A new paradigm for biomonitoring: an example building on the Danish Stream Plant Index

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Summary

1. Despite intensive efforts for more than a decade to develop Water Framework-compliant assessment systems, shortcomings continue to appear. In particular, the lack of reference conditions has hindered the development of assessment systems capturing the heart of the Water Framework Directive (WFD) – that ecological status should be set as the deviation from the natural, undisturbed condition. Recently, the Danish Stream Plant Index (DSPI) was developed. This system contrasts existing systems in that it builds on an expert interpretation of the normative definitions of ecological status classes in the WFD without taking pressure-impact relationships into account.

2. Here, we substantiate the approach taken in the development of DSPI and examine whether the DSPI class decreases with increasing level of anthropogenic stress and, additionally, whether the deviation from the natural undisturbed condition increases with decreasing DSPI class sensu WFD using trait composition of plant assemblages from Danish streams around year 1900 as a reference. We furthermore examine the trait composition of the vegetation in sites classified into different DSPI status classes to explore whether predictable patterns exist that can be used to identify the ultimate cause(s) of failure to meet ecological goals and help guide the selection of appropriate mitigation measures.

3. We observed that DSPI declined with several parameters indicative of environmental stress in Danish streams and, furthermore, that the deviation from the natural undisturbed condition regarding the trait composition of plant communities declined with increasing DSPI, implying that the trait composition of plant communities in the high DSPI status class was most similar to those occurring in Danish streams around year 1900. We also found that trait characteristics capable of disentangling important stressors in Danish streams varied consistently among sites classified into different DSPI classes.

4. Based on our findings, we call for new thinking. We suggest that more effort should be directed at describing reference conditions and interpreting the normative definitions of good, moderate, poor and bad instead of focusing solely on developing assessment systems using pressure-impact frameworks. We find this particularly important with respect to streams as these are seldom impacted by only a single stressor.

Key-words: biomonitoring, expert judgement, lowland, macrophyte, macrophyte index, stream, Water Framework Directive

Introduction

Humans have extensively altered the global environment, changed biogeochemical cycles, transformed land and enhanced the mobility of the biota, which has caused widespread changes in the global distribution of species and unprecedented species loss (e.g. Sala et al. 2000; Foley et al. 2005). Meeting human needs for land cultivation of crops, domestic water, power and transport has come at the expense of natural features of surface waters such as rivers and lakes, and today, these ecosystems are particularly threatened by human activities (Strayer & Dudgeon 2010). Despite increased efforts in recent years to halt and reverse adverse effects on the biota, species loss is an ongoing challenge (Dudgeon et al. 2006). In Europe, the European Water Framework Directive (WFD; European Commission 2000) has been implemented as the main tool for a coordinated protection of aquatic ecosystems. The WFD is highly ambitious. For the first time in history, biota forms the cornerstone of monitoring activities and the status of all surface waters is to be assessed contemplating the biota rather than using a more limited approach based on chemical quality or targeted biological components.

Since the implementation of the WFD, multiple biological assessment systems for surface waters have been developed (e.g. Birk et al. 2012). The prevailing approach used builds on the response of biological indicators to different types of stressors (Hering et al. 2010; Birk et al. 2012; Lyche-Solheim et al. 2013), largely ignoring that ecological status according...
to the WFD should be assessed as the deviation from the type-specific reference condition expressed as the ecological quality ratios derived from a characterisation of the biota of undisturbed or minimally disturbed reference sites. This has a very good explanation: the reference criteria for physicochemical, hydromorphological and pressure criteria cannot be fulfilled (Wallin, Wiederholm & Johnson 2003) when applied in the lowlands (Nijboer et al. 2004; Chaves et al. 2006; Baattrup-Pedersen et al. 2009), making the use of survey data to describe the reference conditions unsuccessful. However, the simplicity of many of the existing assessment systems may entail a risk of not revealing cause–effect linkages that eventually jeopardise the selection of mitigation measures in water bodies that do not fulfil good ecological status. For example, most assessment systems in rivers appear to be better able to detect some types of stressors than others (Marzin et al. 2012), and the selection of mitigation measures may therefore be distorted towards these, despite that these may not be decisive for the assessment (e.g. Demars et al. 2012). Equally important is the fact that most rivers are impacted by multiple simultaneously operating stressors (Hering et al. 2015), making it difficult to identify the most appropriate mitigation measure. Recent reports (EEA 2012 and ETC-ICM 2012) have identified diffuse pollution and hydromorphological degradation as the prevailing stressors in rivers. Both diffuse pollution and hydromorphological degradation are composed of several stressors with complex interactions (Hering et al. 2015). Diffuse pollution embraces increased nutrient loads and associated eutrophication, often in association with elevated contents of fine sediment, pesticides and other toxic substances, and hydromorphological degradation includes hydrological stress from low flows and water abstraction and morphological stress from barriers, channelisation and removal of natural riparian vegetation (Hering et al. 2015).

