abundance of veligers and adults (Rolla et al. 2020) as well as including a broad coverage of environmental conditions within the bay based on a previous monitoring exercise (Alix 2010). Samples were collected over 4 different seasons in January 2016 and in April, June and August 2017. Water samples were collected in sterile 1 L Nalgene bottles at ~30 cm depth in triplicate per site. Each replicate consisted of 1 L of water taken from a boat from the lake surface, using disposable nitrile gloves to avoid contamination. Bottle sterilisation was conducted using 10% bleach solution and rinsing with DNA-free water prior to the field sampling. Water samples were refrigerated, transported to the laboratory, and filtered within 4 h of sampling through a filter funnel attached to a collection bottle and connected to the electronic vacuum pump Welch N. 2522 C- 02, with strength of 20 kpa for 15 s up to 60 s per sample, using Advantec GA55 Borosilicate Glass Fibre Filters with 0.6 µm pore size (47 mm). Negative controls consisting of sterile nuclease-free water taken at the end of each sampling event. To avoid cross-contamination, disposable

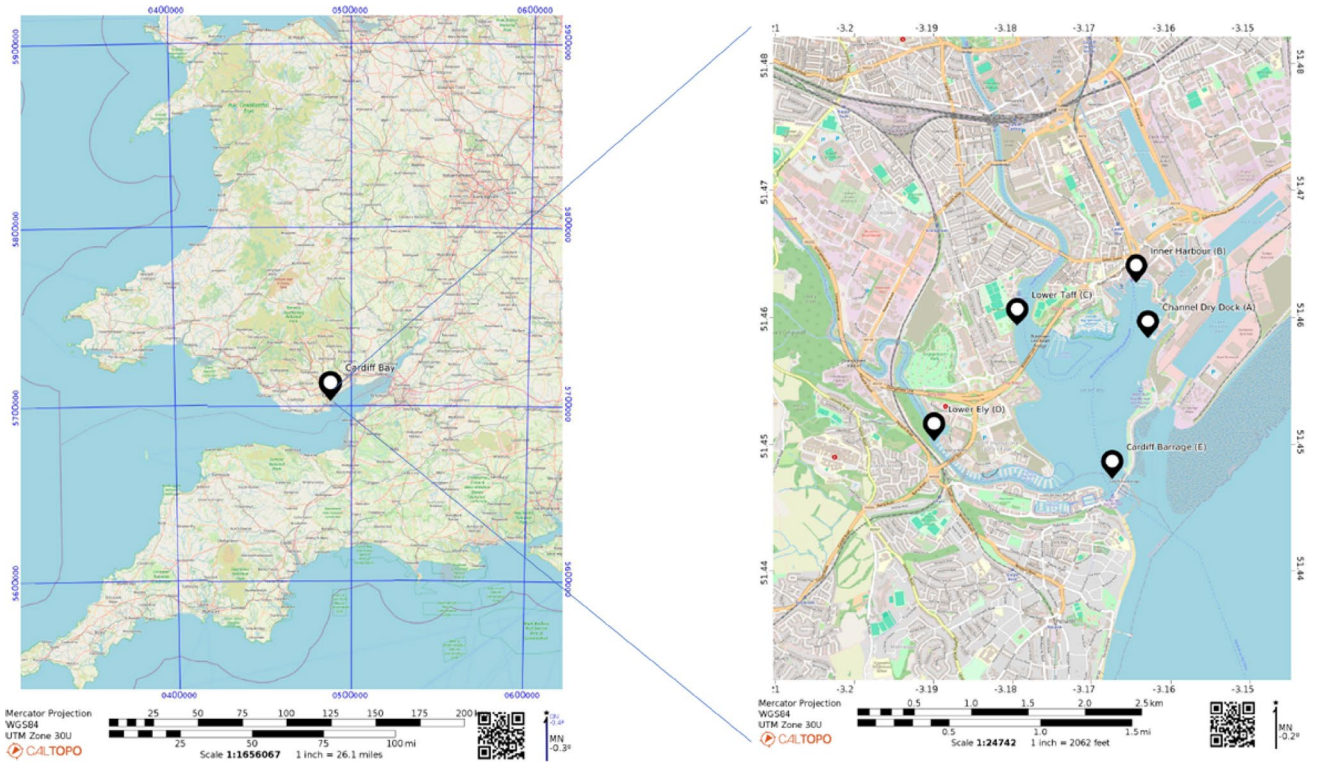

Fig. 1 Map of eDNA sampling locations in Cardiff Bay: Channel Dry Dock (**A**), Inner Harbour (**B**), Sand Wharf- Lower Taff (**C**), Lower Ely (**D**); and Cardiff barrage (**E**)

nitrile gloves were used, filter funnel and handling tweezers were cleaned with a 10% bleach solution and rinsed with 99% molecular grade ethanol and sterile nuclease-free water after each sample. One filter was used for each individual sampling replicate, each stored separately in 1. 5 mL vials at −20 °C until the DNA extraction a week later. All eDNA extractions and amplifications were carried out in a dedicated laboratory, with separate areas equipped with laminated flow hoods and UV light, as well as dedicated equipment/materials for each step. The Qiagen DNeasy Blood & Tissue DNA extraction kit (Qiagen GmbH, Hilden, Germany) was used for DNA extractions, following the manufacturer's guidance of the protocol for dried blood spots, reducing last step to 50 µL of elution. Independent filtering and extraction negative controls were extracted at the same time as environmental samples. All sampling replicates, blanks and tissue extractions were firstly quantified using the Qubit™ 4 Fluorometer (Thermo Fisher Scientific Inc.) applying the high-sensitivity assay (Life Technologies, Carlsbad, CA, USA).

Laboratory validation

An ex-situ based experiment was carried out to validate the molecular assay efficiency based on known biomass quantities and its relationship with eDNA abundance. This included two experimental designs (a) varying the water volume while maintaining constant biomass and (b) varying the biomass in a constant water volume. For these, tanks were pre- sterilised with bleach and washed thoroughly, mussels were collected from Cardiff Bay in August 2018 and brought to the lab where, within 2 h from capture, two experimental settings were prepared: (a) variable biomass: 2.02, 