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Spatial patterns and ecological determinants of the diatom communities in an Alpine flow-regime river (Adige River, North-Eastern Italy). Implications for the ecological status’ assessment

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FOREWORD AND OBJECTIVES OF PhD THESIS

> *We know little about large rivers…defined as those which are large enough to intimidate research workers (D. P. Dodge, 1989)*

My PhD work draws from a project called PlanAdige that has been funded by the Basin Authority of River Adige to the Foundation E. Mach of San Michele all'Adige in the early 2007 for a period of three years. The main objective of this project was the study of the watercourse following an integrated approach that would encompass both its physical, chemical and biological features aiming at assessing its ecological status.

I believe we are facing a particular moment in Ecological Sciences since we have to deal with "hot topics" such as climate change, water scarcity and resources depletion: meeting today's environmental challenges requires gaining reliable knowledge about ecosystems in their deeper details (Schmitz, 2007). It is therefore vital to gather all the knowledge and the science that we know in order to cope with these big issues at our best: every little study or survey that has been made can give a contribution to tackle these concerns. For my PhD Internship I have focused my attention on diatoms and rivers, whose links have been widely used to assess watercourses' general water quality.

Diatoms are very important aquatic organisms: some researchers have estimated that there are 10 million diatom species worldwide but only 11.000 have been identified up to date (Poulin & Williams, 1998) and that they account for 25% of the living matter (Werner, 1977). Because of their abundance in marine plankton, especially in nutrient-rich areas of the world's oceans, diatoms probably account for as much as 20% of global photosynthetic fixation of carbon (ca. 20 Pg carbon fixed per year: Mann, 1999). Diatoms are essential links for energy transfer to upper tropic levels as they are a preferred high quality food source for primary consumers (Brett & Mullen-Navarra, 1997) and are the principal source of biologically induced carbon export from surface to deep waters, therefore playing a central role in nutrient cycling. Some studies have recently showed that it is expected that climate change will affect diatom abundance and community structure (Henson et al., 2010; Widdicombe et al., 2010) as well as increase the density gradient which in turn will suppress the upward flux of nutrients (Behrenfeld et al., 2006). It is expected that warmer climate will preferably favor small-sized diatom cells that show a high surface area to volume ratio (Winder et al., 2009) turning in cell size to be a powerful predictor of optimum dynamic performance (Reynolds et al., 2002).

In earlier times, ecological research on rivers focused on the descriptive research of biological communities in small streams (Minshall, 1988; Cummins et al., 1995). Research on large rivers was limited partly by tradition and partly by methodological problems, considering the large geographical scale of these ecosystems. Nowadays, it is clearer that river ecologists are developing a more integrated view of rivers: this reflects the need to increase the knowledge about these systems in all its components. As for diatoms, unfortunately, we yet do not have a full comprehension of the temporal factors shaping the diatom communities of a watercourse (Passy, 2007) also because only few studies have examined both physical and chemical parameters effects on the communities concurrently (Duncan & Blinn, 1989). Little is known about factors driving diatom species diversity and geographic distribution: studies focusing on the mechanisms generating species diversity are needed (Vanormelingen et al., 2008; Larned, 2010) since force fitting has severe consequences both on ecological and management sides (e.g. the Water Framework Directive 2000/60/CE requires ecological lumping of watercourses for ecoregions' definition).

With these premises, the specific objectives of my PhD thesis, are:

1- Assessment of the main biotic and abiotic drivers affecting the composition and temporal dynamics of the diatom communities in the River Adige. In the view that the abiotic environment has probably set the main stage for evolutionary development of specific traits and associated life-history strategies in the ecosystem (Lytle & Poff, 2004) and that these factors drive a large proportion of the subsequent biotic interactions (Biggs et al., 2005), there is a need to clarify the direct measures of these driving forces together with the associated biotic response variables, at different scales. This ambitious objective rises from the scarcity of studies that look at multiple samples per sites over times and which relate results to contemporary management problems (Kelly, 2002). Benthic diatom communities have a spatial and temporal variation:it follows that we cannot conclude much from such studies (Kelly et al., 2009). In addition, most researches up to date have focused only on a limited component of the diatom community (e.g. the epilithon) almost completely neglecting the other ones (epipelon, epiphyton and epipsammon). In addition, only sporadic comparisons were made between benthic and planktonic algae in a same watercourse (Werner & Köhler, 2005).

2- Evaluation of diatom assemblage structures and its drivers at the light of biomonitoring techniques. The need to use diatom indices for water quality is universally claimed: given that diatom communities vary with abiotic and biotic factors, great potentials exist for refining our assessment of biological and pollution condition by accounting for natural variation. These refinements will turn out to be essential for increasing accuracy, precision and fairness of ecological assessments. Much has been said about this topic but there are still some uncertainties to be discussed: there are few studies which have properly addressed the small-scale (e.g. within-riffle scale) community variations in streams (Soininen, 2007) and even fewer that have studied impaired water courses, such as Adige River.

3- Provide a framework of knowledge on diatoms' autoecology which is the first step to further deal with these organisms. Periphyton communities are solar-powered biogeochemical reactors, biogenic habitats, hydraulic roughness elements, early warning systems of environmental degradation and troves of biodiversity (Larned, 2010) and therefore it is essential to study the rules that govern their components, both individually and among taxa. There is a scarcity of such studies and this turns in consequently hampering of the prediction of changes in resource requirements and endangering of the tout-court application of diatom indices. Little is known about diatom ecology, biology and the factors driving diatom species diversity and geographic distribution (Mann, 1999;

Chepurnov et al.,2004): especially in impacted environments and this could lead to ecological biases. For instance, in the case of Adige River which is highly influenced by discharge regimes (Centis et al., 2010), it is essential to know the impact of this variable on diatom communities . It has to be kept in mind that the relationship between diatom species composition and prevailing hydraulic conditions is one of the original research problems in periphyton ecology (Butcher, 1940; Patrick, 1948).

4- These results will be worked out also as contributions to the exploration of diatom index based on phytoplankton that could help figuring the potential of this community in water quality assessment. Even if this topic has been developed for lakes (Thunmark, 1945; Nygaard, 1949; Stockner, 1971; Catalan, 2003) little has been done for rivers, if we exclude the works by Mischke (Mischke, 2007; Mischke & Behrendt, 2006; Mischke & Behrendt, in prep.), and Borics et al. (2007).

Overall, the aim of this study is to give a contribution to the evolving legacy of stream ecology: at the past two meetings of the North American Benthological Society (Grand Rapids Michigan, 2009; Santa Fè, New Mexico, 2010) has emerged the need to push towards an holistic perspective that would consider global changes occurring in these riverine ecosystems. A number of conceptual models have been proposed to synthesize empirical information (to cite some: Ward et al., 2002; Benda et al., 2004; Thorp et al., 2006) and they are indeed very valuable in organizing what otherwise might be a collection of seemingly unique case studies. This is especially worthwhile since stream ecology is moving towards the disturbance issue (Stanley et al., 2010) and the multiple stressors perspective (Thorp, pers. comm.).

1. INTRODUCTION

1.1 RIVERS AS HABITATS: PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE ENVIRONMENT

Before the development of railways and road networks, the major cities were built close to the sea or to navigable rivers, on the major maritime and river routes. After the age of stone and iron, it could be appropriate to talk about the age of rivers: the Nile, the Tigris and Euphrates and the Tiber are the witnesses and the players of the development and the birth of "hydraulic civilizations". Cities such as Rome, Florence, Turin, Verona, Pavia and Mantova among others, lie just next to a watercourse. But also cities like Milan or Bologna, apparently lying not so close, have been directly connected to watercourses development (Ghetti, 1993). At the end of the 18th century in France, for example, the only towns with a population of more than 50.000 citizens that were not near to sea ports were located on navigable rivers- Paris (between the confluence of the Seine with the Mairne and the Oise), Lyon (at the confluence of the Rhone and the Seine) and Strasbourg (on the Rhine) (Angelier, 2003) and up to the 19th century, the major economic alliances were determined on the basis of water routes. These watercourses were used as hydrological, feeding (e.g. supplying fish), transporting, agricultural resources and were also very useful to get rid of pollution caused by diseases such as epidemics (Wehr & Descy, 1998). Rivers have been a mean for circulation of ideas and unification, especially in a stretched territory such as Italy, since they have allowed navigation and communication with the piedmont zones. These watercourses have been worldwide subject to modification by mankind as reported for example by the ditches built in the Nile river for irrigation by the Egyptians (Wehr & Descy, 1998). Through the years, river systems have been dramatically altered by dams and reservoirs, channelization and land use developments throughout their drainage basins (Petts et al., 1989): some species of flora and fauna have disappeared, exotic species have invaded, the functional characteristics of the river systems have been disrupted and there has been a reduction in landscape quality and loss of wilderness area (Petts & Calow, 1996). Nowadays, the demand of water for consumption, irrigation, land and industry is still high: in the major cities, about 0.5 to 1 m^3 per day per inhabitant is required. Despite the enormous significance of rivers in the development of civilization and the shaping of land masses, the amount of water in rivers at any one time is tiny in comparison to the other water compartments. Only 2.6% of the world total volume of water consists of fresh water. Only 0.009% is stored in lakes, about 0.001% in the atmosphere while rivers contain an amount ten times smaller, 0.0001% of the world's water (Allan, 1995). Freshwater lakes, rivers and wetlands, which are main sources of human consumption and the habitat of other organisms, contain roughly 113×10^3 km^3 of water corresponding to 0.3% of total global freshwater reserves (Kalff, 2002).

What is the status of rivers today? An exaustive answer is lacking: global and national assessments are very approximate (Allan & Castillo, 2007), but nonetheless indicate that most rivers are influenced by human activities, often to a substantial extent (Revenga et al., 2000) and there can be little doubt that most streams and rivers will continue to face a daunting array of threats due to increase of human population and climate change effects (Allan & Castillo, 2007). Damming too will be a threat for watercourses: worldwide the number of large dams (defined as > 15 m in height, or > 5 m and of large reservoir capacity) exceeds 45,000 (World Commission on Dams, 2000) and the number of small dams is around 800,000 (McCully, 1996). The great challenge facing those who study rivers is to contribute to the reconciliation between the needs of humankind and the functionality of ecosystems. Driven by population and economic growth, human demands can only increase. However, increasing efficiencies, redirection of water to higher priority uses and an improved understanding of human benefits derived from healthy rivers, offer pathways towards a better balance among competing needs. Understanding of fluvial ecosystem functions allows not only exciting, conceptual scientific advances, but leads to a proper management, protection and restoration of these systems. In other words, to achieve an holistic perspective of lotic ecosystems it is necessary to identify their major interactive pathways and hierarchical structures. That the stream biota are influenced by the surrounding landscape has been recognized for a long time, notably from a paper written in 1963 by Ross and formalized by H. B. N. Hynes in his Baldi Lecture “The stream and its valley” (Hynes, 1970). The River Continuum Concept (RCC) takes this relationship one step further by focusing on longitudinal resource gradients from the headwaters to the mouth of river systems (Vannote et al., 1980, Minshall et al., 1983): it integrates stream order, energy sources, food webs and to a lesser extent nutrients into a longitudinal model of stream ecosystems. Even though the longitudinal profile of a river can be divided roughly in three zones (e.g. erosion, transfer and deposition of sediments as stated by Schumm, 1997), some studies have underlined the importance of considering a four dimensional conceptualization of lotic ecosystems to facilitate ecosystem-level understanding of the dynamics in natural lotic systems (Ward, 1989; Amoros et al., 1987). Following a four dimensional conceptualization of lotic ecosystems that provides a useful framework to understand ecosystem-level dynamics (Ward, 1989), we can therefore distinguish:

1- The longitudinal dimension. This is an obvious stream characteristics: a natural stream can be conceived as a continuum, where all stream sections are longitudinally linked and abiotic parameters such as temperature, light, slope, flow volume, stream power and size of stream bed sediments change from headwaters to sea. Flow component is very important in rivers’ studies since it allows a pronounced zonation of chemical, physical and biological factors which could be comparable to stratification in lakes (Ward, 1989). Lotic ecologists have devoted considerable attention to longitudinal patterns in biotic and abiotic variables (Minshall et al, 1983; Ward, 1986) but yet few data demonstrate upstream-downstream linkages in ecological processes, despite the fact that such links provide the theoretical foundation of the river continuum (Vannote et al., 1980) and nutrient spiralling concepts (Newbold et al., 1992).

2- The lateral dimension. Interactions along this dimension include active and passive movements of organisms between the channel and the adjacent riparian/floodplain system and exchange of nutrients and organic matter (Ward, 1989). Stream ecologists have long recognized the profound influence that surrounding lands have on the stream ecosystems (“In every respect, the valley rules the stream”- Hynes, 1975). In addition, many indirect interactions occur such as the influence of the flood regime on the composition, productivity and succession state of riparian vegetation, which in turn influences channel morphology, aquatic temperature and light regimes, habitat heterogeneity and the quality, quantity and temporal sequencing of allochtonous inputs. The strength of the interactions between river channels and fringing floodplains that have been so well documented for tropical river systems (Lowe & McConnell, 1987) has not been fully recognized by lotic ecologists because dredging, snag removal, flow regulation and other anthropogenic alterations have severed major interactive pathways in much of the world (Ward & Stanford, 1989). Longitudinal and lateral influences are superimposed since streams are intimately linked to the adjacent lands, especially in watercourses not constrained by topography.

3- The vertical dimension. Lotic ecologists have been slow at recognizing the possibility that significant interactions may occur between running waters and contiguous ground water (Hynes, 1983). In this sense microbial biofilms play an important role in nutrients cycling and energy balance of watercourses. Hyporheic organisms, for example, are essential for nitrification or decomposition of organic material. There is a continuous interaction between these two parts: for instance, floods may reshape the hyporheic zone and, in its absence, it could become isolated from the surface water because of sediment clogging.

4- The temporal dimension. This dimension becomes manifest through the permanent change of the location of gravel bars, islands and channels within a river corridor. This dimension is essential to understand one of the facets of floods' action: it is considered to be the dominant driver of temporal variation in rivers. Its tumbling action on sediments moulds the communities living in the river which in turn affects species diversity and favors species with short life cycles and a high adaptation potential. If floods are rare, poorly competing species become extinct and species diversity is expected to be at its maximum if flood frequency is intermediate (e.g. The Intermediate Disturbance Hypothesis by Connell, 1978).

Lotic ecosystems are therefore open systems that form an integral part of the surrounding landscape with which they are highly interacting: viewing the dynamic and hierarchical nature of lotic ecosystems as interactive pathways along four dimensions is an attempt to provide a synthetic framework for lotic ecology, which has been proven to be very useful for Alpine streams too (Maiolini & Bruno, 2007).

The strict emphasis on the longitudinal dimension of rivers has been amended by emphasizing lateral interactions (e.g. Flood Pulse Concept- Junk et al., 1989; Riverine Production Model- Thorp & Delong, 1994) or by integrating longitudinal, lateral and vertical dimensions on a catchment scale (Lorenz et al., 1997). Another attempt of classification of river ecosystems has been proposed more recently following the legislative needs imposed by the Water Framework Directive 2000/60/CE. Several components such as geology, soils, land use, land surface form and potential natural vegetation are used to divide large, complex landscapes in "ecoregions" which are regions with relative homogeneity in ecological systems and relationships between organisms and their environments (Pan et al., 1999). Ecoregions would serve as a spatial framework that may facilitate establishment of scale-explicit water quality standards for stream bioassessment. This again points to the fact that measurements of river ecosystem functioning requires integration of water quality, hydrology (discharge and stream velocity) and geomorphology describing the structure of the river channel, riparian zones and floodplains, and, finally, ecological information on species diversity and abundance (Lorenz et al., 1997).

The very peculiar functioning of running-water ecosystems brings us back to the definition of limnology given by Wetzel (1983). The study of running water is based on concepts other than those of lacustrine waters- and especially on the fundamental role of hydraulics, the primary limiting factor as will be assessed further. The horizontal gradient of energy and matter flow, the organizing role of current on the substrate and communities and the problems of stability or instability of different substrates make running water a unique chapter in the study of continental waters. Modern-day threats to rivers have been reviewed by a number of authors (among others: Malmqvist & Rundle, 2002; Dudgeon et al., 2006; Strayer, 2006). These works include studies on habitat alteration which causes a reduction in dispersal and migration and therefore on biodiversity because of damming and channelization (Nillson et al., 2005) and non- indigenous species that can result in strong ecosystem-level effects (Olden et al., 2006). But also pollution related effects that harm food chains and give toxic outcomes through biomagnifications (Cheung & Dudgeon, 2006) and climate change effects which alter evapotranspiration patterns and flow regimes therefore introducing disturbance (Tuchman et al., 2002). Causes of the imperilment of river ecosystems and their biota are therefore diverse but ultimate causes can be found in the conflicting demands on fresh waters, changing land use and the many unsustainable practices that characterize growing populations and expanding economies throughout the world. Most human activities that harm river ecosystems show an upward trend throughout the 20th century, and although some aspects of pollution have ameliorated in recent years, other pressures, including species invasions and climate change, are expected to worsen (Allan & Castillo, 2007).

In this chapter I will give a brief introductory description of the main features of the rivers, in terms of their physical, chemical and biological characters since an exhaustive report on their effects would go beyond its scope. These informations will be propaedeutic to the exposition and discussion of results found during my PhD fellowship.

1.1.1 Physical features

STREAMFLOW AND WATER MOVEMENTS. In fluvial systems, the flow of water is a dominant and characterizing variable that influences diverse aspects of stream environment (Biggs & Close, 1989; Ghosh & Gaur, 1998; Hart & Finelli, 1999), best described as a “subsidy-stress” variable owing to both its contemporarily beneficial and detrimental effects (Biggs et al., 1998). Watercourses are always in motion and this characteristics is one of greatest significance for the distribution of heat, gases, nutrients and organisms, both in water and sediments: so current benefits algal growth by enhancing nutrient supply (Borchardt, 1996; Stevenson, 1996). However, current exerts a shear stress on benthic algae, which can cause cell sloughing and high flow disturbs and scours the substrate (Diehl et al., 2005) and this is surely not a benefic effect for algae. Biggs & Smith (2002) have demonstrated that in case of overturned substrates, richness is lower: current velocity can influence the arrival rate of algal cells and therefore the process of colonization, which is low near the substrate and increases towards the centre of the channel. Resistance along the channel and banks results in a spiralling flow pattern that alternates scouring and deposition at relatively uniform distances along the stream channel and determines a meandering channel morphology.

All these different effects played by water velocity and its related variables (such as discharge, water depth, sediment tumbling, turbidity etc) give it a “central role” among the variables in sustaining the ecological integrity of flowing water systems. As in fig. 1.1, five components of the flow regime can be distinguished (magnitude, frequency, duration, timing and rate of change) and these, in turn, influence ecological integrity through cascading effects on other primary regulators of integrity.

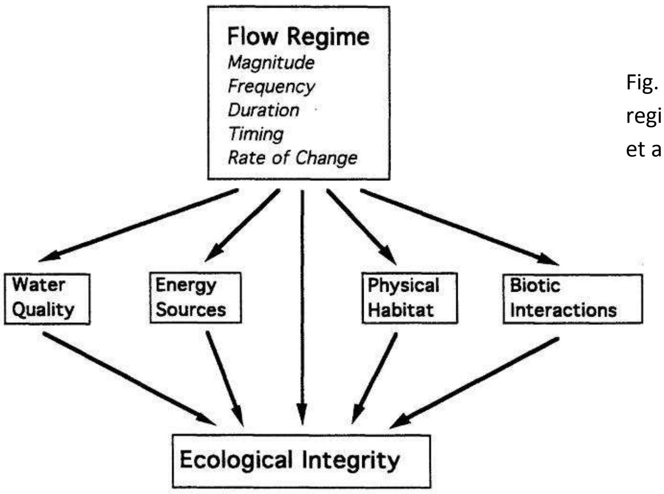


Fig. 1.1- The direct and indirect influence of flow regime’s components on ecological integrity. After Poff et al, 1997

LIGHT. Solar radiation is of fundamental importance to the dynamics of aquatic ecosystems. Nearly all the energy that controls the metabolism of lakes and streams is directly derived from the solar energy utilized in photosynthesis. Light does not only drive the photosynthetic process and allows vision, but also determines the different amounts of solar energy received annually at different latitudes, producing a heating gradient over the Earth: this gradient is ultimately responsible for both latitudinal difference in precipitation and the winds that bring about currents and turbulence (Kalff, 2002). Because streams are so closely linked with the surrounding terrestrial landscape (longitudinal- lateral dimensions), lotic light regimes are highly influenced by terrestrial objects such as trees or geologic features, as reported by Hill et al. (1995) and Minshall (1988): The consequences for stream bioenergetics is an integral part of stream ecosystems as understood earlier by stream researchers (i.e Vannote et al., 1980).

TEMPERATURE. Temperature is a critical environmental variable determining the metabolic rates of organisms, their distribution along a river’s length and over geographic regions and quite possibly their success in interacting with other species (Allan & Castillo, 2007). Regardless the direct or indirect controlling mechanisms in a particular stream, temperature plays a vital role in the presence/absence, life-histories and spatial distribution of stream organisms, from microscopic to macroscopic ones (Ebersol et al., 2001; Hauer & Hill, 2006). Temperature of running waters usually varies on seasonal and daily time scales and among locations due to climate, extent of stream-side vegetation and the relative importance of groundwater inputs. Nevertheless, the temperature of large rivers is unlikely to be affected much by shading, as their size conveys considerable thermal inertia and virtually ensures that they are highly exposed to the sun. In small streams, however, riparian forest moderates stream temperature by reducing the amount of solar radiation reaching the water surface, which also reduces the extent of temperature change between day and night (Beschta et al., 1986). Seasonal changes in water temperature in rivers closely follow seasonal trends in mean monthly air temperature, except that in winter water temperature does not fall below 0℃, and riv ers warm up more slowly in spring that does the atmosphere. The effect of temperature on productivity has been investigated by Morin et al. (1999) who developed empirical models to predict primary production from chlorophyll *a* and water temperatures for stream periphyton as well as lake and ocean phytoplankton. Although stream production was lower in stream periphyton than in lake or marine phytoplankton, presumably because of reduced nutrient diffusion into algal mats, production was more strongly related to water temperature in stream periphyton than for phytoplankton of either lakes and oceans. Human actions can strongly influence stream temperatures in a number of ways. By reducing river volume, water withdrawals reduce the temperature buffering capacity of the river; furthermore reduced flows may result in longer residence times and thus greater heat adsorption (Paul & Meyer, 2001). The impoundments created by dams can greatly affect stream temperatures and, although dams may create a variety of other effects, temperature's impact can be significant: the majority of impoundments are the result of small, surface-release dams and they are most likely to raise downstream temperatures during the summer. These impoundments act like small lakes and beaver ponds that tend to increase stream temperatures because they increase the residence time of water and the surface area exposed to radiation (McRae & Edwards, 1994). Major dams with hypolimnetic releases affect the rivers thermal regimes (Grubbs & Taylor, 2004; Caissie, 2006; Toffolon et al., 2009) and this phenomenon has been studied in the Adige River too (Zolezzi et al., 2010) showing the high impact that these aspects have on the benthic communities.

SUBSTRATE. Substrate is a complex variable of the physical environment that includes everything on the bottom or sides of streams or projecting out into the stream, not excluding a variety of human artefacts and debris, on which organisms reside (Minshall, 1984). Although ecologists borrow freely from the geomorphologists' tool kit, the ecologist's focus is on the characteristics of mineral substrates that may influence their suitability as biological habitats. A greater range of particle sizes and thus substrate heterogeneity, benefits many taxa (Minshall, 1984) and the permeability of the sub-surface region (known as hyporheos) adds to the vertical dimension by allowing water to circulate and transport gases, nutrients and fine organic material. Also diversity seems to be influenced by this variable: abundance and taxa richness are typically low in fine substrate and increase with substrate size at least up to gravel and cobble (Mackay, 1992). It would be inappropriate to forget the interaction between current and substrates in lotic environments: they are inextricably interwoven (Death, 2000; Matthaei et al., 2000). The influence of this variable on biological riverine communities will be developed further in section 1.2.2.

1.1.2 Chemical features

NUTRIENTS. It is long known that nutrients are transported from drainage basins to the receiving waters. The effect of the catchments' geology on the chemistry of lakes and rivers was first noted by A. Forel 100 years ago (Forel, 1892), acknowledged in research by I. Naumann early in the 20th century (Naumann, 1919) and incorporated in limnology by W. H. Pearsall (1932). Some decades later, his ideas were further developed and much better quantified by R. Vollenweider (1968) in response to public concern about increasing algal biomass and turbidity in lakes draining rich agricultural and urban areas. His work and subsequently most other research concerned with the enrichment of waters with plant nutrients (eutrophication) focused on phosphorus and nitrogen. These two elements were already known or suspected to be the nutrients that normally limit production rates and biomass in plant communities. From then onwards, a number of studies have looked at chemical concentrations in watercourses (e.g. Hynes, 1960; Gibb, 1970; Bond, 1979), at how anthropogenic changes to watershed vegetation modified stream chemistry (e.g. Johnson & Swank, 1973; Vitousek & Reiners, 1975) and at how human activities have profoundly altered their dynamics by increasing the available supply in surface waters (Carpenter et al., 1997). Nutrients enter a stream reach from upstream, groundwater and surface runoff and atmospheric inputs: they are incorporated into organisms by biological uptake and assimilation, move through the food web by consumption and subsequently are re- mineralized back to inorganic form by excretion and the decomposition of organic matter. Although the cycling of nutrients between the abiotic and biotic compartments is often thought of as taking place within fixed boundaries, unidirectional flow adds a pronounced spatial dimension to nutrient cycling in running waters: nutrients generated at one location will be typically transported for some distance before subsequent reutilization. The term "nutrient spiralling" describes the interdependent process of nutrient cycling and downstream transport (Webster & Patten, 1979). Nutrients that are in demand relative to their supply should be taken up rapidly, resulting in short transport distances and rapid cycling relative to elements whose supply is less critical. Nutrient dynamics in streams are further complicated by various abiotic uptake and release mechanisms that partially regulate nutrient concentrations and, particularly in the case of nitrogen, by several transformations among inorganic states that yield energy to specialised bacteria. Nutrient supplies can limit rates of photosynthesis and thus the rate at which basal resources for stream food webs are produced. Biological diversity is assumed to decrease with increasing nutrient levels (Wassen et al., 2005) while algal species richness peaks at intermediate nutrient conditions as in the Intermediate Disturbance Hypothesis (Leira et al., 2009).

Phosphorus. Phosphorus occurring in streams enters as orthophosphate (PO_4^{3-}) dissolved in water and attached to inorganic particles in suspension, as dissolved organic molecules and in particulate organic form mainly in bacteria and detrital particles. It is typically high in demand relative to supply and its retention is high when released to receiving water-ways in well-watered and vegetated drainage basins in temperate zones (Wetzel, 2001). The phosphorus that is released is primarily in the dissolved organic form, supplemented by organic phosphorus contained in particles washed from the land with additional dissolved phosphorus from the atmosphere. Total phosphorus (TP) is determined by analyzing unfiltered samples and encompasses all forms of P, including those present in organisms, detritus and absorbed to inorganic complexes such as clays and carbonate (Wetzel, 2001). An operational category known as soluble reactive phosphorus (SRP) based on the reaction of soluble P with molybdate is commonly used as a measure for ortophosphate. However, there is evidence that the SRP fraction can also include polyphosphates and therefore may overestimate ortophosphate concentrations (Dodds, 2003). Therefore, SRP is usually considered the best indicator of what is immediately available for uptake, but because P cycles rapidly among its various states, TP may be a better measure of overall availability of P (Allan & Castillo, 2007). Atmospheric inputs can be significant in areas where P is scarce as in the case of Caura River (Lewis et al., 1987) while phosphorus generated from plant breakdown and stored in the soil organic layer is an important input entering streams by surface runoff and subsurface pathways (McDowell et al., 2001). Anthropogenic sources of phosphorus include municipal and industrial wastewater (Edwards et al., 2000; Goller et al., 2006): dissolved inorganic P often is in the range of 0.05-0.10 mg P L^{-1} in stream receiving agricultural runoff and can reach 0.30 mg P L^{-1} below sewage outlets (Allan & Castillo, 2007). Uptake occurs both by physical adsorption to benthic substrata and to particulate seston as well as by assimilation by attached biota. Although uptake of total dissolved phosphorus by planktonic seston may be small (ca. 1% of the total uptake) in small streams (Wetzel, 2001) , sestonic uptake increases markedly in larger streams, particularly when dominated by phytoplankton (Newbold, 1992).

Nitrogen. Nitrogen, alike phosphorus, is commonly the element in greatest demand by plants and the heterotrophic microbes relative to supply (Kalff, 2002). Nitrogen occurs in freshwater ecosystems in many chemical states: Dissolved Inorganic Nitrogen (DIN) includes ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). Dissolved inorganic nitrogen (DON) consists of amino nitrogen compounds (Polypeptides, free amino compounds) and other organic molecules; while most particulate organic nitrogen (PON) occurs as bacteria and detritus. Based on a review of nutrient concentrations in world rivers, the average of total N, including rivers that are enriched by human activity, is 0.94 mg N L^{-1} (Meybeck, 1982). The sources of the various forms of N in stream include atmospheric deposition, N fixation and terrestrial inputs carried in runoff and groundwater (Hagedorn et al., 2000; Boyer et al., 2002) whom fertilizers have greatly increased since 1950s (Schlesinger, 1997).

Carbon Flux. Rivers carry multiple forms of carbon, including particulate organic (POC) and inorganic (PIC) carbon, dissolved organic (DOC) and inorganic carbon (DIC generally in the form of HCO^-_3 and dissolved CO_2, $CO_2(aq) + H_2CO_3$). The concentration of dissolved inorganic carbon (DIC) is of tremendous importance in aquatic ecosystems because it (1) buffers fresh waters against rapid changes in pH; (2) determines the amount of inorganic carbon available for photosynthesis; (3) provides the great bonding capacity of bicarbonate (HCO^-_3) and carbonate (CO_3^{2-}) ions for cations; (4) makes the ionic carbon concentration an important component of the anion concentration; (5) removes inorganic carbon and adsorbed materials from the water column upon precipitation as $CaCO_3$ aggregates. With a global average content of atmospheric carbon dioxide of 0.037%, about 1.10 mg L^{-1} can dissolve in pure water at 0℃. Solubility is temperature dependent and it declines to 0.65 mg L^{-1} at 15℃ and 0.48 mg L^{-1} at 25℃ (Hutchinson, 1957). The proportion of different ionic forms of DIC is controlled by pH that defines the acidity and the alkalinity of the waters. The pH in the stream will affect the structure and the composition of the community: e.g. low pH is especially harmful to immature fish and insects and it can also speed the leaching of heavy metals which are harmful to fish. The origins of the various forms of the available C are multiple (Garrels & MacKenzie, 1972; Meybeck, 1993) and there are numerous physical, geologic, biotic and anthropogenic forces that have shaped the status of riverborne C over geological and historical times. Anthropogenic change may be an especially important factor in the future although gradual increases in atmospheric CO_2 might not themselves be critical in chemical weathering. Accelerated permafrost melting and change in UVB irradiance, which enhances DOC bacterial degradation after photolysis will be active players in the future of global river C as well. In addition, land-use and land-cover changes, such as wetland filling and the decrease of organic soil carbon content through industrial fertilization of agricultural land, will be important. Land erosion will accelerate particulate carbon transfer but most of it may not ultimately reach the ocean due to storage on slopes, floodplains, lakes and reservoirs. Lake and reservoir eutrophication may also store particulate carbon for 10^2 to 10^4 years (Meybeck, 2005). Direct waste emissions of organic material into rivers represent a large potential C source since the sewage treatment rate does not yet match overall population growth: in fact river eutrophication, now widely developed in low-turbidity, nutrient-saturated rivers, may add up to 6 mg L^{-1} of algal POC, highly labile. This can create near-anoxic conditions when reaching turbid coastal waters like the estuaries of Western Europe. A global database for large reservoirs has already demonstrated a “runoff aging” up to two years for some basins which allows for more in-basin aquatic processing of riverine carbon, as well as an enhanced sediment storage, estimated now to be at least 25% of global river flux (Meybeck & Vörösmarty, 1999).

Nutrient limitation of algae was thought to be less common in streams than in lakes or in the ocean because the continuous flow of water in streams was expected to replenish nutrient supplies to biota (Hynes, 1969). This view began to change at the beginning of the 1980s with the pioneer works by Grimm and Fisher (1986) and Hill & Knight (1988) that culminated in the work done by Francoeur (2001) who did a meta-analysis of 237 stream nutrient-addition experiments and found that co limitation by N and P was more common than limitation by either nutrients alone. Overall, stream ecologists assume an influence played by nutrients on stream biota but this is highly influenced by the high number of abiotic and biotic factors playing simultaneously on a watercourse: especially discharge and its variability have a high effect to determine uptake rates and rule communities’ dynamics therefore nutrients are just “part of the game” (Grace & Malhi, 2002; Allan & Castillo, 2007).

Thus the need for broader ecological knowledge of rivers has allowed ecological awareness to rise: many actions wanted to touch people consciences on the environments they and their siblings will live in. Actions such as the International Biological Program (IBP), aimed at mapping and characterizing all the environments in the 1970s gave some practical measure of the Earth’s capacity to support mankind (Smith, 1968). And from then onwards many scientists became aware of widening their perspectives: Rachel Carson’s Silent Spring (1961) alerted about the use of pesticides and consequently led to government regulation of chemicals in the environment. The investment of resources and brainpower to discover that phosphate pollution from households caused massive algal blooms that choke out other forms of life in major freshwater bodies (Schindler, 1974) was nothing short of an ecological Manhattan project that lead to the

Clean Water Act. As Robert Peters (1991) put it “secured theories, like the operationability of species diversity are, after all, not that water tight” but ecology is the study of interrelations among organisms and their environments and therefore we need to assess the capabilities of this planet as a life-support system. Asking functional questions is a powerful way to study these interrelations and contribute to environmental problem solving because it forces one to think about the root cause of a pattern or process. Understanding these complex interdependencies is the fundamental purpose of ecology and probably the highest duty of ecological research.

SALINITY. Runoff water from catchments is dominated by eight major ions (the cations Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} and the anions HCO_3^{-}, CO_3^{2}-, SO_4^{2-} and Cl^{-}) each normally having a concentration higher than 1 mg L^{-1} (Allan & Castillo, 2007). The remaining elements are either present in non-ionic forms (like silica) or are present in fresh waters in such low quantities (e.g. Fe, N, P, Co, Mo) or trace elements that they have a negligible effect on the salinity of the river (Mulholland & Webster, 2010). In this work, silica should be looked with a deeper focus since it is the major component of the diatom frustule. It strongly affects diatom population dynamics as stated by Lund (1949; 1950; 1954; 1955) and its supply can therefore be expected to become limiting (Borchardt, 1996; Wetzel, 2001). Salinity is assessed through conductivity which is determined as the electrical conductance of water and an approximate measure of total dissolved ions. Conductivity has the ability to integrate several watershed processes, thus indicating its geological nature and has, in several cases, been considered as surrogate for nutrient enrichment, because major ions are not intensively involved in biological processes and relative fluctuations in conductivity are smaller than those for nutrients (Biggs, 1995; Soininen et al., 2004). The observed salinity and component ions in fresh waters are usually determined by chemical reactions that take place in the soils and rocks of the catchments. The ease of breakdown (weathering) and the resulting salinity of the water decrease as rock hardness increases from soft sedimentary carbonate rocks (e.g. limestone or dolomite) or somewhat less soluble sedimentary stones (e.g. siltstone and sandstone) to harder igneous stones (e.g. basalt and dolomite) and ultimately to very hard crystalline rocks (e.g. chert and quartzite). Weathering in non arid regions is not only the result of chemical decomposition but also of biological weathering processes in soils and on vegetated rock surface. The combined processes far outstrip the physical disintegration (physical weathering) of rocks. The three major mechanisms controlling the salinity of the world surface waters are rock dominance, atmospheric precipitation and the evaporation-precipitation process (Eilers et al., 1992; Gibson et al., 1995).

DISSOLVED OXYGEN. The dissolved oxygen (DO) levels in aquatic systems probably reveal more about their metabolism than any other single measurements (Kalff, 2002). Concentrations reflect the momentary balance between oxygen supply from the atmosphere and photosynthesis on one hand and the metabolic processes than consume oxygen on the other. Low DO levels not only affect the distribution and growth of fish and invertebrates, but also have a major influence on the solubility of phosphorus and other inorganic nutrients through an influence on the redox potential. The solubility of DO in rivers is primarily determined by water temperature and, secondarily, salinity and atmospheric pressure (altitude). Oxygen content varies seasonally and from source to mouth (Hynes, 1970). Variation is very often driven by discharge: in the Mississippi and the Amazon rivers, for instance, high water is accompanied by lowered oxygen concentrations and these are brought about by the wash-in of organic matter and the decrease of photosynthesis caused by turbidity (McClain & Naiman, 2008). In many streams there is also a diurnal variation in oxygen content to take in account. As Hutchinson (1957) put it “a skilled limnologist can probably learn more about the nature of a lake or a river from a series of oxygen determinations than from any other kind of chemical data. If these oxygen determinations are accompanied by observations on Secchi disc transparencies, water colour and some morphometric data, a very great deal is known about lakes and rivers”. This statement remains valid today as it was 40 years ago. What has changed during the interval is the ease and precision by which DO concentrations can be measured: there are various ways such as the use of gas-electrode and the optical method but the most widely used technique for determining DO levels in fresh waters is the oxidation-reduction titration technique developed by L.W. Winkler (1888).

Biogeochemical oxygen demand (BOD) is a chemical procedure for determining the amount of oxygen needed by aerobic biological organisms in a body of water to break down organic material present in the water sample at a certain temperature over a specific time period: organic pollution may significantly reduce DO concentrations in entire stream reaches as microbial processes consume the oxygen of the water (Hauer & Hill, 2006). This test is widely used as an indication of the quality of water (Sawyer et al., 2003) as well as chemical oxygen demand (COD). The latter is used to indirectly measure the amount of organic compounds in water (e.g. the amount of organic pollutants found).

1.1.3 Biological features

Numerous studies provide strong evidence of the important influence of grazers in fluvial ecosystems both on benthic algae (Feminella & Hawkins, 1995) and phytoplanktonic algae (Gosselain et al., 1998). Grazers can reduce algal biomass and influence community composition by selectively eliminating some species and growth forms (Rosemond et al., 2000) and can also affect nutrient content and diversity (Poff & Ward, 1995; Hillebrand et al., 2004). In addition, grazing can stimulate algal growth and overall productivity through the removal of senescent cells. A conceptual model of the control of periphyton biomass and composition in unshaded temperate streams considers flood disturbance and nutrient limitation to be the primary controls and grazing as an important factor in stable systems (Biggs et al., 1998). This is primarily because larger and more effective grazers such as caddisflies and snails prefer habitats with slow currents while where the frequency of flooding is higher, mayflies dominate but appear less able to control periphyton (Allan & Castillo, 2007). In the water column, zooplanktonic grazing is mainly caused by small, fast-reproducing organisms like rotifers (e.g. Hynes, 1970). But, as pointed out by Hynes, however, there has been no convincing, experimental evidence of a strong tropic relationship between phytoplankton and zooplankton in rivers. Nevertheless, some observations on the composition of river phytoplankton, along with evidence from model simulations, indicate that changes attributable to grazer control may at times take place in large rivers (Gosselain et al., 1998). However, such biotic interactions can take place only when physical constraints are reduced, i.e. when discharge is low, and when increased transfer time, high temperature and availability of grazeable algae allow high zooplankton biomass. Under the usually moderate environments in which most grazing studies are carried out, top-down control of algae by grazing appears to be at least as strong as bottom-up control by nutrient supply. Disturbance, particularly due to extreme flow, can alter the grazer-algal dynamics by reducing grazer abundance and heavy grazing pressure can reduce algal biomass to a level where it is less vulnerable to scouring due to high flows.

1.2 DIATOMS OF RIVERS AND IMPACTS OF PHYSICAL, CHEMICAL AND BIOLOGICAL VARIABLES

Diatoms are classified as algae, Division Bacillariophyta. They are typically ranging between 5 and 200 µm in diameter or length although sometimes they can be larger than 1 mm. They are unicellular, eukaryotic organisms (heterokont algae with chlorophyll *a* and *c*) characterised by their siliceous cell walls and their yellow-brown pigmentation. Each diatom cell consists of two more or less identical thecae, one slightly larger than the other. The valve face of each theca is intricately patterned allowing even most fossil taxa to be identified at the specific level. Although diatoms may have older origins (Round, 1981; Medlin et al., 1993) the first definitive evidence for diatoms in the geological records is from the early Jurassic (200- 145 million years ago) (Rothpletz, 1900) while the oldest, well-preserved, fully-silicified flora is dated back to the early Cretaceous (that spans from 145 to 65 million years ago) (Harwood & Gersonde, 1990). The earliest known freshwater diatoms do not occur until the Eocene (60- 34 million years ago) (e.g. Lohman & Andrews, 1968) but by the Miocene (23-5 million years ago) both marine and freshwater floras are diverse and many taxa have very similar forms to modern living species. There are 10 million diatom species worldwide and studies have shown that they account for 25% of the living matter (Werner, 1977). The total net primary production of plants on Earth is estimated to be in the order of 1.4 x 10^4 kg dry weight per year, of which at least 40% is produced by the marine phytoplankton species (Golley, 1972) and diatoms would provide 20-25% of globally fixed carbon and atmospheric oxygen (Mann, 1999). This means that about 2 x 10^{13} kg carbon is fixed in organic form per year by the various groups of marine phytoplankton species (Strickland, 1972).

1.2.1 General features

DIATOM FRUSTULE. Much of the uniqueness of diatoms is related to their silica cell-wall: the cell contents are similar to other eukaryotic algae including the nucleus, chloroplasts and mitochondria but silica leads to the rigidity of the cell walls, constraints aspects of reproduction and leads to the preservation of diatom frustules as fossils. The frustule has two different parts (outer-epitheca; inner-epitheca) which are adapted to each other to that of a Petri dish. The remarkable properties of this siliceous cell wall are its transparency, which allows the entrance of the light to the chloroplasts of the cell and its perforated structure, which makes possible the transport of gases and solutes. Throughout the valves there are several structures: in some pennate diatoms (Monoraphidineae and Raphidineae) there is a raphe (longitudinal slot in the theca) in one or two valves, respectively. It has been suggested that this structure is designed to prevent the longitudinal splitting of the valve under turgor.

