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Chapter 7

EPIPELIC BIOFILMS AS INDICATORS OF ENVIRONMENTAL CHANGES IN LOWLAND FLUVIAL SYSTEMS

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ABSTRACT

The epipelic biofilm is a biologic complex of autotrophs (algae) and heterotrophs (fungi, bacteria, microinvertebrates) embedded in a polysaccharide matrix that develops on the fine sediments (silt and clay) of many aquatic ecosystem worldwide of lowland fluvial streambeds. Biofilms play a key role in the energetic balance of the fluvial systems, contributing to the recycling of organic matter and, therefore, to their self-depuration. The structure and function of a biofilm are affected by a variety of factors, both natural and anthropogenic, that, in turn, determine the physical and chemical conditions of the water. The characteristics of biofilms on episammic and epilithic substrates have been widely described in the literature, but the features of epipelic biofilms have been only scarcely documented. In this chapter, we present a review of the use of the structural and functional parameters of epipelic biofilms in order to assess changes in water and habitat quality as a result of human impact. Methodologies for the sampling and analysis of biofilms are described; and selected study cases are discussed in order to provide information about specific composition, density, biomass, biological indices, primary production, respiration, and enzymatic activities of the epipelon in relation to different uses of the surrounding land. The structural and functional parameters of biofilms should be made an integral component in the routine assessment of stream health as well as in the establishment of baseline values for both disturbed and undisturbed systems to be incorporated into monitoring and compliance guidelines.

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INTRODUCTION

An aquatic ecosystem is naturally influenced by stress contributors such as climate, geology, topography, and hydrology. The imposition of stress is vital at every level of biological organization. Consequently, the ability to react to stressful conditions is an important characteristic of all living systems: indeed, no development of the species or the ecosystem as a whole is possible without such natural selective pressures (Schüürmann & Markert, 1998). Through human activity the environment has been confronted with new substances that did not exist previously (xenobiotics) and potentially harmful materials released in quantities unthinkable in the past (heavy metals, natural radionuclides). Moreover, these new stress contributors usually have a multiplying effect, *i. e.*, they add onto the effects of natural systems, or they themselves act in combination with each other, with the result that the tolerance threshold - the limit of the organisms' ability to cope with or to adjust to the stressing condition- is exceeded (Markert et al., 2003).

Aquatic organisms respond to biotic and abiotic parameters that influence their habitat and, in doing so, provide a continuous record of environmental quality. Among the communities that inhabit the rivers, the biofilms -communities of microorganisms attached on surfaces- are the first to interact with dissolved substances such as nutrients, organic matter, and toxicants and therefore can be used to detect the early effects that these disturbances might cause on the ecosystem. The biofilms are designated according to the substrate that they are coating: epilithon over rocks, epixylon over wood, episammon over sand, epiphython over living plants, and epipelon over fine sediments. In rivers and streams that cross depositional areas, as is the circumstance in the Pampean plain (South America) fine sediments (silt and clay) constitute the bottom and are covered by the epipelic biofilms. These latter can be defined as biological-layer developments on organic and inorganic sediments that include bacteria, algae, fungi, and microfauna, embedded in an exopolysaccharide matrix -it consisting of polysaccharides, proteins and lipids-. Biofilms incorporate the effects of environmental conditions over extended periods of time, mainly because of their small size, rapid growth, species richness, and the physiological variety of their constituent organisms. For this reason, biofilms have been widely used for routine monitoring, because they may be very can serve as early warning systems for environmental disturbances (Ector & Rimet, 2005; Sabater et al., 2007).

Several parameters affect the functioning and response of biofilms -some physical, others chemical, and still others biological (Figure 1).

Measurements of the ongoing state of biofilms can be obtained rapidly and at low cost through structural descriptors such as biomass or taxonomic and chemical composition. With both of these variables, the determination of biofilm function can be performed by measuring changes in the status of the system through techniques such as biofilm metabolism, nutrient uptake, and extracellular-enzyme activity (Figure 2).

In the Argentine Pampean plain, biofilms have been used as indicators of natural and anthropogenic disturbances (Gómez & Licursi, 2001; Gómez & Licursi, 2003; Gómez et al., 2003; Gómez et al., 2008; Graça et al., 2002; Herkovits et al., 2006; Licursi & Gómez, 2002; Licursi & Gómez, 2004; Licursi. & Gómez, 2009; Sierra & Gómez, 2007; Tolcach & Gómez, 2002). In these studies ecological, physiological, biochemical, and morphological methods were employed to assess the biotic integrity of the biofilms under study.

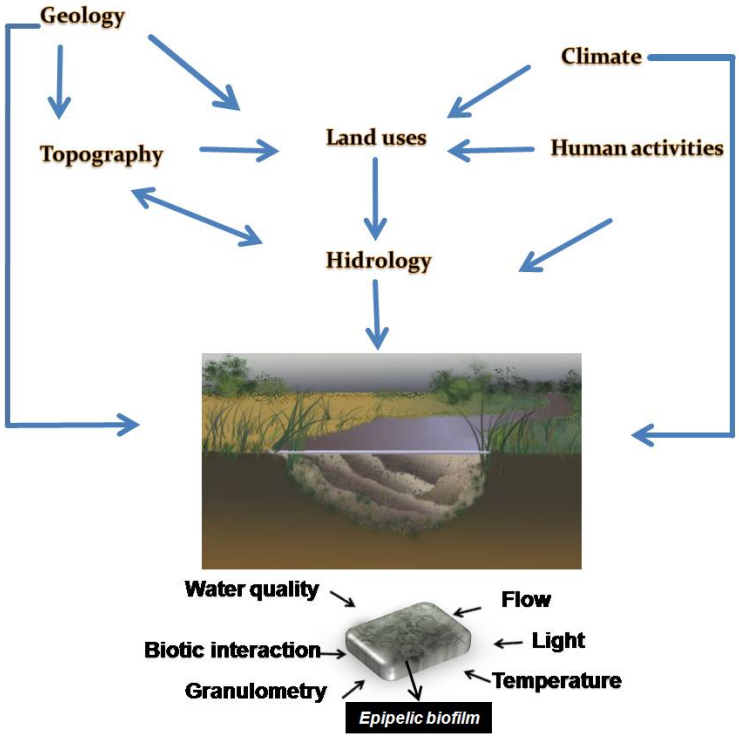

Figure 1. Main parameters affecting lotic ecosystems and consequently the biofilms that develops in the bottom of rivers and streams

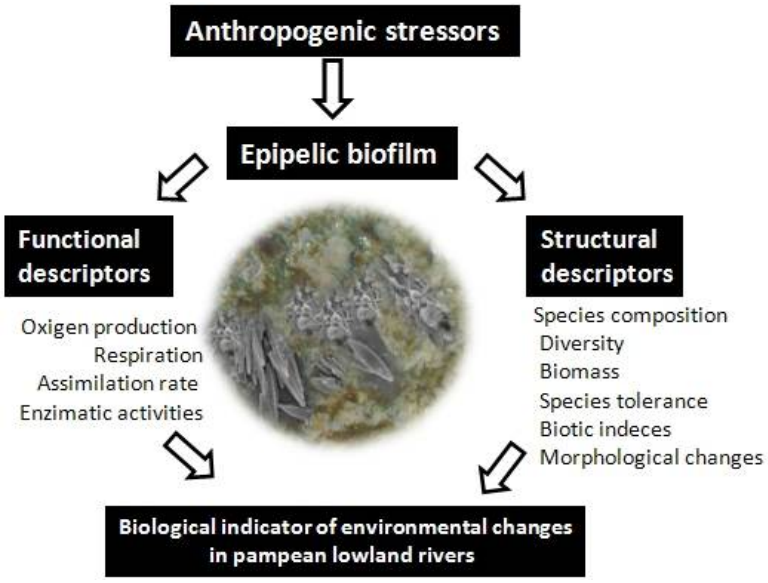

Figure 2. Main Biological indicators employed to assess environmental changes in lowland rivers and streams of the Pampean plain

The area occupied by the Argentine pampas is heterogeneous with respect to geology, climate, and the extent of land-surface relief. This region contains the highest demographic

and industrial concentration in the country and registers the greatest production of agriculture and livestock as well as the most intense use of agrochemicals. In the last 150 years, the pampas has passed through different developmental stages, such as the expansion of crop and cattle farming and the growth of industry. Because of the intensive human activity that arose in certain areas, the Pampean rivers and streams are impacted by point sources of contaminants stemming from sewer effluents and industrial wastes as well as by diffuse sources owing principally to crop cultivation and cattle raising.

The rivers and streams in the Pampean plains are characterized by a minimal flow rate because of the minimal slope of the surrounding terrain, high levels of suspended solids, silty sediment in the benthos, and the development of varied and abundant hydrophytes having discontinuous distributions along the different watercourses.

In this chapter we provide a review of the main techniques for collecting and processing samples for the study of epipelic biofilms that have been employed in Pampean rivers and streams. We furthermore outline the results obtained from the use of structural and functional descriptors in assessing environmental changes in lowland streams exposed to different land uses and stress conditions.

STUDY METHODOLOGY

Field Sampling Procedures

Samples in the river or stream should be collected within a reach of at least 100 meters and removed at a minimum of three sites along a longitudinal transect. To collect an epipelic biofilm sample the instrument of choice is a 10-mL pipette with a rubber bulb attached to the mouth portion for manual aspiration and a rubber or plastic adaptor connected to the truncated lower end that supports the pipette during the removal of 5-10 mm of the sediment surface by suction in a volume of about 4 mL (Figure 3).

