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Pollution Inhibits Predation: Differential Impacts of Metal Contaminants on the Functional Responses of Native and Invasive Gammaridae

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Research Article

Keywords: invasive species, functional responses, resource use, predator-prey, Dikerogammarus, metal contaminants

Posted Date: November 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11432666/v1

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Abstract

Biological invasions and aquatic pollution are among the most significant threats affecting freshwater ecosystems, yet their interactive effects remain poorly understood. Exposure to potentially toxic elements (PTEs) may cause impairments in invasive predators' feeding rates and ability to capture prey, which may in turn influence their ecological impact. This study compared the relationship between consumer feeding rate and prey density, known as the functional response (FR), of two invasive Ponto-Caspian amphipod species (*Dikerogammarus villosus* and *D. haemobaphes*) and one native species (*Gammarus pulex*), following exposure to environmentally realistic PTE mixtures (comprising copper, aluminium, iron and manganese). Predators were assigned to Control, Low and High concentration PTE treatments prior to FR trials and *Daphnia magna* and *Chironomus* spp. larvae (commonly known as bloodworm) were used as prey. Feeding occurrence and intensity were quantified using hurdle models, and density-dependent consumption was modelled via FR analysis and functional response ratios (FRRs; calculated by FR-derived attack rate divided by handling time, a/h). Under Control conditions, all species exhibited Type II FRs towards prey, with both invasive species displaying higher FRRs. PTE exposure suppressed predation across prey types and produced declines in FR curve height under both Low and High exposure. Species-specific responses to PTEs were evident; *D. haemobaphes* maintained relatively higher predatory impact under Low PTE exposure and showed the smallest decline in FRR, whereas *D. villosus* and *G. pulex* exhibited significant declines under Low and High conditions. The relative resilience of *D. haemobaphes* under Low PTE conditions, contrasted with the stronger declines observed in both *D. villosus* and *G. pulex*, highlights a mechanism by which pollution may alter invasion impacts and provide a relative advantage for tolerant invasive species.

Keywords: invasive species; functional responses; resource use; predator-prey; Dikerogammarus; metal contaminants

1. Introduction

Biological invasions and chemical pollution are two of the most significant stressors affecting freshwater ecosystems (Dudgeon *et al*., 2006; Tickner *et al*., 2020), yet their interactive effects

remain poorly understood. Metals and trace elements, hereafter referred to as potentially toxic elements (PTEs), are among the most widespread and persistent aquatic contaminants due to their extensive industrial, agricultural and urban sources and environmental persistence (Briffa *et al*., 2020). Consequently, the addition of PTE pollutants during the invasion process will have a variety of potential consequences upon the physiology, behaviour and populations of invasive species (Crooks *et al*., 2010; Ligocki *et al*., 2025).

The exact consequences of pollutants on the invasion process will depend upon the species traits and the stage of invasion (Ligocki *et al*., 2025), as well as other context-dependent factors. While there is no scientific consensus on whether pollution will uniformly advantage or disadvantage invasive species (Jackson *et al*., 2016), the typical framework proposed is that invaders will be relatively favoured compared to natives (Crooks *et al*., 2010). Traits relating to stress tolerance and phenotypic plasticity are frequently reported in successful invasive species (Devin and Beisel, 2007; Tesfay *et al*., 2023; Varó *et al*., 2015), as these traits are selected for during the invasion process, particularly during the transport and introduction stages (Blackburn *et al*., 2011; Lee *et al*., 2003). If stress tolerance confers some resilience to aquatic pollutants, then tolerant invasive species may be pre-adapted to compete in polluted conditions (Ligocki *et al*., 2025).

Alongside tolerance to stress and varying abiotic conditions, invasive species also frequently exhibit characteristic feeding behaviours. Invasive predators typically have aggressive and highly adaptive feeding, contributing to their ecological impact by depressing prey densities and intensifying resource competition with native predators (Courant *et al*., 2017; Krisp and Maier, 2005; Salo *et al*., 2007). Like stress tolerance, these feeding traits are highly selected within invasive species. Aggressive predation is beneficial during the establishment and spread stages, allowing the invaders to build up energy reserves and grow their population size (Rivera-Estay *et al*., 2024). Predatory traits (and resource-use in general) are key drivers of biological invasions, both in terms of invasion success and ecological impact, and thus serve as reliable predictors of invasion outcomes (Catford *et al*., 2009; Dick *et al*., 2017).

Feeding traits are also highly susceptible to disruption via chemical pollution, as has been seen in a wide variety of native and non-native species (Clements, 1999; Das and Khangarot, 2011; Kasumyan, 2001; Pastorinho *et al*., 2011). Common impacts of pollutants upon feeding traits include reduced consumption rate (Macedo-Sousa *et al*., 2007), reduced boldness (White and

Briffa, 2017), impaired response to food stimuli (Kasumyan, 2001) and disruptions to the digestive system (Lebrun and Gismondi, 2020). However, some species show resilience to contaminants and can maintain typical feeding traits (or minimise the degree of disruption) despite exposure to pollutants (Cunningham *et al*., 2021; Whitehead *et al*., 2017). This raises the possibility that differences in resilience to chemical pollution could influence invasion outcomes for invasive predators. For example, pollutants could potentially hinder the feeding traits of an invasive species and thereby contribute to invasion failure. Alternatively, if an invasive species is more resilient to pollution than a native species, the polluted conditions could potentially provide an advantage to the invader.

The use of functional responses (FRs) can be used to investigate the interactive effects of invasive species and aquatic pollution in the context of feeding behaviour (Alexander *et al*., 2014; Cuthbert *et al*., 2019; Dick *et al*., 2017, 2014; Dodd *et al*., 2014; Haddaway *et al*., 2012; Kestrup *et al*., 2011). The principles of FR were first outlined by Holling (1965), then iterated upon by Rogers (1972), to define three main relationships between resource availability (i.e. the quantity of prey) and consumer uptake (i.e. the consumption of prey). Type I responses show a linear increase in consumption until saturation, with negligible handling time. Type II responses involve significant handling times and a declining proportional consumption with resource density, with feeding eventually plateauing at high prey numbers. Finally, Type III responses are sigmoidal, with low consumption at low prey densities that accelerates as prey become more abundant. These different responses describe how the feeding rate of a consumer changes and scales with prey availability as a result of the consumers' attack rate (a) and prey handling time (h) (Pritchard, 2014). Additionally, these parameters can be integrated into a single comparative metric, the functional response ratio (FRR, defined as the attack rate divided by handling time, a/h) (Cuthbert *et al*., 2019). FR research thus provides a series of quantitative benchmarks with which invasive species can be compared relative to native competitors to predict likely ecological impacts. Integrating ecotoxicological exposure into comparative FR studies can therefore provide insight into how pollution influences these invasion dynamics.