2.54, 5.36, 11.14, 20.76 g of wet weight in 10 L of water, (b) variable volume: 12–15 g wet weight in 3, 5, 10 and 15 L of water. Water temperature was kept between 17 and 17.8 °C. An incubation period of 72 h was selected based on previous studies (Takahara et al. 2012), where as little as 12 h were needed for a steady state at mussel mesocosm experiment under similar conditions to ours (Sansom and Sassoubre 2017). After 72 h, three replicates of water were collected and filtered, including blanks of sterile nuclease-free water. DNA extraction was carried out as for the environmental samples including an additional negative extraction control and extractions were stored at −20 °C until processing.

Primer design, species specificity and amplification conditions

We developed species specific barcoding primers in the mitochondrial cytochrome c oxidase gene subunit I region

(COI), targeting an 80 bp short fragment: ZebMCOI1F: 5′TTACCGGGTCCCTTTGTTCTGC3′; ZebMCOI1R: 3′CCCCAGCTAATACAGGCATTGC5′. We first tested species specificity of the primers in silico, aligning the target sequences using BioEdit (Hall et al. 2011), with cross-referencing the assay by using Primer – BLAST (Ye et al. 2012). Although eDNA primers for *Dreissena polymorpha* were already available (Bronnenhuber and Wilson 2013; Ardura et al. 2016), they were not specifically designed for HRM-qPCR assays. Our newly designed primers ensured high specificity and amplification efficiency suitable for identifying zebra mussel eDNA through that type of analysis, as confirmed by Sanger sequencing. For the assay validation purposes, temperature gradient, linearity, sensitivity, cross- species amplification test and limits of detection tests were performed on genomic DNA from the target species, before the actual qPCR environmental analysis. To estimate the limit of detection (LOD) and confirm linearity we used the method described in Merkes et al. 2019 specifically written for eDNA. A tenfold serial dilution was carried out in triplicate, starting from 1 ng/µL of template DNA down to 10^{-7} ng/µL. Efficiency was 99.9%. The LOD was established as the lowest value with three positive replicate amplifications and a Cq value of 35 cycles or less, within the linear range of the standard curve, in this case 10^{-5} ng/µL (Supplementary material Table S1). The LOQ was determined based on the lowest copy number variation with coefficient of variation < 10%, satisfying the conditions for LOD. The acceptance criteria for both LOD and LOQ included the possibility of LOD being equal to LOQ, as subsequently happened. NTC samples did not show any amplifications. Cross-species amplification was limited to *D. villosus* and *G. pulex*, those being the only available samples from the reservoir at the time. Tissues were extracted using Qiagen DNeasy Blood & Tissue DNA extraction kit (Qiagen GmbH, Hilden, Germany) by manufacturer's instructions, with 50 µL of final elution in Nuclease free water. Species identity of the specimens collected in Cardiff Bay was confirmed with Sanger sequencing.