At each sampling site at least 5 aliquots of 1 cm^2 of sediment should be obtained. Samples should be transferred to labeled plastic vials or bottles. With quantitative samples the label should include information on the date, stream, and surface area sampled. Both formaldehyde and glutaraldehyde at a respective final concentration of 3 or 5% can be used to preserve the sample. Samples that need to be transported to the laboratory without fixatives are placed in the dark and refrigerated.

Samples of epipelic biofilm should be collected during periods of stable stream flow, and after an intense rain a week's time should elapse before sampling.

Laboratory Processing

Two classes of parameters can be analyzed for the biological evaluation of watercourses: the taxonomic (*e. g.*, species composition, diversity, presence and abundance of indicator species) and nontaxonomic (*e. g.*, biomass, oxygen production, respiration, enzymatic activity)

Figure 3. Epipelic biofilm developed on the bottom of a Pampean stream (left) and detail of the sampler used to collect the biofilm (right)

Taxonomic Descriptors

Bacteria

The most widely used method for the analysis of bacterial density and biomass involves dying a liquid sample with DAPI (4',6-diamidino-2-phenylindole), which fluorophore stains the DNA of both active and inactive cells (Porter & Feig, 1980).

There are also alternative procedures that allow us to distinguish between active cells from those either dead or inactive, such as the INT (iodonitrotetrazolium formazan; Bott & Kaplan, 1985; Johnson & Ward, 1993; see further on) or the CTC (5-cyano-2,3-ditolyl tetrazolium chloride; Rodríguez et al., 1992) staining methods.

Although in this chapter we focus on the DAPI techniques (summarized in Figure 4), we will nevertheless give a brief introduction to the INT method.

All epipelic biofilm samples, obtained by pipetting (in a minimum volume of 4 mL), require the separation of bacteria from the sediment to obtain a homogeneous suspended sample. Accordingly, samples can be exposed to either or both of the following procedures:

Sonication: in a sonication bath (40 W power, 40 kHz frequency), twice for 2 min each time. A longer exposure could produce the lysis of the bacterial cells (Romaní et al., 2004). During sonication the samples must be kept on ice. Epipelic samples should be allowed to sediment for a few minutes to decrease the turbidity that might interfere with the counting; the sedimentation time, however, must be the same for all samples.

Sodium pyrophosphate: the salt is added to a final concentration of 0.05 μM and the sample left to settle for 30 min. (Böckelmann et al., 2003).

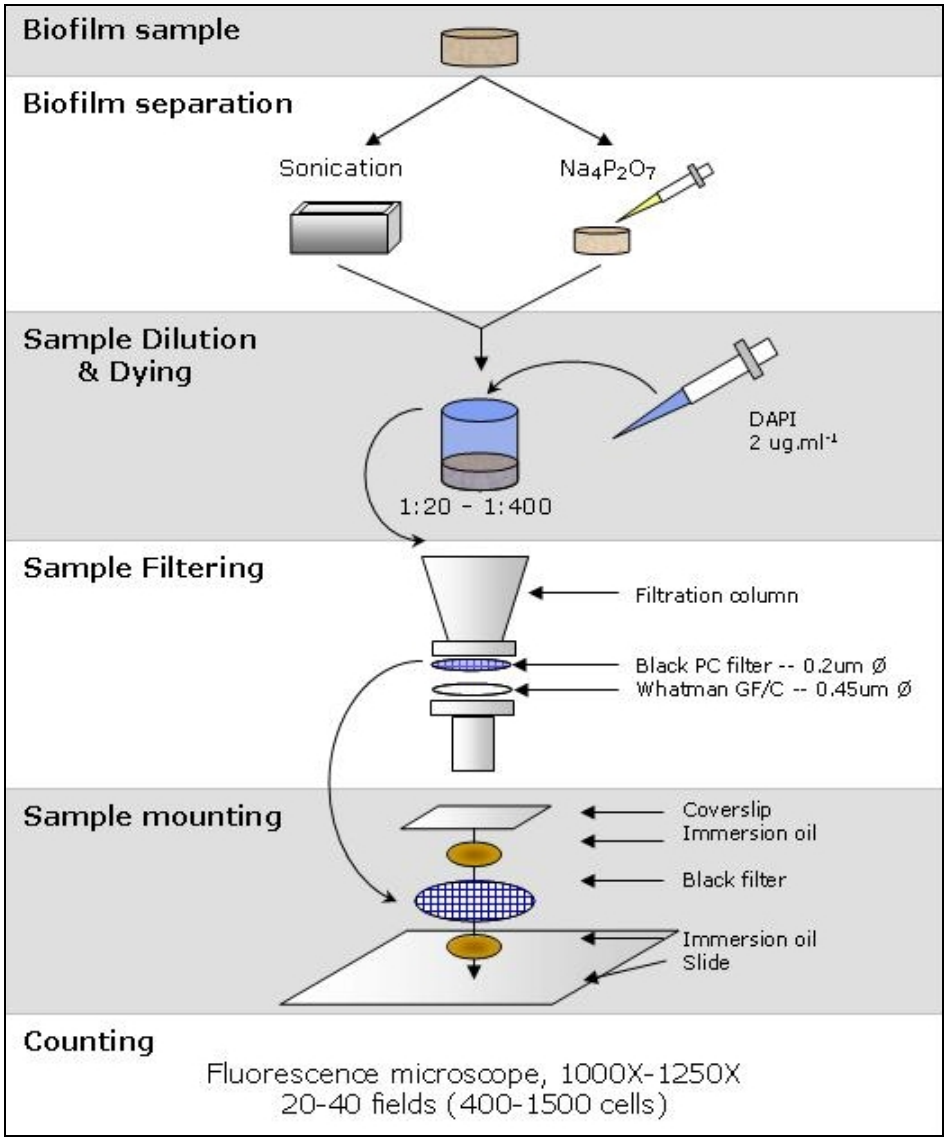

Figure 4. Procedure for counting bacterial cells in epipelic biofilms (DAPI)

The samples will very likely need to be diluted before staining, which step should be done with sterilized water. The degree of dilution has to be determined and recorded for all samples in the study. A convenient final volume for the diluted sample is about 5 mL.

A concentrated stock solution of DAPI at 100 µg mL^{-1} should be kept in the freezer. This stock of the flurophore should be stored in small volumes (*e. g.*, 5-20 mL), to avoid degradation of the compound upon repeated freezing and thawing and since both exposure to light and heat decrease the fluorescence of DAPI.

The diluted samples are then stained for 10 min at room temperature with 2 µg mL^{-1} of DAPI (add 100 µL of the concentrated stock to the 5-mL diluted sample).

The stained samples are then filtered through black polycarbonate filters (0.2 µm, pore diameter) and under low vacuum (about 180 mm Hg). To protect the black filter from

mechanical damage, a Whatman GF/C (1.2μm diameter) or a similar polycarbonate filter can be placed below the black filter in the filtration column.

After filtration, the black polycarbonate filter is mounted on a slide where a drop of immersion oil (of low fluorescence) has been previously added. On top of the filter, another drop of immersion oil is then added followed by a coverslip.

Samples mounted in this way can be stored frozen for two to three months without significant loss of fluorescence, though the best approach is to count them immediately after their preparation.

Counting should be performed with a fluorescence microscope (UV-2A filter, excitation frequency, 330-380 nm), at a magnification of 1000-1250X with an oil-immersion objective. Twenty to forty fields should be counted for each filter, to give a total of 400-1500 cells enumerated. To this end, a reticule mounted on the ocular of the microscope facilitates the counting. An image-analysis system can be used to photograph and perform an automatic counting of the samples, although this approach is more difficult with epipelic samples owing to interference from the sediment present.

The bacterial density per surface (N) is calculated from the total number of bacteria counted per filter (n), the dilution factor (d), the total sample volume (Vm), the diluted and stained sample volume (Vf), the surface of the field sample (S), the filtration area of the individual filter (A, calculated from the diameter of the filtration column), and the total area counted (TA, the number of fields counted times the area of each field):

$$N = [n*(A/TA)*d*(V_m/V_f)] / S$$

Algae

Diatoms, cyanobacteria, and chlorophyta are the main algal groups represented in the epipelon in streams from the Pampean plain (Gómez et al., 2008; Sierra, 2009).

To identify and count the algae there are different methodologies according to which one needs to analyze diatoms or other algae groups. In the latter case, for species identification, an aliquot of the sample is placed on a slide with a coverslip and observed with the appropriate magnification under different types of lighting for visualizing the structures of interest (*e. g.*, interference contrast, phase contrast, dark field). Depending upon the algal group, stains can also be used to facilitate identification (*e. g.*, to reveal the presence of sheaths, mucilage, starch), and local and international taxonomic keys can be utilized to identify the species (Gómez et al., 2009). Before counting, each sample is placed on a shaker for 10 min for resuspension and thorough mixing of the organisms. The method of quantifying algae involves transferring an aliquot of the sample to a counting chamber (Palmer-Maloney or Sedgwick-Rafter) at a density of 10 to 20 cells per field, after dilution if necessary to facilitate observation. A total of 300-400 organisms are identified and counted at a magnification of 400-600X. When the organisms are very small, we recommend placing a smaller volume of sample on a coverslip and carrying out the count at a magnification of 1000X. The density of algae in the counting chamber is the total number of algae counted in all fields of the chamber divided by their total volume. This concentration must then be multiplied by the dilution factor used to calculate the density of the organisms in the original sample.