LIFE CYCLES AND CELLS REPRODUCTION. Diatoms live as diploid cells that progressively increase in volume as they accumulate photosynthetic products. When they reach a threshold volume, they divide unusually since their vegetative cell division involves a successive diminution in population mean cell size as daughter cells are generated by the laying down of daughter thecae back to back with the parent cell, constrained by the relative rigidity of the cingulum wall. This size reduction process was independently reported by MacDonald (1869) and by Pfitzer (1869) and has subsequently become known as the MacDonald-Pfitzer rule. Size is restored after sexual reproduction involving gamete production, auxosporulation and the production of new silicified initial cells (Fig. 1.2).

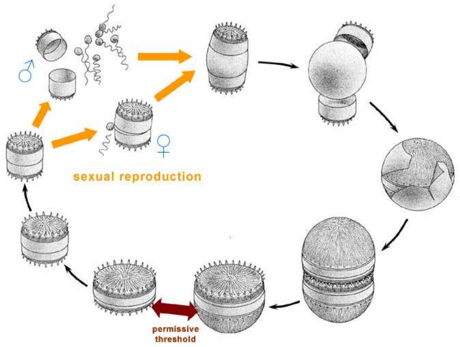


Fig. 1.2- Life cycle of diatoms. Modified from Round et al (1990)

An extensive talk on diatom life-cycles and size reproduction is beyond the aims of this chapter but it is worth knowing that in centric diatoms overall shape is unaltered while in pennate diatoms reduction is greater along one axis so that shape as well as size is altered during cellular mitosis.

LIFE FORMS. Using an “ecological stick yard”, diatoms display different ecological preferences leading to discriminate between those who live in the water column (planktonic diatoms) and those who live in the river bottom (benthic diatoms).

About plankton, a further distinction has to be done: it is appropriate to talk about diatoms that spend their whole life-cycle suspended in the water column (euplankton taxa), diatoms that have some of their life-cycle resting on the sediment (meroplankton) and diatoms that have their true habitat in the benthos but can be found resuspended in the water column (tychoplankton) (Reynolds, 2006). Although this definition is not exhaustive, in the sense that it is difficult to discriminate beneath them (Reynolds et al., 1994) (e.g. one individual taxa can have different living patterns throughout its life), it is very rare to find proper river blooms of diatoms, unless there dead zones with slower currents that could serve as “impoundments” (Reynolds, 2000, 2006). This is the opposite of what happens in lakes (see among others Hutchinson, 1967; Reynolds & Irish, 2000) or for cyanobacteria in warm-waters rivers (see among others Falconer, 1999; Moreno et al., 2003; Guven & Howard, 2006). Whilst most planktonic diatoms are solitary, some other form colonies. These are of different kinds: in the case of *Tabellaria* spp*.* they can be star-shaped, zig-zag, coiled or straight with individual cells linked together at their apices by mucho-polysaccharide strands or pads. Other taxa such as most of the *Fragilaria* and *Aulacoseira* spp. form spines linked cells.

Benthic diatoms, on the other hand, are those associated with substrates: the main habitats are the epilithon (attached to pebbles, cobbles or stones) whose solid current-exposed surfaces favour the establishment of flat cells or those that have strong adherence to the substratum (*Cocconeis, Achnanthes* and *Encyonema*) while under low flow rates stalked and filamentous forms may grow into tufs (*Synedra*). Another kind of habitat is the epiphyton (attached to plants) whose diatom communities are less tightly adherent as they use the hydrodynamic protection of the host: common epiphytes are *Gomphonema, Cocconeis* or *Tabellaria.* Others diatoms are those living on sediments (epipelic) that don't need to be attached but must have a particular motility: *Gyrosygma, Diploneis, Amphora* and *Mastogloia* possess this ability and are common in this habitat. Finally, diatoms living attached to sand grains (epipsammic) are a very distinct community firmly attached by their entire valve to resist the movement and related abrasion of the substrata in relation to water: typical examples are *Cocconeis* and *Amphora* (Patrick, 1977; Sabater, 2009). These taxa are normally capable of surviving potentially long periods in dark, anoxic environments, probably by entering a resting phase until the sand grain is mixed back into the photic zone by wave activity (Jewson & Lowry, 1993). Benthic diatom communities are traditionally considered as being regulated more by local environmental conditions rather than by broad-scale climatic, vegetational and geological factors (Pan et al., 2000; Soininen, 2004) even if the importance of geographical factors in explaining diatom distribution has now been stated to be underestimated (Kociolek & Spaulding, 2000). Periphyton communities are considered to be highly important for bioassessment (see further) and this has profit from several researches done over the last century (i.e. among others: Descy, 1979; Stevenson et al., 1996; Wehr & Sheath, 2003; Azim et al., 2005).

VALVE ULTRASTRUCTURE AND KEY TAXONOMIC CHARACTERS. Although the overall shape and structure of diatom cells are important for generic identification of diatoms, species and variety level taxonomy is based on intricate detail of the cell wall, especially the valve. Whilst most key features of diatom valves can be recognised at the light microscophe (LM), use of the scanning electron microscopy (SEM) since the 1960s has revealed both finer features and new structures and given rise to new terminology.

Almost all freshwater diatoms contain areolae that appear to be simple perforations through the cells wall although internally they are occluded by a finely perforated velum. Areolae are usually arranged in rows (or striae) running at right angles to the valve margin. Striae characteristics are used extensively for identification purposes. The spacing between striae (striae density) is fairly consistent within a species but varies between species and is consequently a taxonomic character used almost universally in diatom floras (usually expressed as the number of striae in 10 microns).

Some diatoms, especially centric forms, have tube-like processes that penetrate the basal siliceous layer. They are often difficult to observe with the LM but are very prominent in internal SEM images. There are two types, labiate processes (or rimoportulae) and strutted processes (of fultoportulae). Labiate processes are restricted to centric and araphid diatoms and usually occur singly on one or both valves in a marginal (centric) or polar (araphid) position. Strutted processes only occur in centric diatoms. They are more numerous than labiates processes and occur both on the valve face and around the margin of the valve.

One of the most important features of some pennate diatoms is the raphe. It is used as a major character in separating sub-orders and classes, in classification systems. It is basically an elongated fissure, or pair of fissures through the valve wall. On each valve, the raphe is divided into two, separated by the central nodule. The ends of the raphe, especially at the apices, often have characteristics terminal shapes and structures that are also of taxonomic value.

DIATOM SYSTEMATICS. Diatom classification progressed rapidly in the 19th century as microscopes and microscope lenses improved, and as collections extended to all parts of the world during the period of European colonialism and global exploration. By the late 19th century and early 20th century, attention was being given to aspects of diatom ecology, to their value as indicators of water pollution and to their potential as indicators of environmental change from sediment records.

In the early 19th century, it was debated whether diatoms should be placed in the animal or plant kingdom because of their mobility: eventually, because of the presence of the chloroplasts, they were placed in the plant kingdom. By the turn of the 19th century, diatomists developed classification systems almost solely based on shape, symmetry and ornamentation of the siliceous cell wall as revealed by cleaned specimen from light microscopy (e.g. Hustedt, 1930). The central dogma of this diatom taxonomy and phylogeny has been an assumption that the diatoms contain two groups, which can be distinguished by their symmetry (Round et al., 1990). The Centrales have valve striae arranged basically in relation to a point and tend to appear radially symmetrical while the Pennales have valve striae arranged in relation to a line and tend to appear bilaterally symmetrical. Using therefore morphological and ecological characters has lead these two groups equal taxonomic ranking and this corresponds to the most used classification system, published by Round et al. (1990). This system gave rise to the most used taxonomic keys and manuals for diatom identification that regard specific areas of the world, like, in the case of Europe: monographs of the series Süßwasserflora von Mitteleuropa, by Krammer & Lange-Bertalot (1986; 1988; 1991a; 1991b) and Diatoms of Europe edited by H. Lange-Bertalot (2004a; 2004b, 2004c; 2009) in addition to many national and regional manuals. With this method, the description of new species was only based on morphological differences between allopatric populations which are purely phenotypic (Cox, 1995). This can lead to the perception that species have restricted geographic distribution patterns but this is not always the case: for example culture experiments can show that two species (*Thalassiosira gravida* Cleve and *Thalassiosira rotula* Meunier) that were considered morphologically different are actually the same because of valve morphology changes with decreasing temperature (Syvertsen, 1977). In addition, the gradual reduction in size associated with the diatom life cycle (see this chapter), can lead to pronounced changes in valve morphology (Round et al., 1990).

The rise of electron microscopy revealed much finer details of the cell wall, such as the importance of processes (Hasle, 1977) (perforations in the cell wall) which lead to further refinements (e.g. Simonsen, 1979). More recent studies are placing greater emphasis on living material (Cox, 1996) and on molecular techniques (Medlin et al., 1993; Medlin & Kaczmarska, 2004) and these are showing faults and weaknesses in the previous systems that has been dominated by a desire to aid identification (explicitly or implicitly) rather than reflect relationships (Williams, 2007). Therefore these studies brought to the creation of new genera and the restoration of old ones: there is in fact a large degree of variation in the diatom cell, which compromises the identification based uniquely in morphological characters of the specimens (Cox, 2009). Molecular techniques using SSU RNA sequence comparison have proven to be a powerful alternative to morphology and ecology for inferring phylogenetic relationships at all taxonomic levels (Woese, 1987; Bhattacharya et al., 1992). This analysis did not support a clear dichotomy between Centrales and Pennales

(Medlin & Kaczmarska, 2004) as in the previous classification, therefore suggesting that the former classification by Round et al., 1990 did not reflect the evolutionary history of diatoms. This discrepancy between the two classifications (the one based on ecological and morphological characters and the one based on molecular evidence) lead to further enquiries and debates that are still not resolved. According to molecular biology diatoms should be split in three new classes: Coscinodiscophyceae, Mediophyceae and Bacillariophyceae.

The class Coscinodiscophyceae (Round & Crawford, emend. Medlin & Kaczmarska) comprises cells with mainly peripheral processes (i.e. labiates processes, colony-linking processes) rarely or secondarily centrally located: cells are usually radially ornamentated from a central point. Sexual reproduction is oogamous. Orders given in Round et al. (1990) that fall into this class are the typical centric taxa such as Melosirales, Aulacoseirales and Coscinodiscales among the most common ones.

Class Mediophyceae (Jousè & Proshkina-Lavrenko, Medlin & Kaczmarska stat. nov.) comprises taxa with tube processes (i.e. labiate, strutted or rhyncho- shaped) that are primarily located in the cell centre or within the annulus, with cells usually bi or multipolar with radial ornamentation. Sexual reproduction is oogamous. A typical order belonging to this class is Thalassiosirales.

Class Bacillariophyceae (Haeckel, emend. Medlin & Kaczmarska) which comprises taxa with central sternum, with or without the raphe. Rapheless species usually have tube processes most commonly located at the cell apices. Others carry a central or marginal raphe system. Cells are typically bipolar usually with bilateral symmetry around an axial rib or raphe system. Sexual reproduction is anisogamous or isogamous. Orders given in Round et al. (1990) that fall into this class are, among the commonest found in rivers: Fragilariales, Tabellariales, Eunotiales, Cymbellales, Achnanthales, Naviculales, Bacillariales and Surirellales.

It seems that the adoption of pure and exclusive morphological criteria has had severe consequences of our understanding of the ecology, diversity and distribution of diatoms, as it not only stretched the morphological boundaries of many species, but also reinforced the idea that most diatom species have cosmopolitan distributions and are ecological generalists (Kociolek & Spaulding, 2000). During the last decade, however, it has become clear that subtle, discontinuous morphological variation patterns, which were hitherto assumed to be of no taxonomic significance, are instead generally correlated with variation in reproductive, molecular-genetic, physiological and ecological characters (Behnke et al., 2004; Lundholm et al., 2006; Amato et al., 2007). These studies suggest that many diatom species contain several subtly distinct, semy-cryptic entities that are worth taxonomic recognition at the species level and that, as a consequence, diatom species diversity has been severely underestimated rather than overestimated. It remains, nevertheless, to be shown whether the application of a fine-grained taxonomy will result in range-splitting or ecological differentiation rather than merely increasing global species diversity.

1.2.2 Physical impacts

STREAM FLOW AND WATER MOVEMENTS.

The pervasive effects of flow on benthic organisms are evident to most aquatic ecologist. The paper that popularized the idea of flow as the master variable in lotic ecosystems (Poff et al., 1997) has been cited more than 300 times while experiments and field surveys have generated relationships between stream flow and periphyton for more than one hundred years (Larned, 2010). Many other studies have addressed this topic (among others Poff et al., 1990; DeNicola & McIntire, 1991; Lake, 2000; Death, 2008). One of the earliest ones, performed by Biggs & Close (1989) on periphyton in the gravel bed rivers of Canterbury (UK), has demonstrated that flow variations in the order of 2.5 to 6 times the average daily flow of the preceding week could result in quite variable effects on periphyton biomass, from up to a 90% increase to a 100% decrease. Flow is the variable that shaped (both literally and figuratively) almost every feature of these systems, including their channel morphology and disturbance regimes, the distributions of organisms in space and time as well as rates of energy transfer and material cycling (Allan, 1995; Gordon et al., 1992).

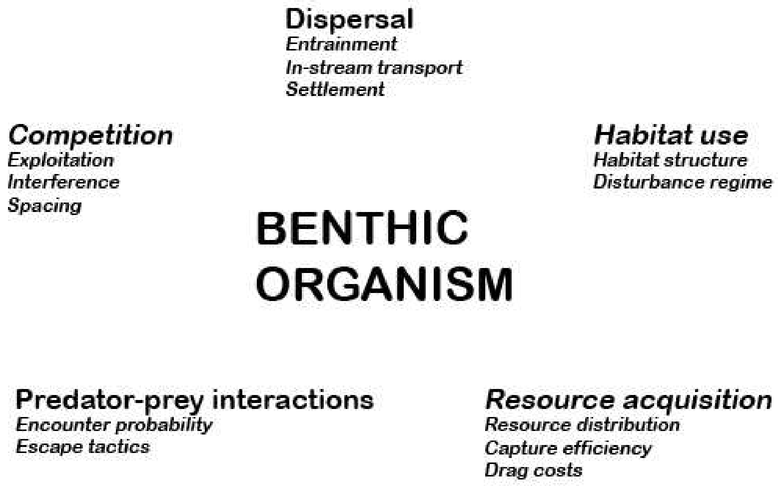


Fig.1.3. Alternative causal pathways by which flow can affect benthic organisms. Potential interactions among pathways not shown. Modified from Hart & Finelli, 1999.

As shown in Fig. 1.3, key components of each ecological process can be modified by flow, thereby affecting the performance, distribution and abundance of organisms:

-Dispersal: stream organisms are often carried downstream via water current (Mueller, 1974). Indeed these suspended or “drifting” organisms can provide a significant supply of immigrants to newly adaptable substrates (Townsend & Hildrew, 1976);

-Habitat use: upon settlement, a benthic organism will encounter a suite of local abiotic conditions. In turn, these habitat characteristics are often affected by flow, which can lead to flow-dependent patterns of habitat use. Of course, many local habitat features ultimately derive from broad-scale variations in climate and geology but regional differences in hydrological disturbance regimes can act as a powerful filter that limits the pool of species and species traits available to colonize a particular catchment (Poff, 1997);

-Resource acquisition: flow can enhance or hinder the rate and efficiency of resource acquisition via its effects on the distribution of resources as well as the ability of organisms to locate and gather these resources (Stevenson, 1996);

-Competition. when flow controls the supply rate of limiting resources such as nutrients, it can also potentially mediate the intensity and the outcome of competitive interactions reducing the availability of resources that might otherwise be used by individuals farther downstream (Wildish & Kristmanson, 1997);

-Predator-Prey Interactions: flow can affect the outcome of predator-prey interactions by altering either predator-prey encounter rates or the predator’s ability to successfully capture the prey following an encounter (Hart & Merz, 1998).

Focusing on susceptibility to hydraulic disturbances (e.g. elevated shear stress), this effect is influenced by periphyton growth form and age, magnitude of disturbance, rate of onset and the hydraulic conditions under which periphyton communities develop (Peterson & Stevenson, 1992; Biggs & Thomsen, 1995). In the case of diatoms, high susceptible forms include chain-forming ones, uniseriate filaments and loosely-attached cells (Passy, 2007). Not much is known about mechanisms of susceptibility of periphyton to hydraulic disturbance and this gap is fairly problematic seen the wide use of diatoms as bioindicators in streams and rivers: information about thresholds for periphyton removal is important for designing environmental flows intended to remove periphyton proliferations in regulated streams (Osmundson et al., 2002). In addition to the direct effects of increased shear stress, elevated flows affect periphyton by increasing sediment mobility which leads to abrasion, by suspended sediments and substrate tumbling (Biggs et al., 1999). Minimum resource requirements are unknown for most periphyton taxa and the consequent scarcity of autoecological studies makes predicting changes in resource requirements difficult (Larned, 2010).

Flow is even an harder player for planktonic algae. A high number of studies has addressed the problem of how open-ended, unidirectional flowing systems might sustain a suspended microflora at all (Butcher, 1932; Blum, 1956; Whitton, 1979; Round, 1981; Reynolds, 1988). But evidence tells us that phytoplankton in rivers is an important component: (see, in the northern hemisphere researches in the Avon River- Moore (1976)- the Danube and its tributaries- Kiss et al.(1996); Kiss & Schmidt (1998)-, the Meuse- Descy (1987); Gosselain et al. (1994)-, the Rhine- Peelen (1975)- and the Thames- Lack(1971)). The phytoplankton of the Mediterranean rivers has not met so much attention: comprehensive studies have been carried out in Spain (Sabater, 1990) and in Greece (Montesanto et al., 2000). Scientific knowledge of the environmental factors that influence phytoplankton has expanded greatly over the last three decades (Tockner & Standford, 2002) emphasising the importance of hydrological processes among other physical factors (Reynolds, 1988). In particular, heterogeneity of flow and river retentiveness have been suggested as the mechanisms for increasing residence time and extending opportunities for development for planktonic organisms (Reynold & Descy, 1996; Reynolds, 1996; Leland, 2005) therefore it seems obvious that planktonic species are by special means governed by physical mixing processes (Bormans & Condie, 1998; Huisman et al., 2004). But as environmental drivers co-act simultaneously, it is not always easy to discriminate which has the most important impact on the riverine community: therefore we should also consider other physical (e.g. water temperature and turbidity), chemical (e.g. mineral content/conductivity and nutrient concentrations) and biological factors (e.g. grazing and competition), like in the following sections.

LIGHT. Light is also an essential regulator of photosynthesis and consequently plays an important role in driving diatom productivity and determining their species composition in watercourses. In temperate lakes, light duration and intensity usually control the timing of the spring diatom bloom (e.g. Maberly et al., 1994) and the attenuation of light with depth, as a result of suspended particulates or water color. It also defines the limit of the euphotic zone in which net photosynthesis takes place. The light regime can affect both diatom community structure (Cox, 1984; Kawecka, 1985, 1986) as well as physiological processes (Guasch & Sabater, 1995) which in turn can influence the response to pollutants (Guasch et al., 1997). It follows that some diatoms are well adapted to dim light environment (such as *Aulacoseira roseana* and *Surirella* spp.) while others take advantage of full light, especially when water temperatures are not excessively high, to produce massive growths (e.g. *Melosira varians*). Most evidence suggests that there are differences in the responses of the major groups of algae to irradiance: green algae are usually associated with high light levels and diatoms and cyanobacteria appear to require lower light intensities (Hill, 1996). In low flowing rivers, motile diatoms are able to avoid extremes by movement along the light gradient. Nonmotile, prostrate taxa that grow near the substrate may decrease in abundance when light levels decline as a result of shading by overgrowth of the algal community (Allan & Castillo, 2007).

TEMPERATURE. There is a substantial older literature (e.g. Hustedt, 1956) that describes the influence of temperature on diatom communities. Most diatoms have their temperature preference at 15-25 ℃: however , a few are able to resist more than 30℃ (*Nitzschia palea,* but photosynthesis declines at 33℃) or to effectively make photosynthesis at 5℃ (*Aulacoseira islandica*) (Sabater, 2009). Also, more modern studies have used contemporary surface sediments that show temperature to be a potentially important variable in explaining differences in diatom composition between lakes (Vyverman & Sabbe, 1995; Lotter et al., 1997). In addition, culture studies often show clear differential growth rate responses of taxa to temperature (e.g. Suzuki & Takahashi, 1995).Unfortunately, temperature change has a major influence on the behavior of other physical and chemical variables in a watercourse such as ice-cover, pH, nutrient cycling (Battarbee, 2000; Anderson, 2000). It is consequently difficult to separate the specific influence of temperature on diatom composition from that of other variables.

SUBSTRATE. The role of substrate has been long investigated in streams: several works, among others Kovàcs et al. (2006), have shown how substrate can be considered as a master- variable in separating diatom assemblages. There is, in fact, an obvious, and to a certain extent inseparable, link between substrate stability and flow effects. This could lead to talk about the effects of these two variables, together Some researchers showed that the taxonomic composition of the benthic diatom mat strongly influenced the extent of biomass removal by moderate/low magnitude events (Death & Winterbourn, 1994; Schwendel et al., 2010). As could be expected, the most disturbance-resistant communities were dominated by low profile, prostrate or adnate diatoms with large celled filamentous green algae being least resistant. Therefore morphological and physiological traits should be highly accounted: for example, prostrate forms (e.g. *Achnanthidium*) and compact (e.g. *Cymbella*) forms represent adaptations to high-drag environments compared with the filamentous and upright forms (e.g. *Fragilaria*). Periphyton communities and their hydraulic environments interact continuously: as communities change in structure (i.e. size, density and flexibility) they modify bed roughness and near-bed water velocity and turbulence (Nikora et al., 2002). In turn, the drag imposed by flowing water leads to changes in community structure. Continual feedback might lead to an equilibrium state with the maximum height and roughness of periphyton communities determined by local hydraulic conditions. For communities that exceed these maxima, drag forces will exceed attachment strength and initiate sloughing. Especially in the benthic compartment, species composition often differs substantially in the different substrates (e.g. rock surface, upper sediments layer or water plants) because some species are better adapted to one microhabitat than to others due to their morphological traits (Round, 1981). This has resulted even in the creation of an index (Siltation Index) by Hill et al. (2001) where the percentages of *Navicula* and *Nitzschia* taxa gave a measure of streambed instability. Researchers, though, do not unanimously agree on this issue. Studies in freshwater (Cattaneo et al., 1997; Soininen & Heino, 2005; Cetin, 2008) and oceans (Jesus et al., 2009) have assessed how finer sediments have been preferably found to host loosely attached diatoms (e.g. *Navicula* spp.) while coarser substrates preferably host attached diatoms such as *Achnanthes* spp. This would of course have huge implications for biomonitoring assessments. Other researchers, instead, believe that even if diatoms could show a sort of segregation, this would not affect biomonitoring results (Kitner & Poulickova, 2000; Rott et al., 1998) since they would give an overall assessment. Whichever the opinion, the effect of discharge on substrates, mentioned earlier in this paragraph, could make the issue even more complicated to disentangle. It follows that we are still far from the truth and we need to have more detailed investigations of the large scale dynamics of lotic assemblages, especially in constrained watercourses.

1.2.3 Chemical impacts

NUTRIENTS. Nutrient limitation is one of the best-studied topics in diatom ecology (Dodds & Welch, 2000; Francoeur, 2001). The high level of interest in nutrient limitation reflects concerns about eutrophication and recognition of the role of nutrient limitation in community and ecosystem processes (Hillebrand, 2002; Holomuzki et al., 2010). Nutrients that are in low demand relative to availability (e.g. K, Mg) are rarely limiting while other nutrients (such as N, P and Si) are frequently limiting because demand is high relatively to availability. For phytoplanktonic diatoms, we cannot exclude that nutrient uptake is dependent upon taxa in the sense that small sized diatoms with high surface areas to volume ratios (i.e. centric taxa) have small diffusion boundary layers that enable efficient nutrient uptake. This way, they have a superior ability to harvest light, lower sinking rates and have the ability to divide more rapidly when compared to larger cells. On the other hand, Bell & Kalff (2001) have shown that large cells are selected for when nutrients are resupplied by turbulent mixing and therefore diatom cell size could be a very powerful predictor of optimum dynamic performance (Reynolds et al., 2002) that has to be taken in consideration when assessing nutrient status. Studies of nutrient requirements and limitations of planktonic diatoms and of their inter-specific relationship under different environmental conditions represent classical ecological issues which have been addressed for many years (e.g. Van Donk & Kilham, 1990). In lakes, Fragilariaceae:Centrales ratio is reported as principally regulated by changes in the Si:P ratio (Kilham & Tilman, 1979; Van Donk & Kilham, 1990) with Fragilariaceae growing well under limitation by phosphorus and Cyclotella taxa performing better when also silica becomes limiting (low Si:P ratio). Chetelat et al. (2006) among others (e.g. Basu & Pick, 1996; Van Nieuwenhuyse & Jones, 1996; Heiskary & Markus, 2001) have proven a positive relationship between potamoplankton and total phosphorus in broad scale studies. This relationships could be connected to a consumption of nutrients during periods that are more favourable for phytoplankton replication (growing season and low discharge) and, possibly, to the existence of negative seasonal relationships between phosphorus concentrations and phytoplankton abundance in the single rivers. This was for example demonstrated by Rossetti et al. (2008), who showed that, from May to October, chlorophyll-*a* in the River Po was inversely related to SRP concentrations, evidencing a potential causal relationship between phosphorus availability and phytoplankton abundance. This confirms that, in this river, the observed algal biomasses are well below the limits imposed by nutrient availability and far from the maximum supportable biomass. The general models linking phytoplankton abundance and phosphorus proposed by the aforementioned studies have showed this relationship but they deal with natural rivers. In the case of constrained or modified watercourses, like the Adige River, the velocity of the water flow has proven to represent an important constraint for the attainment of higher biomasses, imposing lower limit on the supportive capacity of the river.

If increasing the availability of P usually causes most alteration to phytoplankton communities, benthic communities can also be affected as a result of changing habitat availability (e.g. the type and distribution of aquatic macrophytes) and through increased shading by plankton crops. Typically this is reflected by an increase in the ratio of planktonic to non-planktonic diatoms in sediment cores (Battarbee, 1986), even where the production of benthic diatoms also increased (Oldfield et al., 1983). In Italy, for example, several works performed in lakes (Marchetto & Bettinetti, 1995; Marchetto & Musazzi, 2001) gave an estimation of total phosphorus concentration diatom-inferred. Periphyton growth rates are not always positively correlated with environmental nutrient concentrations, as one might expect. Nutrient additions can in fact either increase (e.g. Pringle, 1990; McCormick & Stevenson, 1991) or have no overall effect (Stevenson et al., 1991) on richness and/or evenness of stream benthic algae. More specifically, nutrient excess may favor a certain (low) number of tolerant diatom taxa (e.g. *Navicula gregaria, Nitzschia palea*) but inhibit the growth of others specialized in lower nutrient availability (*Achnanthes biasolettiana, Cymbella microcephala*). Nitrogen limitation favors the growth of *Epithemia* and *Rhopalodia*, since they might have endosymbiontic nitrogen-fixing cyanobacteria. Thus, overall, it is still unclear as to how enrichment of streams might influence mean benthic algal taxa richness (Biggs & Smith, 2002).

SALINITY. Salinity might affect the osmotic pressure within the diatom cell, as well as the nutrient uptake and other physiological processes. As such, salinity is a key factor in the distribution of diatom communities. Some taxa are able to thrive under salinity of 3-4 % (but rarely above this) and are recognized as brackish water species: i.e. *Cyclotella quillensis, Navicula salinarum, Amphora coffeaeformis*. Other taxa are able to tolerate moderate salinities (0.5-2%) and named as halophilic: *Navicula cincta, Cyclotella meneghiniana*

(Sabater, 2009). The use of interference models (essentially a comparison between past and present conditions) for assessing salinity concentrations is becoming more common, generating regional calibrations or datasets (Battarbee et al., 2001; Racca et al., 2004) in many parts of the world, from African lakes (Gasse et al., 1995), to central Mexican lakes (Davies et al., 2002), China (Yang et al., 2003), Australia (Taukulis & John, 2009) and North America (Wilson et al., 1996). In the past decade, a halobiont index (Ziemann, 1991) was assessed with the aim of using salt preferences of diatoms to evaluate water salt concentration in rivers.

pH. pH significance in influencing freshwater systems has been long recognized in the diatom literature (e.g. Hustedt, 1937; 1939) and a lot of indices and models have been studies to reconstruct pH with diatoms (e.g. Smith, 1990; Eloranta, 1990; Battarbee et al., 1997; van Dam, 1997). There is no detailed ecophysiological understanding of how pH influences the growth and the competitive abilities of individual diatom taxa. However, pH controls many chemical and biochemical processes and reactions including the carbonate-bicarbonate balance in watercourses, the availability of nutrients for algal uptake and the solubility of metals, especially toxic ones (e.g. aluminium) and the activity of specific enzymes such as the phosphatases, whom have a great importance in shaping diatom communities features (Smith, 1990). Although rarely used now in any quantitative way this strong relationships between diatoms and pH gave rise to a pH-classification of diatom taxa and the use of diatoms by paleolimnologists to reconstruct past lake pH. This was based on various indices (e.g. Index α, Index β) derived from calculations of the ratio of taxa in different pH groups (Nygaard, 1956; Renberg & Hellberg, 1982) or the use of the pH groups as explanatory variables in multiple regression analyses (e.g. Davis & Anderson, 1985). However, modern studies show that there is a constant diatom species turnover along the pH gradient and that forcing diatoms into pH categories as required by the classification system could be unnatural and eventually lead to anomalies in pH reconstruction.

1.2.4 Biological impacts

Grazing by herbivores affects not only the biomass of the diatom communities (high grazing intensity depletes algal biomass) but it may also determine its preferential growth form. There is a tight relationship between the diatom communities and the herbivore density and morphology of the mouthparts. Depending on the mouthpart morphology of the grazers, the selected growth form to be ingested may change. Mayflies tend to feed on the outer parts (filaments, stalked forms) while snails, caddisflies or tadpoles may feed on tightly attached forms (encrusting or prostrate forms). The type of grazers therefore favors one or the other growth forms, depending on their abundance (Sabater, 2009).

1.3 BIOASSESSMENT OF RIVERS WITH DIATOMS

Diatoms have been widely used to assess environmental water quality because they have a number of prominent distinctive features: (i) high diversity both locally and regionally accounting for much of the freshwater biodiversity, especially in streams; (ii) relatively strict preferences for various environmental factors reflected as a tight coupling between community and environment and (iii) distinct features of the siliceous cell wall for which diatoms are easily to classify. Diatoms distributional patterns respond to a multitude of different factors, ranging from biogeographical to biogeochemical and human influences (McCormick & Stevenson, 1989; Descy & Mouvet, 1984; Potapova & Charles, 2002), as underlined in the previous chapters.. One of the earliest reports from diatoms field studies (Fritsch, 1906) concerns seasonal variation of growth and composition of stream and pond algae. In the 1940s, two new research areas emerged in diatom studies: the effects of abiotic factors on their community composition and abundance and the use of diatom communities as indicators of stream health (Butcher, 1940; Patrick 1948, 1949). From the 1980s onwards, there was a rapid increase in studies of natural disturbances and light and nutrient limitation on diatom communities (Mulholland et al., 1991; McCormick et al., 1996; Biggs et al., 1999). The last decade has seen continued growth in diatom ecology and new conceptual models about the development of diatom communities and their responses to the external environment (Larned et al., 2010). This combination of relatively narrow environmental tolerances for the major part of the species and long-lasting siliceous cell wall also render diatom analysis a useful tool both for environmental reconstruction and for indication of present ecological conditions. A prerequisite for robust and trustworthy indication of ecological conditions is, however, a proper understanding of underlying ecological and evolutionary processes affecting both the species regional distribution and local abundance (Soininen, 2007).

Traditional studies have resulted in the development of the two basic conceptual and analytical approaches used today. At first, Patrick's early monitoring studies (Patrick, 1949; Patrick et al., 1954; Patrick & Strawbridge, 1963) relied primarily on diatom diversity as a general indicator of river health (i.e. ecological integrity) because species composition of assemblages varied seasonally. The diversity of a diatom assemblage reflects the number of species (species richness) and the eveness of species abundance. Diversity indices (e.g. Shannon-Wiener, Margalef's or Simpson's) have been effectively used as indicators of changes in community structure when comparing impacted and reference sites, but did not reliably accounted for phylogenetic, taxonomic and functional variability between species (Heino et al., 2005). This apparent inconsistency is related with internal structuring factors, which affect species abundance and eveness and renders the diversity parameter not suitable for monitoring water quality. Therefore, alternative ways had to be investigated and that led to the development of additional techniques (diatom indices, multivariate statistical techniques and transfer functions) that are nowadays widely used.

1.3.1 Bioassessment techniques

Bioassessing with diatoms can be performed with techniques that rely on the sensitivity of this algal group and by relating changes in the composition of the diatom community to environmental factors.

DIATOM INDICES*.* Indices are one of the most common methods to account for the indicator value of these organisms. They have been developed to reflect the abilities of assemblages of different diatom species in detecting variations in water pH, salinity, nutrient content and total phosphorus. The most tolerant diatom taxa are favoured while the sensitive taxa are depleted or eliminated, in reply to environmental stressors and resources availability, and this might be reflected by accurate diatom indices. They constitute a way of summarizing the information provided by the autoecological preferences of single taxa units. Most of the diatom indices currently used are inspired on the earlier saprobic index of Kolkwitz and Marsson (1908). However, these indices take into consideration the structure of the community and therefore consider not simply the presence of taxa but also their proportion in the community. The majority of indices are calculated according to the formula designed by Zelinka & Marvan, which considers the sum of different species relative abundances (*a*) influenced by their sensitivity (*s*) to the described disturbance and by their indicator value (*v*) (which explains the reliability of the taxon to express that environmental status).

$$ID= \Sigma^{n}_{j=1} a_j s_j v_j / \Sigma^{n}_{j=1} a_j s_j$$

The use of diatom indices is now a question of routine, but they are not exempt of limitations. Uncertainties in their application might occur because of different factors. Sometimes the lack of information about the autoecology of some species may reduce the precision of the index (Sabater, 2009) since accurate knowledge of the autoecological characteristics of the species is necessary to identify the correct reference conditions (Potapova & Charles, 2007). Surely additional taxonomic and autoecological research on sensitive, endemic algal species would further enhance indices performances (Porter et al., 2008) especially when considering untypical and extreme environments (e.g. the Adige River). In addition, the tougher the stressor, the more reliable is the information provided by the index; it has been observed, for instance, that inaccurate prediction of phosphorus loading by diatom communities systematically may occur at lower phosphorus concentrations. Other causes of misuse may occur when there is a mixture of human-produced influences such as nutrient enrichment, habitat alteration, or toxic inputs, where the diatom communities might reflect the overall effect of the disturbance and not the effect of a particular stressor. Indices can be biased by factors other than human-induced components, including biogeographical or biogeochemical factors. A correct diagnosis, in fact, has to consider which part of the information available is related to human influences and which part is related to characteristics defining the ecoregion’s characteristics. Nevertheless, a high number of diatom indices have been proposed in practically each European country (Tab. 1.1), following the pioneer work of Van Dam et al. (1994) that have defined the “tastes” of 948 diatoms for pH, nitrogen, oxygen, salinity and saprobic and trophic levels. Also Rott et al. (2003) have created a similar dataset for 1000 diatom species but they have also defined nine quality classes based on trophic and saprobic tolerances. From then onwards, diatom indices became very popular to express water quality and further taken care for the implementation of the Water Framework Directive as explained further.

METHOD	COUNTRY
Descy Index DES (Descy, 1979)	Belgium, Luxembourg
Index de Polluosensivite Specific IPS (Coste, 1982)	France, Poland, Luxembourg, Finland, Greece; Spain, Portugal
Index Slàdecek SLA (Sládecek, 1986)	Portugal
Index Leclercq and Maquet ILM (Leclercq & Maquet, 1987)	Belgium, Luxemburg, Portugal
Index Diatomic Generic IDG (Coste & Ayphassorho, 1991)	France, Finland, Poland
Index Diatomic Trophic TDI (Schiefele & Kohmann, 1993)	Germany, Finland
Index of Eutophication/Pollution EPI-D (Dell'Uomo, 2004)	Italy, Spain
Index Saprobic (Rott et al., 1997)	Switzerland, Austria
Index Throphic TDI (Kelly, 1998)	United Kingdom
Index Trophic (Rott et al., 1999)	Austria
Index DI-CH (Hürlimann & Niederhauser, 2002)	Switzerland
Index Diatomic Biologic IBD (Prygiel & Coste, 2000)	Finland, Luxemburg, Portugal

Tab. 1.1- Diatom indeces used in Europe. Modified from Rimet et al. (2005)

The diatom index developed in Italy (Epi-D) has been widely used for Italian watercourses even if it was implemented from samples coming from the Chienti River, in southern Italy (Dell'Uomo, 1996). This index is based on diatom sensitivity to nutrients, organic and inorganic matters and it relies on weighted averages since each taxon has its own sensitivity value. The index ranges from 0 ("clean" waters) to 4 ("dirty" waters) and each variation in between has to be interpreted using the following limits imposed by the index (Tab. 1.2):

EPI-D VALUES	QUALITY
0 <= EPI- D <= 1	High quality
1 <= EPI- D <= 1.5	Good quality
1.5 <= EPI- D <= 1.8	Sufficient quality
1.8 <= EPI- D <= 2	Slightly altered quality
2 <= EPI- D <= 2.2	Moderately altered quality
2.2 <= EPI- D <= 2.5	Strongly altered quality
2.5 <= EPI- D <= 3	Highly altered quality
3 <= EPI- D <= 4	Severely altered quality

Tab. 1.2- EPI-D metrics. Modified from Dell'Uomo et al. (1999)

Several works have underlined that metrics don't have the same integration intervals and do not assess the same stresses: the choice of the metric to be used is therefore of prime importance (Rimet et al., 2005; Fejo et al., 2009). While gradient analyses (WA) are commonly employed to elicit overall assemblage patterns (e.g. Biggs, 1990; Leland, 1995; Pan et al., 1996), species optima for key environmental variables which are the fundaments of indices, are most often determined by weighted-averaging (WA) techniques (e.g. Pan et al., 1996; Leland & Porter, 2000; Winter & Duthie, 2000; Potapova & Charles, 2003). WA models have been used to develop species optima and tolerances for conductivity (e.g. Leland, 1995; Leland et al., 2001; Munn et al., 2002; Potapova & Charles, 2003), pH (Kovàcs et al., 2006), phosphorus (e.g. Pan et al., 1996; Winter & Duthie, 2000; Soininen & Niemelä, 2002; Schönfelder et al., 2002; Potapova et al., 2004; Ponader et al., 2007), nitrogen (e.g. Leland, 1995; Ponader et al., 2007), sulphate (Potapova & Charles, 2003), major cations (Potapova & Charles, 2003) and dissolved inorganic carbon species (Schönfelder et al., 2002; Potapova & Charles, 2003). In addition, WA models have successfully been used to reconstruct environmental conditions in lakes (e.g. Birks et al., 1990; Bennion, 1994; Dixit et al., 1999) and wetlands (e.g. Gaiser & Taylor, 1995; Cooper, 1999). However, WA approaches suffer from the simplicity and assumptions of these models, in the sense that they assume that the variable of interest is the sole variable responsible for determining the species distribution: the importance of other environmental variables is implicitly included in the calculation of the optimas. They don't look at the fundamental niche's dimensions but at realised niche's one and this could be biasing since this latter can have different dimensions according to where it is measured (Krebs, 2001). It follows that WA can't explicitly illustrate the interactions among environmental variables and subsequently, environmental variables must be interpreted one at the time. In reality, most studies have displayed interactions among environmental predictors and stream algal assemblages (Leland & Porter, 2000; Soininen & Niemelä, 2002) but these are individual works developed in one specific area and so their applicability is questionable (Weilhoefer & Pan, 2008). A second assumptions of WA modelling is that species abundance forms a unimodal relationship with the environmental variable of interest. While Gaussian responses of diatom abundance to physiological environmental variables (e.g. pH, salinity) have been reported (ter Braak & van Dam, 1989; Juggins, 1992), this relationship is not often the case for resource variables, such as total phosphorus (Potapova et al., 2004) and so, recently, advanced regression techniques (e.g. generalized linear and additive models) have been used to model species-environmental relationships (see Guisan et al., 2002 for a review).

MULTIVARIATE STATISTICAL TECHNIQUES. Such techniques are very common in US (see among others Hill et al., 2000; Leira & Sabater, 2005) and include principal component analysis, canonical correspondence analysis or cluster analysis among the most common. They are used to assess the multidimensional patterns of ordination as well as to relate environmental factors to the ordination patterns. Therefore, these techniques make a useful complement to indices, since they allow determining the ecological factors that are responsible for most of the variation. Often, the information extracted from multivariate approaches may be later used to refine the value provided by the indices. In particular, variance partitioning applied to some of the aforementioned analyses may help to separate the regional versus general factors in the determination of the distribution of diatom assemblages and might be therefore very useful in refining the information provided by the indices (Sabater, 2009). There have been advanced two critics against the use of multivariate methods: i) that they are not easily understood and applied by non-specialists; ii) that a multitude of techniques are available, which presents a further hurdle to the uninitiated (Gerritsen, 1995) but their use has not been hampered yet and continues to be one of the most used methods in the study of diatom dynamics.

TRANSFER FUNCTIONS. These methods have been applied for monitoring pH, salinity and nutrient trends. Transfer functions essentially look for regression between the autoecological value of diatom communities and a master environmental parameter: in many cases they have been used to describe the relationship between sediment diatom assemblage composition and water TP (or water pH) known in a training set and then calibrated with present TP concentrations (Rosén et al., 2000; Bennion et al., 2005). Transfer functions have also been used to infer changes in the environmental conditions of a lake such as lake-water nutrients (Hall & Smol, 1992; Zeeb et al., 1994), dissolved organic carbon (Pienitz & Smol, 1993) and water air temperature (Sporka et al., 2002; Catalan et al., 2002). It is evident that to be reliable, these models require to have been calibrated (tested) in a number of real cases. These types of expressions have been particularly applied in lake ecosystems, where diatoms preserved in the sediments can be used to reconstruct the history of the lake. However, similar transfer functions have been used to detect current trends in acidification or eutrophication in running waters (Sabater, 2009).

1.3.2 Water Framework Directive 2000/60/CE: ecological classification with diatoms

The Water Framework Directive (hereafter WFD; EC Parliament and Council, 2000) was proposed by the European Commission in 1997 (COM(97)49) and was established in 2000 (2000/60/EC). The WFD is a broad concept and aims to develop sustainable management strategies for ground and surface waters in Europe (Padisák et al., 2005). It represents a paradigm shift in the way in which European waterbodies are managed, with the “ecological status” replacing more traditional concepts (e.g. concentration of particular pollutants or needs of end-users) as the primary criterion by which water bodies are assessed. It states the environmental targets or prevention, protection, healing and sustainable uses of the water resource. Ecological status is defined as “an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters”(article 2, clause 21). The aim is to achieve at least good ecological status in all waterbodies by 2015 by comparing its status with the “reference condition” which is the watercourse status previously that being in any way impacted. Reference sites are not single cases but should reflect regional conditions and regional variability under minimal human disturbance (Gerritsen, 1995).

