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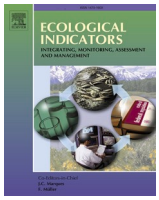

Original Articles

Identification and use of suitable metrics for calling male count-based community assessments in amphibian monitoring in temperate Europe

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ABSTRACT

Calling amphibian male counts (CAMC) is a cost-effective non-invasive acoustic monitoring method that needs amphibian community quality assessment system. In our survey we considered breeding amphibian assemblages as entities having three main ecological traits, size, richness and evenness, and tested the reliability of potentially suitable metrics that each characterize separate ecological trait. We also aimed at finding out the site and surrounding land-use characteristics linked to low or high-quality amphibian community state assessments, and studied differences in responses of community traits to these external factors.

In this CAMC study that covered 400 amphibian breeding sites across Latvia, we tested responses of 11 metrics (5 CAMC for community size, 2 richness metrics, 4 evenness and dominance indices) to five sets of predictors (two sets of anthropogenic land use variables, two natural/mixed effect sets, and a climate variable set) using GLMs, to find out statistically significant anthropogenic factors, site and surrounding land use characteristics and model ranks according to their corrected Akaike information criterions. Then, we used anthropogenic factors to classify sites with natural and strongly impacted site groups, compared their metrics by Wilcoxon rank-sum tests and Box plots, and assessed metric congruences in site classifications.

Based on their properties, we recommend using three metrics, each being the best in representing their own breeding amphibian assemblage trait in the communities of temperate Europe: the total number of calling males ($CAMC_{tot}$), the community completeness, and the reciprocal form of Berger-Parker dominance index.

Despite of good responses to anthropogenic pressure indicators showed by the metrics, local site characteristics were still the highest-ranked factor, with waterbody dimensions being the most important for amphibian population size, and macrophyte vegetation largely determining their taxonomic richness and community evenness. Among anthropogenic threats, the strongest effects were from the transportation corridors (roads) and infrastructure developments that had particularly adverse effects on community richness and evenness, and on CAMC for some taxa. Wetlands in surrounding areas were more important for $CAMC_{tot}$. The climate factor was generally among the least important in ranks, probably due to its general similarity among the study sites throughout Latvia.

Our study shows opportunities for using amphibian-based metrics in ecological indicator systems, where they may substitute fish metrics in some waterbody types, and suggests the necessity of paying more attention to wetland size and location against the road network in amphibian habitat restoration projects.

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1. Introduction

Amphibians play a vital role in aquatic and terrestrial ecosystems, where they are considered as good environmental indicators because of their sensitivity to pollution due to permeable integuments and high rates of contaminant bioaccumulation (Hopkins, 2007). Amphibians can be easily detected by the calls produced by mating males, making them very promising for non-invasive acoustic monitoring (Anthony, 2002, Walker, 2002, Weir and Mossman, 2005). Most established amphibian acoustic monitoring programmes account for species presence/absence (Calderon et al., 2019, van der Hoek et al., 2019), and/or use calling intensity estimations, e.g., the calling index (Pillsbury and Miller, 2008; Calderon et al., 2019) or the call latency (Pierce and Hall, 2013), conducted by trained observers, or have indices derived from the data collected by autonomous recording devices (Crump et al., 2017, Boullhesen et al., 2019). This may limit interpretation of such a data because these measures sometimes may have poor relationships with the true population sizes, e.g., a few very active frogs may produce higher index values than many less active ones (Corn et al., 2011, Bower et al., 2014).

As an alternative acoustic survey method, calling amphibian male counts (hereafter referred as CAMC) was shown to be highly successful (Čeirāns et al., 2020). CAMC surveys allow for collection of amphibian relative population size data that can be used not only in monitoring but also in ecological studies (Čeirāns et al., 2023). However, for this type of calling amphibian data, there are no established system for community quality assessment. To fill this gap, the following questions have to be addressed: What metrics should be used in the CAMC-based community state assessments? How do these metrics respond to human pressure? What are metric values of good versus bad amphibian communities? Do the metrics characterizing different amphibian community traits have similar responses?

There is a wide array of metrics used as indicators of the community state in other vertebrate and aquatic organism groups. Thus, macroinvertebrate indices use taxonomic richness, diversity, community composition and proportions of selected taxonomic groups with certain scores (Mondy et al., 2012, Desrosiers et al., 2020, Vitecek et al., 2021). Fish indices use biomass, relative proportions of selected taxa and the age structure (Blabolil et al., 2017, Ritterbusch et al., 2022). Bird indices refer to diversity, population size, and biomass of selected taxa (Gregory and van Strien, 2010), but macrophyte indices use colonization depth, coverages of taxa with certain scores and community evenness (Croft and Chow-Fraser, 2007, Poikane et al., 2018). Some of these metrics could possibly be used in amphibian assessments as well, but their reliability should be approved.

Local amphibian communities in temperate Europe are moderately rich, with up to 18 species found in some parts of Germany and France (Silero et al., 2014). Our study area of Latvia yields 13 species, and the majority of them (n = 11) belong to the order Anura (tailless amphibians) that possess the male vocalizing activity and can be acoustically detected (Čeirāns et al., 2020). While amphibians demonstrate very diverse reproductive strategies around the globe (Liedtke et al., 2022), all the species found in Latvia have the same biphasic mode, in which both eggs and larvae develop entirely in water, while adults are semi-aquatic or terrestrial outside the spring-early summer breeding season. Here, their breeding assemblages are found mainly in sites belonging to the same broad waterbody class (*sensu* Lyche Solheim et al., 2019), i.e., small lentic waterbodies and very shallow unstratified lakes (Čeirāns et al., 2020). Certain wetland features, such as a shallow littoral zone, high cover of aquatic vegetation, and absence of predatory fish, increase species richness in the amphibian assemblages of temperate zone (Porej and Hetherington, 2005, Boissinot et al., 2019), similar to a wetland interconnections by ecological corridors and wetland clusters (Cunningham et al., 2010; Van Dyke et al. 2017). On the opposite, amphibian species richness declines in urbanized areas (Marsh et al., 2023), near roads and in the agricultural lands with reduced spatial heterogeneity (Boissinot et al., 2019), while vicinity to roads also reduces larval amphibian abundances (Hamer et al., 2021). Besides, urban habitats tend to yield more skewed amphibian communities (Knozowski et al., 2022).

In our survey, we used three major anthropogenic threats to study potentially suitable metric responses to human pressure. The first is agriculture (non-timber crops). Presently, amphibians are the most threatened vertebrates, with agriculture being the top threat affecting 77 % of their species globally (Luedtke et al., 2023). Farmland development contributes to the amphibian habitat loss (Mushet et al., 2014) and chemical fragmentation (Leeb et al., 2020a), while the pesticides used for herb protection increase amphibian mortality (Brühl et al., 2013), morphological and physiological abnormality rates (Wagner et al., 2014), cause behavioural changes (Leeb et al., 2020b) and abandonment of the contaminated breeding ponds (Wagner and Lotters, 2013). Agricultural fertilizers accelerates eutrophication of the breeding waterbodies and promotes pathogenic infections in amphibians (Johnson et al., 2007).

