Mapping the landscape pool of ardeid species to landscape structure associated with Japanese encephalitis virus in Australia

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Abstract

The geographic extent of Japanese encephalitis virus (JEV), a zoonotic, mosquito-borne virus, increased dramatically in south-eastern Australia over the summer of 2021-2022 resulting in outbreaks in piggeries, 45 cases of human disease, and simultaneous detection of JEV by mosquito and arbovirus surveillance programs. Preliminary investigation of the landscape epidemiology of the piggery outbreaks showed that they were associated with particular landscape structure as well as intermediate ardeid species richness. However, no exploration of the ways in which individual species or species composition may couple with JEV-associated landscape composition and configuration has been undertaken and, therefore, key questions regarding the landscape epidemiology and infection ecology of JEV in Australia remain unanswered. The current study sought to interrogate how individual species presence and their functional traits were distributed across the landscape, and how these distributions aligned with the landscape structure of JEV occurrence. Inhomogeneous Poisson point process models and multivariate hierarchical models were used to investigate the landscape-level ardeid species pools and the distribution of their pool-weighted traits with respect to JEV outbreaks. The distributions of six species were strongly associated with the distribution of JEV outbreaks reported in 2022: *Casmerodius albus* (*Ardea alba*), *Ardea pacifica*, *Botaurus poiciloptilus*, *Egretta novaehollandiae*, *Ixobrychus minutus* (*Ixobrychus dubius*), and *Nycticorax caledonicus*. Some JEV-delimiting landscape features, including transient wetlands and proximity to waterways, were associated with the presence of nearly all ardeid species, while others, such as crop cultivation, habitat fragmentation, and water flow accumulation were associated with only a subset of these species. In addition, hand-wing index was associated with species presence in landscapes associated with JEV outbreaks, which may suggest the importance of dispersal capacity in ardeid community assembly in landscapes that share environmental features with JEV occurrence. This study provides a preliminary selection of target species for the development of wild waterbird JEV surveillance, while uniquely accounting for sympatry and the potential for functional modulation of environmental filtering in high-risk landscapes, despite the broad landscape scale that necessarily constrained the analysis. Nevertheless, while the results provide an actionable One Health framework with which to develop wildlife surveillance, it must be noted that they do not describe community assembly rules, nor do they provide a description of community composition at local scale. The findings advance the current understanding of the landscape epidemiology of JEV in Australia by connecting landscapes with individual species from a family of established maintenance hosts and, given the large geographic extent of the potential circulation of JEV in maintenance hosts, provides valuable evidence-based information for targeted surveillance strategies.

Introduction

In early 2022 Japanese encephalitis virus (JEV) was detected for the first time in south-eastern regions of Australia, resulting in the declaration of a Communicable Disease Incident of National Significance. A total of 45 cases of human disease were reported, including seven fatalities(1). Outbreaks were also reported in piggeries with unprecedented geographic distribution, high numbers of affected piggeries, and high incidence within piggeries (2–4). Previous JEV occurrence in Australia had been limited to isolated cases in the Torres Strait Islands(5) and the Cape York Peninsula of northern Queensland(6). The extensive geographic distribution of these outbreaks, which included eastern and southern Australia, was of particular concern because it deviated markedly from anticipated emergence events for JEV(7) and suggested extensive circulation of the virus in maintenance communities prior to emergence in pigs and humans(8). Furthermore, these outbreaks followed an extensive period of anomalous weather due to three consecutive La Niña phases of the El Niño Southern Oscillation (ENSO). The anomalies associated with these shifts included high levels of precipitation and major flooding events across eastern Australia, generating considerable disturbance among natural communities(9). It has been hypothesised that the anomalous La Niña-associated weather patterns may have contributed to the widespread distribution of JEV outbreaks in 2022 due to modulation of the distribution of vector populations or maintenance host populations(8). Notably, an extensive investigation of landscape composition and configuration and La Niña-associated anomalies showed that landscape structure was substantially more influential to outbreak occurrence than were specific precipitation or temperature anomalies associated with La Niña(10). However, certain aspects of high-risk landscape structure, such as the composition of transient wetlands and the propensity to flooding, would also have been affected by both proximal and distal precipitation events, and so La Niña-associated weather anomalies may have influenced outbreak occurrence indirectly, particularly if the influence varied by scale.

Japanese encephalitis virus circulates in wild ardeid bird maintenance hosts(11–17) vectored by mosquitoes (primarily *Culex annulirostris* in Australia(18)) and can spillover to pigs(19–24) and

humans, which are competent and non-competent hosts, respectively. Previous work has examined the distribution of ardeid species richness as a key nidus of the maintenance community, identifying a strong quadratic relationship between richness and JEV piggery outbreak occurrence, whereby intermediate richness was associated with greatest risk(10). Nevertheless, neither the association between outbreaks and individual species, nor the influence of the structure and functional distribution of species assemblages were assessed with respect to the 2022 piggery outbreaks in Australia, and the latter has not been assessed with respect to outbreaks anywhere in the Indo-Pacific region to date. Due to the logistics and resources required to sample birds across such vast landscapes, a better understanding of how particular species and species community structure align with the landscape structure associated with JEV outbreaks would be particularly useful to identify target species to monitor JEV circulation, and other flaviviruses with avian reservoirs, in these landscapes.

The study of the functional ecology of viral maintenance hosts has increased in recent years as a means to help identify potential hosts based on specific biological and ecological traits, frequently focusing on life history due to the association between fast-living and host competence in many r-selected species(25–33). These species, which are typically generalist and frequently more resilient to anthropogenic pressure(34–36), tend to invest more in earlier and more fecund reproductive effort, and less in immune function and adult survival(37). As such, many of these species also present as optimal hosts for pathogens(38). Nevertheless, the association between fast-living species and host competence is by no means universal, with some systems demonstrating the opposite or ambiguous associations, such as ebolaviruses(39), Rift Valley fever virus (RVFV)(40), and Ross River virus (RRV)(41). In addition to life history, other host traits, including population density, diet composition, and dispersal ability, can also be influential to the infection ecology of some pathogens(35,36). Japanese encephalitis virus host functional ecology has hitherto gone unexplored among ardeid species and thus represents a critical knowledge gap for this system.

131 The aims of this study were to identify preliminary target host species in specific landscapes
132 for One Health surveillance monitoring of JEV. Initially, we sought to determine what, if any, ardeid
133 species were distributed in close association with the distribution of JEV piggery outbreaks after
134 adjusting each species' presence for sympatric ardeid species. Secondly, the study interrogated how
135 the structure of species pools at landscape scale were coupled with landscape composition and
136 configuration known to be associated with JEV outbreaks. The study then explored the extent to
137 which pool-weighted species' trait means were coupled with these same JEV landscapes as well as
138 the individual species present therein.

139 **Methods**

140 Data Sources

141 *Animal data*

142 Australian piggery outbreaks (N = 54) reported to the World Organisation for Animal Health
143 (WOAH) by the Australian government between 19 January, 2022 and 4 April, 2022(42) have been
144 described in a previous epidemiological investigation(10). Briefly, outbreak occurrence was defined
145 as one or more cases reported at the level of the piggery. An additional 11 independently-
146 documented JEV occurrence locations (eight piggery outbreaks reported by a commercial pork
147 producer, and 3 positive mosquito pools detected through the NSW Arbovirus Surveillance and
148 Mosquito Monitoring Program(43)) were used to externally validate model performance.