In Italy, the WFD has been transposed at national level with three Emandements Legislative Decreti (152/2006, 131/2008, 56/2009) and several working groups, coordinated by ISPRA (National Institute for Environmental Protection) have been drafting the technical annexes and protocols regarding “Biological Indicators” for water (e.g.macroinvertebrates, macrophytes, fishes and diatoms). To put some order among the dearth of diatom indices used in Italy, a protocol for sampling and analysis has been developed specifically for benthic diatoms of watercourses that gives the directives on methods in the field and to propose an unique methodology for water quality assessment (Intercalibration Common Metrix Index ICMi-Mancini & Sollazzo, 2009). This method arises from several consultation groups called CIS (Common Implementation Strategy) that have been established in order to discuss challenges pushed by the Directive and find common European understandings and approaches for its effective implementation. The ICMi is based on the concept of EQR (Ecological Quality Ratio) which is the ratio between the observed and the reference value of a waterbody. And it is this last value which has been reviewed and decided by the CIS in a rather articulate way. In fact, using the Ecoregions established by the WFD, the CIS has come up with 14 Geographical Intercalibration Groups (GIG) each standing for an area having the same typologies of waterbodies. In Italy there are three geographical areas: Alpine, Central and Mediterranean and in each of these different “Fluvial Typologies” (Fig.1.4) have been established. For the Alpine area there are two, on the basis of their prevalent geological substrate: either calcareous (A1) or siliceous (A2).

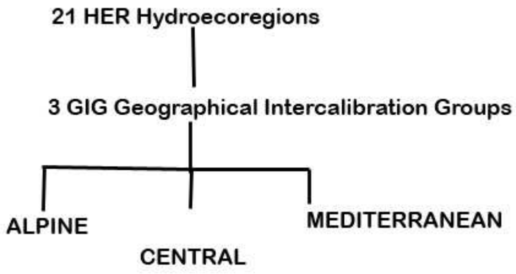


Fig. 1.4- Outcome of the Italian sub-division for the implementation of the WFD

The ICMi is an index that derives from two previous indices: the IPS and the TI. The IPS metric (Coste, 1982) measures general water quality and it is used widely to integrate effects across the entire water quality gradient (Hering et al., 2006) with low values corresponding to high pressure levels and, therefore, low EQR. The TI (Rott et al., 1999) is a trophic index with low values corresponding to low nutrient concentrations so that high values represent high EQR values. For each taxa there is an “i” (reliability) that ranges between 1 and 3 and a “s” (sensibility) value that ranges between 1 and 5 and the index is defined as follows:

$$ICMi= (RQE\_IPS + RQE\_TI)/2$$

The two parameters are calculated as follows:

$$RQE\_IPS= Observed value/Reference value$$

$$RQE\_TI= 4- (Observed value)/ 4- (Reference value)$$

The reference values for each of the two indices are assessed by the protocol on the basis of a national diatom database (Mancini & Sollazzo, 2009). The reference value indicates the waterbody’s pristine conditions. The search for sites at “reference conditions” is, however, not straightforward because of the long history of human settlement across much of the continent especially in rivers. This biases the method already from its very beginning.

The value of the index ICMi obtained shifts into certain ranges that define the ecological quality of the water into High (H), Good (G), Sufficient (S), Insufficient(I) and Scarce(SC) Status (Tab. 1.3).

MACROTYPES	**H/G**	**G/S**	**S/I**	**I/SC**
A1	0.87	0.70	0.60	0.30
A2	0.85	0.64	0.54	0.27

Tab. 1.3- Class limits for the Water Quality Status by the ICMi: High (H), Good (G), Sufficient (S), Insufficient(I) and Scarce(SC) Status

This rather articulated method does not take into account the peculiar characteristics of watercourses. For example, indices assume that one taxa has the same ecological optima in any environment, both freshwater and brackish (i.e. the indices are applicable to any body of water); also they do not take into account the hydrological characteristics of the waterbody (e.g. strong physical gradients such as flow causing mixing and instability of substrates) or morphology of the basins (e.g. different geological substrate of the basin). Frequently cited environmental preferences of diatoms are often based on qualitative best professional judgment which are derived from studies within a limited geographic area or region or are based on synthesis of studies with different study objectives, sampling designs and various spatial and temporal scales (e.g. Lowe, 1974; Beaver, 1981; Van Dam et al., 1994). Not less important is the fact that most of the indices do not consider all centric diatoms in their databases because they have planktonic preferences. It therefore appears that most indices have been developed without a complete understanding of the processes that exist between geographical and environmental factors in a diatom community (Potapova & Charles,2002).

In addition to phytobenthos, phytoplankton is also one of the biological quality elements considered in the classification of the WFD. Water quality assessed with this component must be based on its biomass, composition, abundance and also on any bloom (frequency and intensity). Numerous studies have addressed this issue both in lakes (Padisák et al., 2005; Salmaso et al., 2006; Nixdorf et al., 2008), transitional waters and marine (Revilla et al., 2009; Devlin et al., 2007) and in rivers (Borics et al., 2007; Mischke, 2007) helping to create methods for assessment of ecological status. Specifically for the river biota, the WFD does not consider necessary investigation of the phytoplankton (Annex 5, paragraph 1.1.1) because the rivers are mainly heterotrophic systems (Dokulil, 1996; Skidmore et al., 1998) with a higher predominance of allochthonous organic material over authochtonous one, so, in other words, longitudinal dynamics prevents the reliable use of trophic scales based on phytoplankton biomass. Another reason is that algologists need standardization and intercalibration of different approaches used currently in phytoplankton studies, including techniques for sampling, counting and expressing results. Development of an uniform treatment for phytoplankton analyses is complicated also due to a large number of taxa that might be potentially encountered in water samples (Kelly, 1998).

2. STUDY SITES

2.1 THE ADIGE RIVER BASIN

In this chapter I will report the main morphological characteristics of the river Adige and its three main northern tributaries (Isarco, Noce and Avisio streams). An exhaustive report is beyond the scope of this work so only main features will be detailed.

2.1.1. General features of the river

The Adige River springs eastern the Resia lake, 1586 m above sea level, in the northwestern part of the Trentino Alto Adige region, in northeastern Italy. The river shows a drainage basin of 12.100 km^2 and flows for 409 km to the southeast to enter the Adriatic Sea at Porto Fossone, south of Venice (Zolezzi et al., 2009). Its drainage basin is spread in the regions Trentino Alto Adige and Veneto and, to a smaller extent, in Switzerland. Among the Italian rivers, Adige is one of the few mentioned already from the first centuries of the Christian era: Strabone and Plutarco called it with the names of Atagin and Athisona. Later on, it will be called Athesim and in the Middle Age it will be given its definitive name of Athesis (Miliani, 1937). The general slope of the river, from its source to the mouth, is ranging from 53 to 0.91 % (Basin Authority of Adige River, 2003): the width of its section goes from 40 m between Merano and Bolzano to 269 m close to Zevio, on the southern part of the basin (Basin Authority of Adige River, 2003). In the Adige Basin, there aren't very relevant lakes (Miliani, 1937) except few very small and shallow ones like the Caldaro lake (that is located 20 km southern of Bolzano), the Dobbiaco and the Ledro lakes, the Braies lake, Tovel lake, Terlago lake and the Carezza lake (Basin Authority of Adige River, 2003).

As concerns rain regime, there is a strong variability along the basin. In the Val Venosta (northern part) there is a typical regime of 400-500 mm of rain/year, while the average value in the basin is 900 mm $year^{-1}$. Usually there highest quantity of water falls in the months from May to August and in the months between October and November (Basin Authority of Adige River, 2003). Headwaters are mainly fed by snowmelt and rain by the 185 glaciers (covering around 200 km^2) scattered around the basin that have retreated over the last 50 years (Gumiero et al., 2009). The straightening made for flood-defense purposes during eighteenth and nineteenth centuries have shortened the main course of the river for a length of 14 km (Miliani, 1937; De Antoni, 1989). Seven very relevant floods have occurred in the 19th century and nine in the 20th (Miliani, 1937; Fiorentino & Manfreda, 2004; Gumiero et al., 2009). But it is already from the Renaissance era that mankind is trying to rule this undisciplined river: the Government of the Serenissima Republic created in those years the Eccellentissimo Istituto del Magistrato alle Acque, giving it this hard task (Miliani, 1937). After three centuries, with the cooperation of the Austrian rulers and the Fascist authorities, Adige was provided with high embankments along its course and this caused also its flow above the level of the surrounding countryside in some areas (Miliani, 1937).

2.1.2. Hydrologic and Morphologic features

The Adige basin presents a strong variability according to its geology and lithological characters. Very broadly, a Basin Authority of Adige River (2003) report distinguishes three portions:

i) A northern part dominated by scystic-cristalline substrates, with granites and diorites that are to be considered slightly permeable to water;

ii) A middle part composed by dolomitic rocks standing on a porfiric tableau which is even less permeable;

iii) A smaller southern part laying on carbonate rocks of alluvional origins, very permeable.

On these three parts there is a consistent influence played by the fluvial and glacial actions that has started from the lower Cenozoic period: this action is visible in the metamorphic basement as gentle and uniform slopes whereas it has given rise to high slope variability in the carbonate substrates.

The Basin Authority of Adige River has edited in the middle 1990s, a soil usage chart from Landsat 5 images and from imaged from the Soyuz MK4 probe. From these surveys, fourteen classes of soil usage have been identified and more specifically these have been conducted to:

i) Areas with irreversible human impact like the urban areas (2.8%) that can be found especially in the southern part of the basin and the quarry areas (0.1%) that even if not very consistent in the basin territory, are very crucial in the economic, social and environmental texture of the basin (e.g. the porphyry caves of the Val di Cembra);

ii) Areas with reversible or partially reversible impact like the agricultural areas that are very consistent in this area (e.g. the apple and grapes cultivation is one of the main activities along the river basin) which account for 15%;

iii) Areas scarcely human impacted like the wood (39,2%) and pasture land (3-7%) that are very important for the hydrologic risks connected activities and the rural economy (e.g. 80% of the Trentino woods are devoted to production);

iv) Areas not human impacted such as pioneer vegetation (22.9%), bare rocks (10%), waterbodies (0.3%) and snow covers (5.4%).

Very peculiar about the soil usage of the Adige Basin is the fact that 75% of it (9000 km) has typical mountain characters: woods, pioneer vegetation and bare rocks. Its remaining parts are for valley bottom areas that are therefore more human impacted areas (Basin Authority of Adige River, 2003).

In the northern part of the River Adige 31 major reservoirs have been built over the last 70 years, mostly in the ‘50s (Fig. 2.1). All of them are serving for hydroelectric power, with a total capacity of 571 x 10^6 m^3 of water (Bruno et al., 2009a; Bruno et al., 2009b). Close to the Adige mouth there are the Resia and San

Valentino della Mutta basins (both in the municipality of Curon Venosta) with a total capacity of 125 million m^3. In the highest part of the Val Senales, at 1650 m a.s.l., there is the Vernago basin that has a total capacity of 42 million m^3 (Basin Authority of Adige River, 2003).

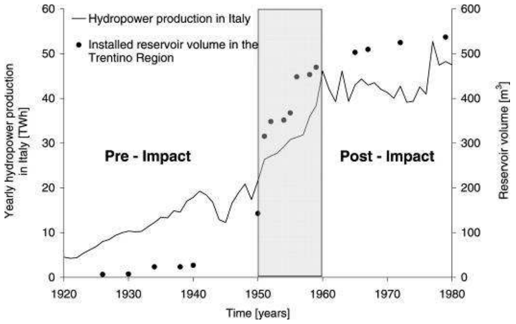


Fig. 2.1- Development, from 1923 to 1980, of the volume cumulated by the dams of the Adige River basin. From Zolezzi et al, 2009.

All these dams have a strong impact of the lamination of the floods of the Adige River but generally have a lesser impact that previous years (in the latest years, smaller quantities of water have been entrapped). Only the Santa Giustina dam (on the lower part of the Noce river) can have a relevant impact because of its high storage capacities (182.8 million m^3 of capacity) (Basin Authority of Adige River, 2003). The hydroelectric plants have nowadays a mean productivity of 7500 GWh (Basin Authority of Adige River, 2003) but the National Electric Authority (ENEL) is considering the opportunity to build some more plants to increase productivity of 1606 GWh, in the years to come.

It follows that the flow in Adige River is, to a large extent, regulated by hydroelectric and irrigation uses (Manfreda & Fiorentino, 2008; Gumiero et al., 2009; Bruno et al., 2009a) and that their effects have already been proven to be very disruptive for the communities living in the watercourse (Bruno et al., 2009a; Centis et al., 2010; Salmaso & Zignin, 2010).

In a recent study of European Rivers, edited by Tockner et al. (2009), Adige River has shown to have a rather peculiar situation: even if it is situated in the Mediterranean area, it has a typically hydrological regime of Northern European rivers. As in figure 2.2, its discharge regime is higher in the warmer months than in the winter ones (as in the case the Tera and the Elbe rivers).

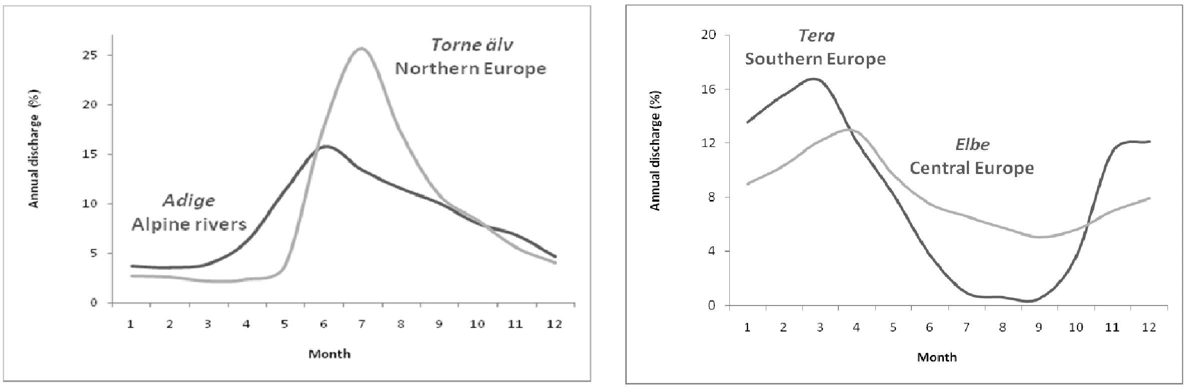


Fig. 2.2- Hydrological regime of four European rivers: Torne älv (Sweden), Adige (Italy), Tera (Spain) and Elbe (Central Europe)

2.2 THE ISARCO, NOCE AND AVISIO STREAMS BASINS

2.2.1. General features of the watercourses

The Isarco River has its source in the Brenner Pass (1990 m a.s.l.), and flows for 90 km until its convergence to the Adige River with a south-west direction. Its drainage basin extends for 4.202 km^2, flowing at an average height of 1760 m a.s.l. that culminates in the peak of the Gran Pilastro (3170 m a.s.l.). It has been estimated that this watercourse has an average discharge of 78 m³/s (Miliani, 1937). Isarco joins the Adige River southern of the city of Bolzano, after having itself received the waters of the Talvera and the Rienza streams. Because of its characteristics, this waterbody has always been given the rank of “river” but it would be better named stream for its intrinsic characteristics (Miliani, 1937).

The Noce river stream is another important tributary to the Adige River. The watercourse is located in the northwestern part of the Trento province and it comes from the Corno dei Tre Signori (3359 m a.s.l.) peak whom receives the Noce Bianco that flows from the glaciers of the Cevedale group in the village of Cogolo (Miliani, 1937). Its basin is 1375 km^2 wide and the average height is 1630 m. Its overall length is 105 km and it shows an average slope of 3.04%. Noce develops along a south-eastern direction (where it flows directly in the Adige River close to the village of Zambana, after the strengthening works made of the middle 19th century to prevent regurgitation events) (Miliani, 1937). This watercourse has quite a high solid transport since it flows among crumbly rocks (Miliani, 1937).

The Avisio stream has been commonly called “rodent of porfids” (Miliani, 1937) since it flows among these materials for 91.47 km with an average slope of 2.02%. Its spring is in the Marmolada glacier (3342 m a.s.l.) and its basin extends for 939.58 km^2 and its perimeter is 228 km. The basin is developed with a North-eastern direction (right after the artificial basin of the Fedaia) and it ends in the village of Lavis where the stream flows in the Adige River. In the past, the influence played by the Avisio stream on the Adige river was noteworthy either for its discharges or for the high quantities of detritus and scattered materials from basin’s rocks and this has threatened the city of Trento and the village of Lavis for many years. Now the danger has lowerd thanks to the hydraulic works done throughout the years by the offices of the Province of Trento (Miliani, 1937).

2.2.2. Hydrologic and Morphologic features

The basin of the Isarco stream is essentially composed of scystic- crystalline rocks with granitic and dioritic nature. These typical impermeable materials are patterned with semipermeable materials around the area of Vipiteno and Val Aurina; in addition, there are few calcareous areas at the end of its basin, with typical permeable nature (Miliani, 1937).

The geology of the lands surrounding the Noce streams have a characteristics alluvional pattern: the materials are generally gross and forms free aquifers connected to the main path of the Noce stream. We can distinguish two main parts along the Basin: a highest portion (Val di Sole) which is very steep and a lower portion (Val di Non) that is generally more plain (Piano Generale di Utilizzazione delle Acqua Pubbliche, 2006). Starting from the ‘50s, three major reservoirs have disrupted the longitudinal continuity of the Noce stream: two in the upper basin (Careser, 2600 m a.s.l. and Pian Palù, 1850 m a.s.l.) and one in the middle part of the stem (Santa Giustina, 530 m a.s.l.) (Carolli et al., 2008).

The land of the Avisio basin generally have a Quaternary filling that is nowadays thicker especially in the lower part because of the water action. In the higher and middle parts of the valley (Val di Fassa and high Val di Fiemme) there are gross sediments, both having sedimentary and volcanic origins, while in the lower portions (medium/low Val di Fiemme and Val di Cembra) there are finer levels. On the confluence between the Avisio and the Adige waters, there is the city of Lavis located on an alluvional conoid with gross materials. The Avisio stream flows between 51 little lakes that as a whole, cover an area of 2.57 km^2. Four of them are regulated. On the basin, there are glaciers that cover an area of 2.99 km^2 that are concentrated mainly around the Marmolada area (Piano Generale di Utilizzazione delle Acqua Pubbliche, 2006).

Several dams have been built in these watercourses: in the Avisio basin, there is the Fedaia (close to the source, on the Marmolada glacier with a total capacity of 16.6 million m^3), the Travignolo (lying at 1400 m a.s.l. with a capacity of 32.1 million m^3) and the Stramentizzo dams (with a volume of 11.5 million m^3) (Basin Authority of Adige River, 2003). On the Noce basin, instead, there are the Careser, Pian Palù, Santa Giustina and Mollaro dams. The Santa Giustina dam is lying 500 m a.s.l. and has a capacity of 182.8 million m^3. Due to its high capacity, Santa Giustina, as well as the Resia lake, are also used for flood control (Gumiero et al., 2009).

3. MATERIALS AND METHODS

3.1 FIELD WORK AND SAMPLING STATIONS

FIRST YEAR SURVEY. In the first year of my survey, I have collected bi-monthly samples of planktonic diatoms from March 2007 to February 2008 in five stations of the watercourse (total distance span of ca. 300 km): station 1- Bolzano, station 2- Cortina all'Adige, station 3- Besenello, station 4- Pescantina and station 5- Boara Pisani (for an easier location, the stations are named according to their closest municipality). The sampling dates are 23: 19/03/2007; 03/04/2007; 18/04/2007; 08/05/2007; 21/05/2007; 04/06/2007; 18/06/2007; 02/07/2007; 28/07/2007; 08/08/2007; 27/08/2007; 10/09/2007; 24/09/2007; 08/10/2007; 22/10/2007; 05/11/2007; 19/11/2007; 03/12/2007; 19/12/2007; 07/01/2008; 21/01/2008; 04/02/2008 and 18/02/2008. Stations have been chosen according to two main reasons: first they needed to be representative of the watercourse area and then they needed to have a bridge or a path where to sample without using boats. The choice ended up in figuring these five stations scattered along an hydrological gradient (Fig.3.1).

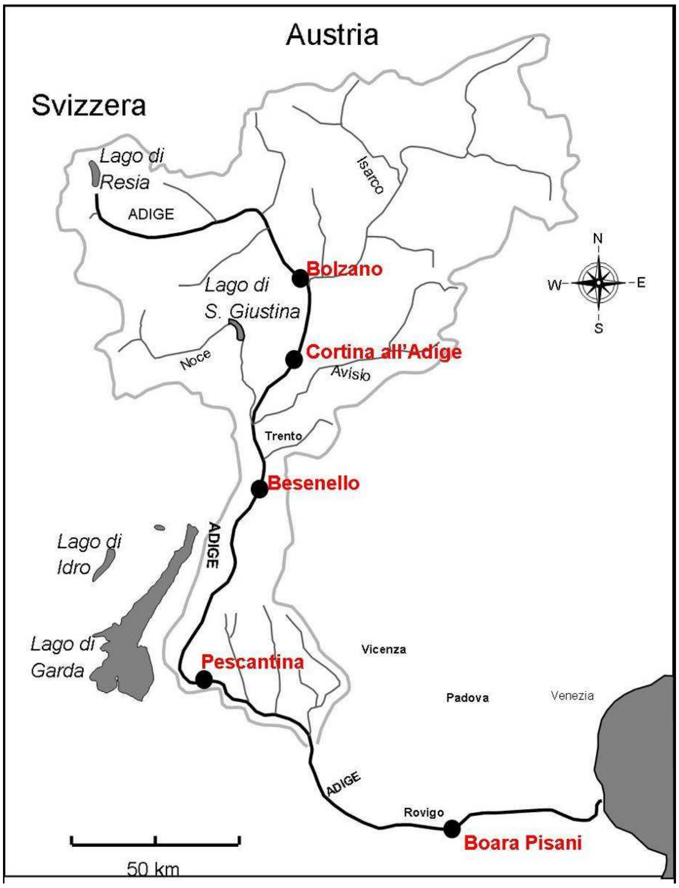


Fig.3.1- First year sampling stations along the Adige River

The stations of Bolzano, Cortina all'Adige and Besenello are located in the mountainous section of the river while Pescantina and Boara Pisani are lying in the piedmont zone. The first station (Bolzano) is lying southward the homonymous city, before the inlet of the Isarco tributary in the Adige. The second (Cortina all'Adige) and the third one (Besenello) are located 15 and 10 km respectively north and south of Trento. In the Besenello station, the Adige River has already received the Avisio and Noce waters. The fourth station (Pescantina) is located north of the city of Verona while the last one (Boara Pisani) is the southethern one (Fig. 3.1). Stations 1, 2 and 3 are characterized by having a typical deep bed with high water depths but differ in their texture (Bolzano having a coarser gravel bed) and shores status (Cortina all'Adige having more natural shores). Even though lying in the valley part, station 4 displays typically low water depths since the river suffers from the loss of high quantities of water which have been taken by the Biffis channel (agricultural and irrigation used) before this station and given back to the mainsteam after the station. Station 5 is representative of the "valley" river since it has a slope of 0,02 ‰ and a width of the banks of 100m ca. with a maximum depth of 8 metres. In the first year survey, the bi-monthly samplings were carried out with the help of the Environment Agencies of Bolzano and Rovigo to effectively cover the long-distance of the river. The Authority of Basin of River Adige and the Autonomous Province of Trento kindly provided the discharge data, obtained by multiplying the mean velocity of the water in the station by the cross-sectional area of the flow (Gore, 2006).

The sampling occurred from bridges using a bucket to collect the water. The work has been done with a careful sequencing: four buckets of water were firstly collected and put on a 50 liters dud so that they were mixed. From there the sub-samples have been collected for the following laboratory analyses:

i) Dry weight

ii) Chlorophyll *a*

iii) Phytoplankton, fixed with Lugol's solution

iv) Diatoms, fixed with Lugol's solution

With another bucket of water collected independently from the previous, I have obtained the 1l sample for the chemical analyses to be stored in a PET plastic bottle. After that, a sample for the dissolved oxygen analyses was collected and stored in Winkler bottles (avoiding the entraining of atmospheric gases) with a subsequent addition of 3 ml of manganese (II) and 3 ml of a solution of potassium iodide and azide sodium.

Samples for zooplankton analysis were collected in the first year survey. This was done using a 50 µm plankton net to filter 50 liters of water. The sample was preserved in 70% ethanol for further analysis.

The collected samples were stored in iced boxes and then brought to the laboratory of San Michele all'Adige where they were processed, the day after.

In each station, several measurements were taken with probes, including:

i) Water temperature (℃) with a Digital Termometre Testo 960;

ii) Conductivity (µS cm^{-1}, a 20℃), pH and dissolved oxygen (mg L^{-1} e % di saturazione) of the water using the multi- parametric probe WTW 450i;

iii) Water turbidity (NTU) with Turbidity probe Hach, Delta OHM HD 25.2.

SECOND YEAR SURVEY. My second year survey has concentrated on a shorter river path (total distance span 40 km ca.) including 8 river stations sampled also of benthic diatom communities. In this second year enquiry, since the stations were located closer, they were not called as previously according to the municipalities, instead named with the progressive letters (A, B, C, D, E, F, G, H) from source to mouth. Stations have been sampled once a month for 11 dates: 16/03/2009; 20/04/2009; 24/06/2009; 13/07/2009; 28/08/2009; 16/09/2009; 20/10/2009; 25/11/2009; 14/12/2009; 26/01/2010 and 16/01/2010.

The experimental design was built in order to investigate the Adige River and its major tributaries (Isarco, Noce and Avisio), right before and after their inlet on the mainstem, having care to find a suitable place to enter the watercourse without major dangers for my colleagues and myself. In addition, I have chosen the sampling points having care to avoid heavy shaded areas that could affect diatoms physiological processes and paying attention to find areas that could have been submerged for weeks (rule of thumb of approximately three weeks) in order to avoid shallower areas without proper diatom communities. Also, I took care to find areas that could present a representative average current speed of the station: in general, I avoided zones of very high current velocity both for safety and for representative reasons. I therefore chose these eight stations (Fig. 3.2):

- STATION A: it is located in the Adige River, before the inlet of the Isarco. This station has typical muddy bed;
- STATION B: this station is on the Isarco stream, before its entrance in the Adige waters. Because of the nature of the stream, the Isarco is dominated by pebbles and cobbles;
- STATION C: it is right after the confluence of the Isarco and the Adige. This station has a typical silty bed and it is characterized by having particularly high banks. These first three stations were in the Autonomous Province of Bolzano;
- STATION D: moving 40 km southward (where no inlet has yet entered the mainsteam) there is this station which has a typical muddy nature and high banks;
- STATION E: this station is lying on the Noce stream right before its confluence with Adige river. It has a typical gravel-cobblish bed that suffers from the effects played by the hydropower plant of Mezzocorona, which roughly discharges 60 m^3/sec during operation;
- STATION F: this stations is positioned upstream of the confluence with the Avisio stream and has a muddy river bed;
- STATION G: this station is lying in the Avisio stream which, differently from the other two previous streams, has a typical silty bed nature;
- STATION H: it is the final station, downstream of all the three tributaries. The substrates is typically silty.

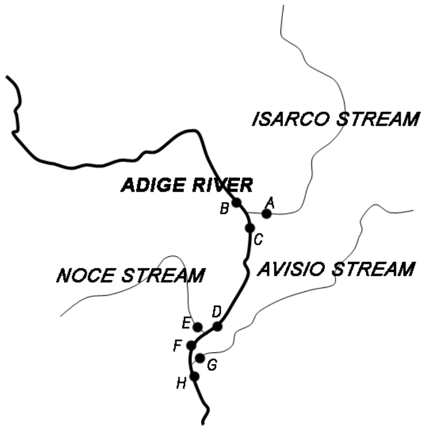


Fig.3.2- Second year sampling stations along the Adige River

The same sample procedure as the previous year (pH, conductivity, dissolved oxygen, alkalinity, turbidity, nutrients, ions, phytoplanktonic diatoms, chlorophyll *a*, dry weight, temperature) was applied but adding four samples of benthic diatoms: epilithon, epipsammon, epiphyton and epipelon. These four different compounds were sampled in each of the 8 stations, with great care in differentiating them while doing it. The epiphytic component was not numerically important in the Adige River so, I did not sample it further than two months. The procedure used is different in regards to the substrate:

- Epilithic community (diatoms on lithic substrates) are usually found in areas of the streams that experience relatively fast currents. Thus, epilithic algae are often tightly attached to the substratum. Five pebbles/cobbles were randomly selected and removed from the stream and placed in a pan for processing onshore. They were scraped into another pan from all the areas of the pebble/cobble exposed to light and not buried in sediments, with a knife to remove most of the algae and then with a toothbrush to remove more tightly attached individuals. The pebble/cobble's surface was then rinsed along with the sampling tools into the pan with distilled water from a squirt bottle. The subsampling bottle was then filled with this material and the total volume of the sample was recorded on the field data sheet;

- Epipsammic community (diatoms living on sand). A small quantity of the sand substrate (obtained directly on the station) was shaken in a bottle containing water so that the sand would quickly fall to the bottom of the bottle after agitation and the suspended algae could be poured off the top into another 100 ml plastic container (Lowe & LaLiberte, 2007). When the substrate was coarser, a swirl-and-pout technique was used to remove algae by repeatedly adding small amounts of water to the sample, swirling it to tumble the sediments and thereby scouring algae from the fine substrate. Afterwards, the suspended algae were gently poured from the sample to a white pan (this step was repeated 5-10 times or until the poured water appeared relatively clean) and then to the subsampling bottle (Stevenson & Rollins, 2006);

- Epipelic community (diatoms living on mud) are often only loosely associated with the substrate: they often occur in areas of no current where fine sediments can accumulate. Epipelon has been collected with a pipette with extreme care to avoid the penetration of the sediments too deeply or with a Petri dish and spatula from at least five representative locations of the station (Stevenson & Rollins, 2006).

Phytoplanktonic diatoms have been analyzed differentiating live cells (living diatoms with live chloroplast) and dead cells (empty frustule diatoms without chloroplast) as in Padisák et al., 2003. Each sampling station was assessed for its granulometry through visual assessments operated making an average of the estimated values collected in 8 different occasions, in different seasons and different hydrological regimes of the watercourses.

3.2 LABORATORY WORK

Oxygen has been directly fixed while sampling and afterwards it has been worked out in the lab using the Winkler method (APHA et al. 1995).

Total phosphorus concentration (TP) has been measured on the samples not yet filtered. Soluble reactive phosphorus (SRP), nitrates ($N\text{-}NO_3\text{-}N$), nitrites ($N\text{-}NO_2$) and ammonium ($N\text{-}NH_4$)have been determined in the filtered samples following the protocols (APHA et al., 1995).

Dry weight (105℃) and the organic substance (550℃) have been determined after the removal of large particles with a 0.2 mm filter, filterering then the samples on Whatman filter GF-C . Chlorphyll a has been determined with the spectrophotometer after filtration with GF-C Whatman filters, breakage of the cells with an Ultra Turrax and pigments extractions in 90% aceton for 24 hours (Lorenzen, 1967).

As concerns the diatom samples (both planktonic and benthic ones), the procedure is more articulated: the sample stored in PET bottles have been concentrated by sedimentation (1:50) using a centrifuge and then cleaned in 30% hydrogen peroxide and 37% hydrochloric acid (Kelly et al., 1998) under a fume hood. Cleaned diatom frustules were then permanently mounted in Naphrax® resin on the glass slide. On each slide 400 valves were counted (European Committee for Standardization, 2004) under a light Leica microscope at 1000 magnification in order to have relative numbers. The use of relative numbers is often preferred for monitoring purposes because it simplifies sampling and processing of algae: the sampled area and subsample volume need not to be precisely determined (Reavie et al., 2010). Taxa were identified following the more recent monographs of the series Süßwasserflora von Mitteleuropa, established by A. Pascher (Gustav Fisher Verlag and Elsevier, Spectrum Akademischer Verlag) and the most updated literature.

3.3 MORPHO FUNCTIONAL DIATOM GROUPS (MFDGs) AS PROXIES OF ASSEMBLAGE STRUCTURE

A good number of studies have proven that a functional group approach in the studies of freshwater communities can give a good description of the community (Irfanullah, 2006; Pan et al., 1999). Using a functional approach can certainly alleviate problems connected to the taxonomic uncertainty easily found using diatom species (Soininen et al., 2004) where the operator has to be very skilled in their identification.

In this thesis I have utilised different Morpho-Functional Diatom Groups (MFDG) as defined, in the frame of my research project, by Centis et al. (2010) (Fig. 3.3).

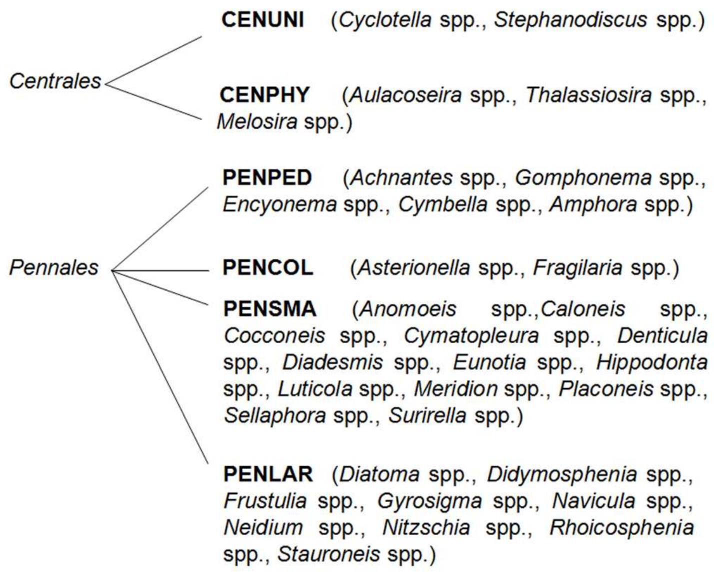


Fig.3.3- Morpho Functional Diatom Groups. From Centis et al (2010)

The criteria adopted to discriminate the groups include morphology and, partly, habitat selection and modality of adhesion to river substrate. The first division separates the two Bacillariophyceae orders (Centrales and Pennales). Within Centrales, the unicellular euplanktonic diatoms (CENUNI) are separated from the filamentous diatoms that can be planktonic or benthic, unattached to any substratum (namely the CENPHY) (Barber & Hawart, 1981). A similar splitting has been applied to the Pennales: PENPED comprehends taxa being attached with stalks better adapted to high current velocities and displaying tychoplanktonic status (Sabater, 2009). PENCOL encompasses colonial diatom taxa. Further subdivisions were based on size ending in two heterogeneous groups mostly benthic or tychoplanktonic (Barber & Hawarth, 1981) (PENLAR and PENSMA).

3.4 NUMERICAL TECHNIQUES

Pearson correlation was used for measuring the strength of the relationship between two variables. Multivariate numerical techniques have been further used in order to assess the main environmental drivers and the dynamics of the diatom communities, both benthic and planktonic ones. The ordination of diatom relative abundances has been carried out by Non Metric Dimensional Scaling (NMDS) (Kruskal & Wish, 1978) applied to Bray and Curtis dissimilarity indices (Podani, 2000) computed on species percentages, after an arcsinus transformation to reduce the weight of the most abundant taxa. The same normalisation procedure was also applied for Principal Component Analysis (PCA) performed both for Morpho-Functional Diatom groups and diatom genera (only genera that display frequencies higher than 10% have been used to avoid noise and sparse matrices). This group of analyses have been carried out with SYSTAT^{TM} 10.2 and CANOCO^{TM} 4.5 packages.

Multiple Responses Permutation Procedure (MRPP) is a non-parametric method that has been used to test the hypothesis of no difference beneath the samples collected in each substrate in the stations (Biondini et al., 1985): this method which is similar to discriminant analysis has the advantage of not requiring assumptions (such as multivariate normality and homogeneity of variances) that are seldom met with ecological community data. The Sorensen coefficient on log(x+1) abundance data has been used as the distance measure in MRPP. Cluster analysis has therefore been used to produce a hierarchical classification of the species beneath the stations based on their similarities (Sneath & Sokal, 1973; Gauch, 1982) in order to analyse the patterns of the three substrates in each station and then results have been further investigated with Indicator Species Analysis (ISA). This method combines information on the concentration of species abundance in a particular substrate and its faithfulness of occurrence, testing the results using a Monte Carlo technique. Dufrene & Legendre (1997) have proposed this method to give ecological meaning to groups in sites and to provide criteria to compare them: this method is also very useful to choose a stopping point in cluster analysis, pointing therefore out the main levels in hierarchical classification of samples and substrates. This method has proven to be effective also with percentage values as shown by many diatom researchers (see Potapova & Charles, 2003; Tornés et al, 2007; Stevenson et al., 2008) and it is has been pointed to be the best to predict species associated to a specific substrate. The indicator value of a taxon varies from 0 to 100 and it attains its maximum value when all individuals of a taxon occur at all sites of a single group. ISA is considered superior to more traditional methods of identifying indicators (e.g. TWINSPAN) on both statistical and practical grounds (Legendre & Legendre, 1998; McGeoch & Chown, 1998). For example, it is robust to differences in within-group sample sizes and abundances across species, therefore it is ideal for my analysis. The significance of the indicator value for each species has been tested with a Monte Carlo randomization procedure with 1000 permutations. All analyses have been conducted prior an arcsinus transformation of diatom abundance data, except ISA which uses untransformed data. These analyses have been performed using the statistical software PC-ORD^{TM} for Windows 4.0 (McCune & Mefford, 1999).

4. RESULTS

4.1 PLANKTONIC DIATOM COMMUNITIES: PATTERNS, PROCESSES AND DYNAMICS

Herein I will present the results of the environmental variables and diatom communities and their interactions during the first year sampling. Graphs have been charted grouping together the first three stations, which can be considered as representative of the upstream reach and the last two stations which are valley stations and therefore downstream-indicative. Station 4 cannot be considerate so reliable of piedmont features though, since agricultural/irrigation withdrawals (e.g. the Biffis channels- see materials and methods session) modify the river's natural condition in this station, giving it similar features of the upstream river reaches (e.g. stations 1, 2 and 3).

4.1.1. Hydrological variables and temperature

In the upstream stations, discharge is showing decreasing values going southward: station 1 has a mean discharge of 130 m^3 sec^{-1} (min=74; max= 250 m^3 sec^{-1}) , station 2 of 92.55 m^3 sec^{-1} (min=45.70; max= 198 m^3 sec^{-1}) and station 3 of 36.68 m^3 sec^{-1} (min=23; max= 74.86 m^3 sec^{-1}). In the lowland stations, station 5 has a mean value of 121 m^3 sec^{-1} (min= 67, max= 121 m^3 sec^{-1}) that could be influenced by the water withdrawals on the Adige waters for irrigation and agricultural purposes (Fig. 4.1). The upstream stations show significant correlations with each other, as expected (r= 0.98, p<0.01 between station 1 and 2 and r= 0.91, p<0.01 between station 2 and 3). In all the stations, it is evident the increase of discharge in spring and summer months (e.g. from May to July) given to the melting and thawing of ice and snow from the glaciers and mountains in the river's basin. Mean discharges measured in Bolzano, Cortina all'Adige, Besenello e Boara Pisani between June and August, in fact, are 153.40, 150.76, 196.17, and 153.57 m^3 s^{-1}, which are quite different from the year's mean values.

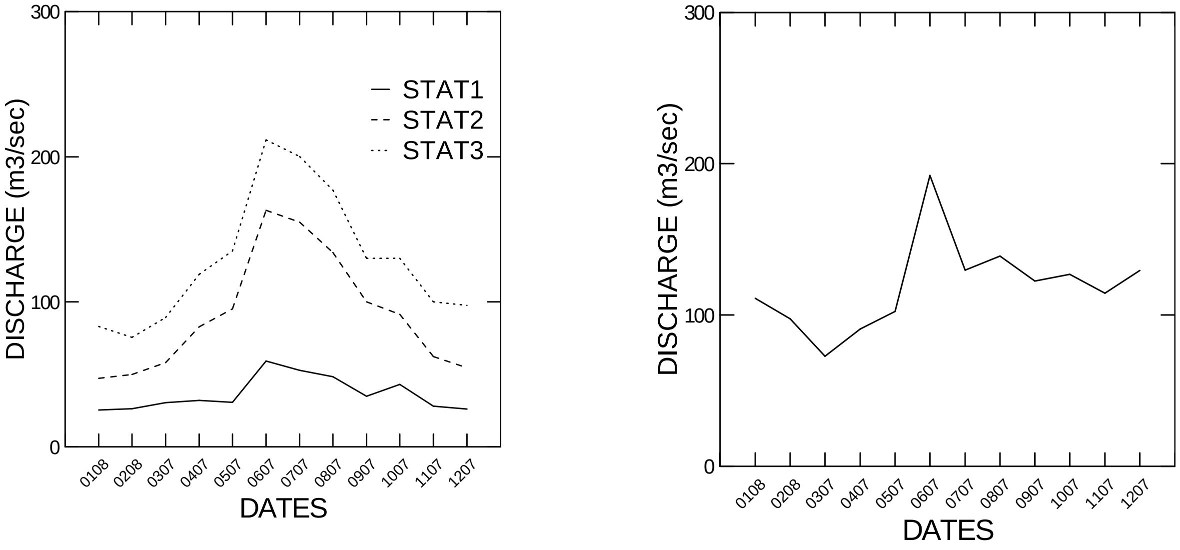


Fig. 4.1- Discharges (m^3 sec^{-1})measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones: discharge values of station 4 are not available.

This pattern is also reflected by turbidity: highest mean turbidity is higher in station 1 (mean= 27.31 NTU; min= 3, max= 256 NTU which is due to the peak recorded in July) and decreases southward (mean= 23 NTU; min= 3, max= 318 NTU in station 2 and mean= 9.53 NTU; min= 2.8, max= 36 NTU in station 3). In the lowland stations, turbidity is higher in station 5 (mean= 15 NTU; min= 4.2, max= 69 NTU) and considerably lower in station 4 (mean= 7.1 NTU; min= 1.3, max= 27 NTU) (Fig.4.2). Highest values have been recorded in the summer months, as noticed for discharge.