Gammaridae provide an ideal model system to study these effects. Firstly, the family contains species of varying invasion status, from abundant native species to highly impactful invaders (Dobson, 2012). In natural or uncontaminated freshwater systems, invasive Ponto-Caspian species such as *Dikerogammarus villosus* and *Dikerogammarus haemobaphes* frequently exhibit higher feeding rates relative to native amphipods (Bovy *et al*., 2015; Cuthbert *et al*.,

2022; Dick *et al*., 1995; Dodd *et al*., 2014), enabling strong suppression of invertebrate prey populations (Jänes *et al*., 2015). Additionally, they are frequently used within ecotoxicological research due to their widespread distribution, both on a global scale and in a diversity of aquatic environments (Kunz *et al*., 2010), their high sensitivity to chemical stressors (Costas *et al*., 2018), and their frequent use as bioindicator species (Navarro-Barranco et al., 2020). However, much of the existing ecotoxicology research has focused on responses within individual species, while comparatively less attention has been given to comparing contaminant exposure effects on native and invasive gammarids. There is a growing body of research comparing the FR of native and invasive species as a predictor of invasive species impact (Alexander *et al*., 2014; Dick *et al*., 2014; Dodd *et al*., 2014; Bovy *et al*., 2015; Landi *et al*., 2022; Cuthbert *et al*., 2022; Howard *et al*., 2022; Faria *et al*., 2023a, 2023b, 2025; Başkur *et al*., 2026; Milano *et al*., 2026), however these studies have typically considered ecological interactions in the absence of chemical stressors. While recent studies have begun to use comparative FR with chemical or environmental stressors in comparisons of native and invasive species (Cruz-Escalante *et al*., 2025; Griffith *et al*., 2023; Laverty *et al*., 2015), this body of research remains limited in scope.

The aim of this study was to compare the FR of the native freshwater gammarid, *Gammarus pulex*, with those of two invasive species, *D. villosus* and *D. haemobaphes,* following acute exposure to environmentally realistic PTE scenarios. The gammarids were exposed to a combination of copper, aluminium, iron and manganese at varying concentrations prior to estimating FRs using *Daphnia magna* and *Chironomus* spp. larvae as prey. These prey species were selected as they are ecologically relevant and are abundant and widely distributed freshwater invertebrates that are known to be predated upon by Gammaridae species (Maier *et al*., 2011; Wilhelm *et al*., 2000). The primary aim was to determine whether predatory feeding rates differed between native and invasive gammarids and whether these differences were modified by PTE exposure. To our knowledge, this is the first comparative FR experiment to contrast the predation rates of native and invasive freshwater Gammaridae with the added effects of environmentally realistic PTE exposure.

2. Methods

2.1 Animal Collection and Maintenance

Three Gammaridae species of differing invasion status (Dobson, 2012) were used in this study, the native *G. pulex*, and the invasive non-native species *D. villosus* and *D. haemobaphes*. *Gammarus pulex* were collected from Biggar Burn, South Lanarkshire (55°37'33.0"N 3°31'35.5"W); *D. villosus* were collected from Grafham Water, Cambridgeshire (52°18'35.6"N 0°19'05.4"W); *D. haemobaphes* were collected from a tributary of the River Great Ouse, Cambridgeshire (52°17'16.5"N 0°13'18.2"W). Sampling was conducted between January and August 2025, and sampled gammarid wet mass overlapped among species (mean ± SD: 39.7–40.7 ± 5.3–6.1 mg). Each species was maintained separately in aerated 20 L acclimation tanks containing charcoal filtered tap water and fed *ad libitum* with sliced carrots and leaf litter. The acclimation tanks were maintained at approximately 12˚C in a controlled-temperature (CT) room with a 12 h:12 h light: dark regime. Aerated water was replaced twice per week to maintain water quality. Gammarids were given a depuration phase under laboratory conditions in the acclimation tanks of a minimum of 8 days, in keeping with previous invertebrate and amphipod metal exposure studies (Maltby, 1995; Vellinger *et al*., 2012a).

2.2 PTE Exposure

Gammarids were individually exposed to one of three PTE treatment concentrations (Control, Low, High) in glass beakers containing 400 mL of treatment medium and ~5 g of pre-washed gravel substrate (2–4 mm). Exposure media were prepared using charcoal-filtered tap water, with metal-spiked treatments created by adding standard solutions of copper ($Cu(NO_3)_2$), aluminium ($Al(NO_3)_3$), iron ($Fe(NO_3)_3$) and manganese ($Mn(NO_3)_2$) to achieve nominal environmentally realistic concentrations (Table 1). The metals and concentrations used in this study were derived from analysis of freshwater contaminant concentrations in the European Environmental Agency (EEA) Waterbase dataset (see supplementary information for procedures on how this was calculated).

Beakers were maintained at a temperature of 12 °C in a CT room with a 12 h:12 h light: dark regime, as above. Size-matched adult gammarids (wet body mass: 39.73 ± 5.30 mg for *D. haemobaphes*; 40.74 ± 6.10 mg for *D. villosus*; 39.83 ± 5.33 mg for *G. pulex*) were transferred individually into exposure chambers and held for 24 h without food to avoid dietary metal

uptake and to standardise hunger levels before feeding trials. Following exposure, individuals were assessed for survival based on spontaneous movement or response to gentle stimulation. Survivors were rinsed in filtered water to remove residual metals and immediately transferred to functional response arenas.

