Diatomededelingen 30

2006

Van de Nederlands-Vlaamse Kring van Diatomisten

<u>Nederlands Vlaamse Kring van Diatomisten</u>

Bestuur:

Bart Van de Vijver	voorzitter
Gert van Ee	secretaris
Peter Vos	penningmeester
Christine Cocquyt	conservatrix en redactiesecretaris
Hein de Wolf	redacteur
Gerhard Cadée	bestuurslid
Herman van Dam	bestuurslid
Bert Pex	webmaster

Redactieadres:

Nederlands Instituut voor Toegepaste Geowetenschappen TNO
t.a.v. H. de Wolf
Laboratorium voor Diatomeeënonderzoek
Postbus 80014
3508 TH Utrecht
h.dewolf@nitg.tno.nl
c.cocquyt@telenet.be

Secretariaat:

Hoogheemraadschap Hollands Noorderkwartier
t.a.v. G. van Ee
Postbus 130
1135 ZK Edam
g.vanee@hhnk.nl

bij de voorplaat: Dr. Bela Jena Cholnoky, auteur van “Die ökologie der Diatomeen in Binnengewässern”, een 699 pagina’s omvattende boek uitgegeven door Cramer (Lehre) in 1968. Zijn studie richtte zich ook op het Afrikaanse continent; een belangrijk deel van zijn materiaal bevindt zich in de “The South African Diatom Collection” (zie http://www.dhec.co.za/dhec/diatoms.php)

INHOUD nr. 30 december 2006

NVKD jaaroverzicht 2005 4

Verslag van de NVKD ledenvergadering op vrijdag 21 april 2006 in Meise 5

Verslag van de jubileumbijeenkomst van de NVKD op 21 april 2003 in Meise 7

Korte impressie van de studiedag te Meise 9

Twintig jaar Nederlands-Vlaamse Kring van Diatomisten
Bart Van de Vijver 13

A short history of the use of diatoms and diatom indices in French networks, and IBD update according to Water Framework Directive requirements
Jean Prygiel, Michel Coste & Juliette Tison 16

On the importance of calibrated diatom concepts for the EU Waterframework Directive - a diatom taxonomist's perspective
Regine Jahn & Wolf-Henning Kusber 26

Phytobenthos and the Water Framework Directive in Hungary and The Netherlands
Herman van Dam, Judit Padisák & Rob Portielje 31

Diatoms in lowland lakes and ponds: WFD's ugly duck needs reliable wings
Luc Denys 33

Diatoms from Bulgarian rivers and their potential for monitoring water quality
Emiliya Kirilova & Plamen Ivanov 36

A stress surveillance system based on calcium and nitric oxide in marine diatoms
Assaf Vardi, Fabio Formiggini, Raffaella Casotto, Allesandra De Martino, François Ribalet, Antonio Miralto & Chris Bowler 40

Nieuw binnengekomen in de bibliotheek van de NVKD 49

Boekennieuws 50

Congressen, symposia, … 53

NVKD jaaroverzicht 2005

Gert Van Ee

In 2005 hebben we één bijeenkomst gehouden en wel op vrijdag 20 mei en zaterdag 21 mei. We waren te gast op het Koninklijke Nederlandse Instituut voor Onderzoek der Zee, het NIOZ, op Texel. De organisatie was in zeer goede handen van Jolanda van Iperen en Gerhard Cadée. Op vrijdag 20 mei waren er lezingen; op zaterdag 21 mei was Gerhard Cadée gids die zeer enthousiast een groep van ca. 15 deelnemers rondleidde op het eiland Texel, dat vele interessante natuurgebieden kent. Het thema van de lezingen was “zoutwater diatomeeën”. Speciale gast was Dr. Wiebe Kooistra, werkzaam bij het Stazione Zoologica "Anton Dohrn (SZN)" in de Villa Comunale, Napels, Italië. De overige lezingen werden verzorgd door medewerkers van het NIOZ. Tijdens de ledenvergadering werd uitgebreid stilgestaan bij de standaardisatie van nomenclatuur bij diatomeeën. Een uitgebreid verslag is reeds rondgestuurd en zal in de komende Diatomededelingen worden afgedrukt. Dit was één van de betere studiedagen die we hebben gehad, zowel inhoudelijk als wat betreft de organisatie: Jolanda en Gerhard: zeer bedankt!

Diatomededelingen heeft, zoals eerder al vermeld, door allerlei omstandigheden lang, te lang, op zich laten wachten. Het bestuur heeft besloten naast redacteur Hein de Wolf een redactiesecretaris aan te stellen in de persoon van Christine Cocquyt. Haar taak is vooral het verzamelen en ordenen van de kopij en gereed maken van de Diatomededelingen. Hierdoor is recent nog een dubbelgenummerd exemplaar 28-29 verschenen met veel samenvattingen van lezingen en nieuwtjes op diatomeeëngebied. Hopelijk volgt nummer 30 spoedig en blijft de snelheid er nu in. Hierbij een oproep aan de sprekers om hun abstract of liever nog een tekst aan te leveren aan Christine of Hein.

Op 28 december 2005 werd ten huize van Peter Vos in Amsterdam een bestuursvergadering NVKD gehouden. Belangrijkste zaken waren de bijeenkomst in 2006, Diatomededelingen 28-29, promotie NVKD en de website, DDT (Deutsch Diatomologen Tagen) in 2007 in Nederland en de samenwerking met de NVKD. Holger Cremer zal in 2007 de DDT organiseren en heeft de NVKD verzocht hierbij te helpen. We hebben besloten ondersteuning (excursies, logeermogelijkheden, congrescentrum) te bieden en onze voorzitter zal hiertoe afspraken maken met Holger Cremer en de DDT organisatie.

Dit jaar gelukkig ook evenals vorig jaar geen overlijdensberichten van leden of ex-leden. Wel een aantal opzeggingen of stopzettingen van het lidmaatschap, maar ook nieuwe leden en instituten: *drs. Emiliya Kirilova*, Palaeoecology Institute of Environmental Biology, Faculty of Science, Utrecht; *Dr. Wiebe Kooistra*, Statione Zoologica “Anthon Dohrn”, Villa Comunicale 80121 Napoli, Italia; *Dr. Bart Schaub*, Hoogheemraadschap van Rijnland; *Geurt Verweij*, Rijks Universiteit Groningen; *Bureau Waardenburg* uit Culemborg. In totaal houden we daarmee exact dezelfde aantallen leden: 55 leden op persoonlijke titel en 7 instituten met een lidmaatschap.

Datum: 21 april 2006.

Verslag van de NVKD ledenvergadering op vrijdag 21 april 2006 in Meise

Gert Van Ee

1. Opening, vaststellen agenda, mededelingen.
Geen bijzonderheden.

2. Notulen ledenvergadering 20 mei 2005 te Texel.
Deze worden goedgekeurd door de vergadering met dank aan de secretaris.

3. Kort jaaroverzicht 2005 (secretaris).
Gert van Ee leest een verslag over 2005 voor. Dit jaaroverzicht staat onder dit verslag afgedrukt.

4. Toelichting financieel jaaroverzicht 2005 en begroting 2006 (penningmeester).
Peter Vos leest voor en vertelt dat de financiële situatie nog steeds goed is. Deze vergadering staat mede in het teken van het twintigjarig bestaan van de NVKD en hiervoor is extra geld gereserveerd. Geen contributieverhoging evenals vorige jaren. Wel moeten we afwachten wat de toekomst gaat brengen i.v.m. pensionering Hein. Hein regelde dat verzendkosten via TNO liepen waardoor de NVKD weinig kosten had. Holger Cremer heeft aangeboden via TNO mogelijk druk- en verzendkosten te organiseren.

5. Verslag kascommissie Marianne Thannhauser en Adrienne Mertens over 2005 en verkiezing nieuw lid van de kascommissie (Marianne Thannhauser treedt af, Adrienne Mertens blijft nog één jaar aan). Op de vergadering wordt een nieuw kascommissielid gekozen.
Helaas waren door omstandigheden de kasgegevens pas laat bij de kascommissie terechtgekomen. Marianne moest zelfs voor het begin van de bijeenkomst in Meise nog even de boeken inkijken. Dit zal de volgende keer beter verlopen.
Ondanks dit schoonheidsfoutje worden de boeken goedgekeurd door de kascommissie en de penningmeester wordt onder algemene dank gedechargeerd voor het gevoerde beleid. Nieuw lid van de kascommissie is Ronald Bijkerk. De kascommissie bestaat voor 2006-2007 uit Adrienne Mertens en Ronald Bijkerk.

6. Web-site NVKD.
Bert Pex doet zeer goed werk als webmaster. Advies aan alle leden: bezoek de website regelmatig. Hierop is voortdurend van alles te zien. www.diatom.nl

7. Verkiezing bestuurslid. Gerhard Cadée treedt af en heeft te kennen gegeven niet herkiesbaar te zijn. Kandidaten kunnen zich melden bij het bestuur.
Er waren geen geschikte kandidaten of tegenkandidaten. Gerhard is verzocht nog een jaar aan te blijven en in 2007 zal het bestuur Holger Cremer van TNO voordragen als nieuw bestuurslid.

8. DDT in 2007 in Nederland. Samenwerking met de organisatie van de DDT: hoe en hoe ver?
Dit wordt niet in 2007 maar mogelijk later. Er zal een en ander worden besproken met Regine Jahn, waarna gekeken wordt of er een gezamenlijke bijeenkomst van Nederlandstalige en Duitstalige diatomisten komt. Dit heeft nogal wat voeten in aarde m.b.t. organisatie. De NVKD (het bestuur) heeft zich positief opgesteld om ondersteuning te bieden bij deze bijeenkomst (excursies, logeeradressen, etc.) We wachten af hoe de ontwikkelingen gaan lopen. Dit punt komt komend jaar weer terug.

9. Reacties op de nieuwe taxonlijst diatomeeën van het RIZA.
Dit punt wordt naar het einde van de vergadering verplaatst.

Aan het eind van de vergadering volgt een discussie. Herman van Dam maakt een statement over het risico dat diatomeeën uit de KRW monitoring worden geschrapt indien het gebruik, mede door problemen met de taxonomie en naamgeving, nog langer aanhouden. Hij pleit voor standaard de 4 groene deeltjes van de Süsswasserflora te gebruiken met noodzakelijke aanpassingen, maar hierin terughouding te betrachten.
Frans Kouwets vertelt dat er bij RIZA hard gewerkt wordt aan een nieuwe naamlijst op basis van de laatste veel gebruikte naamgeving. Deze nieuwe lijst zal als basislijst in heel Nederland gaan fungeren. Dit wordt gesteund door STOWA met tijd en geld. Ronald Bijkerk vindt dat we jaarlijks vanuit de NVKD zouden kunnen aangeven hoe de lijst voldoet en waar knelpunten moeten worden aangepakt. Hierdoor speelt de NVKD een belangrijke rol en is er draagvlak vanuit de praktijk gewaarborgd. Herman pleit voor eens per tien jaar (bijvoorbeeld) een nieuwe lijst (updates iedere twee jaar evt.) te publiceren, maar laten we vooral praktijkgericht blijven. Bart springt wetenschappelijk in en vindt het belangrijk vanuit de wetenschappelijke taxonomische kant goede aansluiting te houden bij te wijzigen naamgeving.

Deze discussie is nog niet beslist. De laatste ontwikkelingen zijn dat recent (juli 2006) is besloten een poging te doen nieuwe maatlatten te gaan ontwikkelen dit najaar voor fytobenthos in Nederland en mogelijk ook in Vlaanderen. Hiertoe heeft STOWA in de persoon van Bas van der Wal het initiatief genomen. Mogelijk wordt ook de NVKD hierin betrokken.

10. Rondvraag.
Bart vertelt dat de Van Heurck collectie naar de Nationale Plantentuin komt. Bart gaat zich inspannen om de collectie voor onderzoek toegankelijk te maken.

11. Sluiting.

Verslag van de jubileumbijeenkomst van de NVKD op 21 april 2006 te Meise

Gert van Ee

De Nederlands-Vlaamse Kring van Diatomisten (NVKD) bestond dit jaar twintig jaar. Ter gelegenheid hiervan werd een bijzondere bijeenkomst gehouden in de Nationale Plantentuin van België, Domein van Bouchout, te Meise nabij Brussel op vrijdag 21 en zaterdag 22 april 2006. Het thema van deze bijeenkomst was: "Diatomeeën als instrument voor de bepaling van de waterkwaliteit in Europa". Sprekers uit verschillende landen gaven hun visie op dit onderwerp vanuit verschillend perspectief. Steeds stond de Europese Kaderrichtlijn Water centraal in de lezingen.

Bart Van de Vijver opende de vergadering, heette iedereen van harte welkom op deze prachtige warme en zonnige dag. In totaal waren ongeveer 50 deelnemers op deze studiedag afgekomen, waaronder veel introducés.

De lezingen werden prima verzorgd door achtereenvolgens Jean Prygiel (die nota bene zijn vakantie hiervoor had onderbroken!) uit Frankrijk, Luc Ector uit Luxemburg, mevrouw Regine Jahn uit Duitsland (zie: http://www.algaterra.org/), Herman van Dam, Luc Denys uit België en Martyn Kelly uit Engeland (zie: www.craticula.ncl.ac.uk/dares). (noot: het openen van deze site gaf op 22 juli problemen). Het programma met de juiste titels staat hieronder vermeld.

Tussen de middag was er een korte ledenvergadering met discussie over naamgeving (zie verslag ledenvergadering 2006). Na de lunch volgde een wandeling door de kassen, die vernieuwd zijn en waarin de meest prachtige planten waren te bewonderen.

Na afloop van de bijeenkomst in late namiddag gingen de blijvers naar hun hotel of logeeradressen en 's avonds werd in Wolvertem vlakbij Meise een heerlijk diner genuttigd.

Op zaterdag 22 april was een excursie georganiseerd en brachten we een bezoek aan het Arboretum in Tervuren, waar behalve bijzondere bomensoorten, bomengroepen en struiken ook waterpartijen (met diatomeeën) te bewonderen waren. Na afloop hebben de deelnemers van een overheerlijke lunch genoten, alvorens de groep uiteenging en meer dan tevreden over deze bijzonder geslaagde bijeenkomst naar huis terugkeerde.

Met bijzondere dank aan Bart Van de Vijver en alle medewerkers van de Nationale Plantentuin en Christine Cocquyt voor de perfecte organisatie van deze bijeenkomst.

PROGRAMMA VAN DE JUBLEUMBIJEENKOMST OP VRIJDAG 21 APRIL 2006 TE MEISE

10.30 u. Ontvangst, koffie, thee.

11.00 u. – 11.20 u. - Opening door de voorzitter van de NVKD, Dr. B. Van de Vijver met een korte terugblik op twintig jaar NVKD.

11.20 u. – 11.50 u. – Dr.Jean Prygiel (Agence de l'Eau Artois-Picardie, Frankrijk): Updating the French diatom monitoring program to WFD requirements. (Lezing in samenwerking met Dr. Michel Coste & Drs. Juliette Tison (Cemagref-Bordeaux, Frankrijk)

11.50 u. – 12.20 u. – Luc Ector (Centre de Recherche Public-Gabriel Lippmann, Luxemburg): Biofilm translocations and intercalibration exercises in rivers: implications for biomonitoring. (Lezing in samenwerking met Frédéric Rimet, Henri-Michel Cauchie en Lucien Hoffmann, CREBS, Lucxembourg).

12.20 u. – 12.50 u. – Ledenvergadering NVKD (agenda en verslag vorige keer op Texel zijn bijgevoegd, evenals het verslag van de discussie over naamgeving).
13.00 u. – 14.30 u. – Lunch in de Nationale Plantentuin van België, aangeboden door de NVKD.
14.30 u. – 15.00 u. – Dr. Regine Jahn (Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität zu Berlin, Duitsland): On the importance of calibrated diatom concepts for the EU Waterframework Directive- examples from Germany
15.00 u. – 15.30 u. – Dr. H. van Dam (Grontmij AquaSense Amsterdam, Nederland): Phytobenthos and WFD in the Netherlands and Hungary.
15.30 – 15.50 u. – Pauze, kopje koffie, thee.
15.50 – 16.20 u. – Dr. Luc Denys, (Instituut voor Natuur- en Bosonderzoek, België): Diatoms in lowland lakes and ponds: WFD’s ugly duck needs reliable wings.
16.20 – 16.50 u. – Dr. Martyn Kelly (Bowburn Consultancy, Durham, Engeland): The conceptual basis for ‘good ecological status’ in the UK.
16.50 u. – Sluiting door de voorzitter van de NVKD.

Vrijdag 21 april 2006: Na de lunch een bezoek aan de vernieuwde kassen van de Nationale Plantentuin van België

Korte impressie van de studiedag in Meise

Bert Pex

Vrijdag 21 april 2006

Ter gelegenheid van het 20-jarig bestaan van de NVKD werd er een bijzondere bijeenkomst georganiseerd. Op vrijdag 21 april 2006 waren we nl te gast in de Nationale Plantentuin in Meise bij Brussel.

Bart Van de Vijver heette iedereen welkom met een korte terugblik op twintig jaar NVKD en belichtte enkele hoogtepunten van de afgelopen 10 jaar. Er werden lezingen gehouden over het thema diatomeeën en waterkwaliteit. Bijzonder was dat er sprekers uit verschillende landen hun verhaal deden over hoe in hun land wordt omgegaan met dit thema, waarbij Europese Kaderrichtlijn Water nadrukkelijk aan bod kwam.

Hieronder een overzicht van de lezingen die door enthousiaste sprekers werden gepresenteerd.

Dr.Jean Prygiel (Agence de l'Eau Artois-Picardie Frankrijk): Updating the French diatom monitoring program to WFD requirements. (Lezing in samenwerking met Dr. Michel Coste & Drs. Juliette Tison (Cemagref-Bordeaux, Frankrijk)

Luc Ector (Centre de Recherche Public-Gabriel Lippmann, Luxembourg): Biofilm translocations and intercalibration exercises in rivers: implications for biomonitoring. (Lezing in samenwerking met Frédéric Rimet, Henri-Michel Cauchie en Lucien Hoffmann, CREBS, Luxembourg).

Dr. Regine Jahn (Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Duitsland): On the importance of calibrated diatom concepts for the EU Waterframework Directive- examples from Germany

De lunch in de Plantentuin werd aangeboden door de NVKD. Aansluitend was er de mogelijkheid om een wandeling te maken in de "koninklijke tuinen" en daarbij een bezoek te brengen aan de grote kas met een rijke collectie aan tropische en subtropische planten. Deze is recentelijk geheel gerenoveerd en nog niet helemaal toegankelijk voor publiek. Maar wij mochten onder leiding van Bart Van de Vijver en Christine Coquyt alvast overal een kijkje nemen. Hoogtepunt in de kas is natuurlijk het meest vochtige gedeelte waar de drijvende bladeren van Victoria amazonica als kleine eilanden in het water liggen en de vliegenvangende Nepenthis hun best doen om een maaltje bij elkaar te scharrelen. Na een korte wandeling langs een beekdallandschap dat in de tuin was ingericht werd het middag gedeelte van de lezingendag ingeluid door Herman van Dam

Dr. H.van Dam (Grontmij AquaSense Amsterdam): Phytobenthos and WFD in the Netherlands and Hungary.

Dr. Luc Denys, (Instituut voor Natuur- en Bosonderzoek, Vlaanderen): Diatoms in lowland lakes and ponds: WFD’s ugly duck needs reliable wings.

Dr. Martyn Kelly (Bowburn Consultancy, Durham, Engeland): Phytobenthos intercalibration for the Water Framework Directive.

Net voor de lunch werd ook de ledenvergadering van de NVKD gehouden.

Zaterdag 22 april 2006: Excursie in het Arboretum van Tervuren onder de deskundige leiding van Bart

Vrijdagavond en Zaterdag

De mensen die bleven voor de excursie op zaterdag werden in de avond verrast door een uitermate smakelijk diner in een restaurant in het kleine plaatsje Wolvertem. Het luistert naar de naam Brazzaville en had naast de naam ook allerlei attributen uitgestald die duidelijk wezen op banden met een rijk koloniaal verleden. Ook de temperatuur in het restaurant paste wel bij de naam. Het eten was zoals gezegd echter overheerlijk en de drank trouwens ook waardoor het restaurant door het internationaal gezelschap van diatomisten overtuigend op de kaart is gezet!

Door enkelen is er die avond in een café in Grimbergen nog erg lang gediscussieerd over de nieuwe Nederlandse biotaxonlijsten onder het genot van dubbele of zelfs driedubbele Grimbergen. De lijsten bleken heel erg lang!?

Op zaterdag 22 april bracht een kleine maar enthousiaste groep mensen een bezoek aan het Arboretum in Tervuren iets ten zuidoosten van Brussel, waar behalve natuurlijk bomen ook waterpartijen (met diatomeeën) te bewonderen zijn en er een koninklijke wandeling werd gemaakt onder deskundige leiding van Bart Van de Vijver. Alleen de route bleek zo nu en dan een probleem voor de GPS of de GPS een probleem voor de route of ....?

Het Arboretum verschilt van andere Arboreta in west Europa door de grootte en de hoeveelheid bomen die er per soort in het begin van de vorige eeuw zijn geplant. Hele scheepsladingen jonge bomen zijn er destijds uit verschillende delen van de wereld, met name uit gematigde streken, geïmporteerd. De bomen zijn er in de natuurlijk aanwezige heuvels en dalen van het park in grotere groepen per soort bij elkaar gezet. Dit met als doel destijds om de groei van de bomen te bestuderen in grotere percelen. Dit natuurlijk met uiteindelijk doel de toepasbaarheid van het hout voor bouw en meubelindustrie te bestuderen. Omdat de bomen in grotere groepen bij elkaar staan komt het minder over alsof ze zijn aangeplant en krijg je een beetje een indruk van hun natuurlijke habitus en habitat. De inrichting van het Arboretum doet hierdoor zeer "natuurlijk" aan en het is zeer de moeite waard om hier nog eens terug te komen als het loofhout blad heeft.

Indrukwekkend waren vooral de Metasequoia's en de Sequoia's met hun enorme hoogte en enorme stam omvang. Het was wel aardig je te realiseren dat wij daar als diatomisten (normaal bestuderen we kleinere familieleden) tussen die giganten liepen.

Na een stevige lunch ging iedereen moe maar zeer voldaan naar huis.

Heden, de achtste januari ------------------------------
negentienhonderd zes en tachtig, verschenen voor mij, --
Jacobus van Nielen, notaris ter standplaats Wijk bij ---
Duurstede: ---

1. de heer Pieter Marcus Houpt, chemicus, -------------
wonende te 's-Gravenhage, Timorstraat 119, geboren --
op acht en twintig november negentienhonderd één ----
en dertig; --
2. mevrouw Gerda Jannetje Jakoba Zonneveld-de Boer, ----
rijksambtenaar, wonende te Santpoort, Kerkweg 36, ---
geboren op vier oktober negentienhonderd vijf en ----
vijftig; --
3. de heer Pieter Catharinus Vos, assistent wetenschap-
pelijk medewerker, wonende te Amsterdam, Singel -----
9, geboren op elf september negentienhonderd --------
drie en vijftig. ------------------------------------

De comparanten verklaarden bij deze een vereniging -----
op te richten, welke zal worden geregeerd door de ------
navolgende ---

---------------------- STATUTEN: ----------------------

NAAM EN ZETEL. ---

Artikel 1. ---

1. De vereniging draagt de naam: "Nederlands-Vlaamse ---
Kring van Diatomisten". ----------------------------
2. De vereniging is opgericht op acht januari negen- ---
tienhonderd zes en tachtig. -------------------------
3. De vereniging is gevestigd te 's-Gravenhage. --------
4. De vereniging is aangegaan voor onbepaalde tijd. ----

DOEL. --

Artikel 2. ---

1. Het doel van de vereniging is het bevorderen van ----
de studie en het onderzoek van diatomeeën. ----------
2. De vereniging tracht dit doel te bereiken door: -----
   a. het organiseren van bijeenkomsten, waar de leden -
   hun ervaring en kennis kunnen uitwisselen; -------
   b. het beheren van een bibliotheek, een archief en --
   een preparatenverzameling, opdat deze aan de -----
   leden beschikbaar gesteld kunnen worden; ---------
   c. het verzorgen en uitgeven van een mededelingen- --
   blad; --
   d. het bevorderen van contacten en samenwerking -----
   tussen diatomisten in binnen- en buitenland; -----
   e. het organiseren van excursies, lezingen en con- --
   gressen; ---
   f. het aanwenden van alle overige wettige middelen, -
   welke voor het doel van de vereniging bevorde- ---
   lijk kunnen zijn. --------------------------------

Twintig jaar Nederlands-Vlaamse Kring van Diatomisten

Bart Van de Vijver

Nationale Plantentuin van België, Domein van Bouchout, B-1860 Meise, België

"Heden, de achtste januari negentienhonderd zes en tachtig, verschenen voor mij, Jacobus van Nielen, notaris ter standplaats Wijk bij Duurstede
de heer Pieter Marcus Houpt, chemicus
mevrouw Gerda Jannetje Jakoba Zonneveld-de Boer, rijksambtenaar
de heer Pieter Catharinus Vos, assistent wetenschappelijk medewerker.

