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Critical appraisal of the indexes of chironomid larval deformities and their use in bioindication

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A recurring positive field correlation of deformity incidence (DI) in sediment dwelling chironomid larvae with environmental contamination suggests that DI is a potential indicator of the impact of contaminants in aquatic systems. Several researchers have developed indexes that take into account the severity of deformities to supplement the information that is obtained from the DI alone. Five such indexes are reviewed in this paper. The indexes can be reformulated into an identical general form that expresses a product of the DI and average severity of deformation. Assessment of the severity of deformities appears to be poorly grounded and subjective. Indexes and the DI are highly redundant, partly due to mathematical necessity. In the data sets of the proponents of the indexes there was a strong linear relationship ($r^2 = 0.77 – 0.99$) between index values and DI. Along with this, other empirical evidence as well as tenuous foundation of the indexes suggest that the presented indexes are likely to be useless, albeit relatively harmless.

1. Introduction

The positive spatial relationship between sediment contamination level and midge (Diptera: Chironomidae) larval deformities in the field is at present relatively well documented (for reviews *see* Johnson *et al*. 1993, Dermott 1991, Vermeulen 1995). Consequently, there appears to be an increasing interest in the use of morphological deformities in bioassessment and monitoring of contaminant stress in lakes and streams. However, few experimental studies have demonstrated exposure-response relationships between contaminants and deformities (Kosalwat & Knight 1987, Madden *et al*. 1992, Hudson & Ciborowski 1996a) and the results are partly confusing (Warwick 1985), and partly controversial (*see* Vermeulen 1995). There is no rigorous evidence of an actual deformity-stress or deformity-fitness relationship. Despite these problems and a considerable amount of unexplained inconsistency (e.g., Bird *et al*. 1995a, Jeyasingham & Ling 1997) the strong and recurring deformity-contamination correlation in the field justifies the view that occurrence of deformities is at least a potential *in situ* indication of sediment contamination or a marker of contaminant-induced stress.

Deformation at an individual level can be considered a simple binary response (Y): a larva (L) is either normal ($Y_L = 0$) or deformed ($Y_L = 1$). A natural and widely used quantification of this response within a population is the proportion of deformed individuals or deformity incidence

$$DI = d/n \quad (1)$$

where d is the number of deformed larvae and n the number of larvae examined. DI is also an estimator of the probability (Pr) of deformation

$$DI = EY_L = Pr(Y_L = 1). \quad (2)$$

However, several authors have further classified deformities according to their 'severity' or 'strength' (Warwick 1985, 1991, Dermott 1991, Lenat 1993, Janssens de Bisthoven *et al.* 1995). Indexes based on these classifications have been thought to be more informative than the DI alone (Warwick 1985, 1991, Dermott 1991, Lenat 1993, Janssens de Bisthoven *et al.* 1995). Although not widely applied, the indexing has attracted some general interest, and improvement of the indices has been considered important (e.g., Rosenberg 1992, Johnson *et al.* 1993, Diggins & Steward 1993). Moreover, adjectives 'mild' and 'severe' or 'weak' and 'strong' are repeatedly used on somewhat obscure grounds to describe deformities (e.g., Janssens de Bisthoven *et al.* 1998a, 1998b). Motivation to write this paper arose after a considerable amount of work in trying to apply the presented indices to my own data (H. Hämäläinen & J. Kukkonen unpubl.) only to realize that it was perhaps of no avail. I shall first examine the structure and some properties of the proposed indexes. Then I shall try to identify existing problems of defining the 'severity' of deformities and consequent difficulties in indexing. Third, using the data published by the proponents (Warwick 1985, 1991, Dermott 1991, Lenat 1993) of the indexes, I shall examine whether the presented indexes do give any information in addition to the DI. Finally, I shall shortly discuss the use of chironomid deformities as bioindicators in general and try to outline an alternative approach for indexing.

2. The structure and properties of indexes

Five separate deformity indexes taken from the literature [Warwick 1985, 1991 (two indexes), Dermott 1991, Lenat 1993] were examined. The sixth index found (Janssens de Bisthoven *et al.* 1995) is not treated here in detail as it was presented as a 'working instrument' not to be applied, and as the provided data do not allow the analyses performed in the present study. However, the approach of Janssens de Bisthoven *et al.* (1995) is essentially similar to the others, and most of that presented below apply to their index as well.