The second threat is infrastructure development (housing, urban, and industrial), which is among top-three global threats for amphibians (Luedtke et al., 2023). It not only contributes to habitat destruction, diminish the breeding site quality through pollution and eutrophication, and enhance amphibian kills by humans (Hamer and McDonnell, 2008), but also possesses many indirect impacts, e. g. reduced amphibian population viability by elevating disease (North et al., 2015) and helminth infection rates (Čeirāns et al., 2023), increased predation pressure by domestic and feral animals (Trouwborst et al., 2020), reduced population recruitment by the breeding behaviour disruption due to light and noise pollution (Hamer and McDonnell, 2008), and enhanced intentional releases of fish that prey on amphibian larvae and cause failure of the breeding success (Reshetnikov, 2003, Pupina et al., 2015, Pupins et al., 2023a).

The third threat, human transportation and service corridors (roads, railways etc.), is particularly significant for the temperate Europe, where it contributes to population fragmentation, reduction of gene flow, lower genetic diversity and higher genetic structuring (Covarrubias et al., 2020). Moreover, roads act as a constant extirpation risk for nearby populations and cause the mass mortality events during amphibian migrations between breeding sites, summer grounds and wintering sites (Elzanowski et al., 2009, Hamer et al., 2021).

Amphibians are susceptible to human-driven climate changes that may affect their survival, growth, reproduction and dispersal capabilities (Blaustein et al., 2010). The local weather in the temperate-zone communities affects amphibian distribution (Pupins et al., 2023a) and demography (Pilliod et al., 2022), and therefore we added the local climate factors in our study as well.

In our study we viewed the breeding amphibian communities as entities with three main dimensions or ecological traits: the overall community size (in terms of specimen abundance), the taxonomic richness, and the community evenness (in terms of distribution of specimens among taxa), which all are determinative for maintaining a high biodiversity and stability of the ecological community (Odum and Barrett, 2004, Mittelbach and McGill, 2019, Keddy and Laughlin, 2021). We hypothesised that the anthropogenically impacted sites should yield smaller and taxonomically poorer communities skewed toward one or two dominant taxa and *vice versa* should be true for the natural sites (Fig. 1). As we wanted to explore each of the traits separately, we chose 11 metrics that can be attributed to a single ecological trait and skipped diversity indices that incorporate several (e.g., Shannon-Wiener, Brillouin, Gini-Simson Index, (Magurran, 2004)).

We tested the responses of the selected metrics to five sets of predictors, that included two sets of anthropogenic pressure indicators (agricultural, human settlement, and road network land-uses within i) 100 and ii) 500 m), one mixed effect set (waterbody features, regarded here as being “mixed effect” due to the combination of anthropogenic or unknown origins and natural site features found in many sites), one natural effect set (wetlands and their features within 500 m), and the

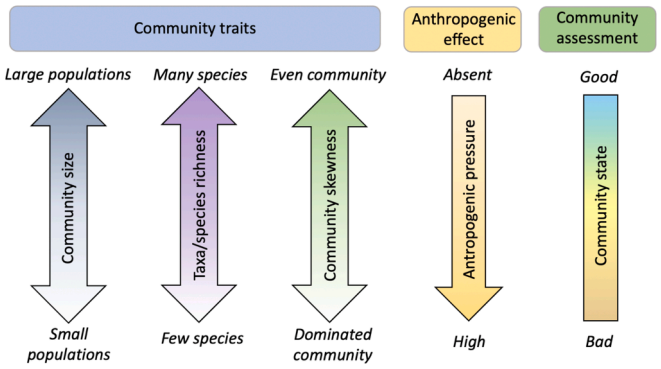

Fig. 1. Hypothesised coherences between breeding amphibian community traits, anthropogenic impact, and community state in our study.

climate variable set. We looked for metrics that i) are most prone to human pressure and ii) have best congruences with metrics characterizing other community traits. We also aimed at finding out the site and surrounding land-use characteristics linked to low or high-quality amphibian community state assessments, and studied differences in responses of community traits to the external factors. The results of our study could be further used in CAMC-based breeding anuran community assessments in monitoring, conservation and habitat restoration programmes and wetland ecological quality state assessments.

2. Methods

2.1. Field work

Field work was carried out from 2016 to 2023 at 400 amphibian breeding sites located all over Latvia to cover as much variation as possible in abiotic and biotical features of both waterbodies and the surroundings (Fig. 1). For each site first two CAMC surveys were made in early–mid April, with 7–10 day intervals between visits, to cover the maximum activity of the early-breeding species. Then, two more surveys were conducted in the warmest periods in mid-May and mid-June, accounting for the later-breeding species during their maximum activity. In each period we selected the warmest nights according to the weather forecasts and conducted surveys during the first half of the night.

The survey method we used (Čeirāns et al., 2020), is based on spotting (by listening sounds) the exact locations of the calling frogs in order to distinguish separate calling males and on counting them accordingly. We approached the wetlands close enough to locate calling male positions, and moved along the section with calling amphibians, where we registered only the calling males and not the amphibians moving around or in water. Typically, this listening observation was done over a period of five minutes, unless we had strong periodic external sounds (traffic etc.) during which we paused the survey. At large wetlands with distant groups of calling males, we spent 5 min for each group. In the cases when overlapping calls were coming from poorly accessible locations, the number of calling frogs (CAMC) in a wetland was recorded as an average between the minimum and maximum estimates of that given night. For each site and each taxa we used the CAMC of the most productive of the 4 nights. The Water Frog (*Pelophylax*) species complex was identified only to the genus level, because their species separation may produce many errors when based solely on audible data (Fedorova and Shabanov, 2023).

All the sites were revisited during daytime after full growth of the vegetation in late June–early September for the waterbody descriptions, and to take pictures of the essential features, such as waterbody and shoreline vegetation. We filled field data forms that included measurements of maximum depth, the composition of the bottom substrates (in categories as follows: 1 – sand, clay worsts, 2 – partially mudded sand, clay, 3 – mud), slopes of banks (1 – steep, 2 – inclined, 3 – flat, 4 – marshy), estimations of percentage covers of submersed, floating and emergent vegetation and proportion of banks overgrown with shrubs and trees.

2.2. Amphibian community metrics

We used three groups of community metrics for population size, as well as species richness and evenness. Although in our study we detected all the anuran taxa found in Latvia, only four of them, the Common Toad (*Bufo bufo* (Linnaeus, 1758)), the Moor Frog (*Rana arvalis* Nilsson, 1842), the Common Frog (*Rana temporaria* Linnaeus, 1758) and the Water Frog species complex (*Pelophylax* spp. = *Pelophylax esculentus* complex), were common and present in at least 150 sites. For the community size measurement, we used CAMC of all the taxa CAMCs summed ($CAMC_{tot}$) and CAMC separately for each of the four common taxa. We also tested the possibility of using calling male density as the community size metric, which was calculated as $CAMC_{tot}$ per maximum surface area of the waterbody (males/ha).

