




A standardized multi-method survey to enhance characterization of riparian invertebrate communities

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Abstract

Riparian invertebrate communities are species rich, but variability in sampling methods hampers assessment of their distributions and inference of the quality of their habitats. To reduce this variability, a standardized, multi-method survey protocol was recently developed. Here, riparian beetle community surveys conducted before and after the protocol's introduction were used to evaluate its effectiveness in characterizing communities and in limiting variability among surveys. Use of the standardized protocol reduced variability in sampling effort, and this had a limited effect on estimates of taxonomic richness. Surveys using the protocol captured significantly more species than surveys done before its introduction, evidencing the benefits of standardized multi-method survey protocols. Our study highlights that standardized multi-method survey protocols may enable identification and prioritization of sites requiring management to improve habitat quality. As such, we recommend the integration of such protocols into monitoring programmes, to enhance protection of biodiverse invertebrate communities in vulnerable riparian habitats.

KEYWORDS

biomonitoring, condition assessment, conservation status, environmental assessment, riparian beetle, riparian zone, river survey

1 | INTRODUCTION

Riparian zones are important transitional habitats between aquatic and terrestrial ecosystems, and enhance biodiversity by supporting species with a wide range of environmental preferences (Décamps et al., 2009; Naiman et al., 2013). Their invertebrate biodiversity has fostered considerable interest in the ecology and conservation of riparian habitats (Manderbach & Hering, 2001; Andersen & Hanssen, 2005; Baiocchi et al., 2012; Ramey & Richardson, 2017). In countries including the UK, riparian habitats support numerous rare, scarce and threatened species of high conservation value, particularly beetles (Coleoptera: Eyre & Lott, 1997; Eyre et al., 2000; Sadler &

Bell, 2000), true flies (Diptera: Godfrey, 1999; Falk & Crossley, 2005) and spiders (Araneae: Eyre et al., 2002). Such taxa are often associated with exposed riverine sediments, which represent relatively natural riparian habitats within increasingly managed floodplain landscapes (Eyre & Lott, 1997; Schindler et al., 2016). In addition, riparian trees, fens and human-made flood meadows support diverse invertebrate assemblages (Hammond, 1998; Lott, 2003), highlighting the need for effective monitoring and management of riparian habitats. However, assessment of riparian biodiversity—which enables inference of habitat quality—is often hampered by the limited evidence available to inform management decisions (Sutherland et al., 2004, 2006; de Sosa et al., 2018).

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Terrestrial beetle communities can reflect variability in environmental factors such as sediment and vegetation (Eyre & Luff, 2002; Eyre et al., 2002; Ramey & Richardson, 2017; Sadler et al., 2004), hydromorphology (Paetzold et al., 2008; Sinnadurai et al., 2016; Wang et al., 2019), land use (Edwards & Huryn, 1996; Stenroth et al., 2015) and management interventions (Januschke & Verdonschot, 2016), and are thus useful for biomonitoring in riparian habitats (Rainio & Niemelä, 2003). To enable the response of terrestrial beetles to habitat conditions to support evidence-informed decision making in the UK, Eyre and Lott (1997) recommended development of a standardized field survey protocol. Since 1997, beetles associated with exposed riverine sediments have been classified, their habitat preferences documented and the effectiveness of methods for their sampling assessed (e.g. Bates & Sadler, 2004; Bates et al., 2007; Petts, 2000; Sadler & Lott, 2006a, 2006b, 2009; Webb & Mott, 2013, 2014). However, a standardized field survey protocol has yet to be introduced in the UK.

Methods for sampling terrestrial invertebrates (e.g. pitfall trapping) have been extensively described and internationally tested (e.g. Andersen, 1995; Brown & Matthews, 2016; Drake et al., 2007; Engel et al., 2017), and the contrasting benefits and limitations of individual methods recognized. For example, pitfall traps are biased towards the capture of large, active, ground-dwelling taxa (e.g. ground beetles: Siewers et al., 2014; Topping & Sunderland, 1992), whereas hand searching more effectively captures organisms that fly (e.g. Bunting et al., 2021) rather than walk (e.g. soldier beetles: Alexander, 2014). Use of multiple methods may thus capture taxa with a wider range of biological and ecological traits, enhancing estimation of biodiversity and enabling inference of habitat quality (e.g. Andersen, 1995; Bunting et al., 2021; Gobbi et al., 2018). However, a consistent approach that both standardizes variation within individual methods and integrates the benefits of multiple methods for sampling riparian invertebrates has not been defined.