The pioneering Index of Severity of Antennal Deformities (ISAD) of Warwick (1985) is based on antennal deformities of *Chironomus* larvae. The second one is a comparable index for *Procladius* (Warwick 1991). The third, Index of Severity of Ligula Deformations (ISLD) (Warwick 1991) is based on ligula deformations of *Procladius*. All three indices (I), using the notation and terminology of Warwick (1985, 1991), take the form

$$I = \Sigma IMR/n \quad (3)$$

where IMR (Index of Morphological Response) is the total of deformity-class values (basic index numbers, BIN) for each larva and ΣIMR the sum over all larvae examined (n). The BIN is assigned to each deformity type according to its apparent severity (discussed below). The values for ligula deformities of *Procladius* are on a geometric scale ranging from 1 for the mildest to 64 for most severe deformities. The BIN for antenna deformities range from 1 to 21 (for details see Warwick 1985, 1991). Minimum value of IMR is zero for normal specimens but there is no defined upper limit for larvae with multiple deformation. Accordingly, ISAD and ISLD could range from zero to some indefinite, large value in populations where all larvae are deformed.

The 'Index of Severity of All Deformities' (ISAD) for *Procladius* (Dermott 1991) is a modification of the preceding indices, but it accounts for deformities in nine different structures instead of ligula or antennae only. The basic value of 1 is assigned to all specimens, including non-deformed larvae, and additional weights ranging from 1 to 4 are given for each deformity according to severity. The minimum for both *IMR* and *ISAD* is thus 1, and the maximum indefinite.

The fifth index proposed by Lenat (1993) and named the *Toxic Score* is based on mentum deformities of *Chironomus* larvae. Abnormality at an individual level is classified into three categories, for which weights 1, 2 and 3 are given according to increasing severity, mainly on the basis of number of deformities per larva. The index is calculated as a severity-weighted sum of deformed larvae (ΣIMR here, for the sake of consistency) divided by the number of specimens examined. As this quotient is multiplied by the factor 100, the index values can range from zero in populations with no deformed larvae to 300 in populations where all specimens are severely (class 3) deformed.

The structure of all indexes (I) is essentially identical. They can be reformulated into a general form

$$I = (d/n) \times (\Sigma \mathrm{IMR}/d) \quad (4)$$

which expresses the product of the DI and the average severity of deformation, $\mathrm{IMR}_{\mathrm{AVE}}$. The general form can thus be shortened as

$$I = \mathrm{DI} \times \mathrm{IMR}_{\mathrm{AVE}}. \quad (5)$$

As Dermott (1991) assigns the basic value 1 for all specimens, his ISAD should read $1 + \mathrm{DI} \times \mathrm{IMR}_{\mathrm{AVE}}$. However, the constant 1 is ineffective, and can be ignored. The multiplier 100 in Lenat's (1993) *Toxic Score* is just a scaling factor. Expressed in terms of expectations over population model

$$I = \Pr(Y_L = 1)\mathrm{E}(W_L | Y_L = 1) \quad (6)$$

where W_L denotes the severity of deformation.

3. The severity of deformities

The proponents of the indexes have not explicitly defined the 'severity' according to which the types of deformities are classified (Warwick 1985, 1991, Dermott 1991, Lenat 1993). The authors do not clearly state whether the deformities are supposed to indicate contamination (exposure), to be reflections of other, more harmful effects or whether deformities are considered to be directly injurious to the larvae and thus, as such the effects of concern. Consequently, the meaning of severity remains obscure. Nevertheless, it seems that the severity of a deformity is, at least partly, considered to be proportional to the apparent magnitude of deviation from normality. This idea might be intuitively justified, but the physical size of deviation from the normal phenotype is not necessarily related to the strength of exposure or stress experienced by the larva nor to the performance of the larva. Further, the basis of consistent quantification of the degree of deformation has not been given, even though e.g., deviations in number of teeth of mentum or number of segments in antennae have been considered (Warwick 1985, 1991, Dermott 1991). Given the large range of deformity types even within a single trait, the assessment of magnitude is inevitably highly subjective. Even more so when deformities in different structures are compared along the same scale (Dermott 1991, Janssens de Bisthoven *et al.* 1995). Furthermore, since the severity has not been objectively dimensioned, the weights or Basic Index Numbers assigned to each deformity type are, as Warwick (1985, 1991) notes, arbitrary. Even if the severity ranking happened to be correct, taking averages ($\mathrm{IMR}_{\mathrm{AVE}}$) of weights on an ordinal scale is questionable and could lead to biased results.

