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Rasmussen *et al.*

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Local physical habitat quality cloud the effect of predicted pesticide runoff from agricultural land in Danish streams

Jes Jessen Rasmussen,* Annette Baattrup-Pedersen, Søren Erik Larsen and Brian Kronvang

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The combination of intensive agricultural activities and the close connectivity between land and stream emphasise the potential risk of pesticide exposure in Danish streams. Benthic macroinvertebrates are applied in the assessment of stream ecological status, and some sensitive species have been shown to respond strongly to brief pulses of pesticide contamination. In this study we investigate the impact of agriculturally derived pesticides on stream macroinvertebrate communities in Denmark. As a measure of toxic pressure we apply the Runoff Potential. We investigated a total of 212 streams. These were grouped into distinct classes according to the magnitude of pesticide contamination in the period from 2003–2006. A total of 24 different macroinvertebrate indices were applied to detect effects of pesticide runoff (e.g. the SPEAR-index and the number of EPT taxa). We found high predicted pesticide runoff in 39% of the streams, but we found no significant effect of predicted pesticide exposure on stream macroinvertebrate indices. We, additionally, examined the influence of a series of environmental parameters ranging from site scale to catchment scale on the macroinvertebrate community. Relative proportions of gravel, sand and silt in bed sediments explained most of the variation in macroinvertebrate indices as well as the upstream riparian habitat quality. We suggest that the Runoff Potential model overestimate pesticide runoff contamination in Danish streams due the presence of buffer strips enforced by Danish legislation. When pesticide runoff contamination is low to moderate, poor physical properties (indirectly related to agricultural activity) are the main impediment for the ecological quality of Danish streams.

1. Introduction

The brief occurrence of pesticides in streams resulting from surface runoff,^{1,2} tile drains^{3,4} and spray drift⁵ from agricultural fields has been documented in several studies. Furthermore, surface runoff and tile drains have often been emphasised as important entry routes for pesticides to streams having a potential impact on benthic macroinvertebrates.^{6,7} However, several macroinvertebrate species declining in abundance during pesticide exposure have been shown to recover within a year.⁶ The

presence of forest on both sides of the stream corridor in the near upstream area has, furthermore, been shown to promote the process of recolonisation by adding some recovering capacity to the system.^{8,9} Pesticide concentrations though rarely reach the level of acute mortality for macroinvertebrates emphasising the potential importance of considering sublethal effects such as feeding behaviour,¹⁰ emergence success and offspring production.^{11,12} Sublethal effects potentially change inter-specific interactions which in time could translate into the displacement of sensitive species. Considering long-term effects is, consequently, important when evaluating effects of pesticides on macroinvertebrates. Recently the SPEAR index was introduced by Liess and von der Ohe⁸ aiming to tackle the challenge of

National Environmental Research Institute (NERI), Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark. E-mail: jr@dmu.dk

Environmental impact

Understanding how pesticides impact stream ecosystems is important in order to conduct proper risk assessment. Actual field data provide the best information about system dynamics during and after pesticide exposure. Collecting sufficient field data is, however, time consuming, expensive and very difficult. Thus there is a scarcity of large scale field studies. In this study we investigate 212 streams and use macroinvertebrate communities as a measure of stream ecosystem health. These parameters are correlated with predicted pesticide exposure. We applied the novel approach of grouping streams according to predicted pesticide runoff contamination consistently recurring every year within a four year period. We hereby expect to enhance the strength of the signal.

detecting effects of pesticides on macroinvertebrate community structure in the field. The SPEAR index classifies species as at risk (SPEAR) or not at risk (SPEnotAR) considering both species sensitivity to pesticides and their ecological traits and thus provides a promising tool for evaluating long-term effects of pesticides on macroinvertebrates in streams.

In Denmark 62% of the total area (43 000 km²) is used for agriculture. The total length of the stream network is 64 000 km, the majority of which are small (<2 metres wide). The combination of intensive agricultural activities and the close connectivity between land and stream enhanced artificially by drainage and narrow buffer strips (2 metres) emphasise the potential risk of pesticide exposure. Intensive agricultural activities, however, have other additional consequences including comprehensive habitat degradation (facilitated by channelisation, dredging activities and siltation) and eutrophication.^{13,14} 90% of all Danish streams are to some extent physically modified which, consequently, translates into low habitat stability and, in more extreme cases, high mud cover. Both habitat degradation and eutrophication have been shown to significantly impact sensitive macroinvertebrate species and the community structure in general.^{14–16} When conducting field studies it is therefore a major challenge to disentangle the effects of one environmental stressor from those of other influencing environmental stressors.