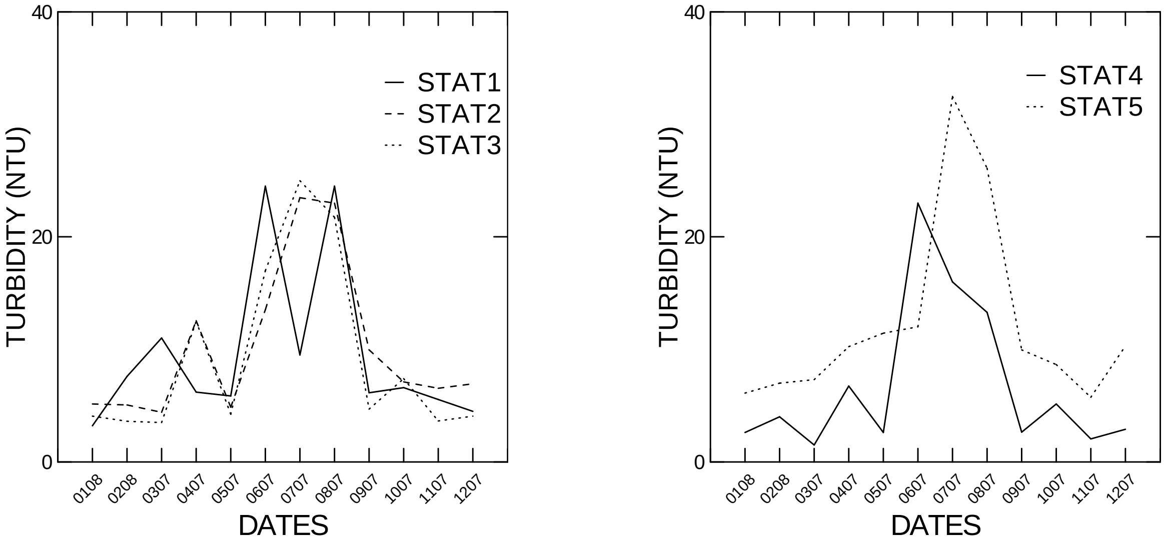


Fig. 4.2- Turbidity (NTU)measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Upstream stations have shown colder temperatures (mean= 9.76, 8.86 and 9.97 ℃ in station 1, 2 and 3, respectively) while in the lowland stations higher values have been recorded (mean= 12.23 and 12.60℃ in station 4 and 5, respectively). Adige water's temperatures range between 0 and 16℃ in the upper river reaches and 3 and 22℃ in the lower reaches: highest fluctuations have been registered in station 5- 2.8℃ in December and 22.4℃ in August (Fig. 4.3). Overall, it seems clear that this watercourse has relatively colder waters. Water temperatures have shown different mean values between the five stations: stations 4 and 5 have shown mean temperatures higher than 4℃ if compared to the first three stations.

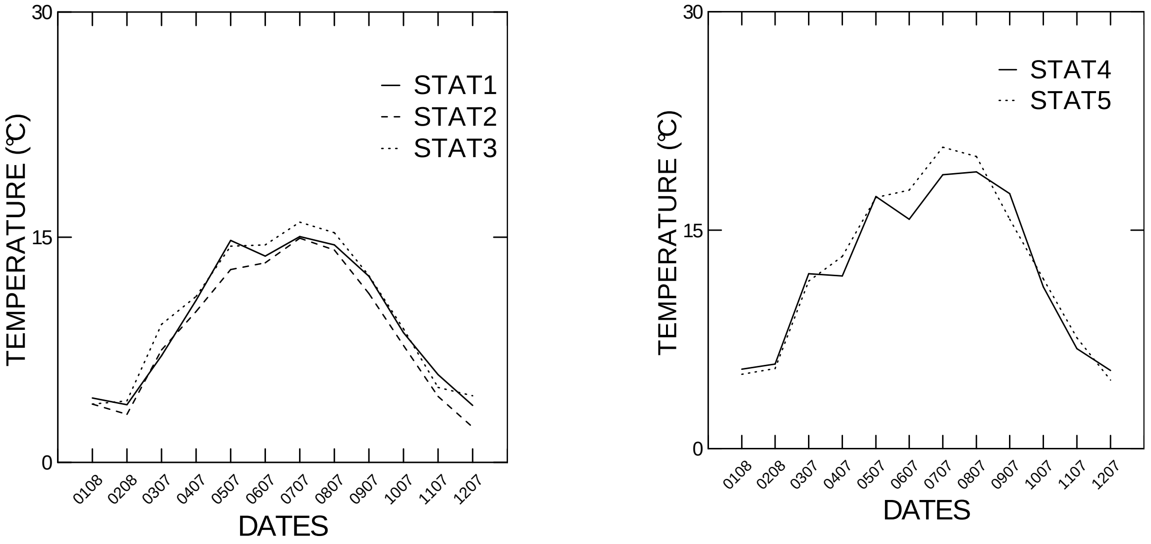


Fig. 4.3- Temperature (°C) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

4.1.2. Chemical variables

Oxygen concentrations has shown decreasing values going southward, with the exception of station 4 (Fig. 4.4) . This variable has proven to be highly dependent on water temperature: in all the sampled stations a non linear negative relation has been recorded between these two variables (station1: $r= -0.85$, $p<0.01$; station2: $r= -0.83$, $p<0.01$; station3: $r= -0.92$, $p<0.01$; station4: $r= -0.70$, $p<0.01$; station5: $r= -0.93$, $p<0.01$). Also oxygen saturation values have been charted in order to get rid of the effect induced by temperature on this variable: mean saturations are higher in station4 (105.78%) while lower in the other stations (95 ≤ oxygen saturation ≤ 96.34%) as from figure 4.5.

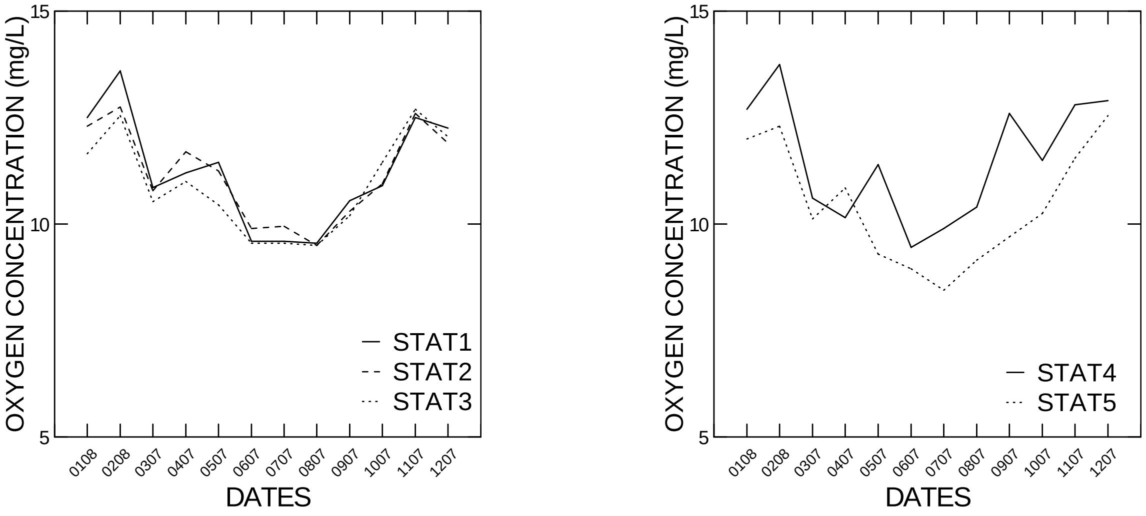


Fig. 4.4- Oxygen concentrations (mg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

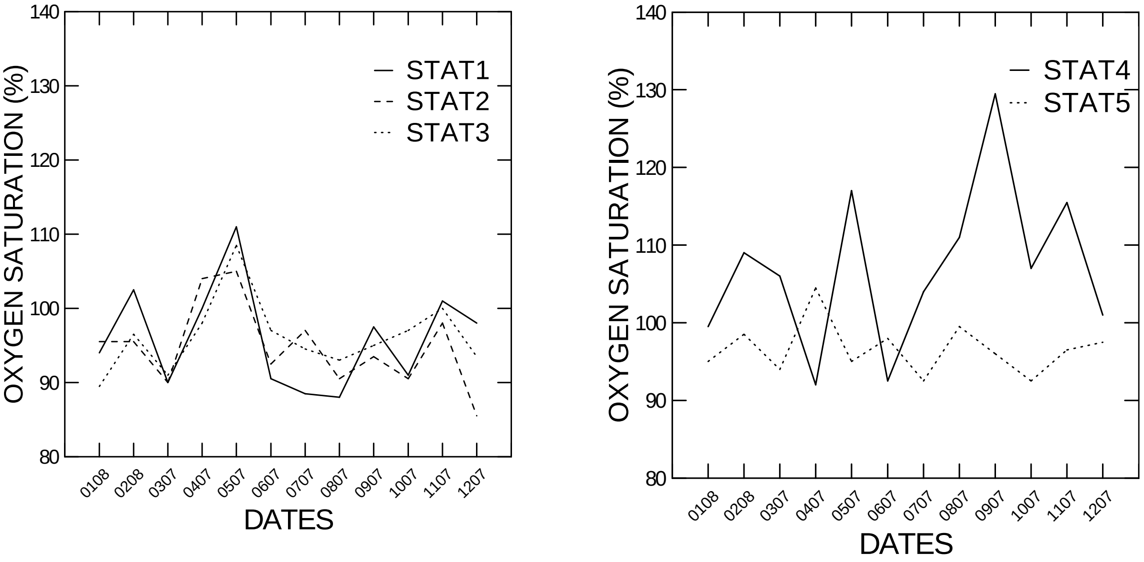


Fig. 4.5- Oxygen saturations ($^{\%}$) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

pH charts of the five stations are represented in fig.4.6. Among the stations, pH mean values range between 7.6 and 9: the highest value has been recorded in station 4 while the lowest in station 1.

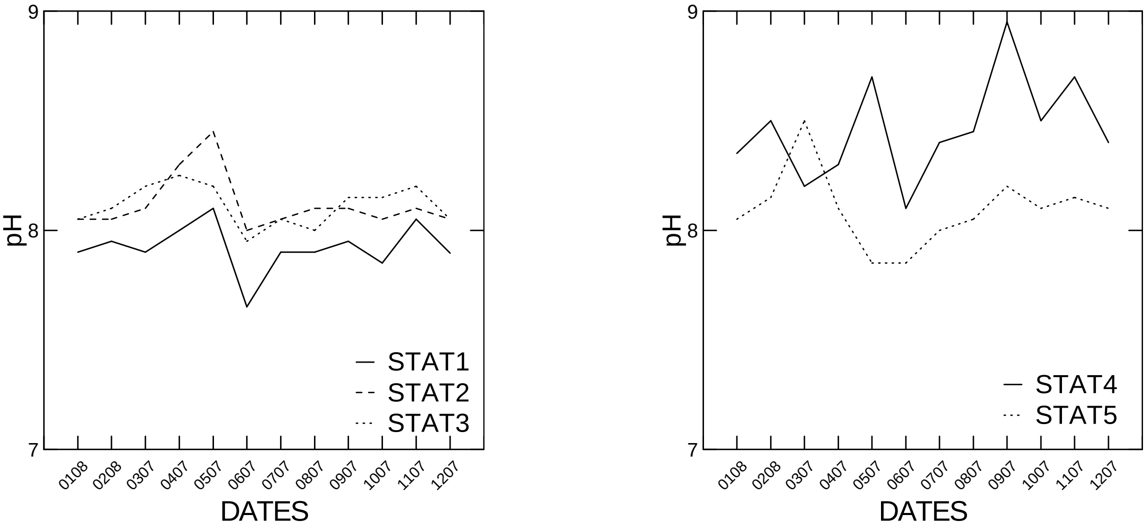


Fig 4.6-pH measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Conductivity measurements at 20℃ show a decrease in the summer months for all the stations (lower peak in the month of September). This is surely due to snow melting and ice thawing from mountains and glaciers of the basin that bring along high amounts of water with lower salinity. In station 1, for example, the mean conductivity value measured between June and September is 194 µS cm^{-1} while throughout the year it is 246 µS cm^{-1}. From station 2 to 5, there is a general linear increase in the mean conductivity value southward the river range (from 237 a 272 µS cm^{-1}) (Fig. 4.7).

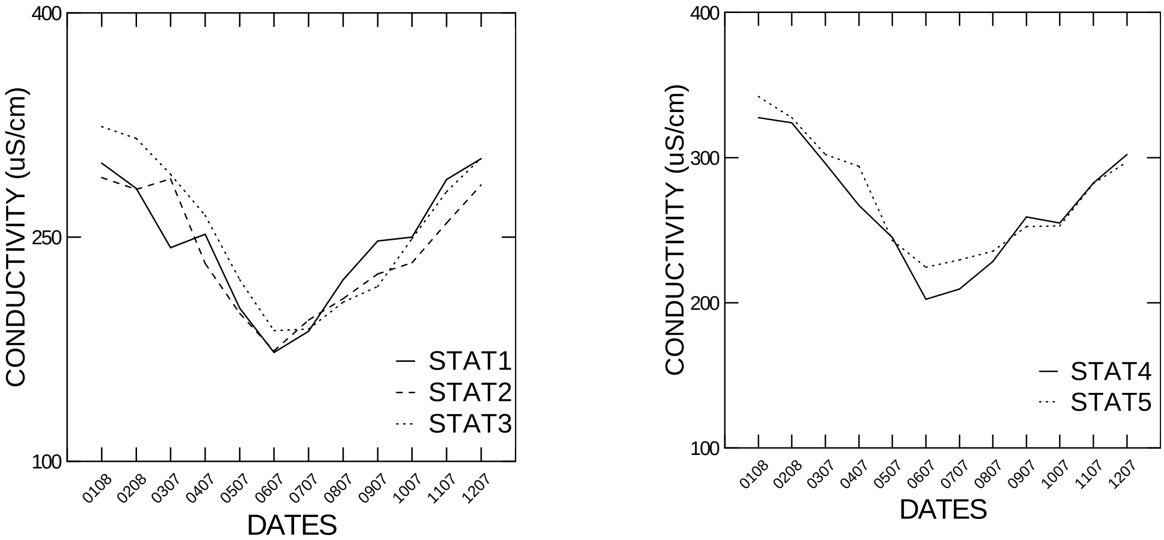


Fig. 4.7-Conductivity (µS cm^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

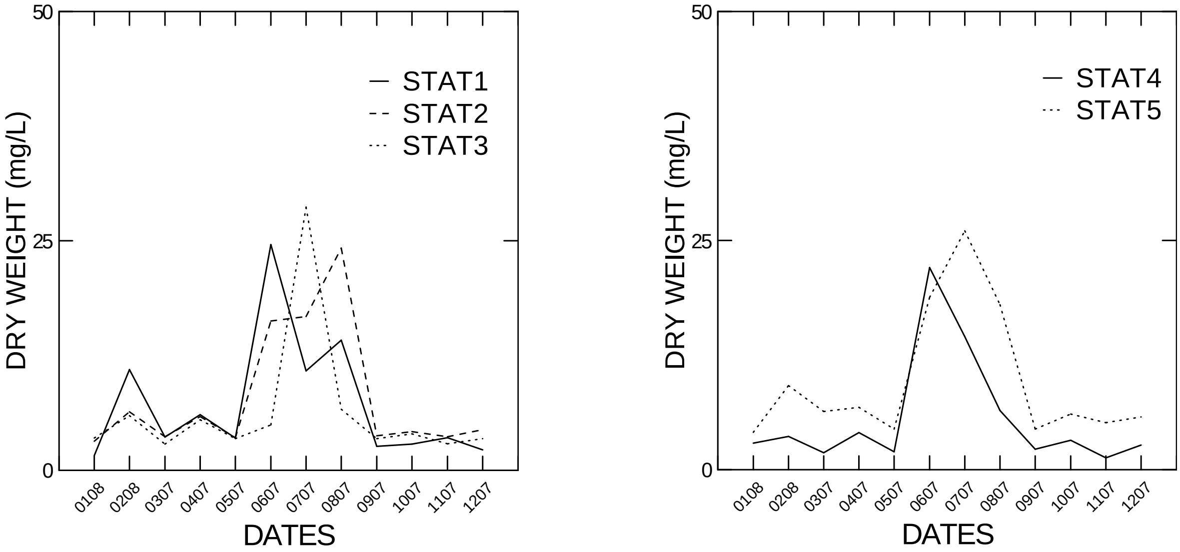


Fig. 4.8-Dry weight (mg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

In all the stations, the highest abundances of suspended material has been registered in the summer months (Fig. 4.8), when the contribution of algal material has started to show numerically important. The highest mean abundances of suspended material have been registered in station 1 (11.33 mg L^{-1}) and 5 (9.72 mg L^{-1}). Station 4 has the lowest mean value registered (mean= 5.72 mg L^{-1}; min= 1.11, max= 35.06 mg L^{-1}).

The upstream stations and station 4 have mean values (18.46; 12.02; 18.60; 18.11 µg P L^{-1}, respectively) half of those recorded in station 5 (36.65 µg P L^{-1}). In the upstream stations, the minimum values recorded are not higher than 1.63 µg L^{-1} which is 20 times less than that recorded in station 5 (21.20 µg P L^{-1}). SRP lowest mean abundance has been recorded in station 2 (mean= 12.02 µg P L^{-1}; min= 1.63, max= 26.09 µg P L^{-1}) (Fig. 4.9).

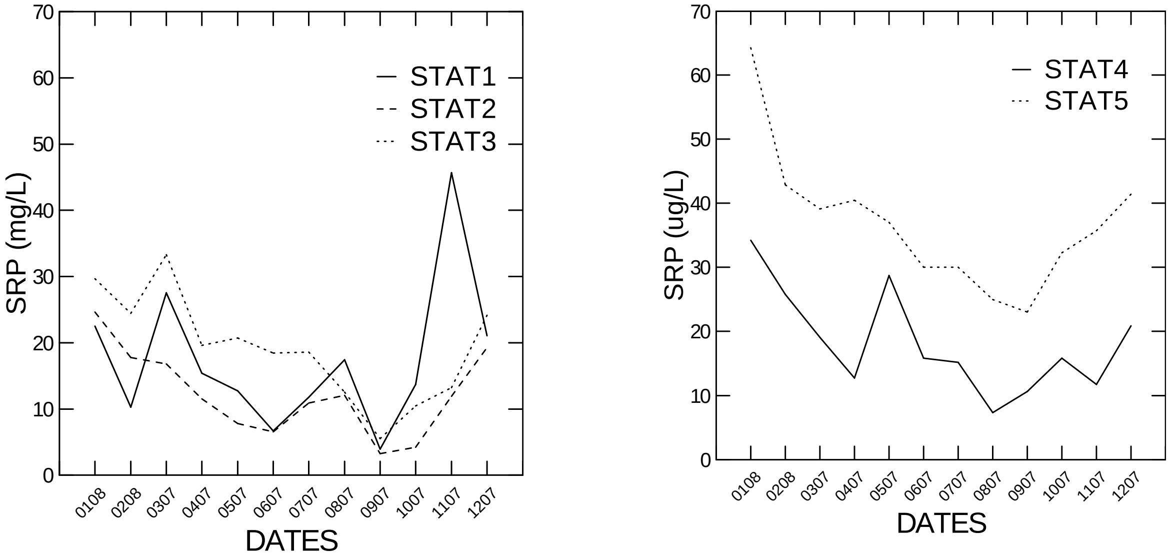


Fig. 4.9-Soluble Reactive Phosphorus SRP (µg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

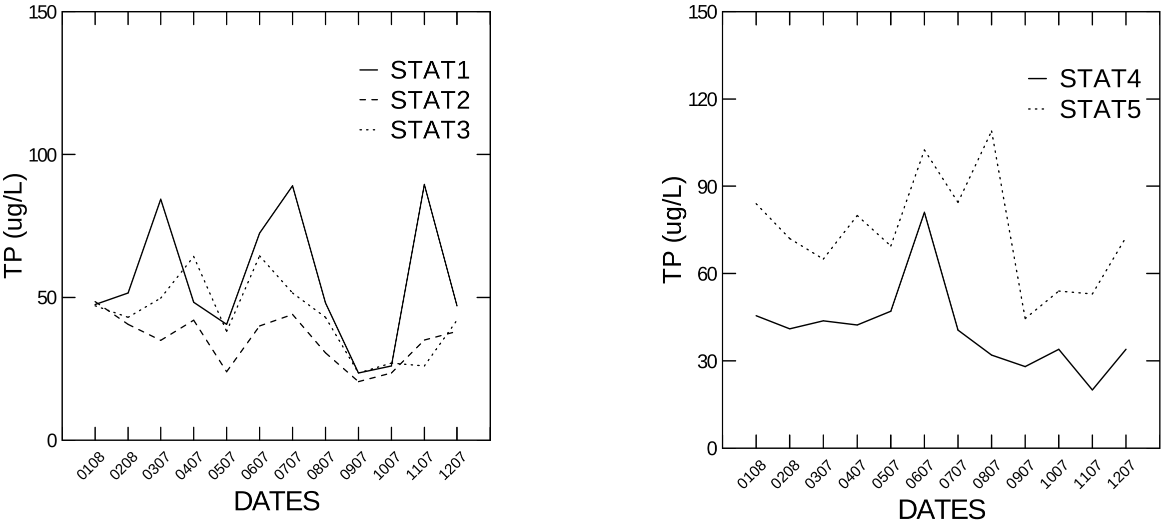


Fig. 4.10-Total Phosphorus TP (µg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Total phosphorus has never been recorded with mean year concentrations higher than 54 µg P L^{-1} in the first four stations (from station 1 to 5: 54.38; 35.12; 43; 48.63 µg P L^{-1}) while station 5 has a mean value of 74.60 µg L^{-1} (min= 44; max= 126 µg P L^{-1} in August) (Fig. 4.10).

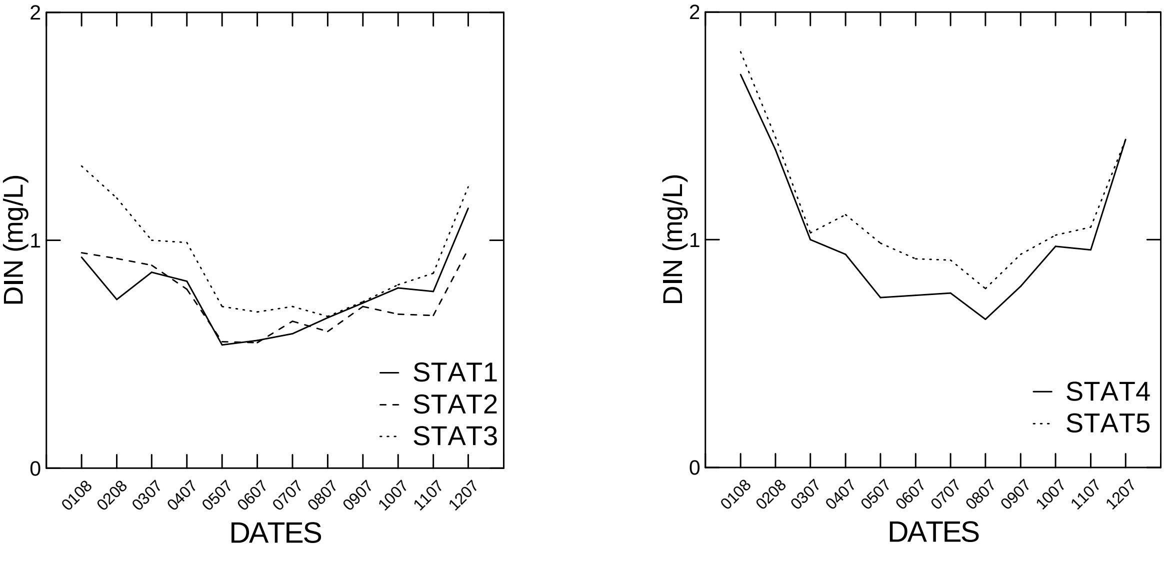


Fig. 4.11-Dissolved Inorganic Nitrogen DIN (mg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Dissolved Inorganic Nitrogen DIN (DIN: N-NO_3 + N-NO_4 + N-NH_4) has ranged between 0.5 and 1.7 mg N L^{-1}, never showing mean abundances higher than 1 mg N L^{-1} in the upstream reaches (0.75, 0.73, 0.90 in station 1, 2, 3 respectively). In the downstream stations, instead, DIN showed higher mean abundances (1.01 and 1.12 mg N L^{-1} in stations 4 and 5, respectively). The dominating fraction of DIN (nitrates) have showed values ranging from 0.5 to 2 mg N L^{-1}, while ammonia and nitrites have showed values lower than 0.2 mg N L^{-1} and 0.003 mg N L^{-1} (Fig. 4.11).

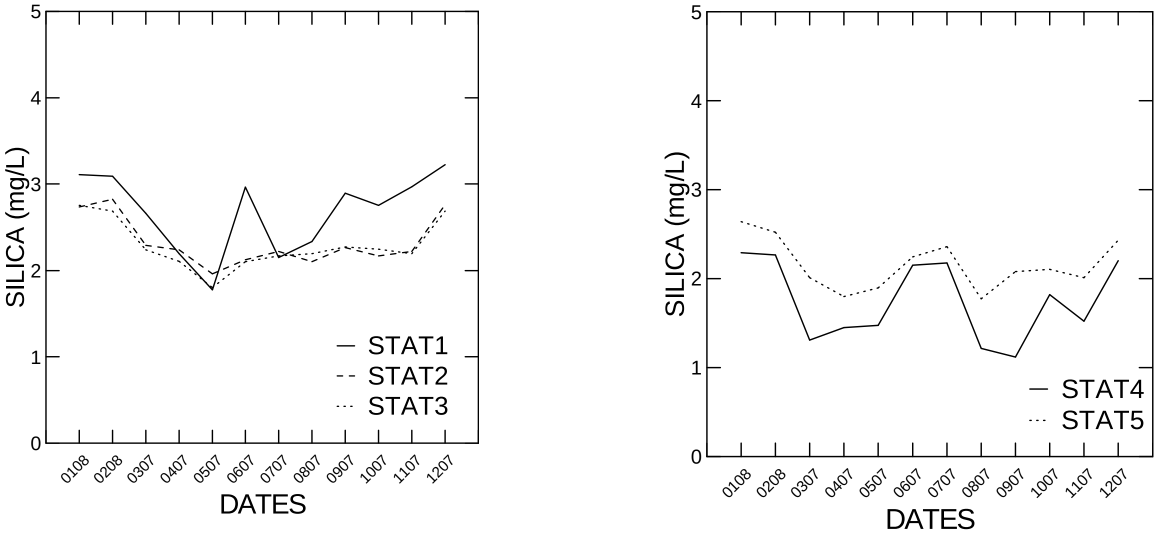


Fig. 4.12- Reactive silica (mg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

DIN, SRP and TP have showed a gradual increase southward: higher abundances have been recorded in station 5. On the contrary, reactive silica shows an opposite trend since it decreases southward. The lowest mean abundance has been registered in station 4 (mean= 1.76 mg Si L^{-1} ; min= 0.14, max= 2.38 mg Si L^{-1}) while the highest in station 1 (mean= 2.67 mg Si L^{-1} ; min= 1.45, max= 3.69 mg Si L^{-1}) (Fig. 4.12).

4.1.3. Algal communities

The highest mean abundances of chlorophyll *a* have been recorded in station 1 and 5 (2.47 and 2.34 µg L^{-1}, respectively). In station 1, there has been registered the highest peak of this variable (15.21 µg L^{-1}) in April due to a massive presence of a typical meroplanktonic taxa, *Diatoma* spp. In the middle stations, mean abundances are lower throughout the year (2.12, 1.4, 1.5 µg L^{-1} in stations 2,3 and 4, respectively). A common pattern between the stations, is the increase of chlorophyll *a* during the spring months (e.g. from April to June). There seems to be a different development in the fall months beneath the upwater and the downwater stations: in station 1, 2 and 3 there is a general decrease of the abundances while in station 4 and 5 there seems to be a different trend (Fig. 4.13).

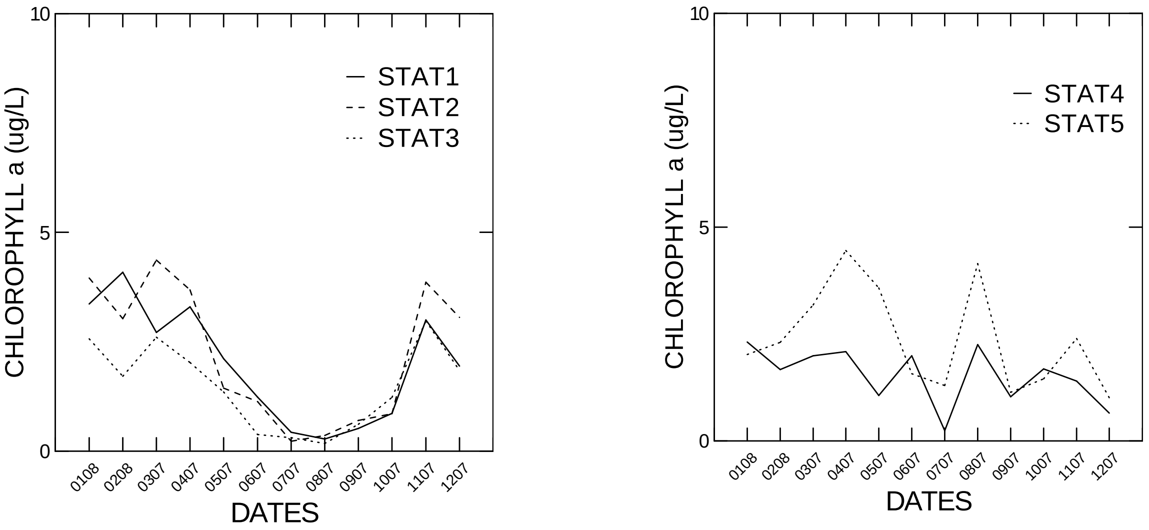


Fig. 4.13- Chlorophyll *a* (µg L^{-1}) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Analyzing the diatom communities using the classification proposed by Medlin & Kaczmarska (2004), different patterns among the communities become evident as in fig. 4.14. The highest mean abundance of Coscinodiscophyceae has been recorded in station 1 (mean= 3.80%; min= 0, max= 9.25%) and 5 (mean= 2.30%; min= 0, max= 5.5%). Higher values have been registered in the month of August in almost all five stations, when the highest algal development is expected to happen because of more favourable environmental conditions (Reynolds, 2006). In station 5, the higher value has been registered in the month of June. In the other stations, mean abundances are never higher than 1% and the lowest abundance has been found in station 2 (mean= 0.82%; min= 0; max= 3%).

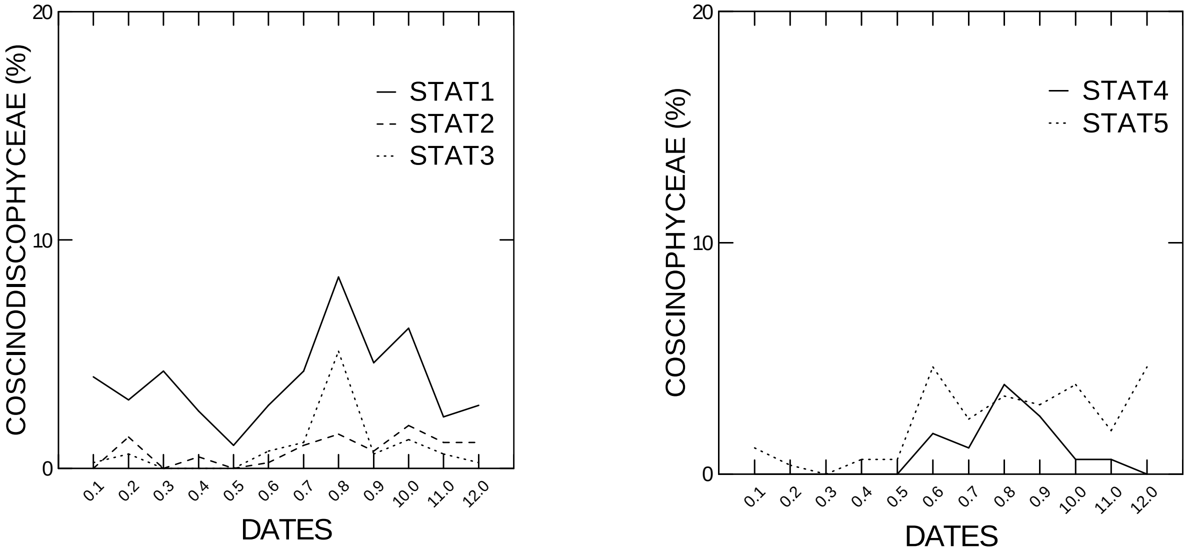


Fig. 4.14- Coscinodiscophyceae abundances (%) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Mediophyceae diatoms are generally more abundant in the Adige River than Coscinodiscophyceae (Fig. 4.15): highest abundances have been found in station 5 (mean= 17.14; min= 3.5, max= 36.25%) with maximum peak values in the month of March and September and minimum values registered in the months between January and May. Lowest mean abundance of Mediophyceae diatoms has been recorded in station 1 (mean= 0.80; min= 0, max= 7.25%) whose highest value has been found in the month of August. Along the river, there is a general increase in abundance of these diatoms, except for station 2 which has a mean value of 6.31%.

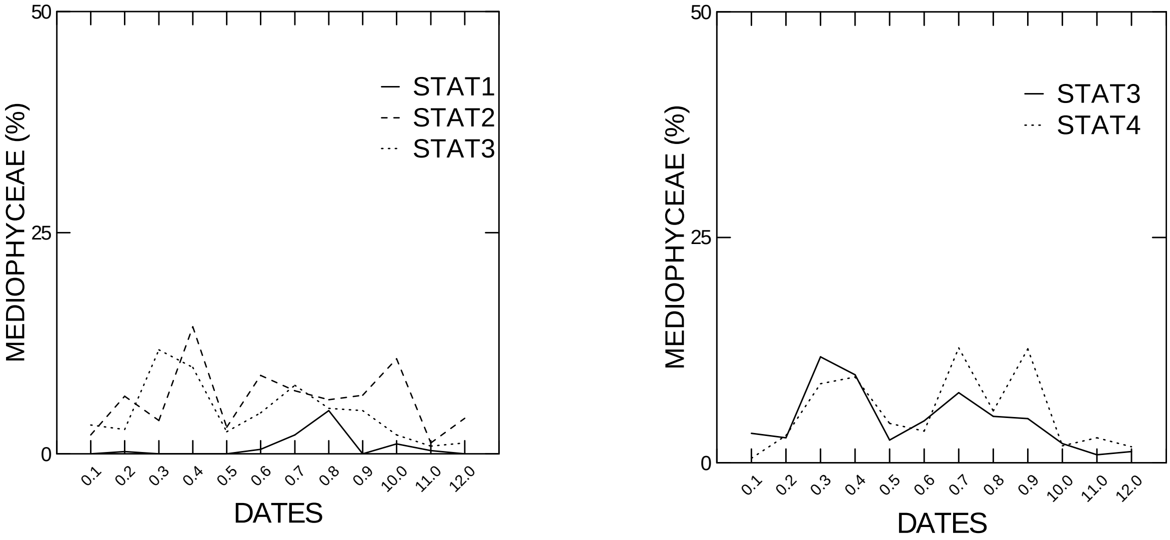


Fig. 4.15- Mediophyceae abundances (%) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

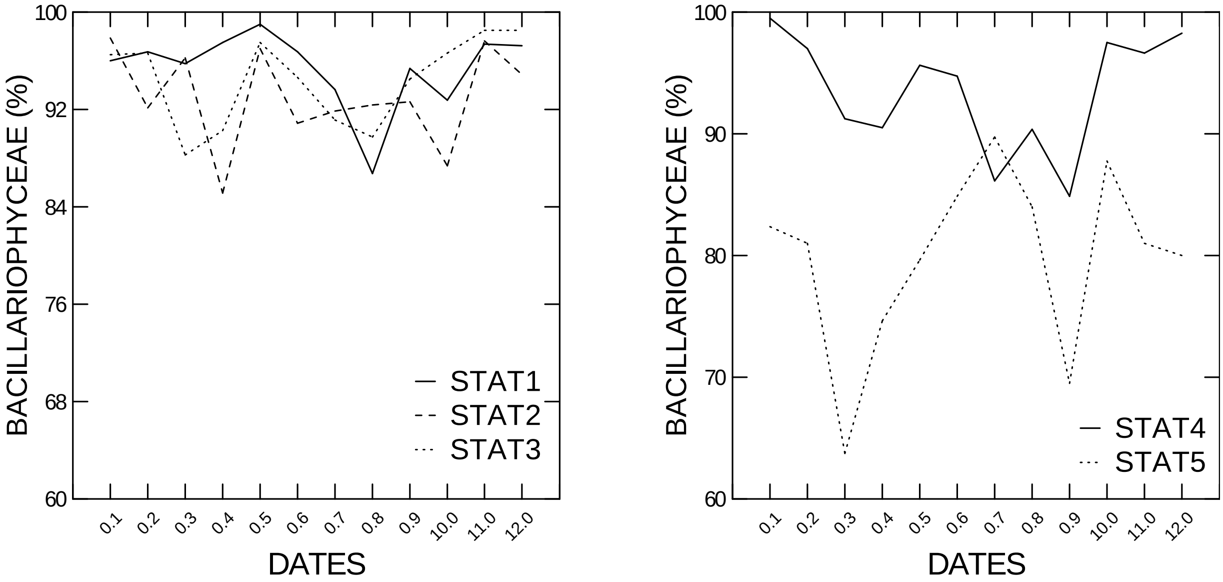


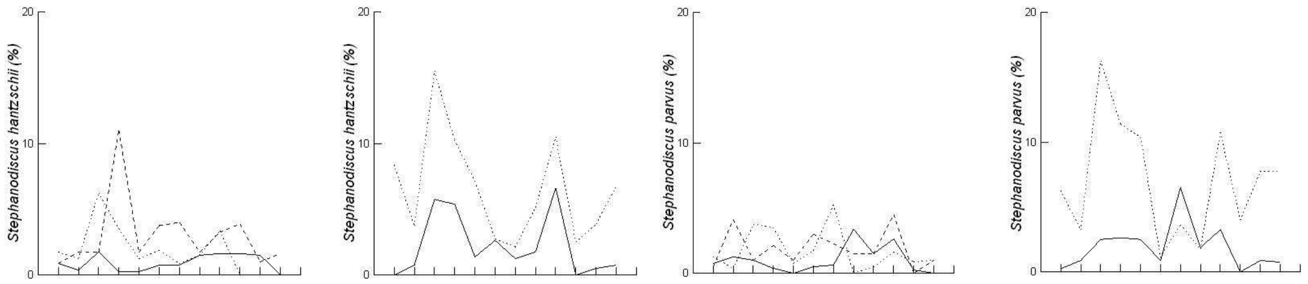
Fig. 4.16- Bacillariphyceae abundances (%) measured in the stations throughout the year. On the left there are plotted upstream stations, on the right downstream ones.

Bacillariophyceae are definitively the most abundant diatoms found in the sampling stations and this is not surprising withstanding that this group encompasses all the Pennales diatoms regardless of their ecology (e.g. Fragilariaceae have different ecological preferences than Achnanthidiaceae). In the first four stations, their mean abundances are always higher than 92% (95.39% in station 1; 92.85% in station 2; 94.66% in station 3 and 93.63% in station 4) whereas in station 5 there is a considerable decrease (80.55%). There is a general increase of these diatoms happening in the months of April and May (Fig. 4.16).

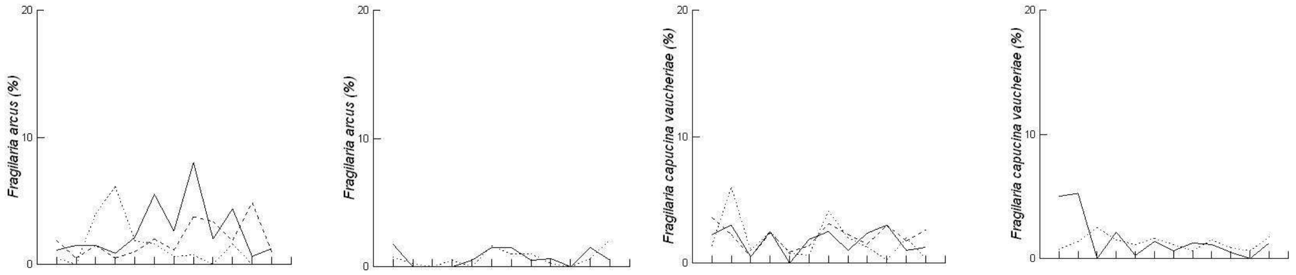
In fig. 4.17 and 4.18, charts of the most abundant diatom taxa found in the water column have been reported. In the upstream and downstream stations, planktonic species are more abundant in station 5 which can be considered the only real potamal station among those sampled: *S. hantzschii* and *S. parvus* have been recorded with percentages higher than 18% and especially during high flow periods (e.g. spring months) (fig. 4.17 a,b,c,d). On the opposite, *Melosira varians* displays higher abundances in station 1 and 4 which have the lower mean water depth level. Fragilariaceae taxa show different among-family patterns: *Fragilaria crotonensis* can be considered as a real planktonic dweller (Reynolds, 2000) and it displays higher abundances in station 5 while *F. arcus* and *F. capucina vaucheriae* seem to be more abundant in station 1.