De comparanten verklaren bij deze een vereniging op te richten, welke zal worden geregeerd door de navolgende statuten.
1. De vereniging draagt de naam 'Nederlands-Vlaamse Kring van Diatomisten'"

With these words, twenty years ago, the association of Dutch-speaking Diatomists was born. The idea for such a language-driven association was not new. The French and English diatomists already grouped themselves at regular meetings where they could share ideas and results and discuss diatom topics. During the International Diatom Symposium in Paris in 1984, that means 2 years before the official start of the NVKD, 8 Dutch participants came together and decided to start an informal group of diatomists in their own language.

A first structural meeting was held on Saturday the 9th of February 1985, on landgoed Broekhuizen in Leersum where Peter Vos, who is nowadays still our most appreciated treasurer, opened the first Convocation. 39 people where present! Besides some formal, organisational discussions, 6 diatom lectures where presented. 7 months later, a second meeting was held and after another 4 months, the founding of the NVKD was a fact.

It was clear that the Dutch-speaking diatomists community really needed a more formal structure. Pieter Houpt became the first president and would remain in function for the next five years. The number of members in these first years quickly rose from 39 to more than 70. Among them were 45 so-called professionals and 20 amateurs, in the positive sense of the word. Unfortunately for today, we lost most of the amateurs but I'm afraid that this was an inevitable but nevertheless regrettable evolution. And to be honest, I don't have the slightest idea at the moment how we can and should combine both groups of diatomists.

In 1991, shortly after the decease of our first honorary member Albert Van der Werff, Herman Van Dam took over the presidency and during the following 10 years, the NVKD steadily has grown into its present form and I am the first to admit that since his presidency, no radical changes where necessary and that we should Herman for all his work and ideas. When he stepped down in 2001, he was celebrated and named honorary member of the association during the symposium at Mont Rigi in the Hautes Fagnes. Today, fortunately, Herman is still a valuable and well-appreciated member of our association and our board. At the same time, Gerhard Cadée became member of the board and although he retires a while ago, he accepted to continue as an active member of our Assocation.

At present the association still has more than 60 members. Most of them are active in the water quality management field, although almost all other topics, ranging from the ecology of marine diatoms to diatom taxonomy have their research interests within the NVKD. It is however a pity that the number of Universities where fundamental diatom research is promoted, still decreases. Correct me if I'm wrong, but the only University in Flanders and the Netherlands where diatoms are one of the major research topics, will be Ghent. It is a general problem and even in France, this tendency has left its traces.

Nevertheless, we can be proud of what we as a diatom association accomplished the past 20 years. There is not only the organisation of the Twelfth International Diatom Symposium in Renesse in 1992 with more than 150 participants and the joint publication in 1994 of Diatomededelingen 16 with the Netherlands Journal of Aquatic Ecology. By the way, one the papers made by Herman Van Dam, Adrienne Mertens & Jos Sinkeldam, and I think still a very important paper for all who are working in the light of water quality monitoring, was awarded the Dresscher price.

There is also our annual journal Diatomededelingen. The first number appeared in December 1985 and was called initially Diatom-times. Our secretary, Gert Van Ee, has counted the pages and told me that we already published 1260 pages of valuable diatom information. The last number, 28-29 appeared a couple of weeks ago. We will try to keep up with this tradition and I have an absolute faith in Hein De Wolf and Christine Cocquyt to continue what we started. I would also launch a serious call for contributions. If you have something to share with your colleagues, do not hesitate to tell Hein or Christine.

There are also the numerous annual convocations held in different places in the Netherlands and Belgium. More than 150 lectures have been presented these 20 years. During the first 11 years, the Dutch-speaking diatomists gathered twice a year to discuss diatoms. Then we celebrated our tenth anniversary in Roermond and from that meeting on, we tried more and more to organize one annual convocation but instead of only a single day, we opted almost each time for a two-days-symposium. One day with formal lectures and a second one reserved for an excursion in the surroundings of the Symposium location. The enthusiasm of the numerous participants during the meeting last year in Texel could only underline that we took the right decision. I strongly believe that these two-day symposia greatly contribute to a better communication among diatomists. Informal contacts during meeting lead in most cases to better science and collaborations. So we will continue on the chosen track.

Modern times ask for modern techniques and when I became president in 2001, one of the first achievements of the Association was the start-up of the web-site *diatom.nl* and I should thank Hein De Wolf initially and later-on especially Bert Pex for all the effort and time that is put into the site. Herman Van Dam already said in Roermond 10 years ago that the NVKD should be more active in the proliferation of diatom research and I think that the website can be an interesting tool in achieving that goal. We will continue to extend the functionality of the website for instance by adding all old Diatomededelingen volumes on the Internet. If you have suggestions or ideas, please contact Bert Pex.

There are a lot of challenges for the near future and I think that the NVKD can, will and has to play an important role in this future. There is of course the European Framework Directive that obliges all members of the European Union to monitor the water quality of their rivers using a wide range of bio-indicators. We will hear more about this theme today and I'm really happy that we were able to invite several international speakers to show us their ideas about what is going on in our neighbouring countries.

Another topic where the NVKD should and can act as a discussion forum is the growing concern about the daily changing taxonomy of our diatoms. I agree that taxonomists on one hand and ecologists and water quality researchers on the other hand are getting more and more separated and I'm afraid that this evolution is only the beginning of all uncertainties. The past few months, a lot of us have been checking and discussing a list that has been provided by RIZA. Although some of us, and I should include myself among these people, have a different vision on diatom taxonomy than most ecologists, I feel that the NVKD should be the one and only leading voice in the discussion with the policy makers regarding the use of the diatoms in water quality monitoring. A consensus on taxonomy and names is thus more than vital and I hope that it can be a satisfying one for everybody. Keep however in mind that diatom science is absolutely not static but continuously in evolution and that we should stay up-to-date, even when it is gets difficult.

Finally, a third challenge is to increase the contacts with our sister associations in France, England and Germany and I'm very happy that we have here today among us the president of the German-speaking diatomists and the vice-president of the French-speaking diatomists. I hope that in the near future we can organize joint meetings to facilitate the contacts and discussions among diatomists from nearby countries but I believe that all of us will benefit from such contacts.

So to conclude, I would like to thank you all for the past 20 years and I think that the NVKD, the Nederlands-Vlaamse Kring van Diatomisten, is heading towards a promising future.

A short history of the use of diatoms and diatom indices in French networks, and IBD update according to Water Framework Directive requirements

Jean Prygiel[1], Michel Coste[2], Juliette Tison[2]

[1] Agence de l'eau Artois-Picardie, 200 rue Marceline, 59508 Douai cedex, France and Université des Sciences et Technologies de Lille, PBDS UMR CNRS 8110, Bât. C8, 59655 Villeneuve d'Ascq Cedex, France.
Author for correspondance: j.prygiel@eau-artois-picardie.fr
[2] Cemagref Bordeaux, 50 avenue de Verdun, 33610 Cestas, France

INTRODUCTION

First diatom studies in French monitoring networks began in the seventies and continued till the nineties with applications in large hydrographical basins. In 1994, French water agencies decided to propose a new diatom index for routine monitoring for the whole French river networks. This index called "Indice Biologie Diatomées or IBD"is used for about ten years now. The 2000 IBD standard has been updated and a modification has been made with the classification system to respect the principle of the deviation to the reference conditions as defined in the Water Framework Directive (WFD). This paper consists in the short history of diatom indices and IBD in French large hydrographical basins monitoring networks. Changes which have been brought to IBD for the WFD surveillance monitoring implementation are also described.

SHORT HISTORY OF DIATOM INDICES IMPLEMENTATION IN FRANCE

Many reviews have been published by Prygiel and Coste (1996a,b ; 1999, Prygiel, 2002). So only the main steps will be presented here.

From a study carried out on the Seine basin in 1974 supported by the Agence de l'Eau Seine-Normandie (39 sites from the springs to Rouen), Coste and Leynaud (1974) proposed a first double entry grid including 55 species (from 350 identified) distributed in 4 groups of 5 euryeceous species of decreasing sensitivity, and 7 subgroups of 5 species describing the river longitudinal profile. The first version of the index of polluosensitivity (IPS) appeared in 1982 (Coste in Cemagref, 1982) and has been built from a study of 189 samples collected between 1977 and 1980 on the National Inventory (the ancestor of the national basin network). It is inspired by the index of Descy (1979). It takes into account the indicator value and the sensitivity for pollution of 263 species and varies from 1 (very bad quality) to 5 (very good quality) but is transformed on a scale from 1 to 20 to make comparisons easier with other biological indices. It is regularly updated and often considered as the reference index by many users and so is largely applied in Europe as well as in other parts of the world. One important question is however the version used. The 2006 IPS update includes now 4590 taxa with varieties and synonyms but is always cited as IPS according to Coste in Cemagref (1982). The publication of Omnidia in 1992 (Lecointe et al., 1993) and following updated versions allowed to stabilise the list of taxa taken into account by users for index calculation. But there is still a large uncertainty related to the version which is or has been used during the studies (historical studies) or when comparing data from different searchers. So there is a big problem of traceability when using IPS. One could add that if IPS allows to take into account all taxa, one can ask himself on the final list of taxa because of the large number of sources of uncertainty. This list of taxa is not limited. New taxa as well as new synonyms regularly appear according to systematics revision and changes in nomenclature, and are added in the IPS data base before being included in Omnidia. One should also remind that users are not equal when identifying diatoms. Omissions, mistakes for rare, small and/or difficult taxa can occur. The exactitude of identification largely depends on the flora and scientific papers the users have referred to, the time he is allowed to put in determining, the possibility or not to use electronic microscopy, and of course the skill and experience of the user. So for a same sample, lists of taxa can be more or less detailed with different names according to users. If differences concern rare taxa, IPS value is not largely affected. If differences concern abundant taxa, everything can happen. That is why IBD which uses a limited number of taxa and a dedicated taxonomy has been proposed for national use in French networks. This argument can be applied to the generic diatom index which has been proposed besides IPS. It included 42 genera in 1982, 88 in 1988 (Rumeau and Coste, 1988), 308 in 1991 (Coste and Ayphassorho, 1991) and about 500 in 2006.

The principle of the 1974 Seine grid has been used again by Descy and Coste (1991) for an EC contract. The new CEC grid included 208 taxa displayed in 4 groups and 8 subgroups and allowed index calculations between 1 and 20. It has been implemented to 155 sites of the national basin Network (French RNB) and to 30 sites belonging to the Rhône-Méditerranée-Corse basin (Descy and Coste, 1989). The CEC grid has been successfully tested on more than 300 European rivers. However, it didn't meet the expected success probably because IPS remained the reference index.
After experiments on small water basins (R. Aa, R. Sensée, R. Somme…), a first monitoring of the whole Artois-Picardie water basin network has been led in 1990 by Coste and Ayphassorho (1991) during two sampling campaigns in June and September. 355 samples have been collected and more than 500 taxa reported. The comparative efficiency of many indices has been evaluated by Prygiel and Coste (1993). It led to confirm the interest of diatoms and diatom indices for water monitoring in large natural and canalised rivers. Even if IPS has been identified as the most efficient, difficulties for implementation led to propose the Artois-Picardie diatom index (IDAP) based on 45 genera together with 91 species (Prygiel et al., 1996). Though obtaining good results, this index has been rapidly abandoned for the IBD which had a national purpose. Another consequence of this first large study was the creation of the first version of Omnidia (from Omnis data basis software and diatoms) by Lecointe et al. (1993) for routine monitoring needs (taxonomy management, index calculations, publishing and printing).

Just after the Artois-Picardie studies, a large application has been carried out in the Rhin-Meuse water basin in 1992 on 220 sites during two campaigns (Lenoir and Coste, 1994). 10 sites have also been monthly sampled for a seasonal variation study. 975 samples collected from various lentic and lotic habitats (mineral, vegetal) allowed to identify more than 650 taxa. After complementary studies in 1993 and 1994, a routine monitoring has been implemented on the whole basin on 120 sites.
Diatom monitoring began in 1993 in the Seine-Normandie water basin with 13 sites from the Ile de France region. The number of sites regularly increased besides the number of users (Diren Ile de France, 1997) to reach 150 sites in 1999 distributed on the whole basin (Agence de l'Eau Seine-Normandie, 2000).

A first monitoring also began on 56 sites of the national network of the Adour-Garonne water basin during summer 1994. To these sites, 15 were added for IBD tests (see below). So, a total of 119 samples belonging to 71 sites and 22 rivers were collected by Coste et al. (1995). 8 diatom indices were used as well as ecological profiles as synthesised by van Dam et al. (1994) and Denys (1991).

As the monitoring experiments increased in large French basins, French water agencies together with the Cemagref decided to collaborate to produce a practical index for use in routine monitoring. IBD was first introduced in 1995 (Lenoir and Coste, 1996) and has been discussed in several papers (Prygiel and Coste, 1998, 1999; Prygiel, 2002). IBD has been built from a national data set including 1332 samples collected from 949 sites between 1977 and 1994 and distributed in whole France (table 1). Samples have been associated to 14 chemical parameters for statistical analyses. IBD takes into account 209 matched taxa. 152 include only one species and 57 include several species and varieties which are morphologically very similar but which can have different ecological characteristics. So IBD is a compromise between efficiency and sensitivity, and practicability. Each matched taxon has an ecological profile which expresses a presence probability in each of 7 water quality classes defined by the statistics. IBD calculation needs first to select taxa which will be taken into account. For this, only matched taxa which abundance is higher than a limit defined for each one (in every case 3 ‰ ie 3 individuals of the requested total of 400 for counting) are retained. Then, their presence is considered as significant. The principle of IBD calculation is to integrate ecological profiles of retained taxa by giving more importance when the taxon is abundant and has an high indicative value. So one obtains an ecological profile of a fictive taxon which represents the whole diatom community of the studied site. The barycentre of the curve is then transformed in a value between 1 and 20 for classification purpose. Tests have been carried out in 15 sites from each large water basin between 1995 and 1998 and adjustments have been made together with a national training program (Prygiel and Coste, 1999). During the development of IBD, diatom monitoring continued in 1996 in the Loire-Bretagne water basin with 260 sites which are monitored since then, and in 1997 in the Rhône-Méditerranée-Corse basin (Ector et al., 2000). In this basin, 52 sites have been studied; 30 of which have been used for IBD finalisation.

With all six large French water basins covered, a first French diatom quality map using IBD has been published in January 2000 with data collected from 1998 and 1999 on 847 sites (RNDE, 2000). The end of the nineties should be considered as a great period for diatoms. Omnidia 3 including the final version of IBD was published (Lecointe et al., 1999) as well as the T90-354 IBD standard (AFNOR, 2000) and the IBD manual (Prygiel and Coste, 2000) in May 2000. A first IBD intercalibration exercise was carried out on the river Loup in vicinity of Nice in September 1999 during the 18° meeting of the French speaking diatomists association (Prygiel et al., 2002). A Cofrac (French Committee for Accreditation) accreditation program for Hydrobiological methods including IBD was also elaborated in 2000 (6 laboratories accredited for IBD in June 2006). Finally, the first monitoring of 9 sites from the Scheldt homogeneous measuring network (CIE, 2002; http://www.isc-cie.com) occurred in September 2000 with 7 French, Walloon and Flemish diatomists (Figures 1 and 2). To make all the material available, a national data basis including preserved material, slides, and sometimes lists of diatoms has been created by the Artois-Picardie water Agency.

Table 1. Number and origin of sites taken into account into IBD. IBD v.1996 (Lenoir and Coste, 1996). IBD v;1999 (Lecointe and Le Renard, 1999). From Ector et al. (2000).

Basin	Nb samplings available	
	IBD v.1996	IBD v.1999
Adour-Garonne	165	165 + 452 = 617
Artois-Picardie	271	271 + 261 = 532
Loire-Bretagne	41	41 + 227 = 268
Rhin-Meuse	740	740
Rhône-Méditerranée-Corse	66	66 + 52 = 118
Seine-Normandie	49	49 + 306 = 355
Total	**1332**	**1332 + 1398 = 2630**

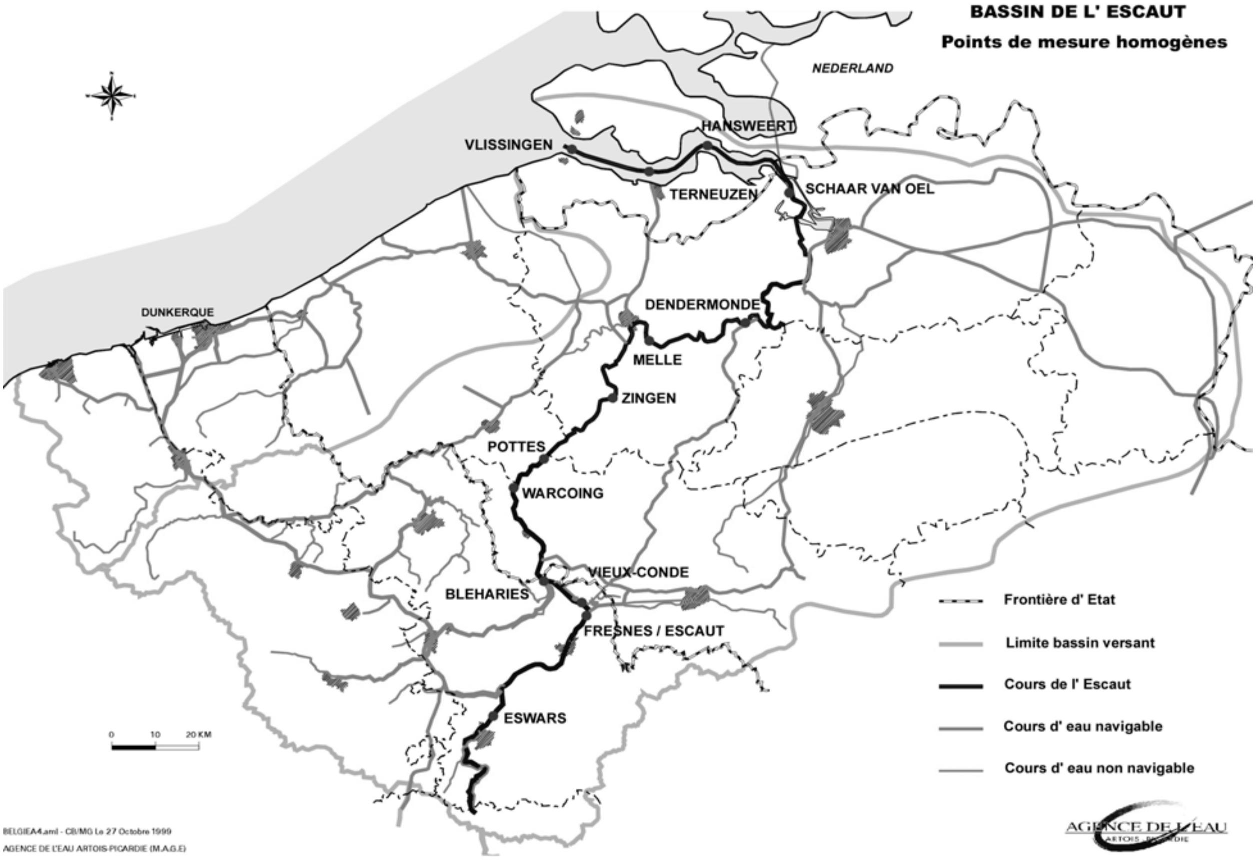

Fig. 1. Location of sites of the Scheldt homogenous measuring network for diatom monitoring (from CIE, 2002).

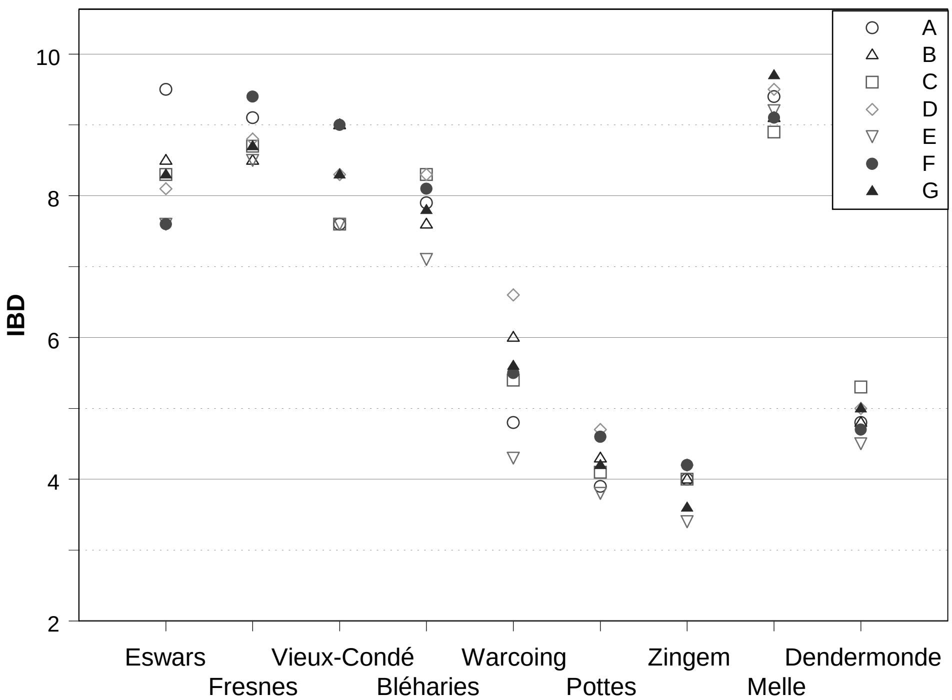

Fig. 2. IBD assessments from 7 diatomists for the Scheldt homogenous measuring network in 2000 (from CIE, 2002).

