Title: The effects of metaldehyde on non-target aquatic macroinvertebrates: integrating field and laboratory-based evidence

Authors

Kieran J. Gething[1,2]*, Alex Pickwell[3], Richard P. Chadd[3] and Paul J. Wood[1].

[1]Geography and Environment, Centre for Hydrological and Ecosystem Science, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK.

[2]School of Science and Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK.

[3]Environment Agency of England, Lincolnshire & Northamptonshire Area, Stepping Stone Walk, Winfrey Avenue, Spalding, Lincolnshire, PE11 1DA, UK.

Corresponding author

*Kieran J. Gething - kieran.gething2015@my.ntu.ac.uk

Abstract:

The use of pesticides has historically helped improve agricultural productivity, although their continued use may have unforeseen effects upon the natural environment when not applied appropriately. Metaldehyde is a commercial pesticide widely used to reduce crop losses resulting from terrestrial mollusc damage. However, following precipitation and runoff it frequently enters waterbodies with largely unknown consequences for aquatic fauna. This study represents one of the first attempts to examine its potential effects on aquatic macroinvertebrate communities at sites known to have experienced elevated metaldehyde concentrations alongside unaffected control sites. In addition, a series of laboratory exposures specifically examined the effects of metaldehyde on the survivorship of non-target aquatic mollusc species. When the entire aquatic macroinvertebrate community and aquatic mollusc community were considered, limited differences were observed between metaldehyde affected and control sites based on field data. Laboratory exposures highlighted that for the molluscs examined, gastropods (*Bithynia tentaculata, Planorbis planorbis, Radix balthica* and *Potamopyrgus antipodarum*) had a greater tolerance to metaldehyde than bivalves (*Sphaerium corneum* and *Corbicula fluminea*). However, the concentrations required to reduce survivorship of all species were much greater than those ever recorded historically under field conditions. The results suggest that the differences in the community composition recorded between sites exposed to elevated metaldehyde concentrations and control sites were probably due to nutrient loading (N and P from agricultural fertilizers) rather than metaldehyde. However, these results do not negate wider concerns regarding metaldehyde use, particularly issues caused when ingested by vertebrate wildlife, livestock or children and pets in domestic settings.

Capsule:

Metaldehyde, a pesticide, frequently enters agricultural water bodies in excess of safe drinking water levels but has limited effects upon non-target invertebrate organisms.

Keywords: Molluscicide, invertebrate, aquatic mollusc, field study, laboratory exposure.

Introduction

Pesticides are a valuable tool in modern agriculture that have historically helped improve productivity (Handford et al. 2015). However, in some instances their use has raised concerns for human health, food and drinking water resource safety (World Health Organization and United Nations Environment Programme, 1990; Nicolopoulou-Stamati et al., 2016; Deng et al., 2019) and more widely in terms of unintended environmental effects (van der Werf, 1996; Carvalho, 2017; Wood and Goulson, 2017). The use of pesticides is heavily regulated in some regions (Karabelas et al. 2009; Handford et al. 2015), such as Europe where the European Union Water Framework Directive 2000/60/EC mandates a maximum permissible concentration of pesticides in drinking water of 0.1 µg/L for a single pesticide (Dolan et al. 2013).

The agricultural sector has benefitted from the use of modern agrochemicals and the availability of plentiful freshwater resources for irrigation, which have collectively helped increased crop yields and minimised damage by pests (FAO 2004; Stewart and Roberts 2012; DEFRA 2013; Handford et al. 2015). However, these practices potentially threaten aquatic biodiversity by reducing water availability, changing the sediment regime and nutrient dynamics, and via the poorly managed use of pesticides (Dudgeon, 2010; Reid et al., 2019; Jiao et al., 2020). Given that biodiversity is a key measure of aquatic ecosystem health (Natural England 2008; Environment Agency 2018), and an important component of freshwater ecosystem service provision (MEA 2005; DEFRA 2011; Parker and Oates 2016; IPBES 2019; UNEP: TEEB 2019), there is an ongoing need to monitor and understand any effects of pesticide use on non-target organisms.

One of the most widely utilised pesticides in the arable agricultural sector is the molluscicide metaldehyde (IUPAC: 2,4,6,8-tetramethyl-1,3,5,7-tetroxocane: Castle et al., 2017). Metaldehyde has been used for over a century and is known to be highly effective against target terrestrial gastropods (slugs and snails), but there have been limited documented effects upon other terrestrial invertebrates (e.g. *Lumbricus terrestris* (Linnaeus 1758): Langan and Shaw 2006; Edwards et al. 2009). In aquatic environments, EC_{50-60} of 100->200 mg/L have been reported for the gastropods *L. stagnalis* (Linnaeus 1758), *Planorbarius corneus* (Linnaeus 1758) and the bivalve *Dreissena polymorpha* (Pallas 1771) (Putchakayala and Ram, 2000; European Food

Safety Authority, 2010; Hallett et al., 2016). However, the tolerances of other aquatic taxa and the effects of metaldehyde upon the wider aquatic ecosystem have not been widely quantified.

Metaldehyde use is permitted throughout the European Union, however, during 2018 the UK Department for Environment, Food and Rural Affairs (DEFRA) announced plans to ban metaldehyde from Spring 2020, owing to the "*unacceptable risks* [posed…] *to birds and mammals*" (DEFRA, 2018). This proposal was subsequently overturned in the UK courts due to lack of evidence regarding metaldehyde's impact upon non-target organisms. Real world studies of metaldehyde pollution have almost exclusively focused on water security concerns (e.g. Kay and Grayson 2014), with most biological studies being undertaken in the laboratory. As a result, limited field studies examining the biological effects of metaldehyde have been undertaken, making it difficult to assess metaldehyde's legacy at the landscape scale. Given that freshwater aquatic molluscs (largely gastropods and bivalves) are evolutionarily and physiologically similar to their terrestrial counterparts (Saleuddin and Wilbur 1983; Thorp and Covich 2009; Moreau et al. 2015; Pyron and Brown 2015) it has been hypothesised that these faunal groups may be disproportionately affected by metaldehyde pollution (e.g. Calumpang et al. 1995; Horgan et al. 2014). As a result, the effect of metaldehyde concentrations recorded in surface freshwater bodies upon aquatic molluscs and the frequency at which these concentrations may be reached needs to be established. Therefore, this study examined a six-year ecological monitoring data set from the east of England (UK) alongside laboratory exposure studies to determine the effects of metaldehyde upon macroinvertebrate communities and specific non-target gastropod and bivalve species.

Methods

Data collection

Field Observations

10 sites in the east of England (53.061025ºN, 0.195007ºW) were selected for detailed investigation in the county of Lincolnshire (Figure S1.). The area has a predominantly arable land use and is serviced by a dense drainage network. Peaks of metaldehyde that exceeded drinking water guidelines (≥0.1 µg/L) were frequently detected between 2012 and 2018 at five locations (D1-D5), with five control sites where metaldehyde had

not exceeded drinking water guidelines in the historical record, but with comparable physical characteristics also being identified (C1-C5) to provide direct comparisons during analysis. The five metaldehyde-affected sites identified were among those monitored by the Environment Agency (the regulatory body for England) during 2014, the year in which 81% of pesticide pollution events in England involved metaldehyde (Pesticides Forum 2016). During 2015, 100% of serious pesticide-related pollution incidents in England involved metaldehyde, 60% of which occurred within the study area (upstream of D4 and D5) (Pesticides Forum 2017).