Functional response trials were conducted in arenas consisting of individual glass beakers (87 - 108 mm diameter, assigned randomly across trials) containing 400 mL filtered tap water and ~5 g of gravel substrate. Arenas were maintained at 12°C and 12 h:12 h light: dark regime as before. Gammarids were presented with a single prey species per trial: either *Chironomus* spp. larvae (hereafter referred to as bloodworm, ~10 mm length) or *Daphnia magna* (hereafter referred to as *Daphnia,* ~2 mm length). Prey were introduced to arenas using a pipette and allowed to acclimate for 15 min before predator addition. Prey were not exposed to PTE treatments during the study as this could have increased prey mortality and induced behavioural changes independent of predation. Isolating PTE exposure to the predator species only allowed for a clearer assessment of PTE impacts upon the predators' feeding behaviour.

Prey densities for bloodworm were 1, 2, 4, 8, 16 and 32 individuals, while *Daphnia* were presented at densities of 2, 4, 8, 16, 32 and 64 individuals. Each treatment combination of prey × predator × density × PTE exposure was replicated a minimum of three times. Prey were not replaced during experimental trials and were therefore depleted over time. Control arenas (n = 30) without predators were run in parallel to quantify background prey mortality.

Functional response trials ran for 16 h and at trial completion, predators were removed and remaining prey counted to determine consumption. Prey were considered consumed if fully or partially eaten. Trials in which predators moulted or died were excluded and repeated. After trials, gammarids were euthanised in ethanol, blotted dry, and weighed.

Table 1 Summary of nominal concentrations of aluminium, copper, iron and manganese used in the multi-metal PTE treatments that were added to charcoal-filtered tap water. Low and High treatments represent nominal concentrations of copper ($Cu(NO_3)_2$), aluminium ($Al(NO_3)_3$), iron ($Fe(NO_3)_3$) and manganese ($Mn(NO_3)_2$). See supplementary information for more information.

Treatment	**Copper (µg/L)**	**Aluminium (µg/L)**	**Iron (µg/L)**	**Manganese (µg/L)**
Control	0	0	0	0

Low	0.43	65.23	447.43	22.22
High	2.54	251.82	512.9	44.35

204

205 ***2.3 Data Analysis***

206 Data were analysed using two complementary analytical approaches: hurdle models and
207 functional response modelling. All analyses were conducted in R (version 4.5.1).

208 *Hurdle models* Hurdle models were applied using the *pscl* package (Jackman, 2024) and with
209 generalised linear models using the *stats* package (R Core Team, 2025). These models were
210 used to assess interspecific differences in (1) whether feeding occurred by the gammarids, and
211 (2) the number of prey consumed conditional upon the gammarid initiating feeding.

212 The zero (hurdle) component was fitted using a binomial distribution with a logit link,
213 modelling the probability that feeding occurred (i.e. at least one prey item was consumed). The
214 count component of each hurdle model was fitted using either a Poisson or negative binomial
215 distribution with a log link function, with the appropriate distribution determined by testing
216 model validation. The model's main effects were assessed using type-II Analysis-of-Variance
217 (*MASS* package: Venables and Ripley, 2002), and pairwise contrasts were conducted using
218 Tukey's post-hoc comparisons (*emmeans* package: Lenth and Piaskowski, 2017).

219 *Functional response models* Functional response analyses were conducted using R packages
220 *frair* (Pritchard, 2014) and *bbmle* (Bolker and R Development Core Team, 2007). FR types
221 were inferred using logistic regression of proportional prey consumption against prey density,
222 where a significant negative first-order term indicates a Type II functional response. Type II
223 dynamics were modelled using Rogers' random predator equation (Rogers, 1972):

224 $$N_e = N_0\,(1 - \exp\big(a(N_e h - T)\big))$$

225 Where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is
226 the handling time (reciprocally the maximum feeding rate, $1/h$) and T is the total experimental
227 period. Attack rate (a) and handling time (h) were estimated for each predator species, prey

type, and PTE treatment. Additionally, Functional Response Ratios (FRR) were derived following the formula FRR = a/h (Cuthbert *et al*., 2019).

Differences in functional response parameters between predator species and metal exposure treatments were assessed using the delta-method framework, implemented via *frair*, to generate pairwise contrasts in attack rate and handling time (Pritchard, 2014). Comparisons were conducted separately for each prey type and were restricted to datasets exhibiting Type II functional responses and stable parameter estimates. To quantify uncertainty around functional response parameters and fitted curves, non-parametric bootstrapping was applied. Bootstrapped confidence intervals around fitted functional response curves enabled visual comparison of feeding dynamics across prey densities.

3. Results

3.1 PTE Effects on Feeding Occurrence

Bloodworm

The survival rate of control prey was approximately 95% for bloodworm, therefore all prey losses in the trials were considered below a threshold for applying survival correction.

Initial hurdle models towards bloodworm prey, including all two- and three-way interactions among predator identity, PTE treatment and prey density indicated no significant interaction effects in either model component. Model comparison supported removal of interaction terms (ΔAIC = 14), and results are therefore presented from reduced main-effects models.

In the zero (feeding probability) component of the model, predator species did not significantly differ in their probability of feeding ($\chi^2 = 1.15$, df = 2, $p = 0.56$), however PTE treatment had a significant suppressive effect on feeding probability ($\chi^2 = 48.29$, $p < 0.001$). Feeding occurred in 93% of Control trials, compared with 52% and 35% under Low and High exposure, respectively. Additionally, increasing prey density reduced the likelihood of zero-consumption events as expected ($\chi^2 = 7.19$, df = 1, $p = 0.007$), with higher probabilities of feeding at higher densities across all predators and PTE treatments.