RIVER DIATOM MONITORING AND WATER FRAMEWORK DIRECTIVE IMPLEMENTATION

The WFD has been published on 20th December 2000 (EC, 2000). This directive highlights two important principles which are a greater role for biology and the principle of the deviation to the reference for status assessment. The annex 5 describes the quality elements for the various water bodies categories. Diatoms are considered as part of phytobenthos and thus are retained to characterize the biological status of rivers. Characterization is based on composition and abundance criteria and should be assessed in relation to reference values identified for each type inside large ecoregions. The annex 2 of the WFD gives the main principles for surface water bodies characterization and proposes two systems called A and B. France chose the system B to establish the typology of continental water bodies. This work has been done by the Cemagref of Lyon (Wasson et al., 2002). The aim was to define and characterise hydroecoregions for metropolitan France (HER) and to assess the validity of these HER to propose reference conditions for invertebrates. Thereafter, this approach has been extended to diatoms by the Cemagref of Bordeaux (Tison et al., 2005a, 2006; Prygiel and Haury, 2006). Cumulatively, 22 HER of level 1 have been identified by combination of geology, relief and climate. A second level of regionalization has been realized to identify inside HER of level 1 variations of some geophysical and climatic determinants which could lead to significant consequences at the local level. 107 HER of level 2 have been identified. HER of level 1 have been combined to a longitudinal description of the river network by using the Stralher ordination (Chandesris et al., 2006). So, by combining HER of level 1 (and in some cases HER of level 2), 5 size classes (very small, small, medium, large and very large rivers) and also exogenous types (rivers which flow on different types from upstream to downstream and which characteristics downstream are inherited from the upstream type), 124 national types are described; 51 of them representing 90% of the rivers' length.

The identification of reference conditions and limits for very good/good status for diatoms has been made for each type using both PAEQUANN (Predicting Aquatic Ecosystem Quality Using Artificial Neural Network) approach (Coste et al., 2004, Tison et al., 2005b) and Cemagref hydro ecoregion delimitation (MEDD, 2005).

836 samples collected from both national monitoring networks and specific campaigns on low or not impacted upstream sites have been used. All come from the Cemagref of Bordeaux and The Centre de Recherche Public Gabriel Lippmann of Luxemburg (CRPGL) and have been collected from stones only between May and August as requested by the IBD standard to minimise the natural variability. Diatom data have been analysed with SOM (Self Organizing Map) neuronal artificial network (Kohonen, 1995) which allows a classification of samples according to the similarity of diatom assemblages. Cluster analysis then allowed identifying 11 groups of diatom assemblages. Each one is representing a specific community linked to particular environmental characteristics (ecotype). Expert analysis on floristic data and human pressure led to distinguish 5 groups of reference assemblages according to ecoregional features and 6 groups of diatom assemblages under human influence. The existence of significant differences between diatom assemblages and differences in IBD values in the 5 reference assemblages justified the necessity to identify reference conditions and status limits for each one. A map of France combining the HER of level 2 and the areas where these reference assemblages could be found has been drawn (figure 3). For each of the 5 reference area, a box plot on IBD data has been made for reference sites. The reference value for IBD is assumed to be the median of IBD values corresponding to reference sites inside of the 5 reference assemblages while the limit very good/good status is assumed to be the percentile 25% (figure 4).

Reference values as well as limits between very good and good status should be considered as provisional values. These proposals are derived from exploitation of data issued from current national networks for which reference sites are underestimated. That is why a reference network is being implemented (MEDD, 2004). 450 sites are proposed and distributed amongst all types for annual sampling from 2005 to 2007. Reference monitoring will continue thereafter on one third of these sites in the following years. Parameters to fill in for diatoms are taxonomic composition, diversity and relative abundance during one summer campaign according to the IBD standard. The level for identification is that of IPS (species and variety). All types are concerned. These new data should make possible to finalise the first reference values and limits proposals.

WHAT FUTURE FOR IBD?

IBD is being reviewed for several years. A first step consisted to revise the standard and especially the sampling and the analysis of diatom following the publication of CEN standards (AFNOR, 2003, 2004). An enquiry has been launched in 2002 to about 40 Omnidia users for a balance in order to improve both IBD and associated tools (programs, technical manual, keys…). Omnidia 4.2 has been published in September 2004 in answer to this enquiry. It proposes an update of taxonomic data bases, the calculation of new European and extra European indices and also new features for page setting, printing, sorting …The standard should also be reviewed to include changes in IBD calculation. Taxa gathering should occur as for example in genera *Achnanthidium* or *Cocconeis,* while disassociation should occur in matched taxa (as for example the complex *Sellaphora seminulum*/*Eolimna minima*). New taxa from acid or brackish environments will be introduced too. Then, the total number should change from 209 to more than 300 (from 740 to about 1000 when taking into account synonyms). Finally ecological profiles and indicative values update are planned for misclassed taxa.

REFERENCES

AFNOR, 2000. NF T 90-354 Qualité de l’eau - Détermination de l'indice biologique diatomées (IBD), juin 2000, Saint-Denis La Plaine, 63 p.

AFNOR, 2003. NF EN 13946 Qualité de l'eau - Guide pour l'échantillonnage en routine et le prétraitement des diatomées benthiques de rivières, juillet 2003, Saint Denis la Plaine, 18 p.

AFNOR, 2004. NF EN 14407 Qualité de l'eau - Guide pour l'identification et le dénombrement des échantillons de diatomées benthiques de rivières, et leur interprétation, octobre 2004, Saint Denis la Plaine, 13 p.

Agence de l’Eau Seine-Normandie, 2000. Qualité hydrobiologique des cours d’eau du bassin Seine-Normandie (méthodes et résultats), Nanterre, décembre 2000, 29 p.

Cemagref, 1982. Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux, Rapport Q.E.Lyon-Agence Financière de Bassin Rhône-Méditerranée-Corse, Pierre Bénite, 218 p.

Chandesris A., Wasson J.-G., Pella H., Sauquet H., Mengin N., 2006. Typologie des cours d’eau de France métropolitaine. Rapport final. Cemagref Lyon, BEA/LHQ, Ministère de l'Ecologie et du Développement Durable, mars 2006, 63 pp. http://www.lyon.cemagref.fr/bea/lhq/dossiers_pdf/Typologie2006.pdf

Commission Internationale de l’Escaut (CIE), 2002. Rapport sur la qualité des eaux de l’Escaut sur la base des mesures du réseau de mesures homogène. Période 1998-2002, octobre 2003 (http://www.isc-cie.com)

Coste M., Leynaud G., 1974. Etudes sur la mise au point d’une méthodologie biologique de détermination de la qualité des eaux en milieu fluvial. Rapport C.T.G.R.E.F., Agence financière de bassin Seine-Normandie, Nanterre, 78 p.

Coste, M., Ayphassorho H., 1991. Etude de la qualité des eaux du bassin Artois-Picardie à l'aide des communautés de Diatomées benthiques. Application des indices diatomiques, rapport final Cemagref Bordeaux - Agence de l'Eau Artois Picardie, Douai, novembre 1991.

Coste M., Lenoir A., Huet F., 1995. Evaluation de la qualité des eaux de 71 stations du bassin Adour-Garonne à l’aide des communautés de diatomées benthiques. Résultats des inventaires 1994. Cemagref Bordeaux - Agence de l’eau Adour-Garonne, Toulouse, mars 1995, 31 p. + annexes

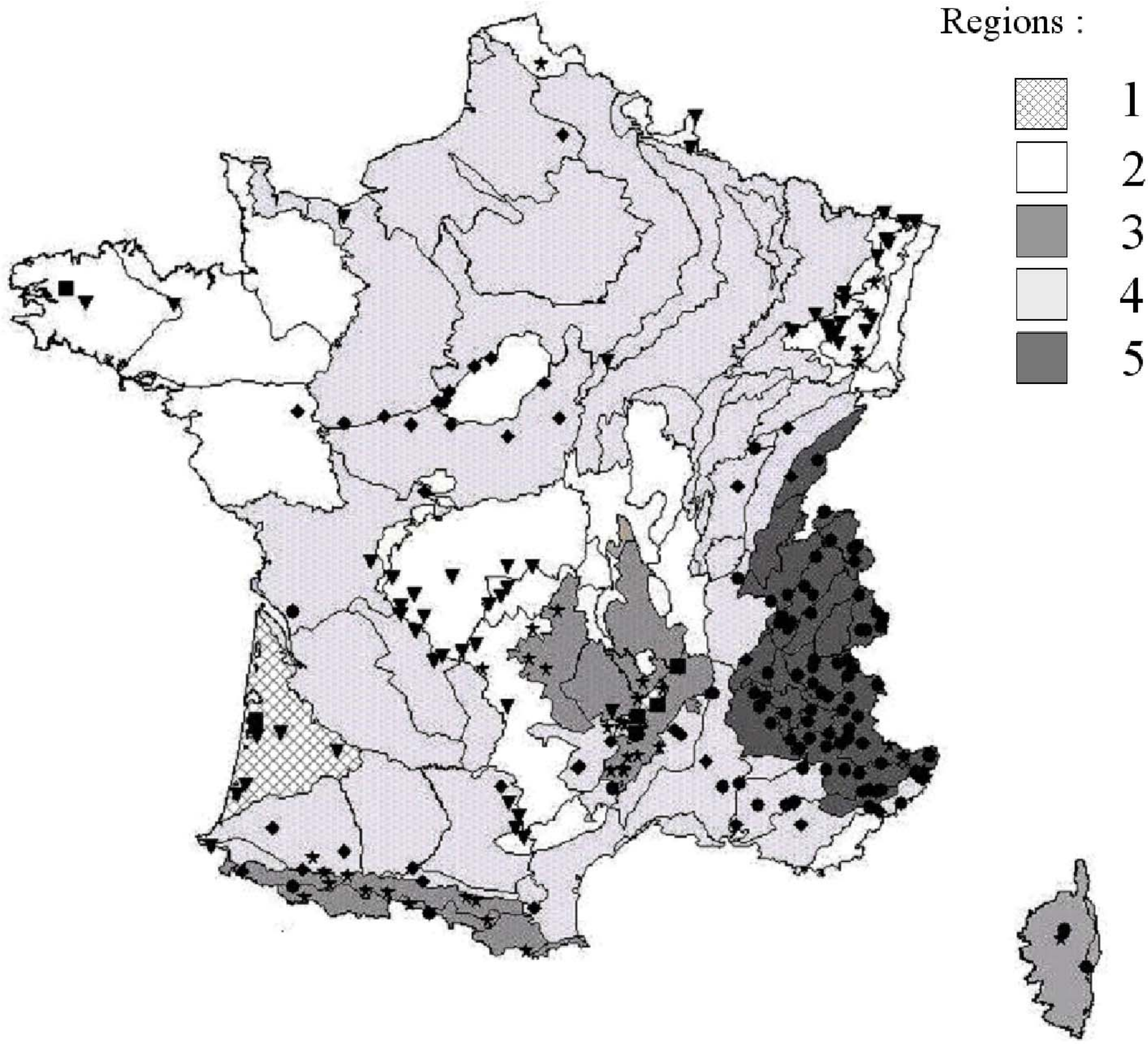

Fig. 3. Localization of first reference sites for diatoms through the 5 French natural diatom regions (from Coste et al. 2004). Region 1: acid rivers (i.e. Landes); Region 2: siliceous river substrates (i.e. Brittany); Region 3: mountain rivers with low mineralization (i.e. Pyrenean rivers); Region 4: calcareous plain rivers (i.e. Northern France); Region 5: mountain calcareous rivers (i.e. Alps) Thin black limits show the borders of French natural level 2 hydroecoregions as described by Wasson et al. 2002. See also explanation in the text.

Denys, L., 1991. A check-list of the diatoms in the Holocene deposits of the Western Belgian coastal plain with a survey of their apparent ecological requirements. I. Introduction, ecological code and complete list., Ministère des Affaires Economiques - Service Géologique de Belgique.: 41 p.

Descy, J. P.,1979. A New Approach water quality estimation using diatoms. *Nova Hedwigia Beiheft* 64: 305-323.

Descy, J.-P., Coste M., 1989. Application d'un nouvel indice (Indice CEE 88) au réseau national de Bassin Rhône Méditerranée Corse, rapport U.N.E.C.E.D., Cemagref Bordeaux, Pierre-Bénite, décembre 1989, 44 p. + annexes.

Descy, J.-P., Coste M., 1991. A test of methods for assessing water quality based on diatoms. *Verh. Internat. Limnol.* 24(4): 2112-2116

Diren Ile de France, 1997. L'apport des indices diatomiques. L'expérience de la Diren Ile de France, Diren Ile de France, Cachan, mai 1997, 19 p.

Ector L., Loncin A., Tudesque L., Dohet A., Rimet F., 2000. Etude sur les diatomées benthiques du bassin Rhône Méditerranée Corse dans le cadre de l'extension de l'Indice biologique diatomées IBD. Campagne 1997. Rapport final, Centre de Recherche Public Gabriel Lippmann – Agence de l'Eau Rhône-Méditerranée-Corse, Lyon, 65 p. + annexes

European Community, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities* L 327/1.

Kohonen T., 1995. Self-organizing maps, 2nd edition, Springer series in Information Sciences, Vol. 30, Springer Verlag : Berlin, Germany

Lecointe C., Coste M., Prygiel J., 1993. "Omnidia": a software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia* 269/270: 509-513.

Lecointe C., Le Renard C., 1999. Amélioration du calcul de l'IBD ou indice Biologique Diatomées. Rapport final CLCI, Etude inter agences, Douai, 18 p. + annexes

Lecointe C., Coste M., Prygiel J., 1999. Le logiciel Omnidia 2, une puissante base de données pour les inventaires de diatomées et pour le calcul des indices diatomiques européens. In Ector L., Loncin A., Hoffmann L. (eds), Compte rendu du 17° colloque de l'Association des Diatomistes de langue Française, Luxembourg, 8 –11 septembre 1998. *Cryptogamie-Algologie* 20(2) : 105-149.

Lenoir A., Coste M., 1994. Estimation de la qualité des eaux du basin Rhin-Meuse à l'aide des communautés de diatomées benthiques. Rapport final. Cemagref Bordeaux – Agence de l'eau Rhin-Meuse, Moulin-lès-Metz, mars 1994, 114 p. + annexes

Lenoir A., Coste M., 1996. Development of a practical diatom index of overall water quality applicable to the French national water Board Network. In Whitton B.A., Rott E. (eds) Use of Algae for Monitoring Rivers II, Institüt für Botanik, Univ. Innsbruck : 29-45.

Ministère de l'Ecologie et du Développement Durable (2004). Circulaire DCE 2004/08 relative à la constitution du réseau de sites de référence pour les eaux douces de surface (cours d'eau et plans d'eau), en application de la directive européenne 2000/60/DCE du 23 octobre 2000 du Parlement et du Conseil établissant un cadre pour une politique communautaire dans le domaine de l'eau. Direction de l'eau, 23 décembre 2004, 8 p. + annexes.

Ministère de l'Ecologie et du Développement Durable (2005). Circulaire DCE 2005/12 relative à la définition du bon état et à la constitution des référentiels pour les eaux douces de surface (cours d'eau, plans d'eau), en application de la directive européenne 2000/60/DCE du 23 octobre 2000, ainsi qu'à la démarche à adopter pendant la phase transitoire (2005-2007). Direction de l'eau, 28 juillet 2005, 17 p.

Prygiel J., 2002. Management of the diatom monitoring networks in France in 2000. *Journal of Applied Phycology 14(1): 19-26.*

Prygiel J., Carpentier P., Almeida S., Coste M., Druart J-C., Ector L., Guillard D., Honoré M.-H., Iserentant R., Ledeganck P., Lalanne-Cassou C., Lesniak C., Mercier I., Moncaut P., Nazart M., Nouchet N., Peres-Weerts F., Peeters V., Rimet F., Rumeau A., Sabater S., Straub F., Torrisi M., Tudesque L.,Van de Vijver B., Vidal H., Vizinet J., Zydek N., 2002. Determination of the

Biological Diatom Index (IBD NF T 90 - 354): results of an intercomparison exercise. *Journal of Applied Phycology 14(1): 27-39*

Prygiel J., Coste M., 1993. The assessment of water quality in the Artois-Picardie water basin (France) by the use of diatom indices. *Hydrobiologia* 269/270: 343-349.

Prygiel, J., Coste M., 1996a. Les diatomées et les indices diatomiques dans les réseaux de mesure de la qualité des cours d'eau français. Historique et avenir. *Bull. Fr. Pêche Piscic.* 341/342 : 65-81

Prygiel, J., Coste M., 1996b. Recent trends in monitoring French rivers using algae, especially diatoms. In Whitton B.A., Rott E. (eds) Use of Algae for Monitoring Rivers II, Institüt für Botanik, Univ. Innsbruck : 87-97.

Prygiel J., Coste M., 1998. Mise au point de l'Indice Biologique Diatomées, un indice diatomique pratique applicable au réseau hydrographique français. *L'Eau, l'Industrie, et les Nuisances* 211: 40-46.

Prygiel J., Coste M., 1999. Progress in the use of diatoms for monitoring rivers in France. In J. Prygiel, B.A. Whitton, J. Bukowska (eds), Use of Algae for Monitoring Rivers III, Agence de l'Eau Artois-Picardie, Douai: 165-181.

Prygiel J., Coste M., 2000. Guide méthodologique pour la mise en œuvre de l'Indice Biologique Diatomées NF T 90-354. Agences de l'Eau-Cemagref Bordeaux, mai 2000, 134 pages + Clés de détermination (89 planches) + cédérom français-anglais (tax'IBD)

Prygiel J., Haury J., 2006. Monitoring methods based on Algae and Macrophytes. In Ziglio G., Siligardi M. & Flaim G. (Eds) “Biological Monitoring of Rivers: Applications and Perspectives”. John Wiley & Sons Ldt, Chichester, 155-171

Prygiel J., Levêque L., Iserentant R., 1996. L'IDP: un nouvel Indice Diatomique Pratique pour l'évaluation de la qualité des eaux en réseau de surveillance. *Rev. Sci. Eau* 1: 97-113.

Réseau National des Données sur l’Eau (RNDE), 2000. La qualité biologique des cours d’eau en France, janvier 2001 (http://www.rnde.tm.fr)

Rumeau, A., Coste M., 1988. Initiation à la systématique des Diatomées d'eau douce. Pour l'utilisation d'un indice diatomique générique [Introduction into the systematic of freshwater diatoms - For a useful generic diatomic index], *Bull. Fr. Pêche Piscic*. 309: 1-69.

Tison J., Giraudel J.L., Coste M., Park, Y.-S., Delmas F., 2004. Use of unsupervised neural networks for ecoregional zoning of hydrosystems through diatom communities: case study of Adour-Garonne watershed (France). Archiv. Hydrobiol. 159(3): 409-422

Tison J., Park, Y.-S., Coste M., Wasson J.-G., Ector L., Rimet F., Delmas F., 2005a. Typology of diatom communities and the influence of hydro-ecoregions: a study on the French hydrosystem scale. Water Research 39: 3177-3188

Tison J., Giraudel J.L., Park, Y.-S., Coste M., Delmas F., 2005b. Classification of stream diatom communities using a self-organizing map. In S. Lek, M. Scardi, P.F.M. Verdonschot, J.-P. Descy & Y.S. Park (eds), Modelling Community structure in freshwater ecosystems, Springer, 5.5: 304-316

Tison J., 2006. Utilisation de la modélisation neuronale et des caractéristiques des communautés diatomiques pour la bio-évaluation de l’état écologique des cours d’eau et l’accompagnement de leur restauration. Thèse de doctorat Université Bordeaux1, 144p.

van Dam, H., Mertens A., Sinkeldam J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Journal of Aquatic Ecology* 28(1): 117-133.

Wasson J.G., Chandesris A., Pella H., Blanc L., 2002. Définition des hydro-écorégions françaises métropolitaines. Rapport final. Cemagref Lyon, BEA/LHQ, Ministère de l'Ecologie et du Développement Durable, Lyon, juin 2002, 190 pp.

Fig. 4. Reference values and limits for good status for French type rivers (from MEDD, 2005).

x -]y-z] : x = valeur de référence, y = limite supérieure du bon état, z = limite inférieure du bon état, En grisé : type inexistant.

: absence de données suffisantes ; Case hachurée : acidité possible, si **pH observé < 6.5**, les valeurs sont alors de **20 -]19 - 17].**

Tableau 3 : ETAT ECOLOGIQUE – DIATOMEES
Indice Biologique Diatomées (norme NF T90-354)

Valeurs provisoires de l'IBD « DCE compatible » par type de cours d'eau

		Classes de taille de cours d'eau ou rangs : bassin Loire-Bretagne	8,7	6	5	4	3,2,1
		autres bassins	**8, 7, 6**	**5**	**4**	**3**	**2, 1**
Hydroécorégions de niveau 1		*Cas général, cours d'eau exogène de l'HER de niveau 1 indiquée ou HER de niveau 2*	**Très Grands**	**Grands**	**Moyens**	**Petits**	**Très Petits**
20	**DEPOTS ARGILO SABLEUX**	Cas général		**16 -]15-13]**		**16 -]15-13]**	**16 -]15-13]**
		Exogène de l'HER 9 (Tables Calcaires)		**16 -]15-13]**			
		Exogène de l'HER 21 (Massif Central Nord)		**16 -]15-13]**	**16 -]15-13]**		
21	**MASSIF CENTRAL NORD**	Cas général				**16 -]15-13]**	**16 -]15-13]**
3	**MASSIF CENTRAL SUD**	Cas général		**18-]17-15]**	**18-]17-15]**	**18-]17-15]**	**18-]17-15]**
		Exogène de l'HER 19 (Grands Causses)			**#**		
		Exogène de l'HER 8 (Cévennes)			**#**		
		Exogène de l'HER 19 ou 8		**16 -]15-13]**			
17	**DEPRESSIONS SEDIMENTAIRES**	Cas général			**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**
		Exogène de l'HER 3 ou 21 (M.Cent.S ou N)	**#**	**#**	**#**	**#**	**#**
15	**PLAINE SAONE**	Exogène de l'HER 3 ou 21					
		Exogène de l'HER 5 (Jura)		**19 -]17-15]**	**19 -]17-15]**		
		Cas général	**16 -]15-13]**		**16 -]15-13]**		**16 -]15-13]**
		Exogène de l'HER 10 (Côtes Calcaires Est)	**16 -]15-13]**				
5	**JURA / PRE-ALPES DU NORD**	Cas général		**19 -]17-15]**	**19 -]17-15]**	**19 -]17-15]**	**19 -]17-15]**
		Exogène de l'HER 2 (Alpes Internes)	**19 -]17-15]**	**19 -]17-15]**			
TTGA	**FLEUVES ALPINS**	Cas général	**#**				
2	**ALPES INTERNES**	Cas général		**19 -]17-15]**	**19 -]17-15]**		**19 -]17-15]**
7	**PRE-ALPES DU SUD**	Cas général		**19 -]17-15]**			**19 -]17-15]**
		Exogène de l'HER 2 (Alpes Internes)	**16 -]15-13]**	**19 -]17-15]**			
6	**MEDITERRANEE**	Exogène de l'HER 2 ou 7					
		Exogène de l'HER 7 (Pré-Alpes du Sud)		**19 -]17-15]**			
		Exogène de l'HER 8 (Cévennes)	**16 -]15-13]**	**19 -]17-15]**			
		Exogène de l'HER 1 (Pyrénées)		**19 -]17-15]**			
		Cas général		**16 -]15-13]**	**16 -]15-13]**		**16 -]15-13]**
8	**CEVENNES**	Cas général		**18-]17-15]**		**18-]17-15]**	
		A-HER niveau 2 n°70			**18-]17-15]**	**18-]17-15]**	
16	**CORSE**	A-HER niveau 2 n°22		**18-]17-15]**	**18-]17-15]**	**18-]17-15]**	
		B-HER niveau 2 n°88			**18-]17-15]**	**18-]17-15]**	
19	**GRANDS CAUSSES**	Cas général				**18-]17-15]**	
		Exogène de l'HER 8 (Cévennes)		**#**			
11	**CAUSSES AQUITAINS**	Cas général				**16 -]15-13]**	**16 -]15-13]**
		Exogène de l'HER 3 (MCN) et/ou 21 (MCS)	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	
14	**COTEAUX AQUITAINS**	Exogène des HER 3, 8, 11 ou 19	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**		
		Exogène de l'HER 3 (MCN) ou 8 (Cév.)			**16 -]15-13]**		
		Cas général		**16 -]15-13]**		**16 -]15-13]**	**16 -]15-13]**
		Exogène de l'HER 1 (Pyrénées)	**16 -]15-13]**	**16 -]15-13]**	**#**	**#**	
13	**LANDES**	Cas général			**20 -]19-17]**	**20 -]19-17]**	**20 -]19-17]**
1	**PYRENEES**	Cas général		**18-]17-15]**	**18-]17-15]**	**18-]17-15]**	**18-]17-15]**
12	**ARMORICAIN**	A-Centre-Sud (HER niveau 2 n° 58 et 117)		**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**
		B-Ouest-N E (HER niveau 2 n° 55, 59 et 118)			**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**
TTGL	**LA LOIRE**	Cas général	**16 -]15-13]**				
9	**TABLES CALCAIRES**	A-HER niveau 2 n°57			**16 -]15-13]**	**16 -]15-13]**	
		Cas général	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**
		Exogène de l'HER 10 (dans l'her2 n°40)		**16 -]15-13]**	**16 -]15-13]**		
		Exogène de l'HER 21 (Massif Central Nord)	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**		
10	**COTES CALCAIRES EST**	Exogène de l'HER 21 (Massif Central Nord)					
		Cas général	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**
		Exogène de l'HER 4 (Vosges)		**16 -]15-13]**	**16 -]15-13]**		
4	**VOSGES**	Cas général			**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**
22	**ARDENNES**	Exogène de l'HER 10 (Côtes Calcaires Est)	**16 -]15-13]**				
		Cas général		**16 -]15-13]**		**#**	**#**
18	**ALSACE**	Cas général			**16 -]15-13]**		**16 -]15-13]**
		Exogène de l'HER 4 (Vosges)		**16 -]15-13]**	**16 -]15-13]**	**16 -]15-13]**	

On the importance of calibrated diatom concepts for the EU Waterframework Directive – a diatom taxonomist's perspective

Regine Jahn & Wolf-Henning Kusber

Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Königin-Luise-Str. 6-8, 14195 Berlin, Germany
r.jahn@bgbm.org
w.h.kusber@bgbm.org

INTRODUCTION

The EU Waterframework Directive is making the member states implementing this directive to standardize their sampling, microscopical, and identification methods and to intercalibrate their analyses. Species lists are being produced whose names are expected to serve as indicator-tags for this biomonitoring. But even if we assume that every identifier is identifying correctly, we need to be aware of the underlying species concept of the authors of the keys. There are big discrepancies between a species name and its concept in such keys as Hustedt 1930, Krammer & Lange-Bertalot 1986-1991, Cox 1996, and in Lange-Bertalot's publications after 1995 (e.g. Lange-Bertalot 2001). In the last two decades species concepts have changed from a conservative taxonomy ("lumping") to a more refined approach ("splitting") and from cosmopolitanism to geographical specificy (e.g. *Stauroneis:* Lange-Bertalot et al. 2003, Vijver et al. 2004; *Surirella:* Cocquyt & Jahn 2005).

