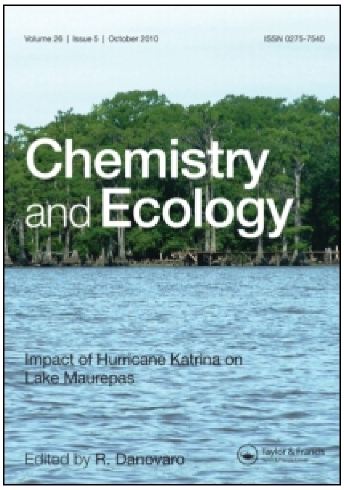

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COMMUNITY TOLERANCE COMPARISON AS A NEW AND SENSITIVE ECOTOXICOLOGICAL TOOL

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The demonstration of an increased tolerance to a pollutant is direct evidence that this pollutant has presented a selection pressure upon a population or community. Recently, pollution-induced community tolerance (PICT) has been applied to the benthic community in a metal-enriched estuary to establish causal linkage between observed changes in community structure and composition and the presence of copper. This paper describes the rationale and methodology behind applying PICT to the marine benthic environment, and shows it to be a sensitive ecotoxicological tool. It also presents a case study, demonstrating how a PICT investigation has aided the interpretation of changes in a benthic community, establishing a causal linkage between these changes in the community and the presence of a specific pollutant.

Keywords: Pollution induced community tolerance; nematodes; meiofauna; ecotoxicology

INTRODUCTION

It has been argued convincingly that only when a pollutant has had a deleterious effect at the level of the population or community, rather than on the individual alone, can it be said that pollution has occurred (McIntyre *et al.*, 1978; Bayne *et al.*, 1985). Therefore many ecotoxicological impact studies have focused on the effect of potential pollutants at these higher levels of biological organisation, often using suites of univariate and multivariate analyses to compare the species composition and community structure of "impacted" and "control" sites (Clarke and Green, 1988). However, while many ecological investigations have ascribed shifts in population or community structure to the presence of a potential pollutant, establishing a clear link between the two has rarely been attempted and has often proved problematical (Gray *et al.*, 1980). This difficulty is often a product of the natural complexity and variability of the ecological community, which makes the attribution of "deleterious effects" to the presence of a specific pollutant, or indeed to pollution at all, highly circumspect (Gray, 1979). However, few if any ecotoxicological tools have been able to link the presence of toxicants and ecological effect at the level of the community. Luoma (1977) has argued that an increased tolerance in a population exposed to a specific pollutant is direct evidence that this pollutant has had a biological impact upon that population. This paradigm has been applied by Blanck *et al.* (1988) to pollution studies at the community level and recently Millward and Grant (1995) have extended its usage into the marine benthic environment. These studies have demonstrated that increases in pollution-induced community tolerance (PICT) can provide that critical link between the presence of specific pollutants and evidence of community impairment and that studies of induced tolerance within impacted populations can provide insights into the effects on pollutants on communities.

The Use of Pollution-Induced Community Tolerance (PICT) in Field Studies

Blanck *et al.* (1988) suggest that a community which has been toxicologically stressed by a pollutant will exhibit an increase in tolerance to that pollutant, due to a combination of extinctions of sensitive species and increases in physiological acclimation or heritable tolerance. They suggest that a demonstration of such a pollution-induced community tolerance (PICT) might provide a sensitive ecotoxicological tool in the field, detecting disturbances at the community level and providing direct evidence of a deleterious selection pressure by that specific pollutant upon the local community. They applied the technique to periphytal communities collected at impacted and reference sites, with community tolerance estimated by the relative photosynthetic activity of the collected community (Blanck *et al.*, 1988). This technique has received some attention in model ecosystems, investigating the effects of pollution on freshwater phytoplankton communities (Wängberg *et al.*, 1991) and marine periphyton communities (Molander and Blanck, 1992) by comparing photosynthetic impairment of algae from exposed and non-exposed communities. However, few studies (*e.g.*, Dahl and Blanck, 1990; Wängberg, 1995) have applied the technique to retrospective studies of chronic pollution in the field, and to date only one study (Millward and Grant, 1995) has extended the PICT rationale into the marine benthic environment.

