Metal induced succession in benthic diatom consortia

Ivorra i Castella, N.

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Chapter VII
CONCLUDING REMARKS

The present study investigated the role of metals in steering diatom succession, and revealed that biofilm characteristics and biological interactions between species play a prominent role. Below the main questions of this thesis are discussed.

*Is metal contamination steering algal succession towards more tolerant forms?*

Chapters III, IV, V and VI demonstrated that diatom species composition and algal density of biofilms are clearly affected by the Zn and Cd contamination of the streams. The diatom species composition of the biofilms differed markedly according to the metal gradient at the field sites (chapter III). The fast adaptation of the reference diatom communities translocated to metal polluted sites contrasted with the slower reaction on the reverse translocation, and provided a first indication of an active metal selection force on the algal consortia of the streams (chapter III). Using a similar approach, Gold *et al.* (2000) also reported inhibiting effects of Zn and Cd on diatom species composition and cell density in biofilms from an uncontaminated stream site after translocation to a polluted site. However, this thesis also demonstrate that metal contamination is not the only factor selecting diatom species.

Shifts in diatom species composition of the reference communities after exposure in the laboratory to Zn and Cd concentrations similar to those found at the polluted sites (chapter IV and V), were consistent with observations on metal exposed biofilms in the field. Also, the higher Zn tolerance of the benthic diatom *G. parvulum* isolated from the metal-polluted environment, when compared to *G. parvulum* isolated from the reference site, confirmed that selection for metal tolerance had taken place in this species (chapter VI).
Selection for increased metal tolerance of diatom communities as observed here is consistent with general ecological theory and analogous to selection for metal tolerance in terrestrial vegetation on e.g. mine sites (Schat and Verkleij 1998). Selection for tolerance also confirms the basics of the PICT concept (Pollution Induced Community Tolerance, Blanck et al. 1988). According to this concept the shifting numerical abundance of tolerant individuals (either belonging to the same or different species) is measurable in overall physiological responses of whole consortia. However, in spite of the large differences in Zn and Cd concentrations and the chronic character of the exposure at the present river sites, the short-term Zn toxicity tests on algal photosynthetic $^{14}$C-incorporation showed an uniformly high Zn-tolerance of all algal consortia (chapter II). Using the same approach, Lehmann et al. (1999) could not only marginally demonstrate selection by Zn or Cd on the microphytic consortia of the polluted sites according to the PICT-protocol. PICT was only found in bacterial consortia of the polluted sites using a very different physiological endpoint i.e. $^{3}$H-thymidine incorporation.

The combined results of the present thesis prove that the fifty percent effect concentration for a biofilm exposed to Zn as obtained from short-term physiological tests, is not an appropiate quantification of the intrinsic tolerance of the films' algal cells. Such an $E_{50}$ value is an apparent value affected by biofilm thickness, diffusion, sorption and (delayed) physiological response based on diffusion of metabolic products. This artifact of short-term tolerance measurements is indicated by $E_{50}$ values for Zn that were one order of magnitude higher in dense and complex field biofilms (chapter II) than in dilute mono-specific *Gomphonema* cultures (chapter VI). Also the inventors of the PICT-concept (Blanck et al. 1988) rely on apparent $E_{50}$ values of intact biofilms to draw conclusions on selection for Zn tolerance. Zn was observed to decrease settlement of micro-algal communities in flow-through aquaria (Paulsson et al. 2000) and created a wide range of densities in terms of chlorophyll and dry weight per surface area. Throughout this range, $E_{50}$ (Zn) for micro-algae were not found to change except for a three-fold increase at the highest Zn concentration; which was interpreted as PICT. Taking the views developed in the present thesis, the apparent $E_{50}$ (Zn) is bound to decrease at lower densities. In Paulsson et al. (2000) such a trend
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can just be detected at intermediate zinc concentration, so that it seems likely that the PICT searched for, was actually much more prominent than thought on first sight. Irgarol, a photosynthetic inhibitor, was found to induce changes in species composition and community density (Dahl and Blanck 1996), but surprisingly not in PICT, measured as EC$_{50}$ in intact biofilms. The confounding effect of decreasing densities may have prevented conclusions on cellular tolerance. In parallel experiments on phytoplankton and periphyton Gustavson and Wångberg (1995) compared tolerance induction by Cu. Increased tolerance to Cu of the phytoplankton assemblages in these enclosures was clearly found by using $^{14}$C incorporation tests, however, a similar effect was not observed for periphyton. This discrepancy is conceivable in view of the differences of $^{14}$C-tests in perfect suspensions of phytoplankton and biofilm layers with diffusion limitations as put forward in this thesis.

The present thesis in conjunction with the published literature show that metals, notably Zn and Cu, do steer the succession of micro-algal consortia. This succession results in predominance of metal tolerant life forms and is detectable in the investigated river system (R. Dommel) with sites exposed to a different degree of metal pollution. However, the way of detecting such a succession via PICT using intact biofilms is fraught with methodological limitations. Species or clone specific techniques have been shown to be effective to overcome these difficulties.