Aquatic plants are mandatory in the biological assessment of surface waters. Existing assessment systems in streams and rivers mainly address nutrient enrichment (e.g. Holmes et al. 1999; Haury et al. 2006; Schneider 2007) and acidification (e.g. Tremp & Kohler 1995), and only few assessment systems address general stream degradation (Schaumburg et al. 2004; Baattrup-Pedersen, Larsen & Riis 2013). Despite intensive efforts in the development of these systems, shortcomings continue to emerge (Demars et al. 2012; Wiegleb et al. 2016), which may be linked to the inherent properties of most assessment systems. These are designed according to the concept of positive, negative and indifferent indicator species, with indicator values being derived from data showing a correlation between the abundance of the species and the impact value of the pressure (Birk & Willby 2010, 2011). If we consider phosphorous, which is the stressor most often targeted in plant assessment systems, this approach is ambiguous since stream water alkalinity, which significantly influences aquatic plant assemblages, and phosphorous often covary in nature (Hutchinson 1975; Demars & Thiebaut 2008; Demars & Trémolières 2009), rendering assessment systems building on ecological indicator values for phosphorous difficult to interpret (Demars et al. 2012). Furthermore plant assemblages are affected by other stressors in streams (e.g. Baattrup-Pedersen et al. 2016a). Consequently, new approaches to assess ecological status of aquatic plant communities should be explored to meet the requirements of the WFD. Recently, the Danish Stream Plant Index (DSPI) was developed (Baattrup-Pedersen, Larsen & Riis 2013). This method contrasts existing methods in that it builds on an expert interpretation of the normative definitions of ecological status in the WFD, that is the deviation from the undisturbed condition. Five experts were provided with data on species abundances from c. 1200 Danish stream sites together with information on the size of the streams and alkalinity as variation in these parameters can be of importance for species segregation (Riis, Sand-Jensen & Vestergaard 2001). Then, the experts were asked to independently classify the sites into five ecological status classes (bad, poor, moderate, good and high) using the normative definitions given in the WFD. Following a number of pairwise comparisons within and among experts, it became clear that the experts generally agreed in their classifications of the stream sites, and a supervised classification model was developed, mirroring the perception of the experts of ecological status. This model can be used to classify new sites into ecological status classes using species scores along the main gradients in a multidimensional space as predictor variables for calculating a probability of site membership to each of the five ecological status classes. The model was later named DSPI (Birk & Willby 2011; Sondergaard et al. 2013).

Here, we take the next important steps. In order to substantiate the approach taken in the development of DSPI, we examine the response of DSPI to anthropogenic stressors in streams, in particular if the ecological status class decreases with increasing level of anthropogenic stress and, additionally, if the deviation from the natural undisturbed condition increases with decreasing ecological status class sensu WFD using plant assemblages from Danish streams around year 1900 as a reference. Secondly, we examine the trait composition of the vegetation in sites classified into different DSPI status classes. We focus on traits previously proven useful to disentangle important stressors in Danish streams (see Baattrup-Pedersen et al. 2016a) to explore whether predictable patterns exist that can be used to identify the ultimate cause of failure to meet ecological goals and, consequently, to guide the selection of appropriate mitigation measures. Specifically, we hypothesised (i) that DSPI varies according to the most important stressors acting in Danish streams and that DSPI is capable of capturing the impact from multiple co-occurring stressors and (ii) that the trait composition of reference plant assemblages shares a high similarity to sites classified as high ecological status sites using DSPI and (iii) that trait characteristics known to respond to the most important stressors acting in Danish streams vary with DSPI, implying that trait composition can be used to reveal the ultimate cause of failure to meet good ecological status.
Materials and methods