Conditional consumption (number of prey eaten given that feeding occurred) varied significantly with predator identity, PTE treatment and prey density. Predator species had a

significant overall effect on conditional consumption ($\chi^2 = 6.05$, df = 2, $p = 0.049$), driven by the higher feeding rates of the invasive *D. villosus* relative to native *G. pulex* ($p = 0.044$), whereas consumption by *D. haemobaphes* did not differ significantly from either species (*D. haemobaphes* vs *G. pulex* $p = 0.21$; *D. haemobaphes* vs *D. villosus* $p = 0.68$). Exposure to PTE treatment also significantly reduced conditional feeding intensity ($\chi^2 = 37.66$, df = 2, $p < 0.001$). Post-hoc comparisons showed that conditional prey consumption was significantly lower under both Low and High exposure treatments relative to Controls (Low vs. Control $p < 0.001$; High vs. Control $p < 0.001$), with mean consumption under Low and High exposure (0.97 ± 0.30 SE and 0.50 ± 0.11 SE for Low and High, respectively) significantly greater than under Control conditions (3.13 ± 0.33 SE). Conditional consumption did not differ significantly between Low and High exposure treatments ($p > 0.05$). These treatment effects were consistent across predator species. Finally, consumption increased with increasing prey density as was expected ($\chi^2 = 52.02$, df = 1, $p < 0.001$).

Daphnia

The survival rate of control prey was approximately 97% for *Daphnia*, therefore all prey losses in the trials were considered below a threshold for applying survival correction.

Initial hurdle models including higher-order interactions predator identity, PTE treatment and prey density provided little consistent support for three-way effects, and model selection supported a reduced model (ΔAIC = 6) retaining main effects and predator × treatment interactions.

In the feeding probability component, there was no evidence that PTE treatment affected feeding initiation differently among predator species ($\chi^2 = 2.803$, df = 4, $p = 0.59$). Predator species itself also had no significant effect ($\chi^2 = 0.186$, df = 2, $p = 0.911$). In contrast, PTE treatment had a strong negative effect on feeding probability ($\chi^2 = 56.58$, df = 2, $p < 0.001$), with *Daphnia* consumed in 98% of Control trials compared with 54% and 39% in Low and High exposure treatments respectively. However, pairwise contrasts did not reveal any detectable significant differences between the probability of feeding on *Daphnia* under different PTE treatments (all $p > 0.05$). Finally, prey density had no significant impact on the likelihood of feeding occurring ($\chi^2 = 1.841$, df = 1, $p = 0.175$).

In the conditional consumption component of the model, feeding intensity was influenced by the interaction of predator and PTE exposure, indicating differing degrees of PTE-induced feeding suppression between the gammarid species ($\chi^2 = 11.20$, df = 4, $p = 0.024$). Conditional consumption by *D. villosus* was more strongly reduced under High exposure than that of the other species, consuming significantly fewer prey than *D. haemobaphes* under High PTE conditions ($p = 0.036$). In contrast, predator-specific differences were not significant under Control or Low exposure treatments (all $p > 0.05$). In contrast, predator identity alone had no significant effect on conditional feeding intensity ($\chi^2 = 4.964$, df = 2, $p = 0.084$). PTE treatment had a significant inhibitory effect on conditional feeding intensity across all gammarid species ($\chi^2 = 99.93$, df = 2, $p < 0.001$), with all species exhibiting significantly greater feeding rates under Control conditions (all $p < 0.004$). The mean consumption of *Daphnia* in Controls was 6.96 ± 0.77 SE, significantly greater than the 1.69 ± 0.35 SE in Low and 1.07 ± 0.26 SE in High treatments (Control vs Low: $p < 0.001$; Control vs High: $p < 0.001$). Finally, as expected, increasing prey density significantly increased the number of *Daphnia* consumed ($\chi^2 = 123.77$, df = 1, $p < 0.001$).

3.2 Functional Responses

Bloodworm

Control treatment Under Control conditions, feeding upon bloodworm was characterised by clear Type II functional responses for each species of amphipod. Logistic regression detected a decelerating relationship between prey density and proportional consumption, with response models returning significant attack rate (a) and handling time (h) estimates for each predator species (Figure 1a and Figure 1d).

The functional response curves revealed some differences in feeding performance, particularly at higher bloodworm densities. In particular, at approximate prey densities above eight bloodworm, *D. villosus* exhibited a significantly greater functional response compared to *G. pulex* and *D. haemobaphes* under Control conditions (Figure 1a). Formal parameter comparisons revealed no statistically significant interspecific differences in either attack rate or handling time parameters (all $p > 0.05$; Figure 2), however the native *G. pulex* had a lower FRR than both invasive species. Under Control conditions, FRR were approximately 1.4-1.7 times higher for both of the invasive *Dikerogammarus* species compared to *G. pulex*, indicating a lower overall predation rate.

Low PTE treatment Under Low PTE treatment conditions, Type II responses were also retained, although parameter estimates were not consistently significant. The functional response curves showed the inhibitory effects of PTE exposure on gammarid feeding performance for bloodworm prey (Figure 1b). Across all three predator species, exposure resulted in significantly depressed FR curves relative to Control conditions (Figure 1a). This was characterised by a marked reduction in the asymptotic consumption rate, with slower increasing attack rates at low densities and lower plateauing consistent with prolonged handling times. Although the magnitude of reduction varied between the gammarid species, the direction of the response was highly similar. Limited overlap between Control vs Low confidence intervals supports a consistent decline in feeding performance for gammarids under contaminant stress.

Interspecific differences in functional response magnitude were evident under Low PTE conditions (Figure 1b). *Dikerogammarus haemobaphes* exhibited the highest FR at particular densities, with prey consumption increasing above those of *G. pulex* and *D. villosus* at densities exceeding ~8 prey. This indicates a comparatively greater predatory rate at higher resource availability under Low PTE conditions. In contrast, both *G. pulex* and *D. villosus* showed strongly suppressed functional responses across the density gradient.

Attack rates of amphipod predators feeding on bloodworm were reduced under Low PTE treatment relative to Control conditions (Figure 2). This pattern was most evident for *D. villosus* (Figure 2c) and *D. haemobaphes* (Figure 2e), both of which exhibited significantly lower attack rates under exposure compared to their Control estimates (*D. villosus*: $\Delta a = 0.769$, $p = 0.03$; *D. haemobaphes*: $\Delta a = 1.280$, $p = 0.033$). In contrast, attack rate estimates for *G. pulex* under Low exposure were associated with substantial variability (Figure 2a), resulting in broad uncertainty around the mean and limiting inference regarding directional change in attack rate.