149 To model the habitat suitability of each of the 14 extant species of ardeid birds in mainland
150 Australia(44), 791,416 observations of these species recorded between 1 January, 2010 and 31
151 December, 2020 were acquired from the Global Biodiversity Information Facility (GBIF)(45). The
152 phylogenetic tree of these species was obtained from the VertLife project using the Hackett
153 backbone and constructed based on the methods described by Jetz et al. (Jetz et al., 2012). Any
154 taxonomic binomial discrepancies between the VertLife tree and the named species obtained from
155 GBIF used for the species distribution models described below were resolved, with alternate
156 binomials appearing in parentheses in Table S1. Data obtained from the national pig herd dataset,

which is used in the Australian Animal Disease Spread Model, were used to quantify domestic pig density and piggery density for the JEV models described below. This dataset comprises >8000 registered pig herds of all types (including commercial, boar studs, smallholder and pig keepers)(46).

Reporting bias can occur with respect to wild bird observations, especially differential reporting between regional and remote locations compared with more accessible locations. Therefore, this study selected background points proportional to the human footprint (HFP), a robust indicator of landscape accessibility(47), to correct for such bias in the ardeid suitability models. The HFP data were retrieved from the Socioeconomic Data and Applications Center (SEDAC) registry maintained by the Center for International Earth Science Information Network (CIESIN)(48). Reporting bias may also affect piggery outbreak data, whereby large commercial herds may be more likely to capture and record cases due to more systematic collection of production data and increased probability of disease detection in larger herds. Therefore, background points for JEV models were selected proportional to herd size to correct for potential piggery reporting bias.

Environmental data for habitat suitability models

Land cover classification based on the European Space Agency and Climate Change Initiative was used to classify wetlands(49,50) and land cover(51) at 3 arc seconds in 2010, as this time point corresponded to the beginning of the period of recorded ardeid observations described above. Waterways were represented by a separate high-resolution (3 arc seconds) image produced in collaboration between CIESIN and the WorldPop project (52,53). Mean annual precipitation, mean annual temperature, and isothermality were obtained from WorldClim (54). The Priestley-Taylor α coefficient (P-Tα) is the ratio of actual evapotranspiration to potential evapotranspiration and was used to describe water stress in the landscape(55,56). The P-Tα data were acquired from the Consultative Group for International Agricultural Research (CGIAR) Consortium for Spatial Information at a resolution of 30 arc seconds(57).

Environmental data for landscape-level metrics

All JEV outbreaks were reported to a scale of at least 5 arc minutes (~ 10 km), which thus represents the landscape-level scale of the current investigation. Land cover classes were represented using the previously validated Copernicus land cover data at 100 m resolution(58,59). The Joint Research Centre's Global Surface Water product (based on Landsat 5, 7, and 8 imagery) was used to represent high resolution (30 m) surface water seasonality in the year prior to piggery outbreaks(60,61). Each pixel in the raster tiles represents the total number of months (0 – 12) with surface water present during 2021. The heterogeneity of water transience across the landscape was based on five derived rasters: 1) permanent surface water presence over the 12 months of 2021, 2) surface water absence over the 12 months of 2021, 3) surface water presence for 1 to 3 months of the year, 4) surface water presence for 4 to 6 months of the year, and 5) surface water presence for 7 to 9 months of the year. Water movement through and accumulation in the landscape was quantified using hydrological flow accumulation, which describes the total upland area draining into each 500m by 500m parcel and was acquired from the Hydrological Data and Maps based on SHuttle Elevation Derivatives at multiple Scales (HydroSHEDS) information system(62).

Ardeid trait data

Bird species' diet composition and body mass were obtained from the Elton Traits database(63). Diet composition may be an important characteristic of host species because this can delineate generalist versus specialist species and potential resilience to human pressure(64). Only the dietary proportions of fish and invertebrates demonstrated heterogeneity among the ardeid species, so only these dietary elements were investigated. Body mass is an important life history characteristic as well as being a potential modulator of immune function(32,33). Body mass was log transformed prior to analysis. The TetraDENSITY database was used to obtain species mean population density(65), the AVONET database was used to obtain the hand-wing index(66), and the Lislevand bird traits database was used to obtain the reproductive characteristic, egg mass(67). These species traits were included since population density may influence the dynamics of JEV

circulation in populations and communities(68–71), hand-wing index has been shown to be the best metric to represent bird dispersal(72,73) particularly with respect to dispersal in response to climate and habitat loss(74,75), and egg mass is an important indicator of reproductive life history. The International Union for the Conservation of Nature (IUCN) Red List was used as a source of an additional life history trait for each bird species: species' generation length (76). The data for population density, hand-wing index, and egg mass were missing for 7, 3, and 3 species, respectively, so a random forest machine learning algorithm, which was previously shown to be a robust approach to this application(31,77), was used to impute the missing data. The rfImpute function in the randomForest package was used to implement the algorithm(78).

Statistical Analysis

Species distribution modelling

All 14 species of ardeid birds extant in mainland Australia were modelled using the GBIF data (Table S1). The modelling of habitat suitability for these species has been previously described(10). Briefly, an ensemble of boosted regression trees (BRT), random forests (RF), and generalised additive models (GAM), each with five-fold cross-validation, was used to model landscape suitability at fine-scale (30 arc seconds). Observation data were thinned so that only one observation per pixel was included in the analysis to avoid overfitting (Table S1). Environmental features in each suitability model comprised mean annual temperature, mean annual precipitation, isothermality, P-Tα, and proximity to surface water. Each model was evaluated according to performance and fit, using the area under the receiver operating characteristic curve (AUC) and deviance, respectively. Suitability estimates for each species were then derived from an ensemble of the three models (BRT, RF, and GAM) using their AUC-weighted mean(79). Potential spatial sampling bias associated with the GBIF observations was corrected by sampling background points proportional to HFP as an indicator of accessibility, and thus occurrence reporting. All species' landscape suitability estimates were then summed as an estimate of local species richness across the geographic extent of Australia.

Adjusted individual species presence at landscape-scale was computed by applying interspecific biotic constraints at a scale of 5 arc minutes (~ 10 km), which was the scale of piggery outbreak reporting as well as the scale at which key landscape structure influencing outbreak occurrence was previously described(10). These biotic constraints were based on spatially-explicit species assemblage modelling (SESAM) to adjust each species' habitat suitability estimate by sympatric species (80–82). The SESAM framework is a frequently used approach to estimate community composition from habitat suitability but is not intended to represent community structure in the current study in which local communities were not observed. Here, the approach is applied to construct a landscape-level species pool, given environmental filtering, which can then be mapped to specific landscapes that are associated with JEV outbreaks.

The SESAM framework was applied as follows. First, each species' habitat suitability was derived from the ensemble species distribution models described above. Second, a crude estimate of species richness was calculated by summing all of the individual species' habitat suitabilities. Third, each species is contrasted with all other species present within each landscape parcel to determine whether a given species should be retained or excluded from the parcel. This final step uses the probability ranking rule(81,83) to rank each species present in the parcel based on their suitability estimate, which was computed in step one. Species are then selected for inclusion in the parcel beginning with the species with the highest suitability and then continuing down the list of lower ranked species until the sum of selected species equals the expected species richness value for each parcel, which is based on the crude richness estimate obtained in step two. After attaining this threshold sum, the SESAM adjusted-species richness has been achieved and no further species will be included in that particular parcel. Subsequently, the species pool available for community assembly was estimated with each species retained in a given landscape parcel under the SESAM model as follows. Each 1 km^2 pixel of each species' habitat suitability estimate (step one, above) that was greater than or equal to the true skill statistic (TSS)(84) was classified as present and summed across all pixels within the 5 arc minute landscape parcel. The TSS was used to designate each parcel

260 as present or absent based on the habitat suitability because it has been shown to be independent of
261 prevalence(84). This summed metric thus provided an estimate for landscape-level species
262 "abundance" available to community assembly within each particular landscape parcel. Similarly,
263 species relative abundance was computed by dividing each individual species abundance by the total
264 abundance of all species for each parcel. The "relative abundance" of each species available thus
265 provides an estimate of the potential influence of a species for community assembly within
266 landscape parcels, rather than an actual estimate of relative abundance of a species in a local
267 community. This proxy of relative influence within the landscape-level species pool is the metric used
268 for identifying ardeid species targets for JEV surveillance in this study. The sdm package(79) was used
269 for fitting all species distribution models and deriving the habitat suitability ensembles. The SESAM
270 analysis was conducted using the ecospat package(82).