In this study we aim to detect the potential impact of agriculturally derived pesticide runoff on stream macroinvertebrate communities in Denmark. As a measure for toxic pressure from pesticide runoff we applied the RP model (Runoff Potential model) which has been validated in German lowland streams both in terms of predictive power for actual pesticide concentrations¹⁷ and its close correlation to the SPEAR index.⁹ We grouped the streams according to the predicted pesticide contamination consistently recurring every year in the period 2003–2006, which we expect to enhance the strength of the signal.

2. Methods

2.1 Study area

Sampling sites were evenly distributed across Denmark (Fig. 1). Denmark is generally characterised by low elevation averaging 31 metres above sea level. The western part of Denmark is characterised by sandy soils with high infiltration capacity whereas the eastern part is characterised by moraine lands with low infiltration capacity. Climatic conditions are temperate and average annual precipitation varies from 500 mm year⁻¹ in the north eastern part of Zealand to 800 mm year⁻¹ in the central parts of Jutland. 62% of the total area (43 000 km²) is used for agricultural activity. Dominating crop types are cereals (70%) followed by grasslands (9%), oilseed rape (5%) and maize (5%).^{18–21}

2.2 Stream characteristics and macroinvertebrates

In total 212 Danish stream sites were included in this study. The distance to source was minimum 1500 metres for all sites. At all sites fauna was sampled using standardised kick-sampling²² in March or April in each of the years 2004–2007. All macroinvertebrates were identified to species level except individuals belonging to Oligochaeta, Tipuloidea, Ceratopogonidae and

Fig. 1 Geographical distribution of sampling sites ($n = 212$).

Chironomidae. Applying the publicly available SPEAR database²³ %SPEAR abundance was calculated as the proportion of species characterised as at risk (SPEAR). DSFI score was calculated after Pedersen *et al.*²² Applying Asterisks 3.1.1 we, furthermore, calculated German Saprobic index value, life index score, total abundance, species richness, separate and summed species numbers and abundance of Ephemeroptera, Plecoptera and Trichoptera, Shannon–Wiener diversity, Simpson diversity, Evenness and r/K relationship. Parameter ranges of selected community descriptive parameters are presented in Table 1.

Site specific physical and chemical parameters in terms of qualitative and quantitative substrate assessments, water chemistry and other physical variation (including species specific coverage of emergent and submergent macrophytes, stream width and distance to source) were extracted from the ODA database (<https://oda.dk>) (Table 1). Substrate composition and macrophyte species specific coverage were surveyed on a representative 100 metre reach according to Pedersen *et al.*²² Based on the detailed substrate data we calculated substrate heterogeneity (SH) after Baattrup-Pedersen and Riis.²⁴

Land cover types (relative proportion of forest, agriculture, permanent grasslands, undisturbed dry areas (*e.g.* heath), undisturbed wet areas (*e.g.* meadows and marsh) and surface water bodies) were scored at three different scales: (i) the total watershed of the stream reach; (ii) a two-sided 50 m buffer along all upstream reaches in the watershed; and (iii) an approximately 100 m × 100 m area located immediately upstream of the sampling site. Land cover types at the different spatial scales were determined using the Analysis Tools in ESRI ArcGIS 9.2.

2.3 Predicted pesticide exposure

We calculated predicted pesticide exposure applying the Runoff Potential (RP) model. The RP is a generic indicator which was

Table 1 Average, std. error, minimum and maximum values for physical and chemical properties for sampling sites, catchment characteristics and selected site specific macroinvertebrate community descriptive parameters. The number of sites for which data were available is indicated