For the quantification of the eDNA samples we used HRM curves based on SYBR Green technology (Bio-Rad, USA) as in (Robinson et al. 2018). qPCR conditions, optimised to avoid cross- species amplification, were as follows: 7 min at 95 °C, followed by 40 touchdown cycles of 95 °C for 10 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s, with an additional melting curve included at the end of qPCR run within a range of 60 °C to 95 °C. The amplification reaction was performed in a total volume of 30 µl with, 15 µL SSO Advanced Syber Green Supermix (2X) (Bio-Rad, USA), 3 µL template, 1.5 µL of each primer (10 µM), adding sterile nuclease- free water to final total volume. For positive controls, 60 ng of tissue-extracted DNA was used, following by a 10-fold dilution series for each plate's standard curve. All samples were run in duplicate (for each of the 3 sub-samples collected per site, total 6 amplifications/site) and included duplicates of non-template PCR controls using nuclease-free water. Data were analysed with Bio-Rad CFX Manager (Bio- Rad, USA). Duplicates of each eDNA sample were used for qPCR quantification. PCR products were directly sequenced using Sanger sequencing to confirm the species. Negative filtration and extraction samples were run in duplicate. Only one of the non-template control replicates resulted in an amplification at 38 cycles, which was higher than the estimated LOD and therefore not considered, all the other filtration and extraction blanks were negative. eDNA starting concentrations (pg/l) were estimated from the quantification cycle (Cq) values using the standard curve slope-intercept equation:

QT (starting quantity of eDNA) = −3.144 [log (Cq)] + 35.466.

Biofouling control experiment

A biofouling experiment was carried out to assess the correlation between mussel colonisation and eDNA. For this we set up two vertical ropes with three mesh boxes 8 cm x 10 cm, with 70 cc distance between them, in each of the five sampling sites in April 2017. Boxes from one rope from each site were sampled in June and mussels were individually counted with second rope being sampled in August, right after the water samplings.

Statistical analysis

Technical replicates (separate qPCR reactions of each sampling replicate) were averaged for the analyses. For the ex-situ experiment we assesses the influence of biomass (mgr/l) and the type of experiment (fixed volume or fixed number of mussels) on the starting quantity of eDNA (QT) with a linear model using the function *lm*, with sampling replicates nested within the experiment type. Model quality and normality were assessed using *checkmodel* from the package performance (Lüdecke et al. 2021). The boxcox function from the package MASS v 7.3–58.4 (Ripley et al. 2013) was used to estimate the best transformation option for QT and the square root was applied to achieve normality. Three outliers identified using the *boxplot* and *rosnerTest* functions in EnvStats v 2.8. (Millard 2013) were removed from the analyses. These data points fell significantly outside the expected range and were technical anomalies.