271 Because of the scaling up of species presence to compute proxies of abundance and relative
272 abundance at the landscape scale of 5 arc minutes, the analyses were limited to a single bird family,
273 the Ardeidae, to limit the degree of assumptions regarding environmental filtering, dispersal, and
274 biotic interactions of species between bird families. A narrower, single family-focus was considered
275 to be particularly necessary because of the direct examination of the associations between the
276 distribution of species pool-weighted mean traits (see description below), species composition, and
277 JEV risk, all at the broad landscape scale of JEV outbreak reporting. Although this was deemed an
278 acceptable compromise given that many of these species have been demonstrated to be competent
279 hosts, it is also recognised that additional non-ardeid waterbirds, such as the Australian white ibis,
280 have been recommended for JEV monitoring(8) despite the current lack of evidence regarding their
281 host status. As such, the current study's narrower focus limits the breadth of the conclusions to the
282 Ardeidae.

283 *Landscape metric analysis*

284 The metrics used to delineate high-risk landscape structure have been described in detail
285 previously in an analysis of the landscape composition and configuration associated with JEV

286 outbreaks in piggeries(10). Briefly, increasing cultivated land and fragmented grassland were both
287 associated with JEV outbreaks and these were computed as follows. Total cultivated land
288 composition was computed by summing the area of all crop patches in each landscape parcel
289 (landscape-level scale of 5 arc minutes). Grassland fragmentation was assessed by measuring mean
290 patch edge relative to patch area for all grassland patches in each parcel using the related
291 circumscribing circle (RCC). The RCC metric has been shown to be robust to patch size(85) in contrast
292 to the perimeter-to-area ratio (PAR), wherein similarly shaped patches can give different values for
293 different sizes and can translate to significant error when summarising PAR at landscape scale(85).
294 These composition and configuration metrics were previously computed for all land cover classes as
295 referenced above, but only those shown to be associated with JEV outbreak occurrence are
296 investigated with respect to ardeid species here as this was the stated aim of the current study. The
297 landscapemetrics package(86) was used to calculate RCC in R v. 4.3.1.

298 *Functional metrics for species traits*

299 As a crude measure of the species pool mean traits at landscape scale, the commonly used
300 community-weighted mean was calculated for each trait among all species identified as present
301 within each landscape parcel(87,88). As with the community composition metrics used to represent
302 abundance and relative abundance in the species pool at landscape scale described above, these
303 "community-weighted means" are not intended to represent local community trait means, but rather
304 to identify whether any species traits shared among the pool stood out as potentially influential for
305 community assembly at landscape scale, and particularly in high-risk JEV landscapes. Because of the
306 limited scope for evaluation of local community functional structure in this context, measures of
307 functional divergence and convergence were not assessed. The FD package(89) was used to compute
308 weighted means for species traits at landscape scale in R v. 4.3.1.

309 *Point process modelling*

310 Each of the 14 individual ardeid species' landscape-level relative abundances within the pool
311 were interrogated with respect to JEV outbreaks by fitting inhomogeneous Poisson point process

312 models (PPMs)(90,91). This allowed the spatial dependence of the outbreaks' distribution to be
313 evaluated against each species' distribution relative to other species across the landscape. To control
314 for potential reporting bias in outbreak surveillance, the background points used in these models
315 were sampled proportional to mean pig density, as described above. For the ardeid species PPMs, a
316 simple univariate PPM of JEV outbreaks was created for each of the 14 extant ardeid species' relative
317 abundance. Similarly, for the species trait PPMs, a simple univariate PPM of JEV outbreaks was
318 created for each of the species' traits; however, this univariate trait analysis was followed by a
319 multiple PPM to determine which traits among the species pool were independently associated with
320 JEV outbreaks. Correlation between the spatial distribution of traits was low (Pearson's $r \leq 0.24$) and
321 all variance inflation factors were ≤ 1.46, indicating collinearity was not a concern for the trait
322 multiple PPM. All PPMs were fit at a scale of 5 arc minutes, which was the scale of outbreak
323 reporting(10). The Akaike information criterion (AIC) was used to evaluate model fit. The spatstat R
324 package was used to fit the PPMs(92).

325 *Multivariate analysis for species assemblage composition*

326 A hierarchical modelling of species communities (HMSC) framework(93,94) was used to
327 assess species pool composition, and individual species presence, in relation to the specific features
328 of landscape composition and configuration that have been shown to delineate JEV outbreak risk
329 (Figure S1)(10). In addition, the HMSC framework accounted for the potential modulation of species
330 presence in high-risk landscape structure by their phylogeny and functional traits, associations with
331 the latter representing species "responses" to the landscape features, however these do not
332 represent estimates of actual responses within communities and so this interpretation must be
333 tempered (see below). Models were fit under two domains: the first comprised each of the specific
334 landscape features shown to be associated with JEV outbreaks (Figure S1) and the second comprised
335 JEV outbreak occurrence itself as the single landscape feature of interest. The two separate domains
336 were evaluated since the landscape features associated with JEV outbreaks did not explain all of the
337 variability in JEV outbreak occurrence, indicating that there are additional unmeasured landscape

338 features that cannot be captured by the first domain, but which may still be relevant to species
339 presence in the landscape. The models were fit with the probit link function to represent species
340 presence, rather than species abundance, as the former is more appropriate given the focus of
341 investigation at landscape scale as already described above. The probit function is preferred over the
342 logit function in HMSC models because it provides a more pragmatic fit to the hierarchical
343 multivariate data(93). These models are fitted using Markov chain Monte Carlo methods and were
344 run here with four chains and 1000 samples each and a burn-in set to 20% of the initial samples. The
345 adjusted r^2 was used to assess model fit for each of the two model domains. Model convergence was
346 demonstrated for each species (Gelman diagnostic ≈ 1 for each species). Although interspecific
347 interaction within communities was not observed in this study, the HMSC framework does allow for
348 further accounting of important phylogenetic and functional influence on the sharing of space
349 between sympatric species given environmental filtering, which cannot be evaluated with the PPMs
350 described above. Nevertheless, it must be noted that since biotic filtering has not been directly
351 observed, which necessarily occurs at the level of the local community, true community-level
352 functional responses to environmental conditions are not captured at this landscape scale. Instead,
353 these multivariate analyses allow correlative associations to be identified that are anticipated to be
354 useful in characterising how community assembly could potentially operate in landscapes associated
355 with JEV outbreaks, and thereby help to inform sampling targets within such landscapes so that
356 community monitoring of JEV hosts can be systematically structured for One Health surveillance
357 across the large geographic extent of eastern Australia. The Hmsc package(94) was used for the
358 HMSC models.

359 **Results**

360 The PPMs identified landscape-level distributions of six species within the pool that were
361 strongly associated with JEV outbreaks: *Casmerodius albus*, *Ardea pacifica*, *Botaurus poiciloptilus*,
362 *Egretta novaehollandiae*, *Ixobrychus minutus*, and *Nycticorax caledonicus* (Table S2, Table 1, and
363 Figure 1). Of all the species traits interrogated under the PPMs, only the distributions of mean

364 population density and mean hand-wing index demonstrated independent associations with the
365 distribution of JEV outbreaks (Table S2, Table 2 and Figure 2), whereby increasing species pool-
366 weighted mean population density and increasing species pool-weighted mean hand-wing index
367 were both associated with increasing JEV outbreaks at landscape scale.