The Application of PICT to Marine Benthic Pollution Studies

It has long been recognised that estuaries and coastal waters face a high risk from anthropogenic pollution and that the monitoring of benthic communities in these localities should be the mainstay of any programme of pollution assessment (McIntyre, 1986). However, the temporal and spatial complexity of these systems often prevents direct attributation of shifts in community structure to the impact of anthropogenic pollution (Gray *et al.*, 1980). With this in mind, Millward and Grant (1995) have recently adapted the PICT rationale to the marine benthic environment, using acute survival as a measurement of tolerance rather than the functional photosynthetic activity assay used by Blanck *et al.* (1988). Using the nematode community as a study group, Millward and Grant (1995) have demonstrated that PICT comparisons can establish causal linkages between the presence of individual pollutants and impact at the community level.

The rationale and methodology employed by Millward and Grant (1995) is deliberately straightforward. Using the theories of Blanck *et al.* (1988), the method assumes that a pollutant which has been present at sufficient duration and concentration to induce a biological impact would lead to a decreased sensitivity of the local community to that pollutant, due to the extinction of individuals and/or species sensitive to that pollutant and, in the surviving populations, an increased activity in mechanisms of acclimation or adaptation. The method therefore compares the relative sensitivity to the potential xenobiotic under investigation of a randomly selected sample of individuals from impacted and non-impacted sites, using a simple acute toxicity test methodology. The method does not attempt to identify or select individual species and readers are referred to the standard ecotoxicological literature for the design of an acute LT_{50} toxicity test relevant to the test group employed (*e.g.*, Sprague, 1969). When selecting the total sample size for the PICT comparisons, Millward and Grant (1995) elected to use 300 individuals, in order to census the community tolerance and also to provide an adequate sample size (30 individuals) from which to calculate relative population tolerance values from all species which comprised more than 10% of the total assemblage. The median survival times for the whole assemblage and for individual common ($\geq$10% of the assemblage) species, were calculated using the SPSS procedure "Survival", treating individuals alive at the end of the experiment as censored observations. The resulting PICT value is a relative index of community sensitivity to the xenobiotic and a significant difference between the indices of different sites was interpreted as direct evidence that this xenobiotic has exerted a higher selection pressure at the site which yielded the more resistant sample than at the reference site, according to the rationale of Luoma (1977) and Blanck *et al.* (1988). The survival data for the more common species was found particularly instructive, presenting clear evidence for the role of the studied xenobiotic in selecting the species composition at impacted sites (see below).

PICT: A Case Study

The interpretation and power of the method is best described with reference to a case study. Restronguet Creek, part of the Fal estuary system in southwest England, has been receiving mining effluent for many hundreds of years and as a result contains sediments chronically enriched with toxic heavy metals, particularly copper and zinc (Bryan and Gibbs, 1983). Somerfield *et al.* (1994) have recently studied the meiofaunal communities in Restronguet Creek and a number of neighbouring creeks in the Fal (see Fig. 1) which yield sediment metal concentrations differing by orders of magnitude (see Tab. I). Using a suite of univariate indices, Somerfield *et al.* (1994) demonstrated that the nematode community is less diverse at Restronguet and Mylor Creeks, the sites of the highest and second highest sediment metal