For the field sampling, first we assessed the influence of the sampling location and month on the eDNA quantity (QT), with sampling replicates nested within location, using a linear model. Model checks and transformations were

done as for the *ex-situ* experiment. A square root transformation was applied to the QT dependent variable. We then assessed the effect of the environmental measurements on QT using a linear model. For this, we built a correlation matrix of all environmental variables using corPlot from the package psych v. 2.3.6. (Revelle and Revelle 2015) to avoid using variables that displayed collinearity. After removing correlated variables, we used a linear model to assess the influence of temperature, dissolved oxygen, salinity and location on eDNA quantity, with sampling replicate nested within location. We then used the *drop1* function to identify the simplest model based on AIC values. The relationship between the number of mussels collected in the biofouling boxes and the eDNA QT was estimated using a Pearson correlation. A linear model was also carried out to roughly assess the effect of temperature and number of mussels on eDNA QT, albeit this could only be run for the four locations (B-E) during June 2017, the only month for which this data was available. All statistical analyses were done using R, version 4.3.0.

Results

Results from the ex-situ experiment indicated that zebra mussel biomass was a good predictor of eDNA concentration, irrespective of whether it was the volume of water or the mussel number that varied (Biomass: df = 1 F = 13.10 $P = 0.004$; Experiment type: df = 1 F = 4.36 $P = 0.061$; Experiment/replicate: df = 2 F = 7.45 $P = 0.497$; Supplementary material Table S2). At Cardiff Bay we found that both sampling month (df = 3 F = 6.75 $P < 0.001$) and location (df = 4 F = 4.39 $P = 0.004$) were significantly related to eDNA quantity but not sampling replicate within each location (df = 5 F = 0.69 $P = 0.633$). Locations B (Inner Harbour) and C (lower river Taff) were the ones with the lowest detection rates, while A (Channel dock) and E (Cardiff barrage) were the ones with the highest quantities of eDNA (Fig. 2), apart from June where samples from A were below the LOD (Supplementary material, Table S3). Zebra mussel eDNA quantity was very low in February, peaked in April and maintained relatively high concentrations in June, decreasing in August. Environmental data was only available for June and August 2017 at locations B, C, D and E (Table 1) and only temperature and pH were significantly variable between months and/or sampling locations. Temperature was variable between months (df = 1 F = 317.23 $P = 0.0003$) and among sites (df = 3 F = 10.44 $P = 0.042$), with sites B and C being the most different (df = 3 t = 5.33 $P = 0.038$); pH also varied significantly between months (df = 1 F = 19.79 $P = 0.021$) but not among sites (df = 3 F = 2.55 $P = 0.230$). After removing highly correlated variables (Table 2), only dissolved oxygen, salinity and temperature remained, with salinity removed from the final model after applying *drop1*. Temperature (df = 1 F = 13.92 $P < 0.001$) and sampling location (df = 3 F = 10.89 $P < 0.0001$) were significant