Data and methods

Data were obtained from the Danish monitoring programme on the aquatic environment, NOVANA (Friberg et al. 2005). We used data from a total of 625 stream reaches covering middle-sized (2–10 m wide) and large (>10 m wide) streams (types 2 and 3 sensu Baattrup-Pedersen & Riis 2004). Aquatic plant data were collected following the protocol described in Pedersen, Baattrup-Pedersen & Wiberg-Larsen (2007). In each stream reach, plant recordings were made in July/August at maximum biomass. Recordings were made in c. 150 plots (25 × 25 cm) placed side by side in cross-sectional transects at a 100-m-long stream reach. Depending on the width of the stream, the number of transects varied from a minimum of 10 to maximum 20 in small streams. A cover score was allocated to each species present in the plots using the following abundance scale: 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100%. Species abundance at each stream reach was then calculated as the sum of cover scores to the maximum score sum (i.e. the number of plots multiplied by the maximum score of five; Pedersen, Baattrup-Pedersen & Madsen 2006), and the DSPI classification model (Baattrup-Pedersen, Larsen & Riis 2013) was applied to classify each of the stream sites into ecological status classes 1–5 (high, good, moderate, poor and bad).

Historic plant data (1876–1920) were retrieved for 27 Danish mid-sized stream reaches distributed throughout Denmark from published literature, excursion reports and herbarium specimens kept in the Botanical Museum, Copenhagen (for further details, see Riis & Sand-Jensen 2001; Baattrup-Pedersen et al. 2008). These data were restricted to species presence data and covered the period 1876–1920, which is well before the period where major construction works took place in Danish streams following the legislation for land reclamation in 1940 (Anonymous 1940).

Description of traits

A total of 55 species were observed in the present data set and 34 in the historic data set. We allocated traits to all species including ecological preference values (Ellenberg N and L; Ellenberg et al. 1991), life-forms (LFs), growth morphology and traits of importance for species dispersal and survival (Table 1). The Ellenberg indicator values offer autecological information on the response of c. 2000 species to a range of climatic and edaphic factors in Central Europe. They have been applied to aquatic vegetation as well, and we therefore decided to also integrate them here and to analyse their variability with DSPI.

Trait data were extracted from online data bases (Willby, Abernethy & Demars 2000). The LFs were divided into six categories: free floating (surface and submerged), anchored with both floating and submerged leaves, and amphibious species with homophyllous emergent leaves and heterophyllous emergent leaves. Growth morphology was divided into three categories: single basal, single apical and multiple apical growth points (Table 1). Plant morphological traits also included a morphology index building on the height and lateral extension of the canopy and the leaf area of the species. Dispersal was characterised by four traits: the ability to disperse by forming extensive root–rhizome systems, the ability to reproduce by fragmentation, the number of seeds and the number of reproductive organs produced by the species. We also integrated traits related to survival in terms of overwintering organs such as tubers, turions and rhizomes.

The LF traits, and traits covering fragmentation, seeds, overwintering organs and rhizomes, were based on presence/absence of the attribute, with a score of 0 for absence, 1 for occasionally but not generally present attributes and 2 for present attributes. The morphology traits describing the meristem growth point type were based on presence (1) or absence (0) of the attribute. The number of reproductive organs was classified into low (<10), medium (10–100), high (100–1000) and very high (>1000), with values ranging from 1 to 4 based on number per individual per year. Leaf area was classified according to the leaf size categories with values ranging from 1 to 4, representing small (<1 cm²), medium (1–20 cm²), large (20–100 cm²) and very large (>100 cm²). The morphology index was also classified into categories (2, 3–5, 6–7, 8–9 and 10) with values ranging from 1 to 5. In some cases, species were classified in-between two categories regarding the number of reproductive organs, leaf area and morphology index (Willby, Abernethy & Demars 2000). In these cases, a classification code in-between was allocated to the particular trait (i.e. 1.5, 2.5, 3.5 and 4.5).