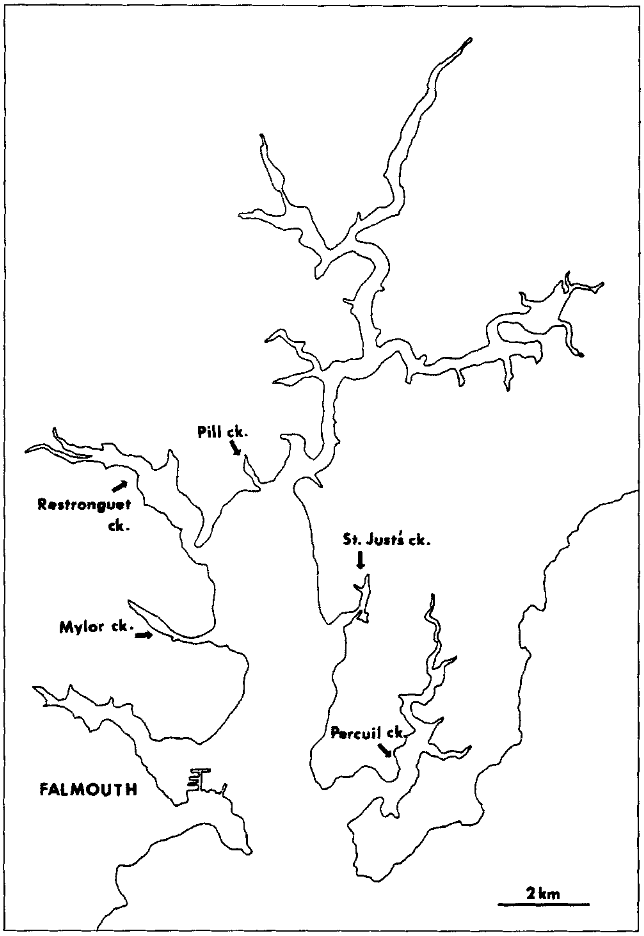

FIGURE 1 Map of the Fal estuary system, Cornwall, S. W. England showing the five estuaries sampled by Somerfield *et al.* (1994) and compared for relative community tolerances by the author.

content respectively, than at other less impacted sites (Fig. 2). Multivariate analysis of the nematode data by multidimensional scaling (MDS) revealed clear distinctions between the nematode assemblages in Restronguet Creek and the other estuaries, which, while contiguous, were in an order consistent with metal contamination (Fig. 3); while an

TABLE I Sediment levels of copper and zinc ($\mu g\ g^{-1}$ dry weight, standard deviations in parentheses) from the five estuaries by Somerfield *et al.* (1994) and compared for relative community tolerances by the author's data from Somerfield *et al.* (1994)

Creek	*Cu*	*Zn*
Restronguet Creek	2532(575)	3814(1295)
Mylor Creek	1272(62)	1431(59)
Pill Creek	697(19)	1006(69)
St. Just's Creek	332(33)	624(33)
Percuil	165(34)	302(62)

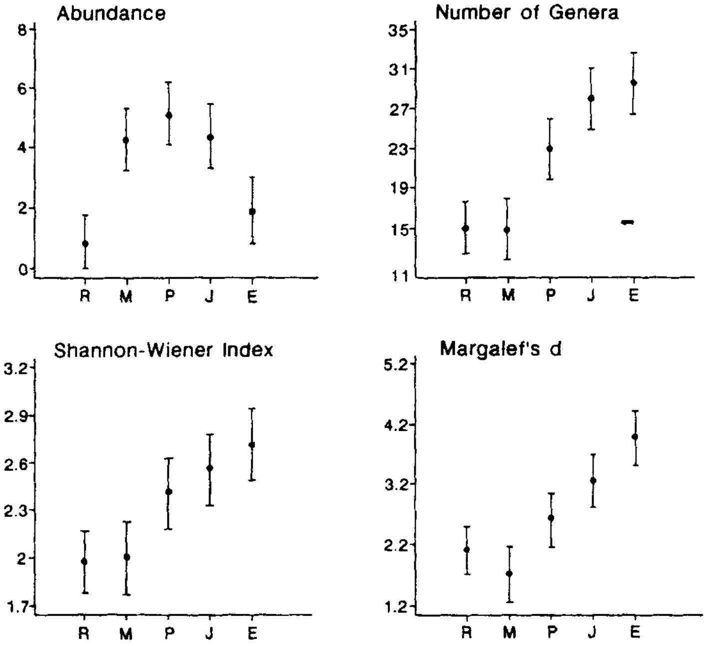

FIGURE 2 Comparison of nematode community diversity and abundance. R = Restronguet Creek, M = Mylor Creek, P = Pill Creek, J = St Just's Creek, E = Percuil Creek. Reproduced with permission from Somerfield *et al.*, 1994.