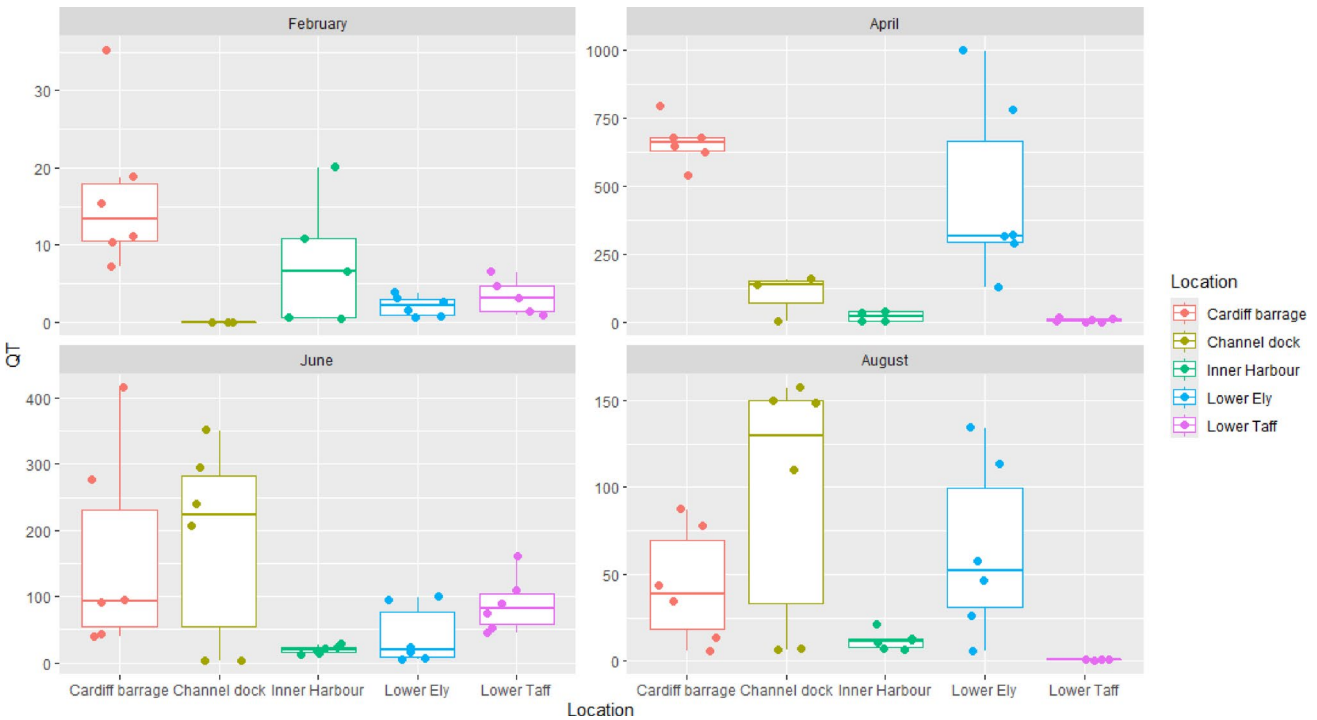

Fig. 2 Starting eDNA concentrations (QT, pg/l), estimated based on the standard curve starting from 1 ng/µL of template DNA down to 10^{-7} ng/µL dilution, at different sampling locations and months

Table 1 Environmental variables measured in sampling locations B-E, from (Rolla et al. 2020)

Location	Month	Temperature	Dissolved Oxygen	Salinity	Conductivity	pH	Turbidity
Inner Harbour	August	20.37	8.88	0.15	333	8.1	7.5
Inner Harbour	June	15.56	8.48	0.18	373	7.82	7
Lower Taff	August	18.87	9.57	0.19	389	8.01	6.1
Lower Taff	June	12.75	10.32	0.08	163	7.87	13.7
Lower Ely	August	20.13	9.01	0.21	427	7.85	5.8
Lower Ely	June	14.81	8.15	0.1	219	7.71	14.9
Cardiff barrage	August	20.12	8.94	0.22	451	8.05	5.4
Cardiff barrage	June	14.32	8.72	0.11	235	7.71	10.9

Temperature- °C, dissolved oxygen- mg/L, salinity- ppt, conductivity- µS/cm, turbidity- NTU

Table 2 Pearson correlation between environmental variables (above diagonal) and significance (P, below diagonal)

	Temperature	Dissolved Oxygen	Salinity	Conductivity	pH	Turbidity
Temperature		−0.13	0.82	0.86	0.73	−0.82
Dissolved Oxygen	0.37		−0.16	−0.21	0.34	0.04
Salinity	<0.001	0.29		1	0.52	−0.94
Conductivity	<0.001	0.17	<0.001		0.54	−0.95
pH	<0.001	0.02	<0.001	<0.001		−0.63
Turbidity	<0.001	0.79	<0.001	<0.001	<0.001	

predictors of zebra mussel eDNA QT but not dissolved oxygen (df = 1 F = 0.12 P = 0.734) neither sampling replicate within location (df = 8 F = 8.89 P = 0.467). Post-hoc (Tukey) analyses indicated that locations D and E differed significantly in zebra mussel eDNA QT from location C (D-C: t-value = 3.28 P = 0.012; and E-C: t-value = 4.439 P < 0.001).