A total of 114 aquatic macroinvertebrate samples (60 from D1-5, 54 from C1-5), collected by the Environment Agency via the kick sampling methodology (see Murray-Bligh, 1999) between 2012 and 2018, were available for investigation. Invertebrates were identified to species level, except Diptera larvae which were primarily identified to family level and Ostracoda, Cladocera, Hydracarina and Oligochaeta which were identified as such. Water temperature (°C), pH, conductivity (µS/cm), dissolved oxygen (DO) (mg/L, %), chloride (mg/L), nitrate (µg/L), orthophosphate (mg/L), phosphorus (mg/L) and metaldehyde (µg/L) data, matched to corresponding date and locations of macroinvertebrate samples, were made available for detailed analysis by the Environment Agency and Anglian Water Plc.

Laboratory exposures

In order to advance understanding of the effects on specific non-target organisms, a series of laboratory exposures were also undertaken to test mollusc survivorship rates upon exposure to metaldehyde under controlled conditions. Six taxa were used in the controlled laboratory exposures: four native species i) *Bithynia tentaculata* (Linnaeus, 1758), ii) *Planorbis planorbis* (Linnaeus, 1758), iii) *Radix balthica* (Linnaeus, 1758) and iv) *Sphaerium corneum* (Linnaeus, 1758) and two non-native species i) *Potamopyrgus antipodarum* (Grey, 1843) and ii) *Corbicula fluminea* (Müller, 1774) that are widely distributed. Organisms were collected from waterbodies around Loughborough, UK (51.395918ºN, 0.734332ºW) using a standard kick net (mesh size 1mm) prior to the commencement of an experimental cycle. All organisms were held in aquaria for approximately 12 h prior to use in the experiment to confirm all individuals were alive and healthy. Exposure tanks were set up and run for 24 h prior to the introduction of organisms to allow the stabilisation of internal conditions.

Each exposure tank contained either 15.0 l of dechlorinated water (control) or 15.0 l of dechlorinated water and a known concentration of metaldehyde (Doff® Slug Killer: 1.5% metaldehyde – all concentrations used were calculated based on reported active ingredient per-unit mass of pellets provided by the manufacturer). Each exposure tank was fitted with an aerator to supply oxygen to the organisms within. Temperature was held at ambient conditions (18-22 °C) within the River Science Laboratory at Loughborough University. At the start of the experiment 20 adult individuals from one of the six tested species were introduced into each exposure tank. All experiments had a 24-hr duration given that metaldehyde peaks ≥0.1 µg/L did not typically exceed this duration in the field data series and reflected typical environmental exposure conditions. On completion of the experimental period, organisms were transferred into a clean aquarium and directly observed over a 3-hr period to determine survivorship rates. Each of the six species was tested at four metaldehyde concentrations, with three replicates of each concentration providing a total of 72 individual experiments. All organisms were first tested at 100 mg/L, reflecting EC_{50-60} concentrations recorded by Putchakayala and Ram (2000), the European Food Safety Authority (2010) and Hallett et al. (2016). If survivorship at 100 mg/L was >80%, the four test concentrations were 0, 100, 200 and 300 mg/L (*B. tentaculata*, *P. planorbis* and *P. antipodarum*). If survivorship at 100 mg/L was <50%, the four test concentrations were 0, 25, 50 and 100 mg/L (*C. fluminea*). One species (*S. corneum*) had a mean survivorship of 58% at 100 mg/L so was tested at 0, 50, 100 and 200 mg/L. *R. balthica*, demonstrated a marked decline in survivorship between 100-200 mg/L, and was therefore tested at 0, 100, 150 and 200 mg/L.

Data analysis

Unless otherwise stated, R Studio v.3.5.1 (R-Studio Team 2016) was used to perform statistical analyses and visualise plots. All faunal and environmental matrices were square root transformed in order to satisfy the assumptions of further statistical analyses and facilitate the use of Vegan package in R language for environment and statistical computing v.2.5.3 (Oksanen et al. 2018; R Core Team 2018).

Field Observations

Spring and autumn macroinvertebrate samples from 2012-2018 with corresponding chemical data were extracted for ordination in Canoco v.5.0 (ter Braak and Šmilauer 2012). Data was plotted in two groups, the first containing the entire faunal community. The second group considered only aquatic molluscs reflecting the fact that there may be more pronounced effects upon this section of the community. Given that both the entire faunal community - all taxa (0.1) and the mollusc community (1.1) displayed linear distributions, Redundancy Analysis (RDA) was conducted with a Monte Carlo random permutations test (n=999) to determine the statistical significance of environmental (water chemistry) variables (Lepš and Šmilauer 2003).

To quantify heterogeneity between metaldehyde detected and control sites for the period of known metaldehyde pollution (2012-2018), analysis of similarity (ANOSIM - utilising the Bray-Curtis dissimilarity metric) was conducted on the entire faunal community (114 samples), and a subset containing only aquatic mollusc taxa (114 samples). Subsequently, similarity percentages analysis (SIMPER) was undertaken on the two ANOSIM groups (all fauna and aquatic molluscs) to determine the species responsible for any observed heterogeneity.

Laboratory exposures

For laboratory experiments, mean survival rate across the three replicates for a given concentration were calculated and plotted for each species.

Results

Field data

A total of 52,286 individuals were recorded from all samples. The relative abundance and number of taxa was lower at control sites (C1-5; mean 415 individuals; 201 taxa) compared to metaldehyde detected sites (D1-5; mean 466 individuals; 245 taxa). The most abundant taxon at sites C1-5 was *Asellus aquaticus* (Linnaeus 1758), followed by *Crangonyx pseudogracilis* (Bousfield 1958), Oligochaeta and Hydracarina. The most abundant taxon at sites D1-5 was also *A. aquaticus* followed by *P. antipodarum*, *B. tentaculata, C. pseudogracilis*, *Pisidium nitidum* (Jenyns, 1832), Oligochaeta, Cladocera and Chironomidae. Molluscs comprised 18.9% and 19.7% of the taxa recorded at control and metaldehyde detected sites and 27% and 32% of the relative abundance respectively. *Pisidium subtruncatum* (Malm, 1855) (801), *Anisus vortex*

(Linnaeus 1758) (789) and *B. tentaculata* (661) were the most frequently recorded mollusc taxa at control sites with *P. antipodarum* (1,856), *Pisidium nitidum* (1,080) and *B. tentaculata* (1,062) being the most frequently occurring mollusc taxa at sites where metaldehyde had been detected.

Redundancy Analysis (RDA) indicated that water chemistry data from samples collected at metaldehyde detected and control sites accounted for 16.8% of the total variance in the faunal data. Orthophosphate was identified as the most significant variable influencing the community composition (**Table 1a.**). Metaldehyde, accounted for only 2.1% of the community variation and was strongly correlated with Axis 2 along with orthophosphate and phosphorus (**Figure 1.**). Cumulatively these three variables accounted for 6.7% of the variance in the faunal community data. Metaldehyde, orthophosphate and phosphorus were negatively correlated with DO, temperature and pH. Samples from sites where metaldehyde had been detected (particularly D2 and D4) predominately plotted in the upper quadrants of the ordination. In contrast, samples from control samples typically plotted on the negative end of Axis 2. However, ANOSIM indicated a high degree of similarity among the assemblages recorded at control and metaldehyde detected sites (ANOSIM R= 0.265: P= 0.001). SIMPER highlighted that 30.7% of the dissimilarity observed was attributable to 10 taxa, three of which were molluscs (*P. nitidum*, *P. antipodarum* and *Valvata piscinalis* (Müller, 1774)) (**Table 2a.**). Hydracarina was the most significant taxon driving dissimilarity between control and metaldehyde detected sites (4.1%) followed by *A. aquaticus* (3.8%).