Current developments in Germany concerning the EU Waterframework Directive use diatoms in the analysis of phytobenthos (Schaumburg et al. 2005) and phytoplankton (see below) whereas diatoms as photoautotrophic organisms have been removed from DIN-list of saprobic indices (see Friedrich 1990). Biovolumes of planktonic algae are being counted for several lakes and rivers in Germany. For diatoms, counts from counting chambers (Utermöhl-technique) are compared to relative counts from diatom permanent slides (for River Odra, see Kasten 2002). The taxonomic concepts, used by the majority of German phycologists today are those published in the Süßwasserflora (Krammer & Lange-Bertalot 1986-1991); only a small minority is using old Hustedt-concepts (e.g. Hustedt 1930) or the most recent literature (e.g. Lange-Bertalot 2001). The conservative concepts of the Süßwasserflora (Krammer & Lange-Bertalot 1986-1991) have also been the basis for the German Flora and Red List of limnic diatoms (Lange-Bertalot 1996) as well as for the diatom part of the list of water organisms in Germany (Mauch et al. 2003).

These lists are now being compared with data reported from plankton countings from all over Germany (Kusber & Jahn, in prep.). The main task of this comparison is to find synonyms, formal inconsistencies, and homonyms. The result is an operational list of planktonic algae, called "Harmonisierte Taxaliste", which is accessible via the Internet (Mischke 2006). Under the same URL some helpfully documents for identification of diatoms are also available.

To show the problems of standardization, intercalibration etc., we present some examples of historical diatom concepts.

EXAMPLES TO ILLUSTRATE HISTORICAL CONCEPTS

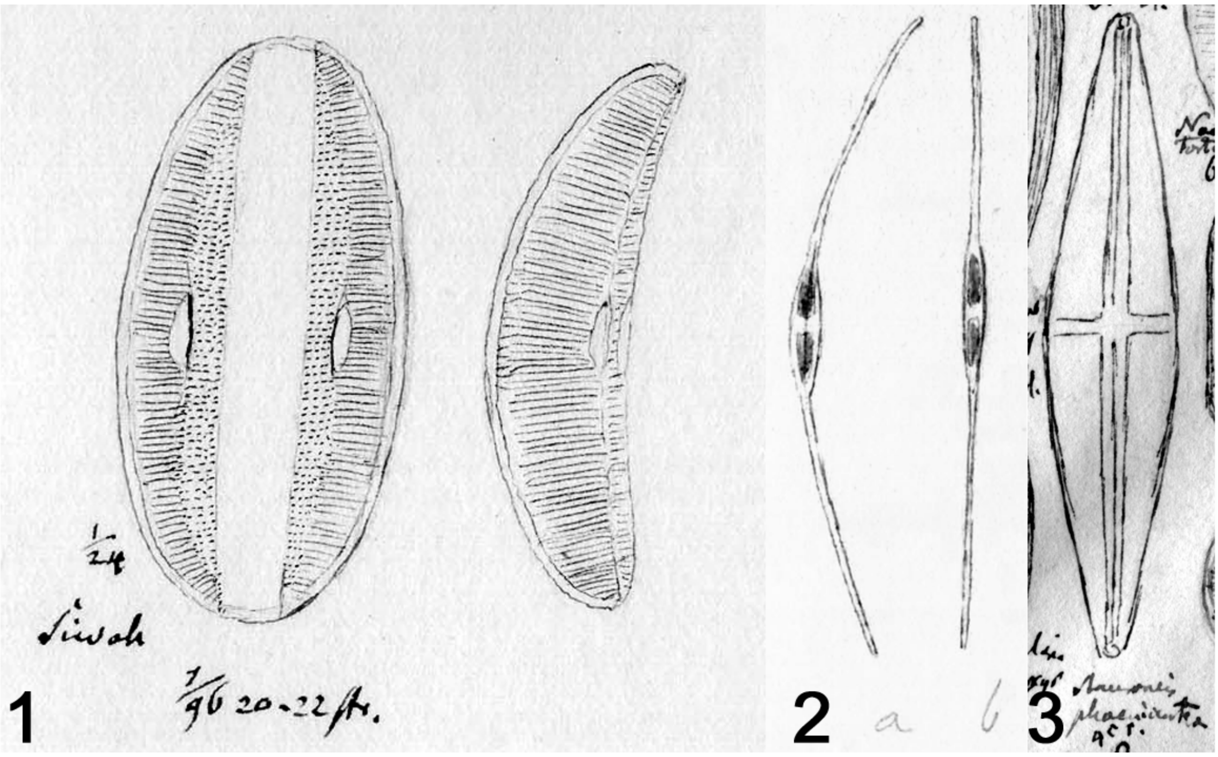

Fig. 1. *Amphora libyca* Ehrenb., drawing no. 112, Ehrenberg Collection, original material; Fig. 2. *Ceratoneis closterium* Ehrenb., drawing no. 235, Ehrenberg Collection, original material; Fig. 3. *Stauroneis phoenicenteron* (Nitzsch) Ehrenb., sample of River Schelde, Antwerp, drawing no. 2136, Ehrenberg Collection, species concept

***Amphora libyca* Ehrenb.**
in Ber. Bekanntm. Verh. Königl. Preuss. Akad. Wiss. Berlin 1840: 205. 1840.
Lectotype: “Siwa 410, 3e-O”, designated by: T. Nagumo (2003).
Original material: Drawing no. 112 in the BHUPM (Fig. 1).
Locality: “Siwa Oasis, Egypt”
Comment: The type specimen is an African species (see also Jahn & Kusber 2006), completely different from the 20th centuries concepts of *Amphora libyca* (e.g. Hustedt 1930, Krammer & Lange-Bertalot 1986) which , according to Nagumo (2003), should be called *Amphora copulata* Kütz.

***Ceratoneis closterium* Ehrenb.**
in Ber. Bekanntm. Verh. Königl. Preuss. Akad. Wiss. Berlin 1839: 157. 1839.
Homotypic synonyms: *Cylindrotheca closterium* (Ehrenb.) Reimann & J.C.Lewin in J. Roy. Microscop. Soc. London 83: 288. 1964; *Nitzschia closterium* (Ehrenb.) W.Sm., Syn. Brit. Diat. 1, p. 42. 1853.
Lectotype: Taxonomical Preparation No. 540032-3 in BHUPM, designated by: Jahn & Kusber (2005).
Locality: “Nordsee bei Cuxhaven ... in dem zur Fluthzeit aus dem hohen Meere anströmenden Wasser”.
Comment: Fig. 2 is an original drawing of C.G. Ehrenberg from live samples he studied in Berlin. With the knowledge of Ehrenberg’s description and, most important, the lectotypification of a specimen it was possible to support the current taxonomic concept. Kützing (1844) published figures of his own taxonomic concept of the species which differs slightly from Ehrenberg’s original observations (Fig. 5).

***Stauroneis phoenicenteron* (Nitzsch) Ehrenb.**
in Ber. Bekanntm. Verh. Königl. Preuss. Akad. Wiss. Berlin 1845: 61. 1845.
Basionym: *Bacillaria phoenicenteron* Nitzsch in Neue Schriften Naturf. Ges. Halle 3: 9. 92, pl. 4. 1816.
Comment: Fig. 3 is part of drawing sheet no. 2136 in BHUPM, showing a specimen of *Stauroneis phoenicenteron* from sediments of the River Schelde in Antwerp, drawn and identified by Ehrenberg. This concept is very broad since at that time that there was only one species within the genus.

***Nitzschia palea* (Kütz.) W.Sm.,**
Syn. Brit. Diat. 2, p. 89. 1856.
Basionym: *Synedra palea* Kütz., Kieselschal. Bacill.: 63, pl. 3: fig. XXVII; pl. 4: fig. II.
Comment: Kützing's (1844) figure, given here as Fig. 5 is insufficient to identify the species. Our current species concept (Krammer & Lange-Bertalot 1988) has evolved as a series of published identifications. Inconsistent results of morphological and molecular studies of clones, identified as *N. palea* indicate the need to re-evaluate both, names and concepts.

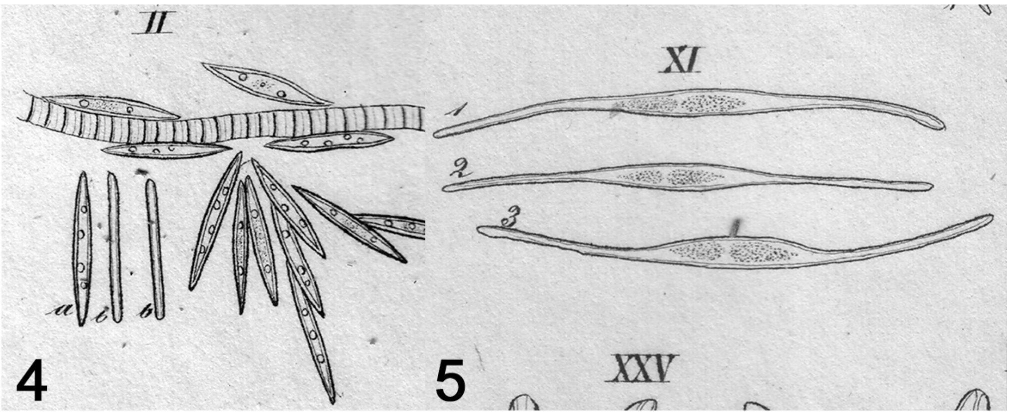

Fig. 4. *Nitzschia palea* (Kütz.) W.Sm. as *Synedra palea* Kützing (1844: pl. 4: fig. II), original material in terms of the ICBN; Fig. 5. *Ceratoneis closterium* Ehrenb. in Kützing (1844: pl. 4: fig. XI), species concept.

HOW TO HANDLE TAXONOMIC INFORMATION IN THE 21ST CENTURY

In order to use diatoms as indicators in biomonitoring it is necessary to rely on species names. Unclear, unprecise or unreconstructable taxonomic entities hinder the optimizing of these systems. To overcome these nomenclatural and taxonomic shortcomings, calibration sets have been invented in a number of regions. But the ultimate 'calibration' is given and institutionalized by the International Code of Botanical Nomenclature: the type of the name of the species. When revising species groups and before describing new species, it is established taxonomic practice to review available names and their types. In diatom research the re-evaluation of types does not have a long tradition and figures (often only drawings) seemed to be sufficient to understand the taxonomy behind a new species (Fig. 1-5). With the advance of the SEM as a routine instrument of research, it became clear that in diatom research we are often dealing with species complexes. The advance of the molecular methods will help us to differentiate look-alike species which will refine our monitoring system even more because so-called ubiquists are turning out to be rather good indicators if defined correctly (i.e. the species complexes *Achnanthidium minutissimum*, *Gomphonema parvulum*, *Nitzschia palea*).

Once we know the type of the name of the species, we know which name tag has been applied to a certain specimen. The next question is then: Can taxonomists agree on a certain species concept to be used for monitoring? The basis of any agreement is standardization. Just as molecular data is standardized and has to be published in a certain place and format on the Internet, we also need to standardize our taxonomy. Nomenclature such as names and types, is ruled by the International Botanical Code and authors (not authorities!) are often standardized according to Brummit & Powell standard (IPNI). But what about taxonomical data such as morphology (outline, length, breadth, striae, costae, raphe, etc.), ecological preferences/tolerances (pH, phosphate, nitrates, etc.), distribution, occurrences, etc.? We need to connect researched data to our taxonomic concepts represented by the name we are using. By visualizing (i.e. figures) our concepts we can clarify which concepts we are using.

But how can we communicate our concepts and findings? We need to be faster in making our new findings known. The fastest and most efficient way is to make our science available on the Internet. This means structuring the different parts of our data to fit database format and to use a powerful relational database. There are a number of projects currently going on that facilitate biodiversity data and taxonomic research going Internet; i.e. GBIF, EDIT and especially for algae: AlgaeBase (Guiry et al. 2006), AlgaTerra, Index Nominum Algarum (INA, Silva 1997-), etc.

AlgaTerra, a joint project of five German research groups, funded by the German Federal Ministry of Education and Research (BMBF) 2001-2005, resulted in the typification of a number of Ehrenberg's taxa, a databasing and digitization of Hustedt's taxa from Germany, the establishment of molecular and ecological facts of a number of freshwater diatoms and green algae which have been databased. Even though the financing of the project has ended, it is sustainable because it is tied to the algae curation at the BGBM. This information system for micro algal biodiversity (Jahn & Kusber 2006) has been online since 2004 and contains currently more than 22 000 algal names, data for 4550 algal types, 4350 facts, figures of more than 250 algal concepts and 12 diatom videos. Morphological information, descriptions, molecular data and bibliographic data are also available. In addition, AlgaTerra is serving GBIF international as type-data-provider and we are continually adding information on newly published types. If you are interested in having your new species visible on the Internet, supply us with your figures!

Even if the water monitoring people are keen on having non-changing harmonized lists of taxa, diatom taxonomy is dynamic and research will produce more findings and more differentiated species concepts in the future with the help of molecular and ultrastructural methods. The only thing that needs to be ensured is that communication is taking place between applied and basic research.

REFERENCES

Cocquyt, C. & Jahn, R. 2005: Rare *Surirella* taxa (Bacillariophyta) from East Africa described by Otto Müller: typifications, recombinations, new names, annotations and distributions. Willdenowia **35:** 359-371.

Cox, E.J. 1996: Identification of freshwater diatoms from live material. – Chapman & Hall, London.

Friedrich, G. 1990: Eine Revision des Saprobiensystems. – Z. Wasser- Abwasser-Forsch. **23:** 141-152.

[GBIF] GLOBAL BIODIVERSITY INFORMATION FACILITY – [12 June 2006]. Available from <http://www.gbif.org/>:

Guiry, M.D. et al. 2006: AlgaeBase **version 4.1.** World-wide electronic publication, National University of Ireland, Galway [12 June 2006]. Available from <http://www.algaebase.org>.

Hustedt, F. 1930: Bacillariophyta (Diatomeae). – In: Pascher, A. (ed.): Die Süsswasserflora Mitteleuropas **10** (2. edition). – G. Fischer, Jena.

[IPNI] The International Plant Names Index [12 June 2006]. Available from <http://www.ipni.org/index.html>.

Jahn, R. & Kusber, W.-H. (eds) 2006: *AlgaTerra* Information System [online]. Botanic Garden and Botanical Museum. Berlin-Dahlem, FU-Berlin [12 June 2006]. Available from <http://www.algaterra.org>.

Jahn, R. & Kusber, W.-H. 2005: Reinstatement of the genus *Ceratoneis* Ehrenberg and lectotypification of its type specimen: *C. closterium* Ehrenberg. – Diatom Research **20:** 295-304.

Kasten, J. 2002: Überschwemmung und Isolation: Die Dynamik der Phytoplankton gemeinschaften einer sailonal überfluteten Fluß-Auen-Landschaft (Unteres Odertal - Brandenburg). – Lehmanns, Berlin.

Krammer, K. & Lange-Bertalot, H. 1986-1991: Bacillariophyceae 1.-4. Teil. – In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds): Süßwasserflora von Mitteleuropa. **2 (1-4)**. – G. Fischer, Stuttgart & New York.

Kusber, W.-H. & Jahn, R. (in prep.): Harmonisierung von Algen-Taxalisten für die Umsetzung der EU-WRRL aus Sicht von Taxonomie und Daten-Management. In: Deutsche Gesellschaft für Limnologie (ed.): Tagungsbericht 2006 (Dresden), Berlin.

Kützing, F. T. 1844: Die kieselschaligen Bacillarien oder Diatomeen. – Nordhausen.

Lange-Bertalot, H. 2001: *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lato, *Frustulia* – Diatoms of Europe **2.** – Gantner, Ruggell.

Lange-Bertalot, H. 1996: Rote Liste der limnischen Kieselalgen Deutschlands. – Schriftenreihe für Vegetationskunde **28:** 633-677.

Lange-Bertalot, H., Cavacini, P., Tagliaventi, N. & Alfinito, S. 2003: Diatoms of Sardinia. – Iconogr. Diatomol. **12:** 1-438.

Mauch, E., Schmedtje, U., Maetze, A. & Fischer, F. 2003: Taxaliste der Gewässerorganismen Deutschlands, Informationsberichte Heft 1/03. Bayerisches Landesamt für Wasserwirtschaft, München.

Mischke, U. 2006: Downloads zum Bewertungsverfahren Phytoplankton nach WRRL. Leibniz-Institut für Gewässerökologie und Binnenfischerei. Available from <http://www.igb-berlin.de/abt2/mitarbeiter/mischke/>.

Nagumo, T. 2003: Taxonomic studies of the subgenus Amphora Cleve of the genus Amphora (Bacillariophyceae) in Japan. – Biblioth. Diatomol. **49:** 1-65.

Schaumburg, J., Schranz, C., Foerster, J., Gutowski, A., Hofmann, G., Köpf, B., Meilinger, P., Schmedtje, U., Schneider, S., Stelzer, D. 2005: Bewertungsverfahren Makrophyten & Phytobenthos. Fließgewässer- und Seen-Bewertung in Deutschland nach EG-WRRL. Informationsberichte Heft 1/05. Bayerisches Landesamt für Wasserwirtschaft, München.

Silva, P.C. 1997-: Index Nominum Algarum, University Herbarium, University of California, Berkeley [12 June 2006]. Available from <http://ucjeps.berkeley.edu/INA.html>.

Van de Vijver, B., Beyens, L. & Lange-Bertalot, H. 2004: The genus *Stauroneis* in the Arctic and (Sub-)Antarctic regions. – Biblioth. Diatomol. **51:** 1-317.

Phytobenthos and the Water Framework Directive in Hungary and The Netherlands

Herman van Dam[1], Judit Padisák[2] and Rob Portielje[3]

[1] Grontmij | AquaSense, P.O. Box 95 125, 1090 HC Amsterdam, The Netherlands
Present address: Consultant for Water and Nature, P.O.Box 37777, 1030 BJ NL-Amsterdam.
herman.vandam@waternatuur.nl
[2] Pannon University, Department of Limnology , P.O. Box 158, H-8201, Veszprém, Hungary.
Padisak@tres.blki.hu
[3] RIZA Institute for Integral Water Management and Waste Water Treatment, P.O. Box, NL-8200 AA Lelystad, The Netherlands
R.Portielje@riza.rws.minvenw.nl

For the implementation of the European Water Framework Directive a lot of investigations done are in several countries. In Hungary typology and indices for the calculation of water quality ratio's (EQR's) are developed and in The Netherlands correlations between EQR's (and the commonly used IPS) and nutrient concentrations are analyzed.

Hungary

Phytobenthos investigations were carried out as a part of the EcoSuv Project (http://www.eu-wfd.info/ecosurv/, Arcadis 2005). A basic ecological survey was conducted in order to create a sound ecological database in lakes and rivers. Only the results on rivers will be discussed here.
In the spring of 2005, samples of phytobenthos were taken in 339 streams. Samples for analysis of water chemistry were taken at most of the stations. In the laboratory, the species composition of samples was investigated using standard methods. About 400 individuals were counted and the percentage contribution of each species was calculated.

In the rivers, 496 species were found. By using ordination (a multivariate statistical technique), it appeared that current velocity, altitude, shading, oxygen and alkalinity are master variables for the species composition of phytobenthos. Nutrients are of less importance in determining the distribution of phytobenthos over the whole country, but regionally they can have a considerable impact. By using classification (another statistical technique), 12 groups (clusters) of phytobenthos types were distinguished which are defined by indicator or character species. Each of the types in the existing river typology supports typical phytobenthos assemblages. Human impact has caused shifts in the assemblages.

For the estimation of water quality in the running waters, the IPS (Index of Pollution Sensitivity), originally developed in France, was calculated and the class boundary limits were adapted to the Hungarian situation. Different class boundaries are set for high-, mid- and low-altitude streams. Using these boundaries, 81% of the streams have a high or good quality. Poor or bad conditions of the streams are often due domestic sewage load, but industrial pollution may also be important. Provisionally, reference sites are selected.
It is recommended to continue the monitoring of rivers in the same way as was done in 2005.

THE NETHERLANDS

EQR's in Dutch surface waters are calculated from the ratio between positive and negative indicator species (Van der Molen 2006, www.stowa.nl/). Ecological pressures like acidification, eutrophication and desiccation enhance the development of negative indicator species, while the growth of positive indicator species is suppressed by these threats.

As trophic state is one of the maior issues in improving the water quality of surface waters, the correlations between EQR's must be known. Therefore results of diatom and chemical analyses of rivers and lakes were retrieved from data bases and correlation coefficients were calculated. Omly the results on rivers are reported here.

From the rivers 196 samples from 101 stations were found. The correlation between the EQR and (log) total nitrogen is –0.32 ($p<0.001$) and between EQR and (log) total phosphate –0.55 for all stations togehther , while the correlations between commonly used Pollution Sensitivity Index (PSI, Cemagref 1982) and the nutrients were slightly higher better, with –0.39 and –0.58 respectively. In specific types (e.g. R05: slowly flowing middle and lower courses of sandy streams) these correlations improved by another 0.5, so the correlation between EQR and phosphate was –0.60. Further improvements for R05 were achieved with a multiple regression model: $EQR = 0.50 - 0.3529\ {}^{10}(\log P\text{-total}) - 0.0.0455\ {}^{10}\log(N\text{-Kjeldahl}) - {}^{10}\log(NH_4\text{-}N)$ ($r = 0.70$).