For the biofouling experiment, only boxes from June contained mussels (Supplementary material Table S4), those sampled in August were mainly colonised by killer shrimp (*Dikerogammarus villosus*) and lake limpets (*Acroloxus sp.*). There was no significant association between the average number of mussels per box and the average eDNA QT (R^2 = 0.312 P = 0.320) but temperature and mussel number were both significant predictors of eDNA concentration (Mussel number: df = 1 F = 1782 P = 0.015, temperature: df = 1 F = 1650 P = 0.016) for the four locations with data for both variables in June 2017.

Discussion

In collaboration with the managing authorities of an artificial lake in Wales, we piloted the use of a cost-effective species-specific HRM-qPCR assay as a monitoring tool. We detected zebra mussel eDNA in all the sampled locations across Cardiff Bay and across all seasons (sampling months), except for the Channel dry dock in February 2016, where eDNA levels were below the LOD. Sequencing of PCR products and cross-contamination checks confirmed the assay specificity.

In terms of spatial differences, eDNA concentrations varied across habitat types. High values at Cardiff barrage and Channel Dock likely reflect high mussel densities on submerged structures, while low levels at Lower Taff (site C) may be due to strong water flow reducing eDNA accumulation—consistent with known low veliger counts at that site (Alix 2010). Areas with stable substrata and slower currents probably favoured the mussel accumulation. The invasive bivalve *Corbicula fluminea* was also consistently detected within its known distribution in Lake Tahoe (Cowart et al., 2018), applying eDNA detection method. Moreover, unlike in riverine systems where downstream transport can lead to eDNA accumulation far from the source, the semi-enclosed nature of Cardiff Bay may enhance site-specific detection, making spatial differences more reflective of local densities.

Seasonally, the highest eDNA concentrations were detected in April 2017 while the lowest corresponded to February 2016. Our validation in the laboratory indicated that, under constant conditions, adult mussels biomass correlated well with the eDNA quantity estimated based on the standard curve, at least at a short term and without taking into account the normal temporal degradation that happens to eDNA in the field (Lance et al., 2017). In contrast, we found no clear relationship between mussel numbers and eDNA concentration in the field-deployed boxes, although we cannot entirely rule out the possibility of PCR inhibition or false negatives, highlighting the need for appropriate controls in future monitoring efforts (Goldberg et al. 2016). The abundance of *D. polymorpha's* eDNA we observed peaked during the month of April, earlier than in previous studies, where veliger abundance peaked in June (Alix et al. 2016) and recruitment peaked in September/October (Rolla et al. 2020). Although invertebrate veliger peaks are common between June and September, we found the maximum

concentrations in April 2017, potentially due to elevated spring temperatures on that particular year (record warm for Wales) accelerating the reproduction above the 12 °C threshold (Nalepa and Schloesser 1992). In contrast, the low abundances observed in February coincided well with mussel growth season dependency, with the lowest growth being in the winter (Durán et al. 2010).

Compared to other zebra mussel assays (Blackman et al. 2020), our HRM-qPCR allows shorter amplicons, lower costs, and efficient quantification in degraded DNA samples, particularly suited to detect eDNA, due to its easily degradable nature (Rees et al. 2014), fulfilling the needs of the managing authorities. In relation to studies of quagga and other invasive mussels, our assay shows comparable sensitivity, detecting as little as 10^{-5} ng/µL targeted DNA (Blackman et al. 2020). Although other eDNA-based assays to detect zebra mussel exist (Feist and Lance 2021), to our knowledge this is first one using HRM-qPCR, which makes it cheaper than probe-based assays, and allows the amplification of shorter barcoding regions (Marshall et al., 2022 null).