How do nutrients modulate metal-steered diatom succession in biofilms?

Phosphate amendment caused an increase in relative abundance of diatom species, such as the chain-forming Melosira varians and motile Navicula spp., considered to be more characteristic of advanced successional stages. Adnate growth forms, *i.e.* Achnanthes minutissima, were observed to be overgrown (chapter IV; Pringle 1990). Metal exposure reduced algal density and thereby suppressed 'overstory' diatom taxa (chapter IV). Similar effects of metal addition on diatoms consortia were seen by Medley and Clements (1998). Early successional taxa such as A. minutissima and F.
capucina var. vaucheriae proliferated under exposure to Zn, Cu and Cd, whereas the growth of the late successional taxa *Diatoma vulgare* and *M. varians* was suppressed.

The effects of metals on the development of biofilm algae appears, at least partly, to be due to disturbed or delayed growth. This indirect effect is different from the direct effect exerted by metals *i.e.* selective killing of sensitive algal taxa and thus stimulation of the fraction of tolerant taxa (see above). The indirect and mainly unselective effect of metals on biofilms compares well with effects caused by perturbations through grazers (Steinman *et al.* 1992), storm events and spates (Blenkinsopp and Lock 1994; Humphrey and Stevenson 1992; Peterson and Stevenson 1992). Indeed these perturbations and the present observations on counteracting effects of metals and phosphate are compatible with the habitat matrix model for stream periphyton by Biggs *et al.* (1998). Following this concept a gradient from low algal biomass in frequently physically disturbed and nutrient poor habitats to high biomass in undisturbed and nutrient enriched habitat is predicted (Biggs *et al.* 1998). 'Climax' communities with high biomass are characterised by large cells (C-selection), slowly colonising with an ability to form tall structures, like the filaments of *M. varians*, that over-top basal cells and thus compete more effectively for nutrients and light (Biggs *et al.* 1998). The utilization of high concentrations of inorganic nutrients in overlying waters by *M. varians* might be enabled by nutrient uptake systems with high half saturation coefficients as determined for other filamentous species in this category *i.e.* *Cladophora glomerata* (Biggs *et al.* 1998). Traits of S-selection taxa partly conform those of C-taxa, but individuals have a reduced size, achieve low densities and are most competitive only at low nutrient and disturbance levels, *i.e.* Meridion and Epithemia. R-selection taxa associated with disturbed and high nutrient conditions, such as *G. parvulum*, and with disturbed and low or high nutrient conditions, like *A. minutissima*, possess one or more combined traits that enable their success over wide ranges of disturbance-nutrient resource supply, namely being small, having high resistance to removal, high immigration/emigration rates, high maximum growth/vegetative reproductive rates and flexible or low half saturation coefficient for nutrient uptake *i.e.* *A. minutissima* (Biggs *et al.* 1998). A low half saturation coefficient for nutrient uptake may enable these taxa also to persist concurrently with other taxa
in mats developing under stable and moderately high \textit{(Navicula cryptocephala, Stigeoclonium tenue)} or high nutrient regimes \textit{(M. varians, Cladophora glomerata)}.

The habitat template of periphyton growth \textit{(Biggs et al. 1998)} allows the disturbance of benthic diatom consortia and their species succession caused by metals to be put into a general ecological context. Reynolds \textit{(1997)} proposed a C-S-R template for the course of ecosystem succession on the basis of observations on disturbance and development of phytoplankton. Planktonic taxa are displaced during succession according to their strategic traits. Climax species are substituted by acclimative or invasive ones according to the timing and magnitude of the disturbing event \textit{(Reynolds 1997)}. The resilience of phytoplankton communities, as in periphyton communities, to lose biomass and structure during disturbing events is bound to their degree of maturity, succession and organisation \textit{(chapter V; Reynolds 1997; Blenkinsopp and Lock 1994)}. In the more complex mature biofilms metal effects are diminished or delayed by structural and chemical barriers created by the presence of taxa, mucus, particles trapped and an altered pH regime with increased biomass and photosynthetic activity \textit{(Ferris et al. 1989; Liehr et al. 1994; Jørgersen and Revsbech 1985)}. The higher tolerance of the mature biofilms to Zn and Cd concentrations conformed to this reduced dependance of mature biofilms on external factors \textit{(chapter V)}. The observations of Paulsson \textit{et al.} \textit{(2000)} are consistent with this argument. Paulsson \textit{et al.} \textit{(2000)} observed that in Swedish oligotrophic waters the settlement of periphyton in flow-through aquaria was extremely sensitive to low Zn amendments. In this case disturbance \textit{(Zn toxicity)} overrules potential effects of any deficiencies of phosphate or micro-nutrients.