For analyses of diatom assemblages, the samples are collected as detailed in the sampling section of this chapter. The identification of diatoms requires the prior removal the cell

contents and -for proper visualization, systematic identification, and counting- is performed on fixed preparations.

While there are many techniques for removing organic matter, a digestion with H_2O_2 is widely used. Thus, after removing the fixative from a given sample by successive washings by centrifugation, H_2O_2 100 vols is added at a ratio of one part H_2O_2 to two parts sample. Afterwards the sample is put in a stove at 60º C for 12 h. Then the samples are washed by centrifugation to remove the peroxide. This methodology has the advantage of reducing the breakage of the material and is as well relatively easy to implement. In calcium carbonate-rich waters, its suggested that the carbonates are eliminated by the use of diluted hydrochloric acid.

If the samples contain a large amount of fine sediments, such as silt or clay (as occurs with samples from the Pampean streams), and these sediments hamper the microscopical observation, hexametaphosphate can be used for clay disaggregation. A solution of 5% (w/v) of the dispersant is added to the sample, followed by incubation for 12 h on a shaker at low speed. The sample is subsequently left for approximately 4 h for the precipitation of the frustules. Subsequent microscopical observations are needed to ensure that diatoms are not left in the supernatant. If not, the supernatant fraction is discarded and the remaining sediment centrifuged. The resulting pellet is washed once by centrifugation.

The washed sample is mounted on microscope coverslip in a synthetic resin with refractive index higher than 1.6 (*e. g.*, Naphrax ®). The diatoms are identified and counted up to a number that can vary between 300 and 500 valves. Species identification is performed by means of specific keys for the group (Gomez et al., 2009) under a magnification of 1000X (Figure 5). Finally, the counts are expressed as the relative abundance of each species (Kelly et al., 1998; Blanco et al., 2008).

Biotic Indices

Diatoms are widely represented in the biofilm biological complex (Allan, 1995) since the large number of species and their adaptation to a wide range of ecological conditions make them sensitive indicators of changes in the general environment and in the specific conditions of their own habitat (Stevenson & Bahls, 1999). Other attributes that make diatoms suitable as environmental bioindicators are their ubiquitousess and ecologically significant contribution to most aquatic ecosystems, their sensitivity to a wide range of environmental stresses, their rapid response to changes in environmental conditions, their short generation times, and the natural preservation in sediments of their frustules, the latter providing a historic record of the water body. Moreover, the cost of sampling and sample analysis is relatively low, while the samples can be readily stored for long periods for future analysis (McCormick & Cairns, 1994; Stevenson & Pan, 1999). Since alterations in species composition is one of the strongest responses of diatoms to changes in environmental conditions (Stevenson & Pan, 1999; Whitton et al. 1991; Whitton & Rott, 1996; Prygiel et al., 1999; Gomez & Licursi, 2001), diatom species composition nowadays is routinely and successfully used for the characterization of ecological conditions in many aquatic ecosystems (Ector et al., 2004).

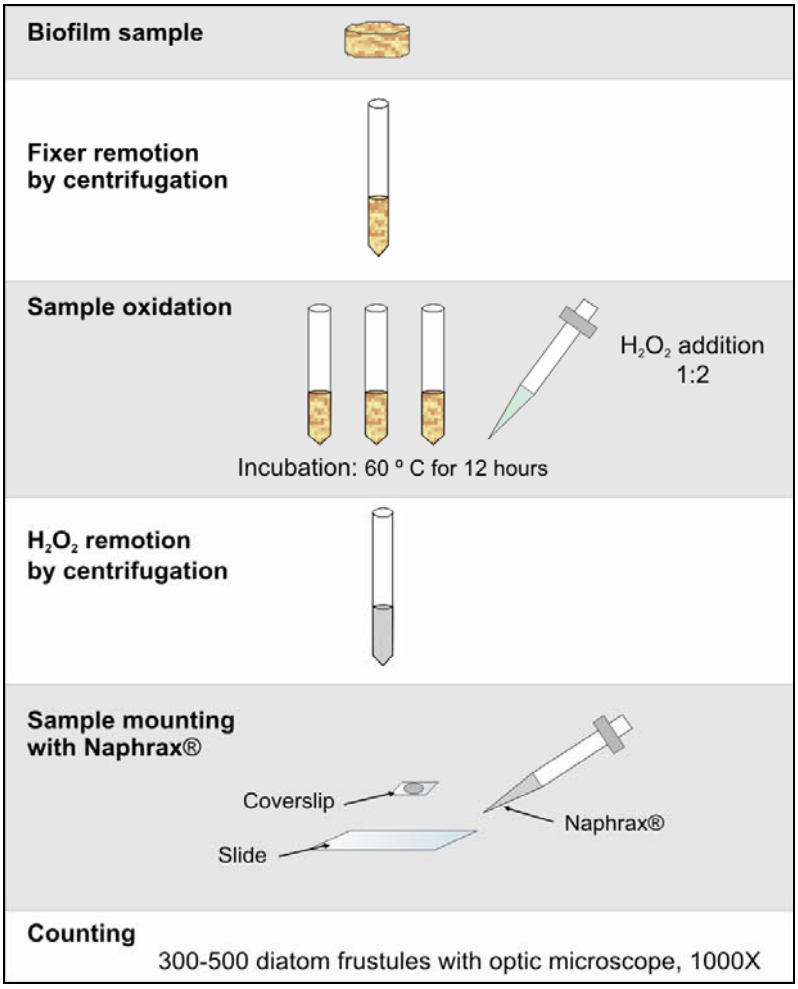

Figure 5. Procedure for cleaning sample, mounting and counting diatoms

There are several diatom indices for the assessment of water quality -the most widely used being the Lange-Bertalot's method (Lange-Bertalot, 1979), Sladecek's Index (Sladecek, 1973), Descy's Index (Descy, 1979), Pollution Sensivity Index (Cemagref, 1982), CEE Index (Descy & Coste, 1990), and the Biological Diatom Index (Prygiel & Coste, 1999). All these indices were developed in Europe on the basis of epilithic diatoms that form biofilms and with the aim at assessing organic pollution and/or eutrophication in rivers. In the Pampas, the beds of lotic systems are dominated by fine sediments and many of the taxa found in epipelic biofilms there exhibit different ecological preferences from those proposed in the lists of indicator species for the Northern Hemisphere. Thus, the design of a local regional index for assessing eutrophication and organic pollution specifically for the Pampean area became necessary, and this index has been designated IDP (Pampean Diatom Index; Gomez & Licursi, 2001). For the creation of this index, 164 epipelic samples were analyzed (from 50 sampling sites with different uses of the surrounding land) and their relationship to the extant physicochemical variables established. For this purpose each taxon was assigned a value of sensitivity to organic pollution and eutrophication (Figure 6) by taking into account the variables closely related to those conditions -*e. g.*, ammonia levels, biochemical oxygen

demand (BOD_5), and soluble reactive phosphorus- and then identifying five classes of water quality (Table 1).

The IDP is calculated by the following formula:

$$IDP = \sum_{j=1}^{n} I_{idp}\, j * A_j / \sum_{j=1}^{n} A_j$$

where:

I_{idp} = specific index value obtained for each species (j), ranging from 0 to 4.

A_j = relative abundance of each species.

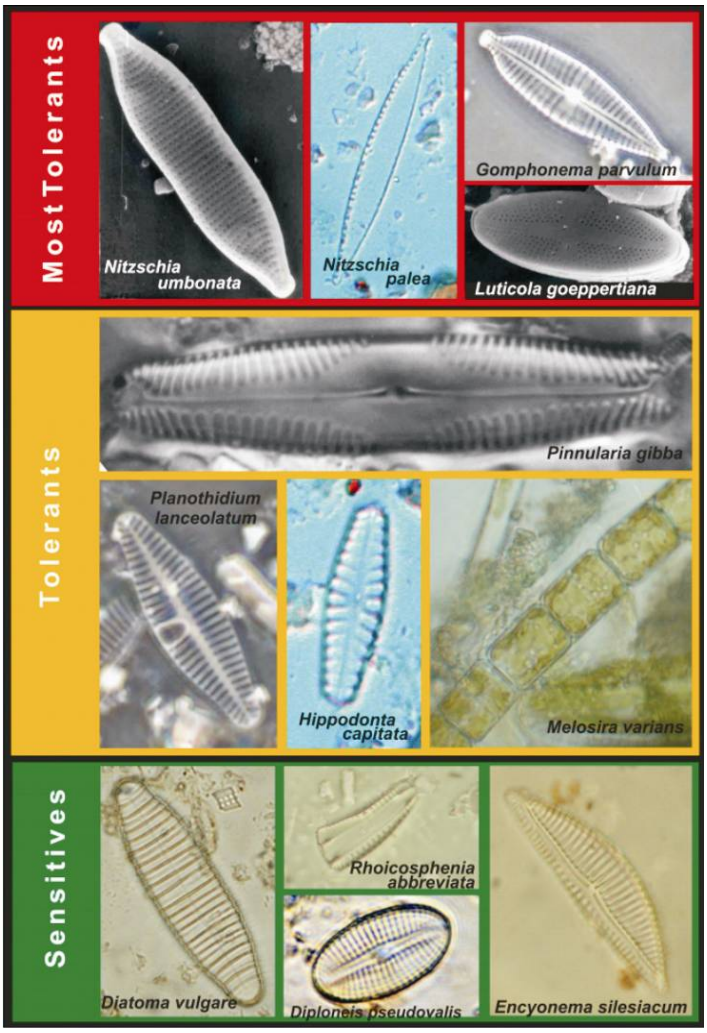

Figure 6. Some diatom taxa identified in the epipelon of Pampean lotic systems grouped according to their tolerance to pollution