368 The HMSC model was in close agreement with respect to the structure of the species pool,
369 identifying the same species presences associated with JEV occurrence as those identified by the
370 PPMs, while also identifying one additional species, *Mesophoyx intermedia* (Figure 3). The median
371 adjusted r^2 for the landscape feature-based model and JEV-based model was 15.8% and 5%,
372 respectively. Furthermore, the HMSC model showed that whilst some high-risk landscape features
373 were common to all ardeids (the presence of transient wetlands and proximity to waterways, as
374 expected), other high-risk features were specific to individual species, or small clusters of species
375 (Figure 3). For example, the presence of *Ardea pacifica*, *Botaurus poiciloptilus*, *Egretta*
376 *novaehollandiae*, and *Ixobrychus minutus* was each associated with increasing presence of croplands,
377 whereas the presence of *Botaurus poiciloptilus*, *Ixobrychus minutus*, and *Nycticorax caledonicus* was
378 each associated in increasing grassland fragmentation. Additionally, the presence of five
379 (*Casmerodius albus*, *Ardea pacifica*, *Egretta novaehollandiae*, *Mesophoyx intermedia*, and *Nycticorax*
380 *caledonicus*) of the seven species associated with high-risk JEV landscapes was specifically associated
381 with greater water flow accumulation (Figure 3). All species whose presence was not associated with
382 JEV outbreaks were not associated, or were negatively associated, with these landscape features
383 (except *Butorides striata*, which was associated with grassland fragmentation).

384 Species presence within these high-risk landscape features did not appear to be modulated
385 by their functional traits after having accounted for phylogenetic structure (left panel, Figure 4).
386 However, interrogating landscapes defined solely by their JEV outbreak occurrence, rather than the
387 individual landscape features associated with outbreaks, showed that species presence in landscapes
388 characterised by outbreak occurrence was associated with greater species hand-wing index and body
389 mass (right panel, Figure 4).

390 **Discussion**

391 This study presents a description of the structure of the ardeid species pool at landscape
392 scale to inform how the landscape and functional structure of ardeid presence may be leveraged for
393 JEV surveillance in this natural nidus of virus circulation. The results showed that six of the 14 extant
394 ardeid species were strongly associated with the distribution of JEV outbreaks in piggeries reported
395 in 2022. While some JEV-associated landscape features, such as transient wetlands and proximity to
396 waterways, were commonly associated with species presence among most ardeids, other features of
397 high-risk landscape structure were specific to smaller groups of species, such as crop cultivation,
398 habitat fragmentation, and water flow accumulation. Interestingly, species traits did not provide
399 additional insight beyond that provided by phylogeny when considering the individual landscape
400 features associated with JEV outbreaks. However, hand-wing index was associated with species
401 presence in landscapes demarcated solely by outbreak occurrence and simultaneously demonstrated
402 an association between the distribution of species-pool weighted mean hand-wing index and JEV
403 outbreaks. These findings suggest that whilst high-risk JEV landscapes can vary with respect to the
404 ardeid species present, JEV surveillance across broad regions of eastern and southern Australia can
405 be informed using a combination of landscape structure and targeting of individual species in these
406 landscapes.

407 Previous work in Australia(10) and across the Indo-Pacific region(95,96) has demonstrated an
408 association between ardeid species richness and JEV outbreaks, albeit one that is quadratic in
409 nature. As such, low risk was associated with low and high ardeid richness, while high risk was
410 associated with intermediate richness. Nevertheless, the ways in which individual species, or species
411 community structure, may respond to specific landscape composition and configuration associated
412 with JEV occurrence has hitherto remained unknown. This marks a critical gap in our current
413 understanding of the infection ecology of JEV and highlights the need for wild bird monitoring to fill
414 that gap. However, given the extraordinarily broad geographic distribution of JEV outbreaks in
415 Australia, developing a systematic sampling strategy to answer key maintenance host ecology

416 questions that is representative of this breadth of occurrence is exceedingly challenging. To help
417 inform the development of such a sampling strategy, the current work sought to describe ardeid
418 composition as a representation of the available species pool for community assembly at the more
419 practical (and translatable) scale of the landscape. This also allowed for species presence to be
420 directly evaluated with respect to the landscape structure previously associated with JEV outbreaks,
421 and therefore that landscape structure can inform species selection for surveillance. The current
422 study identified six species that were individually associated with JEV outbreaks and found that
423 species presence is likely to be heterogeneous across the different landscape features associated
424 with those outbreaks, and that these associations also reflected differences in functional traits.
425 Moreover, the distinct approaches applied using both the PPMs and HMSC models were in strong
426 agreement with respect to the species identified in association with JEV outbreaks.
427 Associations between the dominant traits of species present in the landscape pool may
428 provide insight into potential drivers of species structure in landscapes delineating JEV risk. For
429 example, landscapes associated with JEV occurrence were also characterised by species
430 demonstrating a high level of dispersal capacity, as indicated by greater hand-wing index. This may
431 indicate the importance of dispersal among species in the context of seasonal or transient wetlands,
432 which were strongly associated with species presence in the current study, and JEV outbreaks
433 previously(10). Moreover, both modelling frameworks led to similar conclusions with respect to
434 hand-wing index. Such a scenario is supported by analyses of population structure among birds in
435 Australia following La Nina precipitation anomalies(8,97), and reflects more general evidence for the
436 importance of hand-wing index as an indicator of dispersal capacity in response to climate gradients
437 and habitat fragmentation(73–75). Interestingly, the two modelling approaches did not agree with
438 respect to population density and body mass; the PPM identified a positive association between
439 species pool-weighted mean population density and JEV outbreaks, and the HMSC model showed
440 that the association between species' presence and JEV outbreaks was modulated by species pool-
441 weighted mean body mass rather than population density. The association between JEV outbreaks

442 and higher population density is not surprising as this may reflect pathogen transmission
443 dynamics(68–70), but it is not clear why this association did not persist across modelling frameworks.
444 The discrepancy may result from the ability of the HMSC model to account for phylogenetic structure
445 in species presence, for which the PPM could not account, and the subsequent modulation by traits.
446 It should also be noted that the association between hand-wing index and species presence in high-
447 risk landscapes was not an artifact of body mass because these traits were not correlated (Pearson's
448 r = -0.21), which is as expected since hand-wing index adjusts for body size(72). While these findings
449 are intriguing, it is critical to emphasise that they are only correlative of possible drivers of
450 community assembly at landscape scale and do not describe realised community structure. It is not
451 possible to infer local community composition or functional responses to environmental conditions
452 from the data presented here. Rather, the true utility of this work is to describe preliminary,
453 evidence-based, configurations of ardeid species pools for the coupling of high-risk JEV landscape
454 structure with potential maintenance hosts across extraordinarily broad geographic extent, and
455 thereby inform the development of broad-scale sampling strategies for One Health JEV surveillance
456 in Australia.

457 Beyond the implications of these findings for potential wild bird surveillance programs, there
458 may be further potential utility for the design of other arbovirus surveillance programs by state and
459 territory health authorities in Australia. Arbovirus surveillance is currently conducted in many regions
460 throughout Australia, typically comprising mosquito and sentinel chicken monitoring. The selection
461 of monitoring sites is often informed by historic arbovirus activity or generically according to
462 potential mosquito and waterbird habitats. The results of the current investigation suggest that
463 consideration of the presence of wildlife hosts in specific landscapes may provide additional
464 information in identifying appropriate arbovirus surveillance locations more generally. Nevertheless,
465 whether wild host monitoring is employed specifically in the service of JEV surveillance, or more
466 generally for broad arbovirus surveillance, concomitant consideration of mosquito communities, and
467 specifically the nature of the alignment between their community composition and high-risk

468 landscapes and key maintenance hosts, will also be essential for maximally beneficial surveillance
469 output. The current study was not able to interrogate vector communities specifically, and as such
470 this remains a key knowledge gap in the infection ecology of JEV.