This study presents a previously unpublished protocol for surveying riparian invertebrate communities, which includes two methods (hereafter, 'multi-method'), and offers comprehensive guidance on selecting survey locations, and standardizing and integrating sampling methods. Data characterizing riparian beetle assemblages are used to evaluate the effectiveness of this protocol in reducing sampling variability and characterizing communities, and to identify best practice with the potential to inform wider sampling and biomonitoring programmes.

2 | METHODS

2.1 | Riparian beetle data

Records of riparian beetle assemblages sampled from 1987 to 2017 were collated by the UK invertebrate conservation charity Buglife. Post-2013, surveys were conducted using a draft standardized survey protocol developed by Natural England, a UK public body responsible for environmental protection and improvement, which was finalized in 2017 (hereafter, 'the protocol'; see Appendix S1). The protocol combines two established methods (hand searching and pitfall trapping)

and, to aid assessments of biodiversity and thus inference of habitat quality, suggests how they may be used to maximize representation of the range of invertebrates and riparian habitats present.

Briefly, surveys following the protocol captured organisms using both hand searching and pitfall trapping methods. Replicate 60-min hand searches were conducted at four stations approximately 200 m apart within each survey reach. During hand searches, all surface habitats were disturbed and beetles collected with an aspirator (i.e. a 'pooter'). Each hand search was supplemented by the excavation of a 1-m² area to the depth of the water table, to collect beetles from sub-surface sediments. The sides of the excavated area were collapsed into the water in the hole, and organisms collected from the water surface using a 0.8-mm mesh sieve. Pitfall traps (plastic cups) were buried in sediments above frequently inundated areas. Traps were placed >2 m apart and positioned to represent the range of habitats present. Traps were one-third filled with preservative, covered to prevent infilling by rain or debris, and left in place for 14–28 days prior to collection. From 2013 to 2016, the draft protocol did not stipulate the number of pitfall trap stations, and 1–4 replicate sets of 10 traps per survey reach were used during this period. From 2017, the finalized protocol recommended using a set of 10 traps at only one station per survey reach, because the number of species recorded did not increase with replicate sets. Captures from all hand searches and pitfall traps collected within a survey reach were pooled to form one standardized sample.

Surveys conducted before the protocol's introduction followed variants of the methods described, but typically focused on sampling gravel-dominated sediments within otherwise comparable habitats. Differences in hand searching methods included search durations of 20–60 min. Differences in pitfall trapping included the number of traps, which ranged from 7–12 per station. Surveys also varied the number of hand searches and pitfall trap stations per reach, or only used one of the two methods. In all surveys, captured beetles were identified to species.

2.2 | Data selection and analysis

From the Buglife dataset, 73 surveys which used both pitfall trapping and hand searching were identified for analysis, comprising 56 surveys done before (1997–2012) and 17 done after (2013–2017) the protocol's introduction. Surveys were conducted between April and October in 1–7 reaches per river. Sites surveyed before and after the protocol's introduction represented a comparable range of sediment and vegetative characteristics (Appendix S2), and had comparably low human impact levels.

To compare the assemblages characterized by hand searching and pitfall trapping, total taxonomic richness and the richness of species of conservation concern (SoCC) per survey was calculated. SoCC were identified using Species Quality Scores (SQS) obtained from *Pantheon*, a database detailing the conservation status, biological characteristics and habitat preferences of invertebrates (Webb et al., 2018). SQS scores >1 indicate species that have a national and/or international conservation designation (e.g. *Nationally Scarce* and *IUCN Endangered*),

and thus these species were used to calculate SoCC richness. The total and SoCC richness of each genus containing >2% of species was plotted against sampling method. The biological characteristics (e.g. body size, feeding guild) and habitat preferences of adult beetles were obtained from *Pantheon* (Webb et al., 2018), and species captured by hand searching only, pitfall trapping only and both methods noted.

