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Working title: Flow regimes control the establishment of invasive crayfish and alter their effects on lotic macroinvertebrate communities

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Abstract

1. Invasive non-native species (INNS) threaten biodiversity and ecosystem functioning globally. However, there remains a pressing need to understand the environmental factors controlling the dispersal, successful establishment and subsequent ecological impacts of INNS for receiving ecosystems. Here, we examine how region-wide flow regime magnitudes facilitate the successful establishment of an invasive crayfish species (*Pacifastacus leniusculus*, signal crayfish) in England (UK). We also consider the interactive effects of invasive crayfish with flow regime variations on the structural and functional diversity of macroinvertebrate communities.

2. Low-flow magnitudes increased the likelihood of *P. leniusculus* establishment, with 80% of recorded invasion dates falling in years with flow magnitudes below average (low- and low-moderate flow classes), whilst only 1.6% occurred in high-flow years.

3. Temporal trajectories of structural and functional macroinvertebrate responses in invaded rivers demonstrated reduced diversity compared to control rivers. Lower taxonomic and functional richness measures typically coincided with periods of low discharge in invaded rivers and were greatest during regionally high-flows.

4. Macroinvertebrate communities displayed significant structural and functional responses to the interaction between invasive crayfish and flow regime variations. Specifically, a number of low- and high-flow indices yielded significant associations, highlighting the role of extreme hydrological events in shaping INNS effects on receiving ecosystems. We also detected greater ecological effects of invasive crayfish under hydrologically stable conditions. Importantly, and for the first time, we observed that invasive crayfish reversed macroinvertebrate community responses to flow regime cues (e.g., discharge fall rate and minimum flows in the preceding 180 days).

4. *Synthesis and applications*. Results from this study indicate that low-flow events facilitate the spread/establishment of invasive crayfish and correspond with greater ecological effects for receiving ecosystems. Given that low-flow events are predicted to increase in intensity, duration and frequency over the 21st century, our results highlight the potential threat that invasive crayfish may pose under future hydroclimatic changes. Managing river flow regimes effectively (including

maintaining higher flow events and flow variability) is likely to be vital in conserving ecological diversity following crayfish invasion.

Keywords: structural and functional diversity, hydrological variability, non-native species, river regulation, *Pacifastacus leniusculus,* signal crayfish, low-flow, invasive species

1. Introduction

Invasive non-native species (INNS) represent a significant threat to ecosystem functioning globally and are widely regarded as a key driver of human induced environmental change (Gallardo, Clavero, Sánchez & Vilá, 2016; Reid et al., 2018). Biological invasions are cited as the second major cause of biodiversity loss (behind habitat alteration; Ricciardi, 2004) associated with species eradication or declines. The successful establishment of INNS can modify ecosystem functioning and the provision of services (Pejchar and Mooney, 2017; Simberloff et al., 2013) and the consequences of biological invasions are potentially so far reaching that some biologists have suggested that communities are becoming homogenised by the global redistribution of species (Corlett, 2015). Freshwater ecosystems in particular are some of the most endangered habitats in the world with declines in biodiversity surpassing their terrestrial and marine counterparts (Dudgeon et al., 2006; Martinuzzi et al., 2014; Sánchez-Bayo and Wyckhuys, 2019).

Identifying relationships between environmental drivers and biotic responses is a major goal in ecology. Historically, research has focussed on understanding these drivers as independent entities, but recent efforts have demonstrated that drivers often act in combination and synergistically (Didham, Tylianakis, Gemmell, Rand & Ewers, 2007; Jackson, Loewn, Vienbrooke & Chimimba, 2016). Of increasing importance in the field of invasion biology is understanding environmental factors controlling the successful establishment, spread, and subsequent ecological impacts of INNS for the receiving ecosystem (Bunn & Arthington, 2002; Havel, Kovalenko, Thomaz, Amalfitano & Kats, 2015). Previous research has investigated the effects of anthropogenic and natural disturbances on the successful establishment of INNS (e.g., Johnson, Olden & Vander Zanden, 2008), with regulated rivers and streams more likely to support INNS able to exploit these environments which are typically characterised by less variable flow regimes (Catford, Downes, Gippel & Vesk, 2011; Light, 2003; Olden, Poff & Bestgen, 2006).

Hydrological variability plays a pivotal role in structuring aquatic ecosystems, and collectively the different 'facets' of river flow regimes, namely the magnitude, frequency, duration, timing, and

rate of change in flow conditions (*sensu* Poff et al., 1997; Poff, 2018; Richter, Baumgartner, Powell & Braun, 1996; Richter et al., 1997), are widely regarded as master variables shaping ecological processes. Biota inhabiting riverine environments have adapted to natural hydrological variations over evolutionary timeframes (Lytle & Poff, 2004) and typically possess resistance and resilient traits which enable them to survive extreme flow events within the context of the natural flow regime (e.g. floods, droughts; Bogan, Boersma & Lytle, 2015; Steel, Peek, Lusardi & Yarnell, 2018). However, additional stressors (including biological invasions) occurring simultaneously with extreme flow events may threaten the ecological integrity of these systems, making them more sensitive to disturbances. Despite increasing research considering the role of hydrology as a control on the establishment and subsequent population dynamics of INNS / native species (e.g., Lynch, Leasure & Magoulick, 2018; Rogosch et al., 2019), we are unaware of any studies quantifying the interactive effect of INNS and hydrologic variability upon the composition of the receiving community (with the exception of a mesocosm study; Magoulick, 2014). Understanding the interaction between hydrological variability and invasive species is thus vital, particularly in the face of climate change (Chen & Olden, 2017; Poff, 2018; Rahel et al., 2008).

Freshwater crayfish represent one of the most widely translocated organisms across the globe following either intentional introduction or accidental translocation (Gallardo et al., 2016; Kouba, Buřič & Petrusek, 2015). They are one of the largest freshwater invertebrates, frequently dominating benthic faunal biomass where they occur (Momot, 1995). As such, where they successfully establish, ecological effects can cascade throughout the food web associated with their polytrophic feeding habits. Modifications have been reported in detrital processing rates and algal composition (Carvalho, Pascoal, Cássio & Sousa, 2016; Creed, 1994) through to reductions in the density and richness of macroinvertebrates (Stenroth & Nystrom, 2003; Mathers et al., 2018) and macrophytes (Lodge & Lorman, 1987; Roessink, Gylstra, Heuts, Specken & Ottburg, 2017). The ecological consequences of crayfish invasion can be temporally persistent (decadal) and spatially extensive (regardless of biogeographical context), with modifications in macroinvertebrate community composition representing significant and long-term ecological perturbations (Mathers et al., 2017; Ruokonen, Ercoli & Hämäläinen, 2016). Invasive crayfish have been documented to be more tolerant of stream drying events (Larson, Magoulick, Turner & Laycock, 2009), a factor which may not only enhance their successful establishment but also intensify the impacts felt by the receiving ecosystem during periods of low-flow. Furthermore,

activity levels of crayfish demonstrate a positive relationship with rising water temperatures (Bubb et al., 2004; Johnson et al., 2014); which may exacerbate instream effects but also facilitate their spread throughout waterbodies. Despite the anticipated increase in extreme hydrological events (Ragno et al., 2018; Stephens, Johnson & Marshall, 2018), the interactive effects of invasive crayfish and hydrology on ecological communities are largely unknown.

This study examines the long-term interactive effects of hydrological variability and invasive signal crayfish, *Pacifastacus leniusculus* (Dana), one of the most prevalent non-native crayfish species in Europe (Kouba et al., 2015), on macroinvertebrate communities across multiple rivers in England (United Kingdom). Unlike many invasion studies, the effect of invasive crayfish is investigated using multiple river catchments before and after invasion, and in direct comparison with control sites where long-term biomonitoring has not recorded the presence of *P. leniusculus.* The study also employs rivers free from river regulation and impoundment to ascertain the direct influence of hydrological variability on the subsequent ecological effects of an INNS for the receiving ecosystem. Specifically, we tested the following hypotheses: (1) years with low-flow magnitudes would facilitate the establishment of *P. leniusculus;* (2) temporal trajectories of structural and functional macroinvertebrate diversity metrics would differ between rivers invaded by *P. leniusculus* and those not and; (3) the structural and functional diversity of macroinvertebrate communities would respond to the interactive effects of *P. leniusculus* presence and hydrological variability. For hypotheses (2) and (3) we predicted that the greatest reductions in diversity would occur during periods of low-flow.