4. The applications of indexes

As the severity has not been conceptualized and operationalized, it is impossible to judge, whether the rankings and weights are correct or false. However, a pragmatic attitude can be taken and it can be empirically assessed, whether indexes based on severity classifications provide additional information. As a first step I used the field data presented by the proponents of the indexes (Table 1) and plotted the calculated index values in relation to the observed DI (Fig. 1). In all cases the variation in index values was mainly explained by the DI ($r^2 = 0.77 - 0.99$). In the case of linear relationship

$$I = \mathrm{DI} \times \mathrm{IMR}_{\mathrm{AVE}} = \alpha + \beta \times \mathrm{DI} + \varepsilon. \quad (7)$$

When α is close to zero, $\mathrm{IMR}_{\mathrm{AVE}}$ ($\approx \alpha / \mathrm{DI} + \beta$) is relatively constant ($\approx \beta$), at least when DI is not small. $\mathrm{IMR}_{\mathrm{AVE}}$ thus remains ineffective with regard to discrimination power. The residual variation is proportional to the small amount of additional information that could be gained by the indices. It should be noted, however, that in addition to true information (that is not necessarily associated with contamination or stress), the residuals include also the estimation error. The scatter of data points around the linear fit was somewhat larger in Warwick's (1985, 1991) ISADs and ISLD, compared to the remaining two indexes. This variation, may be attributable to the larger estimation error of $\mathrm{IMR}_{\mathrm{AVE}}$ due to the geometric scaling of severity weights used by Warwick (1985, 1991) in combination with small sample size.

Because the DI and index values are mathematically dependent (Eq. 4), close correlation between the variables is partly endogenous (e.g., Prairie & Bird, 1989). A 'significant' relationship could be expected even if the values were drawn at random (e.g., Krambeck 1995, Berges 1997). A comforting feature of this is that indexes like those presented would be relatively harmless even if the severity ranking and weights had nothing to do with true stress or contamination or were more or less random in nature, as may be the case.

A positive correlation could also be expected between the DI and $\mathrm{IMR}_{\mathrm{AVE}}$ as both are expected to increase with increasing contamination or stress. However, only in the case of *Toxic Score* and Dermott's ISAD, did the average severity increase with increasing DI ($r = 0.87$, $P = 0.01$ and $r = 0.94$, $P = 0.002$, respectively). This is seen as a slight nonlinearity in respective DI-index relationships (Figs. 1d and e) if the slope is thought to go through the origin (as it should). This, however, might not be due to the actual 'severity' or quality of deformities, but simply, due to the increasing *number* of deformities per individual. With increasing incidence of deformities (and supposed stress) the probability for multiple deformation will increase, even if each deformity would be induced independently, providing that the probabilities of induction for different types of deformities increase with stress. As a result the number of deformities and the severity indexes will increase nonlinearily with increasing DI. The formal proof and further analysis is beyond the scope of this article, but to illustrate the effect, I used personal unpublished data (samples of at least 30 larvae from 22 sites in Finnish lakes and rivers) on the mentum deformities of *Chironomus*. The percentage of larvae with multiple deformation increased with increasing DI in a slightly nonlinear fashion (Fig. 2) resembling that which is noted in Figs. 1d and e. Deviation from linearity, however, seems to be small until the DI is very large.

The above analysis of empirical data suggests that the proposed indices are highly redundant with the DI and can give little additional information and are thus unlikely to separate sites according to their contamination or stress level any better than the DI alone. However, the variation in index values that is not associated with the DI, even

Table 1. The data used for evaluation of the deformity indexes. Each observation consists of a deformity incidence and a deformity index value for a *Chironomus* (C) or *Procladius* (P) larval collection. Each collection is from a separate site, unless otherwise stated.

Index	Taxon	Source of data	Nr. of collections
ISAD	C	Warwick 1985: table 8	6 (2 replicates from 3 sites)
		Warwick 1988: table 14.2	1 (additional to the preceding)
		Warwick & Tisdale 1988: table 4	2
ISAD	P	Warwick 1991: table 12	10
ISLD	P	Warwick 1991: tables 10,11	17
ISAD	P	Dermott 1991: figs 6 and 7	5
		Dermott 1991: table 6	2 (III and IV instars, 1 site)
Toxic Score	C	Lenat 1993: table 1	7 (water quality groups, 3–9 sites in each)