Environmental data

Land-use characteristics for each site as well as water chemistry data and data on hydromorphological characteristics (i.e. cross section, planform) were obtained from NOVANA (Friberg et al. 2005). Water chemistry data used for the analyses were based on five yearly

| Short trait name | Explanation | Category |
|------------------|-------------|----------|
| LE               | Ellenberg Light | Ecological preference |
| NE               | Ellenberg Nitrogen | Ecological preference |
| Frfls            | Free floating, surface | Life-form |
| Frfshb           | Free floating, submerged | Life-form |
| Anflle           | Anchored, floating leaves | Life-form |
| Ansole           | Anchored, submerged leaves | Life-form |
| Anemle           | Anchored, emergent homophyllous leaves | Life-form |
| Anhete           | Anchored, emergent heterophyllous leaves | Life-form |
| Meris.sb         | Meristem single basal growth point | Morphology |
| Meris.sa         | Meristem single apical growth point | Morphology |
| Meris.ma         | Meristem multiple apical growth point | Morphology |
| Morph.ind        | Morphology index = (height + lateral extension of the canopy)/2 | Morphology |
| Leaf.area        | Leaf area | Morphology |
| Rhizome          | Reproduction by rhizomes | Dispersal |
| Frag             | Reproduction by fragmentation | Dispersal |
| Seeds            | Reproduction by seeds | Dispersal |
| N.rep.org        | Number of reproductive organs per year and individual | Survival |

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sampling conducted within 5 years of the vegetation surveys. As water chemistry has been rather constant in Danish streams over the last years, we find this approach appropriate (Wiberg-Larsen et al. 2015). The water samples were analysed for phosphate (PO₄-P), nitrate (NO₃-N) and ammonium (NH₄-N) in the laboratory according to European standards. Cross section was determined using the following four categories: heavily modified (0), intermediate modified (1), slightly modified (2) and natural (3). Heavily modified cross sections had a trapezoidal form due to channelisation work with similar depths across the channel profile; intermediate and slightly modified cross sections had signs of earlier channelisation but with, respectively, small and clear signs of physical recovery, whereas natural cross sections exhibited a typical variability in depth characteristics across the transect. Furthermore, contrary to natural cross sections, heavily modified cross sections were deeply positioned relative to the surrounding land, whereas intermediate and slightly modified cross sections were characterised by less deeply positioned channels. Planform was recorded in the field using the following four categories: channelised planform (0), slightly sinuous planform (1), sinuous planform (2) and meandering planform (3). Information on weed cutting frequency was obtained directly from the water authorities. As weed cutting may have changed over time, we focused on sites monitored in 2012 and 2013 with information on cutting frequency in the periods 2007–2011 and 2012–2013 in the analyses. Weed cutting refers to the number of weed cuttings performed annually.

DATA ANALYSIS

Very few sites were classified as bad status sites (class 1), and we therefore decided to treat bad and poor ecological status sites together in the analysis (class 1+2). We used one-way ANOVA (aov in stats) to test for differences between DSPI classes and environmental characteristics using Tukey multiple comparisons of means (TukeyHSD in stats) for comparisons between DSPI classes.

To examine community characteristics, we calculated community-weighted means (CWMs) for each individual trait for both the contemporary and historic data sets (FUnCtComp in R) as:

\[
CWM = \sum_{i=1}^{n} p_i \times trait_i,
\]

where \( p_i \) is the relative contribution of species \( i \) to the community, and trait is the trait value of species \( i \) (e.g. Lavoro et al. 2008).

We then conducted a PCA on the contemporary data set on the CWMs of the individual traits. The PCA constructs a distance matrix based on Euclidian distance to detect linear combinations of the original variables, thus maximising the variance. PCA site scores for the 27 historic stream records were then predicted using species scores as predictor variables (predict.cca in VEGAN). Average Euclidian distances (dissimilarities) between historical stream records and DSPI classes were calculated (singer in VEGAN), and analysis of similarity (ANOSIM; anosim in VEGAN) was used to test for significant differences in trait composition between DSPI classes.