Parameter	# Sites	Average	Std. Error	Minimum	Maximum
Physical					
Catchment area/km ⁻²	212	112.7	1.9	0.4	1223.1
Distance to source/m ⁻¹	212	9188	651.6	1500	208 000
Cobble (%)	164	5.7	0.4	0	69
Gravel (%)	164	19.2	0.8	0	92
Sand (%)	164	41.1	0.9	0	85
Silt (%)	164	26.2	1	0	100
Substrate heterogeneity	164	0.37	0.007	0	0.68
Macrophyte coverage ^d (%)	160	65.6	1.1	0.5	100
# Macrophyte species ^a	160	12.4	0.3	1	50
Chemical					
Ortho-phosphate ^b /mg L ⁻¹	212	0.05	0.004	0	1.67
Nitrate ^b /mg L ⁻¹	212	3.73	0.08	0	14
Ammonia ^b /mg L ⁻¹	212	0.12	0.005	0	1.81
BOD ^b /mg L ⁻¹	212	1.35	0.04	0	9.94
pH ^b	212	7.6	0.03	6.7	8.8
Catchment characteristics					
Agriculture in the near upstream area ^{c,d} (%)	212	62.9	4.1	0	100
Undisturbed area in the near upstream area ^{c,d} (%)	212	23.3	1.1	0	100
Undisturbed area in catchment ^c (%)	212	27.3	0.9	0	88.9
Macroinvertebrate community descriptors					
# Macroinvertebrate taxa ^e	212	36.4	0.4	15	77
%SPEAR ^e	212	30.0	0.3	5.0	52.9
DSFI ^e	212	5.0	0.04	2	7
German saprobic index ^e	212	2.1	0.008	1.5	2.9
# EPT taxa ^e	212	10.5	0.2	0	28
EPT abundance ^e (%)	212	22.7	0.5	0	61.5

^a Measured in months June–September. ^b Measured 1–12 times per year. ^c Analysis based on digital spatial data. ^d Represents a 100 m × 100 m area extending upstream from the sampling site. ^e Fauna samples collected in March–April in each of the years 2004–2007.

developed to quantify risk of pesticide runoff contamination to streams from agricultural land.⁹ The runoff model (eqn (1)) underlying the RP (eqn (2)) predicts runoff contamination of a generic compound instead of predicting actual runoff losses for a specific compound. Due to this simplistic model approach outcome uncertainty is, consequently, increased. However, due to limited data availability, we could not meet data requirements for more detailed models which consider *e.g.* detailed compound application patterns or buffer strip width or quality. For further details on the RP model consult Schriever *et al.*⁹ We calculated RP for all sites for each of the years 2003–2006 applying the 100 m × 100 m area. For convenience this is referred to as the RP-square.

$$\text{gLOAD} = \sum_{i=1}^n \sum_{j=1}^m A_{i,j} D_{\text{generic}} \left(1 - \frac{I_j}{100} \right) \frac{1}{1 + \frac{K_{\text{oc, generic}} \text{OC}_i}{100}} f(s_i) \frac{f(P_i, T_i)}{P_i} \quad (1)$$

where $A_{i,j}$ is the size of agricultural land (ha), index i refers to the respective fields, index j refers to different crop types present on the fields. D_{generic} is the application rate of the generic substance, I_j is the crop- and growth phase specific plant interception of the substance at the time of the precipitation event (%), $K_{\text{oc, generic}}$ is the organic carbon sorption coefficient of the generic compound, OC_i is the soil organic carbon content of a field patch (%), s_i is the mean slope of a field (%), $f(s_i)$ describes the influence of the field slope. P_i is precipitation depth (mm) of the considered event, T_i

refers to soil texture of the field (sandy/loamy), $f(P_i, T_i)$ is a function describing the surface runoff volume for vegetated soils in the middle or late vegetation period. RP is calculated as:

$$\text{RP} = \log \left(\max_{i=1}^n (\text{gLOAD}_i) \right) \quad (2)$$

We parameterised the runoff model as follows. The major crops grown in Denmark were assumed to be uniformly distributed in the country, and agricultural land within the RP-square was assigned values for national relative crop distribution (Danish Environmental Protection Agency, 2003–2006). The soil slope in the RP-square was estimated using DEM (Digitalised Elevation Map) with 1.6 metre resolution in ArcGis 9.2. The soil texture composition (including humus content) within the RP-square was extracted from the Hair database.²⁵ According to Thomas and Goudie²⁶ sandy soil was defined as soils containing <10% clay and >85% sand. The relative organic carbon content of soils was calculated as 57% of the humus content.²⁶ We did not have detailed information concerning the application rate of the generic compound. Consequently, it was set to a constant value of 1 g ha⁻¹ for all crop types. Daily precipitation data were provided by the Danish Meteorological Institute (<http://www.dmi.dk>) (10 km² resolution). Daily recorded precipitation depth was assumed to result from a single precipitation event. Only precipitation events occurring in May or June were considered as these months represent the main pesticide application season. Precipitation depth values <10 mm were assumed