2. Materials and methods

2.1 Datasets characterised

Data from across England formed the basis of the study incorporating the diverse variability of lowland river systems with differing natural biogeographical, geological and hydrological controls. Two ecological datasets were collated: (i) invasion dates of *P. leniusculus* from five English regions ('South East', 'West', 'East', 'North' and 'North West') and; (ii) macroinvertebrate community data from three of these English regions ('North', 'North West' and 'East'; Figure 1). Hydrological data were extracted for the five English regions to enable the statistical derivation of region-wide flow magnitude classes. In addition, detailed facets of flow regime variability (comprising magnitude, frequency, duration, timing and rate of change of hydrological conditions *sensu* Richter et al., 1996; 1997; Poff et al., 1997) were examined in the

East region following invasion; whereby flow gauges were located proximally to macroinvertebrate sampling locations. Each of these datasets is described in detail below. The spatial distribution of the ecological and hydrological datasets are shown in Figure 1. Figure 2 summarises the datasets and subsequent analyses conducted.

2.2 Ecological data

2.2.1 P. leniusculus successful establishment dates

A dataset comprising the year of successful establishment by *P. leniusculus* across multiple rivers in England was collated. All rivers were selected from the Environment Agency (the environmental regulator in England) BIOSYS database following the protocol outlined in Turley et al., (2017). Detecting invasions by signal crayfish is difficult due to their high mobility (Gladman et al., 2010) and there are currently no methods of determining crayfish populations below a density of 0.2 m^{-2} (Peay, 2003). Furthermore, routine sampling of crayfish populations is currently not a standard biomonitoring practice. For the kick-net samples utilised in this study, it is likely that the detection limit is higher, probably approaching higher densities of *c*.1.0 m^{-2}. Consequently, for all sites employed in this study, the successful establishment date represents the time when crayfish population densities were high enough to be detected within kick samples and are used as a reliable surrogate for invasion dates here (*sensu* Turley et al., 2017). Successful establishment dates were derived by performing a systematic search of the BIOSYS database for records of "*Pacifastacus leniusculus*". All sites with "Astacidae" being recorded before the first *P. leniusculus* record were removed to avoid taxonomic ambiguities associated with family-level records of the native British crayfish (*Austropotamobius pallipes*). For each of the remaining sites, the midpoint between the first record of "*Pacifastacus leniusculus*" and the previous sample was designated as the successful establishment date (Turley et al., 2017). Sites are typically sampled every 6 months and therefore provide a reliable estimate of time of establishment. These data comprised a total of 64 invasion dates for independent rivers across England (comprising five regions as defined in this study) distributed across a range of years from 1996 to 2012 (Table S1).

2.2.2 Macroinvertebrate data

Macroinvertebrate data from three regions (North, North West and East; note some of this data was published previously under different region names in Mathers et al., 2016a, Mathers, Chadd, Extence, Rice & Wood, 2016b) were analysed and comprised seven 'invaded' and seven 'control' lowland rivers across England (See Table S2). Rivers were selected to have broadly comparable

physical characteristics within the individual regions (channel size, discharge, water chemistry, altitude and geology; *sensu* Mathers et al., 2016a) and all sites did not support a native crayfish population during any of the time series. All rivers were free from major anthropogenic stressors such as water abstraction, flow regulation or impaired water quality. All benthic macroinvertebrate samples were collected by the Environment Agency during routine biomonitoring. Samples were collected via a standard 3-minute 'kick-sample' (mesh size 1mm) performed across all available habitats, and an additional 1-minute hand search (Murray-Bligh, 1999). Sampling was conducted in spring (March – May) and autumn (September – November). Community abundances were standardised to presence / absence.

The dataset comprised 477 macroinvertebrate community samples collected between 1990 and 2013 encompassing periods before and after the invasion of *P. leniusculus* (209 and 268 samples from invaded and control rivers respectively). For invaded sites, the date of invasion was determined by the first occurrence of *P. leniusculus* in the historical faunal series and for control sites the mean date of invasion was used (*sensu* Mathers et al., 2016a,b). A total of 101 taxa were recorded predominantly to species and genus level, but some taxa were resolved to family level (primarily Diptera larvae); while a small number of taxa (notably the early instars of some aquatic insects) could not be consistently resolved to the lowest taxonomic resolution and were aggregated to a coarser taxonomic level (e.g. *Baetis* sp., *Leuctra* sp.).

2.3 Hydrological data

2.3.1 Region-wide flow magnitude classifications

A network of flow gauges within each of the five regions were utilised to derive changes in region-wide flow magnitudes on an inter-annual basis. Daily discharge data spanning a 25-year period (1989 -2014) were obtained from 39 flow gauges from the National River Flow Archive (NRFA, 2018; Table S3). A total of 10 gauges were employed within three regions (North, East and West) with five used for the South East and North West regions which covered small geographical areas (note that one gauge was used for both the North and North West due to its proximal location to both regions). The final number of gauges employed were selected to account for natural geographic variations in river flow regimes (driven by geological and hydroclimatic controls). Gauges were screened to ensure that hydrological variability was not significantly impacted by flow regulation (by scrutinising metadata and hydrographs) or urbanisation (urban land use coverage <20% of the catchment area in all instances; Table S3). Missing daily discharge

values comprised <10% of the time series in all instances and were interpolated via highly significant (p ≤ 0.001) linear regressions with nearby flow gauges (Table S4). Inter-annual variations in flow magnitudes were highly congruent within each region, and we observed no significant differences between annual flow magnitudes derived from individual gauges within each region (Table S5). These hydrological datasets were used in combination with both *P. leniusculus* establishment dates and the macroinvertebrate data (North West, North and East regions; see Figure 2).

2.3.2 Hydrological variability within individual rivers

Only the East region had flow gauging stations located proximally with macroinvertebrate sampling locations. As such, a second hydrological dataset was compiled for this region to enable specific analyses of macroinvertebrate responses to facets of the river flow regime (magnitude, frequency, duration, timing and rate of change of hydrological conditions; Figure 2). For this, the 'after' invasion period (taken as the mean invasion date from all rivers – 2003) was selected for both control and invaded rivers (a total of 84 macroinvertebrate samples were examined across five rivers). Daily discharge data (2001 – 2014) were obtained from five flow gauges from the National River Flow Archive (NRFA, 2018) in the East region (Table S3). Missing values represented <1% of the total dataset and were interpolated using the *na.approx* function within the "zoo" package (Zeileis & Grothendieck, 2005). All statistical functions and analyses were performed in the R environment (R Version 3.3.1, R Development Core Team, 2016).

2.4 Data preparation

2.4.1 Macroinvertebrate community response metrics

Functional macroinvertebrate compositions were derived from the Tachet, Bournaud, Richoux & Usseglio-Polatera (2010) European trait database (Table S6). Within this database, a fuzzy-coding procedure has been adopted with faunal affinities to individual traits ranging from zero (indicating no affinity) to three or five (indicating high affinity or the upper limit depending on existing scientific certainty; Tachet et al., 2010). In total, 13 'grouping features' (a functional trait category, e.g. 'maximum body size') comprising 76 functional 'traits' (modalities residing within grouping features, e.g. '≤0.25cm', '≥8cm';) were utilized. This included 63 biological traits (11 grouping features) and 13 ecological traits (two grouping features of substrate and velocity preferences); with the latter being selected *a priori* because *P. leniusculus* predate on taxa with specific sedimentological and hydraulic preferences (Mathers et al., 2016a; Usio & Townsend,

2004). Trait values were standardized for all samples so that each grouping feature summed to 1 (to ensure trait affinities were equally weighted between taxa). Taxa resolved to a greater resolution (e.g. species) than available in the trait database (e.g. genus) were aggregated (but maintained in a presence / absence format). All taxa recorded at a coarser resolution than within the trait database were excluded, with a total of 62 taxa being assigned functional traits.

From these standardized trait values, five univariate functional diversity metrics were derived (*sensu* Villéger, Mason & Mouillot, 2008) using the *dbFD* function within the "FD" package (Laliberté & Legendre, 2010). The dbFD function calculates properties of the multidimensional trait space based on a functional trait dissimilarity matrix (Bray-Curtis on the aforementioned 76 standardized trait values) and a dataset characterising the community composition (presence-absence matrix of the 62 taxa assigned traits). Functional richness (the minimum convex hull encompassing all species); functional evenness (the regularity of distances between taxa connected by a minimum spanning tree); functional divergence (the distribution of taxa in relation to the functional centroid) and Rao's Quadratic Entropy (the product of the pairwise distances between taxa; hereafter termed RaoQ) were calculated for each sample (see Schleuter et al. 2010 for more details). A trait-abundance array was calculated by multiplying the presence-absence dataset with standardized trait values, with outputted values being averaged across all taxa and finally standardized across all grouping features (see Gayraud et al., 2003). Subsequently, the functional diversity was calculated using the inverse Simpson's index, which accounts for the fixed number of traits and their lack of independence (White, Hill, Bickerton & Wood, 2017a).