471 In addition to the specific constraints to interpretation of the results described above,
472 further description of additional limitations is included below. First, both the recording of ardeid
473 observations and the reporting of piggery outbreaks may be affected by reporting biases. We have
474 corrected for potential biases by sampling background points for ardeid SDMs proportional to the
475 HFP, and sampling background points for JEV piggery outbreaks proportional to mean pig density, as
476 described above. Nevertheless, it is possible that residual bias may persist. Second, notwithstanding
477 the inclusion of each of the ardeid species found in Australia in the habitat suitability modelling, it is
478 important to note that suitability represents the fundamental niche only, because it does not include
479 direct observational evaluation of biotic interactions to more formally define the realised niche or
480 subsequent community structure. However, this study did adjust the suitability estimates of
481 individual species by the estimates of all other species present at local scale using the SESAM
482 framework to represent species presence more appropriately in the landscape-level species pool.
483 Nevertheless, we must acknowledge that neither local biotic interactions nor dispersal history are
484 represented in this framework and should be considered in future work. Third, as noted above,
485 species other than ardeids may provide additional maintenance hosts to JEV-associated landscapes.
486 However, due to the current study's aim of coupling high-risk landscape structure with individual
487 target species and species pools at landscape scale, the analysis was constrained to the single,
488 established family of maintenance hosts to limit the extent of the assumptions regarding interspecific
489 interactions since direct community observation at local scale was not possible. Therefore, this study
490 is a preliminary, albeit actionable, representation of possible target species for specific landscapes
491 and should be expanded to include additional non-ardeid species as work progresses on demarcating
492 local community structure in high risk-landscapes through the implementation of monitoring
493 programmes. Finally, previous work showed that the piggery outbreaks were not associated with

feral pigs(10). Nevertheless, JEV has been identified in feral pigs, albeit only very sporadically(98). As such, it is possible that there may be multiple landscapes of JEV risk, those pertaining to domestic piggeries and those pertaining to shrub and forested bushlands. If the latter are in fact present, then different surveillance programs targeting different maintenance hosts may be required.

In conclusion, this study has identified an initial selection of target ardeid species for sampling in association with particular configurations of landscape risk. Although preliminary, it is anticipated that this evidence-based approach to early JEV surveillance development will provide a more efficient and productive yield than a simple random or convenience-based approach to maintenance host sampling, particularly given the wide geographic extent in which JEV outbreaks occurred across Australia. As wild bird monitoring progresses and local community structure becomes better articulated with respect to JEV circulation, we expect that this selection will be updated to reflect currently unrecognised local heterogeneities in landscape and community composition, as well as novel maintenance hosts.

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Data Availability Statement: All data described in this study are publicly available for download at the cited sources in the text except the pig density data, which contains sensitive information to the Australian agricultural industry (e.g. locations of piggeries). Provision of this data for third parties requires their application to Australian Pork Limited.

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850 Table 1. Univariable regression coefficients and 95% confidence intervals for the associations
851 between Japanese encephalitis virus outbreaks and each ardeid species as derived from
852 inhomogeneous Poisson models.

Landscape species pool relative abundance	**AIC**	**Estimate**	**95% confidence interval**
Null model	204.70		
Casmerodius albus (*Ardea alba*) – Great egret	202.26	4.55	0.70 – 8.39
Ardea pacifica – White-necked (Pacific) heron	169.57	2.25	1.53 – 2.96
Botaurus poiciloptilus – Australasian bittern	201.13	3.26	1.17 – 5.34
Egretta novaehollandiae – White-faced heron	193.62	2.85	1.49 – 4.21
Ixobrychus minutus (*Ixobrychus dubius*) – Australian little bittern	195.89	10.43	5.69 – 15.17
Nycticorax caledonicus – Nankeen (rufous) night heron	202.12	2.08	0.41 – 3.76

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854 Table 2. Regression coefficients and 95% confidence intervals for the associations between Japanese
855 encephalitis virus outbreaks and species traits as derived from univariable (A) and multiple (B)
856 inhomogeneous Poisson models.

Landscape species pool weighted mean traits	**AIC**	**Estimate**	**95% confidence interval**
Null model	238.05		
A. Univariate PPMs			
Percentage of diet – invertebrates	202.26	4.55	0.70 – 8.39
Hand-wing index	169.57	2.25	1.53 – 2.96
Population density (birds/km^2)	201.13	3.26	1.17 – 5.34
B. Multiple PPM			
Hand-wing index	213.58	0.22	0.104 – 0.335
Population density (birds/km^2)		1.35	0.718 – 1.977

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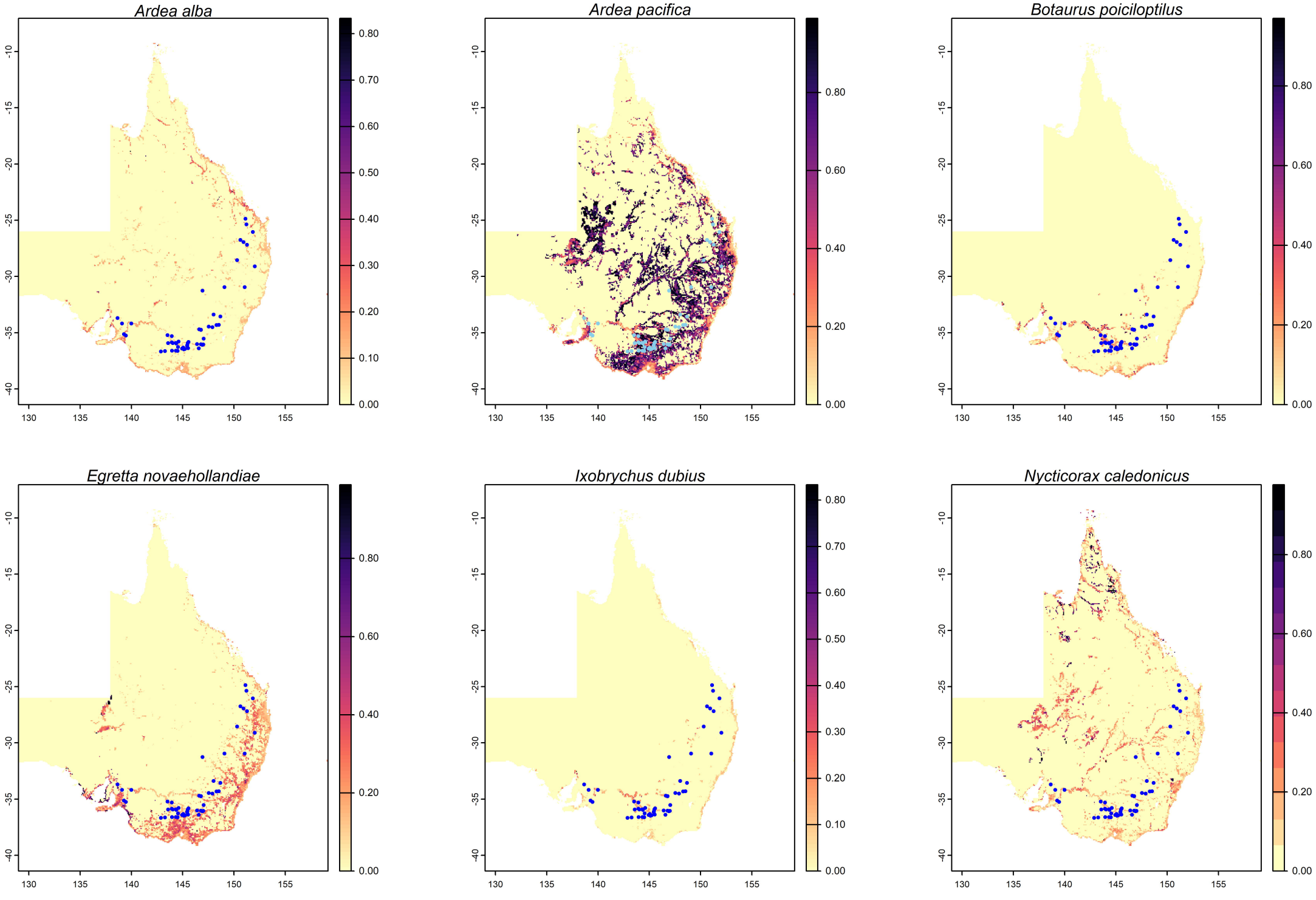
871 Figure legends