The 10 environmental variables were able to account for 21.6% of the total variance in the community composition of mollusc communities from control and metaldehyde detected sites. Orthophosphate accounted for the greatest amount of variance (5.0% - **Table 1b.**); being strongly associated with both phosphorus and metaldehyde concentrations (**Figure 2.**). Metaldehyde was the least significant variable accounting for only 1.2% of the variance in the mollusc data. Most control site samples (with the exception of samples from C3) cluster together and were characterised by higher DO, pH, temperature and conductivity. Many samples from sites D2 and D4 were characterised by higher metaldehyde concentrations. A significant ANOSIM, indicated a higher degree of heterogeneity between metaldehyde detected and control samples

(ANOSIM *R*= 0.467: *P*= 0.001) for the mollusc community compared to the equivalent analysis for the entire faunal community. SIMPER attributed 60.1% of the dissimilarity to 10 taxa (**Table 2b.**), the most influential of being *P. nitidum* (9.4%), *P. antipodarum* (9.1%) and *V. piscinalis* (7.5%). All the mollusc taxa identified by SIMPER, with the exception of *Physa fontinalis* (Linnaeus 1758), were more common at metaldehyde detected sites. Both gastropods and bivalves were identified in the SIMPER, indicating both were significantly associated with differences between communities from control and metaldehyde detected sites.

Laboratory exposures

Gastropods displayed higher survivorship than bivalves when exposed to metaldehyde under controlled laboratory conditions. Operculate gastropods consistently displayed a higher mean survivorship than pulmonates at metaldehyde concentrations ≤200 mg/L; above this threshold results were more variable. The mean survivorship of *C. fluminea* declined rapidly with only a 7% survivorship at metaldehyde concentrations of 100 mg/L (**Figure 3a.**), while *S. corneum* had a survivorship of 3% at 200 mg/L (**Figure 3b.**). The most rapid decline in survivorship for both *C. fluminea* and *S. corneum* occurred at concentrations >50 mg/L. The operculate gastropod *P. antipodarum* had a 100% survivorship at metaldehyde concentrations ≤200 mg/L, but this declined rapidly to a 35% survivorship at 300 mg/L (**Figure 3c.**). *B. tentaculata* displayed very little reduction in survivorship at increased metaldehyde concentrations (**Figure 3d.**) with >90% survivorship at metaldehyde concentrations of 300 mg/L. Whilst *P. planorbis* displayed a similar response to that of *B. tentaculata* at metaldehyde concentrations up to 100 mg/L, the species had lower mean survival rates at concentrations >100 mg/L (**Figure 3e.**). *R. balthica* displayed the most marked response and lowest survivorship of the gastropods studied at metaldehyde concentrations >100 mg/L (**Figure 3f.**).

Discussion

Metaldehyde poses a potential threat to water security and terrestrial vertebrates (Lapworth et al., 2012; Castle et al., 2017; Pesticides Forum, 2017, 2018), however, its potential impact upon aquatic communities has remained largely unexplored until now. Concentrations of metaldehyde in European rivers have exceeded WFD limits for safe drinking water over the past decade (Lazartigues et al., 2012; Kay and Grayson, 2014), although research on the response of aquatic fauna remained rooted in laboratory studies. This study therefore sought to utilise field data from sites known to exceed metaldehyde drinking water standards to examine the legacy of metaldehyde in agricultural catchments upon non-target aquatic macroinvertebrate communities and specifically mollusc species. This research is particularly timely given the recent reversal of the ban on metaldehyde use across the agricultural sector in the UK (Water UK, 2019).

Aquatic macrofaunal community composition at metaldehyde detected and control sites

Differences in macroinvertebrate communities from control sites and sites where elevated metaldehyde had been recorded were marginal, but statistically significant. Control sites were more homogenous than sites subject to elevated metaldehyde concentrations. Three species were recorded in higher abundances at sites subject to elevated metaldehyde concentrations (*A. aquaticus*, *C. pseudogracilis* and *P. antipodarum*), and are noted for their association with nutrient enrichment (Dick et al., 1999, 1998; Simčič and Brancelj, 2006; Alonso and Castro-Díez, 2008). Detailed examination of the mollusc community indicated even larger differences between control sites and those sites subject to greater metaldehyde concentrations than for the entire macroinvertebrate community. Analysis of environmental (water chemistry) variables using the Monte Carlo random permutations test, for both the entire faunal community and the mollusc community, indicated that metaldehyde did not appear to have a significant effect. This analysis indicated that, rather than metaldehyde, phosphate and orthophosphate (probably originating from agricultural fertilisers), and differences in pH and DO were the primary drivers of community composition; with the effects being more pronounced on the mollusc community.

Aquatic molluscs have previously been shown to display limited response to pesticide pollution events (Raven and George, 1989). The maximum metaldehyde concentration recorded during this study (4.4 µg/L) was 22,523 times lower than the >100 mg/L concentration at which lethal effects have previously been observed (e.g. Hallett et al. 2016). Therefore, it is likely that the impact of a single metaldehyde pollution event upon the aquatic mollusc community was limited. Increased duration of molluscicide exposure can increase mollusc mortality (Doherty and Cherry, 1988), however, the duration of metaldehyde peaks ≥0.1 µg/L recorded in the field were short (*c.* 24 h). Operculate and bivalve taxa have been shown to seal their shells to withstand short term exposure to pesticides (Wallace 1990; Gosling 2004; Pandey and Kulkarni 2007). In contrast, pulmonates do not have an operculum and, therefore, may be at greater risk of mortality, even during short-term pollution events. It is however likely that some, if not most, pulmonate taxa possess the ability to withstand high concentrations of metaldehyde over an extended period, with Hallett et al. (2016) noting the ability of *L. stagnalis* to internally detoxify metaldehyde.

Acute impacts of terrestrial molluscicide pollution upon aquatic mollusc species under laboratory conditions

To date, few authors have attempted to quantify the effect of metaldehyde upon non-target aquatic mollusc species. Increasing metaldehyde concentrations reduced the survivorship of all six species examined, although the effect was highly variable. There are three factors most likely to be responsible for the variability observed, reflecting species differences in: (1) feeding mode, (2) respiratory mode, and (3) metaldehyde excretory efficiency.