Figure 4.18 shows the abundances of mero- and tychoplanktonic diatom taxa along the sampling stations: *Cocconeis placentula var. euglypta* is more abundant in station 4 (Fig. 4.18 a, b) and this is not surprising, considered this is typical benthic taxa that lives adherent to the substrate. Low water depth could help the upwelling of these species and tumble them along the water column. Pedunculate taxa such as *Achnanthidium minutissimum, Encyonema minutum* and *E. silesiacum* show higher abundances in stations 1 and 4 while lower values in station 5. Also typical mobile taxa such as *Diatoma ehrenbergii, D. vulgaris, Navicula tripunctata* and *Nitzschia fonticola* show a generalized preference for the first four stations since their mean abundances in the last station are never higher than 6% which is half of that measured in the upstream sampling points (Fig. 4.18 l,m,n,o,p,q,r).

a) Upstream stations b) Downstream stations c) Upstream stations d) Downstream stations



e) Upstream stations f) Downstream stations g) Upstream stations h) Downstream stations



i) Upstream stations l) Downstream stations m) Upstream stations n) Downstream stations

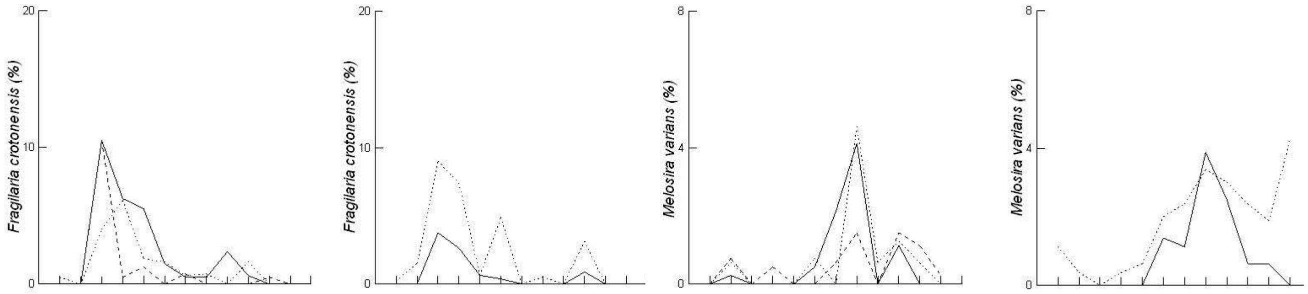
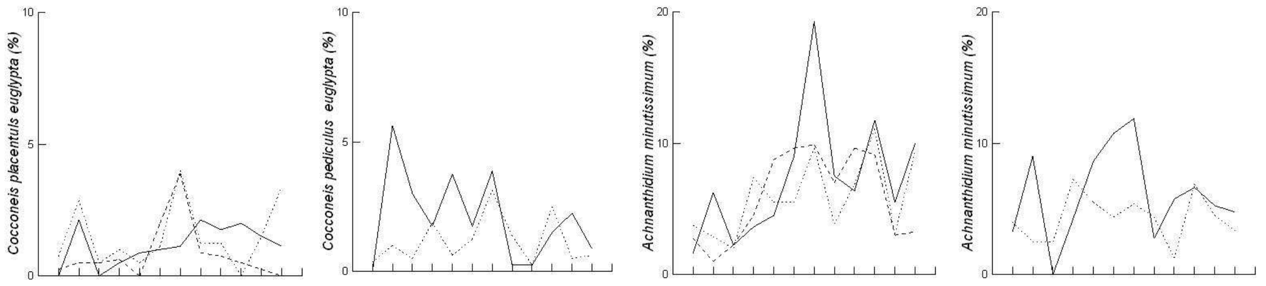


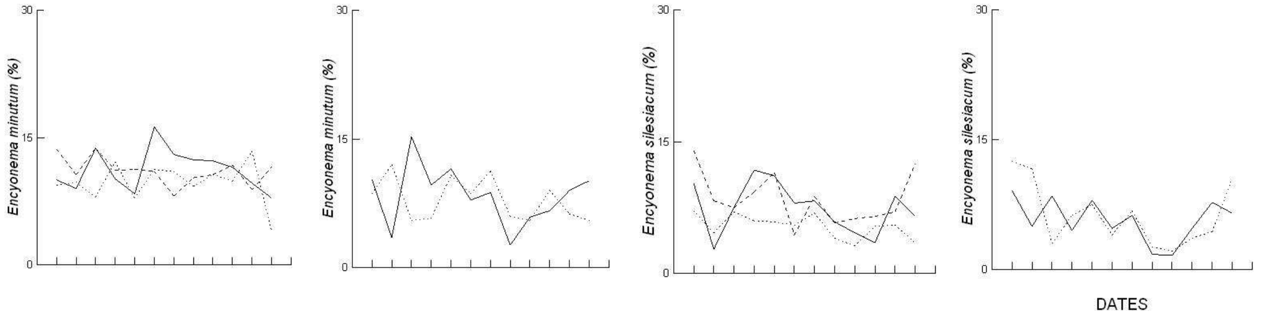
Fig. 4.17- Distribution plots of planktonic diatom taxa that have a higher abundance of 5% in at least 5 sampling dates. On the x axis are the sampling months (from January to December) , on the y axis the species percentages computed for the first three stations (a, c, e, g, i, m) and the last two (b, d, f, h, l, n). Continued line refers to stations 1 and 4, dashed to station 2, dotted to stations 3 and 5, as in the previous figures


a) Upstream stations

b) Downstream stations

c) Upstream stations

d) Downstream stations


e) Upstream stations

f) Downstream stations

g) Upstream stations

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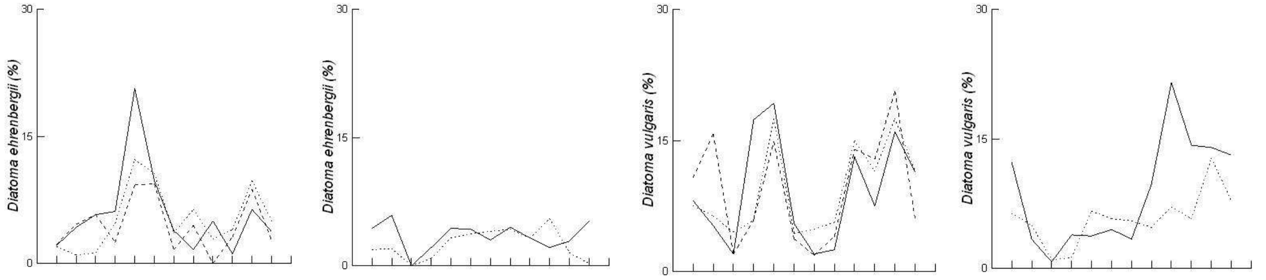
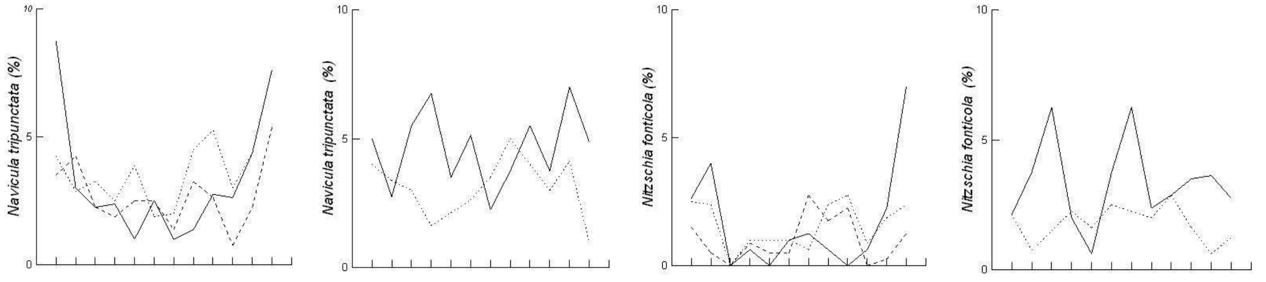

i) Upstream stations

l) Downstream stations

m) Upstream stations

n) Downstream stations


o) Upstream stations

p) Downstream stations

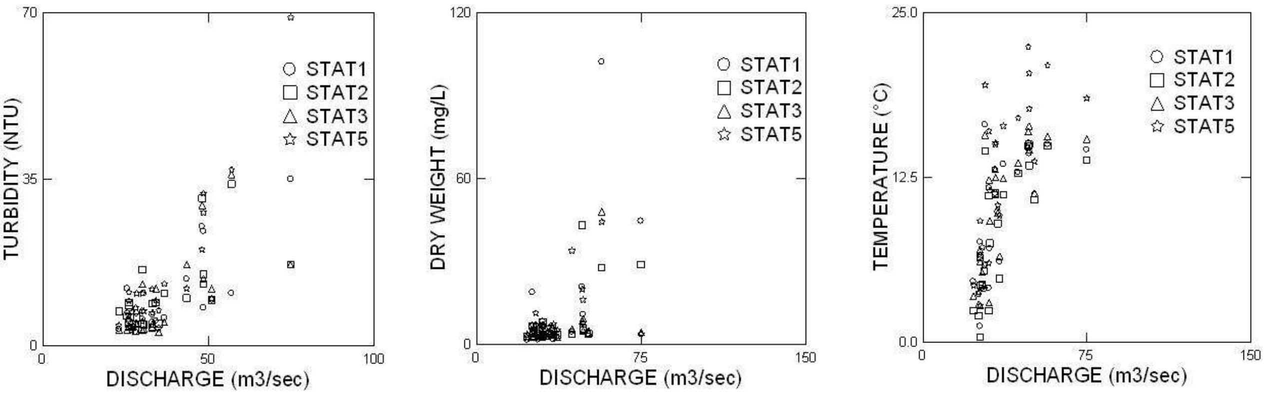
q) Upstream stations

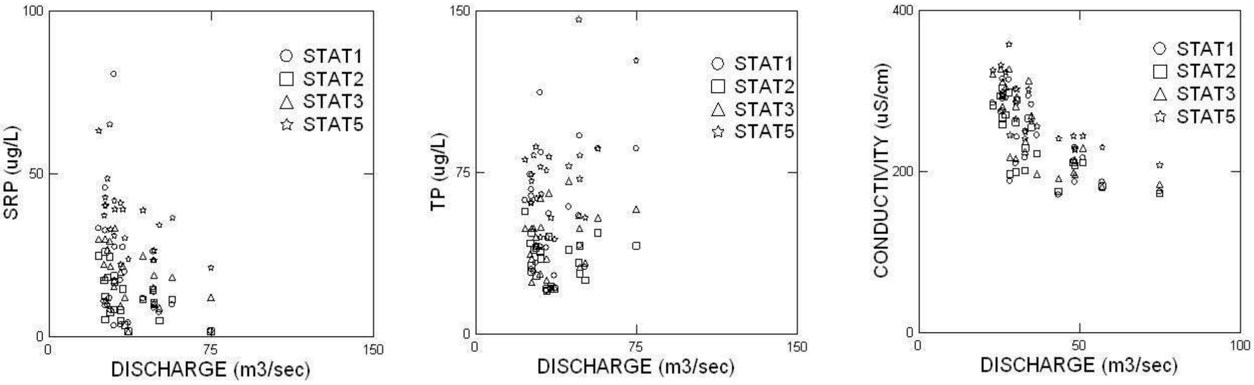
r) Downstream stations

Fig. 4.18- Distribution plots of mero- and tychoplanktonic diatom taxa that have a higher abundance of 5% in at least 5 sampling dates. On the x axis are the sampling months (from January to December) , on the y axis the species percentages computed for the first three stations (a, c, e, g, i, m, o, q) and the last two (b, d, f, h, l, n, p, r). Continued line refers to stations 1 and 4, dashed to station 2, dotted to stations 3 and 5, as in the previous figures

4.1.4. Spatial patterns and ecological determinants of planktonic diatom communities of Adige River

Discharge seems to be the most relevant constraint in this system: the relationships this variable establishes with the other environmental parameters could be considered to act as a unique driver. As from fig. 4.19a, turbidity is highly affected by discharge: positive correlations have been found between these two variables (r= 0.78, p<0.01 in station 1, r= 0.65, p<0.01 in station 2; r= 0.72, p<0.01 in station 3; r= 0.69, p<0.01 in station 5). Also analyzing dry weight, positive correlations have resulted (r= 0.61, p<0.05 in station 1, r= 0.63, p<0.01 in station 2; r= 0.40, p<0.05 in station 3; r= 0.41, p<0.05 in station 5), as expected (Fig. 4.19b). Turbidity and dry weight are collinear variables with discharge, because they show little variability that is independent of discharge: this is why it gets difficult to separate their effects. This effect seems to be more pronounced in the upstream stations rather than in the downstream one. On the other hand, temperature is covariating with discharge: positive correlations indicate a different relationship between these two variables (r= 0.66, p<0.01 in station 1, r= 0.69, p<0.01 in station 2; r= 0.69, p<0.01 in station 3; r= 0.69, p<0.01 in station 5) as from the graph 4.19c. The relationship between discharge and chemical variables is, on the other hand, very different. Discharge and SRP show a negative correlation (r= -0.33, p= n.s. in station 1, r= -0.48, p<0.05 in station 2; r= -0.42, p<0.05 in station 3; r= -0.57, p<0.05 in station 5) (Fig. 4.19d) and it could be due to a diluition effect given by discharge. On the opposite, TP does not show a similar trend (r= 0.25, p= n.s. in station 1, r= -0.01, p= n.s. in station 2; r= 0.26, p= n.s. in station 3; r= 0.50, p<0.01 in station 5) (Fig. 4.19e). Conductivity shows a negative correlation with discharge (r= -0.69, p<0.01 in station 1, r= -0.72, p<0.01 in station 2; r= -0.74, p<0.01 in station 3; r= -0.76, p<0.01 in station 5) (Fig. 4.19f). This last set of parameters has shown higher correlations values in the last station. It seems that upstream stations are driven by hydrolological parameters wheter the downstream stations (station 5) is less constrained by these variables.


a) Discharge and Turbidity b) Discharge and Dry Weight c) Discharge and Temperature


d) Discharge and SRP

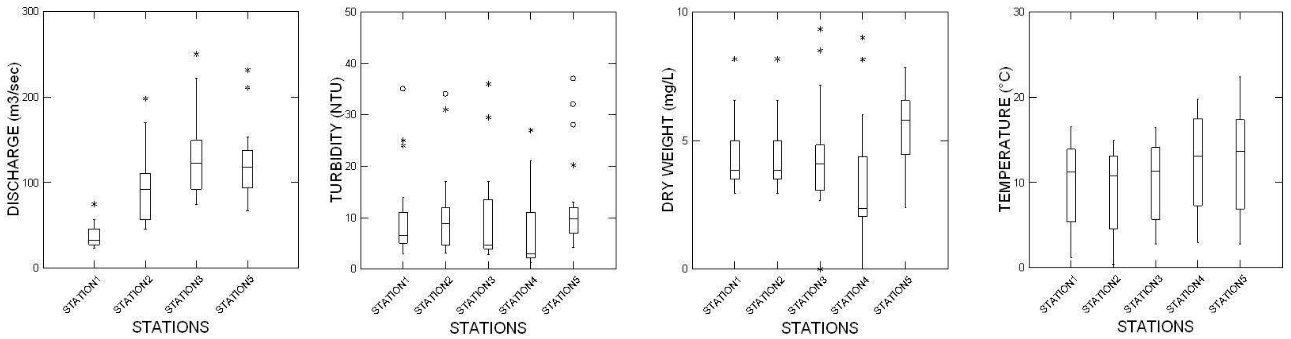
e) Discharge and TP

f) Discharge and Conductivity

Fig. 4.19- Scatterplots of discharge and other environmental parameters recorded in the research, for each station

In the five sampling stations, discharge reaches its maximum mean value in station3. In station2 and 5, mean values are comparable while are lower in station1 (Fig. 4.20a) . Its collinear variables show a similar pattern: higher turbidity have been found in station2 and 5 while it is lower in the others (Fig. 4.20b) while dry weight shows higher mean values in station1, 2 and 5 (Fig. 4.20c). Temperature separates upstream and downstream stations, the first three ones having colder waters (Fig. 4.20d). According to SRP, the first four stations are different from the last one which shows a double mean abundance of SRP. TP mean abundance is higher in station1 and 5 and shows its peak minimum value in station2 (Fig. 4.20f). Conductivity shows similar mean values among the stations: its minimum value has been recorded in station2 while its maximum in station5 (Fig. 4.20g).

a) Discharge b) Turbidity c) Dry Weight d) Temperature



e) SRP f) TP g) Conductivity

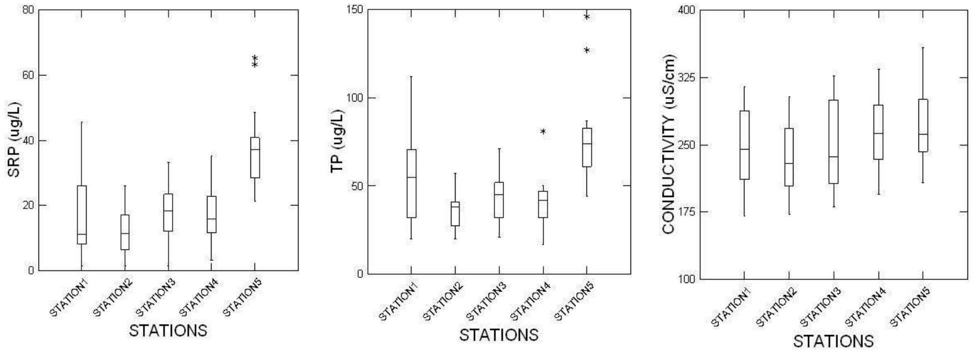


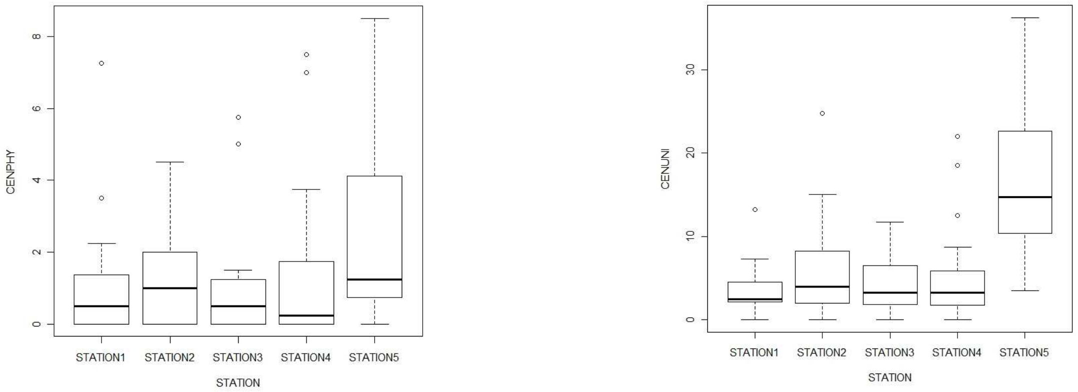
Fig. 4.20- Boxplot od discharge, turbidity, dry weight, temperature, SRP, TP and conductivity recorded in the five stations

The effect of environmental drivers on diatom communities has been studied through a morpho-functional diatom groups approach (MFDG), whom specifically accounts for Adige River diatoms (Centis et al., 2010). In Fig. 4.21, I have charted the six groups in each sampling station. Groups comprising planktonic diatoms (CENPHY, CENUNI) are more abundant in the southern station and this could be due to more natural conditions of the river that allow the development of a real planktonic community in this sampling station (Fig. 4.21a). In fact, higher abundances of CENPHY diatoms have been recorded in station 5 (mean= 2.85%; min= 0, max= 8.5%) while lowest in station 3 (mean= 0.94%; min= 5.75%). The same pattern has been evidenced for CENUNI group (Fig. 4.21b): in station 5 the mean value is 16.58% (min= 3.5; max= 36.25%) and the maximum peak has been registered in the months of March and September. In the other riverine stations, frequencies of these diatoms never exceed 25%: all these maxima have been listed in March and April. PENCOL group comprises two taxa (*Asterionella* spp. and *Fragilaria* spp.) that are generally considered as planktonic dwellers but many species belonging to *Fragilaria* spp. have a mixed ecological nature that would not ascribe them to plankton (e.g. *Fragilaria capucina* var. *vaucheriae* and *Fragilaria arcus* are considered as being tychoplanktonic- Denys 1991 while *Fragilaria crotonensis* is normally considered as a planktonic dweller diatom). This implies that this group has an ecological heterogenic nature and, in the Adige River, it actually shows higher abundances in station 1 (mean= 11.51%; min= 2.5; max= 33.5%) and lower ones in station 4 (mean= 6%; min= 2%, max= 12.75%). In station 5, PENCOL abundances are lower than expected (mean= 6.80%; min= 0; max= 18%) (Fig. 4.21c). Maxima peaks of PENCOL diatoms have been registered in the months of March and April. CENPHY, CENUNI and PENCOL have been called colonial groups because of this mixed nature.

Looking at mero- and tychoplanktonic diatoms groups (PENSMA, PENLAR, PENPED), it is evident how these diatoms are selected for in the first four stations of the river. As in fig. 4.21d, PENSMA diatoms are particularly abundant in station 4 (mean= 8.89%; min= 1.75; max= 23.5%) and 3 (mean= 7.69%; min= 2.75, max= 12.5%) and this is not surprising considering that more than 70% of counted PENSMA diatoms are belonging to *Cocconeis* spp. which is a typical benthic diatom living in the bottom and therefore subject to water tumbling that upholds them in the water column. PENLAR and PENPED are both more abundant in the first four stations: PENLAR diatoms have the highest relative abundances is station 4 (mean= 46.08%; min= 23.75, max= 74.5%) but mean values in the first three stations are always higher than 40% (Fig. 4.21e). The majority of these diatoms belong to the genera *Diatoma* spp. and *Navicula* spp. which are typical tychoplanktonic and mobile taxa. As for PENPED, highest abundances have been recorded in station 1 (mean= 38.46%; min= 22.75, max= 58.75%) but in stations 2,3 and 4 mean values are never lower than 33% (Fig. 4.21f). More than 70% of recorded PENPED diatoms belong to the genera *Encyonema* spp. and *Achnanthes* spp. Both these two groups display higher abundances in the months between March and April, in connection to the high water levels brought by increased discharges in those periods. In station 5, lower abundances of both PENLAR and PENPED have been recorded (mean= 38% and 28%, respectively).

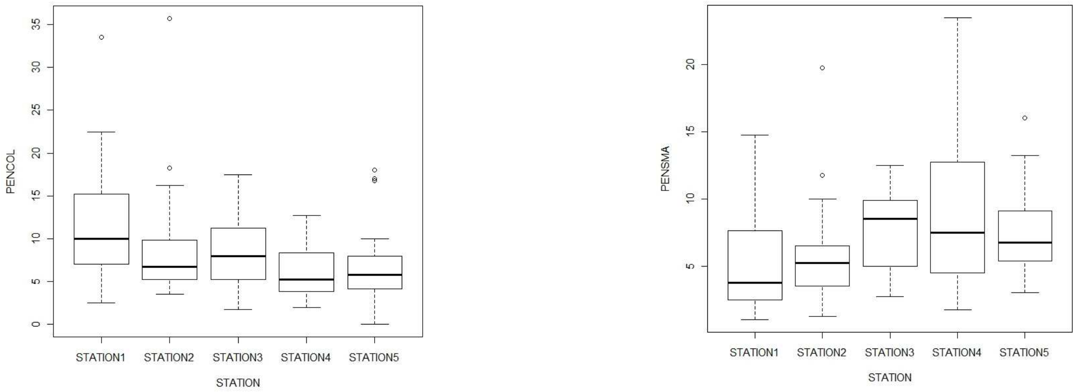
a. CENPHY diatoms

b. CENUNI diatoms



c. PENCOL diatoms

d. PENSMA diatoms



e. PENLAR diatoms

f. PENPED diatoms

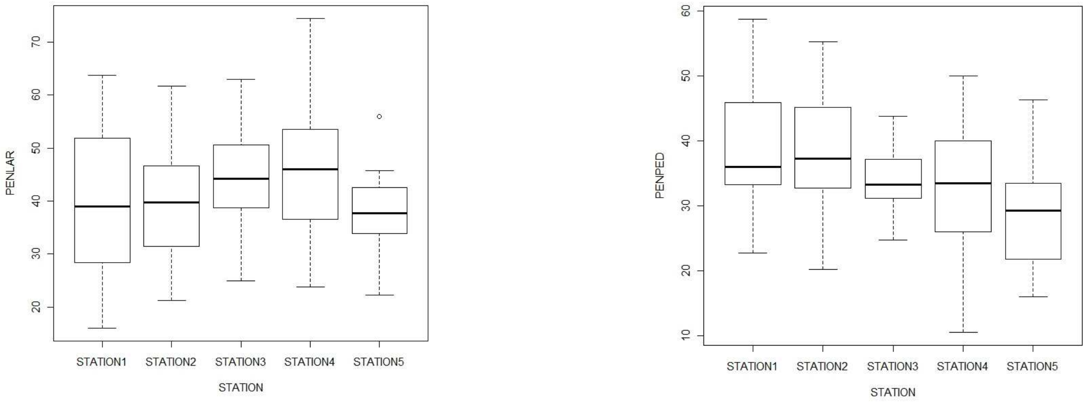
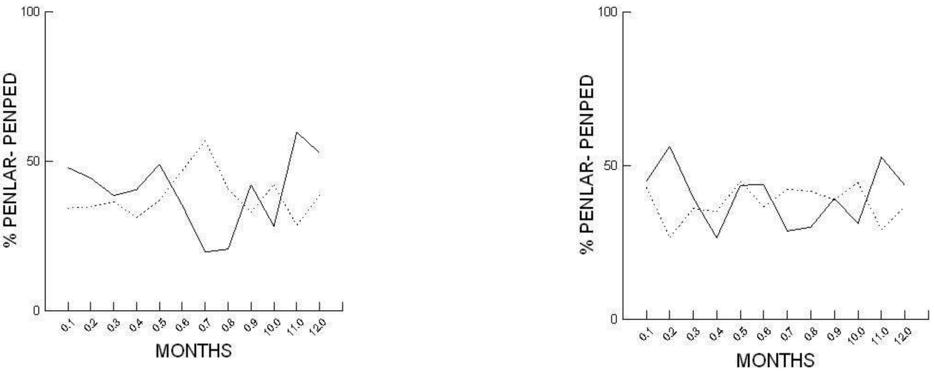


Fig. 4.21- Boxplot charts of morpho-functional diatom groups' relative abundances in the five sampling stations.

In the first four stations, the two most abundant diatom groups (PENLAR and PENPED) show negative non linear correlations with each other (*r*= -0.71, *p*<0.01 in station 1, *r*= -0.66, *p*<0.01 in station 2; *r*= -0.47, *p*<0.05 in station 3; *r*= -0.77, *p*<0.01 in station 4) . Eventhough this could be an effect due to the use of relative numbers, it seems that PENLAR diatoms are more abundant in cold months (i.e. winter) as from fig. 4.22a and 4.22d where the abundances of PENLAR diatoms increase after the summer while PENPED diatoms are more abundant in warm months (i.e. summer) as in fig. 4.22b where PENPED abundances are higher in June and August.



a) Station 1

b) Station 2

c) Station 3

d) Station 4

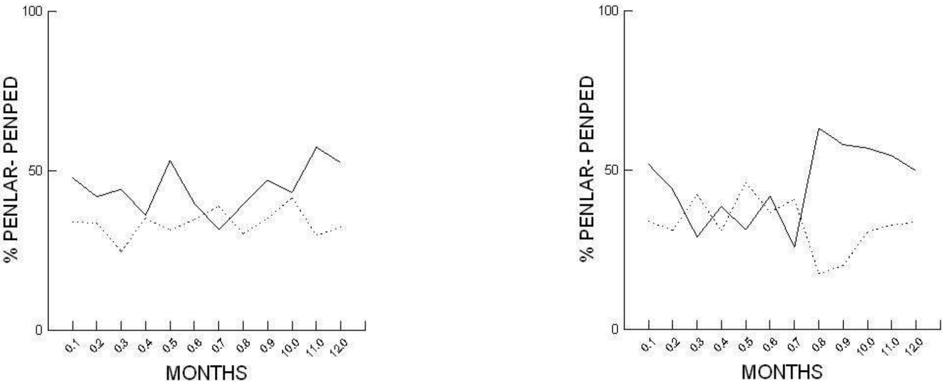


Fig. 4.22- Distribution plot of PENLAR and PENPED diatoms in stations 1, 2, 3 and 4. Continue line refers to PENLAR diatoms while dotted line referes to PENPED diatoms.

This could be due to the different discharge regimes displayed by the Adige River during the year: the general decrease of discharges in the colder months could allow a more stable environment for PENLAR to live. These diatoms are typically mobile taxa that live dislodged from the substratum and move around through the raphe (e.g. *Navicula* spp., *Diatoma* spp. and *Nitzschia* spp.) that therefore could easily be re-suspend them in the water column since they have no grip on the river bottom. Instead, higher discharges happening in spring and summer months, could apply a stronger tearing and tumbling power able to re-suspend PENPED diatoms that live attached to the substrate (mostly lithic one) through a peduncle (e.g.

Encyonema spp., *Achnanthidium* spp. and *Cymbella* spp.) and uphold them in the planktonic river range. It could follow that discharge not only exerts an influence in diatom communities but this influence could be even selective, in the sense that has different effects according to diatom morphological traits. This surely has strong implications for biomonitoring needs. This strong link between diatom communities and discharge values is the starting point of ecological evaluation in this watercourse: in the upstream stations, PENLAR diatoms show a non linear negative correlation with this variable (r= -0.60, p<0.01 in station 1, r= -0.40, p=0.05 in station 2; r= -0.42, p<0.05 in station 3). PENPED diatoms, instead, have shown a non linear positive correlation with discharge (r= 0.54, p<0.05 in station 1, r= 0.26, p= n.s in station 2; r= 0.28, p= n.s. in station 3). This could again imply a strong action played by this variable with different forces applied on each diatom species, depending on its ecological preferences, and it is consistent with the general trend outlined by previous studies in this watercourse (Salmaso & Braioni, 2008; Bruno et al., 2009a; Centis et al., 2010; Salmaso & Zignin, 2010). This correlations has not been found in station 5. This is probably due to the lower abundances of these diatoms in this sampling point and to the consequent higher development of Centric forms (CENUNI and CENPHY that are truly planktonic dwellers) in this stations that has more pelagic conditions. In this case, these centric diatoms have shown significant non linear positive correlations with discharges (r= 0.52, p<0.01 for CENPHY and r= 0.57, p<0.01 for CENUNI) again pointing to the high effect played by discharges on diatom communities. In this case, the effect of discharge applies in a different way to the different morpho-functional diatom groups: PENPED and PENLAR have shown a negative correlation with discharge while CENPHY and CENUNI have shown positive correlation values. This different effect could be a key point to be carefully considered when applying water quality indeces.

The role of discharge has been further investigated in two of the five stations sampled, which represent the extremes of an hydrological gradient: station 2 (northern) and station 5 (southern). These two stations show similar values for discharge and its collinear variables (e.g. turbidity and dry weight) while they differ for temperature, SRP, TP and conductivity (see paragraphs 4.1.1. and 4.1.2). A Principal Components Analysis (PCA) analyses based on MFDG showed a stronger presence of PENPED, PENLAR and PENSMA (e.g. tychoplanktonic, drifted and benthic taxa) in the northern station and a higher abundance of euplanktonic taxa (CENPHY and CENUNI) in the southern station (Fig. 4.23).

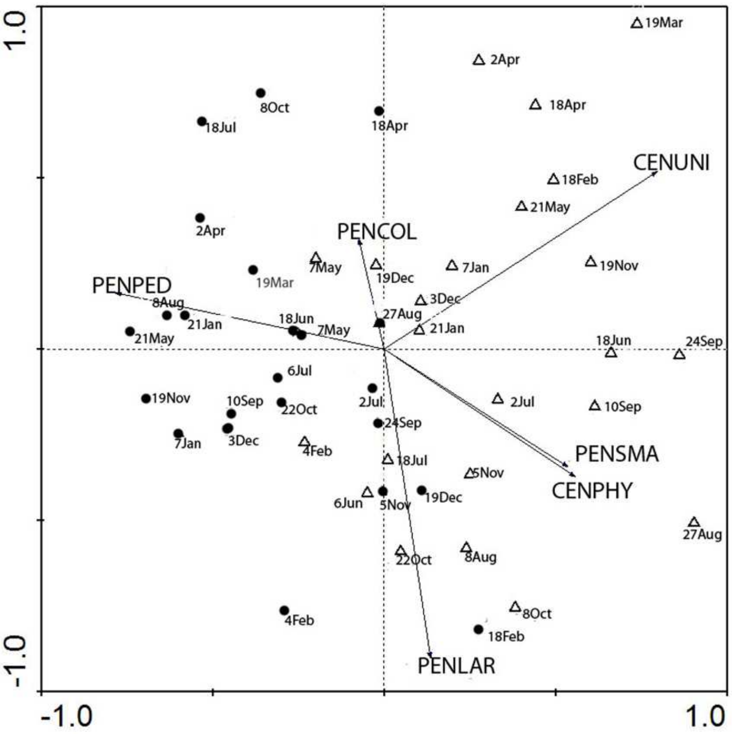


Fig. 4.23- PCA analysis on Morpho-Functional Diatom Groups (MFDG) for Cortina all'Adige (marked with *filled circle*) and Boara Pisani (marked with *open triangle)*

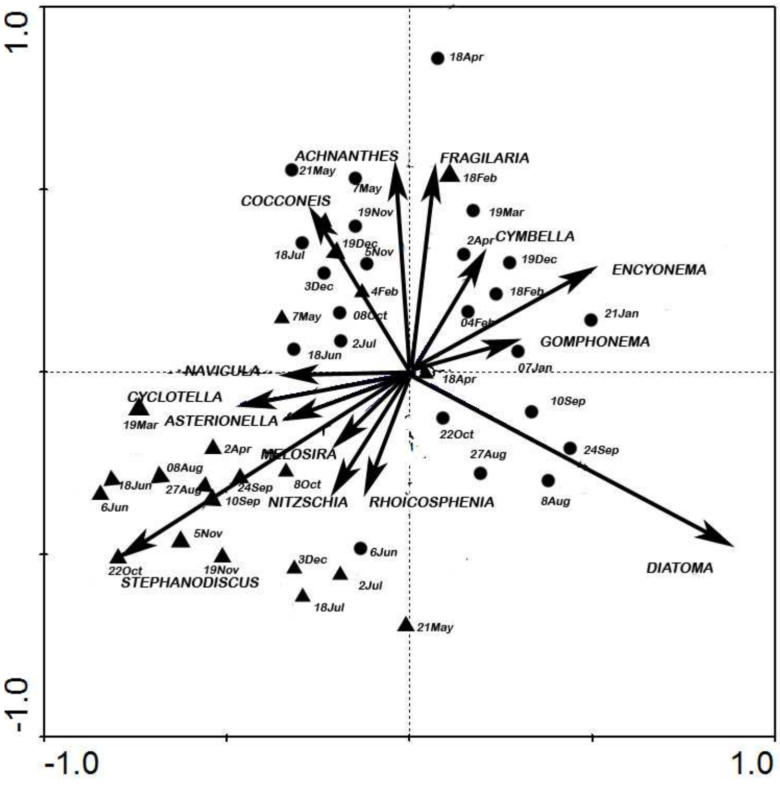


Fig. 4.24- PCA analysis on diatom genera for Cortina all'Adige (marked with *filled circle*) and Boara Pisani (marked with *filled triangle)*

The same results have been found analyzing diatom genera that have been recorded at least with 5% abundance: the northern station is characterised by typical drifted and benthic taxa such as *Diatoma spp., Gomphonema* spp.*, Encyonema* spp.*, Cymbella* spp.*, Achnanthes* spp.*, Nitzschia* spp*.* and *Rhoicosphenia* spp*.* belonging to PENPED and PENLAR groups. On the opposite, the southern station is much more characterised by centric taxa such *as Cyclotella* spp.*, Stephanodiscus* spp*.* and *Melosira* spp. and *Asterionella* spp. which is a typical planktonic dweller (Fig. 4.24).

Non Metric Dimensional Scaling (NMDS) analysis have been performed on these two stations and charted here as a result of a single NMDS analysis but, to avoid superimposition of different samples, the results are presented separately for each station. The chronological order of the diatom samples in the two stations followed very different paths: samples were characterised by pronounced, but not directional, seasonal development. Both the coordinates of the first and second axes of the NMDS configuration showed no significant correlations with the environmental variables (p>0.1, n=46). By converse, when considered separately, the configurations of the two stations showed clear and significant correlations with a few physical variables. The first axis of Station 2 was positively linked (p<0.05, n=23) with D_{3d}, dry weight and turbidity, while the second axis was negatively correlated with D_{3d} and turbidity. The correlation of the NMDS configuration with the physical variables in the Station 5 was apparent only along the second axis, which showed a negative and significant (p<0.05, n=23) relationship with D_{3d}, dry weight and turbidity (Fig. 4.25).

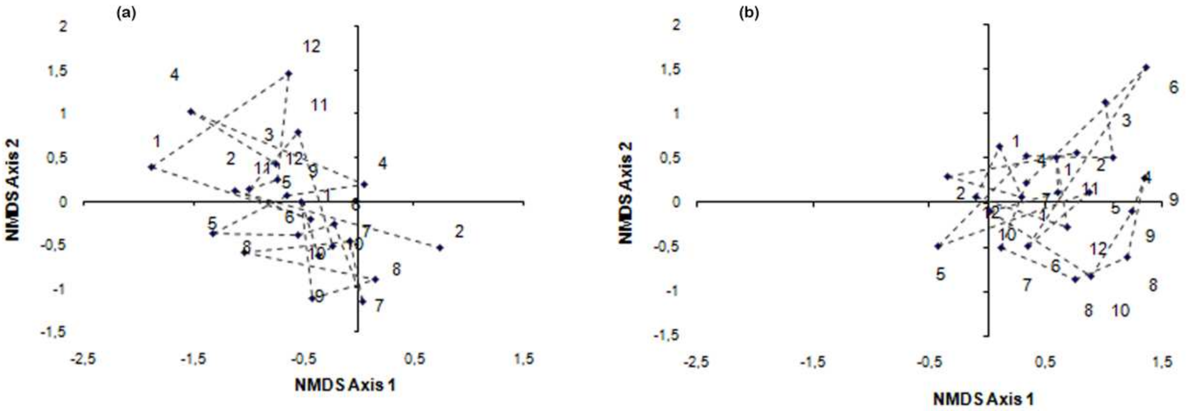


Fig. 4.25- Ordination of diatom samples by Nonmetric Multidimensional Scaling on Morpho Functional Diatom Groups (MFDG); stress=0.22. (a) Cortina all' Adige, (b) Boara Pisani; the Arabic numbers indicate the month of sampling

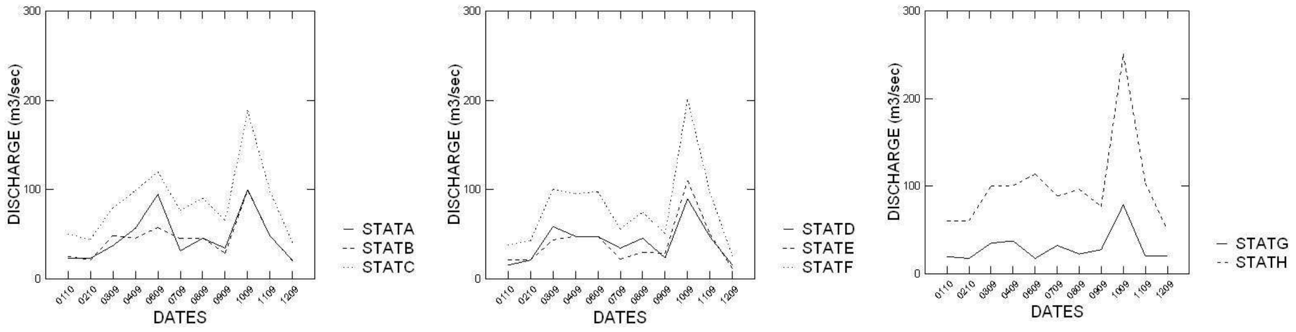
In the upstream stations, algal growth has never been limited by nutrients: the concentrations of TP and DIN have always had non limiting values (cf. Reynolds, 2006). In the first four stations, negative non linear correlations (p<0.05) have been found between DIN and centric diatoms (0,14 ≤ r ≤ 0,45): the same pattern has not been detected in station 5. Nevertheless, cconsidering a few cases of very low concentrations of available SRP, P deficiency for diatoms having higher P requirements (such as the small centric species, Wehr & Descy, 1998) could not be excluded in these stations. As for silica, none significant correlation have been found between this variable and diatom communities in station 5 while correlations have been found in the first four stations (p<0.01; 0,09 ≤ *r* ≤ 0,27).

4.2 BENTHIC DIATOM COMMUNITIES: PATTERNS, PROCESSES AND DYNAMICS

In the following section I will report the results obtained from the samples collected from the 40 km stretch between Bolzano and Trento, comprising the three main tributaries of the Adige River (Isarco, Noce and Avsio streams). The stations are named following an alphabetic order: see materials and methods. I have charted together the first three stations (A, B, C) since they integrate the first tributary system (Isarco river, the previous and the following station on the Adige), the following three (D, E, F) which integrate the Noce stream system and the last two (G, H) which comprise the Avisio stream and the Adige River after its inlet.

4.2.1. Hydrological variables and Temperature

Among the tributaries, Avisio stream (stat G) showed the lowest mean discharge (30.04 m^3 sec^{-1}) while the highest value has been displayed in the Isarco river (44.23 m^3 sec^{-1}). Noce stream showed a mean discharge value of 39.55 m^3 sec^{-1}. All the Adige's tributaries showed an increase in discharge in the month of September (Fig. 4.26). In the first system, station A displayed a mean discharge value of 47 m^3 sec^{-1} while in station C mean discharge is 86.68 m^3 sec^{-1} (Fig. 4.26a). These two stations have shown to correlate to each other (r=0.93 p<0.01) and show an increase in the spring and fall months. Also stations D and F have shown a good correlation (r=0.97 p<0.01) and higher discharges in spring and fall (mean abundances are 40.71 m^3 sec^{-1} and 79.24 m^3 sec^{-1}, respectively) (Fig. 4.26b) . The highest mean discharge was displayed in station H (100.23 m^3 sec^{-1}) which is the final station (Fig. 4.26c)



a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.26- Discharge values (m^3 sec^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

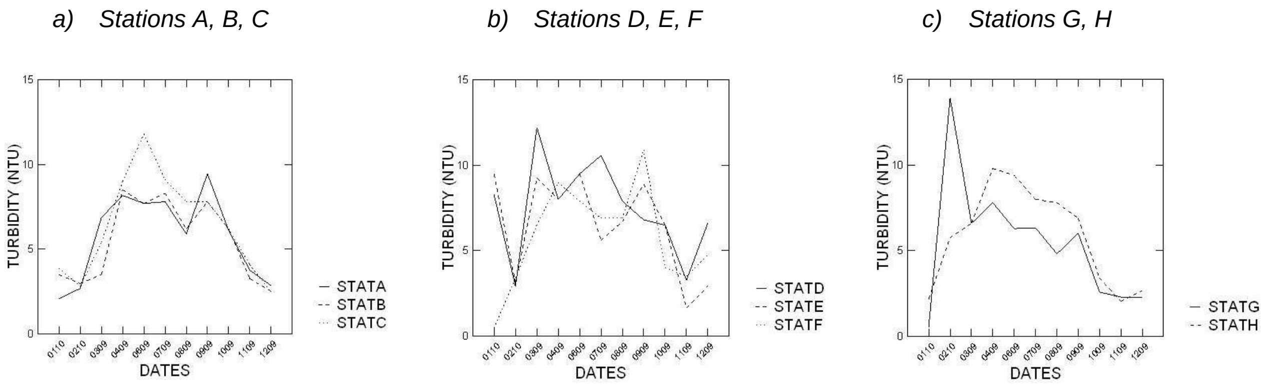

a) *Stations A, B, C* b) *Stations D, E, F* c) *Stations G, H*

Fig. 4.27- Turbidity values (NTU) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

Among the tributaries, the highest turbidity has been recorded in the Noce stream (statE- 6.51 NTU) while the lowest has been found in the Avisio stream (statG- 5.41 NTU). In the first sub-set of stations, the highest turbidity has been found in statC (6.39 NTU) while in statA it was considerably lower (5.76 NTU) (Fig. 4.27a). In statD, turbidity was higher than station F (7.50 and 5.48 NTU, respectively) (Fig. 4.27b) while in statH, the recorded mean turbidity value was 5.87 NTU (Fig 4.27c). As for discharges, higher turbidity values have been recorded in the spring and fall months.