872 Figure 1. The distribution of the relative abundance in the landscape-scale species pool of each
873 species identified as a potential target for Japanese encephalitis virus (JEV) surveillance based on the
874 strong association with the distribution of JEV outbreaks. The overlaid points represent the locations
875 of the JEV piggery outbreaks.

876 Figure 2. The distribution of the species pool-weighted mean (PWM) traits for those traits that were
877 associated with Japanese encephalitis outbreaks in the univariate point process models (proportion
878 of the diet comprising invertebrates, hand-wing index, and population density) or in the hierarchical
879 modelling of species communities model (hand-wing index and log body mass). The overlaid points
880 represent the locations of the JEV piggery outbreaks.

881 Figure 3. The associations between each species and the landscape features under two model
882 structures using hierarchical modelling of species communities (HMSC). The first HMSC model (top
883 panel) shows associations as positive, negative, or neutral "responses" of each species on the y-axis
884 to each landscape feature associated with JEV outbreaks on the x-axis. The second HMSC model
885 (bottom panel) shows these associations for JEV outbreak occurrence only and no other landscape
886 features.

887 Figure 4. The association between species traits and the landscape features, representing
888 modulation of species "responses" to the landscape features, under two model structures using
889 hierarchical modelling of species communities (HMSC). The first HMSC model (left panel) shows
890 associations as positive, negative, or neutral "modulators" for each trait on the y-axis of each
891 landscape feature associated with JEV outbreaks on the x-axis. The second HMSC model (right panel)
892 shows these associations for JEV outbreak occurrence only and no other landscape features.