Feeding mode is potentially of significance, as ingestion has previously been noted to affect the behaviour of aquatic molluscs (Egeler et al. 2007; European Food Safety Authority 2015). Mills et al. (1992) reported that ingestion of metaldehyde resulted in a reduction in feeding and thus the rate at which metaldehyde could be consumed prior to a lethal concentration being reached within an organism. The four gastropod species examined feed via a radula (an arrangement of small teeth located in the mouth: Mendel and Bradley 1905; Saleuddin and Wilbur 1983). This is in contrast to the bivalve taxa, both of which feed predominately via filtration (Thorp and Covich 2009; Pyron and Brown 2015). *S. corneum* and *C. fluminea* displayed lower survivorship rates at lower metaldehyde concentrations. Filter-feeding may have resulted in large volumes of metaldehyde contaminated water passing directly through the organism, thus potentially elevating the effects at lower concentrations. As filtration rates are noted to vary as a function of size in many bivalves (Wagner 1976; Riisgård 2001; Sylvester et al. 2005), it is likely that larger volumes of metaldehyde entered larger individuals more quickly and increased mortality. Further investigation would be required to determine if there is a relationship between body size, filtration and the effects of metaldehyde. The two gastropod species observed to directly ingest metaldehyde pellets (*P. planorbis* and *R. balthica*) demonstrated significant reductions in survivorship at concentrations ≤200 mg/L suggesting that ingestion may be an important factor in determining mollusc survivorship.

Differences in mode of respiration may also help explain the difference in response of the taxa examined. Across the six species examined, two modes of respiration are utilised. First, gills allowing gaseous exchange over an internal membrane to obtain oxygen, are utilised by *S. corneum*, *C. fluminea*, *B. tentaculata* and *P. antipodarum* (Thorp and Covich 2009). Pulmonates (*P. planorbis* and *R. balthica*), also utilise gaseous exchange across membranes underwater via highly vascularised tentacles but can also breathe atmospheric air via a primitive lung when aquatic conditions are unfavourable (Pyron and Brown 2015). However, no difference in survivorship / tolerance was observed in the laboratory exposures and no pulmonate individual attempted to emerge from the water after metaldehyde was added to the exposure tank. This is likely to be because cues which prompt an escape response relate more closely to oxygen availability than a response to a potential biocide.

In addition to the ingestion of metaldehyde, the rate of excretion is likely to influence survivorship. Studies examining the ability of molluscs to excrete metaldehyde are limited. Research on the pulmonate gastropod *L. stagnalis* suggested that it was able to internally detoxify metaldehyde with no observable effect up to 36 mg/L (Hallett et al. 2016); although the mechanism by which detoxification and excretion occurred was not specified. The results of the current investigation demonstrate similarly high survivorship at 36 mg/L, particularly for gastropod taxa with the most marked reductions occurring at concentrations over 100 mg/L for gastropod species. The concentration was an order of magnitude higher than those reported for UK rivers (<10.0 μg/L: Kay and Grayson 2014). Therefore, the results of the laboratory exposures clearly demonstrate that much higher concentrations of metaldehyde than the maximum observed in the field (4.4 μg/L) would be required to reduce the survivorship of molluscs.

Conclusion

The results of field-based research at sites where the greatest concentrations of metaldehyde have be recorded indicate that its effects on non-target aquatic macroinvertebrates within an agricultural setting were so minor that they could not be detected. Although metaldehyde concentrations exceeded drinking water guidelines on multiple occasions at sites examined during the study period, these concentrations were orders of magnitude lower than those documented to influence aquatic faunal communities and individual aquatic mollusc species. The results recorded suggest that the differences observed between metaldehyde detected and control sites probably reflects differences in nutrient concentrations (orthophosphate) resulting from land use practices associated with specific crops (e.g., brassica where agricultural fertilisers and metaldehyde may be used more frequently). Laboratory exposures examining individual mollusc species highlighted a wide range of tolerances. These findings also highlight that real-world metaldehyde concentrations and exposure times recorded in UK rivers were almost certainly lower that those required to have a lethal effect on non-target aquatic macroinvertebrates. The overall results of this research drawing on field based ecological monitoring and laboratory exposures highlight that metaldehyde poses a limited threat to non-target aquatic faunal communities based on current levels of exposure. However, it should be stressed that these results do not negate wider concerns regarding metaldehyde use, particularly issues caused when ingested by vertebrate wildlife, agricultural livestock and domestic pets or the genuine public health concern when accidentally ingested by children when the pesticide is used in a domestic setting.

Acknowledgements

The authors would like to thank the Environment Agency and Anglian Water Plc. for supplying data to facilitate this research. In addition, the authors would like to acknowledge Richard Harland, Rebecca McKenzie and Charlie Springthorpe from Geography and Environment at Loughborough University for supporting this research. Fig S1 contains OS data © Crown copyright database right 2018, free to use under Open Government Licence.

This research did not receive specific grant from funding agencies in the public, commercial or not-for-profit sectors.

References

Alonso, A., Castro-Díez, P., 2008. What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? Hydrobiologia 614, 107–116. https://doi.org/10.1007/s10750-008-9529-3

Calumpang, S.M.F., Medina, M.J.B., Tejada, A.W., Medina, J.R., 1995. Environmental impact of two molluscicides: Niclosamide and metaldehyde in a rice paddy ecosystem. B. Environ. Contam. Tox. 55. https://doi.org/10.1007/BF00196027

Carvalho, F.P., 2017. Pesticides, environment, and food safety. Food Energy Secur. 6, 48–60. https://doi.org/10.1002/fes3.108

Castle, G.D., Mills, G.A., Gravell, A., Jones, L., Townsend, I., Cameron, D., Fones, G.R., 2017. Review of the molluscicide metaldehyde in the environment. Environ. Sci. Water Res. 3, 415–428. https://doi.org/10.1039/C7EW00039A

DEFRA, 2018. Restrictions On The Use Of Metaldehyde To Protect Wildlife [WWW Document]. GOV.UK. URL https://www.gov.uk/government/news/restrictions-on-the-use-of-metaldehyde-to-protect-wildlife (accessed 3.31.19).

DEFRA, 2013. A UK Strategy For Agricultural Technologies. DEFRA, London.

DEFRA, 2011. Biodiversity 2020: A Strategy For England's Wildlife And Ecosystem Services. DEFRA, London.

Deng, S., Yan, X., Zhu, Q., Liao, C., 2019. The utilisation of reclaimed water: Possible risks arising from waterborne contaminants. Environ. Pollut. 254, 113020. https://doi.org/10.1016/j.envpol.2019.113020

Dick, J.T.A., Faloon, S.E., Elwood, R.W., 1998. Active brood care in an amphipod: influences of embryo development, temperature and oxygen. Anim. Behav. 56, 663–672.

Dick, J.T.A., MacNeil, C., Anderson, R., 1999. The distribution of *Crangonyx pseudogracilis* Bousfield, 1958 (Crustacea: Amphipoda) in Northern Ireland, with notes on Its ecology and behaviour. Ir. Nat. J. 26, 236–240.

Doherty, F.G., Cherry, D.S., 1988. Tolerance of the Asiatic clam *Corbicula spp*. to lethal level of toxic stressors—A review. Environ. Pollut. 51, 269–313. https://doi.org/10.1016/0269-7491(88)90167-4

Dolan, T., Howsam, P., Parsons, D.J., Whelan, M.J., 2013. Is the EU drinking water directive standard for pesticides in drinking water consistent with the precautionary principle? Environ. Sci. and Technol. 47, 4999–5006. https://doi.org/10.1021/es304955g

Dudgeon, D., 2010. Prospects for sustaining freshwater biodiversity in the 21st century: linking ecosystem structure and function. Curr. Opin. Environ. Sustain. 2, 422–430. https://doi.org/10.1016/j.cosust.2010.09.001

Edwards, C.A., Arancon, N.Q., Vasko-Bennett, M., Little, B., Askar, A., 2009. The relative toxicity of metaldehyde and iron phosphate-based molluscicides to earthworms. Crop Prot. 28, 289–294. https://doi.org/10.1016/j.cropro.2008.11.009

Egeler, P., Goth, M., Knoch, E., 2007. Metaldehyde – A Study On The Toxicity To The Great Ramshorn Snail (*Planorbarius corneus*) Over 48 Hours.