To assess the influence of variability in sampling effort on total and SoCC richness, an 'effort' score was calculated by summing the number of hand searches and the number of pitfall trap replicate sets in each survey. Each hand search and each pitfall trap replicate set were considered comparable because the two methods can capture a similar number of taxa and, where differences occur, there is no consistent pattern on which to base different weightings (e.g. Melbourne, 1999; Phillips & Cobb, 2005; Privet et al., 2020; Zanetti et al., 2016). Neither hand search duration nor the number of pitfall traps were considered as a weighting factor, because of diminishing returns on increased effort (e.g. 5–9 pitfall traps can capture a similar number of species: Brose, 2002).

The greater number of surveys conducted before the protocol's introduction ($n = 56$ compared with $n = 17$) may have increased variability in effort and richness, influencing conclusions about the protocol's effects. Therefore, randomized resampling of pre-protocol samples was undertaken. Specifically, 17 of the 56 pre-protocol samples were randomly selected 1000 times and then aggregated with the 17 post-protocol surveys to allow direct comparisons (i.e. 1000 subsets, each containing 17 pre-protocol and 17 post-protocol samples). Linear mixed-effect models (LMM) were used to characterize the relationship between total or SoCC richness as the response variable, and protocol use (categorical, used/not used) and effort score (continuous, 2–13) as predictors (i.e. fixed factors) for each of the 1000 subsets. In each model, richness was square-root transformed to meet the LMM assumptions of residual normality and homoscedasticity (Warton et al., 2016). River was included as a random intercept to account for differences in richness caused by spatial variability among survey reaches, this being the finest resolution possible using the subsetting approach. The threshold $P < 0.05$ was used to define statistically significant effects. The variance in richness explained by protocol use, sampling effort and river was determined using goodness-of-fit statistics (marginal and conditional R^2). Marginal R^2 values were partitioned to quantify the variance explained by each fixed factor (i.e. protocol use and sampling effort).

All analyses were conducted in R v. 4.0.3 (R Core Team, 2020). Models were built using lme4 (Bates et al., 2015) and model assumptions checked using DHARMA (Hartig, 2020). Only models meeting all statistical assumptions were considered. Variance in the marginal R^2 was partitioned using variancePartition (Hoffman & Schadt, 2016).

3 | RESULTS

In total, 537 species were captured in the 73 surveys, with 51% of species being predators. Hand searching and pitfall trapping captured 81% and 68% of species, respectively, with 49% recorded by

both methods. Overall, more species were captured only by hand searching (171) than only by pitfall trapping (104), but hand search and pitfall trap captures varied between genera (Figure 1a). Species captured only by one method typically occurred in few samples (mean \pm SE: $1.7 \pm <0.1$), relative to those captured by both methods (20.3 ± 0.6). Species captured only by hand searching were often smaller (max. body size <7 mm), predatory (40% of species) and associated with wetland habitats (54%: e.g. *Bembidion fluviatile*, *Stenus clavicornis*). Species captured only by pitfall trapping were also often predatory (39%), but were larger (>7 mm) and associated with open habitats (49%: e.g. *Carabus monilis* and *Quedius levicollis*).

SoCC comprised 19% (104) of all species, with 35% and 17% being captured exclusively by hand searching and pitfall trapping, respectively (Figure 1b, Table S1). Eight species are Nationally Rare, IUCN Threatened or Vulnerable, three of which (*Coccinella quinquepunctata*, *Lionychus quadrum* and *Negastrius sabulicola*) were captured by both hand searching and pitfall trapping. Two species (*C. monilis* and *Normandia nitens*) are IUCN Endangered and were only caught by hand searching and pitfall trapping, respectively. Most (87%) SoCC were associated with the *Pantheon* broad habitat type 'wetland' (e.g. running water or exposed riverine sediments: Table S1).

3.1 | Effectiveness of the protocol

Variability in sampling effort was greater before (range: 2–13, SD: 2.7) compared with after (mean \pm SE: 5–9, 1.6) the protocol's introduction, but mean effort remained comparable in pre-protocol (6.1 ± 0.37) and post-protocol (6.3 ± 0.39 : Figure 2a) surveys. Protocol use and sampling effort explained $80 \pm <1\%$ (marginal R^2) of the variance in total richness, which increased to $88 \pm <1\%$ (conditional R^2) when differences among rivers were considered. Surveys using the protocol recorded $17 \pm <1$ more species on average, and significantly more species than pre-protocol surveys in all models ($P < 0.001 \pm <0.001$: Table S2A, Figure 2b). Protocol use accounted for $86 \pm <0.1\%$ of the marginal R^2 . Greater effort marginally increased total richness by $<1 \pm <1$ species per additional hand search or pitfall trap replicate set, with this increase being significant in 99% of models ($P = 0.004 \pm <0.001$: Table S2A). Sampling effort accounted for $3 \pm <0.1\%$ of the marginal R^2 .