Taxonomic richness was calculated as the number of taxa present in each sample. Taxonomic variability (*sensu* Swan and Brown, 2017) was quantified using the 'Permutational Analysis of Multivariate Dispersion' (PERMDISP) procedure using each river as a grouping factor. In summary, a total of seven macroinvertebrate community diversity response metrics (hereafter referred to as community response metrics) were employed in subsequent analyses; i) taxonomic richness, ii) taxonomic variability, iii) functional diversity, iv) functional richness, v) functional evenness, vi) functional divergence, and vii) RaoQ.

2.4.2 Hydrological data- regional flow magnitude classifications

Prior to classifying region-wide flow regime magnitudes, the discharge time-series from each flow gauge were standardized via z-scores ($\mu = 0$, $\sigma = 1$). Daily discharge values were averaged to monthly intervals and subsequently averaged across all gauges comprising each of the five

regions. Hydrological years, spanning August- July, were defined to help ensure that the rising limb, annual peak and flow recession were incorporated across a 12-month period (*sensu* Monk *et al.*, 2006; with the lowest annual discharges in the UK typically occurring during summer months; June-August). Four statistical summaries (mean, standard deviation, minimum and maximum values) were obtained for each hydrological year within each of the five regions. These statistical summaries underwent unit-based standardization ($X'=(X-X_{min}/X_{max}-X_{min})+1$) to ensure each possessed equal weighting within subsequent analyses. A hierarchical, agglomerative cluster analysis (Ward's method) was performed on the statistical summary values to quantify the annual flow magnitude within each region (*sensu* Monk et al., 2006). In total, four flow regime magnitude (RM) classes were established for each hydrological year within all five regions; 'low' (RM1), 'low-moderate' (RM2), 'moderate-high' (RM3) and 'high' (RM4) discharges (see Table 1 and Figure S1). RM groups were used in combination with the macroinvertebrate community data (North, North West and East regions) and *P. leniusculus* establishment dates (all 5 regions - see Figure 2).

2.4.3 Hydrological data - hydrological indices

In total, 47 hydrological indices (Table S7) were calculated from daily discharge data in the East region characterising different components of the antecedent flow regime (*sensu* Poff et al., 1997). For this, 33 'Indicators of Hydrological Alteration' were calculated (Richter et al., 1996) in addition to 14 indices which have been shown to be of ecological relevance in the UK (Monk et al., 2006; White et al., 2017b; 2019; Wood, Agnew & Petts, 2000; Wood & Armitage, 2004). Prior to analysis, daily discharge values were transformed to z-scores to ensure standardisation across gauges. Hydrological indices with heavily skewed distributions (visualized from inspection of histograms) were excluded, leaving 39 indices for all subsequent analyses (Table S7).

2.5 Statistical analysis

2.5.1. Flow magnitude influences on P. leniusculus successful establishment dates

The RM group was identified for all successful establishment dates, with a binary response being derived by characterizing non-invasion and invasion occurrences within a specific RM group coded as "0" or "1", respectively. A 'Generalized Linear Model' (GLM) was subsequently fitted using a binomial distribution and tested against RM group and a RM × Region interaction. This GLM provided an assessment of whether flow magnitude influenced the successful establishment of *P. leniusculus* and whether this differed between regions. Post-hoc pairwise comparisons of RM

groups were performed using least-square means and *p-values* were adjusted for multiple comparisons via Tukey tests within the 'lsmeans' package (Lenth, 2016).

2.5.2 Influence of flow magnitude and P. leniusculus invasion on the temporal trajectories of macroinvertebrate communities

To assess the effect of *P. leniusculus* invasion on macroinvertebrate community responses to flow regime magnitude classes (RM groups), temporal trajectories of the seven community response metrics were explored across control and invaded rivers. Generalized Additive Mixed-Effect Models (GAMMs) were constructed in which each community response metric was modelled as a smooth function over time (1989-2014). The smooth functions were fitted using cubic splines and eight knots were created (0.3 times the number of years (*sensu* Vaughan & Ormerod, 2012; 2014). To account for differences in temporal variations in the seven community response metrics between control and invaded rivers ('treatment'), an 'ordered factor smooth interaction' model was created (*sensu* Simpson, 2017). This technique models the difference of a smooth function between factors and was used here to model differences in the temporal trajectories of the community response metrics between invaded and control rivers. In addition, treatment (i.e. control versus invaded), season and RM groups were incorporated as factors into each GAMM and the identity of each river was fitted as a random effect (to reflect community compositions from individual rivers being potentially correlated over time; Mathers et al., 2016a).

2.5.3 Interactive influences of hydrological variability and P. leniusculus invasion on macroinvertebrate communities

In the East region (possessing simultaneous macroinvertebrate and hydrological data), analyses were performed in the 'after' invasion period (for both invaded and control rivers using the mean regional invasion date of 2003). Prior to the construction of flow-ecology relationships, all hydrological indices were standardised to z-scores and extreme outliers identified from interquartile range (IQR) values (defined as observations that fall below $Q1 - 3 \times IQR$ or above $Q3 + 3 \times IQR$) removed. Quantile regression (QR) and Quantile mixed-effect regression (QMR) analyses were performed using the 'lqmm' package (Geraci, 2018). Within each QMR, 'river' was used as a random effect to account for potential temporal autocorrelation (see above). A number of QRs and QRMs were constructed for each of the seven community response metrics (dependent) and hydrological index combinations (independent variable) across a range of quantiles (from 0.20 to 0.80 in 0.05 increments). These statistical models tested the interactive

influence of crayfish invasion (treatment as above) and each hydrological index, as well as various combinations of different spatial and temporal controls. For this, seven null models were created which comprised one constant model (i.e. with no independent variable modelled), and six models comprising different additive combinations of 'season' and 'year' within both QRs and QMRs (see Table S8). These were treated as null models to enable temporal changes in macroinvertebrate communities to be examined separately from hydrological controls, thus providing a basis to determine the significance of the alternative models constructed for each hydrological index and its interaction with treatment (see below).

The alternative QR and QMR models tested each hydrological index (and its interaction with treatment) via four statistical functions (Linear, Exponential, Logarithmic and Quadratic; *sensu* Fornaroli, et al., 2019). Testing for this interaction provided a means of examining whether *P. leniusculus* modified macroinvertebrate community responses to hydrological variability. Various models were constructed that incorporated this interaction alongside different additive combinations of the hydrological index and treatment, as well as season and year. Akaike weights (w_i, derived from Akaike Information Criteria values corrected for the small sample size) were calculated and averaged across all the studied quantiles for each QR and QMR (Fornaroli et al., 2015). For each community response metric, hydrological indices comprising models which possessed an average w_i value less than 2 times the average w_i of the best null model were excluded (where multiple models met this criterion for a community response metric-hydrological index combination, the model exhibiting the highest average w_i was used in subsequent analyses).

Qualifying hydrological indices were tested for collinearity by iteratively calculating 'Variance Inflation Factors' (VIFs) until they were below 3 for each hydrological index (Zuur et al., 2010). Subsequently, the average w_i of qualifying models were again compared against the seven null models described above; only alternative models possessing an average w_i value two times greater than the average w_i of the best null model were subsequently used (as above). This process was necessary given that null models exhibit a greater statistical likelihood when compared against fewer QRs, thus reducing the likelihood of type 2 errors. Finally, to determine the significance of each qualifying QR and QMR, the average w_i was compared against that of a single null model exhibiting the same model structure, but without a hydrological index, treatment or their interactive effect (in a process analogous to a likelihood ratio test). For this, an alternative model exhibiting an average w_i greater than 0.75 was considered significant (Fornaroli et al., 2019).