Environment Agency, 2018. State Of The Environment Report. Environment Agency, Bristol.

European Food Safety Authority, 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance ferric phosphate. EFSA Journal 13, 50.

European Food Safety Authority, 2010. Conclusion on the peer review of the pesticide risk assessment of the active substance Metaldehyde. EFSA Journal 8, 1–71.

FAO, 2004. Fertilizer Requirements: 2012 To 2030 Revisited. United Nations, Rome.

Gosling, E., 2004. Bivalve Molluscs: Biology, Ecology And Culture. John Wiley & Sons, Oxford.

Hallett, K.C., Atfield, A., Comber, S., Hutchinson, T.H., 2016. Developmental toxicity of metaldehyde in the embryos of *Lymnaea stagnalis* (Gastropoda: Pulmonata) co-exposed to the synergist piperonyl butoxide. Sci. Total Environ. 543, 37–43. https://doi.org/10.1016/j.scitotenv.2015.11.040

Handford, C.E., Elliott, C.T., Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integr. Environ. Assess. Manag. 11, 525–536. https://doi.org/10.1002/ieam.1635

Horgan, F.G., Stuart, A.M., Kudavidanage, E.P., 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. Acta Oecologica, Ecosystem Impacts of Invasive Species 54, 90–100. https://doi.org/10.1016/j.actao.2012.10.002

IPBES, 2019. Summary For Policymakers Of The Global Assessment Report On Biodiversity And Ecosystem Services. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn.

Jiao, C., Chen, L., Sun, C., Jiang, Y., Zhai, L., Liu, H., Chen, Z., 2020. Evaluating national ecological risk of agricultural pesticides from 2004 to 2017 in China. Environ. Pollut. 258, 113778. https://doi.org/10.1016/j.envpol.2019.113778

Karabelas, A.J., Plakas, K.V., Solomou, E.S., Drossou, V., Sarigiannis, D.A., 2009. Impact of European legislation on marketed pesticides — A view from the standpoint of health impact assessment studies. Environ. Int. 35, 1096–1107. https://doi.org/10.1016/j.envint.2009.06.011

Kay, P., Grayson, R., 2014. Using water industry data to assess the metaldehyde pollution problem. Water Environ. J. 28, 410–417. https://doi.org/10.1111/wej.12056

Langan, A.M., Shaw, E.M., 2006. Responses of the earthworm *Lumbricus terrestris* (L.) to iron phosphate and metaldehyde slug pellet formulations. Appl. Soil Ecol. 34, 184–189.

Lapworth, D.J., Baran, N., Stuart, M.E., Ward, R.S., 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. Environ. Pollut. 163, 287–303. https://doi.org/10.1016/j.envpol.2011.12.034

Lazartigues, A., Banas, D., Feidt, C., Brun-Bellut, J., Thomas, M., 2012. Pesticide pressure and fish farming in barrage pond in Northeastern France Part I: site characterization and water quality. Environ. Sci. Pollut. Res. 19, 2802–2812. https://doi.org/10.1007/s11356-012-0784-5

Lepš, J., Šmilauer, P., 2003. Multivariate Analysis of Ecological Data Using Canoco. Cambridge University Press, Cambridge.

MEA, 2005. Millennium Ecosystem Assessment - Ecosystems And Human Well-Being: Synthesis. Island Press, Washington, DC.

Mendel, L.B., Bradley, H.C., 1905. Experimental studies on the physiology of molluscs. American Journal of Physiology 13, 17–29. https://doi.org/10.1152/ajplegacy.1905.13.1.17

Mills, J.D., McCrohan, C.R., Bailey, S.E.R., 1992. Electrophysiological responses to metaldehyde in neurones of the feeding circuitry of the snail *Lymnaea stagnalis*. Pestic. Biochem. Phys. 42, 35–42. https://doi.org/10.1016/0048-3575(92)90071-7

Moreau, P., Burgeot, T., Renault, T., 2015. In vivo effects of metaldehyde on Pacific oyster, *Crassostrea gigas*: comparing hemocyte parameters in two oyster families. Environ. Sci. Pollut. Res. 22, 8003–8009. https://doi.org/10.1007/s11356-014-3162-7

Murray-Bligh, J.A.D., 1999. BT001: Procedure For Collecting And Analysing Macro-Invertebrate Samples. Quality Management Systems For Environmental Monitoring: Biological Techniques (No. 2). Environment Agency, Bristol.

Natural England, 2008. State Of The Natural Environment Report. Natural England, London.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public Health 4. https://doi.org/10.3389/fpubh.2016.00148

Oksanen, J., Blanchet, F., Freindly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2016. Vegan - Community Ecology Package: Ordination, Diversity And Dissimilarities.

Pandey, B.N., Kulkarni, G.K., 2007. Biodiversity & Environment. APH Publishing, Delhi.

Parker, H., Oates, N., 2016. How Do Healthy Rivers Benefit Society? UK-WWF, London.

Pesticides Forum, 2018. Pesticides in the UK: The 2017 Report On The Impacts And Sustainable Use Of Pesticides. Health and Safety Executive, London.

Pesticides Forum, 2017. Pesticides in the UK: The 2016 Report On The Impacts And Sustainable Use Of Pesticides. Health and Safety Executive, London.

Pesticides Forum, 2016. Pesticides in the UK: The 2015 Report On The Impacts And Sustainable Use Of Pesticides. Health and Safety Executive, London.

Putchakayala, S.M., Ram, J.L., 2000. Toxic and excitatory effects of the molluscicide metaldehyde on the biofouling bivalve *Dreissena polymorpha* Pallas. Pest Manag.

Sci. 56, 39–42. https://doi.org/10.1002/(SICI)1526-4998(200001)56:1<39::AID-PS84>3.0.CO;2-8

Pyron, M., Brown, K.M., 2015. Chapter 18 - Introduction To Mollusca And The Class Gastropoda, in: Thorp, J.H., Rogers, D.C. (Eds.), Thorp and Covich's Freshwater Invertebrates (Fourth Edition). Academic Press, Boston, pp. 383–421. https://doi.org/10.1016/B978-0-12-385026-3.00018-8

R Core Team, 2017. R: A Language And Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raven, P.J., George, J.J., 1989. Recovery by riffle macroinvertebrates in a river after a major accidental spillage of chlorpyrifos. Environ. Pollut. 59, 55–70. https://doi.org/10.1016/0269-7491(89)90021-3

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol. Rev. 94, 849–873. https://doi.org/10.1111/brv.12480

Riisgård, H.U., 2001. On measurement of filtration rates in freshwater bivalves – the stony road to reliable data: review and interpretation. Mar. Ecol. Prog. Ser. 17, 211–275.

R-Studio Team, 2016. R Studio: Integrated Development For R. R-Studio, Inc., Boston, MA.

Saleuddin, A.S.M., Wilbur, K.M., 1983. The Mollusca: Physiology. Academic Press, London.