Protocol use and sampling effort explained $30 \pm <1\%$ (mean \pm SE: marginal R^2) of the variance in SoCC richness, which increased to $54 \pm <1\%$ (conditional R^2) when differences among rivers were considered. Pre-protocol and post-protocol surveys recorded a comparable number of SoCC on average, only being significantly different in $<1\%$ of models (Table S2B, Figure 2c). Protocol use accounted for $1 \pm <0.1\%$ of the variance explained (marginal R^2) by the predictors. Greater effort had a more consistent effect on SoCC richness (SD: <0.1), relative to protocol use (SD: 0.1), and increased captures in 99% of models ($<1 \pm <1$ species, $P = 0.004 \pm <0.001$: Table S2B). Sampling effort accounted for $34 \pm <0.1\%$ of the marginal R^2 .

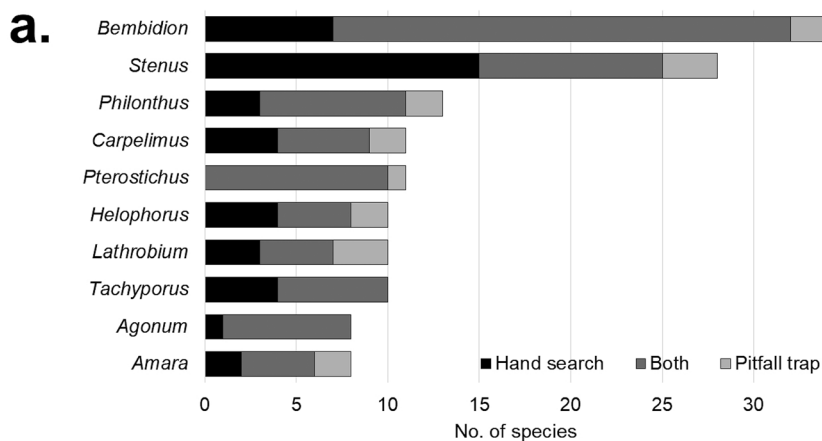
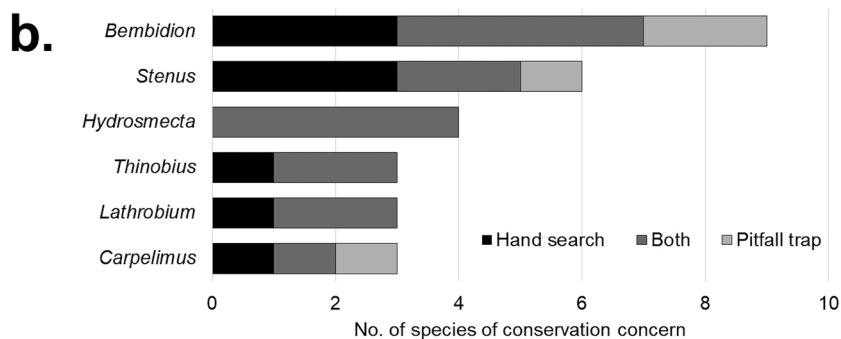


FIGURE 1 Comparison of (a) the total number of species and (b) the number of species of conservation concern captured by each method for genera containing >2% of species



4 | DISCUSSION

The call for a standardized survey protocol to assess the habitat quality of riparian zones using invertebrates (see Eyre & Lott, 1997) has remained unanswered for over 20 years in the UK, hampering informed ecosystem management. This study demonstrates the capacity of surveys done using a standardized protocol that combines two established methods to characterize the taxonomic richness and conservation status of riparian beetle communities, and thus enable inference of habitat quality. Standardized surveys captured more species overall, while expending a comparable effort relative to surveys conducted before the protocol's introduction. However, protocol use and variability in sampling effort did not alter SoCC richness. Differences in the species captured by the two methods (hand searching and pitfall trapping) demonstrate that multi-method surveys increase estimates of total and SoCC richness, thus enhancing assessments of riparian habitat quality.