3. Results

3.1 Flow magnitude influences on P. leniusculus successful establishment dates

In total, 46.9% and 32.8% of all recorded successful establishments occurred during years with low-moderate (RM2) and low (RM1) flow magnitudes respectively. The highest percentage of successful establishment dates occurred within RM1 or RM2 for all regions (Figure 3), although some regions (e.g. West) had a much higher proportion of dates occurring during low magnitude flows (RM1), while others (e.g. East) saw a higher number of successful establishments during low-moderate flows (RM2). Only 1.6% of successful establishment dates (1 of 64) occurred during high-flow years and was in the East region. A binomial GLM highlighted that the number of establishment dates differed significantly between flow regime magnitude (RM) groups (F = 15.59, *p-value* ≤ 0.001). The interaction between flow magnitude (RM) groups and individual regions also exerted a significant influence (F = 1.76, *p-value* = 0.037). Post-hoc analyses revealed that the likelihood of successful establishment was significantly different between low (RM1) and high-flow magnitudes (RM4; z-ratio = 3.29, *p-value* = 0.006), low-moderate (RM2) and moderate-high (RM3) flow magnitudes (z-ratio = 3.30, *p-value* = 0.005) and low-moderate (RM2) and high-flow (RM4) magnitudes (z-ratio = 3.87, *p-value* = 0.001).

3.2 Influence of flow magnitude and P. leniusculus invasion on the temporal trajectories of macroinvertebrate communities

In 13 instances, the long-term temporal trajectories of community response metrics differed significantly between control and invaded rivers (Table 2). Most notably, taxonomic richness and RaoQ exhibited significantly different trends over time between invaded or control rivers in each of the three regions. Within invaded rivers, response metrics demonstrated a decline (relative to control) following *P. leniusculus* invasion (in the mid-1990s for North rivers shown in Figure 4; for corresponding plots in the East and North West see Figure S2). Community response metrics continued to gradually decrease in invaded rivers relative to control rivers until 2005-2010 (dependent on the community variable examined). Taxonomic and functional richness exhibited the greatest reductions within invaded rivers during periods of low discharge (2005, 2006 and 2011; Figure 4b). Evidence of higher relative community values occurred in invaded rivers (relative to control sites) towards the end of the study period (2013-2014) where high regional discharges occurred (Figure 4). Within the East and North region, the functional diversity and functional richness both exhibited significantly different temporal trends associated with invasion.

Functional divergence, functional evenness and taxonomic variability exhibited a significant temporal trend associated with invasion in one region only (East, North and North West respectively; Table 2). A summary of index values for the four BACI levels by region is shown in Table S9.

3.3 Interactive influence of hydrological variability and P. leniusculus invasion on macroinvertebrate communities

In total, 46 significant models comprising an interaction between a single hydrological index and treatment were detected. Six community response metrics responded significantly to hydrological indices and its interaction with treatment, notably taxonomic richness, taxonomic variability, functional richness and RaoQ, which each comprised 11 significant models. Functional diversity and functional evenness demonstrated a significant response to one hydrological index-treatment interaction, whilst no significant models were detected for functional divergence. A large number of the significant associations incorporated hydrological indices characterizing extreme flow conditions, consisting of 13 low-flow and nine high-flow metrics (Table 3). Hydrological indices comprising significant associations characterised different facets of the flow regime. Specifically, 38 described the magnitude of flow conditions, 33 the timing, 12 the frequency, seven the rate of change and four the duration (note some indices reflect multiple facets; see Table S7). In total, 12 hydrological indices (of the possible 47) and their interaction with treatment exerted a significant influence on the seven community diversity response metrics tested. QMar comprised the greatest number of significant associations (five), followed by QDec, QLPD, QFR, QMin180, QJulianMin, QSep, QMax90, QMean7, QNoRises, QJulianMax (each with four significant associations), while QJun comprised one significant association.

When individual associations were examined, RaoQ demonstrated a decrease in invaded rivers when the maximum 90-day discharge values (QMax90) were lower, while control rivers demonstrated the opposite trend (Figure 5a). The minimum discharge occurring in the 180-days prior to sampling (QMin180) did not influence RaoQ in control rivers, but exhibited a negative correlation within invaded rivers (Figure 5b). In control rivers, a steady decline of discharge (indicated by low fall rates in discharge – QFR) was associated with a higher taxonomic richness and decreased taxonomic variability, whilst invaded rivers demonstrated the opposing trend for both responses (Figure 5c and d). A greater reduction in functional evenness was exhibited in invaded rivers compared to control rivers associated with higher average March discharge values

(QMar; Figure 5e). RaoQ demonstrated strong reductions within invaded rivers when average September discharge values (QSep) were low and were highest when flow values increased, whilst control rivers demonstrated no notable difference (Figure 5f).

4. Discussion

A key challenge of invasion biology is understanding how INNS interact with other large-scale agents of environmental change, in this instance hydrological variability (Didham et al. 2007). For the first time, our study investigates the interaction of hydrological variability with invasive crayfish (*Pacifastacus leniusculus)* in their successful establishment and subsequent effects for the receiving riverine ecosystem.

4.1 Flow magnitude influences on P. leniusculus successful establishment dates

Low-flow conditions were found to significantly influence the likelihood of invasion by *P. leniusculus*, with 80% of successful establishment dates (utilized as a surrogate for invasion dates) occurring in years with low-moderate (RM2) and low (RM1) flow magnitudes; providing evidence to support our first hypothesis. In marked contrast, only 1.6% of dates (1 of 64) occurred during high (RM4) flow magnitudes. This can likely be explained by the loss of juveniles that are more prone to displacement during periods of high-flows (Robinson, Thom & Lucas, 2000; Light, 2003). Regional differences in river flow regimes are likely to influence the differences observed in successful establishment date occurrences across each of the flow magnitude groups. For example, discharges were considerably lower in the East compared to other regions (Table S10), providing some explanation as to why an invasion could occur during high-flow periods exclusively in this region.

The high occurrence of successful establishment dates during low-flow magnitude periods may be a result of a number of mechanisms. First, instream movements of crayfish can be triggered or impeded by environmental factors, such as temperature or hydraulic forces, with crayfish activity increasing with rising temperatures (Gheradi et al., 1998; Johnson, Rice & Reid, 2014). As water levels recede during periods of low-flow, water temperatures are likely to increase (Webb et al., 2008), which may stimulate crayfish movements either within the existing waterbody or to colonise new waterbodies. It is therefore likely that crayfish migration and successful establishment may be driven by a strong correlation between changing water temperatures and flow regimes (Webb et al., 2008), rather than the direct influence of flow regime alone (e.g. hydraulic forces). As such, while our study highlights the pivotal role of river flow regimes in

shaping establishment of invasive crayfish, further field and experimental work is needed to disentangle the individual role of each of these environmental factors.

Second, the ability of crayfish to survive streambed drying events is enhanced through their ability to walk overland in order to find proximal perennial waterbodies (Claussen, Hopper & Sanker, 2000). Reductions in available habitat during drying events may lead to increased competition for resources, thereby resulting in crayfish migrating in search of less exploited habitats via instream movements or overland (Ramalho & Anastácio, 2015). These habitats could have previously supported a low population of invasive crayfish or may represent previously uninvaded habitats. Larger individuals are most likely to undertake such migratory activities as they are less vulnerable in exposed habitats then smaller crayfish prone to predation (Wutz and Geist, 2013). Due to such ecological mechanisms, the documented range expansions of *P. leniusculus* are rapid with reported rates of 1.5km per year (Bubb et al, 2004).

Finally, the survey method used in this study (biomonitoring data using benthic macroinvertebrate kick-samples) may also play a small role in enhancing the possibility that signal crayfish are detected during low-flows when wetted perimeters are reduced and a greater proportion of instream habitats are sampled. However, as this study employed flow magnitude groups which characterised annual flow patterns (and not just individual events relating to sample dates), and no summer samples were analysed (when discharges are typically lowest), this influence is likely to be minimal.

4.2 Influence of flow magnitude and P. leniusculus invasion on the temporal trajectories of macroinvertebrate communities

Invasion by *P. leniusculus* resulted in alterations to the temporal trajectories of macroinvertebrate response metrics in 13 instances (of a total of 21). The most significantly influenced metrics were taxonomic richness and RaoQ (a representative of functional diversity), which were affected in all three English regions studied. The temporal trajectories of biotic community responses to *P. leniusculus* invasion has received limited attention to date, with the majority of studies implementing in-situ experimental enclosures or ex-situ mesocosms (e.g., Magoulick, 2014; Parkyn, Rabeni & Collier, 1997; Stenroth and Nyström, 2003); whilst studies which do employ in-situ sampling typically do so over a limited time periods (1-12 months) and where populations are often well established (Crawford, Yeomans & Adams, 2006; Mathers, Rice & Wood, 2018; Ruokonen, Karjalainen & Hämäläinen, 2014); but see Mathers et al. (2016a) and Ruokonen et al.