Handling times estimates for bloodworm prey under Low PTE treatment showed greater variability across species compared to Controls (Figure 2). Formal parameter comparisons indicated no statistically significant changes in handling time relative to Controls. For *D. villosus* and *D. haemobaphes*, the differences in handling time were non-significant ($p = 0.081$ and 0.50 for *D. villosus* and *D. haemobaphes,* respectively). Formal testing for *G. pulex* was not possible due to numerical instability in the model fits.

Although formal parameter comparisons did not reveal any significant differences between the attack rate and handling time under Control and Low PTE conditions, there were noticeable

differences in the FRR between treatments. FRR declined substantially under Low PTE exposure across all gammarid species, with reductions of 70-95% relative to Controls. These declines reflected a combination of reduced attack rates and increased handling times (Table 2). In contrast to Controls, *D. villosus* exhibited the lowest FRR under Low PTE conditions and also had the greatest decline in FRR relative to Controls (ΔFRR = 10.80). *Dikerogammarus haemobaphes* had the greatest FRR value, with *G. pulex* showing an intermediate value between *D. haemobaphes* and *D. villosus.*

High PTE treatment For the High PTE exposure treatments, there was a significant inhibitory effect on functional response expression. No predator species exhibited significant density-dependent feeding responses on bloodworm under High exposure, and therefore functional response models were not parameterised (Figure 1c).

Daphnia

Control treatment All gammarid species exhibited Type II responses when feeding on *Daphnia* under Control conditions. Plots of the FR curves showed high degrees of overlap for predicted prey eaten across low and high prey densities (Figure 1d). At intermediate prey densities (approximately 15-30 individuals), the FR curves of *D. villosus* and *D. haemobaphes* exceeded the upper confidence interval of *G. pulex*, indicating higher prey consumption over this density range. However, the interspecific difference was not significantly evident at higher prey densities as the response curves converged. Formal parameter comparisons indicated no statistically significant differences in a or h (all $p > 0.05$; Figure 2). *Gammarus pulex* exhibited the lowest FRR of the predator species, while *D. villosus* and *D. haemobaphes* had approximately equal values (Table 2). The FRR of each predator for *Daphnia* prey was substantially higher compared to the bloodworm FRR.

Low PTE treatment Type II responses were retained for all predator species feeding on *Daphnia* following Low PTE treatment. Functional response curves for Low PTE treatment significantly declined relative to Controls (Figure 1e), with reduced prey consumption across all prey densities. Comparing the impacts of PTEs on predatory ability between the amphipod species, the FR of *D. villosus* exhibited the greatest decline relative to Controls (Figure 1d), highlighting inhibition of feeding rates. In contrast, *D. haemobaphes* exhibited the lowest level of FR decline relative to Controls, particularly at higher prey densities where there was overlap between the upper and lower confidence intervals (Figure 1d-e). This shows that Low PTE

treatment did not have a significant inhibitory effect on *D. haemobaphes* feeding upon *Daphnia* prey at higher prey densities. Under Low PTE conditions, the plot for *D. haemobaphes* had a higher feeding rate than both *D. villosus* and *G. pulex* at intermediate prey densities (approximately 20-40 individuals), as its plot exceeded both of their upper confidence intervals. This difference disappeared at higher densities as the curves reached their asymptote.

Attack rates declined across all gammarid species under Low PTE exposure (Figure 2b,d,f). *Gammarus pulex* exhibited a significant reduction in attack rate compared to Controls (Δa = 1.102, p = 0.023). Both *D. villosus* and *D. haemobaphes* also showed lower mean attack rate, however formal parameter comparisons with Controls could not be performed because the FR models did not produce stable parameter estimates.

Handling times also increased under Low PTE exposure (Figure 2), with *G. pulex* showing a significant increase compared to Controls (Δh = 0.29, p = 0.021). Correspondingly, FRRs declined substantially relative to Control values (Table 2). *Dikerogammarus haemobaphes* maintained the highest FRR, indicative of a greater attack rate and lower handling time compared to the other predator species, while *D. villosus* and *G. pulex* exhibited approximately similarly low values.

High PTE treatment Following High PTE treatment, there was a substantial reduction of feeding on *Daphnia* across all predator species. Type II responses were retained for the invasive species *D. villosus* and *D. haemobaphes,* though for *G. pulex* no density-dependent response could be detected.

Functional response curves under High PTE treatment showed significant declines (Figure 1f) compared to Controls, with predicted prey consumption remaining low even at higher prey densities. There was overlap of confidence intervals between *Dikerogammarus villosus* and *D. haemobaphes* at all prey densities, indicating no statistically significant difference in their FR behaviour when feeding on *Daphnia* following High PTE treatment.

Attack rates on *Daphnia* prey for *D. haemobaphes* declined significantly compared to Controls (Δa = 1.145, p = 0.021), and their handling time also increased significantly (Δh = 0.608, p = 0.001) (Figure 2f). Formal parameter comparisons could not be completed for High treatment *D. villosus* as the FR models did not produce stable parameter estimates. FRRs declined to