not to offset a runoff event.²⁷ According to Linders *et al.*²⁸ plant interception values (I_p) were assigned to all crop types which were present during the considered precipitation event. No pesticide runoff episodes were predicted if either no agricultural land was present in the RP-square or no precipitation event exceeded 10 mm during May and June. In this case a minimum RP value was assigned indicating potential but minimal pesticide runoff. The assigned value was equal to 50% of the maximum RP calculated for the site with the smallest amount of agricultural land in the RP-square.

2.4 Data analysis

Upstream riparian habitat quality was assessed as the summed relative proportion of all types of undisturbed areas (forest, undisturbed dry areas and undisturbed wet areas) in the two-sided 50 m buffer extending from the sampling site and upstream. We applied catchment size as a measure of stream size, because stream width is influenced by human activities (channelisation and dredging).

Predicted pesticide exposure ranged from -9.96 (indicating potential but minimal risk of exposure) to -0.23 . RP scores were further subdivided into four categories ($RP > -1$ to 0 ; $RP > -2$ to -1 ; $RP > -3$ to -2 and $RP \leq -3$). Sites were grouped into those consistently being in the same RP class and those changing RP class during the period 2003–2006. 175 sites were consistently assigned to the same RP class in the years 2003–2006 whereas 37 sites changed RP class. At sites consistently remaining within the same RP class we correlated macroinvertebrate community structure in March or April (2004–2007) with predicted pesticide exposure in May or June the preceding year. The effect of predicted pesticide exposure on %SPEAR abundance was tested with one-way ANOVA ($P < 0.05$). The effect of stream size and upstream riparian habitat quality on %SPEAR abundance was tested with Pearson correlation, and, subsequently we tested the influence of RP on the correlation between %SPEAR abundance and upstream habitat quality with ANCOVA ($p < 0.05$). Furthermore, we correlated all physical/chemical properties with all macroinvertebrate community descriptors with Pearson correlation. ANOVA's, ANCOVA's and Pearson correlations were conducted using SAS Enterprise Guide 4.2. Performing DCA analyses we down weighed rare species by disregarding all species present in fewer than five samples. Furthermore, samples with five or fewer species were disregarded in order to enhance the signal from the most common trends. DCA and PCA analyses were performed applying PC-ORD 4.25.

3. Results

3.1 Correlation between runoff potential and %SPEAR abundance

We found that %SPEAR abundance was not significantly different among RP classes ($P = 0.433$) (Fig. 2). Furthermore, we found no significant correlation between RP and %SPEAR abundance ($n = 175$, $P = 0.428$). %SPEAR abundance increased significantly with increasing proportions of undisturbed area in the two sided 50 metres buffer extending from the sampling point and upstream ($P = 0.016$) (data not shown). There was no significant effect of catchment area (representing stream size) on

Fig. 2 Relationship between predicted pesticide runoff (RP) and fraction of species at risk (%SPEAR abundance, $n = 175$). RP classes represent sites with RP scores consistently remaining within the RP class during a four year period (2003–2006) and %SPEAR abundance represents averages from the subsequent years (2004–2007) ($n = 4$). Error bars indicate SE.

%SPEAR abundance ($P = 0.24$). We, additionally, tested the potential interaction of RP class on the correlation between proportion of undisturbed area in the two sided 50 metre buffer and %SPEAR abundance and found no significant difference among RP classes ($P = 0.366$) (data not shown).

3.2 Macroinvertebrate communities and environmental parameters

We plotted samples ($n = 656$) in a DCA ordination (considering species qualitatively and quantitatively) with DCA axes one and two in combination explaining 11% of the variation in the data (data not shown). We found no significant differences among correlations between DCA values and RP class ($P > 0.05$). We correlated DCA axis scores with site and catchment based environmental parameters applying Pearson correlation analysis and found that relative proportions of gravel, silt and the upstream riparian habitat quality explained most of the variation in macroinvertebrate community composition (horizontal DCA axis one) ($r = 0.398$, $r = 0.351$ and $r = 0.310$, respectively, $P < 0.001$) (Table 2). The catchment area explained most variation in the macroinvertebrate community along the vertical DCA axis two ($r = 0.425$, $P < 0.001$) followed by the relative proportion of silt and substrate heterogeneity ($r = 0.22$ and $r = 0.215$, respectively, $P < 0.001$).