In conclusion nutrient status of intact biofilms and the resulting vertical differentiation in algal consortia is a major factor determining sensitivity to physical or chemical disturbances. Conversely, acute exposure to toxic substances \textit{(e.g. metals)} affects this vertical organisation and thereby the niches of diatom species, tightly bound to specific biofilm micro-habitats. Nutrient enrichment also affects the vertical organisation and, therefore, the variability in nutrients co-acting with metals. Yet, this indirect effect of metals plus nutrients cannot be discriminated with precision from the direct selection of individuals or taxa for metal tolerance.
Are experiments needed to support the use of benthic diatoms for water quality assessment?

A vast literature on the use of diatoms for assessments of environmental quality has been published in the past decades. Already in the founding studies of Patrick in the 1950s, 1960s and 1970s, physical and chemical determinants of diatom community composition have been extensively documented (see Patrick 1977). The development of diatom indices for these environmental factors have been partly validated by mesocosm studies on stream communities along with a few culture studies on isolated species. Since these early studies several authors have argued that experimental evidence is needed to validate the outcome of correlative studies on the distribution of diatom species. Cox (1991) even questioned the validity of diatom monitoring systems without demonstration of causal relationships. Also Van Dam et al. (1994) noted the lack of any experimental support for 'well established' correlations, such as between diatom species occurring in pH gradients. Consistent with these previous conclusions Winter and Duthie (2000) pointed to the insufficiency of autoecological data to confirm the observed species optima and tolerances.

Essentially, diatom monitoring has been established almost completely empirically. The assumed and widely accepted sensitivity or tolerance of taxa for nutrients, organic pollution, pH, O₂-requirements etc. (Lange-Bertalot 1979; Lowe 1974; Kelly 1998) is based on field observations and not experimentally verified. A series of progress reports on algal monitoring in European rivers (Whitton et al. 1991; Whitton and Rott 1996; Prygiel et al. 1999) shows the prominence of an exclusively empirical approach. This character is also evident in wider scale applications of diatom studies in environmental and earth sciences (Stoermer and Smol 1999). Diatom autoecological indices (DAI) have been listed by Stevenson and Pan (1999) for environmental changes caused by industry, sewage, forestry and mining. For mining DAI factors on heavy metals, pH and SO₄ are categorised. However, field-based ranking of diatom species toward all these factors may not be available. Some of these factors are strongly correlated, especially in the case of acid mine drainage. Studies on metal polluted English rivers have shown, as in the present thesis, that metals are selection factors
modifying species composition of algal communities and affecting the genetic composition of algal populations (Harding and Whitton 1976; Kelly and Whitton 1989). Although these effects may be pronounced, they do not suggest unique metal indicator species. From these studies it seems essential to discriminate the factors associated with metal pollution such as pH and \( \text{SO}_4 \). Even though the correlation between diatom distributions and certain factors are strong, caution is needed in extrapolating or transferring indices to other regions and times (Stevenson and Pan 1999). The general absence of experimental proof introduces the risk that intercorrelated sets of environmental factors (such as for metals, pH and \( \text{SO}_4 \)) lead to false interpretations. In agreement with Stevenson and Pan (1999), it is concluded here that diatom research should be put in a general ecological context allowing experimental testing of hypotheses. This would make this branch of science also more transferable to assessments with other organisms (Admiraal et al. 2000).

Autoecological studies on certain diatom species have shown that these are suitable as indicator of certain conditions. For example the US-EPA listed the percentage of motile diatom species (Surirella, Nitzschia and Navicula) to indicate the degree of siltation in rivers (Bahls 1993; Stevenson and Bahls 1999). Similarly, the percentage anomalies in diatom cells have been proposed as a diagnostic tool to indicate chemical stress (especially of metals, McFarland et al. 1997). In these cases the causative factors may not have been fully proven, but circumstantial evidence is strong and apparently allows further application. However, studies on all diatom species inhabiting a large catchment area tend not to correlate strongly with a battery of environmental factors (Hill et al. 2000). Significant trends may be noted, but the degree of explanation by factor sets is low, contrary to the concept that diatom species are extremely responsive to their environment. Even at the test sites in the present study, extreme differences in one factor (very high metal concentrations) did not produce very distinct patterns of diatom distribution. Is there a common cause of the less distinct response in the study of Hill et al. (2000) and the present one? This thesis shows that multiple factors are involved in determining species succession in diatom communities even when one factor (metals) was supposed to be very dominant. Therefore, it is to be expected that in less extreme environments the confounding effects of multiple determinants of
microphyte development (nutrients, micronutrients, light, space, grazing) are even stronger. If this preliminary conclusion is correct, a critical re-examination of the reported correlations between diatom species and environmental factors is needed. A possible outcome could be that the results of correlative studies are much less transferable between areas than has been assumed until now. This transferability is essential for proper biomonitoring. Summarising, diatom species occurrence correlates with distinct steering factors, but the experimental validation is still needed to avoid misinterpretation of roles played by co-varying factors. In less prominent or extreme aquatic habitats the experimental studies on benthic diatoms, following general ecological theories, are needed to improve understanding of the dynamics of species persistence and interaction. Such studies on cause and effect relationships could lead to new criteria for parameters to be used in diatom monitoring.

REFERENCES


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