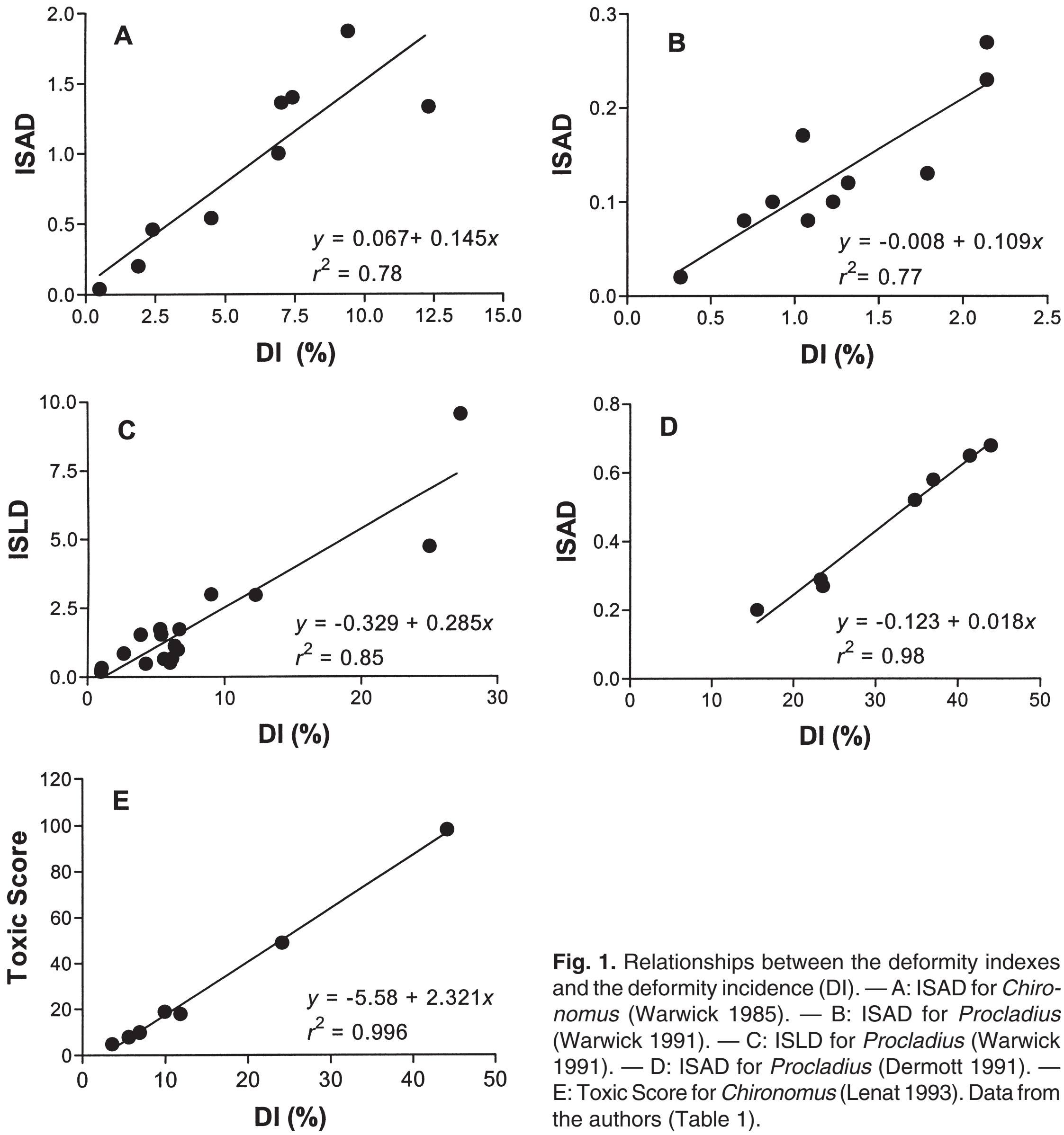

Fig. 1. Relationships between the deformity indexes and the deformity incidence (DI). — A: ISAD for *Chironomus* (Warwick 1985). — B: ISAD for *Procladius* (Warwick 1991). — C: ISLD for *Procladius* (Warwick 1991). — D: ISAD for *Procladius* (Dermott 1991). — E: Toxic Score for *Chironomus* (Lenat 1993). Data from the authors (Table 1).

though small, might be significant. For more rigorous tests, DI and indexes should be related to an observed (measured) pollution or stress level. Two examples were found from the literature. Lenat (1993) compared the deformity response of *Chironomus* larvae between groups of sites representing different water qualities. When differences were found between groups (ANOVA), they were shown by both the DI and *Toxic Score*. Warwick (1985) observed a surprising negative linear exposure-response relationship between the antennal deformities of *Chironomus*, and DDE concentration in water. The relationship was equally strong between DI and DDE ($r = -0.98$, $P = 0.004$) and ISAD and DDE ($r = -0.96$, $P = 0.010$). [The statistics were recalculated using the original data (Warwick 1985)]. Severity of deformities (IMR_{AVE}) did not correlate with the DDE concentration or supposed stress. To further illustrate the meaningless of severity weights in this case, I correlated ISAD, that was calculated from the observed DI, and IMR_{AVE} drawn randomly from an uniform distribution within the observed range (0.05–0.89), to DDE. Eight of the nineteen