(2016). In general, invasion by *P. leniusculus* results in modifications to macroinvertebrate communities associated with selective predation of certain Hirudinea, Gastropoda and Bivalvia taxa, which display limited locomotion and predator avoidance trait affinities (Dorn, 2013; Stenroth & Nyström, 2003; Weber & Lodge, 1990). These modifications in community composition have resulted in reductions in taxa richness following *P. leniusculus* invasion being observed (Crawford et al., 2006; Ruokonen et al., 2014). There have been calls for research to shift from exclusively structurally based ecological descriptors to studies that incorporate functional changes which underlie ecosystem health (Gallarado et al., 2016; Havel et al., 2015). Despite this, and to our knowledge, this study provides the first evidence of structural and functional macroinvertebrate community responses to crayfish invasion (and hydrological controls) over multi-decadal time frames.

Alterations to community response metrics following *P. leniusculus* invasion demonstrated a significant interaction with flow magnitude classes. Community response metrics displayed the greatest post-invasion reductions during periods of low-flow, with the majority demonstrating some recovery to pre-invasion values towards the end of the time series when significant flood events were recorded nationally (Muchan, Lewis, Hannaford & Parry, 2015). Our second hypothesis, that invasion by *P. leniusculus* altered the temporal trajectories of macroinvertebrate response metrics and greatest reductions would occur during periods of low-flow conditions, can therefore be supported.

Contraction and fragmentation of instream habitats during low-flow events can lead to increased predator: prey ratios, thereby altering biotic interactions (Lake, 2003; McHugh, Thompson, Greig, Warburton & McIntosh, 2015) and increasing competition for certain resources (Aspin et al., 2019; Ledger et al., 2013). As a keystone predatory species, the ability of signal crayfish to persist during low-flows and drying events may therefore reduce the tolerance of aquatic invertebrate communities to low-flows (Datry, 2012; Larned, Arscott, Schmidt & Diettrich, 2010), potentially exacerbating the effect of invasive crayfish on the receiving ecosystem during adverse environmental conditions. The ecological implications of invasive crayfish are likely to be particularly affected by changing habitat availability associated with their hierarchal spatially size-sorted instream distributions. Smaller individuals are often confined to shallower riffle areas, whilst larger individuals inhabit deeper pools (Clark et al., 2013; Guan and Wiles, 1996). Reductions in water depths during low-flows are therefore likely to have significant implications

for resource competition, such as the macroinvertebrate communities concentrated in deeper areas (Boulton, 2003; Chadd et al., 2017) occupied by larger crayfish. As high-flow events have been associated with declines in crayfish densities or reductions in activity levels (Light, 2003), higher flow conditions may provide a time period in which macroinvertebrate communities can recover due to reduced predator-prey pressures via the creation of additional habitats not present under lower flow levels or associated with reduced predator abundances (Boulton and Lake, 2008; Lake et al., 2006; Meffe, 1984; Thomson, 2002).

However, the interactive ecological influences of *P. leniusculus* and annual flow magnitudes were not consistent across regions. For example, the East region demonstrated a strong interaction with hydrological controls, whilst the North West displayed a weaker interaction. Geological settings differ between the two regions, with chalk (a fine powdered limestone) deposits dominating river typology in the East region, whilst rivers in the North West are characterised by sedimentary sandstone and igneous rocks; the former produces naturally less flashy flow regimes, whilst rivers in the North West are highly responsive to heavy precipitation (Lavers, Prudhomme & Hannah, 2010; Tables S1, S7). As such, the interactive ecological effects of hydrological controls and crayfish invasion are contextually dependent based on river flow regimes, geological setting and instream habitat conditions. It should be noted that this is a broad-scale study which incorporated a number of English regions. As such, although efforts were made to minimise the influence of other confounding factors (through both our study design and statistical analyses), variations in environmental conditions are still likely to occur. These environmental differences such as landscape characteristics or the presence / absence of predators are likely to have important repercussions when concluding on the ecological influence of the interaction of flow regime and crayfish presence. Future work should seek to assess the role of flow regime and crayfish invasion in association with other environmental variables.

4.3 Interactive influence of hydrological variability and P. leniusculus invasion on macroinvertebrate communities
A number of hydrological descriptors characterizing the facets of river flow regime (i.e. the magnitude, frequency, duration and timing of hydrological events and rate of change between flow conditions; *sensu* Richter et al., 1996;1997) were found to have a significant interactive effect with *P. leniusculus* presence on macroinvertebrate community diversity; providing support for our final hypothesis. A large number of the significant trends incorporated low- and high-flow metrics

(47%), highlighting the role that extreme hydrological events play in shaping INNS effects on receiving ecosystems (Bunn & Arthington, 2002; Magoulick, 2014; but see Ruhi, Holmes, Rinne & Sabo, 2015).

Moreover, the magnitude of hydrological controls (including average conditions) comprised 83% of all significant relationships. As discussed above, *P. leniusculus* are likely to demonstrate strong associations with flow magnitude as a function of their activity levels and population dynamics (Bubb et al., 2004; Johnson et al., 2014). For example, we found that RaoQ demonstrated the greatest reductions in invaded rivers associated with lower maximum discharge values (in the preceding 90 days) and higher minimum discharge values (of the preceding 180 days). Surprisingly, towards the positive limits of these hydrological indices (higher maximum and minimum values), RaoQ vales in invaded rivers surpassed those in control sites. This suggests that biota comprising a functionally diverse, catchment-wide species pool can (re)colonize invaded sites as discharges increase (towards high magnitude events or as discharge increases following low-flow periods). It is possible that crayfish predation may temporarily open new ecological niches for some organisms associated with the reduction in abundance of dominant and highly competitive predatory taxa; for example Hirudinea which can consume a wide variety of macroinvertebrates that occupy a number of functional niches (Young and Ironmonger, 1980).

The timing of flow conditions were also found to be important in influencing the ecological effects of *P. leniusculus*, which comprised 72% of all significant associations (note that some hydrological indices occur in more than one facet). For example, RaoQ demonstrated a strong reduction in invaded rivers when average September discharges were low. This time period coincides with the start of the mating season of crayfish in Europe (Lewis, 2002) and therefore flow conditions during this period likely interacted with breeding behaviours (e.g., low-flows facilitating males locating resident females in fixed points; Gheradi, 2002).

The rate of hydrological change also exerted a strong interactive effect with *P. leniusculus* on macroinvertebrate community diversity. Hydrological stability resulted in reductions in taxa richness and the temporal variability of communities, highlighting that the effects of *P. leniusculus* are greatest when changes in hydrological conditions are more gradual. Within control rivers, flashy regimes typically support heterogeneous communities as taxa are selectively removed during the disturbance and recolonise as flows return to average conditions (Ledger, Harris, Armitage & Milner, 2012). In marked contrast, lower taxa richness was associated with

hydrologically stable conditions in invaded rivers, potentially reflecting that crayfish are able to selectively predate on certain taxa (such as Hirudinea and Mollusca; Crawford et al., 2006; Mathers et al., 2016) without having to react to dynamic hydrological conditions. Although the prevalence of prey taxa are likely to decrease in the presence of invasive crayfish, there is limited evidence to suggest that they become locally extinct and therefore communities may become more variable over time as these taxa become 'rare'.

Greater reductions in functional evenness in invaded rivers (compared to control rivers) were associated with higher average March discharge values. A number of crawling ephemeroptera taxa are preferentially predated on by *P. leniusculus* (e.g. *Caenis sp*., *Habrophlebia fusca*) reducing their densities in invaded rivers (Mathers et al., 2016a; Mathers et al., 2018) which may leave them highly vulnerable to local extirpation during high spring flows (Wood et al., 2000; Adámek et al., 2016). A reduction in functional evenness may have considerable repercussions in controlling future invasion resistance with an even distribution of traits enabling higher invasion resistance (Dukes, 2001) and may therefore make waterbodies supporting invasive species more susceptible to future invasions. While further research is clearly required to unpack the causal mechanisms underpinning ecological responses to INNS establishment and different flow regime facets, this study provides the first evidence (to our knowledge) that biological invasions and flow regimes exert long-term interactive effects on the structural and functional properties of riverine ecosystems.

4.4 Implications for the management and ecological effects of INNS in a changing climate

Our study provides evidence that flow regime variability is critical in the prevention of INNS establishment, as well as the maintenance of structural and functional diversity of receiving ecosystems following biological invasions. Our results have important repercussions when evaluating the future threat that INNS may represent with low-flow events likely to increase in intensity, duration and frequency over the 21st century associated with predicted climatic changes and increasing societal water demands (Döll & Schmied, 2012; Prudhomme et al., 2014). For example, urban water demand is projected to increase by 80% by 2050 (Flörke et al., 2018) and mean summer flows in the South and East of England are 30% lower than the 1961-1990 mean (Arnell, 2004).