Five other anuran species were rare, with part of the study sites being placed outside their natural species ranges in Latvia. As a result, our data set contained only 20 sites with Spadefoot Toad (*Pelobates fuscus* (Laurenti, 1768)), 11 with Oriental Treefrog (*Hyla orientalis* (Bedriaga, 1890)), 4 with Fire-Bellied Toad (*Bombina bombina* (Linnaeus, 1761)), 3 with Green Toad (*Bufotes viridis* (Laurenti, 1768)) and 1 with Natterjack Toad (*Epidalea calamita* (Laurenti, 1768)) record. Therefore, we used two community richness metrics: i) total taxa (species and *Pelophylax* spp.) richness (S) and ii) community completeness (Cc). The latter was calculated by taking into account only the four common taxa, whose natural ranges covered all the study sites, and was expressed as the proportion of the common taxa present in the site.

Among the wide array of published evenness indices, we chose four: i) the Pielou Index, which is the oldest and probably the most often used (Magurran, 2004), ii) the Heip Index, which is better suited for low diversity communities (Heip and Engels, 1974), iii) the Williams Index that possesses mathematically optimal properties (Kvålseth, 2015) and iv) the Berger-Parker index, which is a simple but effective dominance measure (Berger and Parker, 1970). All four indices are defined from 0 to 1, with 1 for perfect evenness in the former three and complete dominance in the dominance index. To have the same vector in all metrics we switched to the reciprocal form in the latter.

The Pielou index (J'), the Heip index (H) and Williams index (W) were calculated as follows:

$$J' = H'/\log S \quad (1)$$

$$H = (e^{H'}-1)/(S-1) \quad (2)$$

$$H' = -\sum_i p_i * \log p_i, \quad (3)$$

$$W = 1 - [(S^* \sum_i p_i^2 - 1)/(S-1)]^{1/2} \quad (4)$$

where H' is the Shanon-Wiener Index, S – taxon richness, p_i – the proportion of individuals belonging to the *i*th taxon.

The reciprocal dominance index (1-d) was calculated as follows:

$$1\text{-}d = 1 - N_{max}/N \quad (5)$$

where N_{max} is the number of individuals of the most abundant taxon and N is the total number of individuals.

2.3. Predictors

We used five sets of predictors in our data analysis, each characterizing a different ecological factor:

i) waterbody – nine variables characterizing site-specific abiotic and biotic properties, which were derived from the field site descriptions (depth, substrate type, shore inclination, coverages of

emergent, floating and submersed vegetation), orthophoto maps (waterbody maximum area, proportion of the permanent part of the waterbody) or both (proportion of the shoreline coverage by the wooded vegetation);

ii) wetland – three variables were considered within a 500 m wide belt around the shoreline: proportion of wetlands (by coverage), average proportion of a permanent water table per wetland and average size of a wetland (ha). A wetland was defined as any site where water covers the soil, or is present either at or near the surface of the soil at least in part of the year, e.g., mires, marshes, periodical or permanent waterbodies that could be identified from orthophoto maps;

iii) and iv) anthropogenic land use within 100 m and 500 m, respectively, with three variables in each: proportions of agricultural land, road, and human settlement coverages. Agricultural lands were namely fields under crop cultivation with visible evidence of regular herbaceous cover removal. As human settlements were defined any buildings with home yards;

iv) the climate factor was characterized by two temperature variables (yearly average minimum and maximum temperatures), three period length variables (days with max temp < 0 °C, vegetation period, days with max temp > 25 °C), and two precipitation variables (total precipitation and snow thickness).

Land use and waterbody area measurements were performed on digital orthophoto maps using Google Earth Pro software (Google LLC, Mountain View, California, USA) online tools. Manual measurements allowed us to verify the land use around each site in map sequences taken from several years. Climate data (averages for 1961–2010) were acquired from the SLLC "Latvian Environment, Geology and Meteorology Centre" free online climate change analysis tool (www4.meteo.lv/klimatariks).

2.4. Data analysis

We used generalized linear models (GLM) of i) negative binomial regression type (NB) (parametrization of dispersion: a function of the expected mean) when the response variables were CAMC with no zeroes ($CAMC_{tot}$), of ii) zero-inflated negative binomial regression (ZNB) type (constant inflation option, logit model for characterizing zeros) for the taxon CAMC with many zeroes (CAMC for separate taxa), and of iii) fractional regression (FR) type (probit identity) for metrics when outcomes were between 0 and 1 (Cc, J', H, W, 1-d indices).

The use of densities over counts could be justified only if the former reflects the relative population size, – in our study the CAMC data. To test this, we first performed NB for both $CAMC_{tot}$ and obtained calling male densities per waterbody area and compared their regression models separately in four waterbody size quartiles. Next, we searched for the statistically significant predictors for our amphibian community metrics and ranked ecological factors accordingly to their corrected Akaike information criterions (AICc) to find out the most and the least important ones. We performed regressions separately for each metric and each ecological factor and for the combination of all the factors. In the model selection, we used the backward elimination approach, where we stepwise removed predictors with the smallest z-scores and $p > 0.05$ from the initial set of ecological factors until we identified models with the lowest AICc values. In our rankings we searched for the best models only and did not include other statistically significant lower ranked models for the same ecological factor.

In the following step, we chose statistically significant anthropogenic variables for the separation of the least impacted (hereafter referred to as natural) vs the anthropogenically most impacted sites (referred as strongly impacted) and applied the best and the worst quartile approach where the natural sites were the sites having all these anthropogenic variables in the lowest quartile while the strongly impacted sites were in the highest quartile. Then, we compared amphibian community metrics of both site groups using box plots and Wilcoxon rank-sum (Mann-Whitney) test. And, finally, we checked the agreement between metrics in how they classified sites for top or the worst-quality states by site arrays and their overlap. The overlap between different metrics was calculated as follows:

$$O = C/(T\text{-}C), \tag{6}$$

where O is the overlap, C is number of common sites and T is sum of sites in both sample sets.

All statistical analyses were performed on STATA 14.2 (StataCorp LLC, College Station, Texas, USA) with the Stata Technical Bulletin insertion 'Scalar measures of fit for regression models' (developed by J. Scott Long, Indiana University and Jeremy Freese, University of Wisconsin-Madison, USA).

The overview of all the data used in the study is given in Supplement 1.