Table 1. Characterization of IDP water-quality classes based on NH_4+-N, BOD_5, and PO_4^{3-}-P (mg l) and their interpretation

Water-quality class	Color code	IDP	BOD_5	NH_4^+-N	PO_4^{3-}-P	Significance	Degree of disturbance
0		0-0.5	≤3	≤0.1	≤0.05	*Very good:* without pollution—natural water, few nutrients and little organic enrichment	Very slight: little human influence
I		0.5-1.5	3-8	0.1-0.5	0.05-0.1	*Good:* slightly polluted and eutrophicated, nutrients and organic matter levels still low	Slight: extensive cattle-raising and agriculture
II		1.5-2	8-15	0.5-0.9	0.1-0.5	*Acceptable:* moderately polluted and eutrophicated; high concentrations of nutrients and organic matter	Moderate: agricultural activity and/or intensive ranching
III		2-3	15-25	0.9-2	0.5-1	*Bad:* highly polluted and eutrophicated, presence of partially degraded organic matter, nitrite, ammonia and aminoacids	Strong: intensive agriculture and cattle-raising, moderate industrial activities and population densities
IV		3-4	>25	>2	> 1	*Very bad:* very highly polluted high concentrations of organic matter, predominance of reductive processes, and presence of industrial products	Very strong: intensive industrial activities and high population densities

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The IDP, as well as the other water quality indices mentioned above, can be calculated through the use of the OMNIDIA 4.2 (Lecointe et al. 1993) software designed specifically for diatoms. The counts are entered into the program as independent inventories for which the software calculates several water-quality indices based on the relative abundances of taxa; the program also generates a table summarizing the ecological character of the inventory.

Another approach providing valuable information about the effects of pollution or physical disturbance on the taxocenosis of benthic diatoms is the analysis of the constancy and dominance of each taxon within the samples since these variables permit the assessment of changes in species composition resulting from a given environmental impact (Acs & Kiss, 1991; Licursi, 2005). The constancy of each species is a value, expressed as a percentage that refers to the frequency with which the species was observed in the samples analyzed. The dominance of the species is defined as its relative abundance in each sample (Acs & Kiss, 1991). Similarity indices can also be calculated to compare the taxocoenoses of sites located upstream and downstream from an effluent or, in experimental treatments, the taxocoenoses of the different treatments compared with control values. One such calculation of great use and simplicity is the percentage of similarity between communities. This index ranges from 0 (no similarity) to 100% (maximum similarity) and is calculated by means of the formula proposed by Whittaker (1952; also *cf*. Stevenson & Bahls, 1999).

Microinvetebrates

Of the microconsumers (of size <1 mm), the most representative in the epipelon are the ciliates, the rotifers and the nematods (Gómez et al. 2008; Sierra 2009). Local and international taxonomic keys can be utilized for specific identification (Lopretto & Tell, 1995). The quantification of consumers can be done in a Sedgwick-Rafter chamber, in which circumstance the whole chamber volume should be counted at a magnification of 400X. A calculation of the number of consumers takes into account the concentration of organisms contained in the counting chamber (1 mL) along with the degree of dilution from the sample collected in the field, the latter accordingly corresponding to a known bottom surface.

Nontaxonomic Descriptors

Ash-free dry weight

Ash-free dry weight (AFDW) is a measurement of the organic matter in samples and includes the biomass of bacteria, fungi, small fauna, and organic detritus in samples. The analysis is relatively simple and measures the difference in weight of a sample after first drying and the incinerating the organic matter in the sample (Stevenson & Bahls, 1999).

To determine the AFDW, place the epipelic samples in porcelain crucibles and leave them in a drying stove at 60 °C for 24 h. When the dried samples are cooled off, first weigh them and then place them in a muffle furnace at 500 °C for 4 h. After turning the furnace off, wait until it has cooled down (approximately another 24 h) before removing the samples and placing them in a desiccator. Once they reach room temperature, weigh them again. Samples are not weighed when they are still hot since the hot-air convection currents can alter the measurements.

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To calculate the AFDW, simply substract the weight of the samples after incineration in the muffle furnace from the initial weight obtained with the dried samples. The results are expressed in whatever weight unit the scale was set to, usually within the milligram range.

Bacterial Biomass

The bacterial biomass is calculated from the densities and volumes of the different cells observed, whose shapes are approximated by the most closely corresponding geometrical figures: thus, the biovolume is calculated with cocci considered as spheres, filaments as cylinders, and bacilli as cylinders with hemispherical tips.

The bacterial biomass of a sample is then calculated from the biovolume and the density of each observed bacterial shape, through the use of the allometric relationship of Norland (Norland, 1993) and a conversion factor. The coefficient we suggest using is $2.2\ 10^{-13}$ g C m^{-3} (Bratbak & Dundas, 1984) since this figure lies in the middle of the data reported in the literature (Psenner, 1990). This value is useful when comparing the bacterial biomass to that in different trophic levels.

Bacterial density and biomass should be expressed per square centimeter of surface area for each stream reach. Specific surface area is calculated by the means of granulometric analysis as described in Marxsen & Witzel (1991), where a conversion factor between the dry mass of sand and the total grain surface area is obtained.

Another method that can be employed is the INT-formazan method, which technique measures the number of actively respiring bacteria (Bott & Kaplan, 1985). For this method, incubate the sonicated samples with 3 mL of 0.02% (w/v) 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) in phosphate buffer. Bring the volume to 5 mL with 0.1 M phosphate buffer (pH 7), and incubate the biofilm samples along with the control samples (biofilms fixed with 40% [v/v] formaldehyde for 30 min before the assay but not shaken) in the dark with shaking for 10-12 h at the same temperature as the stream water was when the samples were collected. After the supernatant is removed by decantation, the incubation is terminated by adding 0.1 mL of 37% (v/v) formaldehyde. The sample is then diluted as necessary for observation by fluorescence microscopy and the results can be used, along with those of the DAPI method outlined above, to measure both the actively respiring and the total bacteria.

Chlorophyll

Algal pigments involved in photosynthesis—the conversion of light energy into chemical energy—are the chlorophylls ("a", "b", "c_1", "c_2", and "d", the latter being found only in the Rhodophyta), the carotenoids (carotenes and xanthophylls), and the phycobilins.

The molecule of chlorophyll "a" constitutes the photosynthesis reaction center and is the specific pigment that excites electrons in order to begin the light-dependent reactions of the photosynthetic process. This pigment either directly utilizes the energy from sunlight itself to excite electrons at its own specific wavelength or, alteratively, receives the solar energy absorbed at different characteristic wavelengths by the other pigment molecules. Chlorophyll "a" is the principal one in all algal groups present in the epipelic biofilms (including the cyanobacteria), ranging in concentration from 0.5 to 2% of the total algal biomass (Eaton et al., 1995; Stevenson & Bahls, 1999); while the other photoreactive molecules are accessory, or so-called "antenna", pigments. The concentration of chlorophyll "a" in a biofilm thus plays

a key role in its primary productivity. The taxonomic composition of algal assemblages influences the amount of chlorophyll "a" and accessory pigments in a given biofilm.

The amount of chlorophyll is often used as an estimate of benthic algal biomass and has been found be highly variable with respect to different scales of either space (vertically as well as horizontally) or time (Azovsky et al., 2004; Murphy et al., 2008; Underwood et al., 2005). Many natural and anthropogenic environmental influences affect the composition, abundance, and physiological state of the algal assemblages in a given biofilm and thus determine its chlorophyll content.

The chlorophyll becomes degraded naturally as algal assemblages age and the constituent cells die. Indeed, since the biofilm is also a sink for dead algal cells—through *in-situ* growth, upstream deposition, and even terrestrial plant debris (Biggs & Kilroy, 2000)—the biofilm necessarily accumulates chlorophyll-degradation products; with the chlorophyllides, pheophorbides, and pheophytins being the most plentiful within the aquatic environment. Pheophorbide "a" and pheophytin "a", two common degradation products of chlorophyll "a", can interfere with the determination of active chlorophyll "a" because they absorb light and fluoresce within the same region of the spectrum as does chlorophyll "a" (Clesceri et al., 1998).

Three different methods are available for pigment analysis: spectrophotometry (Arredondo Vega & Voltolina, 2007), fluorometry (Gregor & Marsálek, 2004) and high-performance liquid chromatography (HPLC; Hernández Sandoval & Ibarra Martínez, 2007), with the first being the most common for measuring algal pigments in streams.

For spectrophotometry, these lipidic pigments are extracted with a nonpolar organic solvent and the optical density or absorbance of the extract read in a spectrophotometer at each pigment's characteristic wavelength. The instrumentation is relatively common and inexpensive, and the protocols are straightforward. This method, according to Yallop et al. (2000), overestimates the chlorophyll "a" content relative to HPLC, but the relationship between the two methods is constant.

Fluorometry is based on the excitation of the algal pigments with light at different wavelengths and their response in the ultraviolet. Since, however, fluorometers must be calibrated against spectrophotometric standards, this method is less straightforward, but can nevertheless be used for *in-vivo* measurements and is more sensitive; which characteristics make fluorometry ideal for the pigment analysis of phytoplankton, when such algae are scarce, as in some surface waters. The relatively high biomass of the benthic algae in streams, however, makes that level of sensitivity seldom needed.