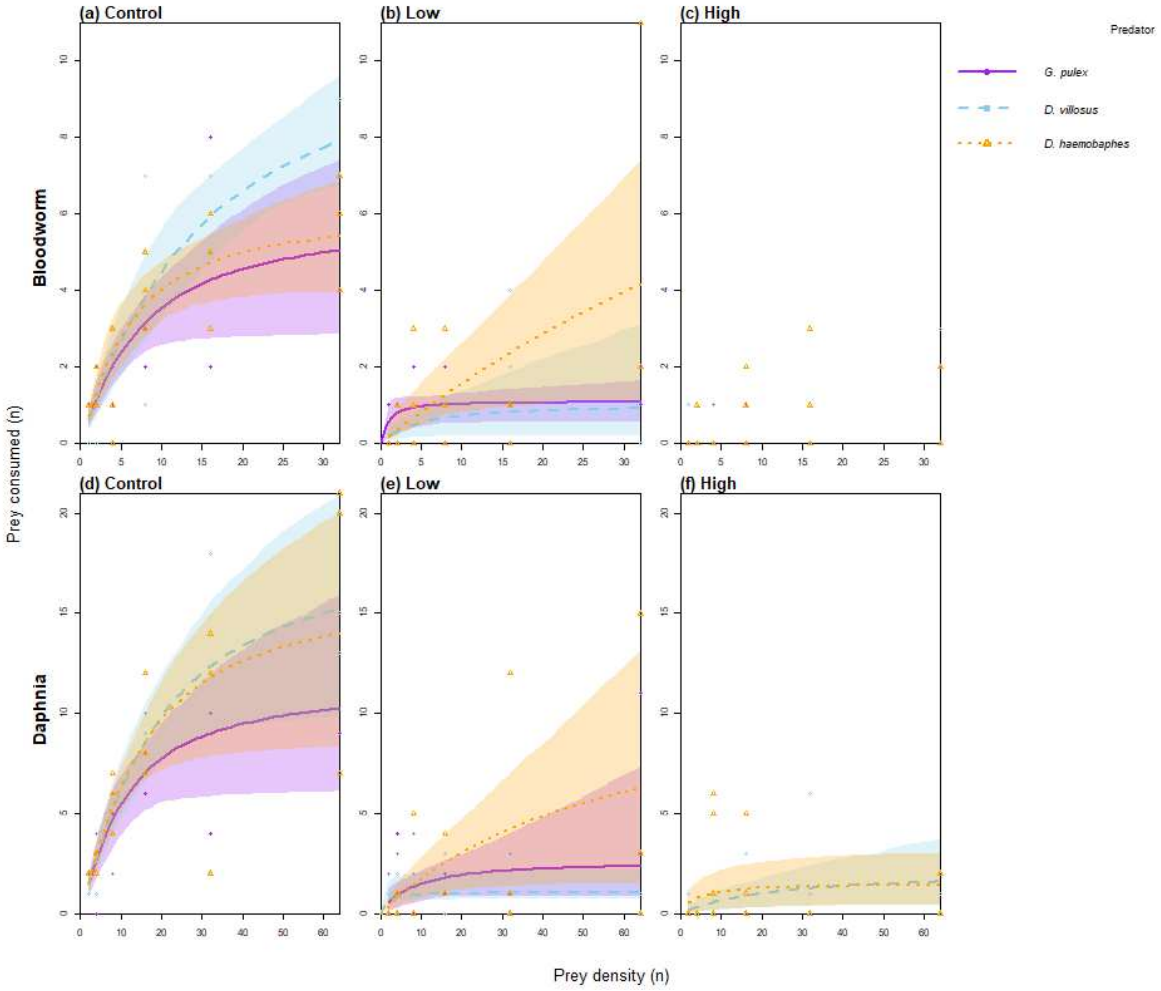
407 near-zero values for both predator species under High PTE conditions, indicating substantial
408 reductions in their predation rate (Table 2).

Table 2 Summary of functional response parameters for gammarid predators feeding upon *Chironomus* spp. larvae (bloodworm) and *D. magna*. Attack rate (a) and handling time (h) estimates are derived from maximum likelihood estimates of model outputs using the *frair* package. Significance codes indicate whether parameter estimates are significantly different from zero: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, . $p \leq 0.1$, unmarked values indicate $p > 0.1$. Values shown in bold indicate these metrics were calculated using statistically significant estimates of attack rate and/or handling time.

Prey	PTE	Predator	FR type	Attack Rate (a)	Handling Time (h)	Max. Feeding Rate (1/h)	Functional Response Ratio (a/h)
Bloodworm	Control	*G. pulex*	Type II	1.064 *	0.166 ***	**6.020**	**6.410**
		D. villosus	Type II	1.005 ***	0.091 ***	**10.989**	**11.044**
		D. haemobaphes	Type II	1.471 *	0.161 ***	**6.211**	**9.137**
Bloodworm	Low	*G. pulex*	Type II	1.704	0.914 ***	**1.093**	1.863
		D. villosus	Type II	0.236	0.964 .	1.037	0.245
		D. haemobaphes	Type II	0.191 *	0.065	15.385	2.938
Bloodworm	High	*G. pulex*	no response	-	-	-	-
		D. villosus	no response	-	-	-	-
		D. haemobaphes	no response	-	-	-	-
Daphnia	Control	*G. pulex*	Type II	1.484 ***	0.086 ***	**11.628**	**17.256**
		D. villosus	Type II	1.434 ***	0.053 ***	**18.868**	**27.057**
		D. haemobaphes	Type II	1.610 ***	0.061 ***	**16.393**	**26.393**
Daphnia	Low	*G. pulex*	Type II	0.382 .	0.380 **	**2.632**	1.005
		D. villosus	Type II	0.799 *	0.933 **	**1.072**	**0.856**

		D. haemobaphes	Type II	0.226 **	0.087 .	11.460	2.598
Daphnia	High	*G. pulex*	no response	-	-	-	-
		D. villosus	Type II	0.099	0.469 .	2.132	0.211
		D. haemobaphes	Type II	0.466	0.669 ***	**1.495**	0.697

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413

414 **Figure 1** Functional response curves showing prey consumed as a function of prey density for three amphipod predators (*G. pulex*, *D. villosus*,
415 *D. haemobaphes*) feeding on two prey types (*Chironomus* spp. bloodworm larvae and *D. magna*) under varying PTE exposure conditions.
416 Coloured lines represent fitted functional response curves for each predator species, with shaded areas indicating 95% confidence intervals
417 derived from bootstrapping. Points represent observed consumption data. No density-dependent response was detected for any predator species
418 with bloodworm prey under High PTE exposure (panel c), or for *G. pulex* under High PTE exposure for *Daphnia* prey (panel f).