Simčič, T., Brancelj, A., 2006. Effects of pH on electron transport system (ETS) activity and oxygen consumption in *Gammarus fossarum*, *Asellus aquaticus* and

Niphargus sphagnicolus. Freshw. Biol. 51, 686–694. https://doi.org/10.1111/j.1365-2427.2006.01522.x

Stewart, W.M., Roberts, T.L., 2012. Food security and the role of fertilizer in supporting it. Procedia Eng. 46, 76–82. https://doi.org/10.1016/j.proeng.2012.09.448

Sylvester, F., Dorado, J., Boltovskoy, D., Juárez, Á., Cataldo, D., 2005. Filtration rates of the invasive pest bivalve *Limnoperna fortunei* as a function of size and temperature. Hydrobiologia 534, 71–80. https://doi.org/10.1007/s10750-004-1322-3

ter Braak, C., Šmilauer, P., 2012. Canoco 5.0. Ithaca, NY.

Thorp, J.H., Covich, A.P., 2009. Ecology And Classification Of North American Freshwater Invertebrates. Academic Press, London.

UNEP: EEB, 2019. United Nations Environment Programme: Economics Of Ecosystems And Biodiversity [WWW Document]. UNEP: EEB. URL http://www.teebweb.org/ (accessed 6.20.19).

van der Werf, H.M.G., 1996. Assessing the impact of pesticides on the environment. Agric. Ecosyst. Environ. 60, 81–96. https://doi.org/10.1016/S0167-8809(96)01096-1

Wagner, R.E., 1976. The effect of size and temperature on the filtration rate of the freshwater mussel, *Elliptio complanatus*. Bios 47, 168–178.

Wallace, J.B., 1990. Recovery of lotic macroinvertebrate communities from disturbance. Environ. Manage. 14, 605–620. https://doi.org/10.1007/BF02394712

Water UK, 2019. Reducing The Impacts Of Metaldehyde On Drinking Water - Water UK Position Paper. Water UK, London.

Wood, T.J., Goulson, D., 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environ. Sci. Pollut. Res. 24, 17285–17325. https://doi.org/10.1007/s11356-017-9240-x

World Health Organization, United Nations Environment Programme, 1990. Public Health Impact Of Pesticides Used In Agriculture. World Health Organization.

Figure captions

Figure 1. RDA biplot of chemical variables and community samples from control (black) and metaldehyde detected (white) sites.

Figure 2. RDA biplot of chemical variables and mollusc community samples from control (black) and metaldehyde detected (white) sites.

Figure 3. Mean survival rate of (A) *Corbicula fluminea*, (B) *Sphaerium corneum*, (C) *Potamoprygus antipodarum*, (D) *Bithynia tentaculata*, (E) *Planorbis planorbis* and (F) *Radix balthica* upon exposure to differing metaldehyde concentrations.

Figure S1. Study sites within Lincolnshire, North-Cambridgeshire and Rutland, UK.

Table 1. Monte Carlo Permutation results of chemical variables impacting (A) all taxa and (B) mollusc taxa recorded 2012-18.

A. Entire faunal community			
Name	**Variation explained (%)**	**pseudo-*F***	***P***
Orthophosphate	3.3	2.1	0.016
Chloride	3.3	2.2	0.020
Conductivity	2.6	1.8	0.040
Metaldehyde	2.1	1.4	0.166
Temperature	2.4	1.6	0.078
DO (%)	1.6	1.1	0.328
DO (mg/L)	2.2	1.5	0.104
Nitrate	2.1	1.5	0.088
pH	1.1	0.8	0.600
Phosphorus	1.3	0.9	0.574

B. Mollusc community			
Name	**Variation explained (%)**	**pseudo-*F***	***P***
Orthophosphate	5.0	3.3	0.004
Chloride	3.5	2.3	0.010
Phosphorus	3.1	2.1	0.026
pH	2.7	1.9	0.044
Nitrate	2.0	1.4	0.152
Temperature	1.7	1.2	0.270
DO (mg/L)	1.5	1.0	0.372
DO (%)	3.8	2.8	0.008
Conductivity	1.6	1.2	0.300
Metaldehyde	1.2	0.8	0.556

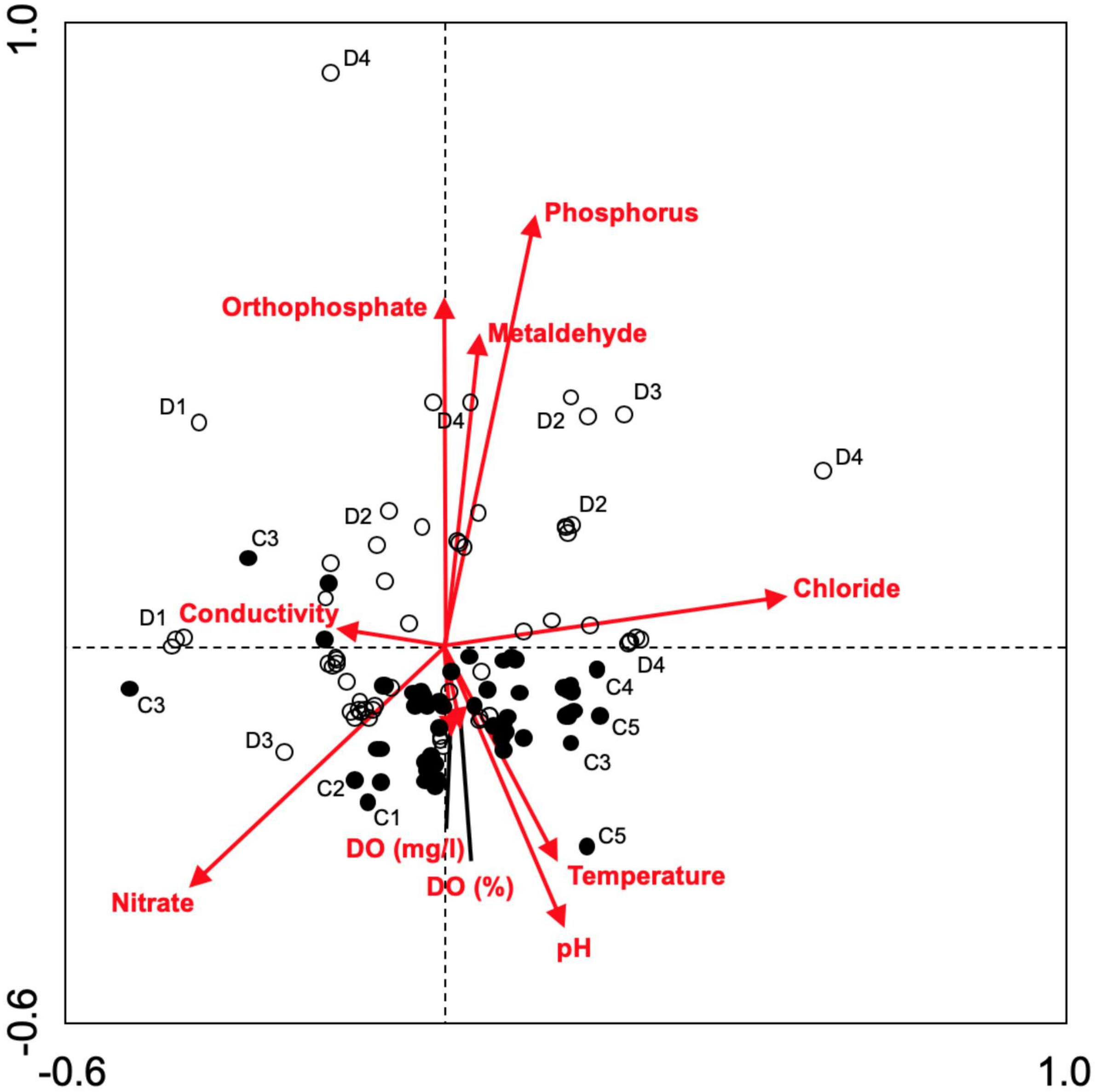
Table 2. Taxa responsible for the largest proportion of dissimilarity (SIMPER) between metaldehyde detected and control sites for the (A) entire faunal community, and (B) the mollusc community.