The relationships between DSPI and trait characteristics were modelled as proportional odds models using cumulative logits (Agresti 1990). A linear model for each logit was established with similar slopes but different intercepts. As DSPI have four ordered categories, a total of three logit models for each trait were established. DSPI was treated as an ordinal variable and the CWMs of the individual traits were treated as continuous variables. All analyses were conducted in R (version 2.15; R Project for Statistical Computing, Vienna, Austria).

Results

Danish Stream Plant Index responded significantly to several parameters indicative of environmental stress (ANOVA; Table 2), including land use (urban area and agricultural area), water chemistry (PO₄-P; NO₃-N) and hydromorphology (planform, cross section and weed cutting). DSPI declined with increasing percentage of agriculture and urban area use in the catchment and with increasing concentrations of PO₄-P and NO₃-N in the stream water (Fig. 1). DSPI was also lower in streams with higher weed cutting frequency (Fig. 1). In contrast, DSPI increased with increasing naturalness in the cross section and planform of the stream profile (Fig. 1).

The PCA clearly separated the stream sites according to the trait composition of the plant communities, with the first three components explaining 27%, 20% and 16% of the total variation, respectively (Fig. 2). The first PCA axis was largely related to reproductive strategy (fragmentation and rhizome) and LF (free-floating submerged and anchored), with free-floating species and species dispersed by fragmentation on the right side and anchored species, species dispersed by rhizome growth and species with large leaf area on the left side. The second PCA axis was also related to LF (anchored species at the lower side and free-floating surface species on the upper side). There were clear differences in trait composition between the ecological status classes (ANOSIM; \( R = 0.09, P = 0.0001 \)). Average dissimilarity was highest between DSPI class 1+2 (comprising sites with poor and bad status) and DSPI class 5 and lowest between DSPI classes 4 and 5 (ANOSIM; \( P < 0.05 \); Table 3).

The trait composition of the plant communities from around year 1900 predominated in the lower quadrant of the PCA diagram (Fig. 2). Average dissimilarity between the trait composition of the plant communities around 1900 and the various DSPI classes varied between 0.407 (DSPI 1+2) and 0.223 (DSPI 5; Fig. 3). Overall, the dissimilarity declined with increasing DSPI class, implying that the trait composition of plant communities around 1900 was most similar to DSPI class 5 (Fig. 3).

Table 2. Comparisons of means of catchment land-use characteristics, stream water chemistry and hydromorphology, including weed cutting practice of streams classified into different DSPI classes using one-way ANOVA

| Source                            | F    | P    |
|-----------------------------------|------|------|
| Land use                          |      |      |
| Urban                             | 3.023| 0.0297|
| Agriculture                       | 2.818| 0.0390|
| Chemistry                         |      |      |
| NO₃–N                             | 3.576| 0.0144|
| PO₄–P                             | 3.446| 0.0171|
| Hydromorphology                   |      |      |
| Planform                          | 4.242| 0.0063|
| Cross section                     | 3.083| 0.0287|
| Weed cutting (2007–2011)          | 5.468| 0.0013|
| Weed cutting (2012–2013)          | 7.285| 0.0001|

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Overall, we observed that several traits were significantly associated with the DSPI classes (logistic regression models with $P < 0.001$ in all cases; Table 4). In particular, growth meristem characteristics (meris.sa and meris.sb), the ecological preference for nutrients (NE) and the degree to which the species possessed overwintering organs varied significantly with DSPI (Fig. 4). Thus, DSPI declined with decreasing abundance of species growing from apical and multi-apical growth meristems, whereas the abundance of species growing from basal growth meristems increased together with the abundance of species with high NE and a high overwintering capacity (Table 4). DSPI also varied with the abundance of the various LFs, with a decline in DSPI as the abundance of free-floating species increased and an increase in DSPI as the abundance of anchored species increased, including both species with floating, emergent, submerged and heterophyllous leaves (Table 4). Additionally, DSPI declined with increasing abundance of species with a high morphology index, high leaf area, extensive rhizome growth and a high number of reproductive organs, whereas DSPI increased as the abundance of species dispersed by seeds and fragmentation increased (Table 4).