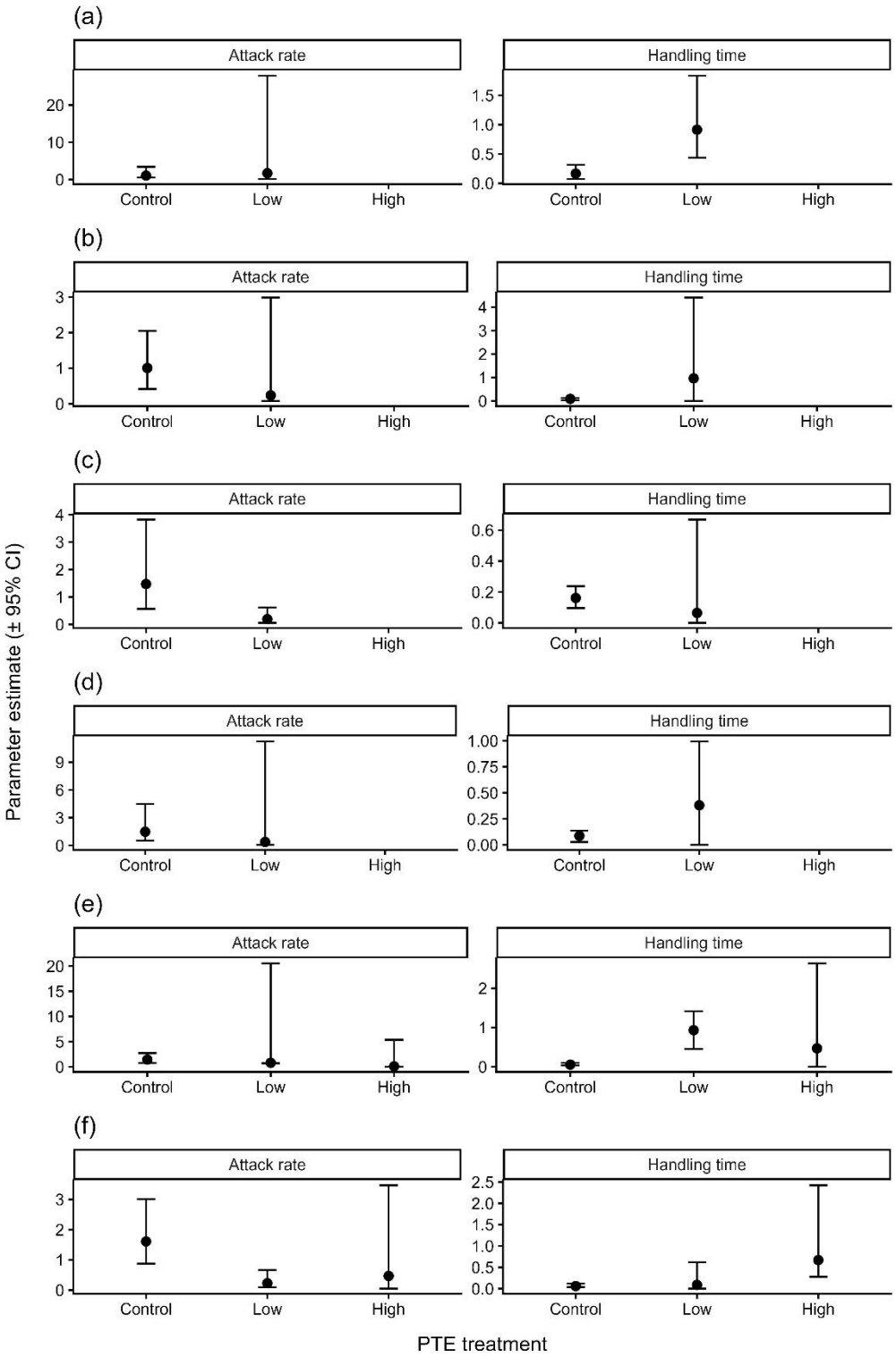


Figure 2 Functional response parameters towards *Chironomus* spp. larvae (bloodworm) and *D. magna* prey by gammarid predators *G. pulex, D. villosus*, and *D. haemobaphes* following PTE exposure treatments. Points represent the estimates for attack rate (*a*) and handling time (*h*), with 95% CI error bars derived through bootstrapping. No density-dependent response was detected for bloodworm under High PTE exposure.

4 Discussion

4.1 PTE Inhibition of Feeding

The results from this study show significant inhibition of gammarid feeding rates on both bloodworm and *Daphnia* following exposure to PTEs, with differences detected among species. Under Low PTE exposure, *D. haemobaphes* exhibited greater predatory impact than *D. villosus* and *G. pulex* on bloodworm at higher prey densities, and greater feeding rates on *Daphnia* at intermediate prey densities. Comparing the FRR, *D. haemobaphes* showed the lowest relative decline from Control to Low PTE conditions, while *D. villosus* showed the greatest relative FRR decline. This indicates that *D. haemobaphes* experienced less functional inhibition under PTE stress, suggestive of a relative predatory advantage for the invader under polluted conditions. Additionally, under High PTE exposure, the native *G. pulex* lost detectable density-dependent feeding responses, whereas invasive species retained functional responses, indicating greater functional resilience under contaminant stress.

Under Low PTE exposure, Type II functional responses were generally retained, but parameter estimates were frequently non-significant or highly variable, particularly for predation on bloodworm. This suggests that while density-dependent consumption was not eliminated, the strength and consistency of predator–prey interactions were reduced, potentially reflecting impaired foraging efficiency or risk-taking behaviour. These results are consistent with *in-situ* observations of predators in polluted environments. For instance, Weis *et al.* (2011) documented severely impaired predator–prey dynamics in crustaceans inhabiting estuaries contaminated with PTEs and persistent organic pollutants, with predation rates significantly lower than at reference sites. Similar patterns have been observed for *G. pulex* exposed to pesticides, with one study showing feeding rates on *Lectura nigra* declining to 0% under sublethal concentrations of cyhalothrin (Rasmussen *et al*., 2013).

Across both prey types, metal exposure strongly suppressed predatory feeding, but effects were prey specific. For bloodworm, prey density influenced both the probability of feeding and the number of prey consumed, and PTE exposure reduced both feeding initiation and conditional consumption. For *Daphnia*, density only affected feeding intensity, while metal exposure produced a near-complete suppression of feeding initiation under both Low and High exposure treatments. These prey-specific patterns indicate that while contaminant exposure consistently impairs predation, the outcome also depends on prey identity.