Ardea alba
Ardea pacifica
Botaurus poiciloptilus
Egretta novaehollandiae
Ixobrychus dubius
Nycticorax caledonicus
-10
-15
-20
-25
-30
-35
-40
130
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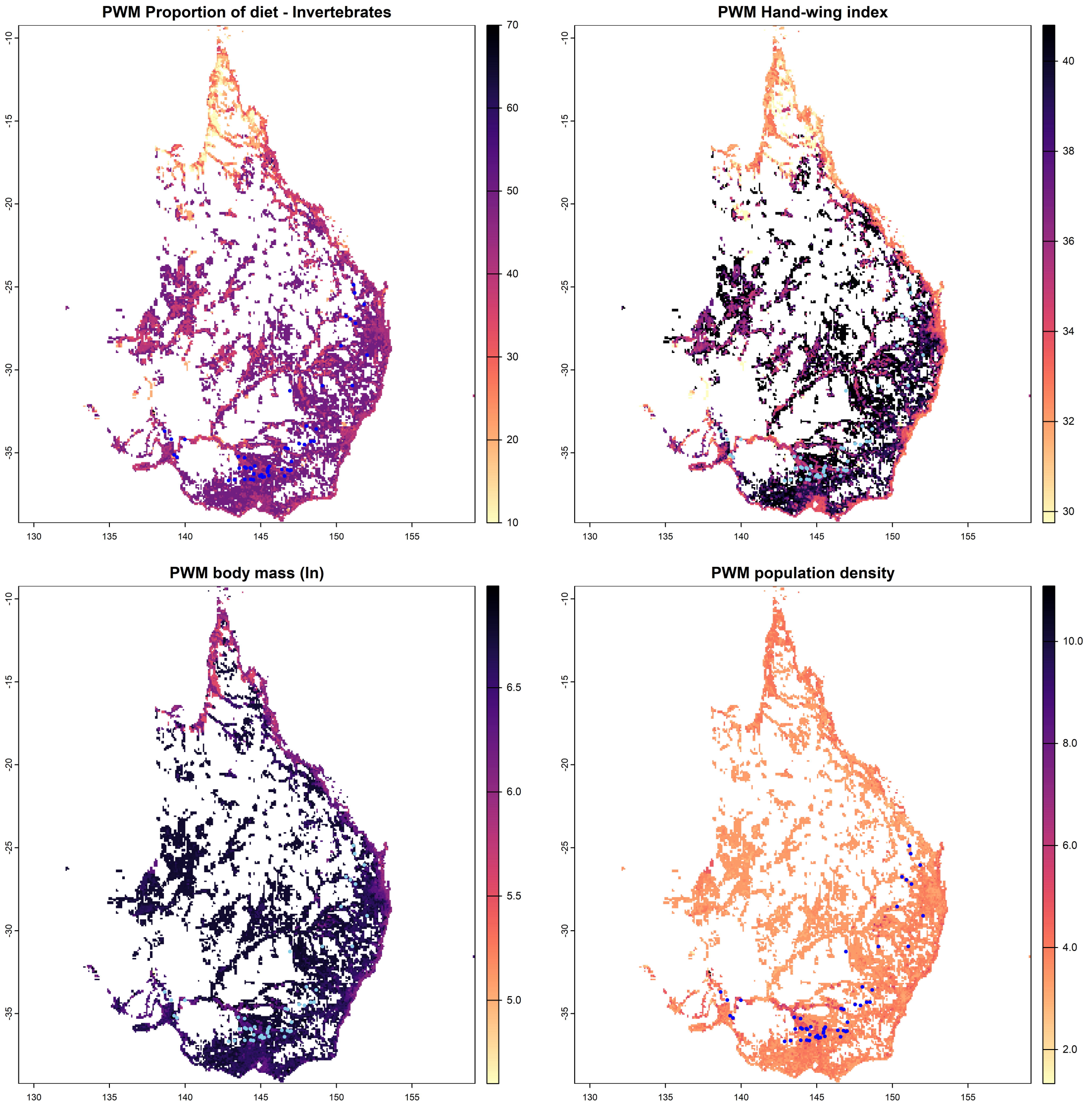
PWM Proportion of diet - Invertebrates
PWM Hand-wing index
PWM body mass (ln)
PWM population density
-10
-15
-20
-25
-30
-35
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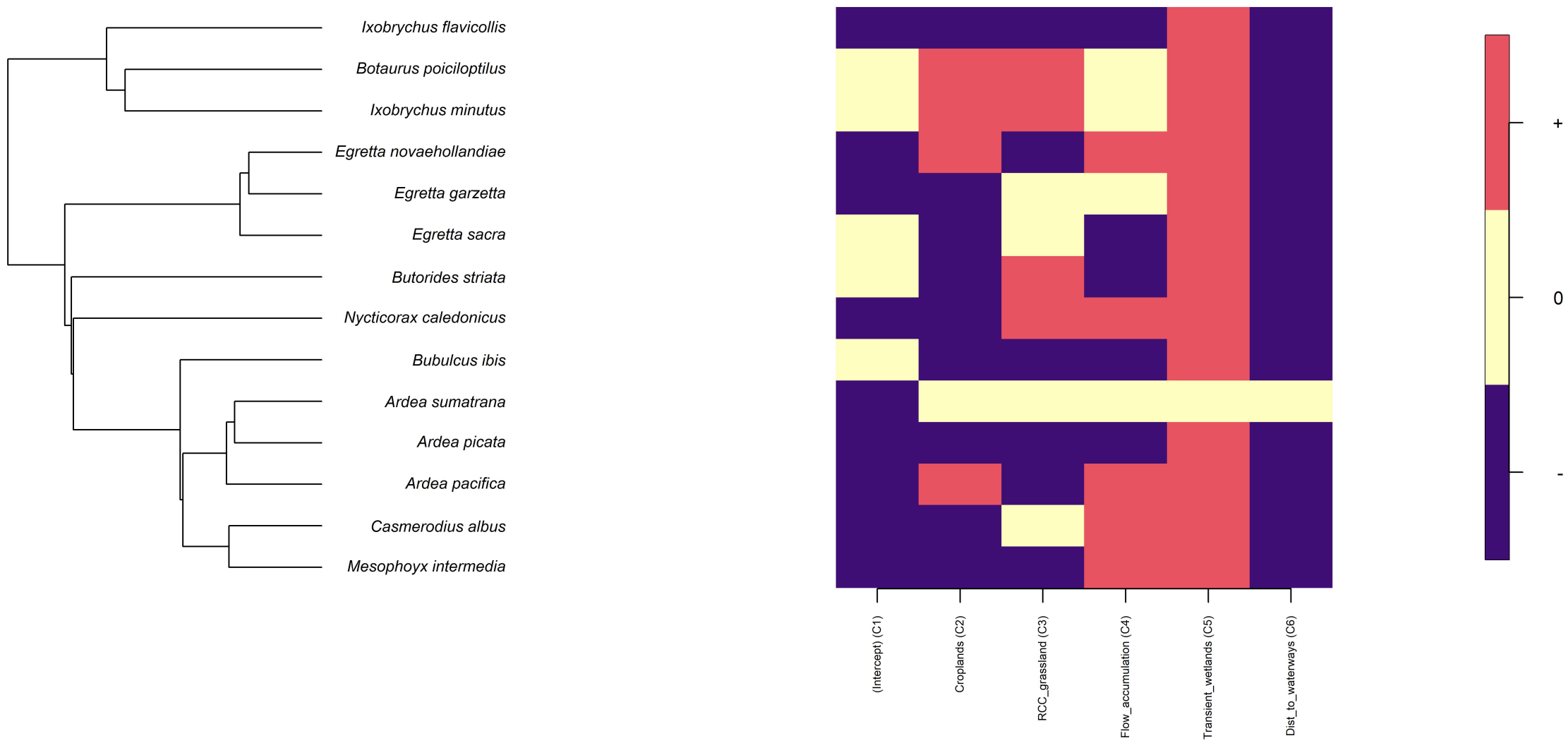
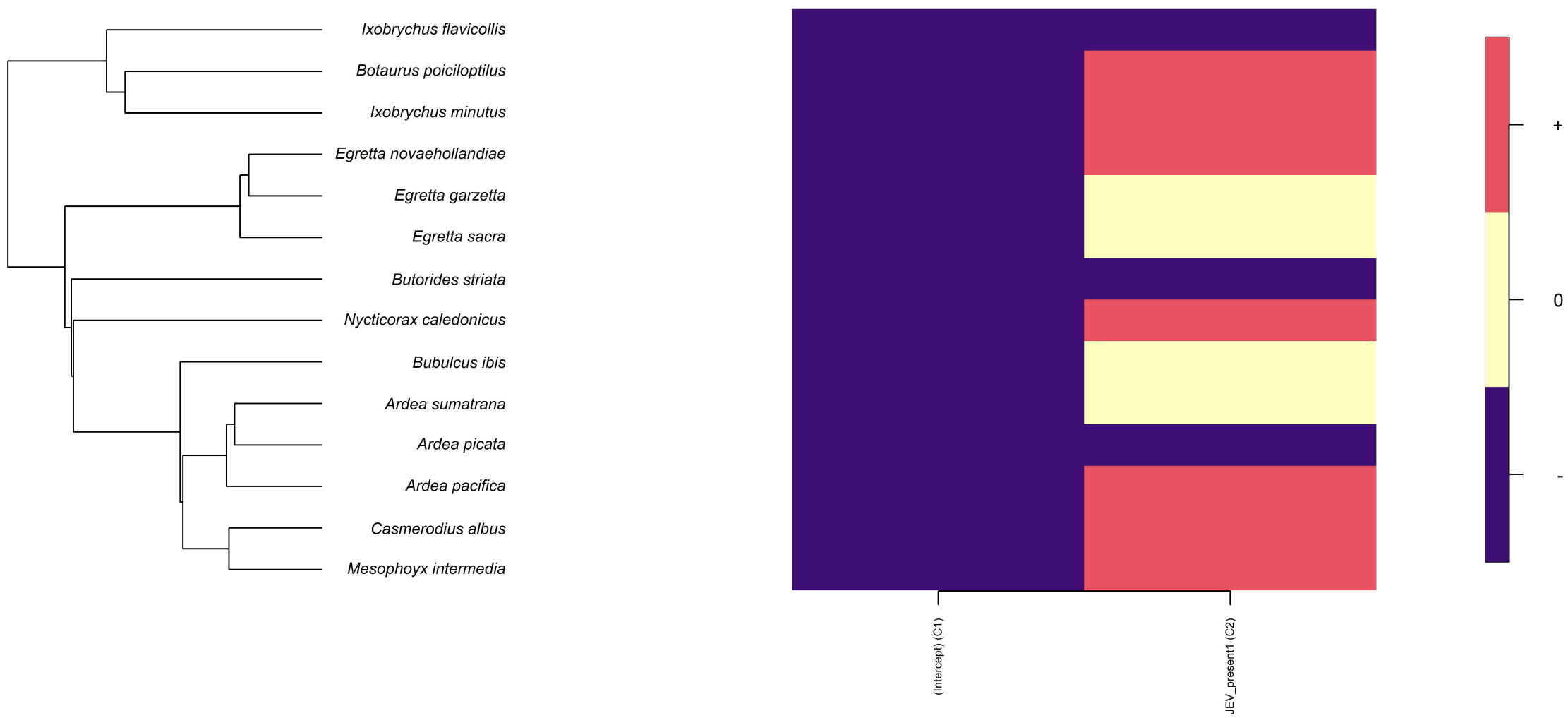

Ixobrychus flavicollis
Botaurus poiciloptilus
Ixobrychus minutus
Egretta novaehollandiae
Egretta garzetta
Egretta sacra
Butorides striata
Nycticorax caledonicus
Bubulcus ibis
Ardea sumatrana
Ardea picata
Ardea pacifica
Casmerodius albus
Mesophoyx intermedia
(Intercept) (C1)
Croplands (C2)
RCC_grassland (C3)
Flow_accumulation (C4)
Transient_wetlands (C5)
Dist_to_waterways (C6)
+
0
-