A. Entire faunal community

Taxa	**Cumulative dissimilarity accounted for (%)**
Hydracarina	4.1
Asellus aquaticus	7.9
Oligochaeta	11.7
Chironomidae	14.8
Ostracoda	17.9
Crangonyx pseudogracilis	20.8
Pisidium nitidum	23.7
Potamopyrgus antipodarum	26.4
Valvata piscinalis	28.6
Sigara dorsalis	30.7

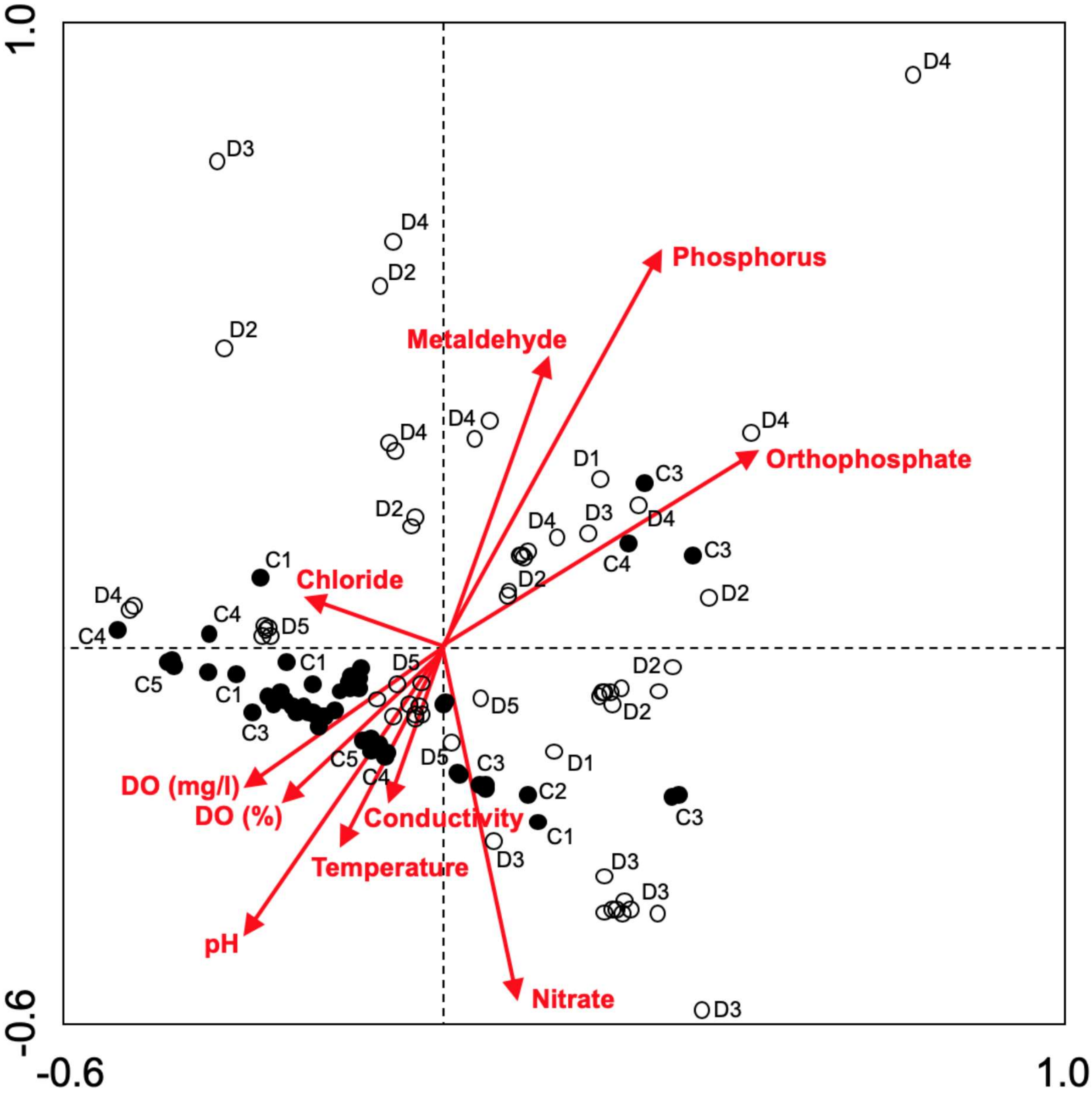
B. Mollusc community

Taxa	**Cumulative dissimilarity accounted for (%)**
Pisidium nitidum	9.4
Potamopyrgus antipodarum	18.5
Valvata piscinalis	26.0
Bithynia tenticulata	32.5
Anisus vortex	38.8
Physa fontinalis	44.9
Radix balthica	49.7
Theodoxus fluviatilis	54.0
Sphaerium corneum	57.9
Sphaerium rivicola	60.1



1.0
-0.6
-0.6
1.0
Phosphorus
Orthophosphate
Metaldehyde
Chloride
Conductivity
Nitrate
DO (mg/l)
DO (%)
Temperature
pH
D1
D2
D3
D4
C1
C2
C3
C4
C5

Figure 1.



1.0
-0.6
-0.6
1.0
Phosphorus
Metaldehyde
Orthophosphate
Chloride
DO (mg/l)
DO (%)
Conductivity
Temperature
pH
Nitrate

Figure 2.