To compound issues, only 37% of rivers globally remain free-flowing (Grill et al., 2019), with alterations to natural flow regimes representing a continuing global threat to ecosystem functioning (Kennen, Stein & Webb, 2018; Poff, 2018). River regulation can lead to reductions in flow seasonality and variability resulting in homogenous hydrological conditions which are typically characterised by reductions in short-term maximum flows and increases in long-term minimum flows (Poff et al., 1997). Flow restoration efforts or 'environmental flows' are being increasingly advocated across the globe in order to balance societal and ecosystem water demands (Theodoropoulos et al., 2019; Webb, Watts, Allan & Conallin, 2018) with recent efforts focused on restoring key components of the flow regime required for wider ecosystem functionality (Poff et al., 1997). Results from this research highlight that these flow components (such as magnitude, rate of change and timing of flow) are likely to be vital in maintaining ecological diversity in the face of a number of current threats including biological invasions.

Data availability statement

All biological data can be obtained freely from the Freshwater and Marine Biological Surveys for Invertebrates England Database at https://data.gov.uk/dataset/ae610ec8-7635-4359-9662-c920046950f7/freshwater-and-marine-biological-surveys-for-invertebrates-england using the site codes found in Table S2. All daily mean discharge data can be obtained freely from the National River Flow Archive at https://nrfa.ceh.ac.uk/data/search using the station codes shown in Table S3.

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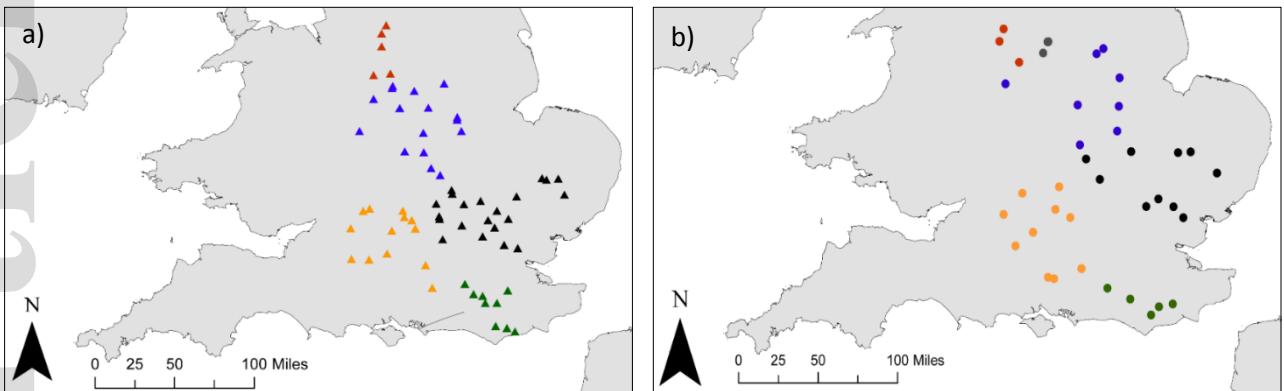
Figures