3. Results

3.1. Testing the usefulness of the density data

Negative binomial regression models showed incongruity in responses between calling male counts (CAMC) and respective male densities (Table 1). In the smallest waterbody quartile (with surface areas of ≤ 0.05 ha), the density was negatively correlated with the waterbody area ($p < 0.0001$) while CAMC showed absence of any relationship ($p = 0.95$). In the larger waterbody size quartiles, density followed the same trend with a weaker relationship in the largest sizes, while CAMC had an opposite general trend with a positive correlation and stronger relationships in the largest waterbodies (Table 1). This meant that the calling male density strongly contradicted relative population size data. Therefore, we excluded calling male density from our further analyses.

3.2. GLM and ecological factor ranking

Full statistics for the highest ranked GLM models for different amphibian community metrics as response variables and ecological factor variables or their combinations as predictors are given in Supplement 2. Model rankings according to their AICc are given in Table 2. Waterbody features (WB) were consistently the highest ranked ecological factor in all the taxa and all the metrics and had the highest average goodness-of-fit among the GLM models for metrics of all three community traits (Table 3). Amphibian relative population sizes were mostly determined by the waterbody dimension predictors, while richness and community evenness metrics were determined by macrophyte coverage (Table 4). Anthropogenic land use predictors were generally next in ranks, as they were second after WB in effect on community richness and evenness, and in two out of four common species population sizes. Wetland factor (WTLD) was the second most important for total amphibian community size and the population sizes of two other common taxa, at the same time having small effects on the Common Toads (*Bufo bufo*), or community richness and evenness. The climate was among the least important factors in all three amphibian community traits, where it was more important for community evenness, followed by richness, and had the smallest effect on community size.

3.3. Discriminating natural vs strongly impacted sites by metrics

Three anthropogenic land-use predictors showed adverse effects on the amphibian communities in the selected GLM models, two of them being road network within 100 and 500 m, and third being human settlements within 500 m (Table 4). Using their best and worst quartiles approach, 32 sites were classified as natural and 42 as strongly impacted. The Wilcoxon rank-sum test showed statistically significant

Table 1
Comparison of negative binomial regression statistics between $CAMC_{tot}$ and the calling male density in their response to waterbody area (maximum area, ha) in four waterbody size quartiles.

Quartile	Predictor range		Counts				Densities			
	Min	Max	LR Chi^2	Prob > chi^2	Pseudo R^2	z	LR Chi^2	Prob > chi^2	Pseudo R^2	z
Q1	0.0018	0.0535	0.00	0.9480	0.0000	0.07	39.15	<0.0001	0.0315	−6.79
Q2	0.0536	0.1400	4.76	0.0292	0.0087	2.19	2.09	0.1480	0.0020	−1.46
Q3	0.1438	0.4801	0.82	0.3652	0.0013	0.91	14.67	0.0001	0.0160	−4.03
Q4	0.4906	13.1265	13.01	0.0003	0.0158	3.08	10.62	0.0011	0.0136	−3.70

Table 2
ΔAICc in generalized linear models (GLM) with different amphibian community metrics as response variables and ecological factor variables or their combinations as predictors; model ranks indicated as exponents.

Response variable	GLM type	Single ecological factor models					Multiple ecological factors models			
		WB	WTLD	A100	A500	CL	WB + A500	WB + A500 + CL	WB + A100 + CL	WB + CL
Population/community size										
Bufo	ZNB	0.051^{2}	0.132^{6}	0.081^{3-4}	0.082^{3-4}	0.122^{5}	0.000^{1}	–	–	–
Pkl	ZNB	0.000^{1-2}	0.124^{3}	0.163^{5}	0.151^{4}	0.175^{6}	0.000^{1-2}	–	–	–
Rarv	ZNB	0.000^{1}	0.061^{3}	0.079^{4}	0.093^{5}	0.119^{6}	–	–	–	0.013^{2}
Rtemp	ZNB	0.005^{2}	0.102^{4}	0.130^{5}	0.095^{3}	0.238^{6}	0.000^{1}	–	–	–
$CAMC_{tot}$	NB	0.000^{1}	0.226^{3}	0.278^{4}	0.287^{5}	0.363^{6}	–	0.062^{2}	–	–
Community richness										
S	NB	0.000^{1}	0.032^{4}	0.017^{3}	0.015^{2}	0.046^{6}	–	–	–	0.034^{5}
Cc	FR	0.000^{1}	10.866^{6}	8.355^{5}	5.329^{3}	7.092^{4}	–	–	–	0.097^{2}
Community evenness										
J'	FR	0.000^{1}	23.516^{6}	14.704^{4}	11.359^{3}	20.229^{5}	–	–	–	3.722^{2}
H	FR	6.751^{2}	25.006^{6}	17.381^{4}	13.916^{3}	21.859^{5}	–	–	0.000^{1}	–
W	FR	0.000^{1}	11.056^{6}	6.499^{4}	2.196^{2}	9.725^{5}	–	–	–	2.392^{3}
1-d	FR	1.806^{2}	8.485^{5}	5.604^{4}	3.367^{3}	9.675^{6}	–	–	–	0.000^{1}

GLM types: ZNB – zero-inflated negative binomial regression, NB – negative binomial regression, FR – fractional regression; ecological factors: WB – waterbody, WTLD – wetlands within 500 m distance, A100 – anthropogenic land use within 100 m distance, A500 – same within 500 m distance, CL – climate; response variables: Bufo – CAMC for Bufo bufo; Pkl, Rarv, Rtemp, $CAMC_{tot}$ – same respectively for Pelophylax, Rana arvalis, Rana temporaria, and all calling amphibians summed, S – taxa richness, Cc – community completeness, J' – Pielou index, H – Heip index, W – Williams index, 1-d – inverse dominance index.

Table 3
Averages of McFadden's adjusted pseudo R^2 (±SD)*1000 for amphibian community trait metrics in single ecological factor GLMs.

Ecological factor	Community size (n = 5)	Community richness (n = 2)	Community evenness (n = 4)
Waterbody	42.8 (±14.9)	19.0 (±4.2)	34.5 (±13.6)
Wetlands within 500 m	12.8 (±8.5)	0.0 (±0.0)	4.8 (±1.7)
Anthropogenic land use within 100 m	10.2 (±5.6)	5.0 (±0.0)	15.7 (±6.4)
Anthropogenic land use within 500 m	11.4 (±4.4)	7.5 (±3.5)	22.5 (±7.0)
Climate	4.4 (±6.3)	5.5 (±6.4)	7.5 (±5.8)

differences between the two site groups in all 11 metrics ($p < 0.01$) (Table 5) and they all had higher values in the natural sites (Fig. 2). $CAMC_{tot}$ were better for discriminating natural vs impacted sites than the CAMC of separate taxa, as was shown by their z-scores (5.4 vs 2.8–4.4) and the boxplots (Fig. 2). Among the two community richness metrics, species richness (S) had a higher z-score (4.2 vs 3.6) but also higher variability among natural sites (Fig. 2). Four community evenness metrics showed similar results in the Wilcoxon rank-sum test, with the highest z-score found in the reciprocal dominance index (1-d) (Table 5). Evenness indices (J', H, W) notably differed in their skewness toward the upper extreme with the most skewed being Pielou (J'), and the least skewed being the Williams (W) Index (Fig. 3).