These correlation is not bad, but the standard error (0.16) is too large to give reliable predictions of the EQR after reduction of nutrient concentrations. The large standard error is partly due to insufficiencies in the data set, like differences in methods of sampling and analyses and a weak empirical base for the EQR. These insufficiencies are to be smoothed next years.

REFERENCES

AquaSense (2005). Relaties tussen nutriënten en kwaliteit van fytobenthos: stuurbaarheid van doelvariabelen voor de Kaderrichtlijn Water. In opdracht van Rijkswaterstaat RIZA. Rapportnummer 05.2499. Amsterdam. 55p.

Arcadis (2005): EcoSurv Final Technical Report, Part A. Ministry of Environment and Water, Budapest. 49p.

Cemagref (1982): Étude des méthodes biologiques d'appreciation quantitative de la qualité des eaux. Rapport Q.E. Lyon - Agence de l'Eau Rhône-Medterrane Corse. 218p.

Van der Molen, D.T. (2006): Referenties en concept-maatlatten voor rivieren voor de Kaderrichtlijn Water. STOWA-rapport 2004/23. Utrecht, 365p.

Diatoms in lowland lakes and ponds: WFD's ugly duck needs reliable wings

Luc Denys

Research Institute for Nature and Forest / Instituut voor Natuur- en Bosonderzoek (INBO), Kliniekstraat 25, B-1070 Brussel, Belgium
luc.denys@inbo.be

Lowland lakes and ponds present particular challenges for the implementation of the Water Framework Directive (WFD). Problematic issues with regard to phytobenthos assessment include the definition of ecological status, as well as the development of appropriate methods. These are considered from the perspective of Flanders, lower Belgium, and in particular with reference to the predominantly non-acid and shallow waters prevailing in this region.

Firstly, some of the difficulties to determine water-type specific reference conditions are illustrated. In general, documenting near-natural diatom communities in terms of taxonomic composition and abundance1 is difficult for water bodies in lower Belgium due to their regional setting and characteristics. In some respects, the pragmatic choice to focus on epiphytic diatom communities for classification purposes even adds to this, e.g., because the historical perspective on their composition at high status is limited. Consequently, substantial reliance on 'expert judgement' can hardly be avoided. Fortunately, the factual basis for defining good status - a societaly more important and challenged concept - is considerably larger, although the use of epiphyton keeps presenting certain difficulties. For instance, it may be less evident to consider the likelihood of undesirable secondary effects in relation to general concepts on lake-ecosystem functioning, e.g., the transition to a turbid state, at this community level. Furthermore, assumed response characteristics of taxa to pressure proxies may not be valid for this specific habitat.

Secondly, the two most important types of monitoring considered by the WFD are discussed. It is argued that status and trend monitoring, on the one hand, and operational monitoring, on the other, differ by goals, frequency as well as scope. Consequently different and complementary assessment approaches are required.

In status and trend monitoring, overall ecological quality of an entire water body needs to be addressed as an Ecological Quality Ratio against the background of water-type-specific unimpacted conditions. In the method proposed for Flanders, this EQR is obtained from the relative abundance of selected impact-associated and impact-sensitive diatoms (Hendrickx & Denys 2005). The abundance of the former is assumed to remain below a certain treshold at good or high status, and increases progressively up to 100 % with decreasing quality, whereas the proportion of the latter allows to distinguish high from good status but is disregarded for the other classes (Figure 1). Matching these changes with an EQR scale divided into equal intervals provides a direct and transparent measure of community integrity.

For each water type, both groups of taxa are identified by comparing historical and recent epiphyton assemblages, evaluating the observed distribution of taxa in relation to pressure-related variables and scrutinizing available information. Only the more unambiguous indicators are retained (e.g., *Achnanthidium minutissimum* s.s. is considered to belong to the impact-sensitive group only for the

1 The conspicuous development of (cyano)bacterial biofilms is considered in the macrophyte assessment module.

most strongly mineralized, naturally eutrophic, shallow freshwaters). Although emphasis for non-acid waters is on nutrient-enrichment and associated effects, other forms of impairment are also taken into account where possible. Overall, some 620 taxa recorded in Flanders are considered as either associated or sensitive to impact at present. Values for the representation of these groups at the class boundaries good/moderate and high/good, respectively, are derived from a selection of water bodies with low total phosphorus or chlorophyll *a* and reference to diatom samples from the period 1852-1945. So far, boundaries for moderate and poor status are set by linear interpolation but a more rational approach is being considered. Spatial and temporal variation, but also the requirement to specify the statistical reliability of the classification, require that several samples are taken into account to classify a water body.

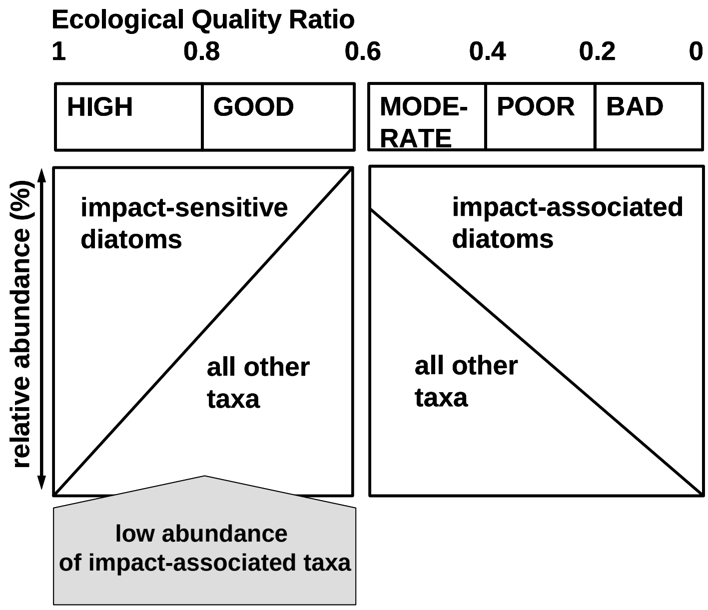

Figure 1. General principle proposed for the assessment of epiphytic diatoms in status and trend monitoring of lakes in Flanders.

A comparison of the classification results for a quality-biased sample of non-acid waters distributed throughout Flanders, mainly from single epiphyton collections, with the outcome of methods proposed for the Netherlands (van der Molen et al. 2004) and Germany (Schaumburg et al. 2004) points out that the former classifies a considerably larger proportion of the Flemish waters into high status and less sites as bad, while the latter generally sets higher standards for acceptable quality (Table 1). These differences probably relate mainly to the choice of indicators and averaging procedure used in the Dutch assessment and the emphasis on very low nutrient conditions in the German method and will need to be addressed in future intercalibration.

Operational monitoring focuses on the evolution of a particular pressure at sites deemed to be impaired or judged at risk. Only the most sensitive biological quality elements will be considered. If this includes the epiphytic community, its response to this pressure needs to be sufficiently specific and strong; interference by environmental background variation must be minimal. Good discrimination should extend from the moderate to the poor quality level. Compared to the EQR used for general screening, operational assessment methods are precision tools to document whether measures have the desired effect. As such, they are closer to the conventional approaches to infer water quality from diatoms in running waters. Robust transfer functions can measure community response to pressure-related variables reliably and are a possible way to interpret biotic trends. Two examples, obtained by

WA-PLS calibration of c. 190 taxa from 144 samples to total phosphorus and a compound PCA score for eutrophication, are presented. The moderate quality of these models implies that, in spite of the high-rated merits of diatoms to track eutrophication, only quite substantial trends in assemblage composition will actually allow conclusions. Clearly there is some scope for further development in this area also. It may be noted that the consequences of 'false positives' can be substantial in operational monitoring, i.e., continuation of non-effective management. Whereas other quality elements are likely to provide corrective information in trend and status assessment thanks to the 'one out, all out' principle, reliance to a single set of biological indicators in operational monitoring might be equally injudicious.

Summing up, the need for a sensible and differentiated, but always critical, approach to the assessment of diatoms in the WFD is stressed.

Table 1. Classification of 162 non-acid water bodies from lower Belgium according to different WFD-proposals for phytobenthos assessment (epiphytic diatoms only; subtotals for the proportion of sites with acceptable status between brackets).

% of water bodies	Flanders	Netherlands	Germany
high	1.9	25.9	4.3
good	48.1 (50.0)	21.6 (47.5)	9.3 (13.6)
moderate	24.1	32.1	30.9
poor	14.2	16.0	50.6*
bad	11.7	4.3	-
undetermined	-	-	4.9**

* no distinction of poor and bad status

** no indicative taxa for trophic classification present

REFERENCES

Hendrickx A. & L. Denys (2005) Toepassing van verschillende biologische beoordelingssystemen op Vlaamse potentiële interkalibratielocaties overeenkomstig de Europese Kaderrichtlijn Water – Partim "Fytobenthos". Rapport Instituut voor Natuurbehoud IN.R.2005.06, Brussel.

Schaumburg J., Schmedtje U., Schranz C., Köpf B., Schneider S., Stelzer D. & G. Hofmann (2004) Handlungsanweisung für die ökologische Bewertung von Seen zur Umsetzung der EU-Wasserrahmenrichtlinie: Makrophyten und Phytobenthos. Bayerisches Landesamt für Wasserwirtschaft, München.

van der Molen D.T., Altenburg W., Arts G., Baretta-Bekker J.G., van den Berg M.S., van den Broek T., Buskens R., Bijkerk R., Coops H.C., van Dam H., van Ee G., Franken R., Higler B., Ietswaart T., Jaarsma N., de Jong D.J., Joosten A.M. T., Klinge M., Knoben R.A.E., Kranenbarg J., Noordhuis R., Twisk F. & K. Wolfstein (2004). Referenties en maatlatten voor meren ten behoeve van de Kaderrichtlijn Water. STOWA, Utrecht.

Diatoms from Bulgarian rivers and their potential for monitoring water quality

Emiliya Kirilova[1] and Plamen Ivanov[2]

[1] Utrecht University, Department of Biology, Laboratory of Palaeobotany and Palynology, Budapestlaan 4, 3584 CD Utrecht, The Netherlands.
E.P.Kirilova@bio.uu.nl
[2] Sofia University "St. Kliment Ohridsky", Faculty of Biology, Department of Botany, 8 Dragan Tzankov blvd., 1164, Sofia, Bulgaria
plivanov@ns.biofac.uni-sofia.bg

INTRODUCTION

The diatom flora of Bulgarian rivers is poorly studied (Kawecka 1980a, b, 1981, Temniskova-Topalova & Misaleva 1982, Passy-Tolar et al. 1999, Ivanov et al. 2003a, b) and more studies are urgently needed for the river system in Bulgaria. On the basis of the distribution and ecology of diatoms in rivers and given their practical use as water quality indicators such studies should be carried out also for the purpose of a National Ecological Monitoring System as well as for the implementation of the European Water Framework Directive (EWFD). The composition of diatom communities should play a significant role in choosing monitoring reference sites.

The study of periphytic (epilithic, epiphytic, epipsammic and epipelic) diatoms from three of the longest Bulgarian rivers started in 1998 with the aim to determine the diatom flora from different substrata in these rivers and to describe the seasonal dynamics of the diatom assemblages. The evaluation of these data has led to an assessment of ecological status and water quality for these rivers. Consequently, a proposal was put forward to include diatom-based assessment of the ecological state of rivers in the National Ecological Monitoring System of Bulgaria (Ivanov et al. 2003a, b).

STUDY AREA

The studied rivers are located in the Eastern Balkan Ecoregion of the EWFD. They range from high altitude (>800 m a.s.l.) to mid-altitude (200 to 800 m a.s.l.) locations as defined by the EWFD (Fig. 1).
The Iskar River (368 km long) is a tributary of the Danube River. The study area includes a part of the river (60 km) that is located within the limits of the town of Sofia and its adjacent suburbs, at altitudes of between 700 and 500 m a.s.l. (Fig. 1).
The Strouma and Mesta Rivers are the longest south-western Bulgarian rivers. Sampling sites are located between 1000 and 200 m a.s.l.
Almost all catchments of the Iskar and Strouma Rivers flow trough urbanized regions, whereas the watercourse of the Mesta River is situated in a less populated mountain region.

MATERIALS AND METHODS

Epilithic diatoms have been collected seasonally from the Strouma (8 sampling points: S1-S8) and Mesta Rivers (9 sampling points: M1-M9) and epilithic, epiphytic, epipsammic and epipelic diatoms have been collected monthly from Iskar River (5 sampling points: I1-I5). Samples were collected according to the Rapid Biomonitiring Protocols of the U.S. Environmental Protection Agency (http://www.epa.gov/). Laboratory processing of the samples was carried out according to Hasle &

Fryxell (1970). The taxonomic identification of the diatoms follows mainly Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot (2001) and in some cases Round et al. (1990). The relative abundance of taxa in each sample was determined by counting at least 500 valves in each slide. A total of 400 samples of periphytic diatoms were collected. The identified species were classified according to their sensitivity to organic pollution due to calculation of the Trophic Diatom Index (TDI) and the same index was used to assess the water quality (Kelly & Whitton 1995, Kelly 1998).

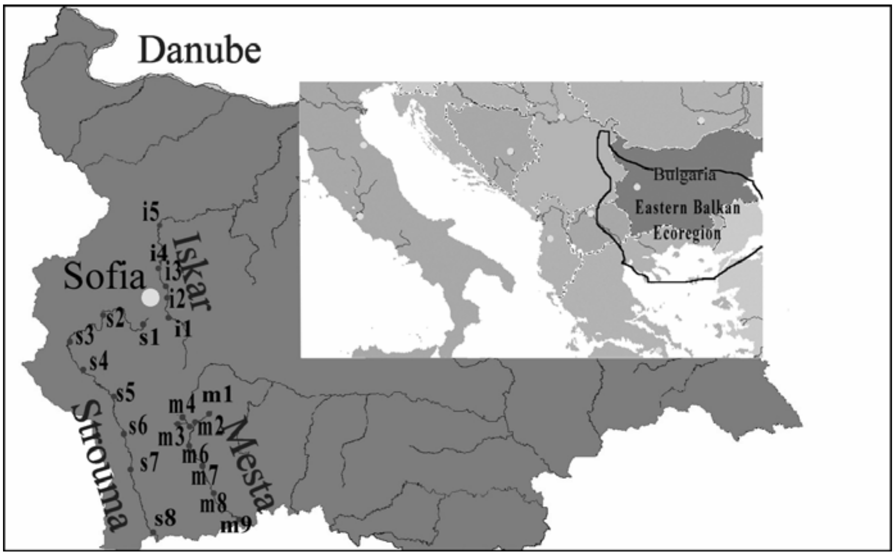

Fig.1. Study area and sample points along the three Bulgarian rivers

RESULTS

A relatively rich diatom flora was identified in the 245 studied samples that included 332 taxa, many of which were found for the first time in Bulgarian rivers. Navicula s. l. spp., Nitzschia spp., Gomphonema spp., Fragilaria s. l. spp., and Cymbella s. l. spp. were the most species-rich genera.

Deterioration of water quality and ecological status of the rivers was reflected in the TDI and the percentage of pollution tolerant taxa (or PTV (pollution tolerant valves) according to Kelly 1998) (Figs 2 and 3). Two ‘clean sites’ were found along the Mesta River sampling sites (M1 and M3) with TDI values of 2.8 and 22.83, and PTV values of 0.69 and 0.28. Strouma River has one ‘clean’ site (S1) with a TDI of 34 and a PTV of 2.38. The Iskar River region has no ‘clean’ sites. The ‘clean’ sites were dominated by: Achnanthidium minutissimum (Kützing) Czarnecki, A. pyrenaicum (Hustedt) Kobayasi, Cocconeis placentula Ehrenberg, C. placentula var. euglypta (Ehrenberg) Grunow, C. placentula var. lineata (Ehrenberg) Van Heurck, Hannaea arcus (Ehrenberg) Patrick, Diatoma ehrenbergii Kutzing, Cymbella excisa var. excisa Kützing, Encyonema minutum (Hilse) D. Mann, Reimeria sinuata (Gregory) Kociolek et Stoermer, Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot., and G. tergestinum Fricke. The PTV values of the Strouma River increased from 2.38 at S1 to 78.20 at S8, and for the Mesta River from 0.69 at M1 to 69.06 at M9. The most polluted parts of the rivers are dominated by Nitzschia palea (Kützing) W. Smith, Ulnaria ulna (Nitzsch) Compère, and Gomphonema parvulum (Kützing) Kützing.

DISCUSSION

Almost all the main watercourses of the Strouma and Mesta Rivers are subject to strong anthropogenical pollution. The “clean” sites are located above the first settlements in the valleys of these rivers. In its studied part, the Iskar River is moderately to very highly pollute following its passage through the city of Sofia, but particularly in the vicinity of the city.

For Strouma and Mesta Rivers all suitable referent points are located at high altitudes (>800 m, S1, M1, M3), whereas there are no mid-altitude type (200 to 800 m) reference sites for Strouma, Mesta and Iskar Rivers among the studied sites. However, more comparable studies are needed, especially from the smaller tributaries of the rivers in order to find suitable reference points.

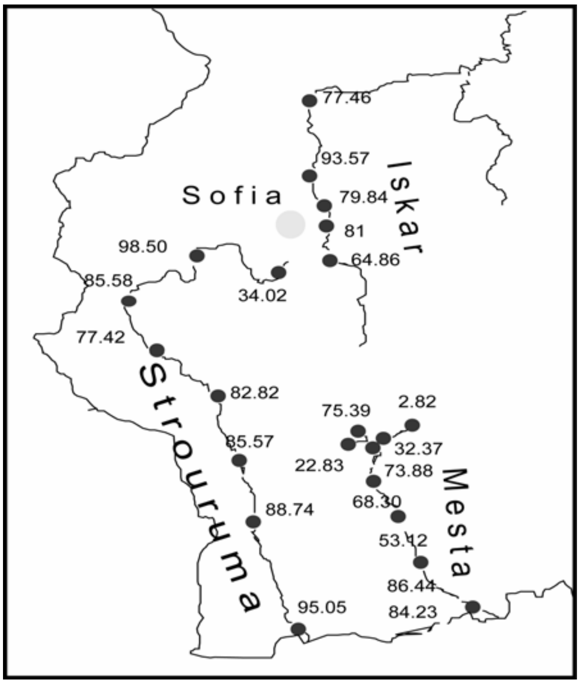

Fig. 2. Values of the Trophic Diatom Index (TDI) for the Iskar, Strouma and Mesta Rivers. TDI values vary from 0 (no organic concentration) to 100 (very high organic concentration).

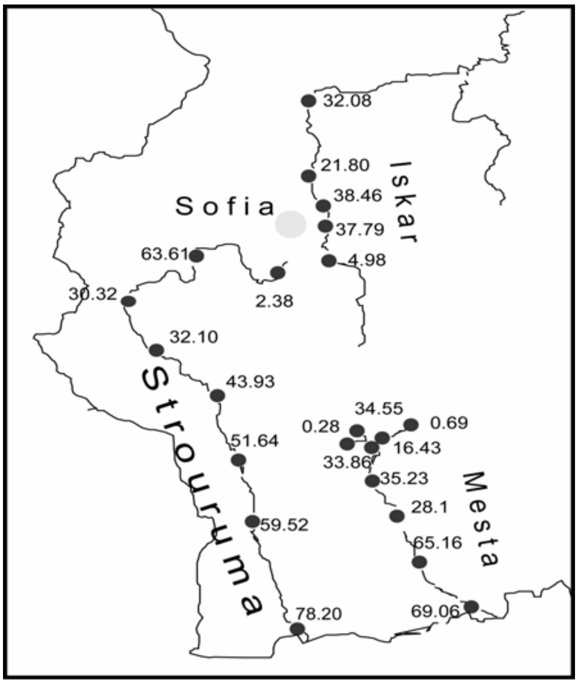

Fig. 3. Percentage of the Pollution Tolerant Taxa (PTV) for the Iskar, Strouma and Mesta Rivers. PTV values vary from 0% (no organic pollution) to 100% (strong organic pollution).

ACKNOWLEDGEMENTS

We are grateful to Dobrina Temniskova, André Lotter and Holger Cremer for valuable comments on the manuscript, and to Yasen Mutafchiev for the assistance during fieldwork.

REFERENCES

Ivanov, P., Chipev, N. and Temniskova, D. (2003a). Diatoms of the river Iskar (Sofia Plain) and their implication for water quality assessment, Part 1. The diatom flora, ecology and community structure. Journal of Environmental Protection and Ecology, 2: 288-300.

Ivanov, P., Chipev, N. & Temniskova, D. (2003b). Diatoms of the river Iskar (Sofia Plain) and their implication for water quality assessment, Part 2. Diatom indices and their implication for water quality monitoring. Journal of Environmental Protection and Ecology, 2: 301-310.

Kawecka, B. (1980a). The ecological characteristics of diatom communities in the mountain streams of Europe. In: R. Ross (ed.) Proceedings of the Sixth Symposium of Recent and Fossil Diatoms. Koeltz Scientific Publishers, Koenigstein, p. 425-434.

Kawecka, B. (1980b). Sessile algae in European mountain streams. 1. The ecological characteristics of communities. Acta Hydrobiologica, 22: 361-420

Kawecka, B. (1981). Sessile algae in European mountain streams. 2. Taxonomy and autecology. Acta Hydrobiologica, 23: 17-46.

Kelly, M. G. (1998). Use of the Trophic Diatom Index to monitor eutrophication in the rivers. Water Research, 32: 236-242.

Kelly, M. G. and Whitton B. A. (1995). The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. Journal of Apply Phycology, 7: 433-444.

Krammer, K. and Lange-Bertalot, H. (1986). Bacillariophyceae 1. Teil: Naviculaceae. *In*: Ettl, G., J. Gerloff, H. Heynig, D. Mollenhauer (eds.): Süßwasserflora von Mitteleuropa. Band 2/1. Gustav Fischer Verlag, Heidelberg. 876 pp.

Krammer, K. and Lange-Bertalot, H. (1988). Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl., G., J. Gerloff, H. Heynig, D. Mollenhauer (eds.): Süßwasserflora von Mitteleuropa. Band 2/2. Gustav Fischer Verlag, Heidelberg. 611 pp.

Krammer, K. and Lange-Bertalot, H. (1991). Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In: Ettl., G., J. Gerloff, H. Heynig, D. Mollenhauer (eds.): Süßwasserflora von Mitteleuropa. Band 2/3. Gustav Fischer Verlag, Heidelberg. 599 pp.

Krammer, K. and Lange-Bertalot, H. (2004): Bacillariophyceae 4. Teil: Achnanthaceae. Kritische Ergänzungen zu *Achnanthes* s.l., *Navicula* s.str., *Gomphonema*. - In: Ettl., H., G. Gärtner, H. Heynig, D. Mollenhauer (eds.): Süßwasserflora von Mitteleuropa. Band 2/4. Gustav Fischer Verlag, Heidelberg. 468 pp.

Lange-Bertalot, H. (2001). Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Volume 2. *Navicula* sensu stricto. 10 Genera separated from *Navicula* sensu lato. *Frustulia*. A.R.G. Gantner Verlag K.G. 526 pp.

Passy-Tolar, S., Pan, Y and Lowe, R. L. (1999).Ecology of the major periphytic diatom communities from the Mesta River, Bulgaria. International Review of Hydrobiology, 84: 129-174.

Round F., Crawford, R. and Mann, D. (1992). The diatoms. Biology & Morphology of the Genera. - Cambridge University Press, 745pp.

Temniskova-Topalova, D. and Misaleva, N. (1982). Diatoms from the Bulgarian part of Mt. Odrazhden. Phytoecology, 20: 50-63.