HPLC effects the separation of the different molecules in a sample: each molecular species is transported in the mobile phase (liquid) at a specific, and thus characteristic, retention time through the support particles of the stationary phase (solid or gel) packed in the chromatographic column. As a consequence, the components of the sample are recorded in a resulting chromatogram, where each molecule appears as a peak (whose area is used for the quantification of its relative abundance) at a specific and characteristic retention time (therefore used for that molecule's identification). The calibration is performed by comparison with control chromatograms of standard pigment molecules. HPLC is thus an extremely sensitive method capable of measuring a wide spectrum of algal pigments. As with fluorometry, this technique offers a distinct advantage over the other methods for the analysis of very low concentrations of algae. The instrumentation, however, is expensive and the

procedure time-consuming, so that HPLC is not standard equipment in many ecology laboratories.

The spectrophotometric method used for the case studies analyzed in this chapter is detailed here. The following steps are proposed for estimating the concentration of chlorophyll "a" or pheopigments in a given sample of epipelic biofilm:

1. From the riverbed collect 10 aliquots by pipetting (as detailed above) at a distance of 1 m from each other to obtain a sampling from 10 cm^2 (approximately 40 mL). Collect at least three such replicates along the reach.
2. Keep the samples in the cold and dark until processed on the day of collection.
3. At the laboratory, place the samples in glass vials and sonicate them for 3 2-min bursts.
4. Filter the supernatant by vacuum through a glass-fiber prefilter (Sartorius 13400-47-Q, or the equivalent Whatman GF/C). Add tap water (or if heavily chlorinated, Biggs & Kilroy (2000) recommend the use of distilled water) to the remaining sediment. Shake and filter the supernatant again in. Filters can be plugged by the presence of fine sediments, so that more than one filter often must be used for a single sample (from 2 to 4, in fact, is common).
5. Remove the filter, fold the pad in half, and dry it off with absorbent paper. Wrap the filter corresponding to each sample in aluminum foil and label the sample.
6. Store the sample (for up to 3 weeks) in a container with silica gel at -18 to -20 ºC. Freezing the samples and using glass-fibre filters (during crushing) helps break down the algal cells, thus facilitating the extraction of the pigments with the solvent.
7. After thawing, place the filter/s corresponding to each sample in a glass vial; cover them with 5 mL of 90% (v/v) aqueous acetone; keep them in the dark at 4°C for 20 h.
8. Submit the vials to a sonication bath for 3 2-min bursts.
9. Add 3 mL of 90% (v/v) aqueous acetone to the vials and grind the samples. If there are several filters or the extract seems to be very concentrated (*i. e.*, is very deep green in color) add a greater volume of acetone 90%. Place the samples in test tubes of volume 15 mL or greater and with screw caps. Centrifuge the tubes to remove particulates from the solution at 3000 rpm for 1 h. The centrifugation of the samples should be as long as is necessary to reduce turbidity caused by fine sediments.
10. Transfer 3 mL of the clarified extract to a 1-cm–path-length spectrophotometric cuvette.
11. Read the absorbance of the sample extract at 665 and 750 nm.
12. To determine the concentration of active chlorophyll "a" and pheophytin, measure the absorbance of the sample extract at 664/750 nm before acidification and 665/750 nm after acidification of the contents of the cuvette with 0.1 mL (2 drops) of 0.1 N HCl, in order to convert all the active chlorophyll of the extract into its degradation products (Clesceri et al., 1998). The reading at 664 nm should be within an absorbance range of 0.100-1.000; higher readings than the upper value would correspond to concentrations no longer within the range of linearity between absorbance and concentration (Biggs & Kilroy, 2000; Clesceri et al., 1998). Because the degradation products of chlorophyll "a" absorb light in the same region of the spectrum as chlorophyll "a", some researchers prefer to follow step 12 to allow the calculation of active chlorophyll "a" and the degradation product, pheophytin "a".

After mixing, always leave the samples for at least 90 s before reading to ensure that acidification has occurred throughout. The correct volume of acid and time after acidification is critical to avoid the development of products which might interfere with the pheopigment-absorbance peak (Clesceri et al., 1998). Nevertheless, according to Gómez et al. (2009), the HPLC method is recommended for the accurate determination of the chlorophyll-degradation products in natural samples.

13. If out of step 12, estimate the concentration of chlorophyll "a" by the following empirical formula (Gómez et al., 2009):

$$\text{Chl “a”} [\mu g\ cm^{-2}] = 11.4*(665-750)*V / S*L$$

where:

665/750 = 665/750-nm absorbance value

If you have followed step 12, substract the 750-nm absorbance value from the readings before (664 nm) and after acidification (665). Using the corrected values estimate the concentration of chlorophyll "a" and pheophytin "a" as follows (Steinman et al., 2007):

$$\text{Chl “a”} [\mu g\ cm^{-2}] = 26.7*(664b-665a)*V / S*L$$

$$\text{Pheo “a”} [\mu g\ cm^{-2}] = 26.7*[(1.7*665a)-664b]*V / S*L$$

where:

A = the absorption coefficient of chlorophyll "a" at 664 nm: 11.0

K = the ratio to correct for the reduction in absorbance from the initial chlorophyll concentration as a result of acidification: 1.70/0.70 or 2.43

26.7 = the absorbance-correction result from A*K

664b = reading before acidification

665a = reading after acidification

In the three formulas:

V = the volume of the extract in mL

S = the area of substrate sampled in cm^2

L= the length of the light path—*i. e.*, the width of the cuvette—in cm

During the different steps of this process, beginning with the collection of samples for the determination of pigments, keep the samples cold and in the dark to avoid degradation of the different chlorophylls.

Metabolism

Production and respiration are measurements of metabolism that distinguish between autotrophic and heterotrophic dominance within biofilms (Burns & Ryder, 2001). Algae consume carbon dioxide from, and release oxygen into, the overlying water during the

daylight through photosynthesis. Algae, microinvertebrates, bacteria, and fungi consume oxygen through respiration. In fluvial systems, both metabolic processes, photosynthesis and respiration, alter the concentrations of these two gases along with the biomass (through carbon uptake or loss (Rutherford & Cuddy, 2005)).

Primary production may be defined, in broadest terms, as the conversion of solar energy to reduced chemical energy; but more specifically, as the amount of organic matter formed from inorganic carbon by photosynthetic organisms during a specified time interval. Some of this fixed energy is, in turn, lost through the organism's respiration; but the portion remaining stored in the biomass is termed the net primary production (NPP), while the total (respired plus stored) the gross primary production. Respiration measurements include the metabolism of both heterotrophs and autotrophs and this total is thus termed community respiration -CR- (Bott, 2007).

The studies on the metabolism of stream communities that included these parameters have been conducted through the use of different methods: (1) measurements of pH changes, (2) open-system measurements of gases, (3) closed-system measurements of gases, and (4) measurements of ^{14}C uptake (Bott et al., 1978, 1997; Fleituch, 1999; Rutherford & Cuddy, 2005). The methods (2) and (3) are employed to determine changes in dissolved O_2 or CO_2 concentrations, but the O_2 measurements are most often used with the carbon metabolism being indirectly estimated through those O_2 changes (Acuña et al., 2009). Even though each method has advantages and disadvantages, all four approaches are generally accepted and are useful in the assessment of productivity (Stevenson, 1996), Figure 7.

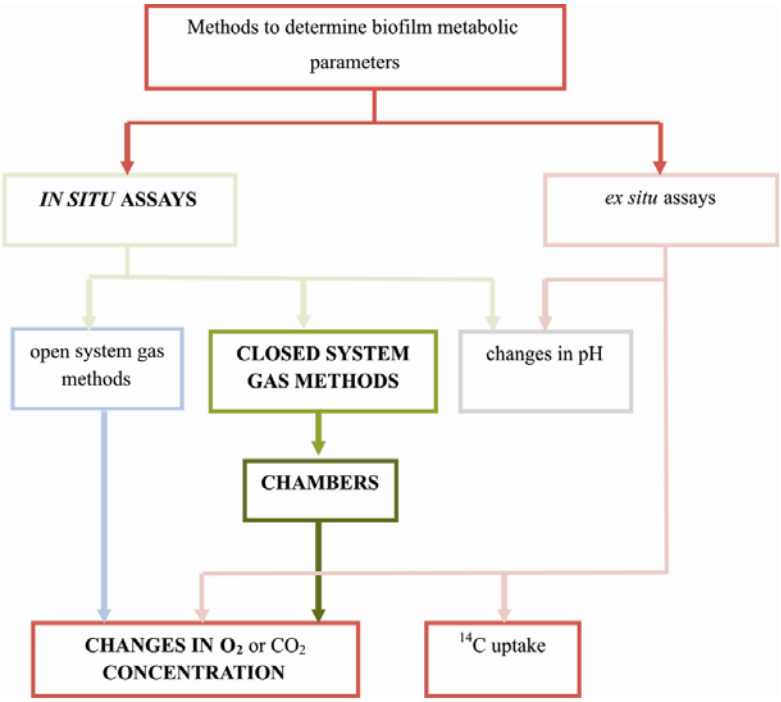

Figure 7. Methods employed to determine biofilm metabolic parameters. Boldface and capital letters designate the methodology employed in the study cases discussed in this chapter

The case studies discussed below show data on metabolic variables, such as NPP and CR, that were obtained through the use of metabolism chambers *in situ*. Information about this latter methodology will be provided below.