3.4. Agreement between metrics

We set top-quality and worst-quality community state thresholds by the metrics' tenth best and worst percentiles in natural and strongly affected site pools, respectively (Table 5). CAMC of four common taxa all had 0 as a threshold for the worst quality-state that resulted in 90–250 such sites out of 400 (or 23–63 %) sites in total. That suggested that $CAMC_{tot}$ with only 37 such sites (or 9 % from the total) was a much more reasonable community state indicator. As for attribution to the top-quality state, there was a much better agreement with 9–15 such sites found in CAMC of individual taxa and 12 in $CAMC_{tot}$.

There was a good overall agreement between two taxonomic richness metrics for highest and worst quality sites, with 20 % more sites classified as top-quality accordingly to taxon richness (S) than by community completeness (Cc) (57 vs 47, overlap 0.82), making the latter a stricter metric, and almost full agreement between both for the worst-quality state (overlap 0.99).

All four evenness metrics were identical in site attribution to the worst-quality state, where they all produced a value of 0 (Table 5). As for the top-quality state, the Pielou Index classified the largest number of sites for such a state and had better overlap with total calling male counts and community completeness than the less skewed Heip and Williams Indices (Table 6). However, the best results in terms of coherence with both had the reciprocal dominance index (Table 6).

The general pattern was that many more sites could be classified as being in the worst-quality state than in the top-quality state, with the community size being detrimental for reaching consensus on site classification for the bad state. The top-quality sites had no such detrimental qualifier as they showed small overlap between the community size and the community evenness assessments, with both having good overlap with the community richness assessment (Fig. 4).

4. Discussion

4.1. Reliability of metrics

The population density was an unreliable metric for describing

Table 4
Highest Z-scores for the statistically significant predictors in the GLMs.

Ecological factor	Predictor	Bufo	Pkl	Rarv	Rtemp	Tot_c	S	Cc	J'	H	W	1-d
WB	a_max		6.67	4.44	5.12	8.11		3.53				
	a_perm			−4.54	−5.79	−6.73						
	banks		3.63	4.37		6.64	3.08		4.02			4.38
	depth	6.18										
	vg_subm					4.64	4.23	6.74	5.24	5.31	5.10	5.76
	vg_emerg									3.63	3.70	2.13
WTLD	wtld_prc		5.12	4.42		6.77	2.44	2.35	3.00	2.92	2.71	3.64
	wtld_prm	2.32	−3.69		−4.19	−5.22						
	wtld_size				3.77							
A100	road1	−5.14	−4.27	−5.18	−4.00	−7.66	−3.34	−4.25	−4.34	−4.25	−4.05	−4.23
A500	hum2	−5.12		−4.48	−5.78		−3.28	−6.45	−4.18	−4.22	−4.49	−4.58
	road2		−4.93			−7.48						
CL	t_min	−4.90		−4.02	−2.11	−5.98	−3.06	−4.71				−4.55
	t_max	−3.65				−5.26		−3.83	−3.38	−3.35	−3.11	−4.35
	freez_d	−5.47		−4.09		−5.43	−2.45	−4.61				−5.45
	veget_d				−2.21							
	summer_d					5.58						3.32
	precip				3.20							
	snow		−3.59		−3.63							

For abbreviations of response variables from the top row and ecological factors from the first column see Table 2; predictors: a_max – waterbody maximum area, a_perm – permanent part of the waterbody (min/max area), banks – shore inclination category (from 1 – steep to 4 – marshy), depth – max depth of the waterbody, vg_subm – submersed vegetation cover (%), vg_emerg – emergent vegetation cover (%), wtld_prc – coverage of wetlands within 500 m distance, wtld_prm – proportion of permanent water table in these wetlands, wtld_size – average size of these wetlands, road1 – coverage of roads within 100 m distance, hum2 – coverage of human settlements within 500 m distance, road2 – coverage of roads within 500 m distance, t_min – yearly average min temperature, t_max – yearly average max temperature, freez_d – days with max temperature < 0 °C, veget_d – length of vegetation period, summer_d – days with max temperature > 25 °C, precip – yearly total precipitation, snow – average snow cover.

Table 5
Some threshold values and summary statistics for a natural vs strongly-impacted site comparison.

Metric	Natural sites (n = 32)			Strongly impacted sites (n = 43)			Wilcoxon rank-sum (Mann-Whitney) test	
	10th %ile	Median	90th %ile*	10th %ile **	Median	90th %ile	z	Prob > |z|
Bufo	0.00	1.25	7.50	0.00	0.00	1.00	2.959	0.0031
Pkl	2.55	6.25	12.50	0.00	2.50	5.40	4.359	<0.0001
Rarv	0.00	0.00	11.35	0.00	0.00	1.40	2.775	0.0055
Rtemp	0.00	1.50	15.80	0.00	0.00	3.40	3.129	0.0018
$CAMC_{tot}$	0.25	14.50	46.80	1.00	4.50	11.20	5.391	<0.0001
S	1	3	4	1	1	3	4.212	<0.0001
Cc	0.000	0.75	1.00	0.25	0.25	0.75	3.565	0.0004
J'	0.000	0.896	0.975	0.000	0.000	0.945	4.095	<0.0001
H	0.000	0.837	0.960	0.000	0.000	0.901	4.028	0.0001
W	0.000	0.682	0.851	0.000	0.000	0.763	4.061	<0.0001
1-d	0.000	0.488	0.672	0.000	0.000	0.531	4.211	<0.0001

For abbreviations of metrics see response variables under Table 2. * Top-quality community lower threshold ** Worst-quality community upper threshold.

amphibian breeding waterbody communities despite being widely used in macroinvertebrate community assessments (e.g., Gjoni et al., 2017; Rumschlag et al., 2023) and evaluation of fish stocks (e.g. Willis et al., 1993, Blabolil et al., 2017). For amphibians, such unreliability may be due to relatively small population and wetland habitat sizes where the calling male density can be more affected by random effects than by the population size itself. Thus, the amphibian density may strongly fluctuate simply due to waterbody area changes driven by the weather conditions of a given year. The density also lacked ecological plausibility: for instance, a population of one calling male in a 0.01 ha (10 x 10 m) waterbody produces the same density as 15 calling males in a 0.15 ha (50 x 30 m) waterbody, even though the first indicates a bad population state and the latter a good one. Hence, using the number of calling males (CAMC) appears to be the only reliable option for characterizing the community size component in our study.