A Stress Surveillance System Based on Calcium and Nitric Oxide in Marine Diatoms

Assaf Vardi[1,2], Fabio Formiggini[2,3], Raffaella Casotti[4], Alessandra De Martino[1,2], François Ribalet[4], Antonio Miralto[4], Chris Bowler[1,2*]

1 Laboratory of Diatom Signalling and Morphogenesis, Ecole Normale Supérieure, Paris, France, 2 Laboratory of Cell Signalling, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy, 3 Section of Molecular Cytology & Centre for Advanced Microscopy, University of Amsterdam, Kruislaan, Amsterdam, Netherlands, 4 Laboratory of Ecophysiology, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy

Diatoms are an important group of eukaryotic phytoplankton, responsible for about 20% of global primary productivity. Study of the functional role of chemical signaling within phytoplankton assemblages is still in its infancy although recent reports in diatoms suggest the existence of chemical-based defense strategies. Here, we demonstrate how the accurate perception of diatom-derived reactive aldehydes can determine cell fate in diatoms. In particular, the aldehyde (2*E*,4*E/Z*)-decadienal (DD) can trigger intracellular calcium transients and the generation of nitric oxide (NO) by a calcium-dependent NO synthase-like activity, which results in cell death. However, pretreatment of cells with sublethal doses of aldehyde can induce resistance to subsequent lethal doses, which is reflected in an altered calcium signature and kinetics of NO production. We also present evidence for a DD–derived NO-based intercellular signaling system for the perception of stressed bystander cells. Based on these findings, we propose the existence of a sophisticated stress surveillance system in diatoms, which has important implications for understanding the cellular mechanisms responsible for acclimation versus death during phytoplankton bloom successions.

Citation: Vardi A, Formiggini F, Casotti R, de Martino A, Ribalet F, et al. (2006) A stress surveillance system based on calcium and nitric oxide in marine diatoms. PLoS Biol 4(3): e60.

Introduction

Diatoms are major components of phytoplankton blooms in aquatic ecosystems and are central in the biogeochemical cycling of important nutrients such as carbon, nitrogen, and silicon [1,2]. Unraveling the factors that regulate the fate of blooms is therefore of great importance. During a bloom succession, phytoplankton are thought to utilize chemical signals to enhance their defense capacities against grazers [3] and pathogens [4,5], and for outcompeting other phytoplankton for available resources [6,7]. The evolutionary and ecological success of diatoms in the contemporary oceans might suggest that they utilize sophisticated mechanisms to monitor and adapt appropriately to changing environmental conditions [8]. Indeed, previous reports have implicated the role of a chemical defense based on diatom-derived aldehyde products of fatty-acid oxidation [9,10], which impair the normal development of grazers such as copepods and other invertebrates [11,12]. Furthermore, it has now emerged that these same aldehydes are toxic to the diatoms themselves and can trigger a process bearing the hallmarks of programmed cell death [13]. We therefore explored the hypothesis that they may function as infochemicals in the marine environment, and so we investigated how diatoms perceive and respond to diatom-derived antiproliferative aldehydes such as (2*E*,4*E/Z*)-decadienal (DD). DD was chosen as a model aldehyde because its reactive properties are currently being tested on various animal, plant, and unicellular systems [14–16].

Results/Discussion

One of the early responses of plants and algae to pathogens and allelochemicals is thought to be the generation of reactive oxygen species (ROS) [7,17,18]. Our results indicated that DD did not stimulate detectable increases in general ROS production (assayed by dihydrorhodamine 123; data not shown), but rather induced the generation of nitric oxide (NO). NO exerts crucial physiological and developmental functions in both animals and plants, and is also involved in defense responses [19–21]. We monitored NO generation in two representative diatom species, *Thalassiosira weissflogii*, representing a cosmopolitan diatom genus, and *Phaeodactylum tricornutum*, which has become a central model for molecular and cellular studies of diatom biology [22,23]. Endogenous NO generation was measured by flow cytometry, fluorometry, and subcellular real-time imaging using the NO-sensitive dye 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM) [24]. Microscopic analysis of *T. weissflogii* cells revealed that NO began to accumulate within 5 min after

Academic Editor: Jeffrey Dangl, University of North Carolina, United States of America

Received August 8, 2005; **Accepted** December 27, 2005; **Published** February 21, 2006

DOI: 10.1371/journal.pbio.0040060

Abbreviations: DAF-FM, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate; DAPI, 4′,6-diamidino-2-phenylindole; DD, (2*E*,4*E/Z*)-decadienal; DEANO, diethylamine nitric oxide; NMMA, *N*G-monomethyl-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; ROS, reactive oxygen species, SNP, sodium nitroprusside

* To whom correspondence should be addressed. E-mail: cbowler@biologie.ens.fr

These authors contributed equally to this work.

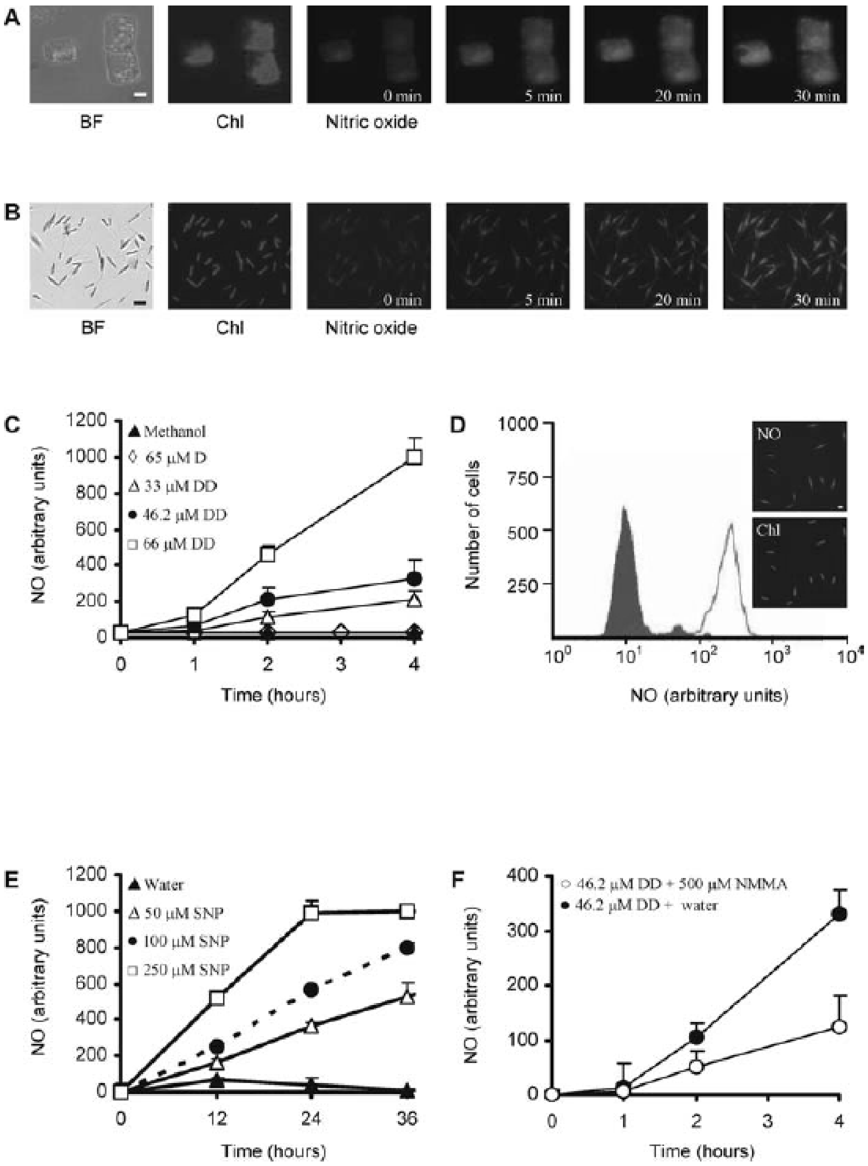

Figure 1. DD Induces NO Generation in Diatoms

Micrographs depicting NO generation over time in response to DD (66 μM [10 μg/ml]) in *T. weissflogii* (A) and *P. tricornutum* (B). (C) Monitoring of NO production in *P. tricornutum* in response to a range of DD concentrations; (D) Cytogram showing NO generation 15 min after addition of DEANO (2 mM) to *P. tricornutum* cells (filled violet indicates the KOH control; open green indicates DEANO). Insets show epifluorescence micrographs of the DEANO-treated cells. (E and F) Relative accumulation of NO in *P. tricornutum* cells following treatment with SNP (E) or NMMA prior to exposure to DD (F).

In all experiments, NO generation was assayed using the fluorescent probe DAF-FM. Data in (C), (E) and (F) are means plus standard deviation from four experiments. Representative data from at least four experiments are shown in (A), (B), and (D). Experiments shown in (C), (D), and (F) were performed by flow cytometry, and in (E) using a fluorescence microplate reader.

BF, bright field; Chl, chlorophyll-derived red autofluorescence; D, (2*E*)-decenal. Scale bars represent 5 μm.

DOI: 10.1371/journal.pbio.0040060.g001

exposure to DD and increased significantly thereafter (Figure 1A). Furthermore, the DAF-FM fluorescence was localized close to the nucleus and was excluded from the plastid. A similar response was observed in *P. tricornutum* cells, in which the NO burst was also detected 5 min after exposure to DD (Figure 1B). In these short-term experiments, production of NO was proportional to DD concentration (between 33–66 μM [5–10 μg/ml]) with respect to the percentage of DAF-FM–positive cells, the extent of DAF-FM staining, and the lag time until significant numbers of cells emitted green fluorescence (Figure 1C). Treatments with methanol (1%), acetaldehyde (247 μM [10 μg/ml]), and other C10-unsaturated aldehydes such as (2*E*)-decenal (65 μM [10 μg/ml] failed to induce NO production (Figure 1C).

We used two NO donors, diethylamine nitric oxide (DEANO) and sodium nitroprusside (SNP), as positive controls to verify the reliability of DAF-FM as a probe for NO detection in *P. tricornutum* cells (Figure 1D and 1E). To further demonstrate DD-dependent NO production, we treated *P. tricornutum* cells with the NO synthase (NOS) antagonist *NG*-monomethyl-L-arginine (NMMA) prior to addition of DD (Figure 1F). This inhibitor reduced significantly the production of NO, implicating the possible involvement of NOS-like activities in NO generation (see below).

A recent study in *T. weissflogii* showed that DD causes cell cycle arrest and induction of cell death, which was accompanied by morphological hallmarks of apoptosis [13]. Similarly, treatment of *P. tricornutum* cells with DD for 4 h led to cell death in more than 90% of the population, as evidenced by assaying plasma membrane integrity with the fluorescent dye Sytox Green, which is commonly used to detect dead cells [13,25] (Figure 2A). We further analyzed the kinetics of diatom cell death in response to a range of DD concentrations using flow cytometry (Figure 2B). DD was found to induce cell death in a dose- and time-dependent manner, and increased dramatically above a distinct threshold below which, although cell division was arrested, no cell death occurred. In these short-term experiments using cell densities of 2×10^5 cells/ml, the threshold concentration of aldehyde required to induce cell death was around 19.8 μM (3 μg/ml). Treatments with methanol (1%), acetaldehyde (247 μM [10 μg/ml]) and (2*E*)-decenal (65 μM [10 μg/ml]) failed to induce significant cell death (Figure 2B).

To further examine the role of NO in determining diatom cell fate we examined cell death in response to an NO donor in the absence of DD, and treated cells with a NOS inhibitor prior to exposure to DD (Figure 2C and 2D). Treatment with the NO donor SNP led to an increase in the number of Sytox-positive cells, which coincided proportionally with NO accumulation (Figures 2C and 1E), in agreement with the threshold nature of the response to a range of DD concentrations (Figure 1C). Conversely, the NOS inhibitor NMMA could reduce DD-dependent cell death (Figure 2D). These data implicate the involvement of NO in the cell death cascade.

To investigate the intracellular origin of NO, we double stained *P. tricornutum* cells with DAF-FM and 4′,6-diamidino-2-phenylindole (DAPI) to label the nucleus (Figure 3A). Analysis of the images acquired by fluorescence microscopy showed that DAF-FM–derived fluorescence localized in neither the chloroplasts nor the nucleus, although it was closely associated with the latter. This could suggest that NO accumulates within a specific subcellular compartment, although one should caution that this observation could be a consequence of dye localization (DAF-FM fluorescence is nonetheless pH insensitive [24]). To further decipher the source of NO in diatoms, we assayed diatom extracts for NOS enzymatic activity using a conventional citrulline/arginine assay [26]. Basal NOS activity was 4 pmol • min^{-1} • mg^{-1} and increased significantly around 2.5-fold within the first 15 min after exposure to DD (Figure 3B). Analysis of the whole genome sequence of the diatom *Thalassiosira pseudonana* [27] (http://genome.jgi-psf.org/thaps1/thaps1.home.html), as well as the draft genome sequence of *P. tricornutum*, revealed several candidate genes with homology to genes encoding NO-generating enzymes from bacteria and plants [26,28–30], of

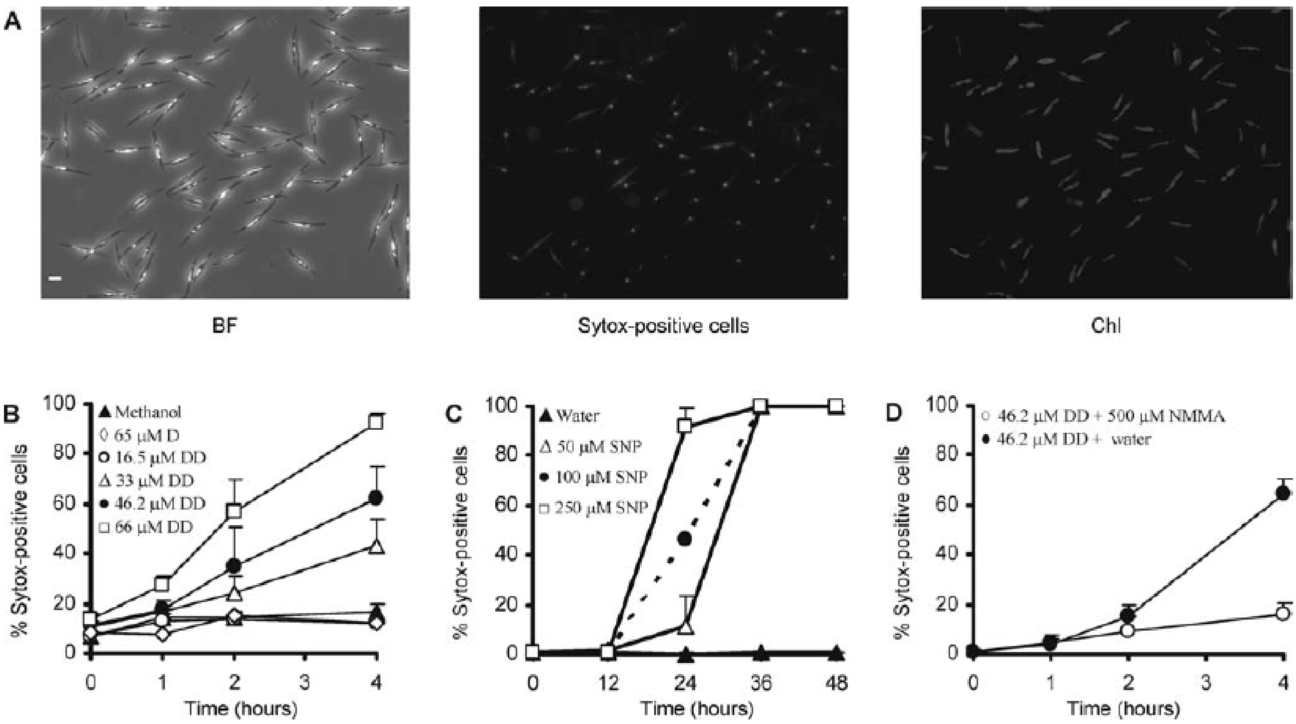

Figure 2. DD-Dependent NO Production Induces Cell Death

(A) Micrographs of *P. tricornutum* cells treated with DD (66 μM [10 μg/ml]) for 4 h, which resulted in 90% cell death (assayed by Sytox Green fluorescence). Chlorophyll autofluorescence (shown in red) was significantly reduced in Sytox-positive cells, giving a further indication of cell death. (B–D) Quantification of cell death kinetics induced by DD or (*2E*)-decenal (B), SNP (C), and NMMA added prior to DD application (D). Data in (B–D) are means plus standard deviation from four experiments. Representative data from four experiments are shown in (A). Experiments shown in (B–D) were performed by flow cytometry. Abbreviations are as in Figure 1. Scale bar represents 5 μm.

DOI: 10.1371/journal.pbio.0040060.g002

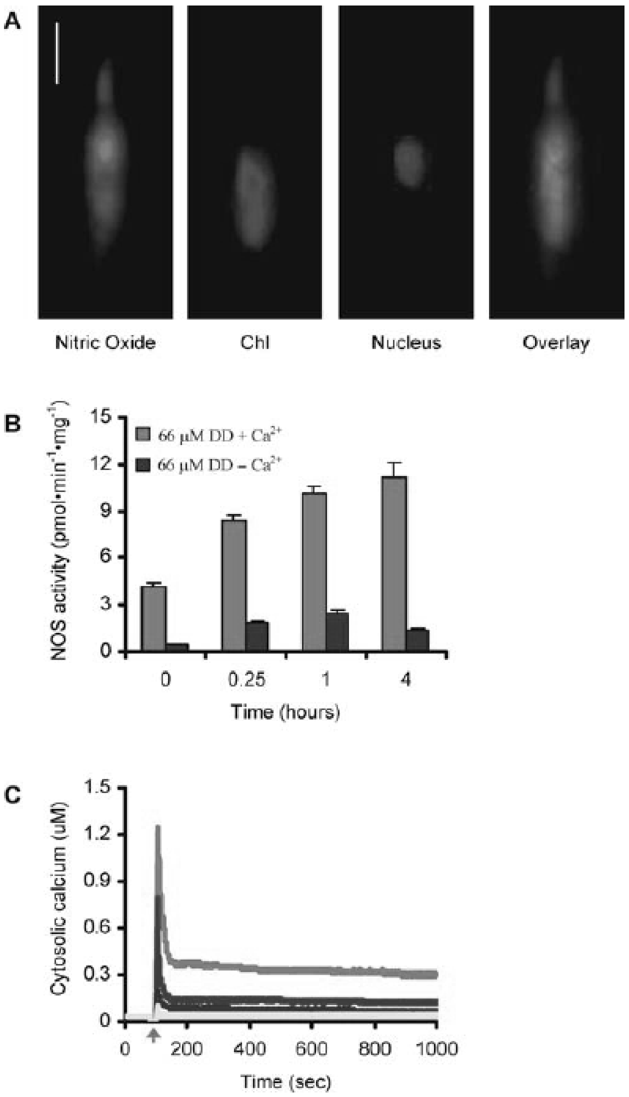

Figure 3. The Origin of NO in *P. tricornutum* and Its Interplay with Calcium

(A) Intracellular localization of DAF-FM-derived fluorescence (green) compared with DAPI-staining (blue) and chlorophyll (Chl) autofluorescence (red) in *P. tricornutum*.
(B) NOS enzymatic activity in cell-free extracts induced by DD (66 μM [10 μg/ml]), in the presence or absence of calcium.
(C) Ca^{2+} transients in response to addition of 1, 3, and 5 μg/ml (6.6, 19.8, and 33 μM) DD, depicted in blue, pink, and green respectively and of 10 μg/ml (65 μM) (2*E*)-decenal (yellow) in transgenic *P. tricornutum* cells expressing the calcium-sensitive photoprotein Aequorin. Addition is indicated by arrow. Data in (B) are means plus standard deviation from four experiments. Representative data from at least four experiments are shown in (A) and (C). Scale bar represents 5 μm.
DOI: 10.1371/journal.pbio.0040060.g003

which the diatom ortholog of the plant enzyme AtNOS1 (present in both diatom genomes) appeared to be the most likely candidate, based on overall similarity (data not shown). Indeed, diatom extracts exhibited NOS activity that was similar to the plant NOS enzyme [31] in that activity was strongly calcium dependent (Figure 3B).

Calcium is known to be an important second messenger for a wide variety of environmental stimuli in both plant and animal cells [32,33]. Previous studies have revealed that *P. tricornutum* displays sophisticated sensing systems for perceiving abiotic environmental signals that involve calcium-dependent signal transduction mechanisms [34]. We used transgenic *P. tricornutum* cells expressing the calcium-sensitive photoprotein Aequorin to detect transient changes in cytosolic calcium in response to reactive aldehydes. Application of DD stimulated a dramatic increase in intracellular calcium that persisted for several minutes before returning to basal levels, whereas its monounsaturated form, (2*E*)-decenal, or methanol, its solvent, did not provoke any substantial response (Figure 3C). As seen both for DD-dependent NO production and cell death, DD triggered Ca^{2+} release with maximal amplitude proportional to the applied dose (Figure 3C). In an attempt to identify the source of the cytosolic calcium increase, we exposed *P. tricornutum* cells to the impermeant form of BAPTA (1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, tetrapotassium salt), a known highly selective calcium chelating reagent, prior to addition of DD. This chelator had no effect on the DD-dependent calcium transient, but suppressed the cellular response to hypo-osmotic shock (Figure S1). These data suggest that internal calcium stores are responsible for the Ca2+ release in response to DD, contrasting with the external origin of cytosolic calcium induced in response to the abiotic stress.

To our knowledge this is the first time that NO has been detected in marine phytoplankton, although it has been detected in sea water and was suggested to originate from abiotic nitrite photolysis and from bacterial denitrification/nitrification cycles [35,36]. Neither short- nor long-term exposures to NO donors (DEANO and SNP) led to any detectable increases in cytosolic Ca^{2+} (data not shown), suggesting that NO acts downstream of Ca^{2+} in the signaling cascade, in agreement with the earlier response of calcium compared with NO following addition of DD, and the Ca^{2+} dependency of NOS activity (Figures 1B, 3B, and 3C). Furthermore, several control compounds (see above) failed to induce either calcium transients or NO production, and did not induce cell death. Conversely, other pharmacological agents that amplified the calcium response (e.g., nifedipine) also amplified changes in NO and increased cell death (data not shown), implying a causal link between calcium and NO in the induction of cell death. Our results therefore suggest a signaling pathway in which DD-induced cell death in diatoms is preceded by accurate perception of the aldehyde, followed by changes in intracellular calcium that may activate a plant-type NOS to subsequently generate NO.

Real-time imaging of NO generation in *P. tricornutum* cells treated with DD revealed that after 30 min, intracellular levels of NO were at least 10-fold higher in reacting cells with respect to basal levels (Figure 4A). Furthermore, some of the cells displayed higher sensitivity and responded to DD earlier than in adjacent cells (see Video S1). Neighboring cells in the proximity of these early-responding cells exhibited significantly delayed responses (Figure 4B), suggesting the generation of a diffusible NO-inducing signal from reacting cells. These observations suggested a DD-derived intercellular communication system that could propagate within the diatom population.