**Discussion**

DSPI AND ANTHROPOGENIC IMPACTS

It has previously been suggested that DSPI is in accordance with the EU WFD as the classifications are set relative to a deviation from the natural, undisturbed conditions (Baattrup-Pedersen, Larsen & Riis 2013). Here, we are able to substantiate this suggestion. First, and in accordance with our first hypothesis, we found that DSPI responded to several of the environmental parameters integrated as measures of the level of impact in the streams and that sites fulfilling at least good ecological status were those least affected by these stressors. We observed that the DSPI was lower in sites with high impact from morphological alterations in terms of channelisation (cross section, planform) and increasing impact from weed cutting. Similarly important, we observed that DSPI was lower in sites with high intensity of agricultural and urban land use in the catchment as well as in sites with high levels of phosphate and nitrate in the stream water, indicating that DSPI also captures impact from eutrophication. Taken together, these findings confirm that DSPI is capable of capturing the impact from several co-occurring stressors. Secondly, and according
to our second hypothesis, sites classified as high ecological status sites showed the highest resemblance to plant communities in Danish streams around 1900, that is before anthropogenic stress became serious in Danish streams. Furthermore, we found that the resemblance to the historic plant communities diminished as the DSPI ecological status class declined. We acknowledge that the variability in dissimilarity is high among sites classified in the different ecological status classes, especially those in DSPI class 1+2, but the trend is unambiguous.

We propose that it is the inherent features of DSPI, that is the simultaneous use of several DCA axis scores, together reproducing the covariation in species occurrence as predictor variables for the classification, which make it suitable for capturing multiple stressors in stream environments. Thus, using several DCA axis scores, we capture a majority of the environmental variability responsible for species segregation in the streams, including both variability associated with natural environmental variation (like $\text{HCO}_3$, $\text{CO}_2$ and slope; Riis, Sand-Jensen & Vestergaard 2001; Demars & Trémolières

Table 3. Average Bray–Curtis dissimilarity calculated from community trait composition in stream sites classified as either DSPI 1+2, 3, 4 or 5

|          | DSPI 3 | DSPI 4 | DSPI 5 |
|----------|--------|--------|--------|
| DSPI 1+2 | 0.415***| 0.413***| 0.454***|
| DSPI 3   | 0.295***| 0.323***| 0.241*  |

*** $p < 0.0001$; * $p < 0.05$. 

Fig. 2. Results of a principal component analysis (PCA) based on community trait composition calculated from CWMs of aquatic plants in 625 streams. To the left is shown the position of the streams with different colour symbols for sites classified into DSPI 1+2, 3, 4 and 5. To the right is shown the trait loading plot. Predicted PC site scores for 27 historic mid-sized stream sites distributed throughout Denmark are superimposed on the figure as old Danish sites (OD) based on the co-occurrence of species in the historical and contemporary data set. Abbreviations for traits are explained in Table 1.
species distribution patterns, and furthermore, they can be insight into the underlying biological mechanisms determining occurring stressors. Traits have the advantage of providing
sequence, that different types of mitigation measures can be moderate ecological status sites. This result clearly illustrates
stream water), whereas the level of hydromorphological catchment and high levels of phosphate and nitrate in the
sites (DSPI 1
2009) and variability associated with environmental stressors. Interestingly, sites classified as poor and bad ecological status sites (DSPI 1–2) seemed to be most affected by eutrophication (high percentages of agriculture and urban land use in the catchment and high levels of phosphate and nitrate in the stream water), whereas the level of hydromorphological impact was comparable with those observed in sites classified as moderate ecological status sites. This result clearly illustrates that different stressors may set the upper limit for the ecological status that can be achieved in different sites and, in consequence, that different types of mitigation measures can be needed in sites failing to fulfill at least good ecological status.

REVEALING CAUSE–EFFECT LINKAGES

While DSPI seems to be a successful assessment system able to capture stressors related to anthropogenic impacts in streams, it does not reveal why sites may fail to reach good ecological status. Therefore, in continuation of an assessment revealing that a site fails to fulfill good ecological status, the ultimate cause should be identified in order to select appropriate measures to improve the status (Fig. 5). As indicated, this step may be particularly important in streams that are subjected to co-occurring stressors. Traits have the advantage of providing insight into the underlying biological mechanisms determining species distribution patterns, and furthermore, they can be compared consistently among different sites independent of differences in spatial constraints on species distribution (Townsend & Hildrew 1994; Poff 1997; Friberg et al. 2011). According to the third hypothesis, we discovered that plant traits previously found to respond to key stressors in Danish lowland streams, including eutrophication and hydromorphological degradation, varied with DSPI. In particular, we observed that

DSPI declined with increasing abundance of species growing from basal meristems and with increasing abundance of species having high productivity (Ellenberg N, morphology index).