The methods employing the closed-system measurements of gases are those wherein biofilm metabolism can be estimated from the changes in O_2 and CO_2 of a particular stream community enclosed in test chambers or bottles (Bott et al., 1978). The chambers have been designed to incubate small intact portions of substrates along with their biofilm either directly in the stream or back in the laboratory under controlled conditions (Sabater et al., 1998; Summer & Fisher, 1979). The design of the chambers (their size, the internal current velocity) varies according to the objectives of the research, the water velocity of the aquatic system, and the substratum size, among other variables. For example, in streams or rivers where the discharge and current is high the chambers are equipped for water recirculation with submergible pumps, while in bodies of water where the flow is minimal the chambers have no such pumps. Since this latter circumstance characterizes the rivers and streams in the pampean plain, the metabolic parameters in the study cases discussed here were measured through the use of metabolism chambers without water recirculation (Figure 8a).

Figure 8. a) Metabolism chambers employed to measure metabolic variables, b) Artificial substrata used for biofilm attachment, c) Artificial substrata after the 4-week period necessary for colonization of the biofilm

Using metabolism chambers to obtain metabolic variables from epipelon, it is necessary to use artificial substrates placed in the aquatic habitats for subsequent colonization over a

period of time (Stevenson & Bahls, 1999). There are many possible types of artificial substrate (*e. g.*, Petri dish, aluminium-foil plates); and depending on the nature of the sampling sites (current velocity, degree of turbidity, depth, surrounding population), the most suitable needs to be choosen. For the lotic systems of the Pampean plain the recommended substrates would be like those shown in the Figure 8 b. Each sampling tray (135 cm^2 of surface) has perforations over its entire perimeter (with holes 0.2 - 0.3 mm in diameter) at about 2 cm below the upper rim in order to allow the free circulation of water over the epipelon. Likewise, each tray has a central hole that permits it to be anchored to the river bottom with a stake. For these trays to remain submerged, cement was added to the lower half of their volume. The Figure 8c shows artificial substrates with biofilm attachment after remaining 4- weeks in a pampean stream, the period recommended to insure an optimal colonization by the microbenthic organisms (Descy & Coste, 1990; Stevenson & Bahls, 1999). Once colonization is completed, the measurements of the metabolic parameters can begin.

The appropriate *in-situ* assays take into account the following:

1. Considerable replication is required to compensate for the heterogeneity of lotic systems. The placement of chambers in at least triplicate is recommended. Control chambers containing stream water only should also be used, but with uncolonized substrates in order to keep the water volume the same as in the other chambers; the purpose of this control being to correct later for the changes in gas concentration that occur as a result of *in-situ* plankton metabolic activity.
2. Fill the chambers with the stream water, place the artificial substrates in the chambers (in the case of sampling trays, two per chamber), and close the lid of the chamber after submersion to avoid the retention of air bubbles.
3. Both the production and the respiration of a community in the same chamber can be measured. To obtain biofilm respiration the chambers are covered with a black plastic sheet and then to obtain biofilm production they are incubated in the sunlight.
4. The O_2 or CO_2 levels are measured at the beginning and end of the incubation period, which time can vary between 30 to 120 min. An estimation of the appropriate incubation time to avoid oxygen oversaturation is also essential. For example, if an accumulation of gas bubbles is observed on the wall of the chambers incubated in the light, the incubation should be stopped immediately (Bott et al., 1978).
5. The O_2 or CO_2 concentration can be measured with microelectrodes, or the dissolved O_2 can be determined by the Winkler technique.
6. The values of NPP and CR of the epipelic biofilms can be obtained employing the modified Fellows et al. (2006) equation:

   $\mathbf{CR_{biofilm}}$ (mg O_2 cm^{-2} h^{-1}) = ([OD_{idch}]- [OD_{fdch}] * V) / (T * S) - [OD_{idcch}]- [OD_{fdcch}] * V) / (T * S).

   $\mathbf{NPP_{biofilm}}$ (mg O_2 cm^{-2} h^{-1}) = ([OD_{fcch}] - [OD_{icch}] * V) / (T * S) - ([OD_{fcccch}] - [OD_{icccch}] * V) / (T * S).

   where:

   OD_{idch} = initial oxygen concentration in the dark chamber [mg O_2 L^{-1}]

   OD_{fdch} = final oxygen concentration in the dark chamber [mg O_2 L^{-1}]

   OD_{idcch} = initial oxygen concentration in the dark control chamber [mg O_2 L^{-1}]

OD_{fdcch} = final oxygen concentration in the dark control chamber [mg O_2 L^{-1}]
OD_{icch} = initial oxygen concentration in the the clear chamber [mg O_2 L^{-1}]
OD_{fcch} = final oxygen concentration in the clear chamber [mg O_2 L^{-1}]
OD_{iccch} = initial oxygen concentration in the clear control chamber [mg O_2 L^{-1}]
OD_{fccch} = final oxygen concentration in the clear control chamber [mg O_2 L^{-1}]
V= chamber volume (L)
T= incubaction time (h)
S= substrate surface area (cm^2)

7. To facilitate comparison with other studies, these NPP and CR estimates can be converted from mg O_2 cm^{-2} h^{-1} to mg O_2 cm^{-2} d^{-1} (Crossey and La Point, 1988; Fellows et al., 2006; Hill et al., 1997). Even these values can be further transformed into units of carbon assuming that one mole of carbon (*i. e.*, 12 g) is equivalent to one mole of O_2 (*i. e.*, 32 g) for both respiration and photosynthesis (*Ergo*: 1 mg O_2 = 12/32 = 0.375 mg carbon; Fellows et al., 2006).
8. The gross primary production and the assimilation rate -the latter being a measure of the fraction of the biofilm biomass that is photosynthetically active- are parameters obtained from the value of NPP and/or CR. The gross primary production is calculated as the sum of the NPP and CR, while the assimilation rate is calculated by dividing the NPP h^{-1} by the amount of chlorophyll "a" present (Crossey & La Point, 1988; Hill et al., 1997).

Despite concerns about nutrient limitation in chambers (though certain chamber modifications overcome this problem), departure of the internal flow pattern from that found *in situ*, and other aspects of chamber design that may affect metabolic rates (*e. g.*, increases in temperature caused by some types of pumps); this closed-system method gas measurements has definite advantages: (1) This experimental model does not require a correction for gas exchange, whereas open-system–gas-measurement methods require an accurate determination of the gas-diffusion rate. (2) A detailed analysis of the biofilm constituents contributing to the O_2 or CO_2 fluxes (standing crop of algae, detritus, or animals) is possible. (3) Working with chambers allows to compartmentalize the environment so as to be able to relate metabolic parameters to standing crops of organisms on particular substrata with considerable confidence (Bott et al., 1978; Bott, 2007). Furthermore, given the technical simplicity of the approach to measuring metabolic parameters through the use of closed chambers -as opposed to gas-production measurements with open systems, which models necessarily involve sophisticated instrumentation- it is possible to transfer communities to metabolism chambers for the purpose of metabolic measurements.

Extracellular Enzymatic Activities

The mineralization of organic N and P and the uptake of dissolved organic matter within stream biofilms is carried out by the extracellular enzymes produced by bacteria, fungi, and, in some instances, algae (Sinsabaugh et al., 1991). These enzymes are attached to the surface of the microbial cell (or in the periplasmic space in Gram-negative bacteria) and react on extracellular polymeric substrates (Chróst, 1991). The term *extracellular enzymatic activity*, refers not only to those enzymes that are attached to the exterior cell surface (ectoenzymes) but also to those that become released free into the environment (to form part of the polymeric matrix). These enzymes convert high-molecular-weight molecules to low-

molecular-weight ones, which species are then available for microorganisms (Chróst, 1994). The decomposition rate of the organic matter depends directly on these enzymatic activities, which catalyses involve both hydrolysis (ß-glucosidase, phosphatase, leucine-aminopeptidase, cellobiohydrolase) and oxidation (peroxidases, phenoloxidases).

In this section we describe a fluorescence-based method for measuring hydrolytic enzymes, utilizing the methylumbelliferyl (MUF) or aminomethylcoumarin (AMC) as substrates (the artificial substrate used depends on the enzyme measured) with which the enzymes react to release a fluorescent product that can be measured fluorometrically.

Replicate field samples must be collected by pipetting, placed in sterile vials at a final volume of at least 12 mL (3 4-mL aliquots), and kept cool and in the dark for processing on that same day.

Epipelic samples should be sonicated in an ultrasound bath (40 W power, 40 kHz frequency), twice for two min each burst.