Figure 1. Site locations of: a) *Pacifastacus leniusculus* establishment dates and; b) flow gauges employed in the study. For specific locations of macroinvertebrate community data sites see Figure 1 in Mathers et al., 2016. Red symbols = North, blue = North West, black = East, orange = West and green = South East as defined in this study. Note the two flow gauges in dark grey were used in both the North and North West regions.
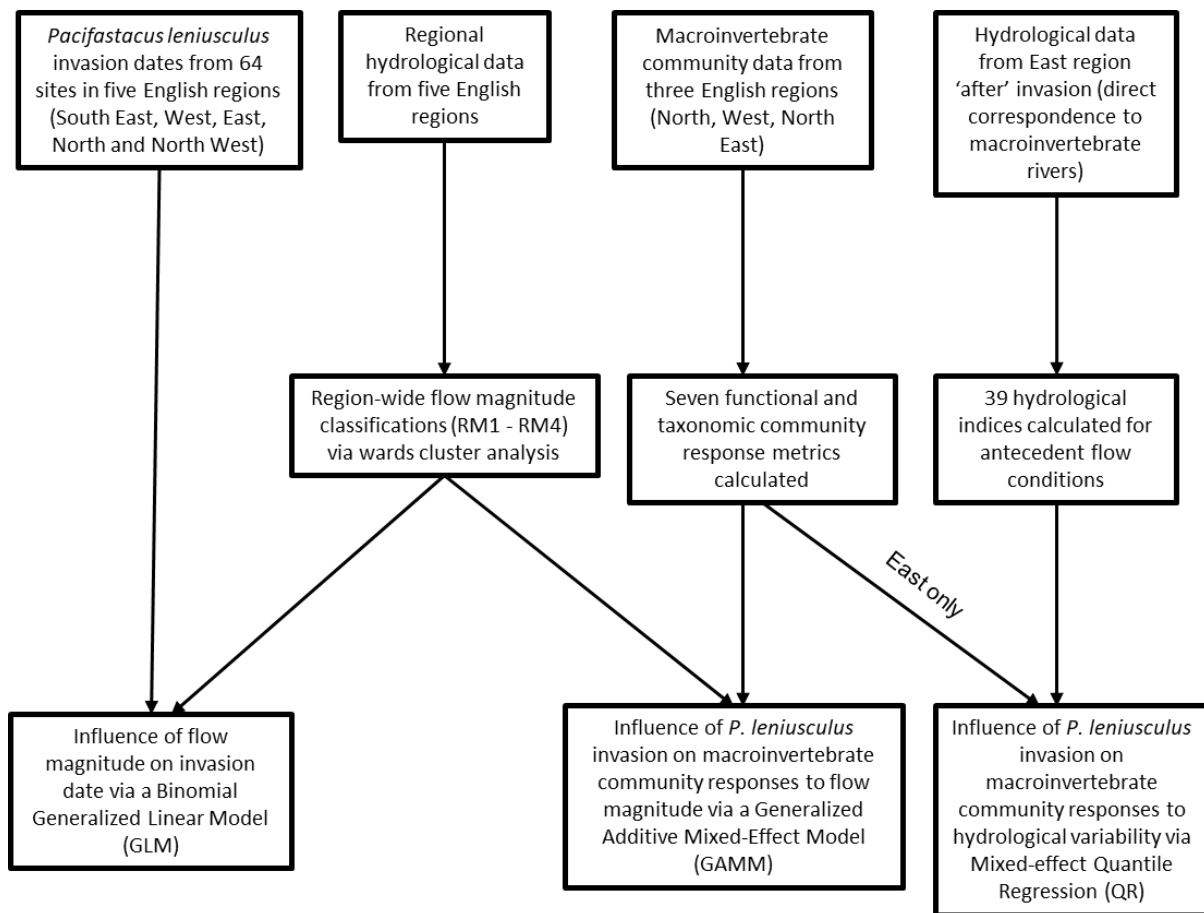
Figure 2. Flow chart depicting the analytical framework adopted within this study.
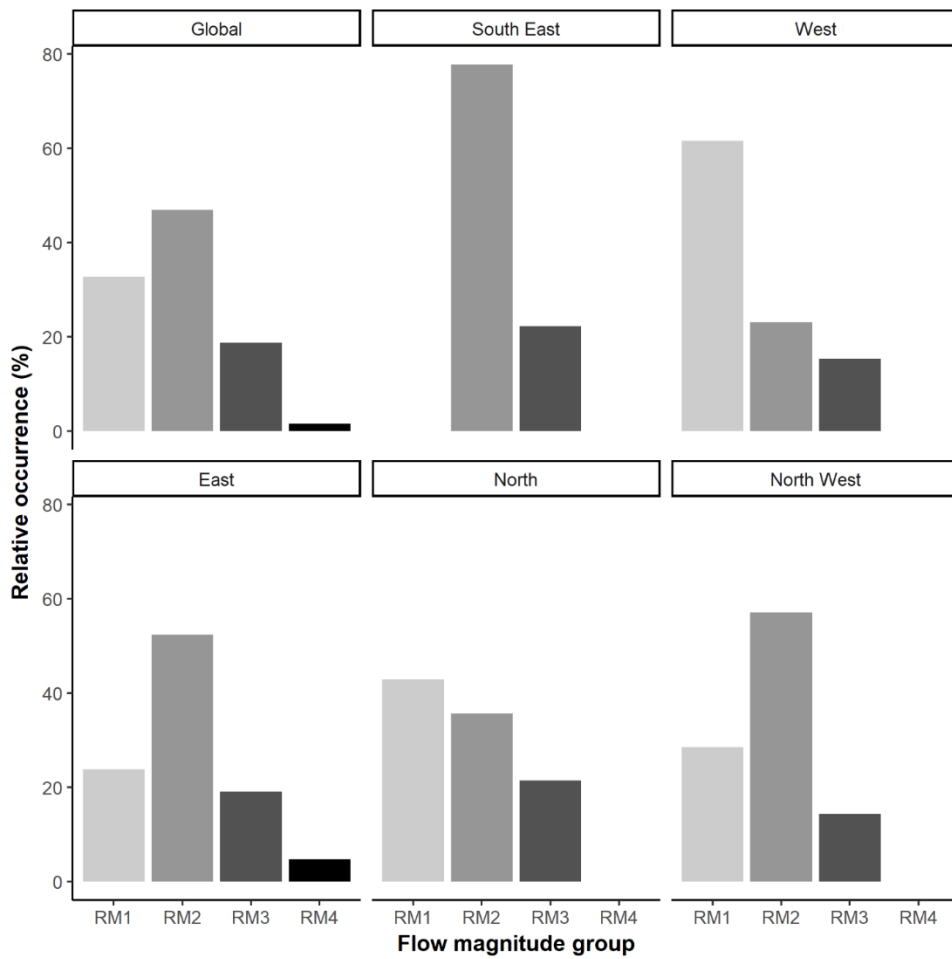
Figure 3. Occurrence of *Pacifastacus leniusculus* establishment dates during the different region-wide flow regime magnitude (RM) groups for all (global) and each of the five English regions. RM1 = low magnitude flows, RM2 = low – moderate magnitude flows, RM3 = moderate - high magnitude flows and; RM4 = high magnitude flows. See Table 1 for values detailing how each RM group differs from the long term regional mean.
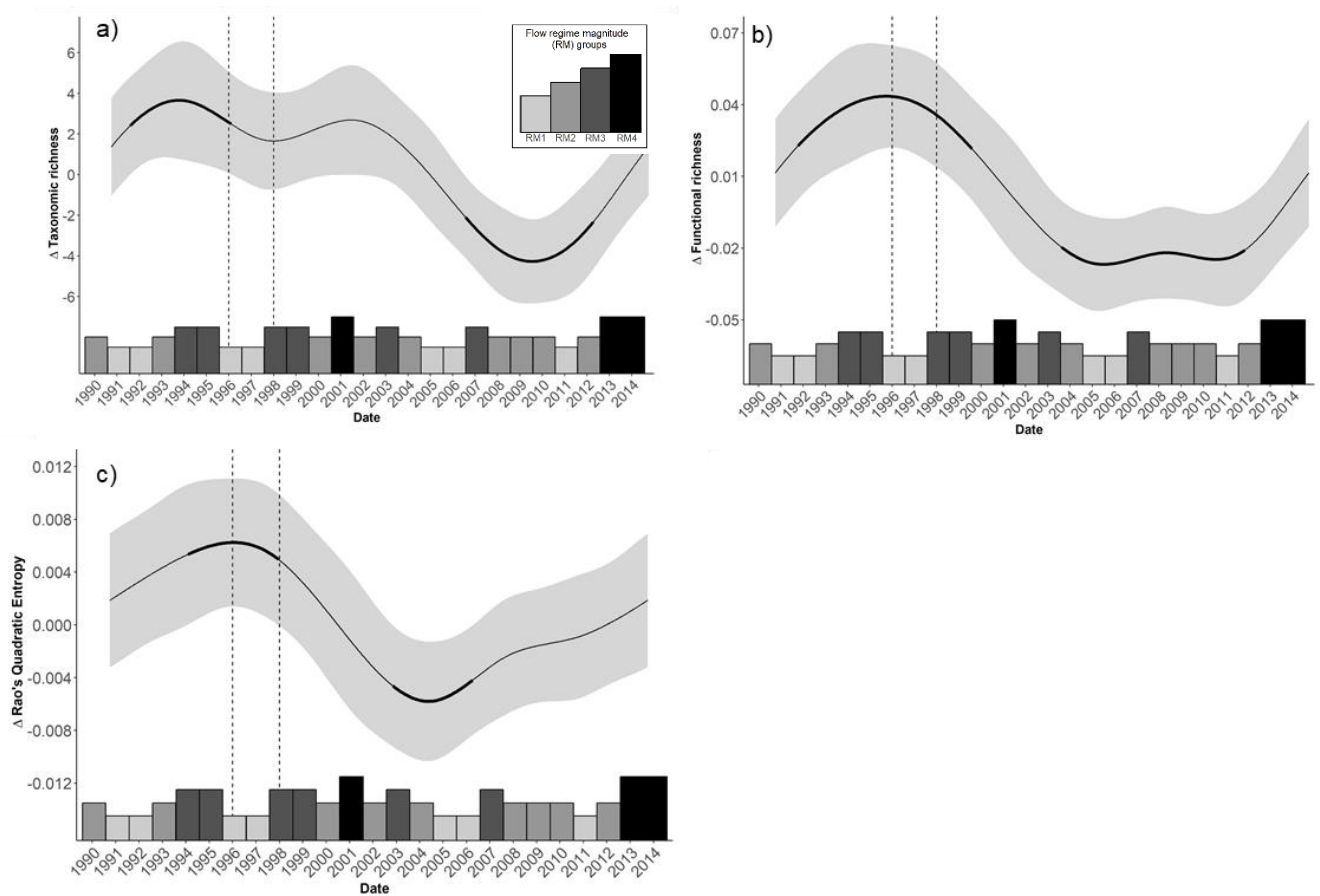
Figure 4. Difference in temporal trajectories of a) taxonomic richness; b) functional richness and; c) Rao's Quadratic Entropy between control and invaded rivers as a function of the GAMM outputs for the North. Corresponding graphs for the other regions are shown in Supplementary material (Figure S2). The dashed line represents dates of invasion by *P. leniusculus*. The annual flow regime magnitude (RM) classification is displayed at the base of each plot. RM1 = low magnitude flows, RM2 = low – moderate magnitude flows, RM3 = moderate - high magnitude flows and; RM4 = high magnitude flows. See Table 1 for values detailing how each RM group differs from the long term regional mean.
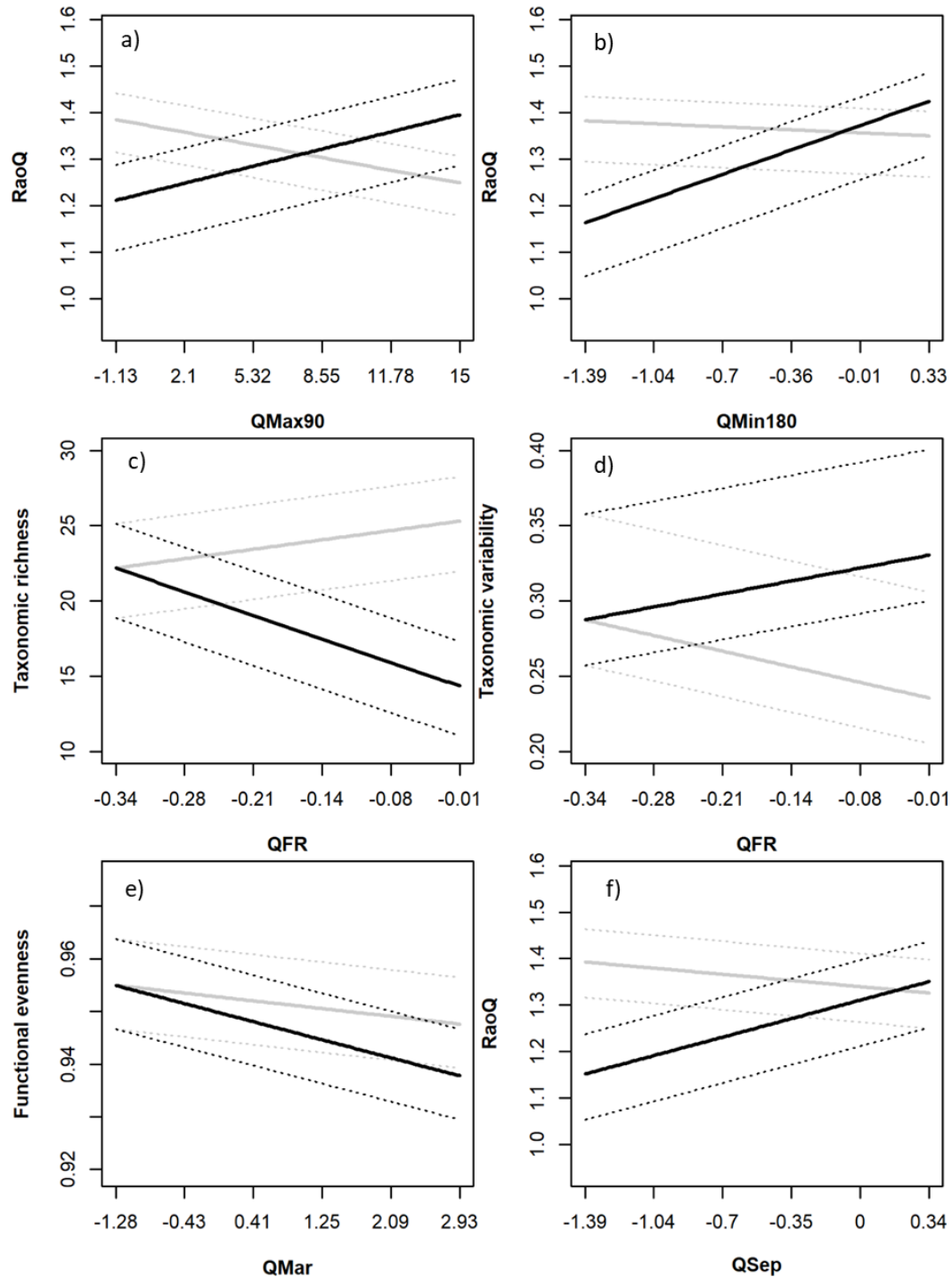
Figure 5. Macroinvertebrate community responses (a-f) to a number of hydrological indices derived from quantile regression (QR) models associated with *Pacifastacus leniusculus* presence. Control = grey and invaded = black. Shown are 20th (dashed), 50th (solid) and 80th (dashed) percentiles.