Ixobrychus flavicollis
Botaurus poiciloptilus
Ixobrychus minutus
Egretta novaehollandiae
Egretta garzetta
Egretta sacra
Butorides striata
Nycticorax caledonicus
Bubulcus ibis
Ardea sumatrana
Ardea picata
Ardea pacifica
Casmerodius albus
Mesophoyx intermedia
(Intercept) (C1)
JEV_present1 (C2)
+
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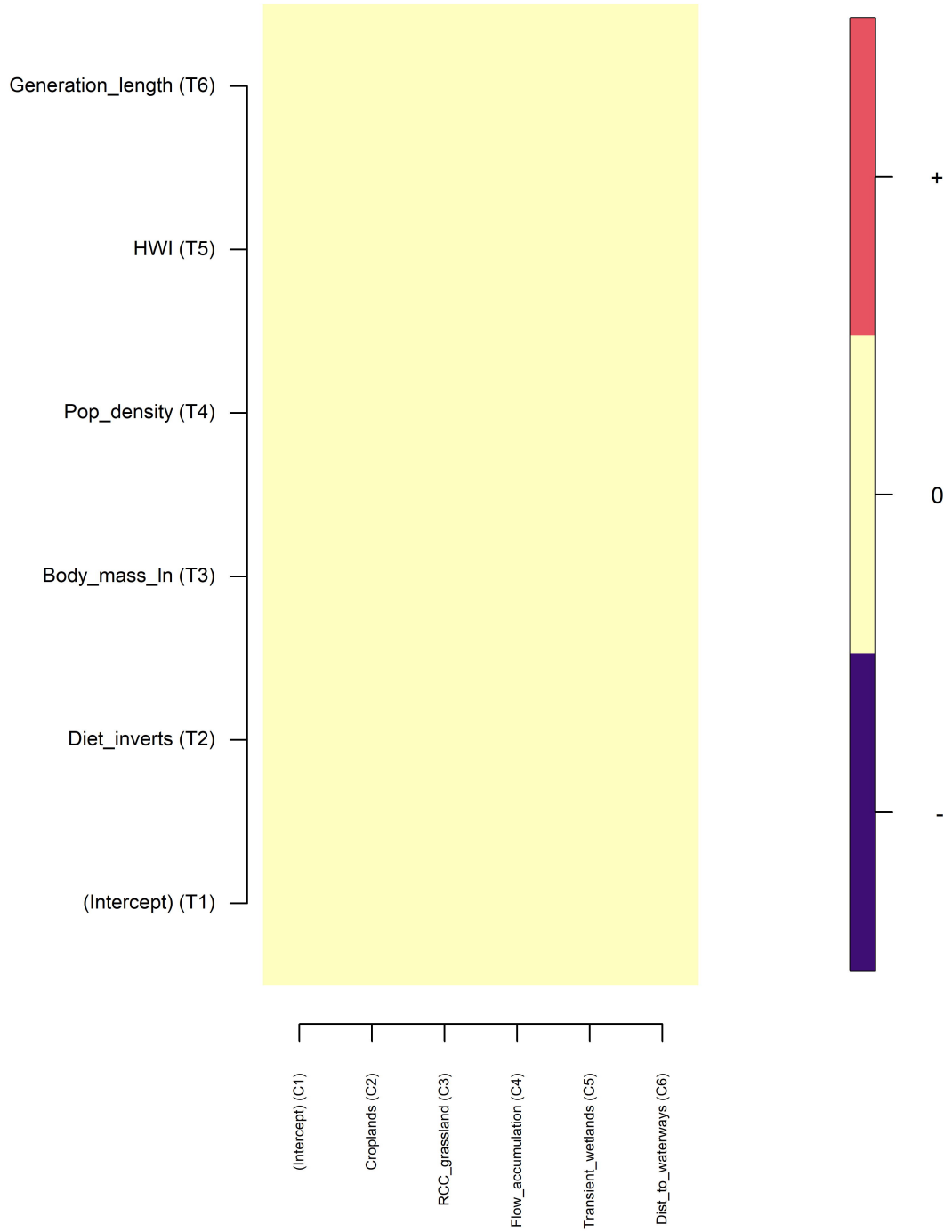
Generation_length (T6)
HWI (T5)
Pop_density (T4)
Body_mass_ln (T3)
Diet_inverts (T2)
(Intercept) (T1)
(Intercept) (C1)
Croplands (C2)
RCC_grassland (C3)
Flow_accumulation (C4)
Transient_wetlands (C5)
Dist_to_waterways (C6)
+
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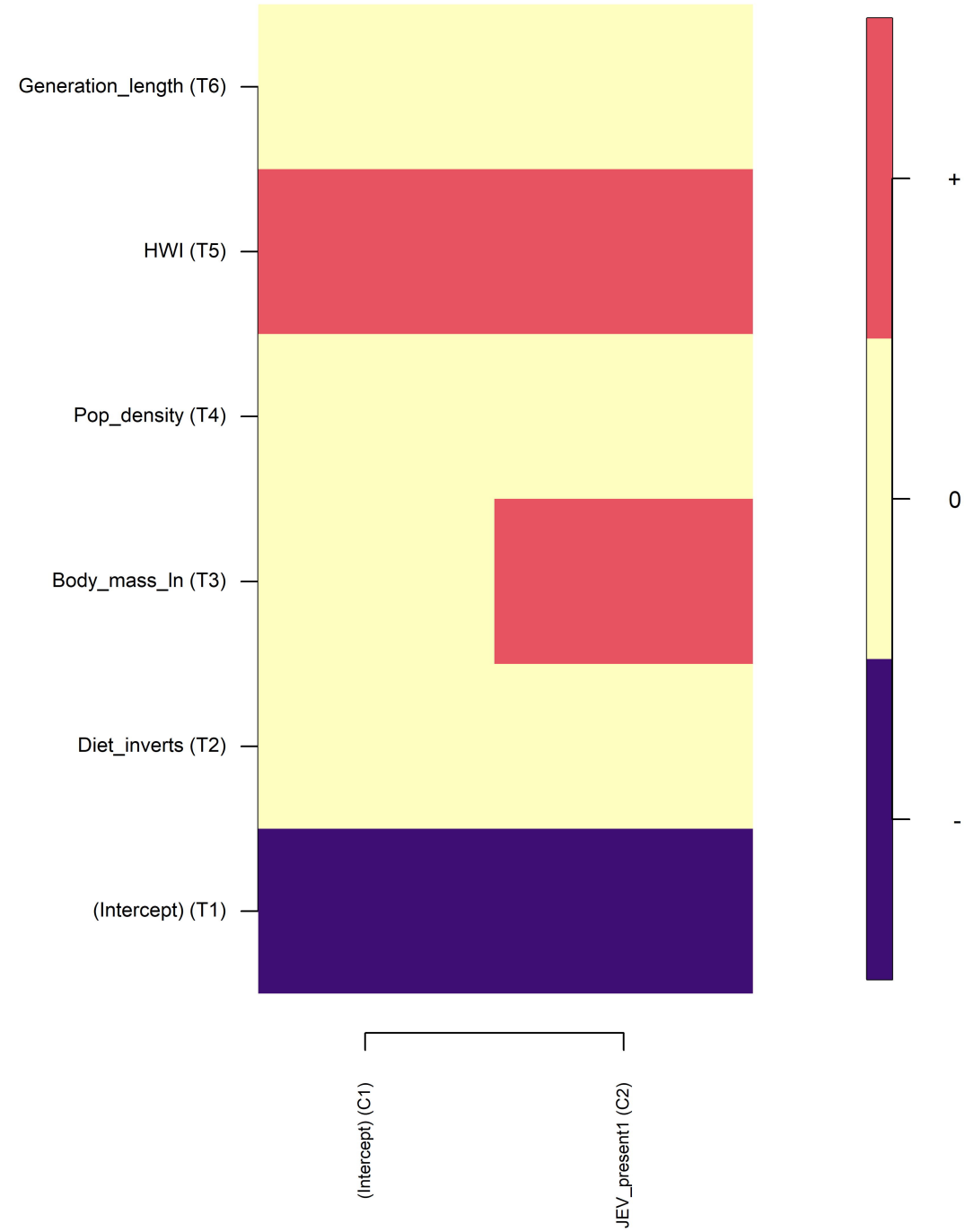
Generation_length (T6)
HWI (T5)
Pop_density (T4)
Body_mass_ln (T3)
Diet_inverts (T2)
(Intercept) (T1)
(Intercept) (C1)
JEV_present1 (C2)
+
0
-