The following steps should be performed:

1. Estimate the saturation concentration of the enzyme with respect to the substrate. This concentration is around 0.3 mM, but should be calculated through a determination of the kinetics of the enzyme: To this end, the fluorescence is recorded at increasing substrate concentrations (*e. g.*, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mM) and the results plotted to generate the Michaelis-Menten hyperbolic curve: here the reaction velocity is plotted on the *ordinate* and the substrate concentration on the *abscissa*. The saturation concentration will be the minimum substrate concentration producing the maximum velocity, while the Michaelis constant is the substrate concentration giving a half-maximum value.
2. Prepare the following (minimal final volume, 2 mL):
   Epipelic subsample: the supernatant from the sonicated sample.
   Blanks: Use pure Milli-Q water, to measure the abiotic degradation of the artificial substrate.
   Controls: Use a water sample from the stream to measure the natural fluorescence of the water without the artificial substrate.
   Concentration standards: To quantify the fluorescence obtained for the epipelic samples, replicate standards of known MUF or AMC concentrations should be prepared and measured along with the samples.
3. A volume of the artificial substrate at saturation concentration, obtained on a previous run as described in Step 1, has to be added to the samples at a 1:1 sample:substrate volume ratio.
4. Place the samples in the dark on a shaker with the temperature is either adjusted to that of the stream or maintained at the ambient temperature (*ca*. 20ºC, 68ºF) throughout. The incubation time should not exceed 90 min and is usually 1 h.
5. Add glycine to all samples at a concentration of 0.05 M and pH 10.4, at a 1:2 glycine:sample volume ratio.
6. Since epipelic samples tend to have high turbidity, centrifuge the samples for a few minutes at low velocity (2,000-3,000 rpm).
7. Measure the fluorescence of the supernatant at 365/455 excitation/emission frequency for the MUF substrates and 365/445 excitation/emission frequency for AMC substrates in a fluorometer with a 10 mm quartz cuvette.

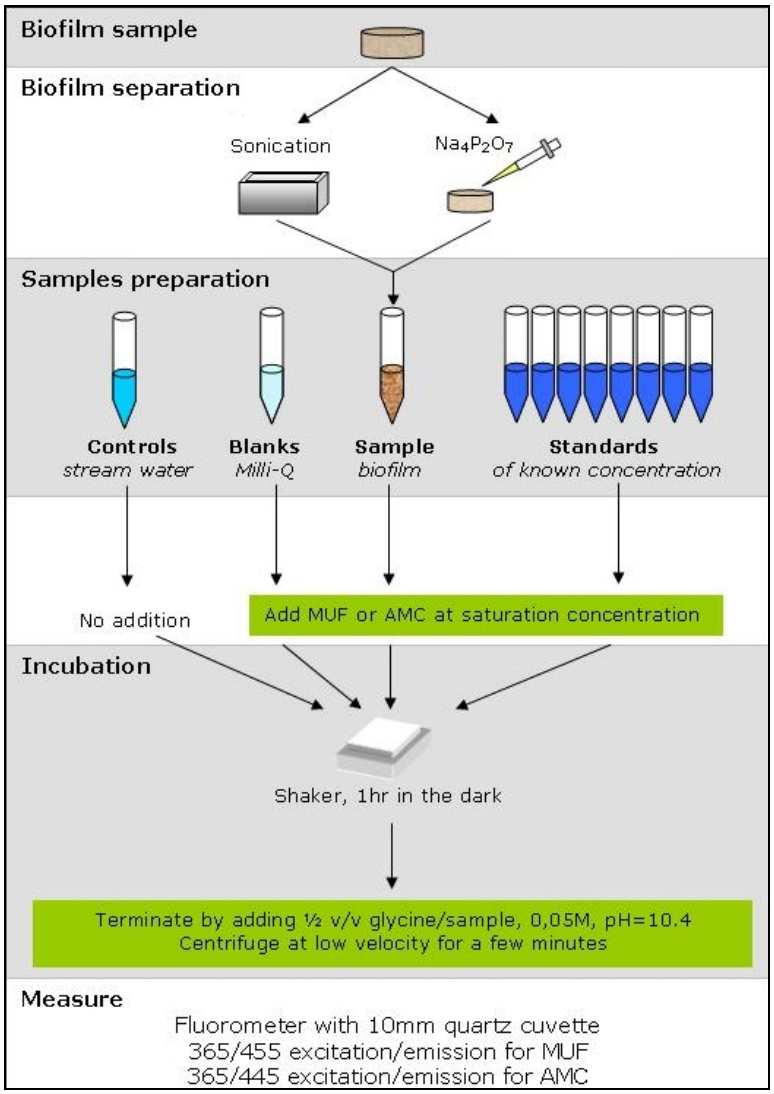

Figure 9. Extracellular activities measurement by the fluorometric method

To correct the obtained absorption values, the values of the blanks and controls should be subtracted from those of the epipelic samples. The values obtained for the standards are used to determine the linear portion of the fluorescence *vs.* concentration curve. The enzymatic activities are expressed in nmoles of MUF or AMC released per h (incubation time) per cm^2 of biofilm.

The values obtained can also be expressed as enzymatic activity per bacterial cell (with those enzymes produced only by bacteria, such as the ß-glucosidase, if the bacterial density is known), per microbial biomass (from the microbial carbon content of the biofilm, if known), or per mg of protein.

STUDY CASES

Epipelic Biofilms *vs.* Agricultural Land Use

Agricultural land use degrades streams by increasing diffuse inputs of pollutants, thus impacting the riparian and stream-channel habitat and altering flow rates. Higher inputs of sediments, nutrients, and pesticides and/or herbicides occur with increased agricultural land use.

The Pampas contains some 21 million inhabitants, accounting for 90% of the country's soybean production, and has accordingly been the most highly affected by the degree of expansion of this crop. Among the principal consequences of this growth in soybean cultivation has been the marked decline in the fertility of the soils (principally through decreases in nitrogen and phosphorus), which result has necessitated the addition of fertilizers to the soil.

With an aim at observing the changes manifested by epipelic biofilms, an experiment was carried out involving nutrient enrichment in a Pampean stream, the La Choza. To this end, we employed a Before-After-Control-Impact (BACI) experimental design (Stewart-Oaten & Bence, 2001), selecting two 100 m stretches, with similar characteristics, and separated by a distance of 5.3 km; one, designated Control (at 34° 44.36 'S; 59° 6.42' W) and located upstream from the other, the second, referred to as Treated (34° 42.08' S; 59° 4.53' W). At this latter point, a fertilizer frequently used in that area (Compo Nitrofoska Azul® with added urea) was added and in doing so elevated by an average value of threefold the basal concentrations of phosphorus (PRS: 0.210 ± 0.14 mg L^{-1}) and nitrogen (DIN 1.01 ±0.70 mg L^{-1}) in that localized portion of the stream. The study lasted a total of 18 months. Table 2 summarizes the changes in the descriptors pertaining to the epipelic biofilm that reached statistical significance ($p < 0.05$) after exposure to this increment in the concentration of nutrients.

The fertilization effected changes in the density and biomass of the producers and bacteria along with a decrease in the production of ß-gucosidase per bacterial cell. Alterations in the diatom assemblages were also observed that favored those species preferring more eutrophic milieux and having metabolisms cosistent with higher concentrations of organic nitrogen (*e. g.*, *Nitzschia frustulum*, *Nitzschia inconspicua*, *Nitzschia amphibia*) (van Dam et. al., 1994). The results obtained permitted not only an evaluation of the impact of such an increment in nutrients on the responses of epipelic biofilms but also a preview of the inconstancies that the Pampean lotic systems will likely suffer in the face of the new climatic-change scenarios predicted by the IPCC (IPCC, 2007) for the Argentine Pampas. Among these effects, alterations in the land runoff as a consequence of higher levels of rainfall will produce an increased input of nutrients into the rivers through fertilizer drainage from the fields under cultivation.

Table 2. Epipelic-biofilm descriptors that exhibited statistically significant changes in the fertilization experiment, expressed as the mean value with the standard deviation shown in parentheses

Variable	Control	Impact
Chlorophyll "a" (μg cm^{-2})	2.51 (± 1.86)	3.62 (± 3.58)
Bacterial density (cells cm^{-2})	2.70 10^8 (± 2.25 10^8)	5.24 10^8 (± 5.49 10^8)
ß_Glucosidase / Bacterial density (nM MUF cells cm^{-2} h^{-1})	4.99 10^6 (± 6.2 10^6)	4.05 10^6 (± 6.8 10^6)
Diatom density (cells cm^{-2})	7.70 10^6 (± 3.20 10^6)	2.10 10^7 (± 9.40 10^6)
Diatom relative abundance (%)		
Nitzschia frustulum (Kützing) Grunow	9.62 (± 9.20)	25.89 (± 18.44)
Nitzschia amphibia Grunow	10.48 (± 9.40)	7.72 (± 4.08)
Nitzschia inconspicua Grunow	0.63 (± 1.05)	3.36 (± 3.49)
Achnanthidium minutissimum (Kützing) Czarnecki	2.54 (± 3.92)	0.64 (± 0.87)

Table 3. Physicochemical characteristics of the water upstream (Site 1) and downstream (Site 2) from a textile discharge in Don Carlos stream

	Site 1	Site 2
Physicochemical variables		
Nitrate (mg L^{-1})	1.27 ± 0.88	1.57 ± 1.93
Nitrite (mg L^{-1})	0.11 ± 0.03	0.18 ± 0.38
Ammonia (mg L^{-1})	0.05 ± 0.03	0.34 ± 0.14
Phosphate (mg L^{-1})	0.90 ± 0.12	0.13 ± 0.14
BOD_5 (mg L^{-1})	3.83 ± 4.57	71.00 ± 5.31
COD (mg L^{-1})	7.66 ± 5.24	7.00 ± 1.54
Heavy metals		
Pb (μg g^{-1})	10.30	13.30
Ni (μg g^{-1})	11.25	10.00
Cr (μg g^{-1})	9.00	9.30
Cd (μg g^{-1})	< 0.125	< 0.125
Cu (μg g^{-1})	19.00	23.00
Phthalic-acid esters (phthalates)		
benzylbutyl phthalate (ng g^{-1})	< 1	< 1
diethyl phthalate (ng g^{-1})	< 1	< 1
dimethyl phthalate (ng g^{-1})	< 1	< 1
di-n-butyl phthalate (ng g^{-1})	< 1	20