analysis of similarity test revealed the sites to be significantly different in nematode species composition. Somerfield *et al.* (1994) used rank correlation analyses to show that sediment metal content best explained the separation of creek nematode communities by MDS and infer that sediment metal content is the best explanation for the ordination. However, whilst the inferential evidence of such data is

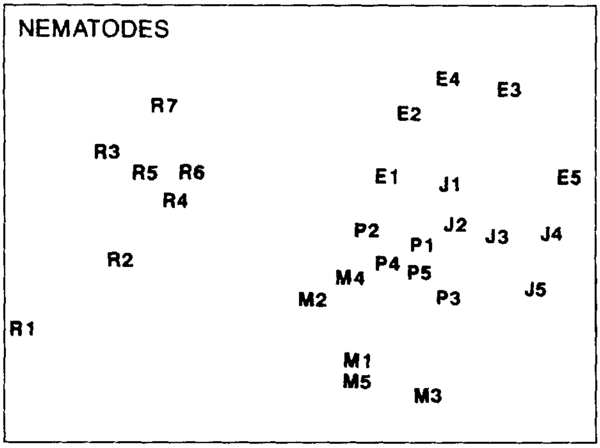

FIGURE 3 Multidimensional Scaling (MDS) ordinations for fourth root transformed nematode abundance (stress = 0.13) in Restronguet Creek (R), Mylor Creek (M), Pill Creek (P), St Justs Creek (J) and Percuil Creek (E). Reproduced with permission from Somerfield *et al.*, 1994.

strong, ecological monitoring alone cannot establish a direct causal link between such observations and the presence of any xenobiotic. Likewise, such ecological studies are often hampered by the presence of 'nuisance factors'; natural or non-toxic parameters which correlate with the xenobiotic and whose effects are often difficult to identify (Gray *et al.*, 1988).

Millward and Grant (in press) have used the PICT methodology to investigate a direct cause and effect link between the presence of copper in the Fal system and changes in community structure as described by Somerfield *et al.* (1994). Nematode samples were collected from each of the five estuaries addressed by Somerfield *et al.* (1994) and were exposed to 200 ppb copper in a controlled acute toxicity test (refer to Millward and Grant, in press for details of the methodology). The pairwise multiple comparisons of median survival times for assemblages from each of the five estuaries following exposure to 200 ppb copper are presented in Figure 4. This figure shows significant differences within the sites of medium contamination (sites Pill and St Just's) and both the most contaminated (sites Restronguet and Mylor) and least contaminated sites (site Percuil). The figure also demonstrates that the relative community tolerance revealed by these toxicity tests orders the five

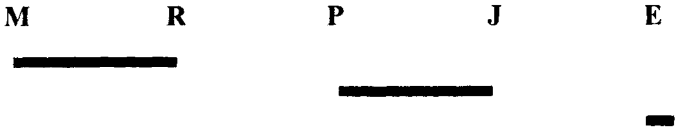

FIGURE 4 Results of pairwise multiple comparison using the Wilcoxon (Gehan) statistic between sites of median time to death for nematodes exposed to 200 ppb copper. R = Restronguet Creek, M = Mylor Creek, P = Pill Creek, J = St Just's Creek, E = Percuil Creek.

estuaries according to copper content, although Restronguet and Mylor Creeks are switched. Potentially, this data offers direct evidence that copper has indeed presented a selection pressure within these estuaries, evidence not provided by current, more traditional ecological surveys and monitoring methods.