As for the community richness component, both taxonomic richness (*S*) and community completeness for the common taxa (*Cc*) were reliable and showed similar results. Although the maximum possible scores of the former varied from 4 to 7 depending on site geographical position, it probably did not have a notable effect on the results due to the overall rareness of the taxa with limited ranges. Still, for surveys containing many sites with strong natural variation in potential taxonomic composition (e.g., sites from several biogeographic regions), community completeness should be preferred over simple taxonomic richness.

All the evenness metrics used in our study discriminated natural and strongly impacted site pools reasonably well, although mathematically the best metric (Williams Index, W) (Kvålseth, 2015) was not the most suitable in the natural communities (see below). The most skewed metric among evenness indices (J', H, W) was the Pielou Index (J'), which overestimated the number of top-quality scores (15 % of all the sites), while the least skewed, the Williams Index (W), showed poor agreement with metrics of other community traits, and the Heip Index (H) showed intermediate properties. Our recommended choice as a single evenness measure would be the Berger-Parker dominance index (in its reciprocal form), which classified much smaller number of sites as being at the top-quality state (2.5 % of all), while also showing by far the best agreement with community completeness and relative population size metrics (Table 6). The dominance index performed better probably because it weights towards a single dominant taxon and not the identical distribution of all individuals that permits more variation among non-dominant taxa, thus better fitting natural communities where large multi-species assemblages almost never contain identical numbers of

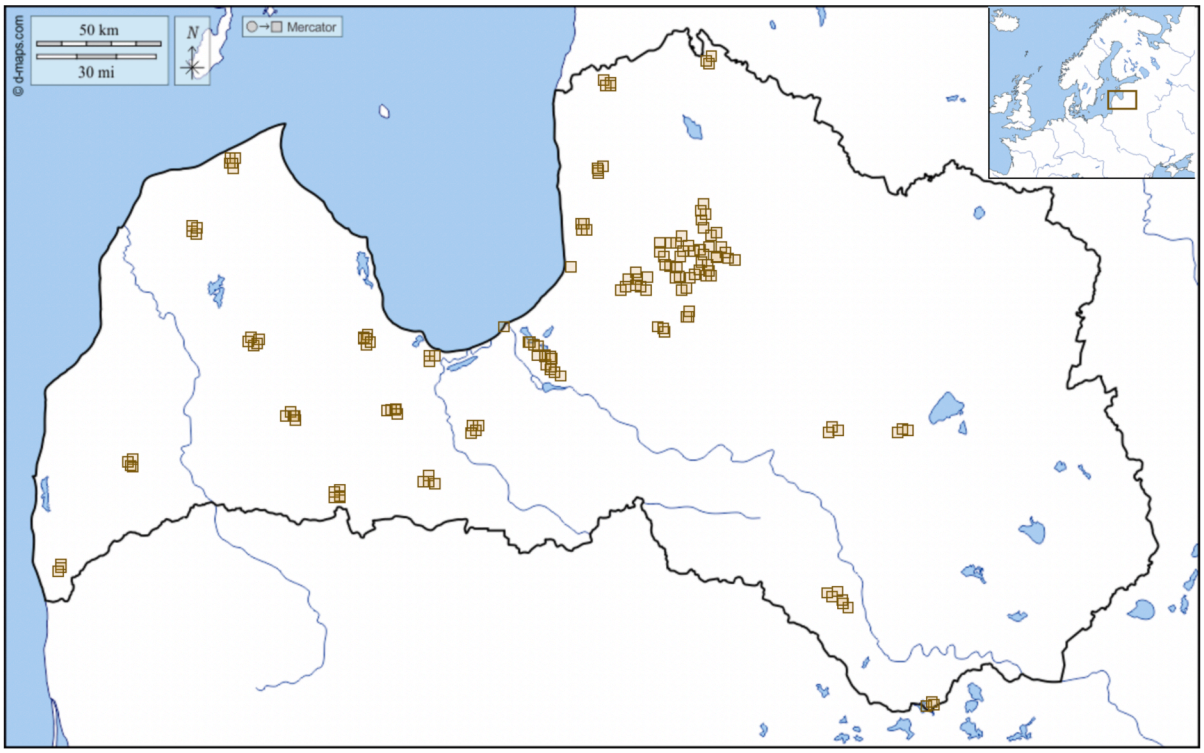

Fig. 2. Location of the study sites in Latvia (base map taken from the free online resource d-maps.com).

individuals in each taxon.

4.2. Community responses to ecological factors

The predictors in the GLM models allowed us to identify several sub-factors or the same factor that could be traced in several predictor sets. Thus, relative population sizes of three taxa (both *Rana* species and *Pelophylax* sp.) positively correlated to predictors characterizing large but semi-permanent waterbodies with flat banks suggesting the presence of a vast littoral zone. One taxon (*Bufo bufo*) preferred deeper waterbodies, while community taxonomic richness and evenness responded to the development of macrophyte (especially submersed) vegetation. The road factor was adverse for communities within the 100 m and 500 m zones, with much more negative responses in the closer range. The climate factor was among the least important ones, probably due to its general similarity among sites throughout Latvia, and combinations of predictors emerged in its GLM models that were more difficult to interpret, e.g., contradicting negative responses by communities to both maximum temperatures and many cold days that suggested a preference for the medium climate with no extreme heat or cold.

Community members showed good coherence between their ecologies and responses to predictors. Thus, the presence of wetlands within 500 m was among the high-ranked factors in semi-aquatic *Pelophylax* sp. and *Rana arvalis* frogs, but was the lowest-ranked factor in the toad *B. bufo*, following the gradient in their "terrestriality" (Čeirāns et al., 2021). In *B. bufo*, road and human settlement predictors were higher ranked than in the other taxa, corresponding to its higher vulnerability to roadkills during mass breeding migration events (Elzanowski et al., 2009; Kolenda et al., 2019).

There were common and distinctive patterns among the community traits in their responses to ecological factors (Fig. 5). While the site characteristics were the most important factor for all the traits, macrophyte vegetation had a greater effect on community evenness and taxonomic richness, while waterbody dimensions largely determined overall community size. Evenness was more affected by the human pressure than the other community traits, which agrees with the generally accepted concept of dominance of a few generalists in anthropogenically disturbed habitats (Hillebrand et al., 2008; Nordberg and Schwarzkopf, 2019). *Pelophylax spp*. was the only taxon regularly present in strongly impacted sites (Table 5), probably being the most "generalized" among them. Still, in our study we did not have sufficient data for some other species (*Pelobates fuscus, Bufotes viridis*) that are frequently found in anthropogenically-transformed habitats in other parts of the range (Bossman and van den Munckhof, 2006; Landler et al., 2023). However, as an isolated ecological indicator the community evenness should still be used with some caution due to the possibility of type I errors. For instance, a site with two taxa containing one specimen of each will score as a perfectly even community, even when it represents the presence of a couple of taxa in a bad population state.