4.1 | Protocol use, not sampling effort, enhanced assessment of riparian ecosystems

The standardized protocol reduced variability in effort between surveys, increasing comparability of the sampled assemblages. Thus, any spatial and temporal differences in richness observed while using the protocol were more likely to reflect habitat conditions, rather than variability caused by sampling methods (Brown & Matthews, 2016;

Magurran et al., 2010; Ward et al., 2001). Protocol use did not eliminate variability in effort because the draft protocol used between 2013 and 2016 did not stipulate the number of pitfall trap stations. From 2017, the finalized protocol removed this remaining variability which, in conjunction with the already limited effect of effort, further enhanced comparability of surveyed assemblages.

In addition to standardization, most biomonitoring seeks to maximize efficiency by gathering sufficient ecological information while minimizing effort expended (Hoffmann et al., 2019; Stenzel et al., 2017). Mean sampling effort was comparable pre-protocol and post-protocol, but the overall number of species captured increased, reflecting an increase in sampling efficiency. Sampling effort accounted for 3% of the explained variance (marginal R^2) suggesting that effort did not cause this increased efficiency, which is consistent with findings that relatively few samples are required to capture the most common species (e.g. Preston, 1948; Schneck & Melo, 2010). However, SoCC are less common overall and may occur at low densities, even at sites meeting their habitat preferences (Harmer, 2015; Sadler et al., 2004). Therefore, surveys seeking specifically to characterize rare species and not the wider assemblage may need to adapt the protocol, for example by increasing the number of hand searches, pitfall traps and stations.

The limited influence of sampling effort on total richness suggests that increased sampling efficiency (i.e. increased total richness for comparable effort) reflects other guidance in the protocol, specifically the broader range of habitats considered. Surveys conducted before the protocol's introduction focused primarily on gravel-dominated

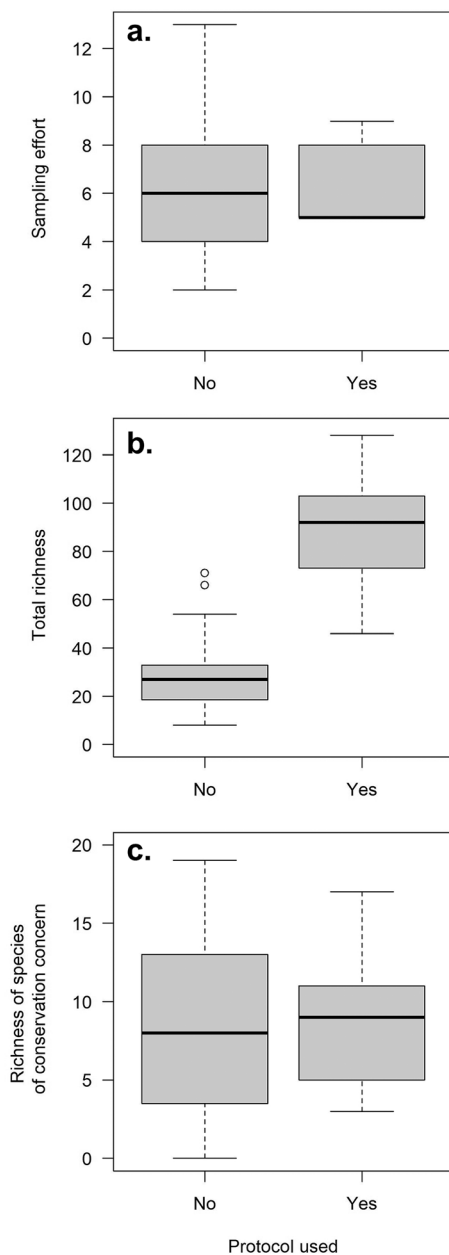


FIGURE 2 Riparian beetle assemblage surveys conducted while not using and using a standardized multi-method protocol: (a) sampling effort, (b) taxonomic richness and (c) richness of species of conservation concern, per survey. The centre line represents the median; boxes represent the interquartile range; whiskers represent the minimum/maximum values which are within $1.5 \times$ the interquartile range of the first and third quartiles; circles represent outliers