In addition to comparing the relative tolerances of the whole communities, Millward and Grant (1995) disaggregated the assemblage survival data by species at the most impacted (Restronguet Creek) and least impacted (Percuil River) sites to compare the relative sensitivities of individual species to copper. The ranked species median survival times from the two sites are presented in Figure 5. The figure reveals that the metal-impacted Restronguet Creek assemblage was comprised of fewer species and that these species were markedly more resistant to copper stress than were the species present at the less impacted Percuil River. These results suggest that copper has been an important factor controlling species composition at the site, demonstrating that copper enrichment has selected for copper-resistant types and the local demise of copper-sensitive species. In addition, previous work has indicated that the Restronguet Creek population of at least one of species exhibits a significantly higher tolerance to copper than the population at Percuil River (Millward and Grant, 1995), indicating that the higher community tolerance at the impacted sites is also a product of acclimation or genetically inherited adaptation. It is therefore clear that this disaggregation of species survival data is a potentially powerful tool in establishing the direct effect of a xenobiotic on benthic community structure and composition and that the method is instructive in interpreting the univariate and multivariate data commonly presented by ecotoxicological community level studies (Millward and Grant, 1995).

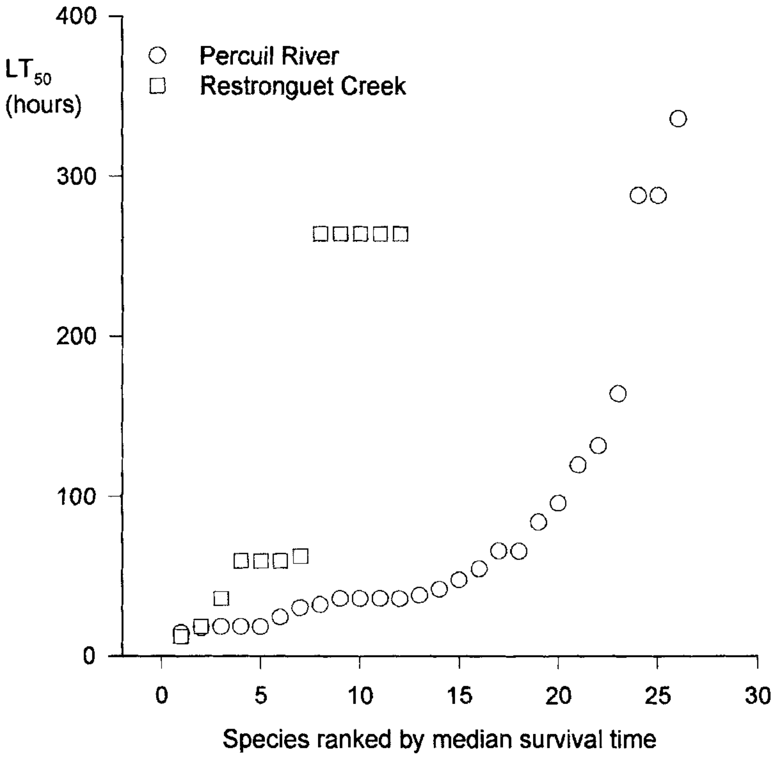

FIGURE 5 Ranked median survival times following copper exposure of nematode assemblages from Restronguet Creek (metal enriched) and Percuil River (non-impacted) sites.

Millward and Grant (1995) have found that the nematode community offers many advantages in studies of relative tolerance. The Nematoda are often the most species rich taxon in freshwater and marine sediments (Heip *et al.*, 1985), especially in species-poor biotopes such as estuaries and exposed beaches, which are often susceptible to pollution (Giere, 1993). This high diversity offers an almost unparalleled source of potential variability in pollution sensitivity and Millward and Grant (1995) have shown that estuarine nematode species do indeed express a range of sensitivities to a pollutant; making them promising candidates for use in PICT studies. Nematodes are benthic invertebrates with no dispersive pelagic larval stage and a relatively short generation time, characteristics which assure exposure of individuals to local pollution episodes and a rapid expression of acclimation or adaptation. They are therefore ideal candidates for PICT assessments in marine benthic situations.