4.3. Implications for conservation efforts

While wetland restoration projects can play important roles in amphibian conservation (Burrow and Lance, 2022, Pupins et al., 2023b), they seldom set quantitative targets for desirable population sizes, especially regarding species assemblages and not separate target or "umbrella" species. While at its worst quality state in our study calling male assemblages contained only one male (most often from the Water Frog species complex [*Pelophylax* sp.]), the community top state could be described as a taxa assemblage of 4 species or more, with more than 45 calling amphibians counted in total and at least 7–15 ones from individual taxa, where the dominant species yields no more than one-third of all individuals. This can be set as a kind of general target for amphibian conservation efforts in wetland restoration projects of Baltic region, whose achievement can be verified by a few relatively brief and resource-efficient CAMC acoustic surveys.

Target features for the restored amphibian breeding waterbodies typically are the presence of a vast shallow-sloped littoral zone with macrophyte vegetation and the absence of fish (Brown et al., 2012, Shulse et al., 2012). Our study suggests that the wetland size should always be considered as well, because larger wetlands can sustain larger amphibian groups that may act as a core for the *meta*-population in a

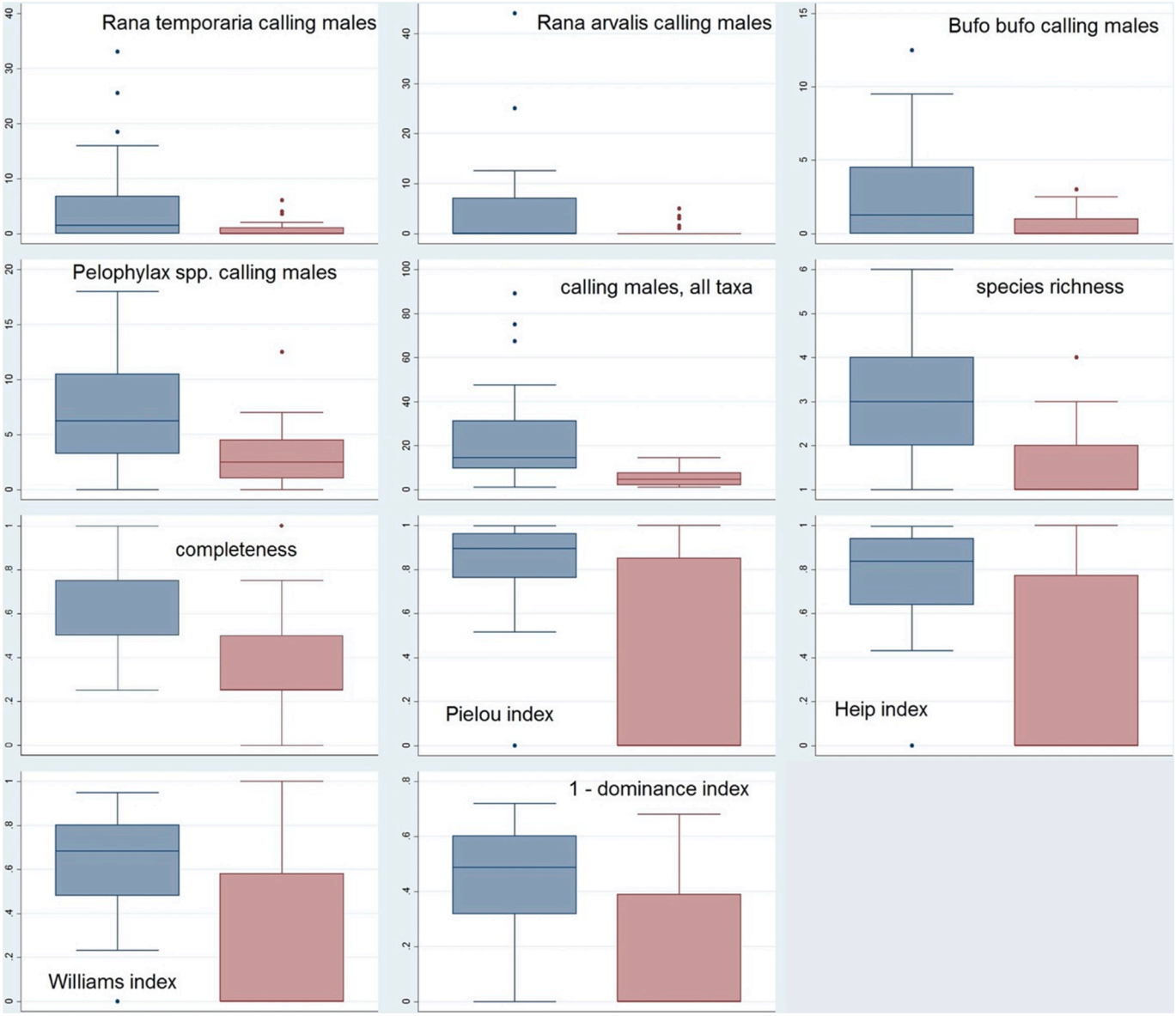

Fig. 3. Box plots for natural (blue) and anthropogenically impacted (red) site comparison in 11 metrics registered or calculated in our study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Comparison of agreement of four evenness metrics with population size (Tot_c) and completeness (Cc) metrics for the top-quality community state.

Group	Metric	Sites classified as yielding the top-quality community			
		By the given metric only (n)	Overlap with $CAMC_{tot}$	Overlap with Cc	Overlap with both $CAMC_{tot}$ and Cc
Community evenness metrics	J'	60	0.000	0.039	0.000
	H	48	0.000	0.032	0.000
	W	17	0.000	0.016	0.000
	1-d	10	0.018	0.140	0.015
Selected other metrics	$CAMC_{tot}$	12	–	0.157	–
	Cc	47	0.157	–	–

larger area (Marsh and Trenham, 2001, Schooley and Cosentino, 2016). However, the wetland size factor is typically neglected in restoration projects, probably because the creation of a larger wetland is more costly and often does not contribute to the fulfilment of quantitative targets (i.e., the number of restored wetlands) set by project applications. While small wetlands are still important (Semlitsch and Bodie, 1998, Kim et al., 2022), those with areas of less than 0.05 ha showed random amphibian population sizes in our study (Table 1), which may indicate their sub-optimal properties.

Further, the markedly adverse effect of nearby roads that was found in our study is sometimes ignored in restoration site selection, since roads ease access for the site management. The road network is usually outside the lists of the main large-scale anthropogenic threats (Cordier et al., 2021) despite much evidence for their devastating effect on amphibian populations (Clipp and Anderson, 2014; Hamer et al., 2021; Puky, 2006). In our study, the transportation corridors (roads) was a major anthropogenic threat comparable to the infrastructure development (housing, urban, and industrial), and more important than the agriculture, whose land-use variables were absent in our top-selection GLM models. In the study area of Latvia, where roads lack amphibian passages and their construction is not forced by the national legislation, it is a factor of particular concern since it contributes to population declines (Čeirāns and Pupins, 2019).