Compared to eDNA patterns observed in other invasive mussels or in lotic systems, our study reveals distinct spatial and temporal dynamics influenced by the artificial lentic environment of Cardiff Bay (Amberg and Merkes 2019; Bedwell and Goldberg 2020). While standard curves can be used to roughly estimate starting eDNA concentrations (Takahara et al. 2012), the correlation with biomass can be considerably less straight forward, particularly in flowing waters (Shogren et al. 2019), and also depends on the DNA region analysed (Mauvisseau et al. 2019) and other environmental factors. Even if eDNA shedding and decay rates were similar across mussel densities, as in other freshwater mussels (Sansom and Sassoubre 2017), the number of eDNA copies detected in the field ultimately depends on the conditions of the molecular assay, such as target fragment size, efficiency of the assay (De Ventura et al. 2017; Katano et al., 2017) or volume of water filtered (Muha et al. 2019), and also on biotic factors including water temperature and ultraviolet radiation (Klymus et al. 2015; Pilliod et al. 2014). Zebra mussel eDNA release can also vary depending on the life stage, adults size and the presence of veliger (Amberg et al. 2019), which can constitute up to 20% of the animal plankton in Cardiff Bay (Alix et al. 2016) and can increase the eDNA signal at the spawning season (De Ventura et al. 2017). Additionally, the earlier seasonal peak in eDNA we observed, coinciding with a warmer than average spring, contrasts with the later peaks reported for zebra mussels in natural lakes or rivers (Rolla et al. 2020), and highlights the role of local thermal regimes in shaping eDNA signals. Recent zebra mussel mesocosm experiments applied eDNA: eRNA ratio as a useful tool to assess eDNA saturation rates in time (Marshall et al. 2022), which could be further applied in future assessments of spatio- temporal environmental variation of the invader. Although based on limited sample size, our results indicate that the amount of eDNA could be related to the combined effect of the temperature and the number of mussels in the different sampling locations. Environmental variation is known to affect eDNA detection, for example over half of the variation in eDNA-based abundance models for salmonid smolt migration Upper Salmon River (Canada) could be explained by environmental covariates (Morrison et al. 2023), and also species' survival, e.g., low dissolved oxygen strongly limits veliger distribution (Gantz et al. 2022). Taking all this into consideration, our results indicate that for an accurate quantification of zebra mussels in the field, environmental variables need to be considered, even at a small scale (location site), particularly water temperature.

The management and control of zebra mussel is complicated as larval stages can easily spread, particularly through recreational navigation, unless strict controls are in place (Robertson et al. 2020; Rodríguez-Rey et al. 2021). Most of the control measures are based on chemical control or mechanical cleaning, combined with the establishment of preventive measures such as disinfection stations and avoiding uncontrolled access to recreational areas at risk (Alix 2010; Durán et al. 2010). However, these strategies rarely achieve full eradication due to the difficulty of locating mussels when they are at low densities, particularly in large invaded areas (Lund et al. 2018), and rely on regular monitoring to assess presence, spread and intervention timings. For example, in the Ebro basin regular larval monitoring established that the months of May, June and July, which coincide with the reproductive season, where those with the highest detection levels, although extending the monitoring to September is advised to cover the period with more nautical activity (Durán et al. 2010). These also coincide with the peak seasons previously detected in Cardiff Bay (Alix et al. 2016), but our study indicates that in warmer years peaks can be found earlier if, potentially, reproduction occurred earlier.

In summary, in collaboration with the public agency responsible for the managing of an artificial lake (Cardiff Bay), we have developed a fast and cost-effective assay to monitor the spatial and temporal fluctuations of one of the most widespread aquatic invasive species, the zebra mussel. Our pilot monitoring analysis indicated that zebra mussel densities can shift outside the common seasonal ranges, particularly if the conditions are adequate for early reproduction (e.g., high temperature), suggesting that monitoring activities should be planned considering environmental conditions, particularly when there are extreme variations, and that small scale spatial variation should also be considered.

Establishing fast, accurate and affordable methods for zebra mussel monitoring, such as the one developed here, is particularly relevant in relation to climate change, as zebra mussels are sensitive to high temperatures and may shift their distribution to milder areas (Griebeler and Seitz 2007), potentially increasing the risks of further spread.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12686-025-01406-5.

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Author contributions TPM: investigation, methodology, data curation, original draft; CGL: conceptualisation, supervision, funding acquisition, review and editing; DH: conceptualisation, supervision, resources, review and editing; SC: conceptualisation, supervision, funding acquisition, methodology, final manuscript.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval The study was approved by the Swansea University Biosciences Department Ethics Committee (4th February 2016).

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