Tables

Table 1. Summary of mean hydrological conditions per region-wide flow regime magnitude (RM) group relative to the long term mean (%) by region

	South East	West	East	North	North West
RM1	-16.92	-17.86	-16.83	-20.34	-24.20
RM2	-3.54	6.81	-0.79	-3.12	-8.26
RM3	11.94	4.79	3.96	12.17	4.99
RM4	41.14	35.00	21.52	32.47	18.77

Table 2. Summary values from the Generalized Additive Mixed-Effect Models. Significant terms are denoted in bold.

	Treatment		Season		RM		Temporal differences between treatment	
	F	P-value	F	P-value	F	P-value	F	P-value
East								
Taxanomic richness	20.02	**<0.001*** **	0.44	0.506	3.11	**0.028***	6.37	**<0.001*** **
Taxanomic varaibilty	5.05	**0.026***	1.01	0.317	0.58	0.629	0.39	0.145
Functional diversity	0.45	0.501	1.3	0.256	6.40	**<0.001*** **	1.36	**0.012***
Functional richness	57.40	**<0.001*** **	0.55	0.458	2.78	**0.043***	6.78	**<0.001*** **
Functional evenness	2.37	0.126	0.11	0.738	3.29	**0.022***	0.00	0.439
Functional divergence	1.15	0.285	4.12	**0.044***	3.57	**0.016***	2.25	**0.002****
Rao Q	3.39	0.067	6.53	**0.012***	3.07	**0.030***	3.04	**<0.001*** **
North								
Taxanomic richness	27.69	**<0.001*** **	6.61	**0.011***	3.25	**0.024***	2.20	**0.001****
Taxanomic varaibilty	12.69	**<0.001*** **	0.16	0.69	0.82	0.483	0.00	0.877
Functional diversity	0.12	0.734	25.18	**<0.001*** **	1.59	0.195	3.34	**<0.001*** **
Functional richness	18.54	**<0.001*** **	11.33	**0.001***	2.95	**0.035***	3.35	**<0.001*** **
Functional evenness	1.71	0.194	0.01	0.909	1.12	0.344	2.27	**<0.001*** **
Functional divergence	5.05	**0.026***	0.26	0.611	1.07	0.366	0.00	0.752
Rao Q	0.45	0.501	23.95	**<0.001*** **	3.42	**0.019***	1.37	**0.012***
North West								
Taxanomic richness	25.31	**<0.001*** **	3.35	0.07	1.87	0.138	2.78	**<0.001*** **
Taxanomic varaibilty	14.00	**<0.001*** **	0.02	0.89	4.02	**0.009****	3.58	**<0.001*** **
Functional diversity	2.73	0.101	2.53	0.115	1.29	0.279	0.00	0.391

Functional richness	18.36	**<0.001*****	0	0.995	1.07	0.366	0.19	0.172
Functional evenness	1.24	0.269	0.01	0.924	0.74	0.532	0.00	0.623
Functional divergence	0.56	0.457	0.35	0.558	2.67	0.05	0.00	0.44
Rao Q	1.55	0.216	1.55	0.216	0.54	0.659	2.96	**<0.001*****

* p ≤ 0.05, ** P ≤ 0.01, *** p ≤ 0.001

Table 3. Summary table of significant relationships between macroinvertebrate community diversity response metrics and the interactive effect of different hydrological indices and (invasion) treatment modelled from quantile regression and quantile mixed-effect regression analyses. The formula underpinning the model exhibiting the highest statistical power (average wi) for each paired combination between ta community response metric and hydrological index × treatment interaction is shown. LIN = linear, EXP = exponential and LOG = logarithmic statistical functions. Low flow indices are indicated in bold, and high flow in italics.

Community response	Average w_i	Hydrological index	Function	Formula
	1.00	*QDec*	EXP	x × Treatment
	1.00	**QLPD**	EXP	x × Treatment
	1.00	QFR	LOG	x × Treatment
	1.00	**QMin180**	EXP	x × Treatment
	1.00	**QJulianMin**	EXP	x × Treatment
Taxonomic richness	1.00	QSep	LIN	x × Treatment
	1.00	*QMax90*	EXP	x × Treatment
	1.00	QMean7	EXP	x × Treatment
	1.00	QNoRises	EXP	x × Treatment
	1.00	QMar	EXP	x × Treatment
	1.00	*QJulianMax*	LIN	x × Treatment
	0.96	QFR	LOG	x + (x × Treatment)
	0.84	**QJulianMin**	LOG	x + (x × Treatment)
	0.79	**QLPD**	EXP	x + (x × Treatment)
	0.78	*QDec*	EXP	x + (x × Treatment)
Taxonomic variability	0.83	QSep	EXP	x + (x × Treatment)
	0.96	**QMin180**	LIN	x + (x × Treatment)
	0.88	QMar	EXP	x + (x × Treatment)
	0.86	QNoRises	LIN	x + (x × Treatment)
	0.83	QMean7	EXP	x + (x × Treatment)
	0.97	*QMax90*	EXP	x × Treatment
	0.93	*QJulianMax*	LIN	x × Treatment
Functional diversity	0.98	**QJun**	LIN	x × Treatment
	1.00	**QLPD**	LIN	x + (x × Treatment)
	1.00	*QMax90*	EXP	x + (x × Treatment)
	1.00	QMar	LIN	x + (x × Treatment)
	1.00	QMean7	LIN	x + (x × Treatment)
	1.00	QNoRises	LIN	x + (x × Treatment)
Functional richness	1.00	*QDec*	EXP	x + (x × Treatment)
	1.00	QSep	LIN	x × Treatment
	1.00	**QMin180**	EXP	x × Treatment
	1.00	*QJulianMax*	EXP	x × Treatment
	1.00	**QJulianMin**	EXP	x × Treatment
	1.00	QFR	EXP	x × Treatment
Functional evenness	0.80	QMar	LOG	x × Treatment
	0.92	*QMax90*	LIN	Treatment + (x × Treatment)
	1.00	QMean7	EXP	x + (x × Treatment)
	0.99	QMar	EXP	x + (x × Treatment)
	0.95	**QJulianMin**	LIN	x + (x × Treatment)
	0.99	*QDec*	LIN	x + (x × Treatment)
Rao's Quadratic Entropy	0.96	QNoRises	LIN	x + (x × Treatment)
	0.95	*QJulianMax*	EXP	x × Treatment
	0.97	**QLPD**	LIN	x × Treatment
	0.99	**QMin180**	EXP	x + Treatment + (x × Treatment)
	0.98	QFR	LOG	x × Treatment
	0.97	QSep	EXP	x + Treatment + (x × Treatment)