surface sediments, but many riparian beetle species require other habitats (e.g. decaying wood: Alexander, 2004; Fowles et al., 1999). The protocol offers guidance on selecting survey reaches, spacing sampling units and sampling different habitats (e.g. subsurface sediments, water margins, decaying wood and emergent vegetation), thus maximizing the range of habitats sampled and the range of species captured (Brown & Matthews, 2016; England et al., 2019; Ward et al., 2001). Therefore, the identification and surveying of other key habitats likely

contributed to a more complete characterization of the assemblages present, thus explaining the higher total richness observed while using the protocol. SoCC richness was unaffected by protocol use, likely because most of these species are associated with gravel-dominated surface sediments (i.e. exposed riverine sediments: Table S1), and therefore had comparable capture probabilities in pre-protocol and post-protocol surveys. The similar number of SoCC captured supports the suggestion that protocol use increased sampling efficiency, because a similar number of SoCC were captured despite the comparable sampling effort being spread over a greater range of habitats.

As in other studies (e.g. Andersen, 1995; Bunting et al., 2021; Gobbi et al., 2018), hand searching and pitfall trapping sampled different assemblages. Species captured by only one method typically occurred in few samples; their absence from samples collected by the other method may have multiple causes. First, absences may reflect a bias against the trapping of some species due to their biological traits or habitat preferences (Engel et al., 2017; Lang, 2000). For example, smaller species which typically reside on riparian vegetation, such as *Stenus clavicornis* (Webb et al., 2018), may have been absent from substrate-level pitfall traps, which were outside their preferred habitat. Pitfall traps can more effectively capture larger, ground-dwelling species such as *C. monilis* (Lang, 2000; Siewers et al., 2014). Second, low population densities and/or activity levels may have reduced some species' probability of capture (Brown & Matthews, 2016; Luff, 1975; Spence & Niemelä, 1994). Third, other factors influence beetle captures, such as trap design, ambient temperature, and the seasonal and diurnal timing of sampling (Work et al., 2002; Knapp et al., 2020). Regardless of cause, using both methods provided a more comprehensive assessment of the assemblage present (as assessed using total and SoCC richness), demonstrating pitfall trapping and hand searching as complementary, not alternative, methods for biomonitoring riparian communities (also see Bunting et al., 2021; Gobbi et al., 2018; Knapp et al., 2020).

4.2 | Monitoring and management implications

Management intended to protect riparian habitats is often ineffective due to the limited evidence available to inform decision making (de Sosa et al., 2018). Non-standardized surveys can hamper attribution of observed biotic differences to habitat factors rather than sampling methods (Brown & Matthews, 2016; Magurran et al., 2010), emphasizing the need for a consistent biomonitoring approach to guide more effective management. A consistent multi-method biomonitoring approach could generate reproducible evidence to inform objectives that enhance riparian habitat quality and protect valuable sites, enable evaluation of the effectiveness of management interventions, and allow site quality to be tracked over time. In addition, holistic monitoring of riparian zones could be enabled by concurrent consideration of biotic and physical habitat survey data. Specifically, the consistent effort and more complete species list provided by this multi-method protocol may complement standardized physical habitat assessments, such as the River Condition Assessment (Gurnell

et al., 2020) or Modular River Survey (Shuker et al., 2017). Such a combination of standardized biotic and physical surveys is needed to characterize riparian habitats that support diverse invertebrate assemblages including species of conservation interest, and thus identify priority sites for both protection and restoration.

5 | CONCLUSIONS

1. The multi-method protocol tested here reduced variability in both methods and sampling effort, enhancing inter-survey comparability.
2. Pre-protocol and post-protocol surveys expended comparable sampling effort, but post-protocol surveys sampled more beetle species, enhancing estimation of biodiversity.
3. SoCC richness was not influenced by protocol use or sampling effort, because habitats suitable for such species were represented in pre-protocol and post-protocol surveys.
4. Use of multiple methods enhanced characterization of total and SoCC richness, because ~50% of species were captured by only one of the two methods used.
5. The widespread adoption of standardized multi-method approaches may allow management and conservation priorities to be identified, and promote the protection of biodiversity within high-quality riparian habitats.

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CONFLICT OF INTEREST

The authors declare that there are no conflicting interests.

DATA AVAILABILITY STATEMENT

The data used in this study are available from the authors upon reasonable request.

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