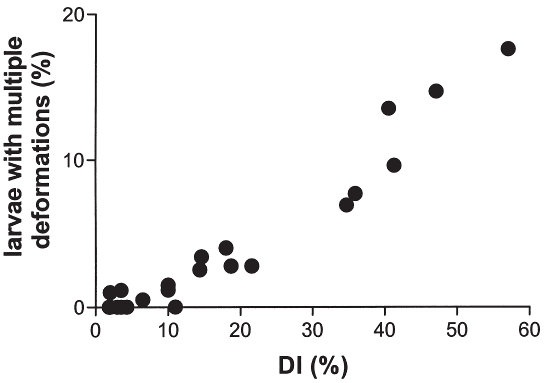

Fig. 2. Percentage of larvae with multiple (more than one deformation per larva) mentum deformations *vs.* percentage of deformed larvae (DI) in *Chironomus* spp. from 22 sites in Finnish lakes and rivers.

first iterations produced correlation that was stronger than the observed. Both examples suggest that the DI is as sensitive an indicator of contamination or supposed stress as the indexes accounting for the 'severity'.

5. What to do?

In conclusion, it seems likely that the existing indexes are useless, but relatively harmless. The indexes just tend to convert the deformity incidence into an arbitrary scale which is difficult to perceive. The introduction of severity weights also impede statistical treatment of the data. For example, relatively easy methods (e.g., simple logistic regression) to relate binary response to predictor variables should be replaced by complicated techniques to model hierarchical response (Eq. 6) (e.g., McCullach & Nelder 1989). Additionally, much extra effort is needed to classify the deformities, and in practice, it is impossible to assign all deformity types to the predefined categories. There seems to be nothing in the presented indexing approach that could compensate for these costs.

The idea of using the qualitative information of deformities may not be totally disregarded but since the approach presently applied is poorly grounded and obviously does not work, some other measures should be considered. First, the goal in using deformities in assessment and monitoring should be decided. Environmental degradation (Diggins & Stewart 1993, Bird 1994, Bird *et al.* 1995a, 1995b, Hudson & Ciborowski 1996), contamination (Cushman 1984, Diggins & Steward 1993), effects of contaminants (Dickman & Rygiel 1996, Hudson & Ciborowski 1996), contaminant, toxic or environmental stress (Rosenberg 1992, Warwick 1988, 1991, Janssens de Bisthoven *et al.* 1992), quality of sediments (Janssens de Bisthoven 1995) and toxic conditions (Lenat 1993) represent the wide variety of variables or processes that have been mentioned to be (potentially) indicated. Thus, relatively little progress has taken place since the pioneering phases, when Hamilton and Saether (1971) concluded that 'the presence of deformed larvae is certainly indicative of something'.

In order to use chironomid deformities as indicators of 'something', that something should be first explicitly defined in measurable terms. Relationship of the deformities to that variable or process should then be modelled and the predictive or indicative power of the model subsequently assessed using independent data. Considering the vast array of possible causal agents and the apparent non-specificity of the deformity response, trials to develop models to infer concentrations of, or even identify specific pollutants from, the incidence of chironomid deformities in the field (e.g., Hamilton & Saether 1971, Johnson *et al.* 1993, Vermeulen 1995, Janssens de Bisthoven *et al.* 1998b) are likely to be unsuccessful. More fruitful at present might be to establish the deformity-stress or deformity-fitness relationships, i.e. relate the deformities to responses of ecological significance (effect) as proposed by Wiederholm (1984). The few attempts to do so (Gerhardt & Janssens de Bisthoven 1995, Janssens de Bisthoven 1995, Janssens de Bisthoven *et al.* 1998a) have not yet yielded conclusive results.

Those who do not rely on the DI or simple binary response, but attempt to make use of the severity of deformities, should first assess the severity in relation to the target variable (either specified stressor or effect) using quantitative methods, instead of ranking the deformities according to their magnitude or apparent seriousness from the researcher's perspective or along an intuitive 'horror scale'. The next step will be to develop means to use that information and show its additional value, bearing in mind the extra effort needed.

For the time being, the proportion of deformed individuals or DI, seems to be the most useful measure of deformity response. As noted by Warwick (1988) and shortly discussed and exemplified above, in populations with very large DI the proportion of individuals with multiple deformities can be high, and important information could be masked. Accounting for the number of deformities (when countable) might deserve closer examination.

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