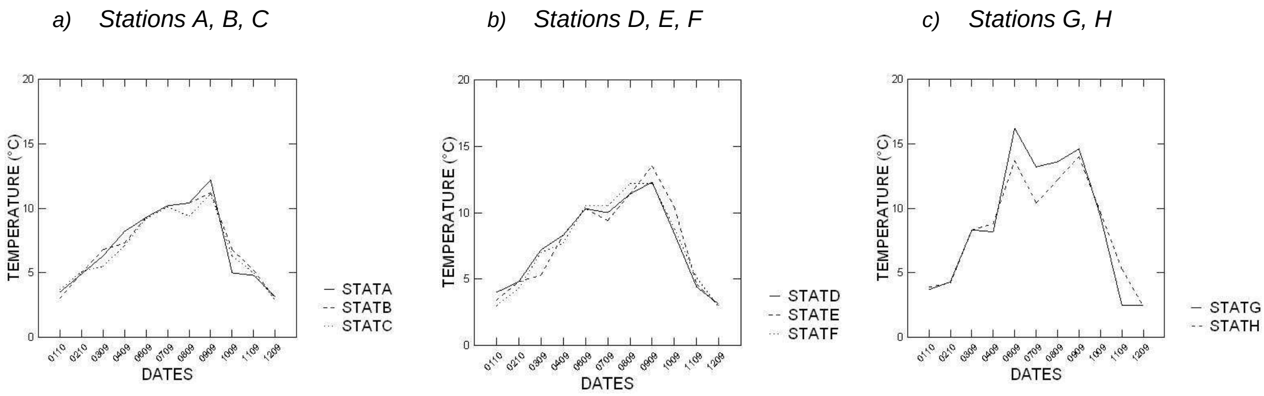
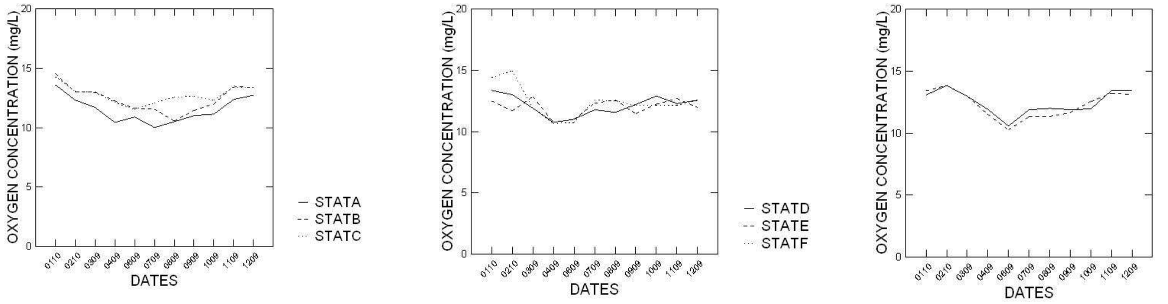

a) *Stations A, B, C* b) *Stations D, E, F* c) *Stations G, H*

Fig. 4.28- Temperature values (°C) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

The warmed-water tributary is the Avisio stream (statG) whose mean temperature value is 8.77℃: the colder one is the Isarco river (statB- mean temperature is 7.10℃). The Noce stream (statE) has a mean temperature value of 7.67℃ (Fig. 4.28b). As for the Adige mainsteam, the colder station is statC (mean temperature value of 6.85℃) (Fig. 4.28a) while the warmer one is statH (mean water temperature is 8.44 ℃) (Fig. 4.28c). From station A to F, temperatures peaks have been recorded in September while in the last two, in June.

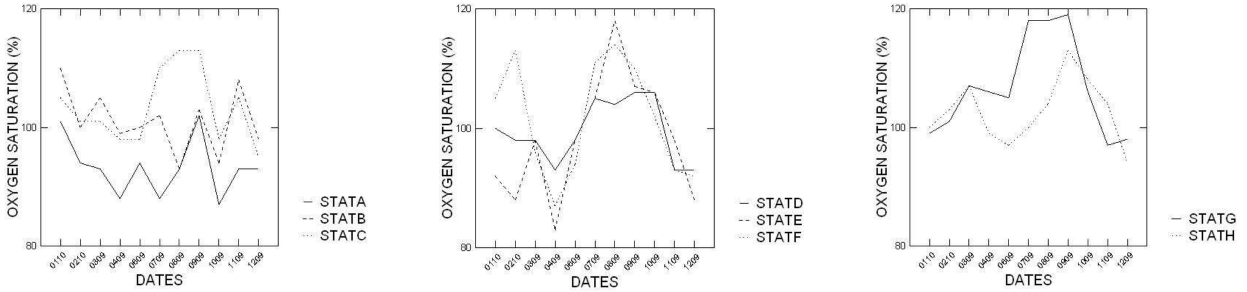
4.2.2. Chemical variables

Oxygen concentrations measured with the Winkler method have given similar mean values among all the eight stations. The minimum mean value has been recorded in statA (11.51 mg L^{-1}) while the maximum in statC (12.75 mg L^{-1}) (Fig. 4.29). These data have been confirmed by the oxygen saturation's measurements, plotted in figure 4.30.



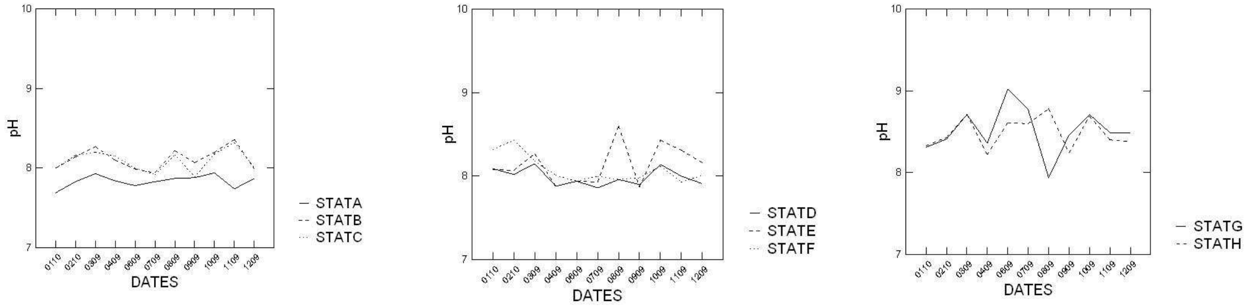
a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.29- Oxygen concentrations (mg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)



a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.30- Oxygen saturation (%) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)



a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.31- pH values measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

The three tributaries showed a pH value higher than 8: in particular, the highest mean value has been recorded in statG (Isarco stream- pH 8.51) and this is not surprising considering the geological nature of the its basin (Fig. 4.31c). As for the other stations, there is a progressive increase of the value southward: the lowest mean one has been recorded in statA (7.83) and statD (7.98) (Fig. 4.31a,b).

The highest mean conductivity has been recorded in statG (Isarco stream- 293.18 µS cm^{-1}) (Fig. 4.32c) while the lowest in statE (Noce stream- 235.36 µS cm^{-1}) (Fig. 4.32b). In the Adige river the lowest value has been measured in statC (243 µS cm^{-1}) (Fig. 4.32a) while the highest in statH (290 µS cm^{-1}) (Fig. 4.32c). In almost all the stations, conductivity decreases in summer apart from statG and statH that show a peak in the month of June.

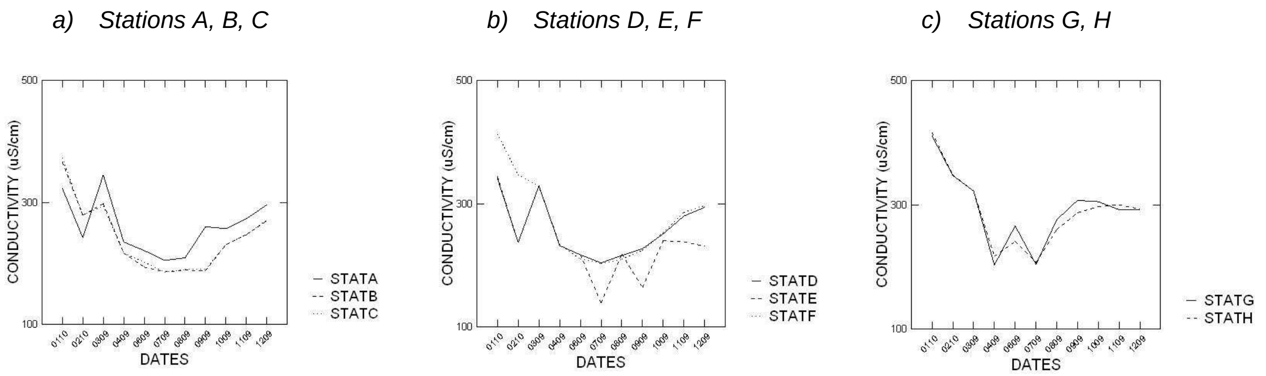


Fig. 4.32- Conductivity values (µS cm -1) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

Among the tributaries, dry weight maximum values have been registered in statB (mean= 4.21 mg L^{-1}; min= 1.85; max= 7.76 mg L^{-1}) while the lowest ones in statG (mean= 3.06 mg L^{-1}; min= 0.95; max= 5.79 mg L^{-1}) (Fig. 4.33a, c). Along the Adige River, dry weight seems to increase southward: the lowest value has been measured in statA (mean= 4.06 mg L^{-1}; min= 1.89; max= 12.33 mg L^{-1}), the highest in statH (mean= 5.98 mg L^{-1}; min= 0.70; max= 14.95 mg L^{-1}), as for discharge (see fig. 4.26). In all the stations, dry weight increases in the warmer months.

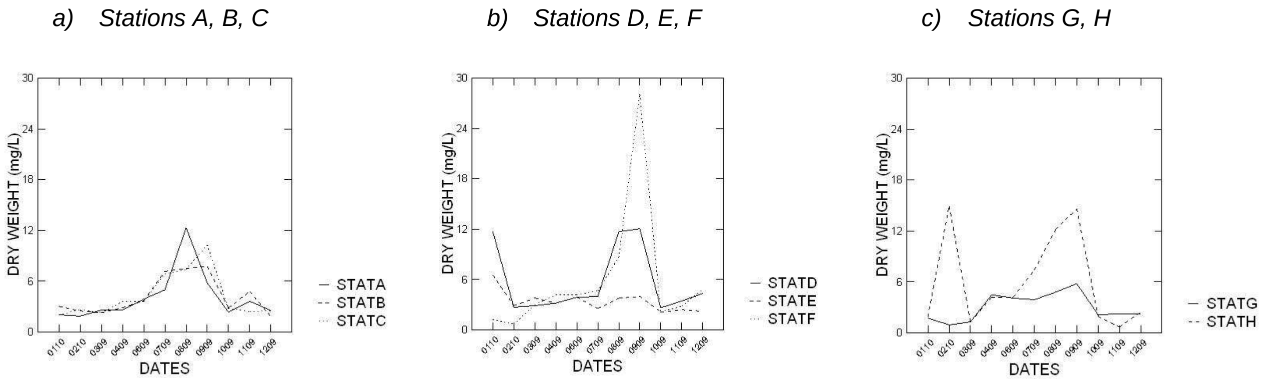


Fig. 4.33- Dry weight values (mg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

StatE has displayed the highest mean SRP value (15.77 µg L^{-1}; min= 7.8 µg L^{-1}, max= 29 µg L^{-1}) while statB has displayed the lowest one (12.78 µg L^{-1}; min= 6 µg L^{-1}, max= 21 µg L^{-1}) (Fig. 4.34a, c). Among the Adige stations, the highest mean value has been registered in statH (10.85 µg L^{-1}; min= 5 µg L^{-1}, max= 28 µg L^{-1}) while the lowest in statA (8.14 µg L^{-1}; min= 2 µg L^{-1}, max= 12 µg L^{-1}) which is the only station that has displayed SRP values lower than 10 mg L^{-1} (Fig. 4.34a, b).

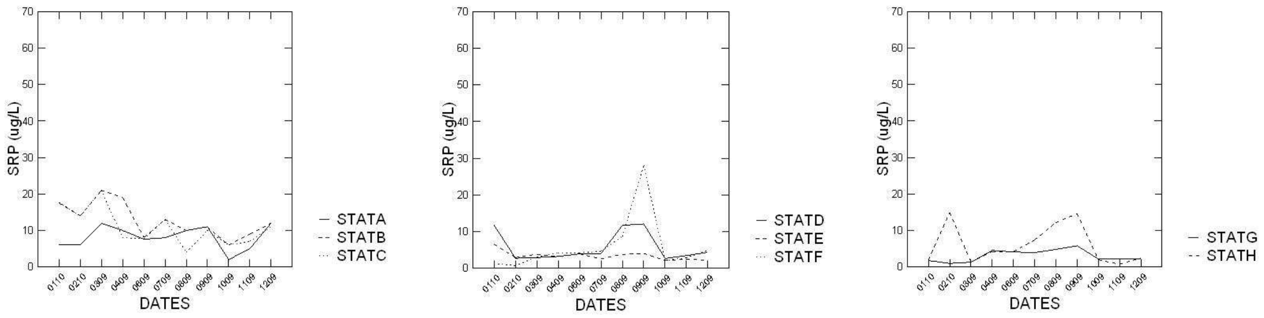

a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.34- Soluble Reactive Phosphorus SRP values (µg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

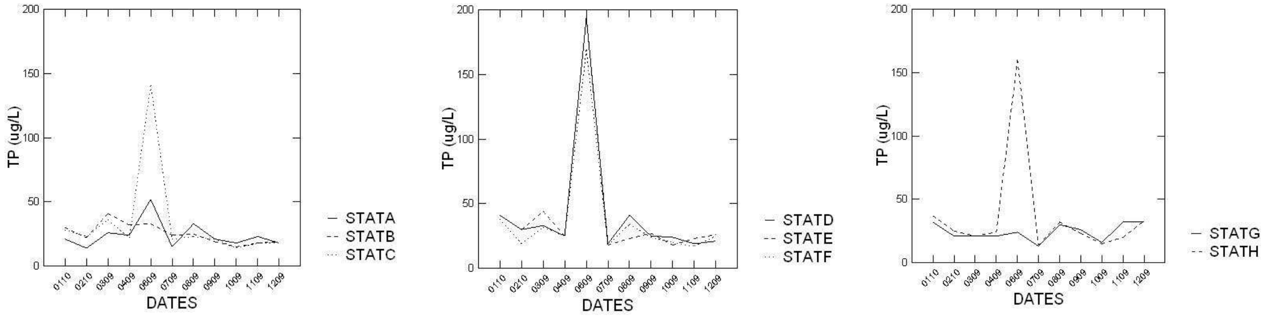

a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.35- Total Phosphorus TP values (µg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

StatB and statG have shown similar mean TP values (25.27 and 24.36 mg L^{-1}, respectively) while statE has a higher mean value (42.63 mg L^{-1}). Along the Adige stations, the lowest mean value has been displayed in statA (24.09 mg L^{-1}) while there is an increase southward, with the maximum value recorded in statD (42.90 mg L^{-1}). In almost all the sampled stations, there seems to be an increase in the summer months (Fig. 4.35a, b, c).

There is a general increase in DIN mean abundance southward: lowest mean abundance has been found in statA (0.66 mg L^{-1}) while the highest in statH (0.93 mg L^{-1}); this applies also for tributaries, the lowest being in the Isarco (statB- 0.70 mg L^{-1}) and the highest in the Avisio stream (statG- 0.93 mg L^{-1}) (Fig. 4.36a, b, c).

This could be due to an “additive” effect due to the strong agricultural use of the soil and inputs due to fertilizers in the studied zone, and in particular in the southern stations (cf. Fig. 4.36c).

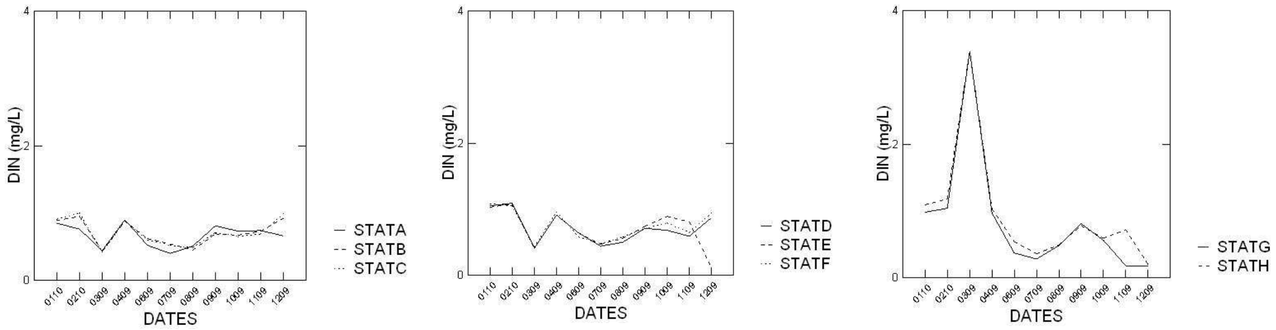

a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

Fig. 4.36- Dissolved Inorganic Nitrogen DIN values (mg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

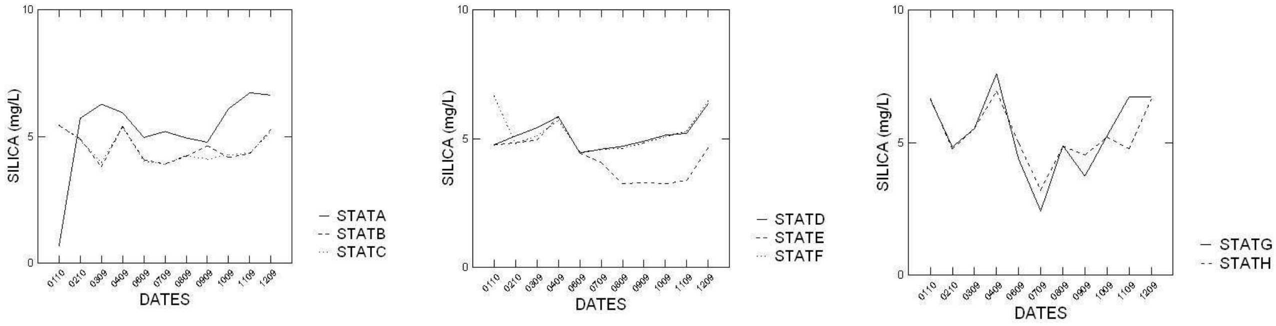

a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

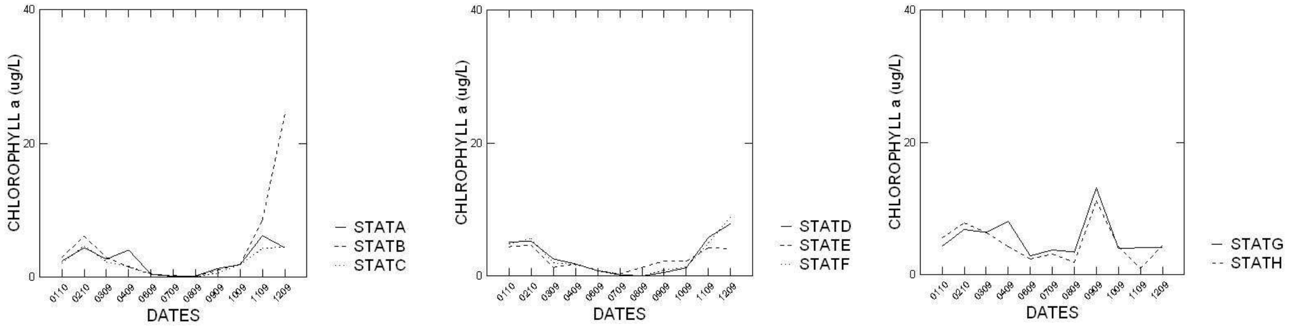
Fig. 4.37- Silica values (mg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

Among the tributaries, the highest silica abundances have been found in statG (mean= 5.33 mg L^{-1}, min= 2.42 mg L^{-1}, max= 7.06 mg L^{-1}) while in the other two stations, maximum abundances were never higher than 5.84 mg L^{-1} (Fig. 4.37a, b, c). On the Adige mainsteam, mean silica abundances have always been higher than 5.13 mg L^{-1} except in statC where lower mean values have been recorded (4.52 mg L^{-1}).

4.2.3. Algal communities

This paragraph deals with benthic diatom communities found in the Adige river and in the Isarco, Noce and Avisio streams. Except for chlorophyll *a* who has been charted according to a spatial criteria (watershed induced- like the physical and chemical variables), the results of each sampling station have been plotted separately, using the morpho-functional diatom groups in Centis et al. (2010) to better explicit the results given by the different substrates.

In the eight stations, mean chlorophyll values are overall higher in the tributaries than in the mainsteam: in the Avisio and Isarco streams (stat G and B, respectively) the highest mean values (4.55 and 5.52 µg L^{-1}) have been recorded (Fig. 4.38a, c). The Noce stream (statE) is an exception since its mean chlorophyll a value is 2.48 µg L^{-1} (Fig. 4.38b). In the mainsteam, there is a general spatial increase with the longitudinal gradient since the lowest mean value has been registered in statA (2.54 µg L^{-1}) and the highest in statH (4.73 µg L^{-1}). The general decreasing pattern of chlorophyll a in warmer months (from April to August) does not apply in the last two sampling stations since their mean values are generally higher in those months (see figure 4.38c).



a) Stations A, B, C *b) Stations D, E, F* *c) Stations G, H*

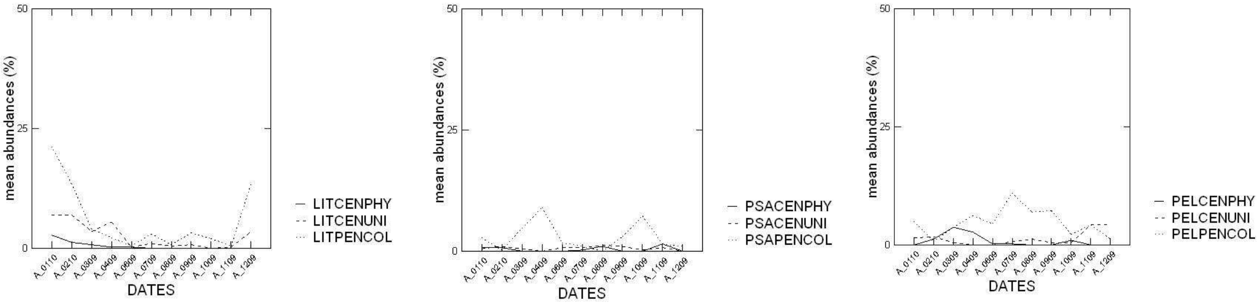
Fig. 4.38- Chlorophyll *a* values (µg L^{-1}) measured in the eight sampling stations. The first chart (a) refers to the first three stations (A, B, C); the second one (b) to the following three stations (D, E, F) while the last one (c) refers to the last two stations (G, H)

In the following part of the chapter I will report the results obtained from each sampling station, to better appreciate diatoms' relative contributions and ecological meanings. For each station, I have reported morpho-functional diatom groups mean abundances according to each substrate and Principal Component Analysis of these groups and environmental variables recorded. These analysis are the result of an unique analysis performed on the MFDGs and on environmental parameters but results have been presented separately for each station, to avoid superimposition of different samples. A comparative study of their spatial patterns and environmental drivers will be assessed in the following chapter.

STATION A (Adige before Isarco)

Beneath the substrates, the relative contributions of the different morpho-functional diatom groups varies according to their texture. CENPHY diatoms are more abundant in the finer samples (mean abundances are 0.84% and 0.38% in psammon and pelon, respectively). In the lithic samples, on the opposite, higher abundances of CENUNI and PENCOL diatoms have been registered (2.65% and 5.86% respectively) (Fig. 4.39a,b,c). The percentages of benthic groups are substantially higher in all the substrates: PENSMA diatoms show comparable frequencies (8.20 ≤ mean abundance ≤ 9.68%) while there is a higher percentage of PENPED diatoms recorded in the lithic samples (mean abundance is 60.11%- doubles that found in the finer habitats) and of PENLAR diatoms in the psammon and pelon (mean abundances are 53.93% and 49.77%, respectively) (Fig. 4.39 d,e).

a) LITHIC "Colonial groups" *b) PSAMMIC "Colonial groups"* *c) PELIC "Colonial groups"*



d) LITHIC "Benthic groups" *e) PSAMMIC "Benthic groups"* *f) PELIC "Benthic groups"*

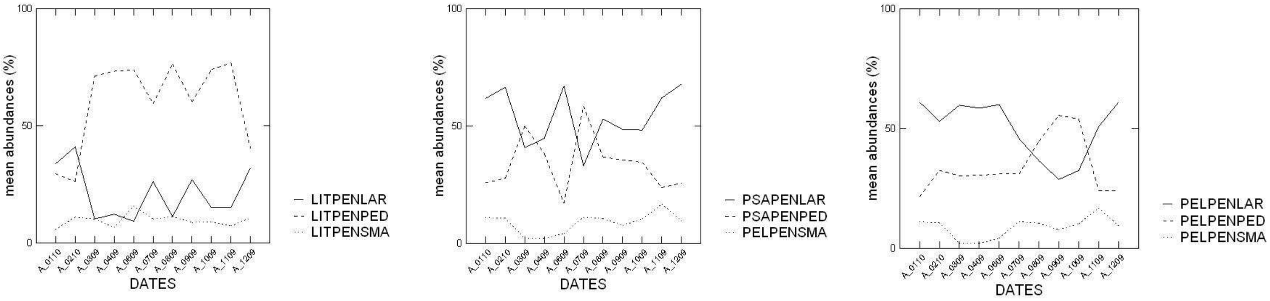


Fig. 4.39- Diatoms mean abundances (%) measured in statA in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

These results have been confirmed by a PCA analysis where samples distribute according to a gradient that separates finer and coarser habitats. In this analysis, coarser substrates seem to better host PENPED diatoms while finer ones seems to be preferably inhabited by PENLAR diatoms (Fig. 4.40a). Cluster Analysis (Fig. 4.40b) confirmes these results and roughly separates the samples according to their textures. These results have been confirmed also by the MRPP analysis (delta variance of 0.92 and chance-corrected within-group agreement value of 0.07).

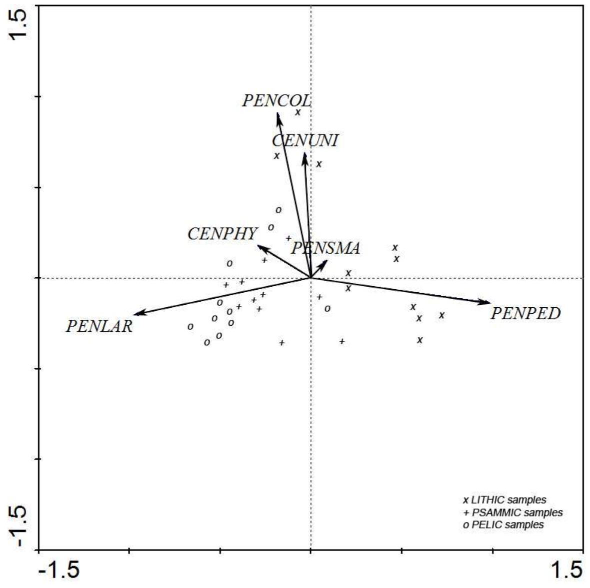


Fig. 4.40a- PCA analysis with MFDG on the three substrates sampled in station A. 93% of variance explained

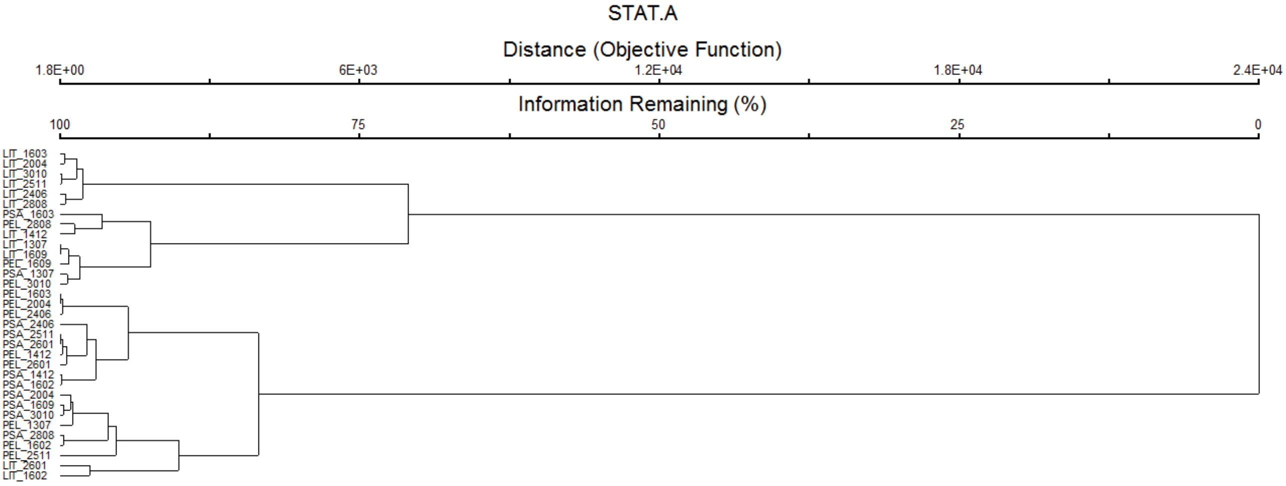


Fig.4.40b- Cluster analysis of samples collected in each substrate. Almost 75% of the variance is explained by the separation of coarser (LIT) and finer samples (PEL and PSA). Percent chaining= 3.31

Also the ISA analysis (Tab. 4.1) performed both on groups and on the most abundant species belonging to each group has confirmed these results. CENPHY diatoms show a higher value for finer substrates while CENUNI and PENCOL for coarser ones. There seems to be a neat prevalence of PENPED group and species in the lithon and PENLAR group and species in the pelic and psammic substrates, respectively.

	LITHON	**PELON**	**PSAMMON**
CENPHY	*13*	***31***	*12*
MELVAR (CENPHY)	8	**24**	4
CENUNI	***52***	*24*	*11*
STEPAR (CENUNI)	**26**	3	1
PENCOL	***43***	*36*	*20*
FRAULN (PENCOL)	***50***	*9*	*0*
PENLAR	*7*	***45***	*30*
DIAVUL (PENLAR)	11	**44**	24
NAVLAN (PENLAR)	7	**45**	30
NAVTRI (PENLAR)	11	41	**44**
PENPED	**47**	27	26
ACHMIN (PENPED)	**44**	20	34
ACHBIA (PENPED)	**47**	23	23
ENCMIN (PENPED)	**42**	33	24
ENCSIL (PENPED)	**46**	27	27
PENSMA	***36***	*33*	*31*
COCPLE (PENSMA)	**53**	24	18

Tab. 4.1- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

PCA analysis performed on environmental variables recorded in statA is an extract of the whole PCA performed for all the stations. The choice of the variables used will be explained further in paragraph 4.2.4 where the PCA analysis of all the stations is plotted. From the graph, it seems evident that the hydrological variables such as discharge and its collinear variables like turbidity and dry weight and temperature, play a joint effect in the diatom population (Fig. 4.40c) living in this station. Samples of relatively calm waters (e.g lower discharge regime, such as those sampled in winter months) seem to be driven more by other environmental parameters (e.g. SRP and conductivity) than those sampled in high water regime (months of April, June, September and October) where hydrological variables seem to be the main drivers. In statA, positive correlations have been found between discharge and PENPED diatoms (p<0.01; 0,22 ≤ r ≤ 0,65) while negative ones between discharge and PENLAR diatoms (p<0.01; -0.22 ≤ r ≤ -0.68) and this could be due to the different habitus of these two groups. This effect has been noticed in all the substrates but it is influenced by the different contributions of MFDG in each substrate (e.g. the lithic compartment is more highly inhabited by PENPED diatoms while the psammic and the pelic ones by PENLAR diatoms as explained above). Generally, during high water periods, a higher amount of dead planktonic diatoms have been recorded in the water column of this station while in the calm water periods an increase of colonial groups has been noticed.

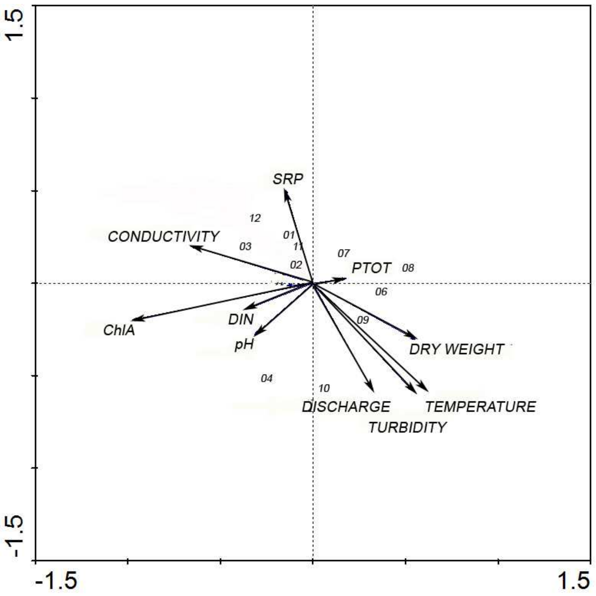
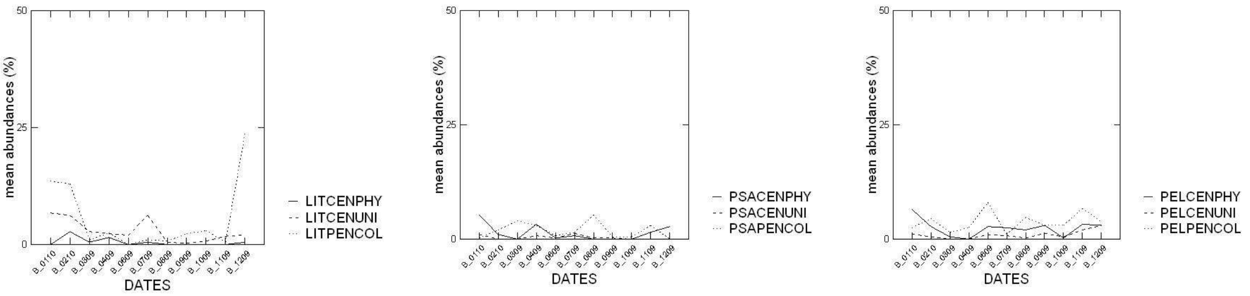


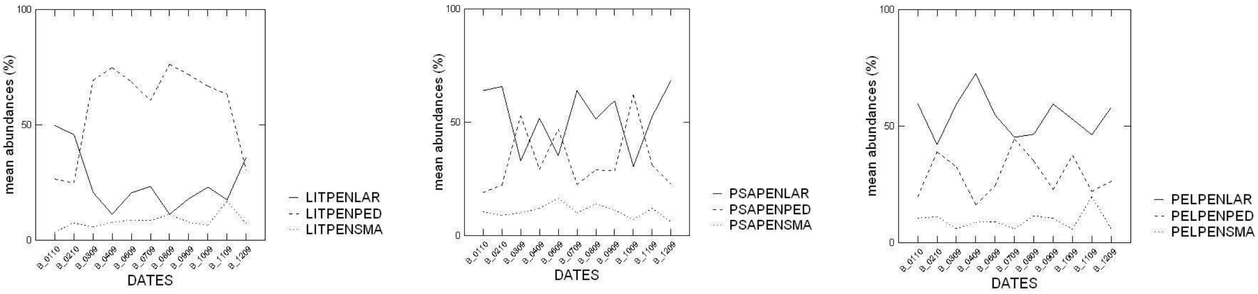
Fig. 4.40c- PCA analysis on the MFDGs of statA with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION B (Isarco stream)

In station B, excluding the peak of PENCOL diatoms recorded in the lithic substrate in the months of December, January and February (Fig. 4.41a), the mean abundance of these diatoms in the pebbles is 1.37%. In the finer substrates, instead, these diatoms' mean abundances is 0.85% (Fig. 4.41b, c). The relative contribution of centric taxa (CENPHY and CENUNI) never exceeds the value of 3% in these station. On the other hand, benthic groups have higher relative frequencies and their mean abundances are always higher than 10%. PENSMA diatoms show comparable lowest relative abundances while PENPED and PENLAR diatoms seem to distribute according to the texture of each substrate. This is shown by a relatively higher abundance of PENPED diatoms in the lithic samples (Fig. 4.41d) where the mean abundance of this group is 58% while higher mean abundances of PENLAR diatoms have been recorded in the finer substrates (52% in the psammon and 54% in the pelon) (Fig. 4.41e,f).



a) LITHIC "Colonial groups" *b) PSAMMIC "Colonial groups"* *c) PELIC "Colonial groups"*



d) LITHIC "Benthic groups" *e) PSAMMIC "Benthic groups"* *f) PELIC "Benthic groups"*

Fig. 4.41- Diatoms mean abundances (%) measured in statB in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

MRPP analysis points at a homogeneous set of data in this station (delta variance= 1.35; chance-corrected within-group agreement= 0.27) and PCA and Cluster Analysis confirm the previous results. In the cluster analysis, there is a neat clustering of samples according to their textures, with the exception of winter samples in the lithic compartment (Fig. 4.42b) while the PCA analysis shows a gradient between PENLAR and PENPED diatoms where samples distribute according to the texture where they have been recorded: PENLAR diatoms seem to be more abundant in the finer habitats (pelon and psammon) while PENPED diatoms seem to be more frequent in the coarser habitat (lithon) (Fig. 4.42a).

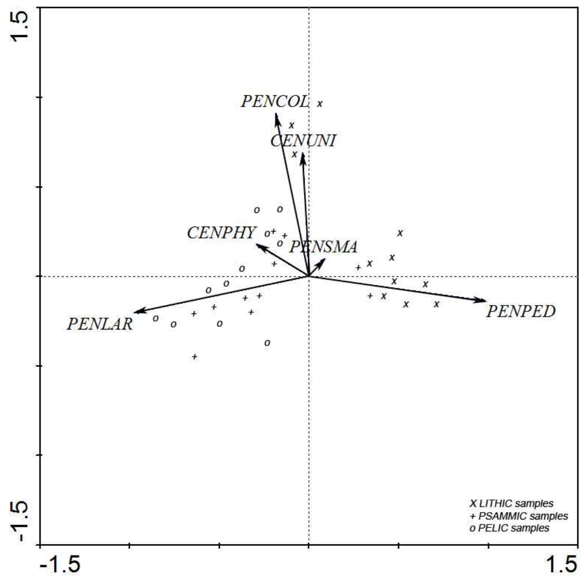


Fig. 4.42a- PCA analysis with MFDG on the three substrates sampled in station B. 93% of variance explained

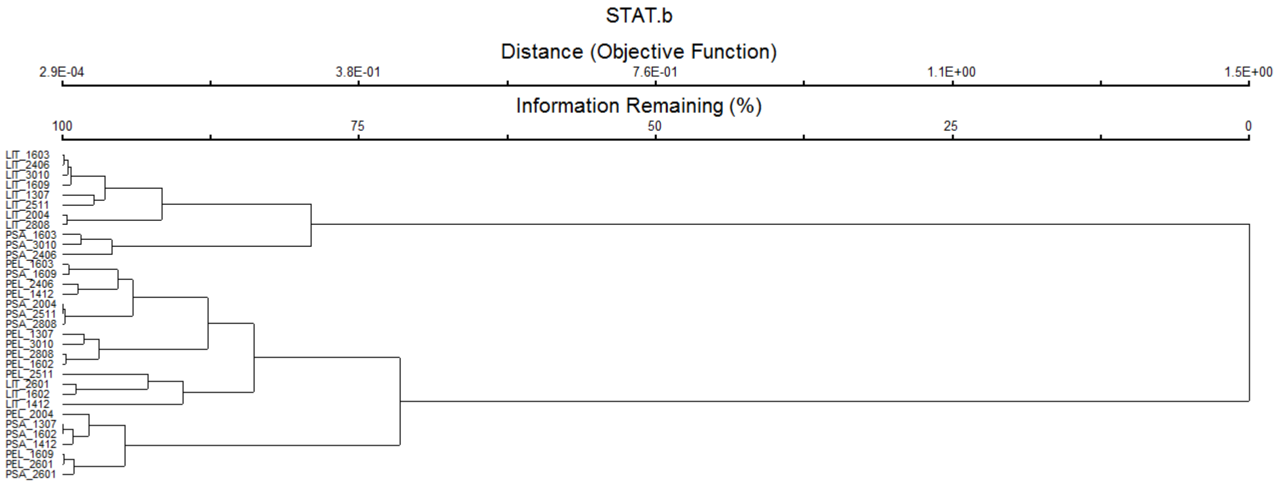


Fig.4.42b- Cluster analysis of samples collected in each substrate. Percent chaining= 3.82

ISA performed on morpho-functional diatom groups and most abundant species have confirmed the above results. CENPHY diatoms have a higher indicator value in the pelic samples as well as *Melosira varians* which is the most abundant species belonging to that group. CENUNI, on the other hand, has a higher indicator value in the lithic samples as shown by *Stephanodiscus parvus*. PENCOL diatoms are more abundant in the pebbles while PENSMA show similar values among the substrates. The indicator values recorded for PENPED and PENLAR diatoms show a neat separation according to textures: the first group being more abundant in the lithon and the second one in psammon and pelon (Tab. 4.2) as also shown by abundant species belonging to each of them.