A Critique of PICT in Ecotoxicology

Theoretically, PICT offers a number of conceptual advantages over the range of ecotoxicological methods currently employed in investigating pollution impact at the community level (Blanck *et al.*, 1988):

- PICT might provide direct evidence that a community is, or has been, affected by the specific toxicant under investigation.
- The results of a PICT study aid the interpretation of the multivariate and univariate results from pollution impact assessment studies.
- The PICT data could be correlated with the levels of a pollutant along a concentration gradient to determine the levels of pollution which have induced a toxicological stress *under field conditions.* The high sensitivity of PICT suggests that it might be used to calculate the no-effect concentration of the community as a whole.
- PICT might be of use in conditions of complex pollution mixtures, providing information on which potential pollutants have exerted a selection pressure on the local environment.
- PICT should be more sensitive than single species toxicity tests or censuses of indicator species, since it involves no prior selection of a key indicator species within a community but censuses any changes in or among all populations within an impacted community.
- PICT should also prove sensitive since the method inherently records any significant changes in the community members most susceptible to the toxicological challenge.
- Since PICT directly assesses the impact of specific pollutants on a community the method should not be hampered by abiotic or biotic ‘nuisance’ factors which often hinder interpretation of ecotoxicological monitoring results (Gray *et al.*, 1980).
- PICT would differentiate between the primary effects of a pollutant (for example, increased mortality or decreased fecundity) and secondary effects (*e.g.*, a decreased predation due to predator extinction).

- The PICT comparison is relatively straightforward, requiring a low degree of expertise and expenditure, since it employs a simple toxicity trial methodology.

There are a number of areas which require consideration in the application of the PICT methodology:

- The specificity of tolerance has been questioned, with cases of co-tolerance (tolerance for one toxicant eliciting tolerance for a second toxicant) being cited. Documented examples of co-tolerance suggest that it might occur between similar chemicals, or chemicals which affect the same target area in a similar way (Blanck *et al.*, 1988). This would suggest that a measured increase in community tolerance to one toxicant would infer toxicological challenge by that chemical, or a chemical acting in a similar manner and further investigations of potential co-tolerance might elucidate the specificity of the response.
- The specificity of the PICT response would also be impaired if a toxicological challenge resulted in the selection of generally resistant species, rather than species adapted to the specific pollutant. For example, it has been noted that estuarine benthic communities, tolerant of salinity and other abiotic stresses, are also more resistant to a host of pollution challenges than communities from more stable environments (Boesch and Rosenberg, 1981). It is therefore conceivable that a community which has been challenged by a pollutant might have a higher proportion of such resistant species, which would register an increased non-specific community tolerance for other pollutants to which it has not previously been exposed. Disaggregation of the PICT data by species and comparing population tolerances will address this issue, by presenting evidence of an elevated tolerance between populations of such potential resistant species at impacted and reference sites.
- The method also requires that populations not impacted by a pollutant should not register significantly different PICT values. Preliminary work (Millward, unpubl.) suggests that this is the case, but care should be taken in the design of PICT trials to include more than one non-impacted reference site.
- The method is still under development, in particular addressing its usage with non-metallic pollutants and the role of habitat on baseline community tolerance.

In conclusion, the theoretical advantages of PICT suggest that it might offer a straightforward method for attributing pollution impacts at the level of the community to pollution. Intensive validation of the method as a retrospective ecotoxicological tool in the field has yet to be done, but the few published studies which have used the method suggest that it might prove a useful adjunct to other community-level analyses, particularly when the data is disaggregated by species (Millward and Grant, 1995, in press). It is suggested that PICT be subjected to further testing and development, in particular addressing the specificity of increases in community tolerance to individual toxicants. This paper argues that the nematode community is a suitable group in marine and benthic PICT assessments and that survival is a more suitable measurement of tolerance than any individual function.

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