The PCA analysis of calculated macroinvertebrate community descriptors ($n = 175$) explained 62% of the total variation in the dataset (Fig. 3). Vectors depicting the six macroinvertebrate community descriptors explaining most of the variation in the dataset are added (SPEAR, The Saprobic index, DSFI, #EPT taxa, EPT abundance and total #taxa). Selective criterion was $|r| > 0.2$. Vectors representing Shannon–Wiener diversity and Evenness are, furthermore, added to the plot despite weaker explanatory power. The vector length represents explanatory power. The Saprobic index and %SPEAR abundance demonstrated strongest explanatory power and together with DSFI and EPT abundance primarily explained variation along the plane

Table 2 Pearson correlations with DCA ordination axes. Only the strongest correlations of 29 variables analysed are included in the table. All environmental variables not included here but described in Methods did not correlate significantly ($p < 0.01$) with any of the DCA axes. Values indicate $|r|$ and asterisks indicate significant correlations (** $p < 0.01$ and *** $p < 0.001$). In the case of autocorrelation characterised by $|r| > 0.5$, the environmental parameter with the strongest correlation coefficient is presented

Parameter	DCA 1	DCA 2
Catchment size	—	0.425***
Substrate heterogeneity	—	0.215***
Silt (%)	0.351***	0.220***
Gravel (%)	0.398***	—
Sand (%)	0.215***	—
Agriculture in the 100 m × 100 m quadrant (%)	0.112**	0.150***
Upstream riparian habitat quality	0.310***	—

from top left to down right of the ordination space. Community descriptors based on the presence/absence of species explained variation along the plane from top right to down left.

Considering all environmental parameters the local physical characteristics in terms of substrate types were most strongly correlated to macroinvertebrate community descriptors (Table 3). Especially the relative proportion of silt, sand and gravel was strong governing factors applying a negative (silt) or positive impact (sand or gravel) on macroinvertebrate community structure. Increasing concentrations of *ortho*-phosphate and BOD, additionally, had a negative impact on the macroinvertebrate community structure (especially %SPEAR abundance) whereas predicted pesticide runoff (RP) only was weakly correlated with %SPEAR abundance ($r = 0.08$, $P > 0.05$). RP

Fig. 3 Scatter plot of streams with consistent predicted pesticide exposure during pesticide spraying season from 2003–2006 in a PCA ordination ($n = 175$). PCA axes one and two together explained 62% of the total variation in the dataset. Vectors representing selected macroinvertebrate community descriptors (%SPEAR abundance, the Saprobic index, EPT abundance, the number of EPT taxa, the total number of taxa, DSFI, Shannon–Wiener diversity and evenness) are depicted. The vector length is congruent with explanatory power of the community descriptor.

Table 3 Pearson correlation coefficients are presented for a selected subset of macroinvertebrate community descriptive parameters and environmental variables. Asterisks indicate correlation significance level (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). Positive or negative values indicate whether correlations are positive or negative, respectively

Physical/chemical properties	%SPEAR abundance	DSFI	Saprobic index	# Macroinv. taxa	# EPT taxa	EPT abundance	Shannon–Wiener diversity
Sand	0.29***	0.30***	−0.34***	0.16***	0.17*	0.24***	0.09
Silt	−0.42***	−0.41***	0.45***	−0.18***	−0.31***	−0.44***	−0.05
Gravel	0.38***	0.45***	−0.26***	0.06	0.27***	0.21***	0.12*
<i>ortho</i> -P	−0.25***	−0.11*	0.15***	−0.05	−0.16***	−0.13**	−0.1*
BOD	−0.22***	−0.2***	0.21***	−0.11*	−0.21***	−0.06	−0.07
Macrophyte coverage	−0.26***	−0.2***	0.28***	0.31***	−0.13**	−0.23***	0.22***
# Macrophyte species	0.08	0.22***	0.02	0.37***	0.32***	0.03	0.24***
RP	0.08	0.07	0.06	0.08	0.10	0.07	0.02

was, furthermore, correlated with both *ortho*-phosphate ($r = 0.313$, $P = 0.034$) and BOD ($r = 0.197$, $P = 0.044$). DSFI score, saprobic value, %SPEAR abundance, the number of EPT taxa and EPT abundance were negatively correlated to macrophyte coverage, whereas the number of macrophyte species was positively correlated to DSFI scores, the number of EPT taxa and Shannon–Wiener diversity. The macrophyte coverage was, furthermore, correlated to both relative proportions of silt ($r = 0.374$, $P < 0.001$) and sand ($r = 0.223$, $P < 0.001$).