Evidence suggests that predators exhibit greater PTE tolerance than non-predatory taxa. Field surveys have shown that increasing aquatic metal contamination is associated with declines in in herbivorous and detritivorous species, while predatory taxa are comparatively less affected (Clements, 1994; Leland *et al*., 1989; Qu *et al*., 2010). For example, Liess *et al.* (2017) demonstrated that the ratio of predators to other functional feeding groups was strongly linked to metal toxicity across multiple rivers. There are a variety of likely mechanisms for the lower sensitivity of predators to PTE impacts compared to lower trophic taxa, predominantly the difference in dietary uptake. For example, there is a significantly lower metal load in invertebrate prey compared with algae and detritus from the same site (Bossuyt and Janssen, 2005). This increased tolerance of predators to PTE stress provides important context for the present findings. It suggests the invasive *Dikerogammarus* species, particularly *D. haemobaphes* which showed the strongest predatory response under Low PTE conditions, could be less exposed to PTE uptake through dietary pathways compared to other amphipods.

4.2 Feeding Comparisons of Native and Invasive Gammaridae

Although formal statistical differences were not detected under Control conditions, invasive species (particularly *D. villosus*) consistently exhibited higher Functional Response Ratios compared to the native. Additionally, once feeding was initiated, invasive gammarids consumed significantly more bloodworm prey than the native *G. pulex*, indicating greater *per-capita* predatory impact rather than higher encounter frequency. Comparison of the functional response plots also revealed several instances where invasive species showed a significantly higher predation rate. The FR curve for *D. villosus* exceeded the upper confidence limit of *G. pulex* under Control conditions for bloodworm, and *D. haemobaphes* exceeded *G. pulex* confidence limits at certain prey densities following Low PTE treatment, for both bloodworm and *Daphnia*.

Comparative functional response studies offer a mechanistic framework for evaluating differences between the feeding behaviours of native and invasive species. Bollache *et al.* (2008) used this method to compare the predation intensity of *D. villosus, Gammarus duebeni celticus, Gammarus roeseli* and *G. pulex* predating on *Asellus aquaticus*, a benthic water louse species. The results showed a Type II functional response for all gammarids (i.e. the consumption rate increased with prey density then decreased to an asymptote) but significantly greater responses for the invaders, with *D. villosus* in particular exhibiting the greatest

predation rate. Of additional note, the comparison by Bollache *et al*. (2008) was made between size-matched individuals, however *D. villosus* can grow to twice the size of other gammarids in the wild (Macneil and Platvoet, 2005) and reach higher population densities (Devin *et al*., 2003), therefore its likely these results underestimate the actual scale of *D. villosus* predation intensity. There have been several repeated gammarid comparative FR studies demonstrating a greater predatory rate for the invasive species (Cuthbert *et al*., 2022; Kestrup *et al*., 2011; Laverty *et al*., 2015). However, this pattern is not universal, with native species sometimes exhibiting higher feeding rates depending upon prey type and interspecific interactions (Dodd et al., 2014; Médoc et al., 2015). Thus, while invasive gammarids frequently exhibit enhanced predatory performance, FR can vary with context-dependent factors.

4.3 Mechanisms of Feeding Rate Inhibition

There are a variety of possible mechanisms explaining the inhibitory effects of PTEs observed in this study. Once predators have been exposed to PTEs, they will suffer a variety of detrimental effects which can offset their predation success. Predatory foraging requires olfaction as an essential source of information (Ferrari *et al*., 2010). Pollutants can interfere with the natural olfactory information by masking predator/prey cue signals, adjusting signal chemistry, or even causing damage to the organism's receptors and sensory organs (Lürling, 2012; Morelia Camacho-Cervantes and Wong, 2023). This has been documented to occur for fish and crustacean species, with metals causing direct effects on the nervous system which disturbs a variety of behaviours (Olsén, 2011). This was well documented for rusty crayfish *Orconectes rusticus* after exposure to sublethal concentrations of copper (Lahman *et al*., 2015). Exposed organisms showed a reduced response to food stimuli, with a lowered overall orientation ability to locate a food source. A reduction in encounter rates and handling success caused by damage to olfactory organs may explain the dramatic decline in feeding rates during functional response trials in the current study.

Generalist invasive species may gain a competitive advantage over specialist predators by switching to alternative, more easily acquired resources while under PTE-induced stress. Following exposure to contaminants, typically predatory species may shift their diet to alternative food sources: for example, crabs in polluted estuaries shift to nutrient-poor detritus, as the pollutant stress impairs their ability to capture animal prey (Weis *et al*., 2011). Similarly, stream salmonids in metal-polluted rivers consume more terrestrial invertebrates as aquatic

prey become smaller and less abundant (Kraus *et al*., 2016). Conversely, predators invading polluted environments may benefit from encountering prey populations that are themselves behaviourally impaired by contaminants, showing reduced anti-predator behaviour and thereby increasing prey vulnerability (Faria *et al*., 2010).

5 Conclusion

Exposure to environmentally realistic PTE mixtures caused substantial inhibition of amphipod predation, reducing both the likelihood of feeding and the number of prey consumed when feeding occurred, and depressing density-dependent functional responses across prey types. The findings also confirm the established greater predatory rates exhibited by invasive *Dikerogammarus* species. Under Control conditions, both invasive species exhibited greater functional responses than the native *G. pulex*, indicating higher baseline predatory ability. *Dikerogammarus villosus* lost this predatory advantage under PTE exposure, with feeding rates becoming similarly suppressed to *G. pulex*, whereas *D. haemobaphes* maintained significantly higher predatory rates and showed comparatively greater functional resilience. Additionally, under High exposure, *G. pulex* lost detectable density-dependent predation, whereas invasive species retained functional responses, indicating greater functional resilience under contaminant stress. These results provide some support for the hypothesis that invasive species exhibit greater tolerance of aquatic pollutants compared to native species, however responses were clearly species- and context-dependent. PTE exposure has the potential to alter predator-prey dynamics in ways which could compound the ecological changes driven by highly invasive species such as *D. haemobaphes.*

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Statements & Declarations

Funding

This work was supported by the Scottish Universities Partnership for Environmental Research Doctoral Training Partnership (SUPER DTP).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

The first draft of the manuscript was written by Connor Wood. Previous drafts of the manuscript were reviewed and commented on by Mhairi Alexander, Jennifer Dodd, and Katherine Sloman. All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Data Availability

Datasets generated during the current study are available from the corresponding author on request.

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