	LITHON	**PELON**	**PSAMMON**
CENPHY	*6*	***51***	*20*
MELVAR (CENPHY)	7	**32**	4
CENUNI	***69***	*19*	*4*
STEPAR (CENUNI)	**70**	13	5
PENCOL	***45***	*33*	*15*
FRAULN (PENCOL)	***50***	*11*	*7*
PENLAR	*19*	***41***	***40***
NAVLAN (PENLAR)	5	**50**	40
NAVTRI (PENLAR)	9	49	**51**
PENPED	***48***	*24*	*28*
ACHMIN (PENPED)	**48**	21	22
ACHBIA (PENPED)	**49**	20	21
ENCMIN (PENPED)	**45**	23	24
ENCSIL (PENPED)	**51**	30	19
PENSMA	*29*	*33*	***38***
COCPLE (PENSMA)	20	20	**30**

Tab. 4.2- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

In statB, maximum discharge values have been recorded in the months of September and October: this is evident from the PCA analysis of the environmental variables that separates these samples from the other ones collected during the year. Discharge and its related variables are the main drivers in these samples while their effect is negligible in the calm waters months (December, January and February) where conductivity and SRP play a much more important role (Fig. 4.42c). Also in this case, a correlation has been found between discharge and diatom populations: positive ones between discharge and PENPED diatoms ($p<0.01$; $0,84 \le r \le 0,24$) and negative ones between discharge and PENLAR diatoms ($p<0.01$; $-0,22 \le r \le -0,86$). Correlations are higher in specific texture samples: discharge and LITPENPED diatoms and discharge and PSAPENLAR diatoms have shown the highest correlation values and this effect could be due to the preferences shown by these two groups for a specific texture. As for the water column, during high water periods, higher abundances of colonial groups have been recorded as live cells while higher frequencies of planktonic benthic taxa have been sampled during high water regimes.

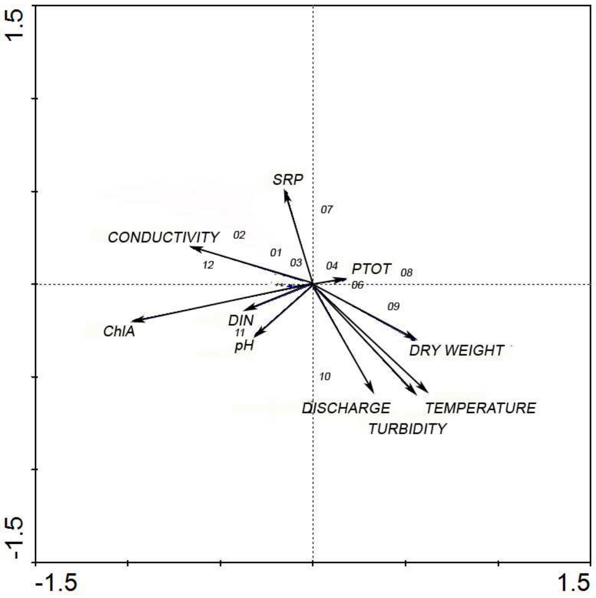
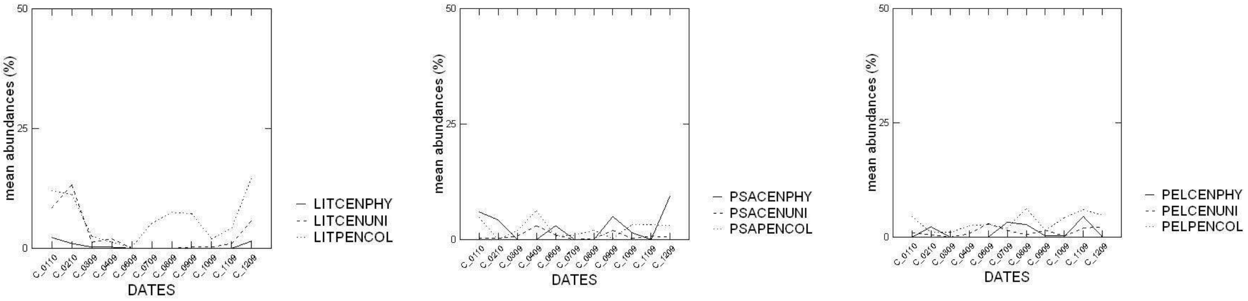


Fig. 4.42c- PCA analysis on the MFDGs of statB with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION C (Adige after the Isarco)

The relative contribution of colonial diatom groups in this station is never exceeding mean abundances of 3%: only in the lithic substrate, PENCOL diatoms shows an higher abundance of 6% (in the second part of the year) (Fig. 4.43a). CENPHY diatoms seem to have higher mean abundances in the finer substrates (Fig. 4.43b,c) while CENUNI cells seems to be more preferably hosted in the lithic samples (Fig. 4.43a). Analyzing benthic groups, comparable abundances of PENSMA have been found among the substrates while a different pattern has been found for PENPED and PENLAR diatoms. As in the previous stations, in fact, higher frequencies of PENPED have been recorded in the lithic substrate with a mean abundance of 59% which doubles those found in the finer substrates (Fig. 4.43d). On the other hand, PENLAR diatoms have largely higher abundances in the finer substrates (e.g. pelon and psammon).



a) *LITHIC "Colonial groups"* b) *PSAMMIC "Colonial groups"* c) *PELIC "Colonial groups"*

d) *LITHIC "Benthic groups"* e) *PSAMMIC "Benthic groups"* f) *PELIC "Benthic groups"*

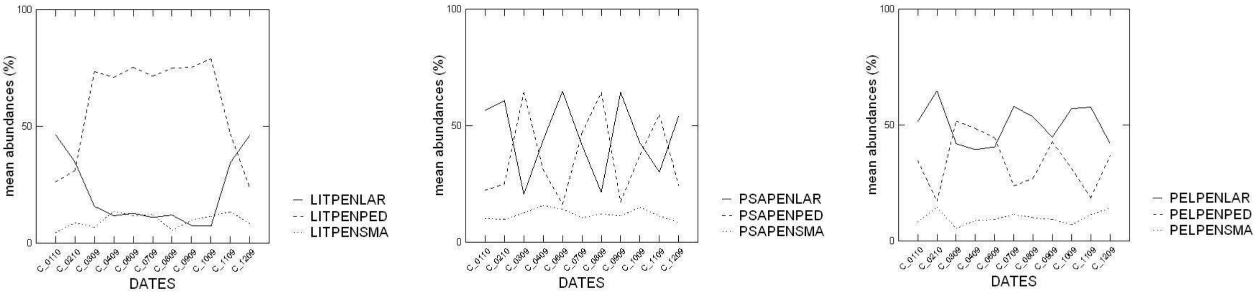


Fig. 4.43- Diatoms mean abundances (%) measured in statC in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

The same results are reflected by the PCA analysis which shows a strong gradient on the first axes between PENLAR and PENPED diatoms and the consequent distribution of these diatoms to finer and coarser substrates, respectively (Fig. 4.44a). This effect is evident also by Cluster analysis, which shows a neat separation between the samples collected form coarser and finer habitats (Fig. XXb). MRPP analysis performed on substrates attests that these groups can be considered significantly different (delta variance= 0.88; chance-corrected within-group agreement= 0.17).

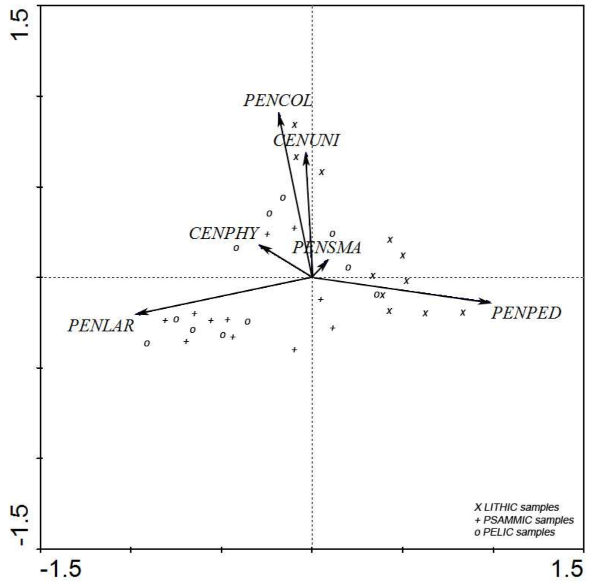


Fig. 4.44a- PCA analysis with MFDG on the three substrates sampled in station C. 93% of variance explained

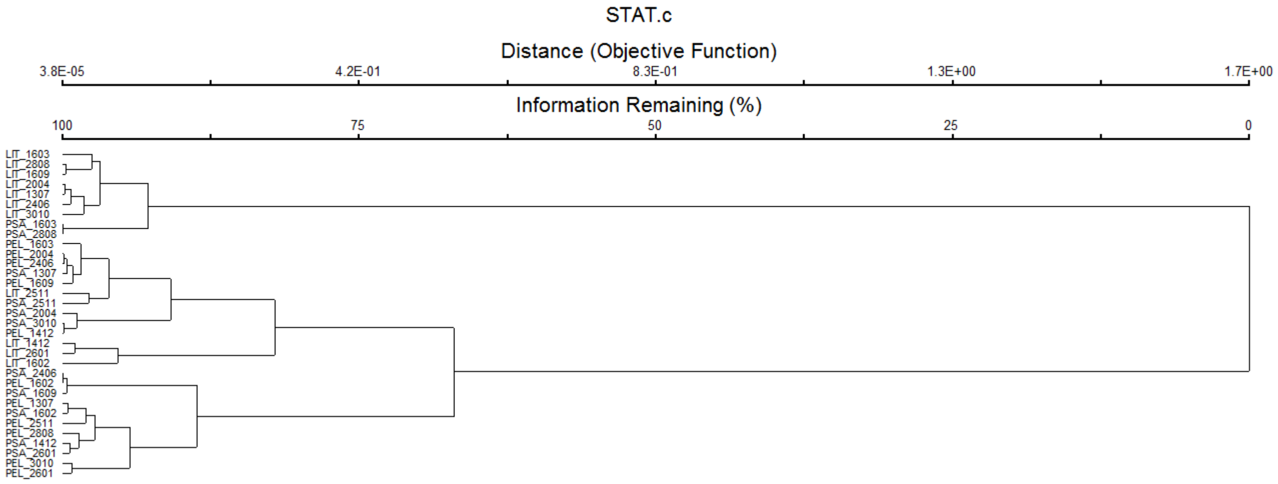


Fig.4.44b- Cluster analysis of samples collected in each substrate. Percent chaining= 5.09

ISA analysis confirms the results of PCA and Cluster Analysis by showing higher indicator values for CENPHY in the psammon (not confirmed by *Melosira varians*) and for CENUNI and PENCOL for lithic habitats. PENSMA diatoms show comparable indicator values while there is a neat difference between the indicator values displayed by PENLAR and PENPED on the three habitats. In fact, as evidenced with PCA and Cluster Analysis, stalked diatoms (PENPED) preferably inhabit lithic substrates while not stalked diatoms (PENLAR) are more frequently found in the psammon and pelon (Tab. 4.3).

	LITHON	PELON	PSAMMON
CENPHY	*5*	*15*	***33***
MELVAR (CENPHY)	**23**	0	2
CENUNI	***43***	*22*	*15*
STEPAR (CENUNI)	**29**	0	4
PENCOL	***52***	*28*	*18*
FRAULN (PENCOL)	***58***	*2*	*5*
PENLAR	*19*	***43***	*39*
DIAVUL (PENLAR)	19	**41**	36
NAVLAN (PENLAR)	14	**28**	16
NAVTRI (PENLAR)	5	**45**	14
PENPED	***45***	*26*	*28*
ACHMIN (PENPED)	**46**	28	24
ACHBIA (PENPED)	**48**	25	22
ENCMIN (PENPED)	**43**	26	33
ENCSIL (PENPED)	**41**	26	33
PENSMA	*31*	*32*	***38***
COCPLE (PENSMA)	34	**38**	22

Tab. 4.3- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

In this station, the highest discharges have been recorded in the months of June and September-October and in those periods discharges and its related variables were the main drivers of the diatom communities sampled in all the three substrates. On the other hand, samples collected in low water regime (e.g. in December, January and February) seem to be driven by conductivity and SRP (Fig. 4.44c). As in the previous stations, also in this one, a close link between discharge and the more abundant diatoms groups have been found: positive correlations have been found between this variable and PENPED diatoms ($p<0.01$; $0,08 \le r \le 0,65$) and negative ones between discharge and PENLAR diatoms ($p<0.01$; $0,03 \le r \le 0,60$), pointing to a side effect due to the hydrological regime of the watercourse. This effect must take account of the different inhabiting rate of diatoms on each substrate as shown previously (e.g. PENPED diatoms are more frequently found in the coarser habitats). The high role played by discharge is evident also if analyzing the suspended water diatom community: during high discharge periods a higher frequency of benthic taxa (especially PENLAR and PENPED diatoms) have been drifted as dead frustules while in the winter months, with low water, a higher percentage of colonial taxa (e.g. Centric ones) have been detected.

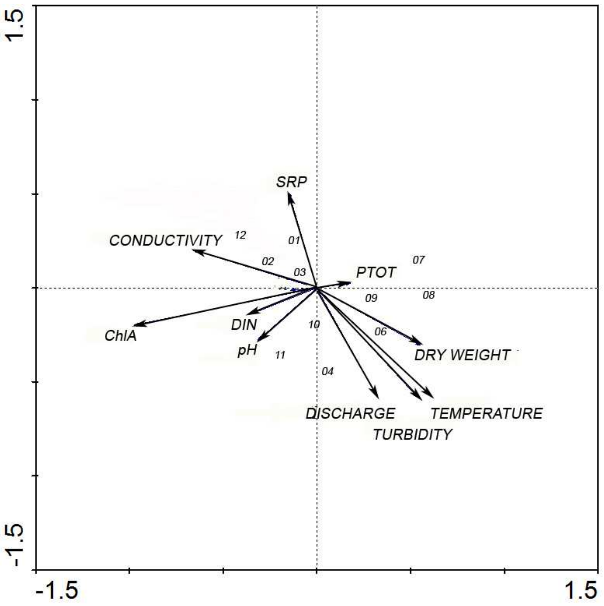
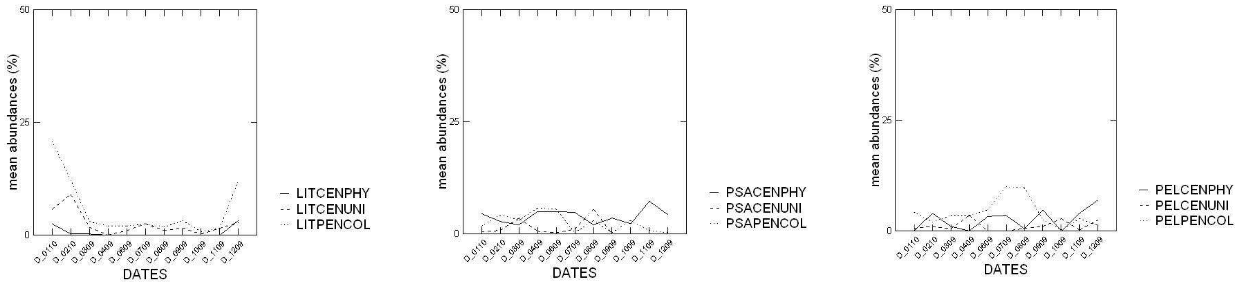


Fig. 4.44c- PCA analysis on the MFDGs of statC with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION D (Adige before the Noce stream)

Also in station D, mean relative abundances of colonial diatom groups (e.g. CENPHY, CENUNI and PENCOL) are lower than those recorded for the benthic groups (e.g. PENLAR, PENPED and PENSMA). CENPHY diatoms seem to be more abundant in the psammic and pelic samples (Fig. 4.45b,c) while CENUNI diatoms seems to be more abundant in the lithic ones and especially in the months of December, January and February (Fig. 4.45a). Among the benthic groups, PENSMA diatoms have comparable abundances between the substrates as evidenced also in the previous stations. PENPED diatoms are more abundant in the lithic samples since their mean abundance is 55% while their frequencies are half in the psammon and pelon (Fig. 4.45d). PENLAR diatoms are instead more abundant in pelon and psammon (mean abundances are 50% and 48%, respectively) especially in the spring and summer months (Fig. 4.45 e,f).


a) LITHIC "Colonial groups" *b) PSAMMIC "Colonial groups"* *c) PELIC "Colonial groups"*

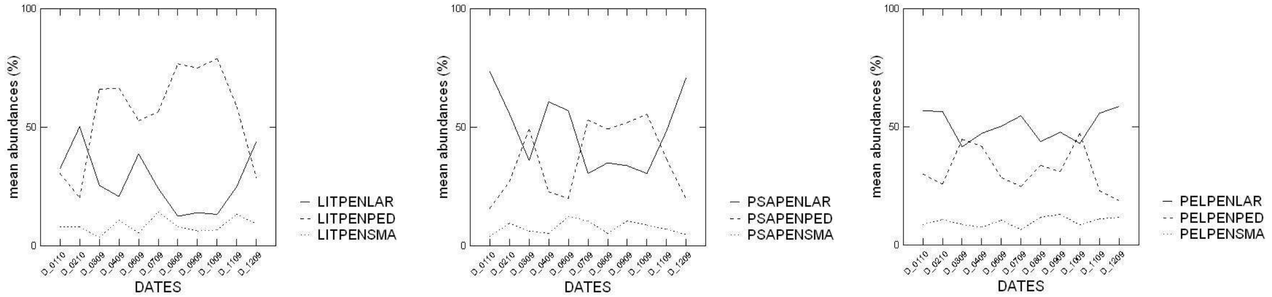

d) LITHIC "Benthic groups" *e) PSAMMIC "Benthic groups"* *f) PELIC "Benthic groups"*

Fig. 4.45- Diatoms mean abundances (%) measured in statD in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

In this station, MRPP analysis returns a delta variance of 0.60 and a chance-corrected within-group agreement of 0.17, showing that each substrates displays a significantly different diatom community than the others. PCA analysis confirms the different colonization patterns of PENLAR and PENPED diatoms showing a strong gradient on the first axis (Fig. 4.46a). This result is also confirmed by the Cluster Analysis that separates samples according to textures (Fig. 4.46b).

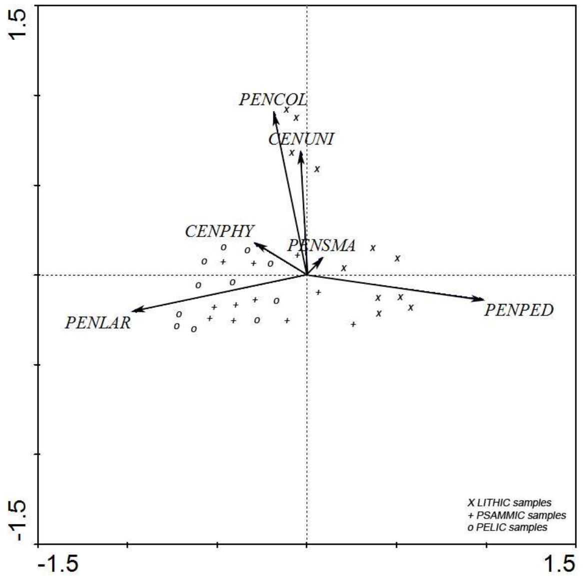


Fig. 4.46a- PCA analysis with MFDG on the three substrates sampled in station D. 93% of variance explained

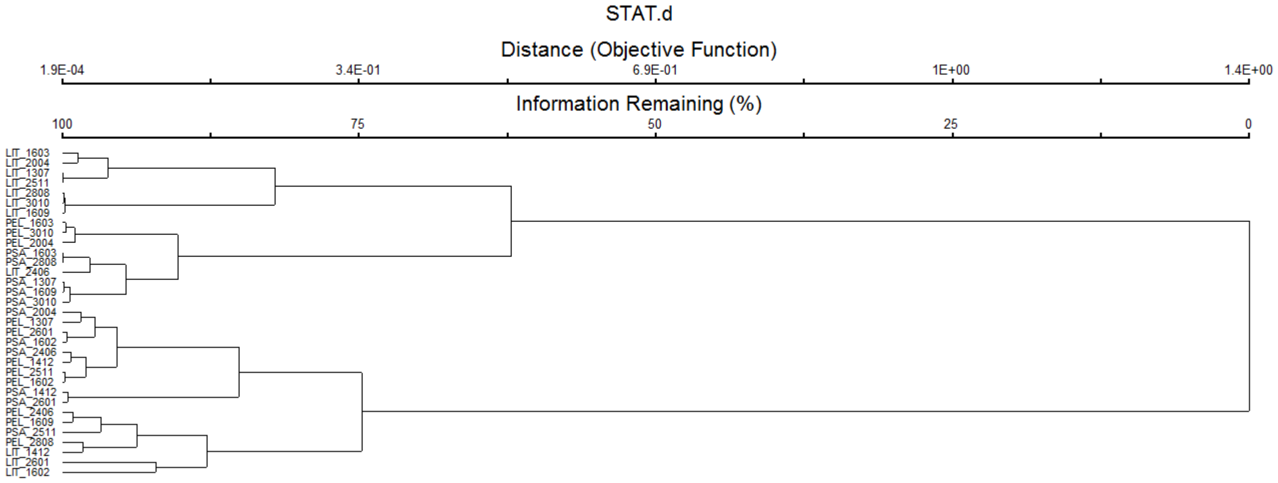


Fig.4.46b- Cluster analysis of samples collected in each substrate. Percent chaining= 1.53

ISA shows higher indicator values for CENPHY for psammic habitat while higher ones for CENUNI and PENCOL for lithic samples. PENSMA diatoms, in this station, do not seem to show a preference for a particular substrate on the opposite as what happens for PENPED and PENLAR. These groups, in fact, show different indicator values and these results are confirmed by the most abundant species belonging to each (Tab. 4.4), as pointed out by the previous analysis.

	LITHON	**PELON**	**PSAMMON**
CENPHY	*3*	*26*	***56***
MELVAR (CENPHY)	**27**	0	0
CENUNI	***47***	*24*	*15*
STEPAR (CENUNI)	**24**	3	15
PENCOL	***46***	*33*	*21*
FRAULN (PENCOL)	***15***	*7*	*5*
PENLAR	*22*	***40***	*38*
DIAVUL (PENLAR)	17	**45**	24
NAVLAN (PENLAR)	24	22	**27**
NAVTRI (PENLAR)	1	31	**34**
PENPED	***45***	*26*	*29*
ACHMIN (PENPED)	**47**	34	19
ACHBIA (PENPED)	**51**	30	19
ENCMIN (PENPED)	**42**	31	27
ENCSIL (PENPED)	**38**	24	28
PENSMA	***33***	***38***	*29*
COCPLE (PENSMA)	**38**	33	23

Tab. 4.4- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

PCA analysis performed on the environmental data acting in this station underlines the effect played by discharge and its related variables (e.g. dry weight, temperature and turbidity) on the diatom communities. Diatoms collected in high water regimes (e.g. months of June, September, October) are driven by such variables while others are the environmental variables acting in low water regime, as in the winter months (Fig. 4.46c). A different effect played by discharge has been noticed in the communities: PENPED diatoms have a positive correlation with discharge (p<0.01; 0,51 ≤ r ≤ 0,75) while PENLAR diatoms have a negative correlation value ($p<0.05$; -0,54 ≤ r ≤ 0,73). Discharge not only affects phytobenthic diatoms but also phytoplanktonic ones: in the months of June, September and October (e.g. high discharges) higher frequencies of empty frustules have been registered and 90% of them belonged to the benthic groups (e.g. PENSMA, PENLAR and PENPED) while in the winter months, with relatively lower water discharges, higher abundances of colonial groups have been found in the water column (e.g. CENPHY, CENUNI and PENCOL diatoms).

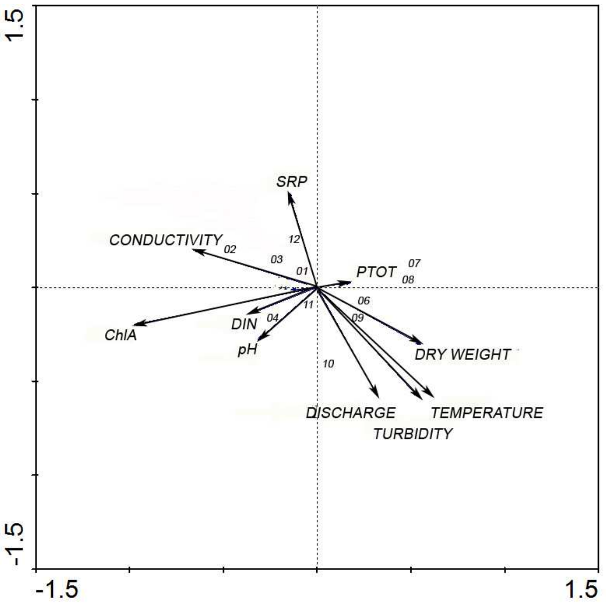
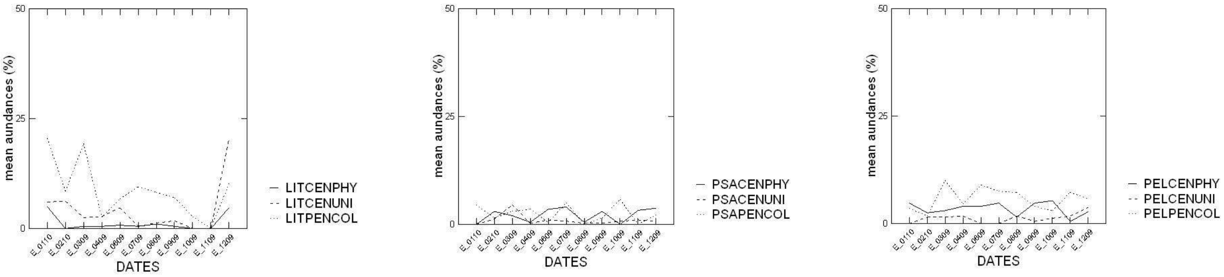


Fig. 4.46c- PCA analysis on the MFDGs of statD with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION E (Noce stream)

In this station, as in the previous ones, colonial diatoms (e.g. CENPHY, CENUNI, PENCOL) show lower frequencies than benthic diatoms (e.g. PENLAR, PENPED, PENSMA). CENPHY diatoms have a higher mean abundance in the finer substrates (Fig. 4.47b,c) and in the warmer months. CENUNI and PENCOL diatoms are more abundant in the lithic samples since their abundances are almost double in pebbles than in the sediments and sand. PENSMA diatoms do not seem to have a preference habitat among the three substrates sampled. As in the previous stations, PENPED diatoms are more abundant in the lithic habitat (mean abundance= 51%) than in the finer ones (mean abundances of 30%) (Fig. 4.47d). PENLAR diatoms are instead, more abundant in the pelon and psammon: mean abundances in these habitats are 51% and 47% in the pelon and psammon, respectively (Fig. 4.47e, f).



a) LITHIC "Colonial groups" *b) PSAMMIC "Colonial groups"* *c) PELIC "Colonial groups"*

d) LITHIC "Benthic groups" *e) PSAMMIC "Benthic groups"* *f) PELIC "Benthic groups"*

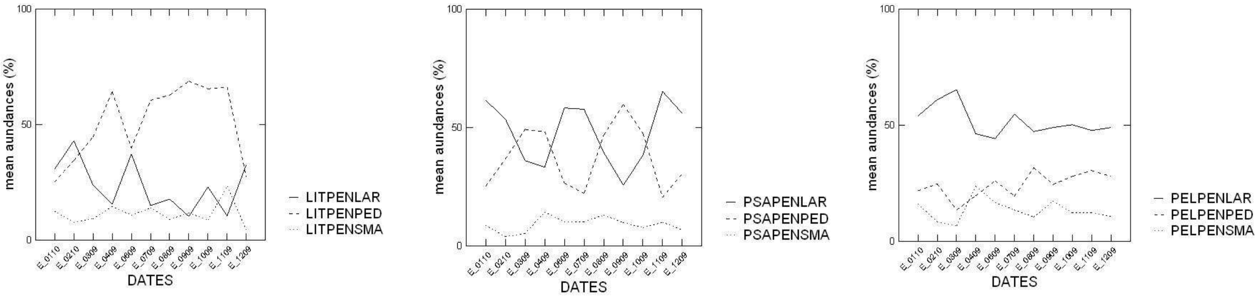


Fig. 4.47- Diatoms mean abundances (%) measured in statE in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

PCA analysis in fig. 4.48a confirms the above results: there seems to be a strong gradient that separates the samples according to the textures they have been sampled in. Higher abundances of PENPED diatoms have been found in the lithic samples while higher frequencies of PENLAR diatoms have been registered in the psammon and pelon. This pattern though, seems to be less "evident" than in the previous stations as also stated by the Cluster Analysis (Fig. 4.48b) which does not show a definitive cut between the three substrates. MRPP points at a significant difference between the samples (delta variance of 0.53 and a chance-corrected within-group agreement of 0.25).

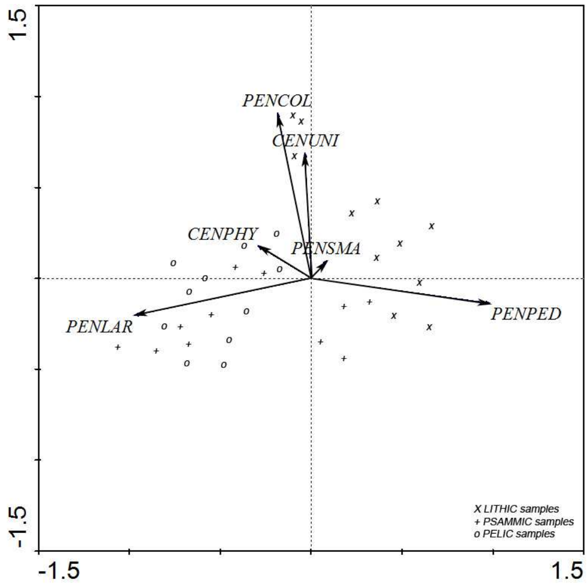


Fig. 4.48a- PCA analysis with MFDG on the three substrates sampled in station E.
93% of variance explained

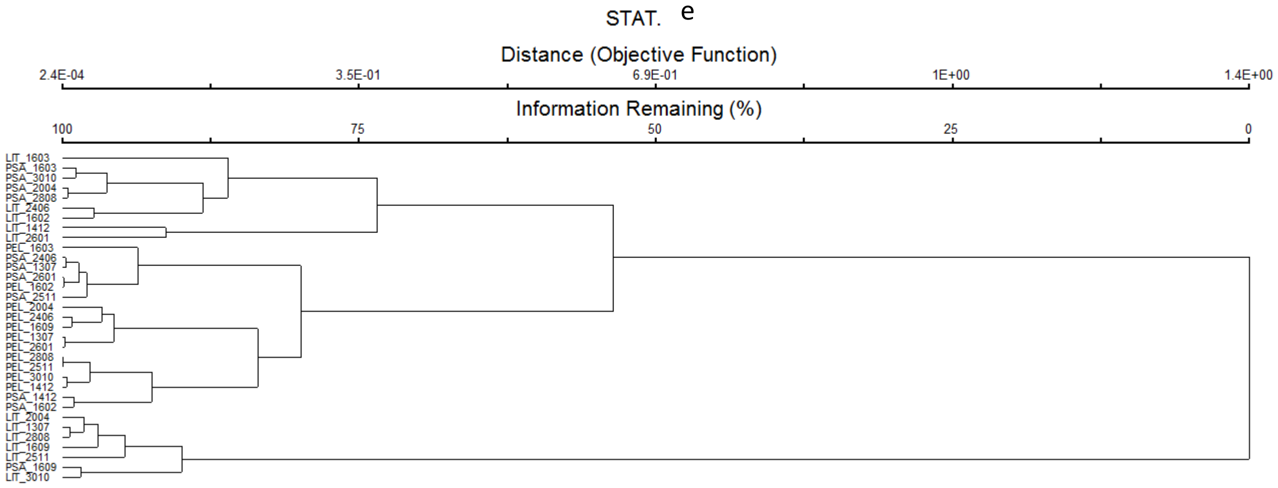


Fig.4.48b- Cluster analysis of samples collected in each substrate. Percent chaining= 6.11

ISA analysis shows higher indicator values of CENPHY diatoms in the psammon and higher values of CENUNI and PENCOL diatoms in the lithic samples. Same results can be deduced by the indicator values of the most abundant species belonging to these groups. PENSMA diatoms show higher values in the lithon and pelon samples and a lower one in the psammon. As in the previous stations, PENLAR diatoms have higher indicator values in the finer substrates (pelon= 42; psammon=39) while PENPED diatoms have higher indicator values in the lithon (45). Same results can be deduced from indicator values of the most abundant species belonging to each group (Tab. 4.5).

	LITHON	**PELON**	**PSAMMON**
CENPHY	*13*	***51***	*26*
MELVAR (CENPHY)	**55**	0	0
CENUNI	***54***	*14*	*14*
STEPAR (CENUNI)	**21**	2	8
PENCOL	***46***	*34*	*12*
FRAULN (PENCOL)	***48***	*2*	*9*
PENLAR	*19*	***42***	*39*
DIAVUL (PENLAR)	29	**40**	26
NAVLAN (PENLAR)	12	18	**40**
NAVTRI (PENLAR)	7	**34**	31
PENPED	***45***	*22*	*33*
ACHMIN (PENPED)	**42**	31	25
ACHBIA (PENPED)	**43**	31	26
ENCMIN (PENPED)	**48**	14	38
ENCSIL (PENPED)	**47**	20	33
PENSMA	***34***	***40***	*27*
COCPLE (PENSMA)	24	29	**34**

Tab. 4.5- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

PCA analysis performed in statE underlines the role of discharge and its related variables as drivers of diatom communities: samples collected in the months of April, June, September and October are highly influenced by these drivers. On the opposite, samples collected in the other months seem to be driven by other environmental variables, such as conductivity and phosphorus (Fig. 4.48c). Nevertheless, only weak correlations have been found between discharge and PENPED (p= n.s.; $0,14 \leq r \leq 0,47$) and discharge and PENLAR (p= n.s.; $-0,18 \leq r \leq -0,28$) and this could be due to a specific hydrological regime found in this station that will be discussed in the next paragraph.

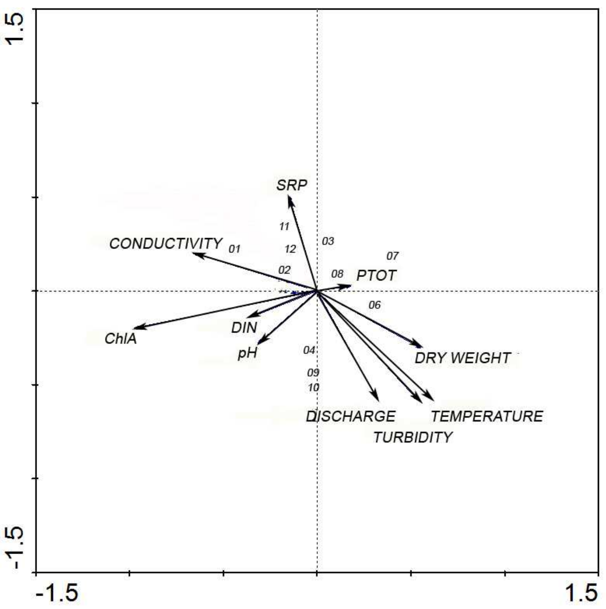
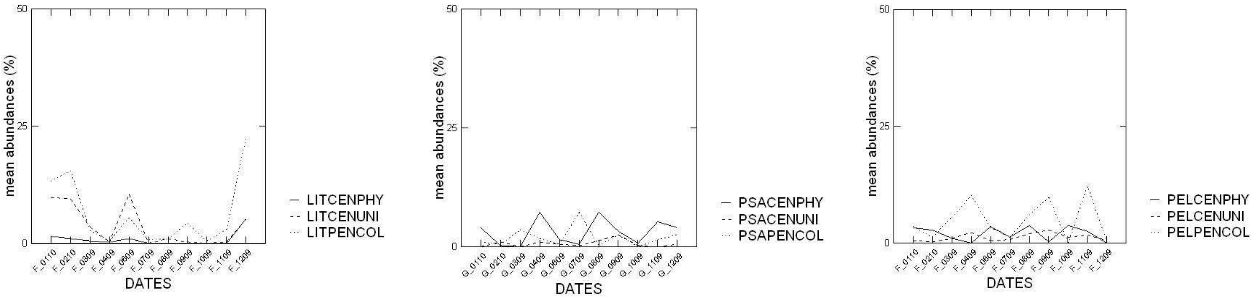


Fig. 4.48c- PCA analysis on the MFDGs of statE with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION F (Adige after the Noce stream and before the Isarco stream)

In this station, CENPHY diatoms have displayed higher mean abundances in the finer substrates (2.71% and 2% in psammon and pelon, respectively) (Fig. 4.49b, c), as in the previous sampling points. On the opposite, CENUNI diatoms have shown higher abundances in the lithic substrates (mean abundance= 3.69%) with a peak in the first months of the year and in June (Fig. 4.49a). Benthic diatoms are more abundant than colonial ones: PENSMA diatoms show comparable abundances beneath the textures with mean frequencies ranging around 11%. As for the other benthic groups, there is a neat prevalence of PENPED diatoms in the lithic samples (mean frequency= 55%) which doubles the ones recorded in the finer substrates (Fig. 4.49d); PENLAR diatoms are instead more abundant in the sand and sediments samples (mean abundances are 46% and 52% in the psammon and pelon, respectively) as underlined in the previous stations (Fig. 4.49e, f).

a) LITHIC "Colonial groups" *b) PSAMMIC "Colonial groups"* *c) PELIC "Colonial groups"*



d) LITHIC "Benthic groups" *e) PSAMMIC "Benthic groups"* *f) PELIC "Benthic groups"*

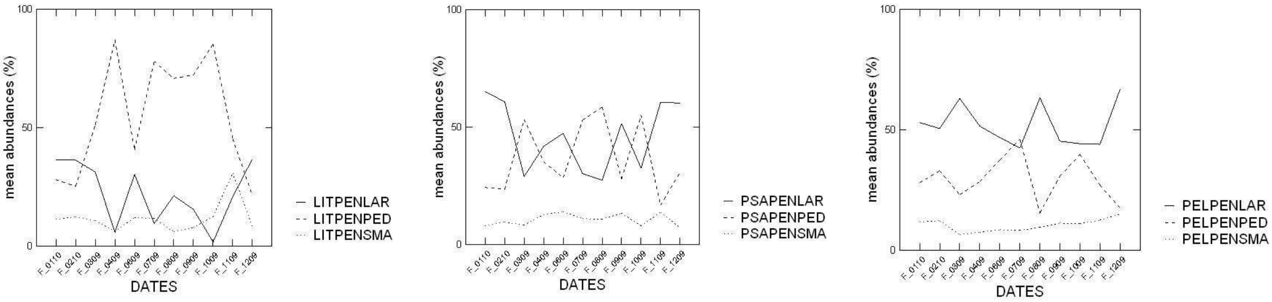


Fig. 4.49- Diatoms mean abundances (%) measured in statF in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

PCA analysis performed on the morpho-functional diatom groups in this station clearly shows a gradient determined by PENLAR and PENPED diatoms (Fig. 4.50a). Cluster Analysis returns a rather branched tree, evidencing a "chaotic" situation where samples are clearly divided according to their textures (Fig. 4.50b): this is the only station where the samples are packed according to substrates they have been collected from. MRPP as well, shows a significant difference between the samples (delta variance of 0.42 and a chance-corrected within-group agreement of 0.11).

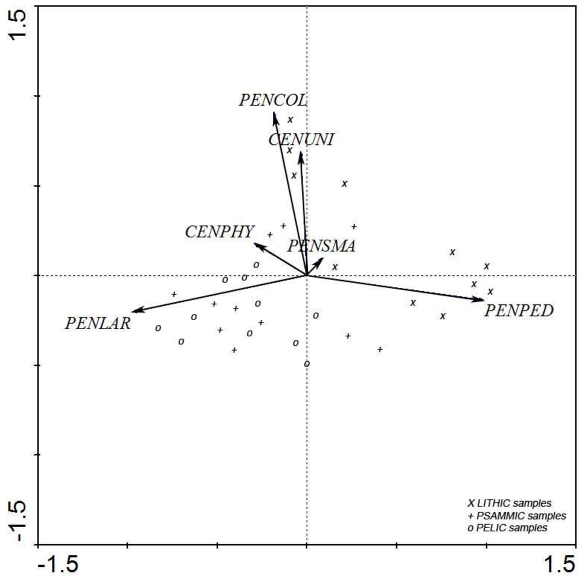


Fig. 4.50a- PCA analysis with MFDG on the three substrates sampled in station F.
93% of variance explained

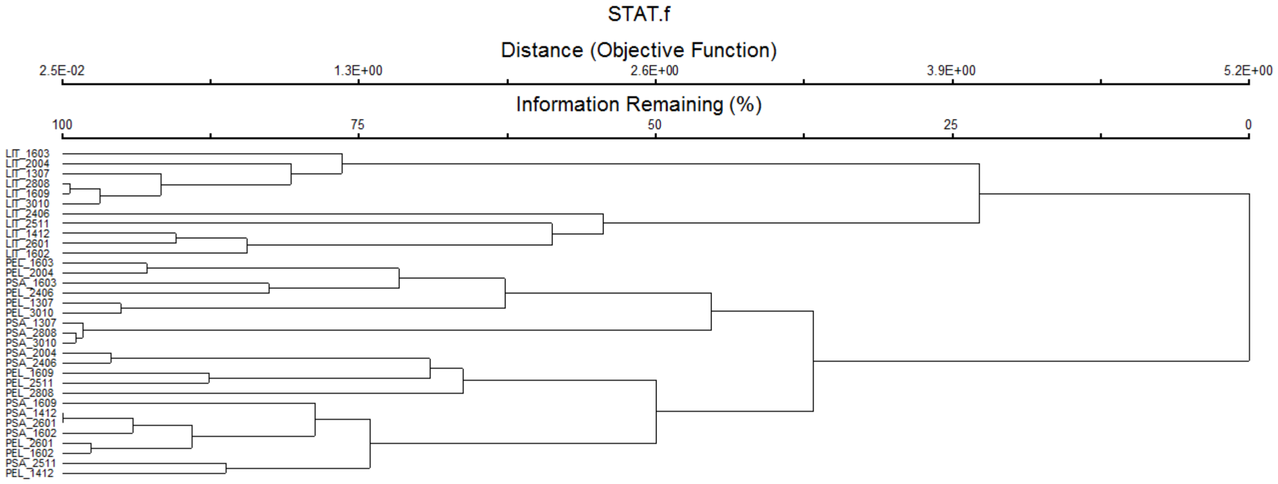


Fig.4.50b- Cluster analysis of samples collected in each substrate. Percent chaining= 4.33

ISA gives a higher indicator value for CENPHY in psammic samples but its most abundant species do not confirm this datum. CENUNI and PENCOL diatoms have instead been found to have an higher indicator value in the lithic samples: while *Stephanodiscus parvus* (CENPHY) confirms this datum, *Fragilaria ulna* shows a higher indicator value in the pelic samples. PENSMA diatoms show comparable indicator values beneath the samples while PENLAR has higher indication for pelic diatoms, as its most abundant species. PENPED diatoms show higher indicator values for lithic samples as its most abundant species (Tab. 4.6).