4. Discussion

4.1 Predicted pesticide runoff and SPEAR abundance

Predicted pesticide runoff (RP) ranged approximately ten orders of magnitude from -9.96 to -0.23 . Schriever *et al.*⁹ applied the runoff potential model for predicting pesticide runoff in German lowland streams generating RP values ranging from -9.67 to -0.36 . Worst case scenario and range of predicted pesticide runoff are therefore comparable between these two studies.

We did not find any significant effect of consistent predicted pesticide runoff on %SPEAR abundance. One main explaining factor is probably that fauna samples were collected in early spring prior to the main pesticide application season. The potential impact from agriculturally derived pesticides was, consequently, related to exposure scenarios the preceding year leaving ten months for the macroinvertebrate community to recover. Several studies confirm that the signal of pesticide impact on the macroinvertebrate community structure in streams is strongest during exposure (pesticide spraying season) and in the following post-exposure months.^{6,29,30}

There was no significant effect of stream size on %SPEAR abundance, indicating that the increasing connectivity between stream and land with decreasing stream size did not translate into increasing impact of predicted pesticide runoff from agricultural fields. However, other studies confirmed that the footprint of pesticides is more distinct in small streams where the interaction between stream and land is more intense.^{8,31} Furthermore, the dilution effect decreases with decreasing stream size due to decreasing discharge.

The proportion of undisturbed area in the two sided 50 metre buffer extending from the sampling site and upstream had a significant positive effect on %SPEAR abundance. The presence of undisturbed areas in the stream corridor potentially acts as large scale spatial refugia for macroinvertebrates promoting the process of recolonisation and system recovery. This is facilitated by increased net downstream transport of especially Ephemeroptera, Plecoptera and Trichoptera (EPT) as these insect orders often maintain high densities at such habitats due to higher habitat quality and complexity of both stream and riparian zones.^{32,33} Our findings are supported by Liess and von der Ohe⁸ and Schriever *et al.*⁹ who, additionally, found increased %SPEAR abundance at stream sites with forest on both sides of the stream corridor in the near upstream environment.

When comparing %SPEAR abundance grouped according to consistent predicted pesticide runoff to those found by Schriever *et al.*⁹ there is a difference between sites with highest predicted pesticide runoff. At sites where $RP > -2$ average %SPEAR abundance was 31% in Danish streams whereas %SPEAR

abundance was 30% and 16% in German streams with upstream forested reaches being present or absent, respectively. Assuming that the near stream environment is not significantly different among streams from these studies, %SPEAR abundance was generally lower in the German streams. One possible explanation could be that the most extreme runoff incidents in reality are characterised by higher pesticide concentrations in Germany than in Denmark. Consequently, the maximum impact on benthic macroinvertebrates would be smaller in Danish streams as the maximum concentration is the primary factor determining severity of effects.³⁴ Such a difference could be mediated by a higher pesticide application rate, which generally increases with decreasing latitude.³⁵ This is not considered in the RP model as application rate was set to a constant value of 1 for a generic compound. Additionally, quantitative assessments of buffer strips are not considered in this model but have major impact on the amount of pesticide reaching surface water recipients *via* runoff.^{36,37} In Denmark, streams are often surrounded by small buffer strips, partly due to legislative requirements. In the Braunschweig area, Germany, where Schriever *et al.*⁹ conducted their study, streams were not surrounded by buffer strips (Matthias Liess, personal communication). Due to the presence of buffer strips and lower pesticide application rates, we suggest that Danish streams in general are not impacted by heavy pesticide runoff contamination. Because these factors are not included in the runoff model, predicted pesticide contamination is probably overestimated, which could explain the impaired correlation between RP and %SPEAR abundance found in this study. However, relatively high pesticide concentrations have been detected previously in both water and sediments in Danish streams,^{4,38} and in more extreme cases pesticide concentrations have been significantly correlated to variation in macroinvertebrate community structure.³⁹ We therefore infer that agriculturally derived pesticide contamination at least locally can pose a threat to stream macroinvertebrate communities.