Table 4. Results of a series proportional odds models using cumulative logits conducted to identify relationships between the categorical dependent variable DSPI and the trait composition of the aquatic plant community. For each model, significance (Rob > χ²) is given as well as the estimate for each trait and the intercepts between the ecological status classes. LE is not included as no significant relationships between DSPI and LE could be identified. Abbreviations for traits are explained in Table 1.

| Trait     | χ²  | Rob > χ² | Intercept | Estimate |
|-----------|-----|----------|-----------|----------|
| NE        | 69.19 | <0.0001 | y_1 = −11.646 | −1.599 |
| Frhr      | 17.91 | <0.0001 | y_1 = −1.473 | −1.508 |
| Frhsb     | 11.47 | 0.0007  | y_1 = −0.276 | 1.089 |
| Anflle    | 21.83 | <0.0001 | y_1 = −0.651 | 0.927 |
| Anhete    | 22.79 | <0.0001 | y_1 = −0.905 | 1.416 |
| Meris.ma  | 6.925 | 0.0085  | y_1 = −0.559 | 0.512 |
| Meris.sb  | 102.46 | <0.0001 | y_1 = −2.829 | −1.927 |
| Meris.sas | 111.40 | <0.0001 | y_1 = −0.500 | 3.32 |
| Morph.ind | 14.09 | 0.0002  | y_1 = −3.430 | −0.642 |
| Leaf.area | 11.63 | 0.0006  | y_1 = −2.678 | −0.684 |
| Seeds     | 10.38 | 0.0013  | y_1 = 0.987  | 1.214 |
| Rhizome   | 8.87  | 0.0029  | y_1 = −1.960 | −0.605 |
| Frag      | 17.87 | <0.0001 | y_1 = −0.347 | 0.833 |
| N.rep.org | 9.00  | 0.0027  | y_1 = −3.559 | −0.860 |
| Overwintering | 162.21 | <0.0001 | y_1 = −5.173 | −2.711 |

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Fig. 3. Average (SD) Bray–Curtis dissimilarity based on the trait composition between 265 stream sites classified into DSPI 1–2, 3, 4 and 5 and 27 historic stream sites calculated from presence-absence data of aquatic plants. The historical records cover the period 1876–1920, that is before the time period of significant human impact in Danish streams.
It has previously been shown that community trait characteristics of the vegetation in streams can be used to disentangle the most important stressor (Baattrup-Pedersen et al. 2015, 2016a). Using the framework proposed by Baattrup-Pedersen et al. (2016a) on sites failing to meet good ecological status, applying DSPI can provide insight into the underlying mechanisms (Fig. 5). For example, stream sites characterised by high abundance of species growing from basal meristems are likely to be impaired by hydromorphological impacts, in particular weed cutting, which is commonly applied in Danish streams to improve run-off from adjacent fields (Baattrup-Pedersen et al. 2009). The likely mechanism behind this response is, as previously suggested, that species growing from basal meristems are more resilient towards cutting because they can start regrowth immediately after the intervention as opposed to species growing from apical meristems (Baattrup-Pedersen, Larsen & Riis 2002; Baattrup-Pedersen et al. 2016a). Similarly, low abundance of species with heterophyllous also indicates that hydromorphological impacts are prevailing, but the mechanism is likely different. Thus, heterophyllous has been shown to respond negatively to both channelisation such as straightening and deepening of the stream channel and weed cutting, probably reflecting that habitats of heterophyllous species are negatively affected by these interventions due to impairment of fluvial geomorphological processes, thereby preventing depositional areas from developing fully during low flow in summer (Garcia de Jalón et al. 2013).