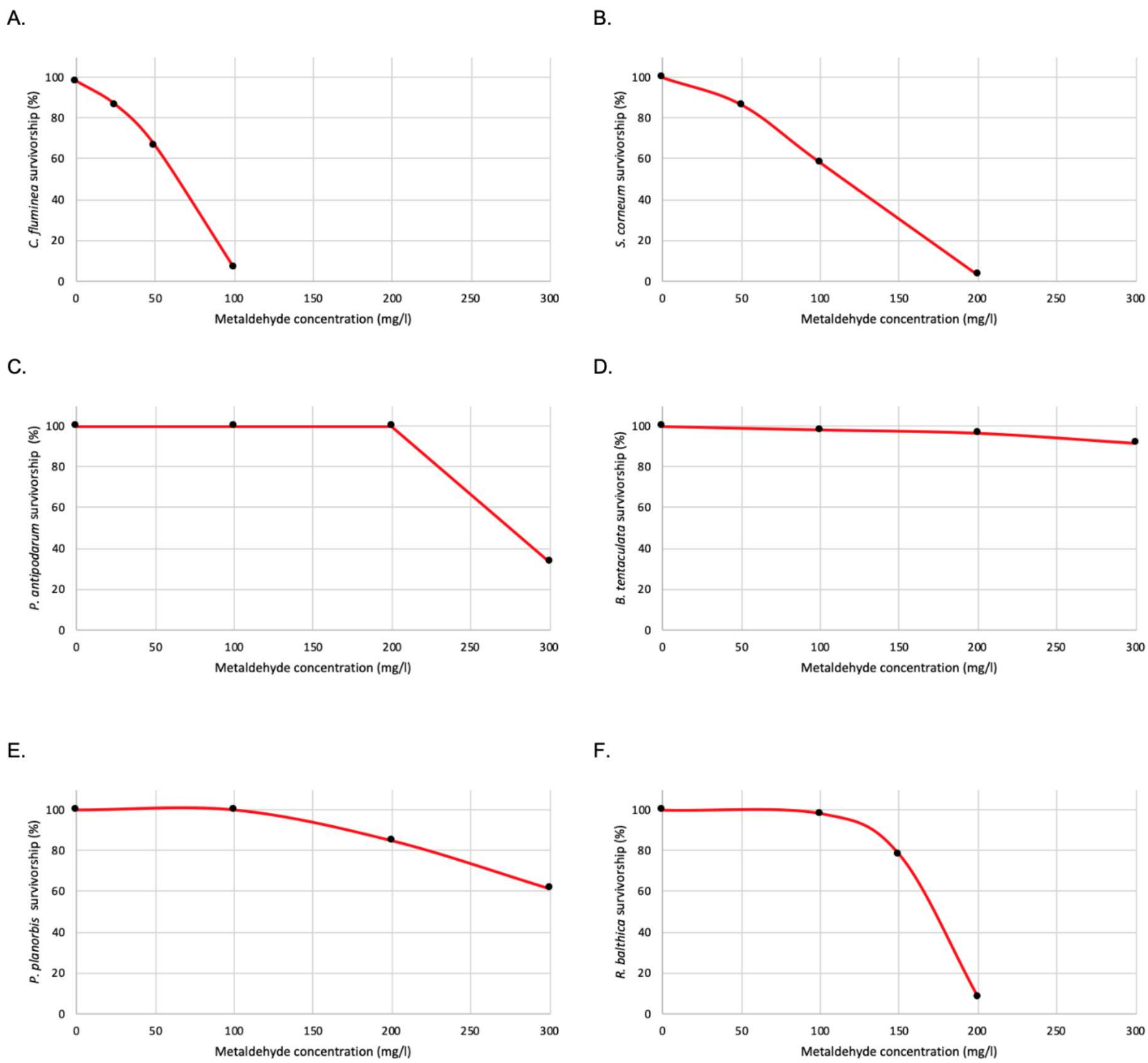
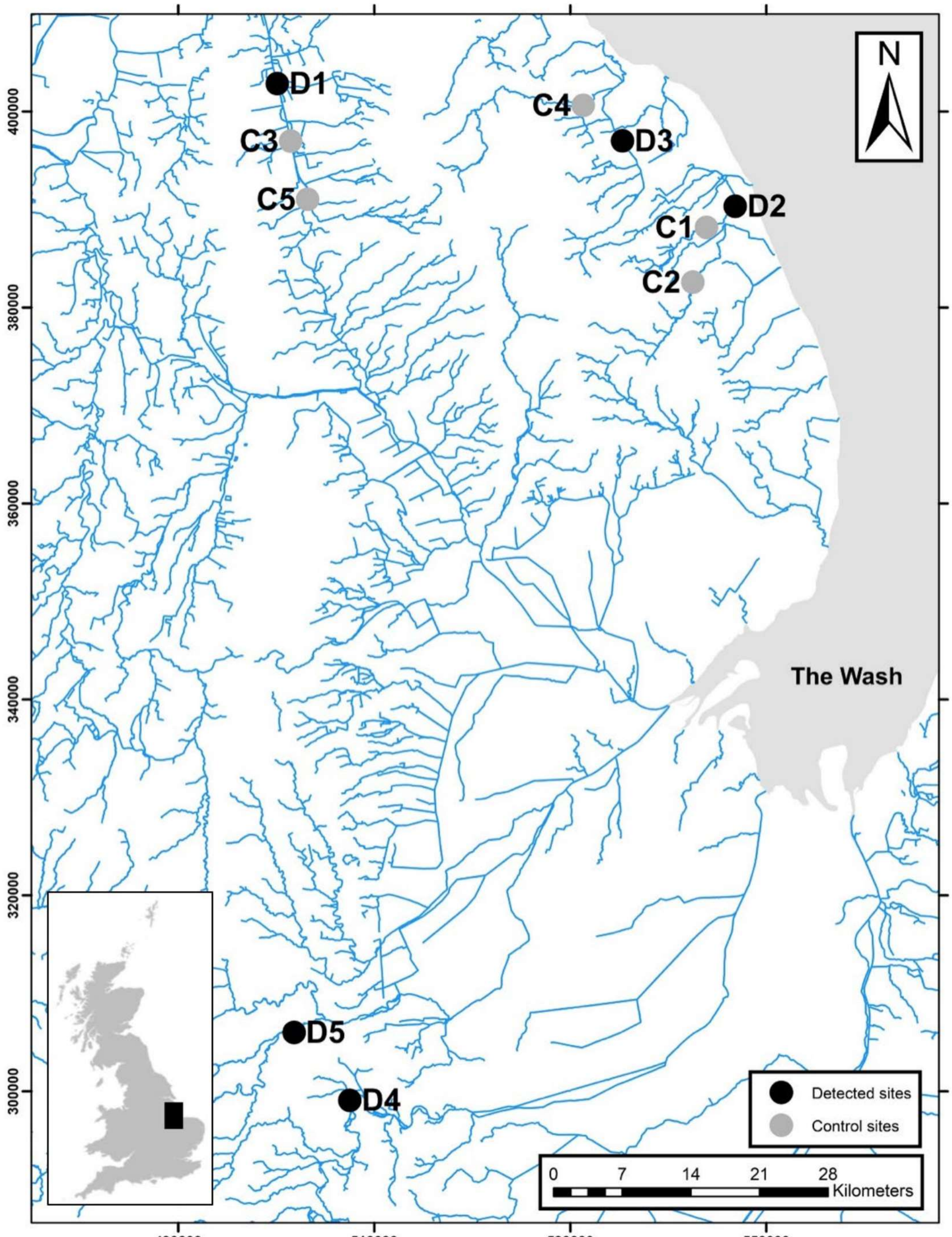

A.
C. fluminea survivorship (%)
Metaldehyde concentration (mg/l)
B.
S. corneum survivorship (%)
Metaldehyde concentration (mg/l)
C.
P. antipodarum survivorship (%)
Metaldehyde concentration (mg/l)
D.
B. tentaculata survivorship (%)
Metaldehyde concentration (mg/l)
E.
P. planorbis survivorship (%)
Metaldehyde concentration (mg/l)
F.
R. balthica survivorship (%)
Metaldehyde concentration (mg/l)

Figure 3.



N
D1
C3
C5
C4
D3
D2
C1
C2
The Wash
D5
D4
Detected sites
Control sites
0
7
14
21
28
Kilometers
400000
380000
360000
340000
320000
300000
490000
510000
530000
550000

Figure S1.