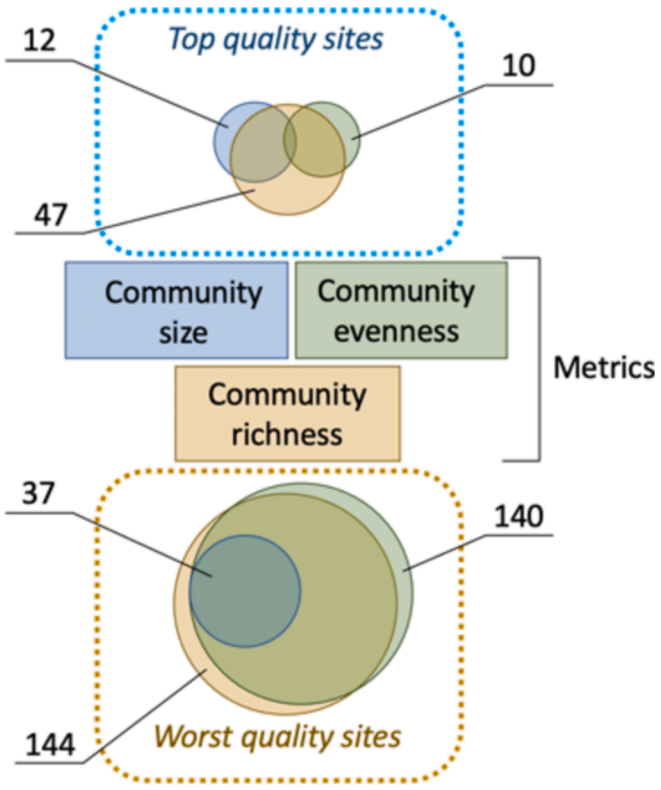

Fig. 4. Venn diagrams for sites classified as having the top and worst quality states by selected community size (represented by $CAMC_{tot}$), richness (Cc) and evenness (d-1) metrics.

4.4. Use in the ecosystem quality assessment systems

Being a prominent part of freshwater ecosystems, amphibians are potentially good bioindicators since they possess a set of suitable properties, such as good response to anthropogenic impact, abundance, well-studied biology and public recognition (Holt and Miller, 2010). There are many other freshwater indicators as well, which can be arranged according to the concept of an ecological pyramid and energy flow, where different trophic levels and ecological niches can be described by different indicators, thus better characterizing the whole ecosystem. An example of such an approach is the sets of indicators used in ecological state classification in the European Water Framework Directive, where, for instance, lake ecosystems are evaluated by a metric set containing of phytoplankton, macrophyte, phythobentos, benthic macroinvertebrate and fish metrics (Poikane et al., 2015). For such assessment systems, amphibians could replace fish as bioindicators in smaller, especially semi-permanent or ephemeral waterbodies of temperate Europe, where fish communities can be largely depauperated (Kutsokon et al., 2021) or absent. Another interesting option is to use CAMC-based metrics in the ecosystem service value indicator systems, e.g., for the open-air semi-natural aquaculture and fishery ponds, where fish are stocked and should not be used as part of the evaluation system; such commercially-operated ponds can still be good amphibian habitats under certain circumstances (Kloskowski, 2010).

4.5. Conclusion

The use of calling amphibian male counts (CAMC) produces amphibian relative population size data in a cost and resource efficient way that allows coverage of many sites, does not require any special devices and yet still utilize standardized metrics to characterize the community state of anuran amphibians in temperate Europe. Unless the aim is to study/monitor taxa separately, we recommend using three metrics that cover three main dimensions of breeding amphibian associations; the total number of calling males in all taxa ($CAMC_{tot}$) as a measure of the community size, the community completeness (Cc) as a measure of the taxonomic richness and the reciprocal form of the Berger-Parker dominance index (1-d) as a community evenness measure.

It should also be considered that local site characteristics can strongly affect the results, especially in achieving scores characterizing high community quality. In contrast, strongly anthropogenically impacted sites will most likely show poor amphibian communities even in dimensionally and structurally perfect sites, meaning that calling amphibian assemblages perform better as human pressure indicators than ecosystem natural state indicators. Our CAMC-based evaluation system could be used as a basis for the in-depth studies on amphibian community responses to various environmental factors, development of amphibian metric-based wetland type-specific ecological state classifications, and could also be tested for suitability in other regions and climate zones.

CRediT authorship contribution statement

Andris Čeirāns: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mihails Pupins:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Arturs Skute:** Writing – review &

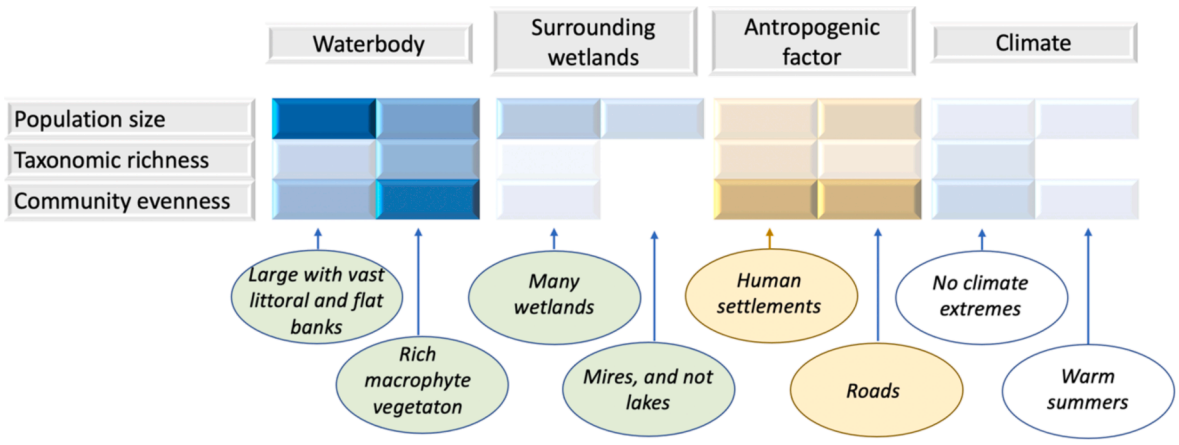

Fig. 5. Synopsis of the most important ecological factors affecting breeding amphibian associations in our study; blue colour indicates a positive effect, brown indicates a negative effect; intensity of the colour corresponds to the strength of the factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Oksana Nekrasova:** Writing – original draft, Investigation, Conceptualization. **Muza Kirjusina:** Writing – review & editing, Conceptualization. **Isabelle Combroux:** Investigation. **Corinne Grac:** Investigation. **Yuriy Kvach:** Writing – review & editing, Conceptualization. **Karina Anna Elisabeth van der Zon:** Investigation. **Kathrin Theissinger:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Jean-Yves Georges:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.112771.

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