Table 4. Epipelic-biofilm descriptors that exhibited statistically significant changes in the fertilization experiment, expressed as the mean value with the standard deviation shown in parentheses

	Site 1	Site 2
Biotic index (IDP)	2.28 (± 0.40)	3.38 (± 0.25)
Bacteria (cell cm^{-2})	$9.11\ 10^5$ (± $1.30\ 10^5$)	$2.75\ 10^6$ (± $5.53\ 10^5$)
Density (autotrophs and microinvertebrates) (org cm^{-2})	$1.32\ 10^7$ (± $5.42\ 10^7$)	$2.75\ 10^5$ (± $5.77\ 10^7$)
Morphological deformations in diatoms (%)	-	2.8
Phosphatase (nM MUF h^{-1} cm^{-2})	79.42 (± 47.50)	376.15 (± 99.20)
Net primary production (g O_2 m^{-2} d^{-1})	1.48 (± 0.75)	-2.74 (± 1.44)
Gross primary production (g O_2 m^{-2} d^{-1})	3.37 (± 1.99)	0.59 (± 2.11)
Assimilation rate (mg O_2 mg Ch *a* m^{-2} h^{-1})	0.83 (± 0.81)	-3.32 (± 1.71)

Epipelic Biofilms *vs.* Urban and Industrial Land Uses

Major changes associated with urban and industrial land uses cause an increase in the amounts and varieties of pollutants along with changes in habitat structure owing to sediment inputs, bank destabilization, channelization, and restricted interactions between the river and its land margin.

As a case study, two sites were selected on a Pampean stream that received an industrial effluent (Gómez & Licursi, 2003; Gómez et al., 2008; Sierra, 2009). In this stream, called Don Carlos (34° 55' - 34° 50' S to 58° 00' - 58° 03' W), we analyzed structural and functional descriptors upstream and downstream from the effluent out of a textile plant that modified the physicochemical characteristics of the water (Table 3).

The results obtained demonstrate that in the face of a deterioration in water quality the epipelic biofilm manifested statistically significant changes (Table 4). Although the density of the organisms increased at the site downstream from the industrial effluent, the biofilm exhibited a lower concentration of chlorophyll, a decrease in both gross and net primary production, and consequently a lower assimilation rate. The biofilm response with respect to these descriptors as well as the observed somatic deformations within the taxocenosis of the constituent diatoms emphasize the degree of deterioration in the quality of the water. These changes reflect the stress-producing influences of levels of heavy metals and phthalates that are prohibitive for the protection of aquatic life: these chemical inputs necessarily inhibit key physiologic processes and impede the normal development of the populations that otherwise would comprise the biofilm. Of importance also was a marked development at the site downstream from the industrial discharge of filaments of bacteria of the genus *Beggiatoa*, it being characteristic of reducing environments. Moreover, the biofilm at this sampling site was thicker, with this characteristic being associated with a defense reaction against the presence of toxic substances (Sabater et al., 2002).

Biotic Indices *vs.* Water Quality

Diatoms

The application of biotic indices employing diatoms is recognized worldwide as a valuable tool for the reliable monitoring of aquatic ecosystems for the purpose of identifying the various adverse consequences resulting from contamination. In the Pampean area information provided by diatom taxocenosis has been employed for the characterization of biotic quality within the Matanza Riachuelo Basin (35° 06' S – 58º 49' W and 34° 38' S - 58º 21' W). About 30% of the basin's total area is occupied by urban settlement, 22% devoted to agriculture, and 42% containing pastureland.

In the middle and lower portion of the Matanza Riachuelo River is located one of the densest urban areas, that of metropolitan Buenos Aires, which city also coincides with a complex industrial zone. The basin accordingly has a population of roughly 3 million people.

Studies performed by Gómez (1998, 1999) demonstrated that the taxocenosis descriptors of epipelic diatoms—such as species richness, the degree of species tolerance to contamination, the diversity and saprobiety indices—described the extent of deterioration in the quality of the water in that basin.

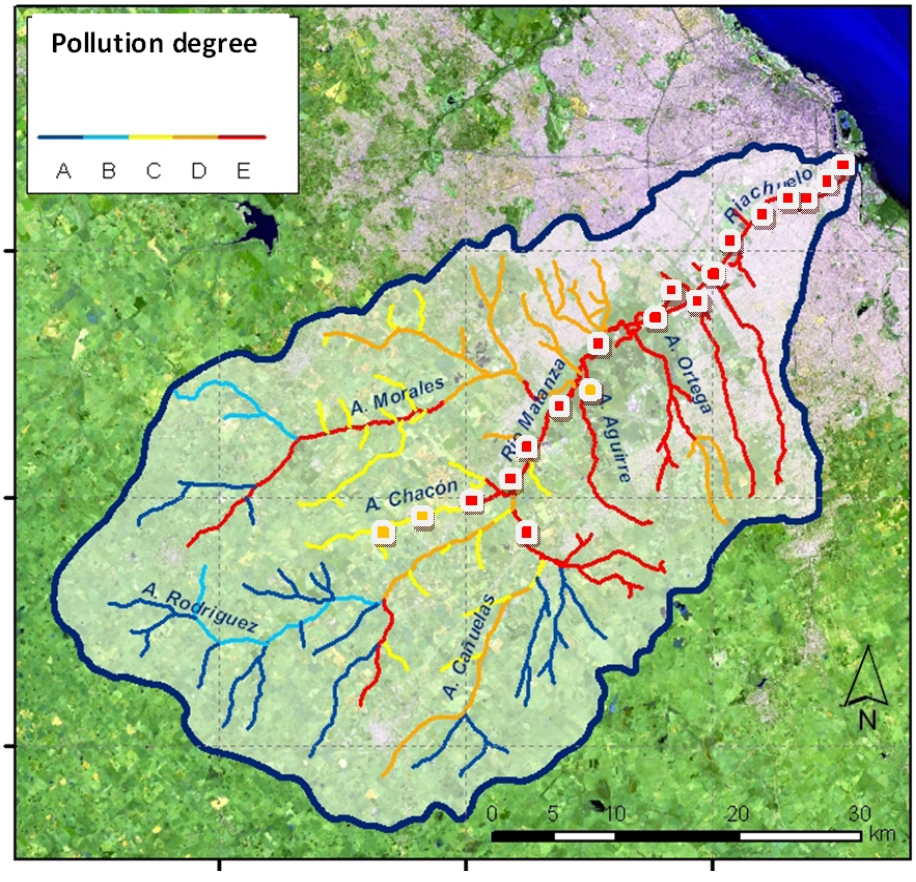

Figure 10. Diatom Pampean Index (red square, very bad water quality and orange square, bad water quality) *vs.* pollution degree in Matanza-Riachuelo basin.

Table 5. Degree of water pollution in the Matanza Riachuelo basin with respect to dissolved oxygen (DO) and biochemical oxygen demand (BOD_5)

Pollution degree	Environmental indicator
A	DO ≥ 7 mg L^{-1}; BOD5 ≤ 3 mg L^{-1}
B	DO < 7 -5 mg L^{-1}; BOD_5>3 - 5 mg L^{-1}
C	DO < 5 -4 mg L^{-1}; BOD_5 >5 – 10 mg L^{-1}
D	DO <4 - 2 mg L^{-1}; BOD_5>10- 20 mg L^{-1}
E	DO < 2; BOD_5 >20 mg L^{-1}

During the monitoring program carried out in 2008, the Pampean Diatom Index, described here in the study methodology, was employed to evaluate the state of eutrophication and organic pollution in the middle and lower basin. Figure 10 shows the values of the IDP Index and the degree of contamination of the basin in relation to environmental indicators such as DO and BOD_5, Table 5 (Acumar, 2008).

The IDP values obtained correspond to a condition of bad to very bad water quality and reveal a strong to very strong level of eutrophication and organic pollution. These findings, of course, coincide with the extremely high degree of urban settlement and industrial activity in the basin.

CONCLUDING REMARKS

The use of biota provides a comprehensive measure of all environmental impacts on a water body as well as a potential historical record of past pollution levels.

To this end, biofilms consist in a biotic complex that provides a multiplicity of information permitting the evaluation of both natural and anthropogenic stress influences. The organisms that make up the epipelon are in permanent contact with the sediments, are capable of accumulating different types of contaminants, and therefore are extremely sensitive indicators of the environmental quality of watercourses allowing them to provide early warning signals of ecological ill health.

A standardization of the basic methods and procedures is indispensable for the establishment of protocols that will permit the more frequent use of this microcommunity in biomonitoring programs for the plains' rivers. Likewise, we need to investigate the intrinsic mechanisms functioning in this type of biofilm in order to better elucidate the cause-effect interactions with the xenobiotics that the new technological industries generate.

A combination of studies that involve additional epipelon structural as well as functional descriptors will permit not only a more refined and detailed diagnosis of the Pampean watercourses' state of health but also provide knowledge that will contribute to our ability to eventually engineer the self-purification of the plains lotic systems.

Additional effective investigations -particularly of the molecular-biological type to identify of new biomarkers- in a world experiencing rapid changes involving an increasing number of known and unknown contaminants, in combination with adverse climatic shifts

and loss of biologic diversity, is the challenge confronting researchers today in the face of these mounting threats to aquatic ecosystems. This network of complex interactions will accordingly require an intensification of our efforts in order to muster the necessary information to allow, with the least degree of uncertainty, a reestablishment of high levels of environmental quality.

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