We found that Danish streams in general were characterised by high macrophyte coverage (65%). Previous studies confirm that macrophytes are very abundant in small lowland streams in Denmark.^{15,40} Other studies indicated lower macrophyte abundance in European streams; *e.g.* in France (19%), Finland (14%) and Germany (4–13%).^{8,9,41} Within macrophyte patches flow is reduced,⁴² and the vast majority of a pesticide pulse, consequently, passes in the main flow channel outside/between patches. Beketov and Liess⁴³ showed that thiacloprid concentration was reduced up to 60% within macrophyte patches during a 10 hour pulse exposure compared to the non-vegetated main flow channel. Macrophyte patches therefore potentially provide important small scale spatial refugia sheltering resident macroinvertebrates from pesticide exposure. In consequence, benthic macroinvertebrate community resistance and recovery potential after pesticide exposure could be improved in macrophyte rich streams. This could be an additional explaining factor for the observed high %SPEAR abundance in Danish streams despite high predicted pesticide runoff exposure. However, as macrophyte data were collected from June to September it probably overestimates macrophyte abundance during the main insecticide application season in May and June. Further studies are needed to clarify the role of macrophytes as small scale physical refugia for macroinvertebrates during pesticide exposure.

4.2 Importance of local physical stream properties

Applying all supplementary environmental variables we found that local physical habitat properties most strongly explained variation in macroinvertebrate community structure compared to chemical properties and land-use characteristics. Stream bed coverage of silt was the most important environmental parameter having a negative impact on abundance and species richness of EPT taxa. Stream bed coverage of gravel and sand were the two most important parameters having a positive effect on abundance and species richness of EPT taxa. Most EPT species are associated with hard substratum or macrophytes and are depending on high oxygen concentrations resulting from the turbulent flow prevailing around these structures.^{15,44} Our findings are supported by Pedersen *et al.*⁴⁵ and Pedersen and Friberg¹⁵ who conducted comprehensive studies linking environmental variables at different scales to ecological stream quality in Danish streams. They found that local physical stream properties primarily were explaining variation in macroinvertebrate communities in Danish streams. We therefore consent to the fact that habitat degradation is a major problem in Danish streams.

The applied macroinvertebrate indices (SPEAR, DSFI, Saprobic index) responded congruently with abundance and species richness of EPT taxa to physical habitat property characteristics. As many EPT species are sensitive to various types of pollution they are often applied as indicators for high stream ecosystem quality in various macroinvertebrate based biological indices.^{14,44}

Overall, a multitude of environmental parameters at different scales co-explain variation in macroinvertebrate communities as many environmental parameters at different scales are directly or indirectly inter-correlated. The stream habitat quality (substrate composition and heterogeneity) is tightly linked to land use in the catchment with streams draining agricultural land being characterised by increasing relative coverage of silt and decreasing relative coverage of coarse substrate probably due to stream channelisation, dredging and weed cutting.¹³ Depending on spatial and temporal location of the stream, present biota is influenced by both small and large scale environmental parameters⁴⁶ as the biota has to pass through several environmental filters at different spatial scales to potentially persist at any site. Additionally, local scale filters are influenced by changes in large scale filters (*i.e.* at the catchment or regional scale).^{16,47}

5. Conclusion

In this study the negative impact of poor physical habitat properties overclouded the impact of pesticides as predicted from the runoff potential model. The runoff potential model, however, probably overestimated pesticide runoff contamination due to the presence of buffer strips surrounding Danish streams and due to the moderate pesticide application rate in Denmark. We therefore suggest that heavy pesticide contamination from agricultural non-point sources in Danish streams is only a potential problem locally. Streams influenced by agricultural activities are often severely impacted by other agriculturally related activities (*e.g.* channelisation, dredging and weed cutting) resulting in physical habitat degradation which, eventually, translates into

the disappearance of sensitive macroinvertebrate species. At least in the absence of heavy pesticide runoff contamination, physical habitat properties are primarily governing stream macroinvertebrate community structure in Danish streams. When pesticide runoff contamination is low to moderate, it is therefore important to consider physical habitat properties in risk assessment in order to avoid overestimating toxic impact of pesticides in streams. This study emphasises the importance of considering the hierarchical contribution of all potential environmental stressors when conducting risk assessment of agricultural streams.

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