In order to examine this intercellular signaling phenomenon further, we designed an experiment in which cells were exposed to a range of DD concentrations (660 nM–13.2 μM [0.1–2.0 μg/ml]) for 24 h (population A) and then were mixed

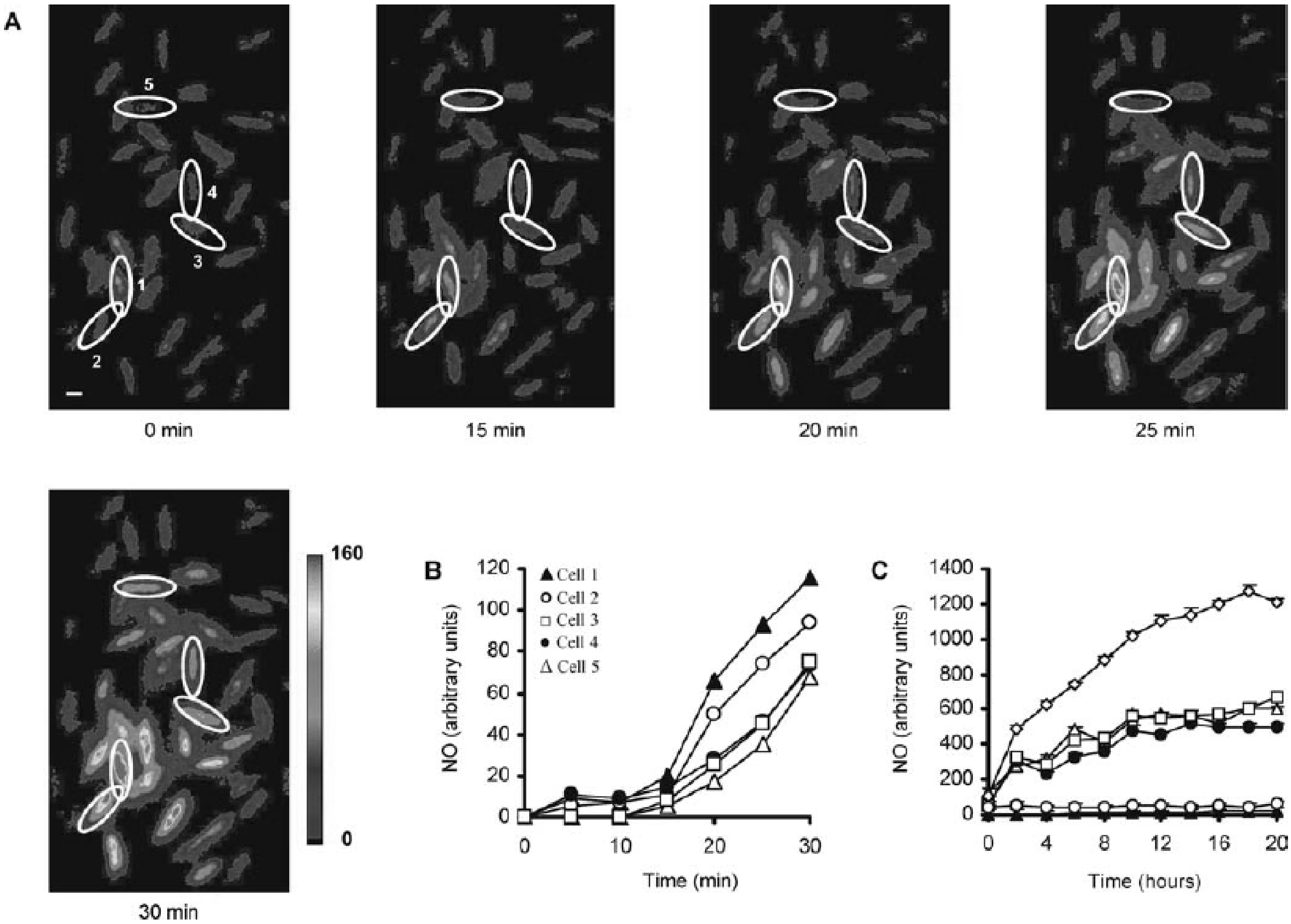

Figure 4. Intercellular Transmission of a DD-Derived Signal that Induces NO in Neighboring Cells

(A and B) In vivo imaging of DD-induced NO burst in *P. tricornutum* cells.

(A) Time course of NO production in single cells based on corresponding real-time movie (see Video S1).

(B) Relative accumulation of NO in selected cells of the micrographs shown in (A).

(C) NO accumulation in an untreated population of DAF-FM-loaded cells that were inoculated with a non DAF-FM–loaded population that had been exposed to DD at different concentrations (open circle, methanol solvent; open square, 660 nM; filled circle, 3.3 μM; open triangle 6.6 μM, and open diamond, 13.2 μM) for 24 h prior to the mixing. Incubation of fresh medium with 13.2 μM DD for 24 h (filled triangle) prior to addition to DAF-FM-loaded cells did not provoke any detectable increase in NO. Data in (C) are means plus standard deviation from four experiments.

DOI: 10.1371/journal.pbio.0040060.g004

with DAF-FM–preloaded cells that were not exposed directly to DD (population B). Monitoring DAF-FM fluorescence in the untreated population B revealed that NO accumulated over the next 24 h (Figure 4C). Interestingly, the response correlated with the level of DD pretreatment of population A and was already detectable in response to cells treated with very low sublethal concentrations (e.g., 660 nM [0.1 μg/ml]). Furthermore, the pattern of NO production resembled a threshold response: Population A cells treated with DD concentrations between 660 nM and 6.6 μM (0.1–1.0 μg/ml) provoked equivalent NO production profiles in population B cells, whereas treatment with 13.2 μM (2.0 μg/ml) DD generated much higher changes in NO (Figure 4C). Such responses are in agreement with the EC50 value for *P. tricornutum* cell growth of 7.06 μM ± 2.44 μM (1.07 ± 0.37 μg/ml) calculated by Probit analysis for initial cell densities of 10^5 cells/ml after exposure to DD for 24 h (data not shown). These data suggested that diatom cells could detect the level of stressed cells within the population by sensing a DD-derived diffusible signal (or signals) transmitted by wounded cells to neighboring healthy cells. To exclude the possibility that the observed response was simply due to the residual presence of DD in the medium, fresh cell free medium was incubated with the same DD concentrations for 24 h prior to addition to population B cells. Such treatments failed to induce any detectable increase in NO (Figure 4C), confirming that the aldehyde was likely to be degraded over the 24-h period [37].

A recent report [11] has demonstrated arrested larval development in copepods fed on dinoflagellates that were treated with DD concentrations in the range of 9.9 μM (1.5 μg/ml). In these experiments it was proposed that the dinoflagellates acted as DD carriers by absorption of the molecule to the cell surface. However, the amount of DD absorbed by the dinoflagellate carrier cells ranged from only 0.4 fg $cell^{-1}$ to 36 fg $cell^{-1}$, much lower than the total aldehyde concentration initially inoculated in the culture medium. Considering this finding and considering that even lower

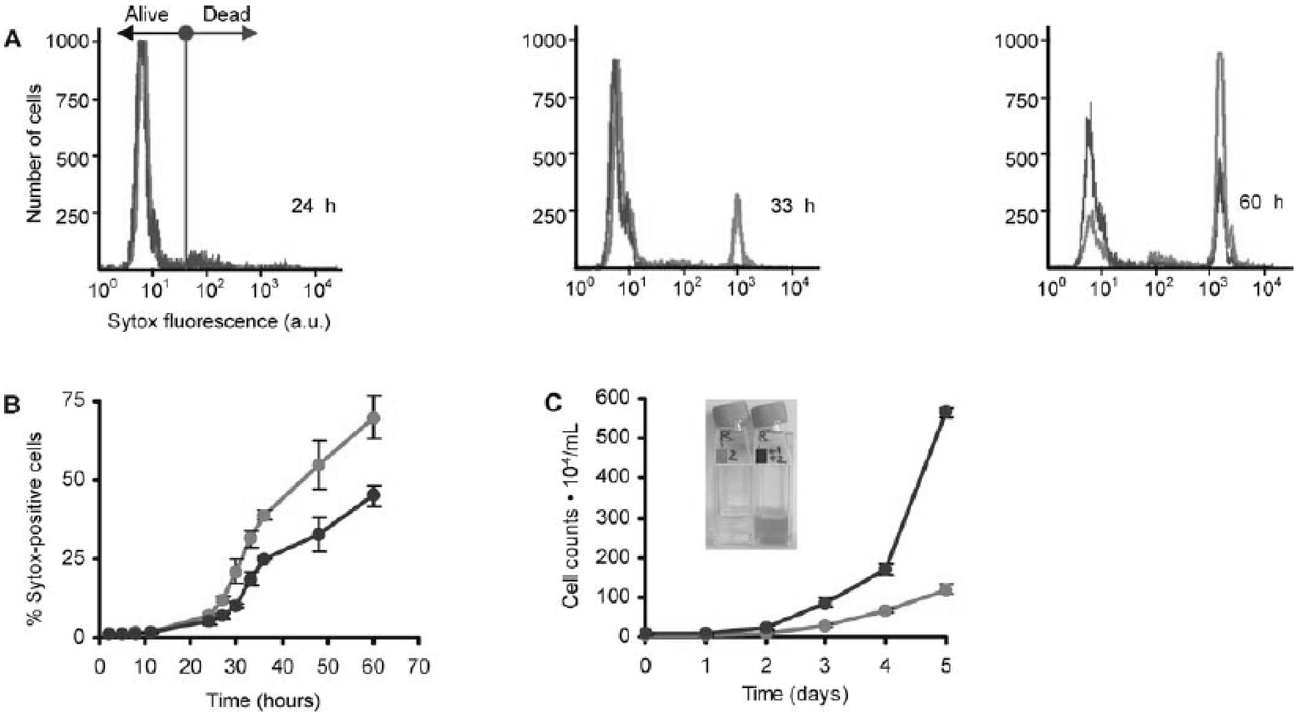

Figure 5. Sublethal DD Concentrations Can Induce Resistance to Lethal Doses in *P. tricornutum*

Cells were pretreated with 0.1 μg/ml (660 nM) DD for 2 h prior to subsequent addition of 2 μg/ml (13.2 μM) DD (blue) and compared to a single dose treatment of 2 μg/ml (13.2 μM) DD (green).

(A and B) Cell death was assayed by flow cytometry both qualitatively in cytograms (A) and quantitatively (B) using Sytox Green at the indicated time points.

(C) Cell growth curves following resuspension of pretreated and non-pretreated cells in DD-free fresh medium 60 h after the 2 μg/ml (13.2 μM) DD treatment. The time scale indicates the days following resuspension. Inset shows photograph of the two cultures taken 2 wk after resuspension starting from an initial inoculum of 5×10^4 cells/ml.

Representative data from at least five experiments are shown in (A–C).

a.u., arbitrary units.

DOI: 10.1371/journal.pbio.0040060.g005

concentrations of DD (660 nM [0.1 μg/ml]) led to responses in our untreated population B cells, we propose that unsaturated aldehydes such as DD could play an important role in diatom assemblages during blooms, in which local densities can reach as high as 5×10^5 cells/ml [38]. Benthic diatoms living in dense microbial mats and biofilms are also known to produce fatty acid derivatives [39]. Such microenvironments, consisting of high cell densities within polysaccharide matrices, are likely to further facilitate the potential for cell–cell communication and to further enhance defenses against grazers [40].

Based on our observations presented in Figure 4, we further explored the signaling role of unsaturated aldehydes in diatom populations. We aimed to mimic conditions in which diatoms could be exposed to different doses of DD on successive occasions, as may be the case in natural environments. We performed long-term experiments in which cells were exposed to sublethal doses of DD (660 nM [0.1 μg/ml]) for 2 h prior to subsequent addition of a higher dose (13.2 μM [2 μg/ml]), and compared the responses to a single dose only. The two populations displayed remarkable differences. Substantial cell death was already visible after 33 h in the non-pretreated culture, whereas cell death in the pretreated culture was significantly delayed (Figure 5A). After 60 hr, cell death in the preconditioned population was around 40%, whereas more than 70% of cells in the non-preconditioned population were positively stained with the cell death indicator Sytox Green (Figure 5B). Furthermore, resuspension of the same cultures in DD-free fresh medium revealed a notable difference in growth rates, with a 6-fold increase in cell density in the acclimated population compared with cultures from non-acclimated cells after 5 d (Figure 5C). Interestingly the pretreated culture could fully recover, whereas the non-acclimated culture failed to be viable and ultimately collapsed (Figure 5C, inset). These data demonstrate the potential of DD as an infochemical for regulating cell fate in diatom populations at doses one order of magnitude lower than used in previous reports [10,11,13]. Specifically, it appeared that pretreatment with sublethal doses of DD could stimulate resistance to normally lethal concentrations.

To examine the molecular mechanism responsible for these contrasting responses (induced resistance versus death), we examined the role of calcium and nitric oxide. Preconditioned *P. tricornutum* cells treated with 660 nM (0.1 μg/ml) DD for 2 h, which did not provoke any changes in intracellular calcium (data not shown), were dramatically sensitized to a successive administration of 13.2 μM (2.0 μg/ml) DD (Figure 6A). The initial peak in cytosolic calcium was increased almost 2-fold compared with cells that were not preconditioned, and a second more-sustained peak was

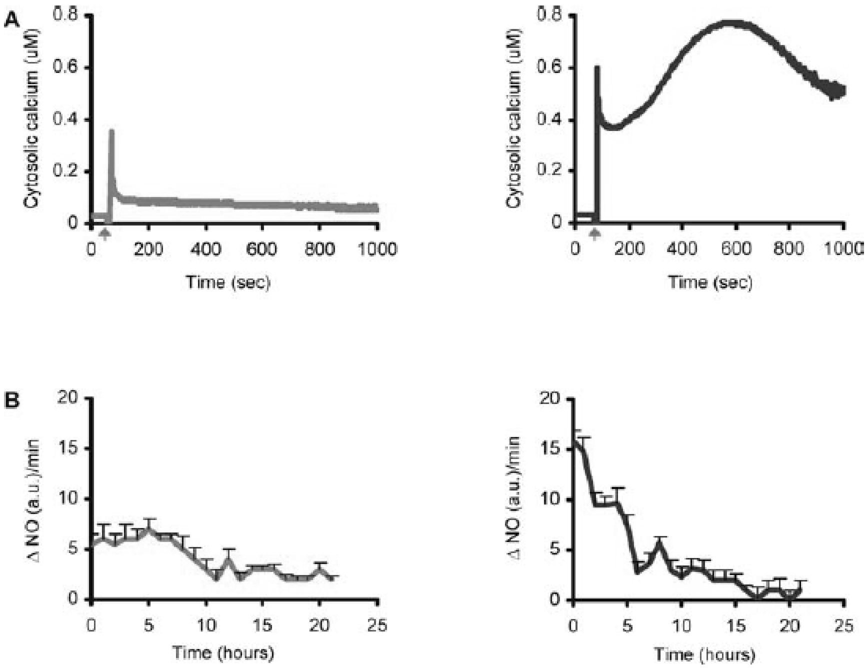

Figure 6. The Role of NO and Calcium in Acclimation to Sublethal Doses of DD

(A) Cytosolic calcium transients in transgenic *P. tricornutum* cells expressing Aequorin. Left panel shows calcium response in non-pretreated cells (green) following addition of 13.2 μM (2 μg/ml) DD (indicated by arrow), whereas right panel shows the calcium signature of cells that had been pretreated with 660 nM (0.1 μg/ml) DD for 2 h prior to addition of 13.2 μM (2 μg/ml) DD (blue).

(B) Relative rate of NO production in pretreated (blue) and non-pretreated (green) cells. Experimental conditions as in (A).

Representative data from five experiments is shown in (A). Data in (B) are means plus standard deviation from five experiments.

a.u., arbitrary units.

DOI: 10.1371/journal.pbio.0040060.g006

apparent that was not seen in non-pretreated cells. Interestingly, this modulation in the calcium signature correlated with a 3-fold increased rate of NO production in the acclimated population during the first 2 h after exposure to the second treatment (Figure 6B). Only after 5 h did the rate of production return to steady-state levels that were similar in the two populations. Such a pronounced increase in the preconditioned population could be due either to a higher production rate of NO per cell or to a higher number of reacting cells, as also observed in the real-time studies of NO propagation within a DD-treated population (see Video S1). In either scenario this result implies that NO is associated not only with cell death but also with induced resistance. The results in Figure 4C confirm this, in that NO production increases both in response to lethal and sublethal DD concentrations. However, in both this and other experiments (see Figures 1–4) we observed a clear threshold response, suggesting that sublethal doses of DD may trigger signaling phenomena that lead to induced resistance, whereas higher doses induce cell death. In terms of calcium, the different signatures of cell populations destined to die and those with induced resistance may suggest that the first acute response may trigger active cell death, whereas the second sustained response may override this and induce resistance responses. The mechanisms whereby the same molecule can mediate such contrasting responses must be the subject of future study. Interestingly, a similar phenomenon has been reported for NO in plants [41].

In these studies we used the best-characterized unsaturated aldehyde, DD, to study effects on diatom cell-fate regulation, and responses to very low concentrations could be observed. However, previous reports demonstrate that a variety of unsaturated aldehydes are produced by different diatom species, and variability can also be found among different strains of the same species [10,42,43]. Hence, local concentrations of reactive aldehydes may be considerable and they may display synergistic effects not yet tested. Furthermore, analysis of the amount of aldehydes produced by different diatom species has revealed a high variability ranging between 30 and 869 fg per cell [11,44], and it appears that aldehyde production is continuous once cell membrane integrity is disrupted [9]. A variety of factors may therefore contribute to raise actual concentrations further, and so it is reasonable to believe that the observations reported here may have ecological relevance. Recent attempts to develop a sensitive method for the detection and quantification of diatom-derived aldehydes in cultures and in natural populations will clearly help determine the ecological role of these molecules in aquatic habitats [44].

In conclusion, our results demonstrate that diatom cells can sense local DD concentrations and integrate this information in a temporal context. Because aldehydes such as DD are released by wounded diatom cells [9], we propose that they are used as infochemicals to provide a surveillance system to evaluate stress during bloom conditions. Indeed, in chrysophytes, aldehyde concentrations also increase following exposure to abiotic stress (e.g., light and nutrients [45]).

Furthermore, perception of some aldehydes could be used for the detection of other phytoplankton competitors, analogous to the cross talk (allelopathy) observed between a bloom-forming dinoflagellate and toxic *Microcystis* sp. in Lake Kinneret [7]. Perception of sublethal levels of aldehydes by cells in the locality of bystander damaged cells could sensitize calcium- and NO-based signaling systems to induce resistance to successive aldehyde exposure, providing an early-warning protective mechanism, as clearly observed in Figure 5. In a somewhat analogous fashion, plants use volatile organic compounds (VOC) for chemical communication to provide immunity during plant–plant–herbivore interactions [46].

When stress conditions aggravate during a bloom, and cell lysis rates increase, aldehyde concentrations could exceed a certain threshold, and may function as a diffusible bloom-termination signal that triggers population-level cell death [25,47]. It is now established that coordination of stress responses, cell survival, and death can also operate in unicellular organisms and can orchestrate multicellular-like behavior [48,49]. Based on our observations, we therefore propose that differential production and sensitivity to reactive aldehydes by diatoms may determine the fitness and succession of phytoplankton communities in the marine environment through mechanisms regulated by intracellular calcium and NO signals. Such a hypothesis is further supported by observations that different species and even different strains of the same species display qualitative and quantitative differences in aldehyde production [10,42,43].

A recent field study has evaluated intraspecies genetic variability in populations of the harmful diatom *Pseudo-nitzschia delicatissima* and indicated a high genetic variability in pre-bloom conditions whereas only one major clade dominated during the peak of the bloom [50]. Future studies should therefore determine the function of infochemicals such as unsaturated aldehydes in mediating selection in intra- and interspecies interactions during bloom succession.

Materials and Methods

Diatom growth conditions. *Phaeodactylum tricornutum* Bohlin strain CCMP 632 and *Thalassiosira weissflogii* clone CCMP 1336 were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (West Boothbay Harbor, Maine, United States). Transgenic lines of *P. tricornutum* expressing the *Aequorin* gene were obtained as previously described [34] and were grown axenically in artificial sea water (ASW) at 20 °C in a 12-h photoperiod (100 μmol • m^{-2} • s^{-1}). *T. weissflogii* cells were grown axenically in filtered sea water (SW) enriched with nutrients as in f/2 medium. Exponentially growing cultures at cell densities from 1×10^5 to 5×10^5 cells/ml were used for all experiments.

Chemicals. Dihydrorhodamine 123 (5 mg/ml stock in ethanol), coelenterazine (1 mM stock in methanol), DEANO (10 mM stock in KOH [pH 12]), DAF-FM (5 mM stock in DMSO), SNAP (*S*-nitroso-*N*-acetylpenicillamine; 100 mM stock in DMSO), NMMA (100 mM stock in water), DAPI (4′,6-diamidino-2-phenylindole [5 mg/ml stock in water]), impermeant BAPTA ((1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, tetrapotassium salt; 1M stock in MOPS [pH 7.2]), and Sytox Green nucleic acid stain (5 mM stock in DMSO) were purchased from Molecular Probes-Invitrogen (http://probes.invitrogen.com). SNP (sodium nitroprusside; 100 mM fresh stock in water), acetaldehyde, (2*E*)-decenal, and DD were obtained from Sigma-Aldrich (http://www.sigma-aldrich.com). DD from Sigma-Aldrich was used for all experiments except for Aequorin assays, in which we used a purified preparation of diatom-derived DD, kindly provided by Dr. Georg Pohnert (MPI, Jena, Germany). DD was dissolved in methanol, and concentrations were determined by measuring absorption at the lambda max for DD of 274 nm, using a Hewlett-Packard 8453 spectrophotometer (Hewlett-Packard Company, Palo Alto, California, United States).

Fluorescence detection. Fluorescence microscopy was performed using the following filters from Omega Optical (http://www.omegafilters.com): XF104–2 (for DAF-FM and Sytox Green detection), XF39 (for chlorophyll detection), and XF06 (for DAPI detection). Image acquisition was performed using a Hamamatsu ORCA-100 CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan). For the video (see Video S1), DAF-FM-loaded cells were embedded in 0.5% low melting point agarose (Bio-Rad, Hercules, California, United States) in ASW. Image acquisition in the microscope was begun 5 min after addition of DD (10 μg/ml), using a 20× objective and an intensified CCD camera, I-PentaMAX Gen III ICCD:HB, from Princeton Instruments (Roper Scientific, Tucson, Arizona, United States).

For NO measurements, *P. tricornutum* or *T. weissflogii* cells were incubated in the dark with 10 μM DAF-FM for 60 min followed by two washing steps (incubation for 30 min after the first wash to allow de-esterification). Efficiency of loading was tested by examining DAF-FM–dependent fluorescence in the microscope following addition of the NO donor SNAP (0.5 mM). To quantify NO accumulation, DAF-FM fluorescence was measured either with a Bio-Tek FL600 Fluorescence Microplate Reader using a GFP filter set (excitation 485/30, emission 530/30), or using a FACScalibur Becton-Dickinson flow cytometer (Becton-Dickinson, Palo Alto, California, United States) equipped with a 488-nm laser as excitation source. A 530/30BP emission filter was used for detection of DAF-FM–derived fluorescence. Cell death was assayed using Sytox Green [13,25], and fluorescence was monitored both microscopically and using flow cytometry or a fluorescence microplate reader, as for NO.

Determination of NOS activity. DD-treated cells were harvested and sonicated with lysis buffer (10 mM Tris-HCl [pH 7.5], 0.5 mM EDTA, 50 mM NaCl, 1 mM DTT, 0.1% Triton X-100, and protein inhibitor cocktail). The protein solution was then used to measure NOS activity with a NOS assay kit from Cayman Chemicals (http://www.caymanchem.com) as described in Guo et al. [26]. In order to determine whether the reaction was calcium dependent, 5 mM EDTA was added prior to the assay. Protein quantification was determined using the Bio-Rad Lowry kit.

Supporting Information

Figure S1. DD Triggers Calcium Release from Internal Stores

P. tricornutum cells were treated with impermeant BAPTA (50 mM) prior to exposure to either DD (A) or a hypo-osmotic shock (25% ASW) (B). Traces from BAPTA-treated cells are in blue, traces from untreated cells are in green.

Found at DOI: 10.1371/journal.pbio.0040060.sg001 (46 KB PDF).