In contrast to the above-mentioned examples, where failure to meet good ecological status is related to hydromorphological stress, failure can also relate to other types of stressors or multiple stressors. Applying the same framework (Baattrup-Pedersen et al. 2016a), high abundance of species with low light requirements and apical growth meristems is likely to be impacted by eutrophication, reflecting that it is the ability to maximise the biomass in the upper waters that provides an advantage in eutrophic streams due to an impoverished light
climate (Sand-Jensen 1990; Hilton et al. 2006; Baattrup-Pedersen et al. 2015), whereas high abundance of productive species (high Ellenberg N and high morphology index) likely reflects combined stress from eutrophication and weed cutting. That is, productivity in itself may not guarantee success in nutrient-rich waters, reflecting that it is the availability of light that controls species abundance patterns and not the ability to rapidly convert nutrients into biomass, as shown in a recent study embracing lowland streams in Europe (Baattrup-Pedersen et al. 2015). In that way, trait characteristics can reveal the ultimate cause of failure to meet at least good ecological status applying DSPI.

**PERSPECTIVES FOR BIOMONITORING**

In recent years, use of trait-based approaches in biomonitoring as an alternative to species-based approaches has been suggested, in particular because trait-based approaches can provide a mechanistic understanding of biological responses, thereby offering a clear predictive power (e.g. Friberg et al. 2011). In many cases, predictable trait-abundance patterns have been established in response to natural and anthropogenic stressors (e.g. Doledec & Statzner 1994; Pollard & Yuan 2010), but examples also exist of trait responses differing from a priori predictions derived from hypothesised mechanisms of response (Statzner & Béche 2010; Orlofske & Baird 2014). It has been suggested that this may link to methodological issues (described in Verbek, van Noordwijk & Hildrew 2013) and to the quality of the existing trait data bases (Orlofske & Baird 2014). The simultaneous occurrence of several stressors may, however, be additionally important and complicate predictions using trait-abundance-based approaches (Verbek, van Noordwijk & Hildrew 2013). For example, low abundance of species possessing apical growth meristems in Danish streams not necessarily implies that eutrophication is an insignificant stressor; it may just as well indicate that weed cutting combined with eutrophication determine trait-abundance patterns (Baattrup-Pedersen et al. 2016a).

We therefore see major obstacles associated with the use of trait-abundance patterns as a backbone in ecological assessment systems as combinations of traits rather than single traits determine species success under different levels and combinations of stressors. Consequently, substituting taxonomic approaches with trait-based approaches may not provide a solution to existing challenges regarding cause–effect linkages in water bodies that do not fulfil good ecological status. Equally important, trait-based approaches do not secure compliance with WFD. Thus, independent of the approach taken, deviation from the natural conditions (i.e. the reference conditions) is needed to comply with the WFD. We therefore call for new thinking. We find it important to put more effort into describing reference conditions and also into interpreting the normative definitions of good, moderate, poor and bad in the sense of the WFD (see Willby 2011) as a framework for developing assessment methods. Only few attempts have been made to thoroughly investigate the scientific strength of using expert judgments to assess community conditions and only for benthic macroinvertebrates (but see Teixeira et al. 2010; Thompson et al. 2012; Keeley, Macleod & Forrest 2012). Some of these efforts have been quite successful and may provide a better starting point than focusing on identifying or developing assessment systems based on indices or multi-metrics using a pressure–impact framework, especially in multi-stressor environments characterised by highly complex stressor interactions. Then, as a next step, knowledge about the interplay

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between trait-abundance patterns and environmental stress can be used as a diagnostic framework to identify cause-effect linkages and to select appropriate mitigation measures in streams not fulfilling good ecological status.

Acknowledgements

The study was supported by the European Union Seventh Framework Project REFORM under contract no. 282656 and MARS under contract no. 603378. We thank Anne Mette Poulsen for manuscript editing and Tinna Christensen for figure layout.

Data accessibility

The data are stored at the Dryad repository titled ‘Data from: A new paradigm for bio-monitoring – An example building on the Danish Stream Plant Index’ with the provisional DOI http://dx.doi.org/10.5061/dryad.h3s8 (Baattrup-Pedersen et al. 2016b).

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Received 24 June 2016; accepted 2 October 2016

Handling Editor: Jana McPherson

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