Video S1. In Vivo Imaging of DD-Induced NO Burst in *P. tricornutum* Cells

Real-time movie of DAF-FM–loaded *P. tricornutum* cells treated with 66 μM (10 μg/ml) DD and imaged for 35 min for NO detection. Frame interval: 10 s. Movie time: approximately 35 min (QuickTime).

Found at DOI: 10.1371/journal.pbio.0040060.sv001 (3.2 MB MOV).

Acknowledgments

We thank A. Ianora for critical comments on the manuscript, and T. W. J. Gadella and S. Mazza for experimental support, and dedicate this work to W. Takkenberg, who passed away tragically during preparation of this manuscript.

Author contributions. AV, FF, and CB conceived and designed the experiments. AV, FF, RC, ADM, and FR performed the experiments. AV, FF, and RC analyzed the data. RC and AM contributed reagents/materials/analysis tools. AV and CB wrote the paper.

Funding. This work was supported by funding from the EU MARGENES, DIATOMICS, and MARINE GENOMICS EUROPE projects and a Centre National de la Recherche Scientifique (CNRS) Actions Thématiques Initatives sur Programmes (ATIP) Blanche grant to CB and a European Molecular Biology Organization (EMBO) Short-Term Fellowship followed by a Marie Curie Intra-European Fellowship to AV.

Competing interests. The authors have declared that no competing interests exist. ■

References

1. Falkowski PG, Barber RT, Smetacek V (1998) Biogeochemical controls and feedbacks on ocean primary production. Science 281: 200–206.
2. Nelson DM, Treguer P, Brzezinski MA, Leynaert A, Queguiner B (1995) Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. Global Biogeochem Cycles 9: 359–372.
3. Wolfe GV, Steinke M, Kirst GO (1997) Grazing-activated chemical defence in a unicellular marine alga. Nature 387: 894–897.
4. Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399: 541–548.
5. Nagasaki K, Tomaru Y, Takao Y, Nishida K, Shirai Y, et al. (2005) Previously unknown virus infects marine diatom. Appl Environ Microbiol 71: 3528–3535.
6. Keating I (1977) Allelopathic influence on blue-green bloom sequence in eutrophic lake. Science 196: 885–886.
7. Vardi A, Schatz D, Beeri K, Motro U, Sukenik A, et al. (2002) Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Curr Biol 12: 1767–1772.
8. Smetacek V (2001) A watery arms race. Nature 411: 745.
9. Pohnert G (2002) Phospholipase A(2) activity triggers the wound-activated chemical defense in the diatom *Thalassiosira rotula*. Plant Physiol 129: 103–111.
10. Miralto A, Barone G, Romano G, Poulet SA, Ianora A, et al. (1999) The insidious effect of diatoms on copepod reproduction. Nature 402: 173–176.
11. Ianora A, Miralto A, Poulet SA, Carotenuto Y, Buttino I, et al. (2004) Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. Nature 429: 403–407.
12. Caldwell GS, Olive PJW, Bentley MG (2002) Inhibition of embryonic development and fertilization in broadcast spawning marine invertebrates by water soluble diatom extracts and the diatom toxin 2-trans,4-trans decadienal. Aquat Toxicol 60: 123–137.
13. Casotti R, Mazza S, Brunet C, Vantrepotte V, Ianora A, et al. (2005) Growth inhibition and toxicity of the diatom aldehyde 2-trans, 4-trans-decadienal on *Thalassiosira weissflogii* (Bacillariophyceae). J Phycol 41: 7–20.
14. Tosti E, Romano G, Buttino I, Cuomo A, Ianora A, et al. (2003) Bioactive aldehydes from diatoms block the fertilization current in Ascidian oocytes. Mol Reprod Dev 66: 72–80.
15. Adolph S, Bach S, Blondel M, Cueff A, Moreau M, et al. (2004) Cytotoxicity of diatom-derived oxylipins in organisms belonging to different phyla. J Exp Biol 207: 2935–2946.
16. Trombetta D, Saija A, Bisignano G, Arena S, Caruso S, et al. (2002) Study on the mechanisms of the antibacterial action of some plant alpha,beta-unsaturated aldehydes. Lett Appl Microbiol 35: 285–290.
17. Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM (2003) Allelopathy and exotic plant invasion: From molecules and genes to species interactions. Science 301: 1377–1380.
18. Potin P, Bouarab K, Salaun JP, Pohnert G, Kloareg B (2002) Biotic interactions of marine algae. Curr Opin Plant Biol 5: 308–317.
19. Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394: 585–588.
20. He YK, Tang RH, Hao Y, Stevens RD, Cook CW, et al. (2004) Nitric oxide represses the *Arabidopsis* floral transition. Science 305: 1968–1971.
21. Nathan C, Xie QW (1994) Nitric-oxide synthases: Roles, tolls, and controls. Cell 78: 915–918.
22. Falciatore A, Bowler C (2002) Revealing the molecular secrets of marine diatoms. Annu Rev Plant Biol 53: 109–130.
23. Montsant A, Jabbari K, Maheswari U, Bowler C (2005) Comparative genomics of the pennate diatom *Phaeodactylum tricornutum*. Plant Physiol 137: 500–513.
24. Itoh Y, Ma FH, Hoshi H, Oka M, Noda K, et al. (2000) Determination and bioimaging method for nitric oxide in biological specimens by diaminofluorescein fluorometry. Anal Biochem 287: 203–209.
25. Vardi A, Berman-Frank I, Rozenberg T, Hadas O, Kaplan A, et al. (1999) Programmed cell death of the dinoflagellate *Peridinium gatunense* is mediated by CO_2 limitation and oxidative stress. Curr Biol 9: 1061–1064.
26. Guo FQ, Okamoto M, Crawford NM (2003) Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science 302: 100–103.
27. Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, et al. (2004) The genome of the diatom *Thalassiosira pseudonana:* Ecology, evolution, and metabolism. Science 306: 79–86.
28. Kers JA, Wach MJ, Krasnoff SB, Widom J, Cameron KD, et al. (2004) Nitration of a peptide phytotoxin by bacterial nitric oxide synthase. Nature 429: 79–82.
29. Desikan R, Griffiths R, Hancock J, Neill S (2002) A new role for an old enzyme: Nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 99: 16314–16318.
30. Crawford NM, Guo FQ (2005) New insights into nitric oxide metabolism and regulatory functions. Trends Plant Sci 10: 195–200.
31. Zeidler D, Zahringer U, Gerber I, Dubery I, Hartung T, et al. (2004) Innate immunity in *Arabidopsis thaliana:* Lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci U S A 101: 15811–15816.
32. Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: Dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 4: 517–529.
33. Bowler C, Fluhr R (2000) The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci 5: 241–246.
34. Falciatore A, d'Alcala MR, Croot P, Bowler C (2000) Perception of environmental signals by a marine diatom. Science 288: 2363–2366.
35. Zafiriou OC, McFarland M, Bromund RH (1980) Nitric-oxide in seawater. Science 207: 637–639.
36. Naqvi SWA, Yoshinari T, Jayakumar DA, Altabet MA, Narvekar PV, et al. (1998) Budgetary and biogeochemical implications of N_2O isotope signatures in the Arabian Sea. Nature 394: 462–464.
37. Adolph S, Poulet SA, Pohnert G (2003) Synthesis and biological activity of alpha,beta,gamma,delta-unsaturated aldehydes from diatoms. Tetrahedron 59: 3003–3008.
38. Guillard R, Kilham P (1978) The ecology of marine planktonic diatoms. In: Werner D, editor. The biology of diatoms. Berkeley (California): University of California Press. pp. 372–469.
39. Juttner F (2001) Liberation of 5,8,11,14,17-eicosapentaenoic acid and other polyunsaturated fatty acids from lipids as a grazer defense reaction in epilithic diatom biofilms. J Phycol 37: 744–755.
40. Pohnert G (2004) Chemical defense strategies of marine organisms. Topics Curr Chem 239: 179–219.
41. Delledonne M (2005) NO news is good news for plants. Curr Opin Plant Biol 8: 390–396.
42. d'Ippolito G, Romano G, Iadicicco O, Miralto A, Ianora A, et al. (2002) New birth-control aldehydes from the marine diatom *Skeletonema costatum:* Characterization and biogenesis. *Tetrahedron Lett* 43: 6133–6136.
43. Pohnert G, Boland W (2002) The oxylipin chemistry of attraction and defense in brown algae and diatoms. Nat Prod Rep 19: 108–122.
44. Wichard T, Poulet SA, Pohnert G (2005) Determination and quantification of alpha,beta,gamma,delta-unsaturated aldehydes as pentafluorobenzyl-oxime derivates in diatom cultures and natural phytoplankton populations: application in marine field studies. J Chromatogr B Analyt Technol Biomed Life Sci 814: 155–161.
45. Watson SB, Satchwill T, Dixon E, McCauley E (2001) Under-ice blooms and source-water odour in a nutrient-poor reservoir: Biological, ecological and applied perspectives. Freshw Biol 46: 1553–1567.
46. Baldwin I, Kessler A, Halitschke R (2002) Volatile signaling in plant-plant-herbivore interactions: What is real? Curr Opin Plant Biol 5: 351–354.
47. Bidle KD, Falkowski PG (2004) Cell death in planktonic, photosynthetic microorganisms. Nat Rev Microbiol 2: 643–655.
48. Ameisen JC (2002) On the origin, evolution, and nature of programmed cell death: A timeline of four billion years. Cell Death Differ 9: 367–393.
49. Arnoult D, Tatischeff I, Estaquier J, Girard M, Sureau F, et al. (2001) On the evolutionary conservation of the cell death pathway: Mitochondrial release of an apoptosis-inducing factor during *Dictyostelium discoideum* cell death. Mol Biol Cell 12: 3016–3030.
50. Orsini L, Sarno D, Procaccini G, Poletti R, Dahlmann J, et al. (2002) Toxic *Pseudo-nitzschia multistriata* (Bacillariophyceae) from the Gulf of Naples: Morphology, toxin analysis and phylogenetic relationships with other *Pseudo-nitzschia* species. Eur J Phycol 37: 247–257.

Nieuw binnengekomen in de bibliotheek van de NVKD

Christine Cocquyt

CREMER, H. & B. VAN DE VIJVER, 2006. On *Pliocaenicus costatus* (Bacillariophyceae) in Lake El'gygytgyn, East Siberia. European Journal of Phycology 41 (2): 169-178.

STOYNEVA, M., G. GÄRTNER, C. COCQUYT & W. VYVERMAN, 2006. *Eremosphaera tanganyikae* n.sp. (Trebouxiophyceae), a new species from Lake Tanganyika. Belgian Journal of Botany 139 (1): 92-102.

SYMOENS, J.-J. & K. WOUTERS (eds), 2003. Biological evaluation and monitoring of the quality of surface waters. Cercle Hydrobiologique de Bruxelles, 129 pp.

Inhoud :

SYMOENS, J.-J. Foreword: From the ancient naturalists' observations to the European Water Framework Directive. 5-8.

NIXON, S. An overview of the biological assessment of surface water quality in Europe. 9-16.

VERHAEGEN, G. Initiatives taken in Flanders in function of the implementation of the Water Framework Directive concerning biological water quality. 17-24.

GERARD, P. Eléments de référence pour la mise en place de réseaux d'évaluation biologique de la qualité des eaux de surface en Région Wallone. 25-28.

THIRION, A. Evaluation et monitoring des eaux de surface en Région de Bruxelles-Capitale. 29-30.

TRIEST, L. Perspectives for macrophytes and diatoms as indicators of the ecological status in headwaters in Flanders (Belgium). 31-44.

GOSSELAIN, V., C. FAUVILLE, S. CAMPEAU; M. GEVREY & J.-P. DESCY. Typology and prediction of diatom assemblages in rivers: building of database and first predictive model. 45-58.

PAULISSEN, M. & L. LECLERCQ. Relation entre la chimie de l'eau et la flore algale des palses de la fagne du Brackvenn (Hautes-Fagnes, Belgique/Allemagne). 59-64.

VANDERPOORTEN, A. Using aquatic bryophyte communities as freshwater biodindicators: results, issues and perspectives. 65-70.

BERVOETS, L., R. SMOLDERS, J. VOETS & R. BLUST. Active biomonitoring (ABM), using aquatic invertebrates. 71-76.

VANDEN BOSSCHE, J.-P. A G. JOSENS. Macrozoobenthos biodiversity and biological quality monitoring of watercourses in Wallonia (Belgium). 77-80.

ADRIAENSSENS, V., A. DEDECKER, T. D'HEYGERE, P.L.M. GOETHALS & N. DE PAUW. Relations between structural characteristics and macroinvertebrate communities in the Zwalm river basin at different spatial scales. 81-86.

ADRIAENSSENS, V., A. DEDECKER, T. D'HEYGERE, P.L.M. GOETHALS & N. DE PAUW. Relations between structural characteristics and macroinvertebrate communities in the Zwalm river basin at different spatial scales. 81-86.

ADRIAENSSENS, V., F. SIMONS, P.L.M. GOETHALS, B. GODDEERIS & N. DE PAUW. Ecological analysis of the Chironomid communities in the Zwalm river basin. 87-94.

DE PAUW, N. & S. HEYLEN. Biological sediment quality assessment of rivers and lakes. 95-102.

DETEMMERMAN, L., S. HEYLEN, B. GODDEERIS, F. OLLEVIER & N. DE PAUW. Sediment quality assessment of watercourses based on mentum deformations of Chironomus larvae. 103-110.

GOFFAUX, D., N. ROSET, J. BREINE, J. DE LEEUW, T. OBERDORF, P. GÉRARD, J.-C. MICHA & P. KESTERMONT. Selection of the most appropriate sampling technique and compilation of a common data set as a basis for standardizing a fish-based index between three European countries. 111-129.

Boekennieuws

Bahls, Loren L., 2005. Northwest Diatoms: A Photographic Catalogue of Species in the Montana Diatom Collection. With Ecological Optima, Associates, and Distribution Records for the Nine Northwestern United States. Volume 1.
84 photographs. 153 distribution maps. 4 figs and 54 tabs.

Contents: Introd./ The Montana Diatom Collection/ Geography of the Pacific Northwest/ Format of Northwest diatoms/ Taxonomic Considerations/ Microscope and Camera/ Dedication/ Acknowledgements/ Literature Cited/ Register of Taxa/ Species Profiles/ Distribution Maps.- Will be continued in form of annually published volumes, loose-leaf, punched to fit in a standard 3-ring binder. The binder is not provided. – Northwest diatoms is a photographic catalogue of species in the Montana Diatom Collection (MDC) and encompasses the NW quarter of the coterminous US, which includes large portions of the Great Plains, Rocky Mountains, Cascade Mountains, Coast Range, Columbia Plateau, and Great Basin regions. For each species the catalogue gives basionyms, synonyms, varieties found in the region, similar species and references used by the author to identify the species. Also given are ecological data, geographical distribution incl. maps, a partial checklist of diatom species from inland habitats of the Pacidic Northwest region. ND is offered as a supplement to the diatoms of the United States (Patrick and Reimer 1966,1975).

Iconographia Diatomologica 16. Levkov, Zlatko, Svetislav Krstic, Ditmar Metzeltin and T. Nakov: Diatoms of Lakes Prespa and Ohrid (Macedonia). 2006.
2650 figs (LM & SEM) on 242 plates. 649 p.

Chapter headings: Abstract/ Quickfinder for the plates/ Introduction/ Meterial, methods and investigated area/ Observations/ New Combinations/ Characteristics of diatoms from Lake Prespa and Ohrid/ Acknowledgements/ References / Index of Taxa. - Lakes Preska and Ohrid belong to the old tectonic oligomictic deep isolated lakes, which have a high percentage of endemic biota and which are also considered as centers of speciation. This volume offers the results of a three year basic taxonomical research in the area. A total of 450 taxa is presented, 78 (17.3%) being described as new taxa (1 new genus, 73 species and 4 varieties) and 9 taxa gained new combinations or status. Presence of relict species has been confirmed. Navicula turris HUSTEDT is reported for the first time outside the Kopecz Tertiary deposits in a recent lake.
In preparation. Orders will be recorded. The price is tentative.

Crawford, Richard M., Brian Moss, David G. Mann and Hans R. Preissig (eds.): Microalgal Biology, Evolution and Ecology. Volume dedicated to Professor Frank E. Round. 2006.
(Nova Hedwigia, Beiheft 130).
illus. XV, 382 p.

Twenty five contributions dealing mostly with diatoms.

Graneli, Edna and Jefferson T. Turner (eds.): Ecology of Harmful Algae. 2006. (Ecological Studies, Volume 189). 385 p.

Contents (main headings only): An Introduction to Harmful Algae/ Molecular Taxonomy of Harmful Algae/ The Biogeography of Harmful Algae/ Importance of Life Cycles in the Ecology of Harmful Microalgae/ The Ecology of Major Harmful Algae Groups/ The Ecology of Harmful Flagellates Within Prymnesiophyceae and Raphidophyceae/ The Ecology of Harmful diatoms/ Ecology of Harmful Cyanobacteria/ The Ecology and Physiology of Harmful Algae/ Ecological Aspects of Harmful Algal In Situ Population Growth Rates/ Harmful Algae and Cell Death/ The Diverse Nutrient Strategies of Harmful Algae: Focus on Osmotrophy/ Phagotrophy in Harmful Algae/ Allelopathy in Harmful Algae: A Mechanism to Compete for Resources?/ etc.
Due in August 2006. Orders will be recorded.

Iconographia Diatomologica 17. Antoniades, Dermot, Paul B. Hamilton, Marianne S. V. Douglas and John P. Smol: Diatoms of North America: The freshwater floras of Prince Patrick, Ellef Ringnes and northern Ellesmere Islands from the Canadian Arctic Archipelago. 2006.
1500 figs. on 131 plates. Approximately 530 p.

Contents: Acknowledgements/ An Introduction to Ellef Ringnes Island, Prince Patrick Island and Nothern Ellesmere Island: Freshwater Ponds and Lakes (Overview/ History of Diatom Research Across the Canadian Arctic)/ Ellef Ringnes, Price Patrick and Northern Ellesmere Islands: Study Area (Climate/ Limnology/ Site Description/ Methods)/ The Diatom Flora (General Information and Terminology/ The Taxa)/ Literature Cited/ Photographic Plates/ Appendices/ Index.- (From the preface:) This project was initiated in 1998 with the development of a surface diatom calibration set for selected regions from the Arctic islands as part of the PhD dissertation of D. Antoniades at the University of Toronto, Canada. Under this study, surface sediments, mosses, rock and other available habitats were studied from 91 ponds and lakes along with sediment cores from several sites. It quickly became clear that many of the diatoms observed from this study were either poorly documented or new and a taxonomic treatment of these diatoms from high latitudes in the Arctic was waranted. Detailed taxonomic studies continued at the Canadian Museum of Nature ussing addition SEM generated images and the extensive reference library.
Due November 2006. Price not yet available. Orders will be recorded.

Colin Reynolds, 2006. The Ecology of Phytoplankton
Cambridge University Press

Communities of microscopic plant life, or phytoplankton, dominate the Earth's aquatic ecosystems. This important new book by Colin Reynolds covers the adaptations, physiology and population dynamics of phytoplankton communities in lakes and rivers and oceans. It provides basic information on composition, morphology and physiology of the main phyletic groups represented in marine and freshwater systems and in addition reviews recent advances in community ecology, developing an appreciation of assembly processes, co-existence and competition, disturbance and diversity. Although focussed on one group of organisms, the book develops many concepts relevant to ecology in the broadest sense, and as such will appeal to graduate students and researchers in ecology, limnology and oceanography.

Arnold G. van der Valk, 2006.The Biology of Freshwater Wetlands

Oxford University Press

This introduction to freshwater wetlands describes those abiotic features of wetlands that make them unique as a habitat and examines in detail the adaptations, distributions, and interactions of various organisms (microbes, invertebrates, plants, and vertebrates) that collectively form wetland ecosystems. All kinds of freshwater wetlands are covered including lacustrine, palustrine, riverine and tidal forms. The management, conservation and restoration of wetlands are also covered. This is an accessible text suitable for both undergraduate and graduate students taking courses in wetland ecology as well as professional researchers in the fields of limnology and freshwater ecology requiring a concise overview of the topic.

Giuliano Ziglio, 2006. Biological Monitoring of Rivers

Wiley Publishers

The contents are presented as follows: The river environment - including floodplains, instream and bankside habitats Biological monitoring of rivers - considers the monitoring and assessment methods based on macroinvertebrates, fish, algae and macrophytes. In addition it covers the organisation of biological monitoring with a focus on the EU and North America. Case studies in biological monitoring - features alpine rivers, North European rivers, Mediterranean and running waters in Eastern and Central European countries as well as bioassessment development in the North America. New tools and strategies for river ecology evaluation - presents decision making on what constitutes a significant environmental change; predictive modelling approaches; evaluating fluvial functioning (FFI), planning the integration of urban and ecological processes and concluding with a discussion 'Beyond Biological Monitoring: An Integrated Approach'

C. E. Cushing, 2006. River and Stream Ecosystems of the World

The University of California Press

Rivers and streams around the world that once flowed wild and unchecked are rapidly disappearing into dams or being channelized between concrete banks. This valuable sourcebook, now available to a wide audience in a paperback edition, is an important comparative documentation of what is being lost: naturally flowing river and stream ecosystems. No other single olume brings together so much critical information on rivers and streams worldwide. Each chapter is packed with a wealth of raw data on waterways including the prominent rivers of North America, Central and South America, Europe, Africa, Australia, and Oceania. The volume evaluates the usefulness of the River Continuum Concept and ecosystem-level measurements for evaluating the structure and function of rivers and streams. The new introductory chapter examines the relevance of other useful concepts including Nutrient Spiraling, Patch Dynamics, the Flood Pulse Concept, the Network Dynamics Hypothesis, and the Hyporheic Corridor Concept.

Congressen, symposia

30th Congress of the International Association of Theoretical and Applied Limnology

Montréal, Canada, 12-18 August 2007

Five days of exciting talks, of seminal plenary lectures, of poster sessions, of workshops and of informal scientific gatherings. This is what awaits you at the Montréal SIL congress. Global change and increasing environmental pressures are confronting the limnological community with new and unprecedented scientific challenges and we can only face the challenges through the sharing of our collective advances gleaned from around the world.

The Organizing Committee is making every effort to diversify the Congress' established activities, by emphasizing new fundamental and applied research themes, and by promoting special sessions on emerging concepts and problems, and on new methodological developments. We also want to ensure that students will play an active role in all aspects of the congress, including the organization of special student forums on a range of topics and other more social events. During the traditional mid-Congress excursions, you will be able to visit the various nearby lake districts, the UNESCO biosphere reserves, or other more cultural venues such as Old Montréal and Québec City.

http://www.sil2007

Symposium for European Freshwater Sciences 5

Palermo, Italy. 8-13 July 2007

The Organizing Committee is pleased to invite all those interested in freshwater sciences to join the Fifth Symposium for European Freshwater Sciences (SEFS5). From the 8th to the 13th of July 2007, Palermo, its University, and in particular the Department of Botanical Sciences and Botanical Garden, have the pleasure to host this meeting organised in cooperation with the Freshwater Biological Association (FBA) and the Italian Association of Oceanology and Limnology (AIOL).

These Symposia, coordinated by the Freshwater Biological Association in collaboration with other European freshwater and limnological Associations, are held every two years: the first took place in Antwerp (Belgium) in 1999, the second in Toulouse (France) in 2001, the third in Edinburgh (Scotland) in 2003 and the fourth in Krakow (Poland) in 2005.

Like the previous Symposia, SEFS5 will be devoted to basic questions in freshwater biology and various aspects of applied freshwater science. We would like to bring together all European scientists working on freshwater organisms, freshwater habitats, and freshwater systems. Special attention is devoted to students and young scientists who are at the beginning of their career: the Symposium is an ideal occasion to meet their colleagues and look for reciprocal inspiration, share knowledge, exchange experience and ideas about freshwater ecosystems in the broadest sense and…last but not least, have a nice time.

http://www.sefs5.it