	LITHON	**PELON**	**PSAMMON**
CENPHY	*8*	*29*	***40***
MELVAR (CENPHY)	**36**	0	0
CENUNI	***55***	*22*	*8*
STEPAR (CENUNI)	**30**	0	5
PENCOL	***44***	*31*	*20*
FRAULN (PENCOL)	*7*	***35***	*8*
PENLAR	*19*	***43***	*28*
DIAVUL (PENLAR)	20	**37**	35
NAVLAN (PENLAR)	30	**44**	1
NAVTRI (PENLAR)	14	**39**	12
PENPED	***45***	*24*	*30*
ACHMIN (PENPED)	**44**	30	26
ACHBIA (PENPED)	**37**	30	30
ENCMIN (PENPED)	**40**	25	35
ENCSIL (PENPED)	**42**	21	37
PENSMA	***36***	***32***	***32***
COCPLE (PENSMA)	26	**37**	28

Tab. 4.6- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

PCA analysis performed on environmental variables acting on morpho-functional diatom groups points at a higher role played by discharge and its related variables during the high water regime months, which in this station have been registered from March to June and from September to November (Fig. 4.50c). During low water periods, instead, conductivity and phosphorus seem to be the main drivers acting on the communities. Correlations have been detected between discharge and diatom groups, as discharge correlates positively with PENPED ($p \leq 0.05$; $0{,}31 \leq r \leq 0{,}43$) while it does negatively with PENLAR diatoms ($p \leq 0.05$; $-0{,}32 \leq r \leq -0{,}61$). Discharge seems to influence also the water column diatom community: higher frequencies of benthic groups (e.g. PENSMA, PENPED and PENLAR diatoms) have been found as empty frustules in high waters regime while higher frequencies of colonial groups (e.g. CENPHY, CENUNI and PENCOL diatoms) have been detected during low water regimes.

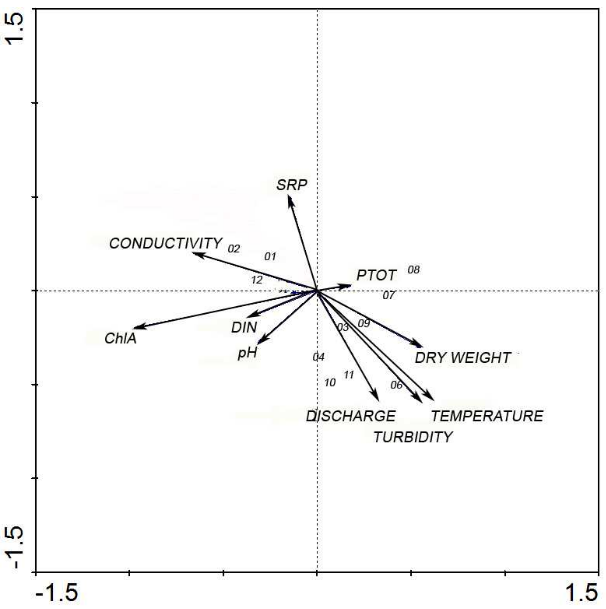
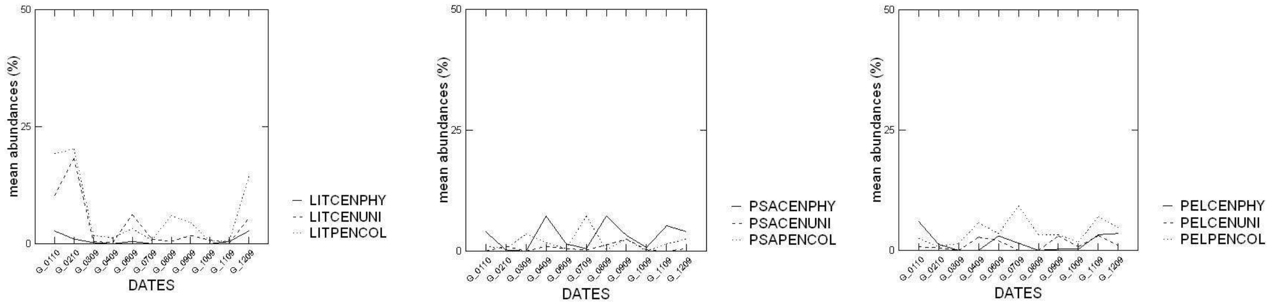


Fig. 4.50c- PCA analysis on the MFDGs of statF with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge ($m^3 sec^{-1}$); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION G (Isarco stream)

In this station, CENPHY diatoms are more abundant in the psammic samples while CENUNI taxa are in the lithic samples (mean abundances are 3% and 4.11%, respectively) (Fig. 4.51a,b, c). Also PENCOL diatoms are more abundant in the lithic samples, being that its mean abundance there (6.61%) doubles those recorded in the finer substrates. Frequencies of PENCOL diatoms in the lithon are usually higher in the colder months (e.g. from December to February). Comparable abundances of PENSMA have been recorded in all the samples (mean abundances of 9 ± 0.86%). Also in station G, higher frequencies of PENPED diatoms have been recorded in the lithic samples (mean abundance is 53.68%) with a peak in the months of April and August (Fig. 4.51d). On the other hand, high abundances of PENLAR diatoms have been found in the finer samples (mean abundances of 53.65% and 50.50% in the psammon and pelon, respectively) (Fig. 4.51e, f).

a) LITHIC "Colonial groups" *b) PSAMMIC "Colonial groups"* *c) PELIC "Colonial groups"*



d) LITHIC "Benthic groups" *e) PSAMMIC "Benthic groups"* *f) PELIC "Benthic groups"*

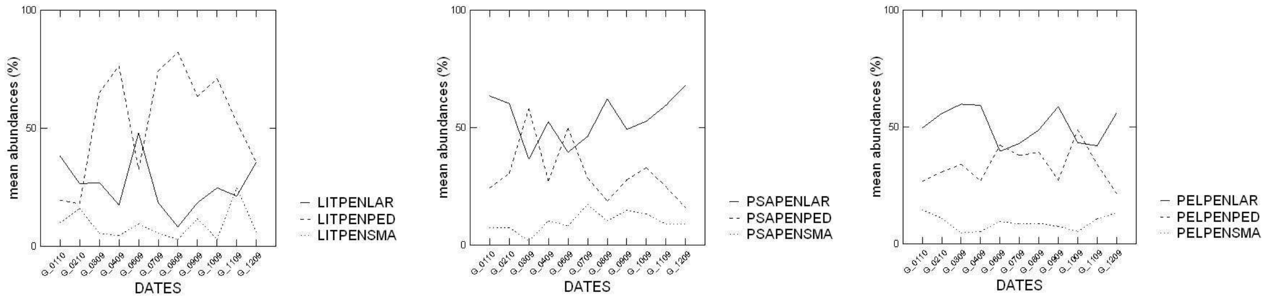


Fig. 4.51- Diatoms mean abundances (%) measured in statG in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

In this station, PCA analysis shows a gradient formed by PENLAR and PENPED diatoms but samples are distributed more randomly than in the previous stations (Fig. 4.52a). It seems in fact that there is less sharp separation between samples collected in different habitats as also shown by the Cluster Analysis (Fig. 4.52b). MRPP shows a less significant difference between the samples than in the previous sampling points (delta variance of 0.53 and a chance-corrected within-group agreement of 0.20).

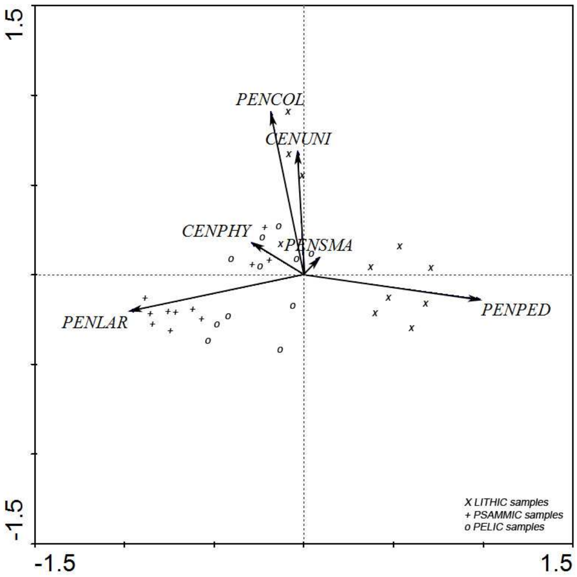


Fig. 4.52a- PCA analysis with MFDG on the three substrates sampled in station G. 93% of variance explained

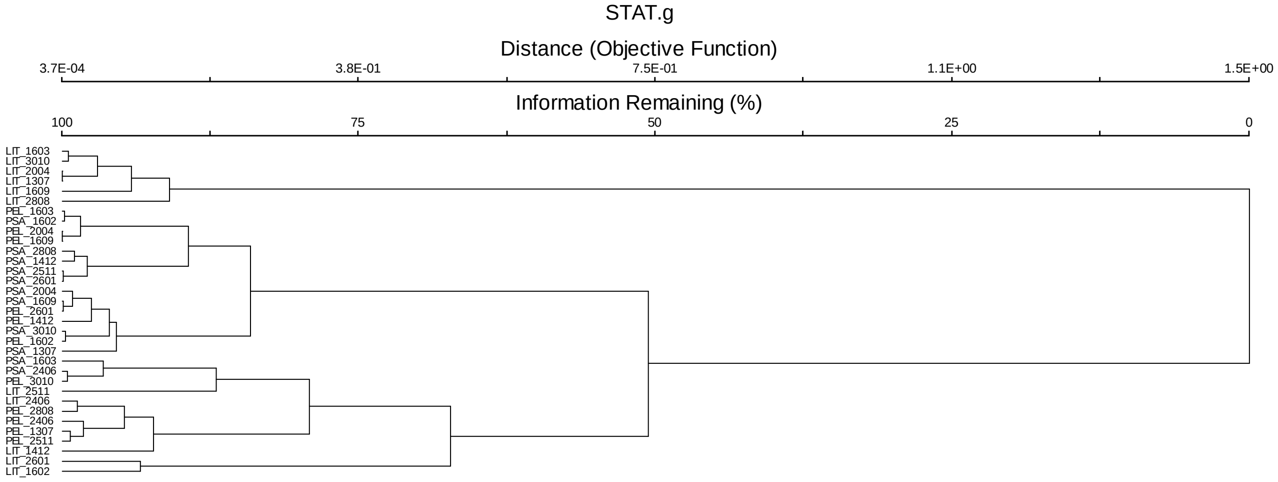


Fig.4.52b- Cluster analysis of samples collected in each substrate. Percent chaining= 6.62

ISA shows higher indicator values for CENPHy in psammic environments and higher for CENUNI and PENCOL for lithic substrates (Tab. 4.7). PENSMA diatoms show similar indicator values among the samples while PENPED diatoms seem to better indicate lithic environments. PENLAR diatoms have higher indicator values in the finer habitats.

	LITHON	**PELON**	**PSAMMON**
CENPHY	*7*	*23*	***51***
MELVAR (CENPHY)	0	0	**36**
CENUNI	***68***	*15*	*8*
STEPAR (CENUNI)	**35**	4	3
PENCOL	***53***	*32*	*14*
FRAULN (PENCOL)	***24***	*11*	*1*
PENLAR	*20*	*39*	***48***
DIAVUL (PENLAR)	24	**35**	22
NAVLAN (PENLAR)	30	**44**	1
NAVTRI (PENLAR)	14	**39**	12
PENPED	***45***	*28*	*26*
ACHMIN (PENPED)	**63**	16	19
ACHBIA (PENPED)	**29**	27	30
ENCMIN (PENPED)	**49**	21	30
ENCSIL (PENPED)	**41**	30	30
PENSMA	***32***	***35***	***35***
COCPLE (PENSMA)	25	**27**	26

Tab. 4.7- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

In this station, PCA performed with environmental variables shows a very weak interaction between discharge (and its related variables) with diatom communities (Fig. 4.52c). Very low discharge values have been recorded here: mean discharge is of 30.04 m^3 sec^{-1} while maximum values have been registered in April and October (of 38 and 40 m^3 sec^{-1}) therefore the station is permanently in low water status, easing the mixing between water column and benthic river bed. This is also evident if considering the higher quantities of colonial diatoms found in the phytobenthic compartment (see graph XX) and the high frequencies of living frustules found in the water column. It comes that communities seems to be driven by other variables, such as Phosphorus, conductivity, DIN and pH. Nevertheless, the effect of discharge cannot be completely discarded here since positive correlations have been found between discharge and PENPED diatoms (*p*

=n.s.; 0,15 ≤ *r* ≤ 0,55) and negative ones between discharge and PENLAR diatoms (*p* =n.s.; -0,11 ≤ *r* ≤ -0,23).

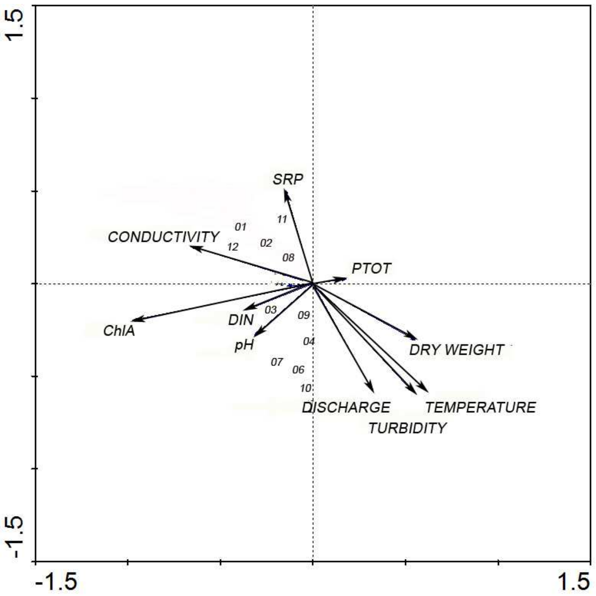
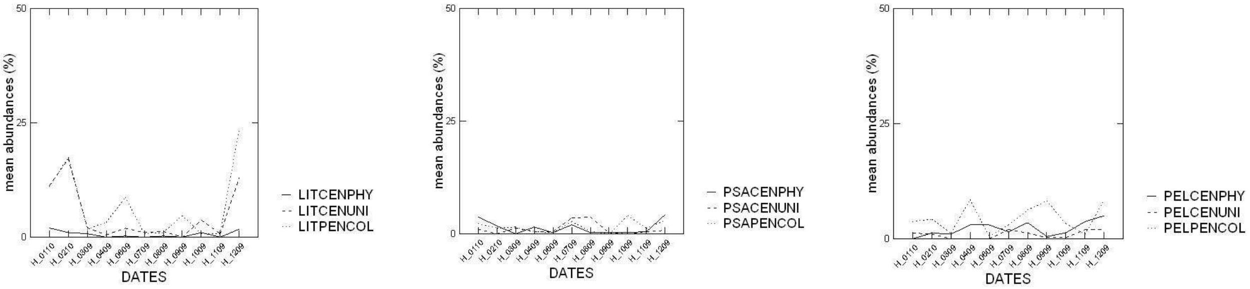


Fig. 4.52c- PCA analysis on the MFDGs of statG with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

STATION H (Adige after the Isarco stream; end of the pathway)

In this last station, CENPHY diatoms are more abundant in the finer substrates (mean abundances of 1.35 and 2% in psammon and pelon, respectively) while CENUNI and PENPED diatoms are in the lithic samples (mean abundance of 4.77% and 6.27% respectively) (Fig. 4.53a, b, c). PENSMA diatoms seem to be more abundant in the finer substrates since their mean abundances are three percentage points higher than in the lithic samples. Also in this station, in the lithon, more than 50% of the sample is populated by diatoms belonging to PENPED groups, except for a few sampling dates: nevertheless the mean abundance of this diatoms in the lithon is 54.20% which doubles those found in the finer substrates (Fig. 4.53d). On the other hands, finer substrates seems to be most preferably inhabited by PENLAR diatoms (mean abundances of 46% and 51.5% in psammon and pelon, respectively) (Fig. 4.53e, f).

a) LITHIC “Colonial groups” *b) PSAMMIC “Colonial groups”* *c) PELIC “Colonial groups”*



d) LITHIC “Benthic groups” *e) PSAMMIC “Benthic groups”* *f) PELIC “Benthic groups”*

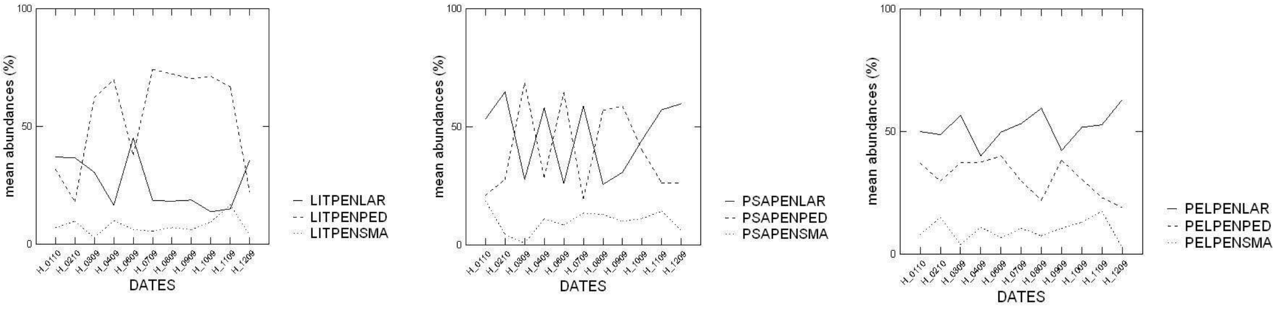


Fig. 4.53- Diatoms mean abundances (%) measured in statH in the different substrates. The first three charts (a, b, c) refer to the LITHIC, PSAMMIC and PELIC colonial diatom groups (CENPHY, CENUNI and PENCOL). The last three charts (d,e,f) refer to the LITHIC, PSAMMIC and PELIC benthic diatom groups (PENLAR, PENPED and PENSMA)

PCA analysis of morpho-functional diatom groups shows a gradient given by PENLAR and PENPED diatoms where sampled are displayed according to their textures (Fig. 4.54a). Cluster Analysis shows clusters according to each texture, even if not exclusively based on that (Fig. 4.54b) since lithic samples are quite scattered. MRPP confirms these results since it shows a delta variance of 0.73 and a chance-corrected within-group agreement of 0.18.

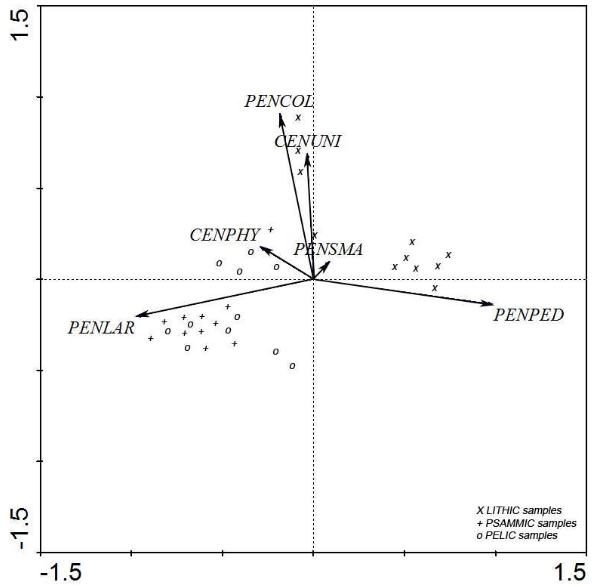


Fig. 4.54a- PCA analysis with MFDG on the three substrates sampled in station H. 93% of variance explained

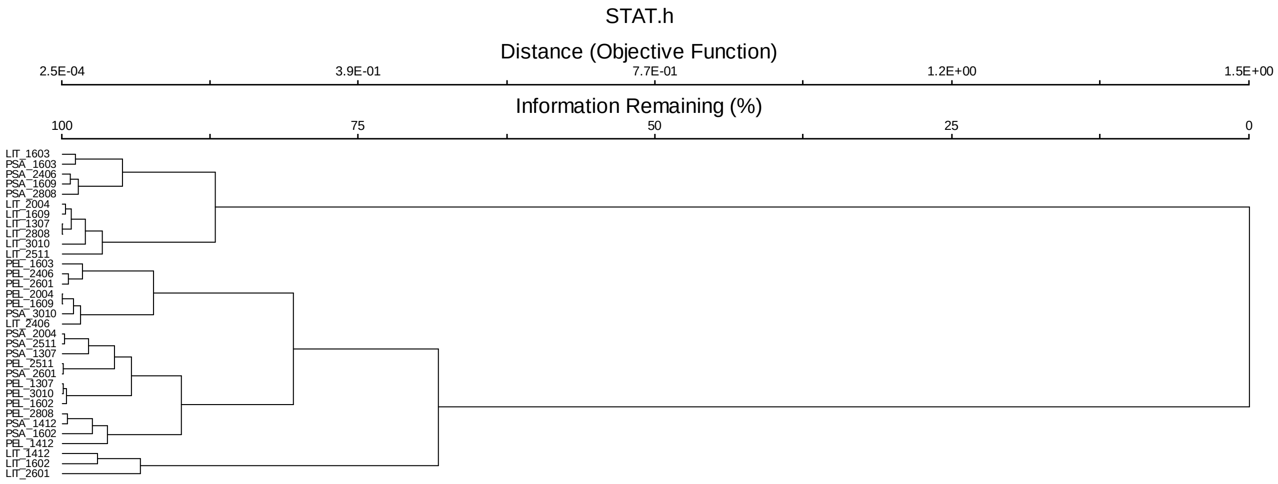


Fig.4.54b- Cluster analysis of samples collected in each substrate. Percent chaining= 5.34

ISA shows higher indicator values of CENPHY diatoms for pelic assemblages and of CENUNI and PENCOL diatoms for lithic samples, as also shown by their most abundant species. PENSMA diatoms have higher indicator values for the finer substrates as well as PENLAR diatoms. On the other hand, PENPED diatoms have higher indicator value for lithic samples (Tab. 4.8).

	LITHON	**PELON**	**PSAMMON**
CENPHY	*10*	***48***	*29*
MELVAR (CENPHY)	**55**	0	0
CENUNI	***64***	*10*	*13*
STEPAR (CENUNI)	**24**	6	3
PENCOL	***53***	*35*	*11*
FRAULN (PENCOL)	***12***	*11*	*4*
PENLAR	*21*	***42***	*37*
DIAVUL (PENLAR)	22	**41**	32
NAVLAN (PENLAR)	22	**31**	20
NAVTRI (PENLAR)	1	**46**	38
PENPED	***43***	*25*	*32*
ACHMIN (PENPED)	**51**	20	24
ACHBIA (PENPED)	**41**	30	24
ENCMIN (PENPED)	**39**	19	**42**
ENCSIL (PENPED)	**38**	24	**38**
PENSMA	*28*	***35***	***37***
COCPLE (PENSMA)	25	11	**49**

Tab. 4.8- Indicator Values of the each morpho functional diatom group (CENPHY, CENUNI, PENCOL, PENLAR, PENPED, PENSMA) and their most abundant species in the three substrates sampled: MELVAR stands for *Melosira varians*; STEPAR for *Stephanodiscus parvus*; FRAULN for *Fragilaria ulna*; DIAMON for *Diatoma moniliformis*; DIAVUL for *Diatoma vulgaris*; NAVLAN for *Navicula lanceolata*; NAVTRI for *Navicula tripunctata*; ACHMIN for *Achnanthes minutissima*; ACHBIA for *Achnanthes biasolettiana*; ENCMIN for *Encyonema minutum*; ENCSIL for *Encyonema silesiacum*; COCPLE for *Cocconeis placentula* var. *euglypta*

PCA analysis performed using environmental variables show a influence played by discharge and its related variables (e.g. dry weight, turbidity and temperature) on the diatom communities: in June and October (e.g. high water regime) and December-January- February (e.g. low water regime) variables driving communities are different (Fig. 4.54c). Also in this station, positive correlations have been found between discharge and PENPED diatoms (p =n.s.; 0,08 ≤ r ≤ 0,47) and negative ones between discharge and PENLAR diatoms (p =n.s.; -0,06 ≤ r ≤ -0,44): the highest correlation values have been found for the lithic samples.

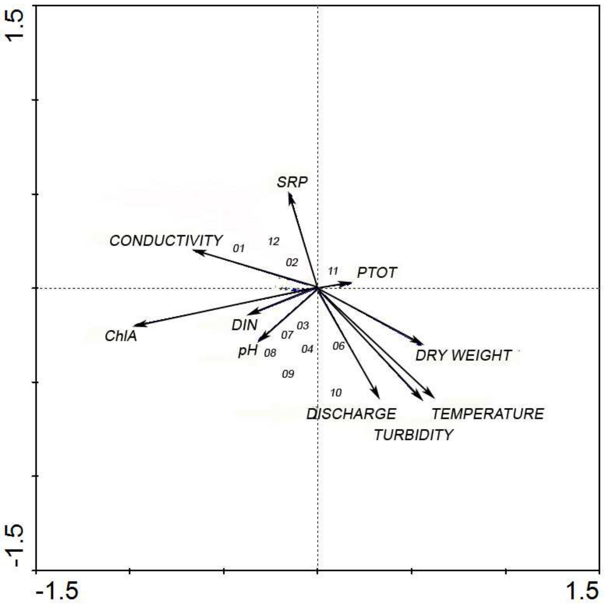


Fig. 4.54c- PCA analysis on the MFDGs of statH with the environmental variables recorded. This analysis is an extract of the PCA performed on all the samples recorded in the eight stations of the research (97.8% of variance explained). Discharge (m^3 sec^{-1}); Turbidity (NTU); Temperature (°C); Dry Weight (mg L^{-1}); PTOT is Total Phosphorus (µg L^{-1}); SRP is Soluble Reactive Phosphorus (µg L^{-1}); Conductivity (µS cm^{-1}); Chlorophyll *a* (µg L^{-1}); DIN is Dissolved Inorganic Nitrogen (µg L^{-1}). Progressive numbers refer to the months of the year.

4.2.4. Spatial patterns and ecological determinants of benthic diatom communities of Adige River

Some environmental parameters have shown higher within-site variation: discharge and its collinear variables (dry weight and turbidity) and discharge and temperature show different records among the stations (Fig. 4.55 a, b, c, g) and this has been expected to highly influence the diatom community of the site. On the opposite, other variables have shown less ample variations, such as oxygen (Fig. 4.55d) whose mean average is ranging around 12 mg L^{-1}. Conductivity and pH are considered to be important drivers since they are thought to exert a control on diatom growth: therefore their within-site variability is expected to influence diatom communities (Fig. 4.55 e,f). Among the nutrients, there is a high within-site variation: tributaries (stations B, E and G) display different values than the mainsteam (Fig. 4.55 f, g, h, i) and this could affect the resulting diatom population living in it as it seems evident from the chlorophyll *a* boxplot (Fig. 4.55 l).

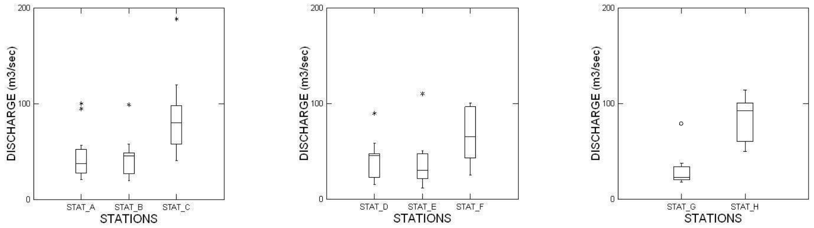


Fig. 4.55a- Boxplot of discharge values (m^3 sec^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

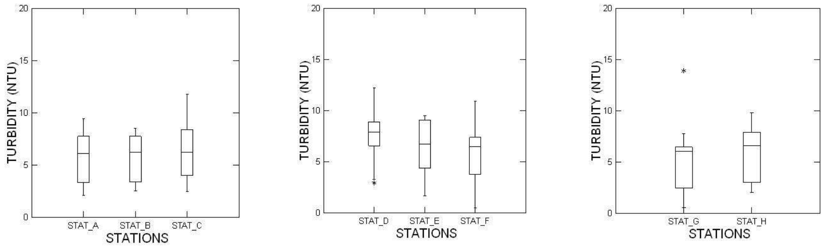


Fig. 4.55b- Boxplot of turbidity values (NTU) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

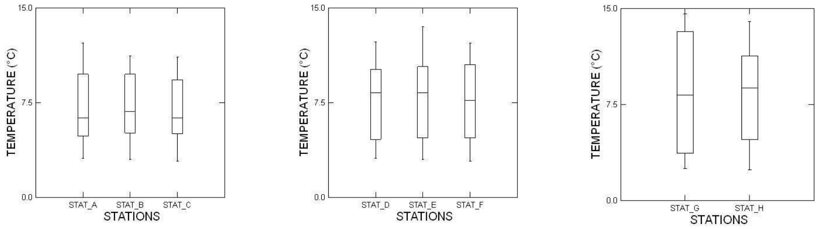


Fig. 4.55c- Boxplot of temperature values (°C) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

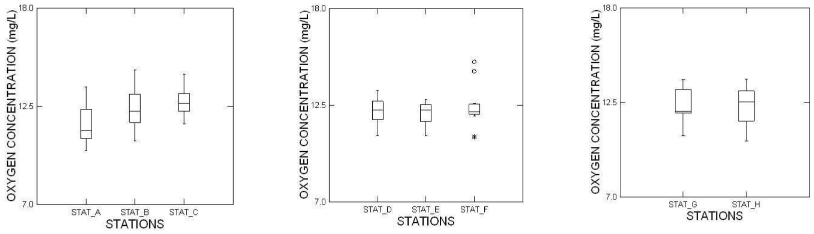


Fig. 4.55d- Boxplot of oxygen concentrations(mg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

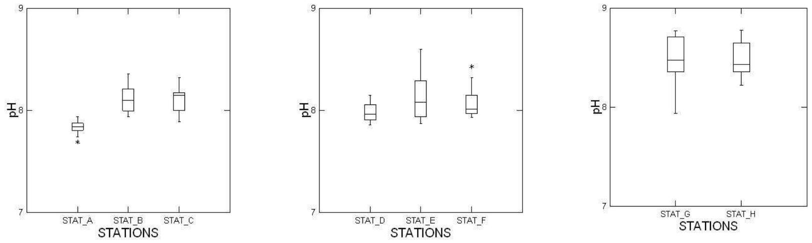


Fig. 4.55e- Boxplot of ph values measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

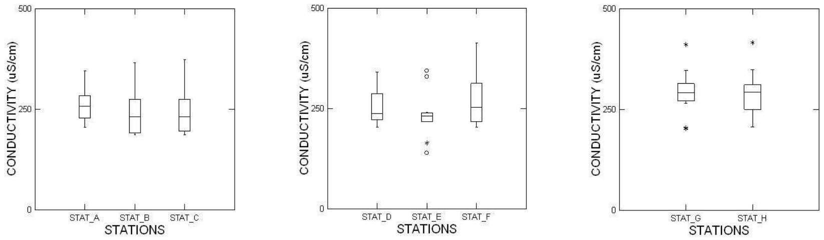


Fig. 4.55f- Boxplot of conductivity values (µS cm^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

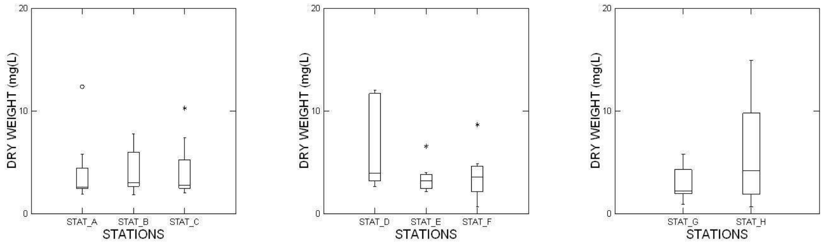


Fig. 4.55g- Boxplot of dry weight values (mg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

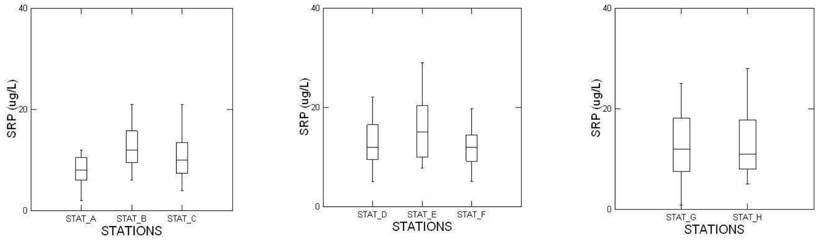


Fig. 4.55f- Boxplot of SRP values (µg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

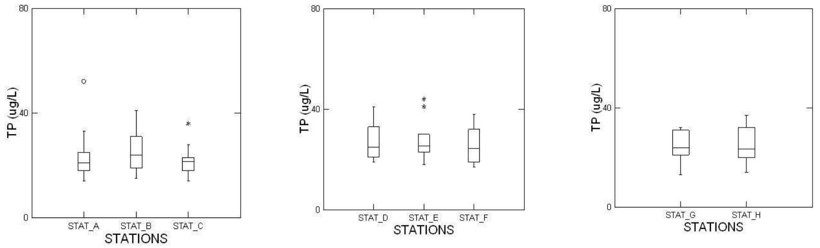


Fig. 4.55g- Boxplot of TP values (µg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

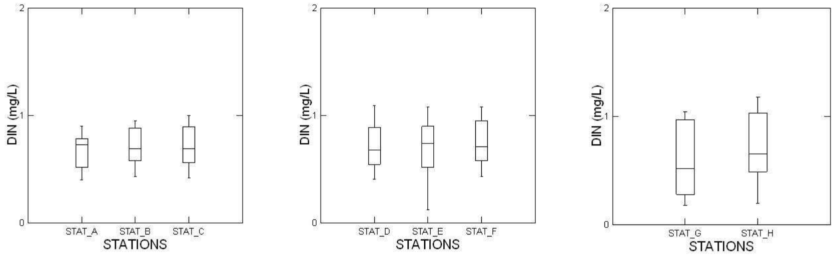


Fig. 4.55h- Boxplot of DIN values (mg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

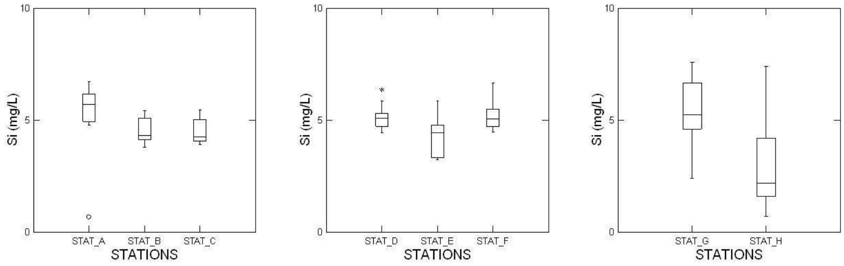


Fig. 4.55i- Boxplot of silica values (mg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

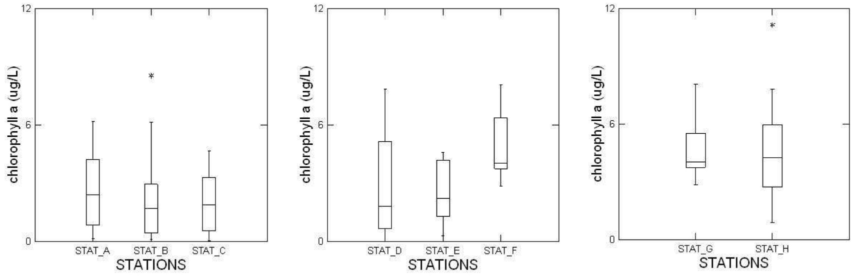


Fig. 4.55l- Boxplot of Chlorophyll *a* values (µg L^{-1}) measured in the eight sampling stations, plotted for each sub-basin (stations A, B, C for the Isarco; stations D, E, F for the Noce and stations G and H for the Avisio sub-basin)

Among the stations,centric taxa show different distributions according to each substrate: CENPHY diatoms are more abundant in the finer substrates (psammon and pelon) while CENUNI diatoms are more abundant in the lithic ones. Also in the case of PENCOL diatoms, higher abundances have been recorded in the lithic samples in all the stations, expecially in the colder months of the year (i.e., from December to February) when the river system shows its lowest discharge values. PENSMA diatoms have shown comparable abundances among the substrates in all the eight stations (see previous paragraph). PENLAR diatoms have been found to be more abundant in the finer substrates (i.e., psammon and pelon) while PENPED diatoms have been shown higher relative abundances in the coarser substrates (i.e, lithon), in all the eight sampling points.

These common patterns, have been confirmed by Principal Component Analysis performed on the Morpho-Functonal Diatom Groups (results have been reported in the previous paragraph according to each station, but they are the result of an unique analysis) and by the Indicator Species Analysis (ISA), Cluster Analysis and Multi Response Permutation Procedure (MRPP). In the PCA, a gradient has been detected between PENLAR and PENPED diatoms in all the stations: samples from finer habitats (psammon and pelon) showed a higher abundance of taxa belonging to the first group while samples from coarser habitats (lithon) showed higher abundances of taxa belonging to the second group. These results have been confirmed by ISA which has shown higher indicator values for PENLAR diatoms in the psammic and pelic samples and, on the other hand, higher indicator values for PENPED diatoms in the lithic samples (see previous paragraph).

PCA analysis performed on environmental variables (with the exception of oxygen, seeing its similar value along the river pathway) has been reported for each station but it is the outcome of an unique analysis like in the revious case. The enquiry has shown a different temporal pattern among the samples, with high water samples more driven by hydrological variables and low water samples more driven by other variables. In order to elicit this patterns, these results from the previous paragraph, have been summarized according to the three “sub- basins” (stations A, B, C: stations D, E, F; stations G, H). (Fig. 4.56a, b, c).

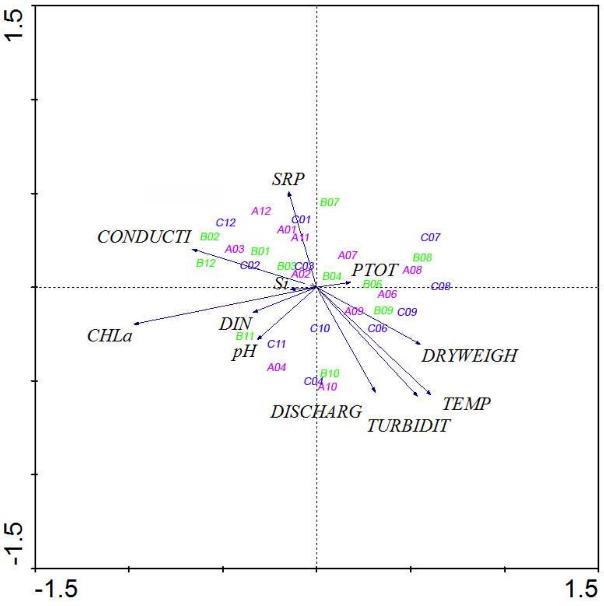


Fig. 4.56a- Extract from the eight-stations PCA analysis accounting for stations A, B and C. Stations are indicated by their progressive letter and by the sampling month

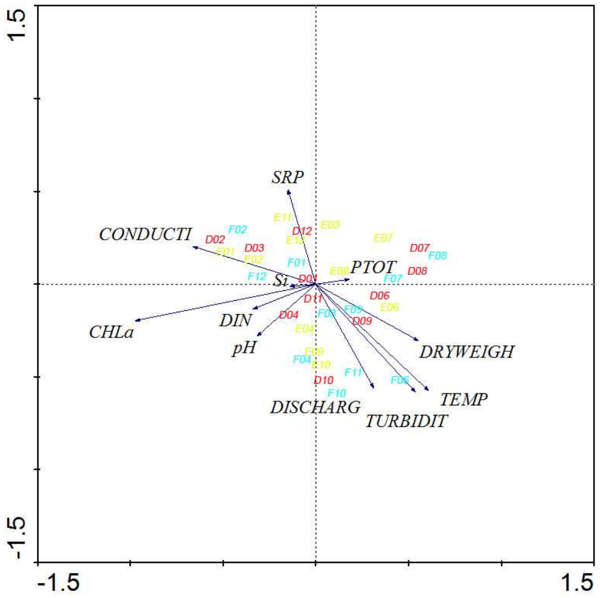


Fig. 4.56b- Extract from the eight-stations PCA analysis accounting for stations D, E and F. Stations are indicated by their progressive letter and by the sampling month

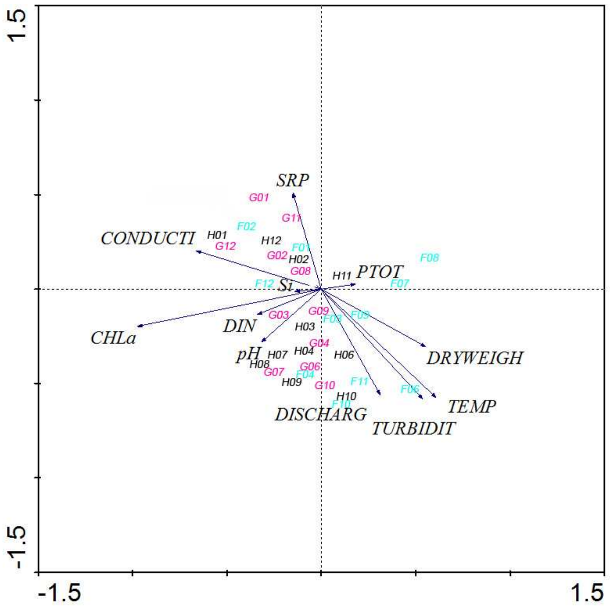


Fig. 4.56c- Extract from the eight-stations PCA analysis accounting for stations F, G and H. Stations are indicated by their progressive letter and by the sampling month. Stations F has been repeated also in this graph

As from the figures, it seems evident that diatom communities developing during high-water months are driven by different variables than low-water months (e.g. months of October and February), in the three sub-basins. In the sub-set of stations D, E and F there is a particular situation imposed by the tributary Noce (statE). This stream has a particular hydrological regime (see introduction) causing fast and sudden changes of discharge (a phenomenon called "hydropeaking"- Salmaso et al, 2010) and this surely affects the benthic community. In the PCA analysis, in fact, it seems evident that consequent samples have a common pattern, especially in the high-water months, but there are cases, such as the month of November, where statE seems to be driven by other variables than the other two stations (Fig. 4.56b). The sub-basin of stations F, G and H was expected to be influenced by the low discharges recorded in statG (Avisio stream). This watercourse displays very low fluxes (mean average is 30.04 m^3 sec^{-1}) and this can have profound implications on the diatom community. This is evident in the months of August and November where the diatom community of the station seems to be driven by other variables than the other two (Fig. 4.56c). There could be also an implication due to the geological nature of the Avisio basin (the watercourse is called “the rodents of porfids”- see introduction) which, in turn, could influence the following station (statH) as in the sample collected in the month of July. In order to better appreciate the monthly variations among the stations, figure 4.57 presents PCA results according to each station (being nevertheless the results of an unique analysis) and the seasonal patterns intercorring in each sampling point.

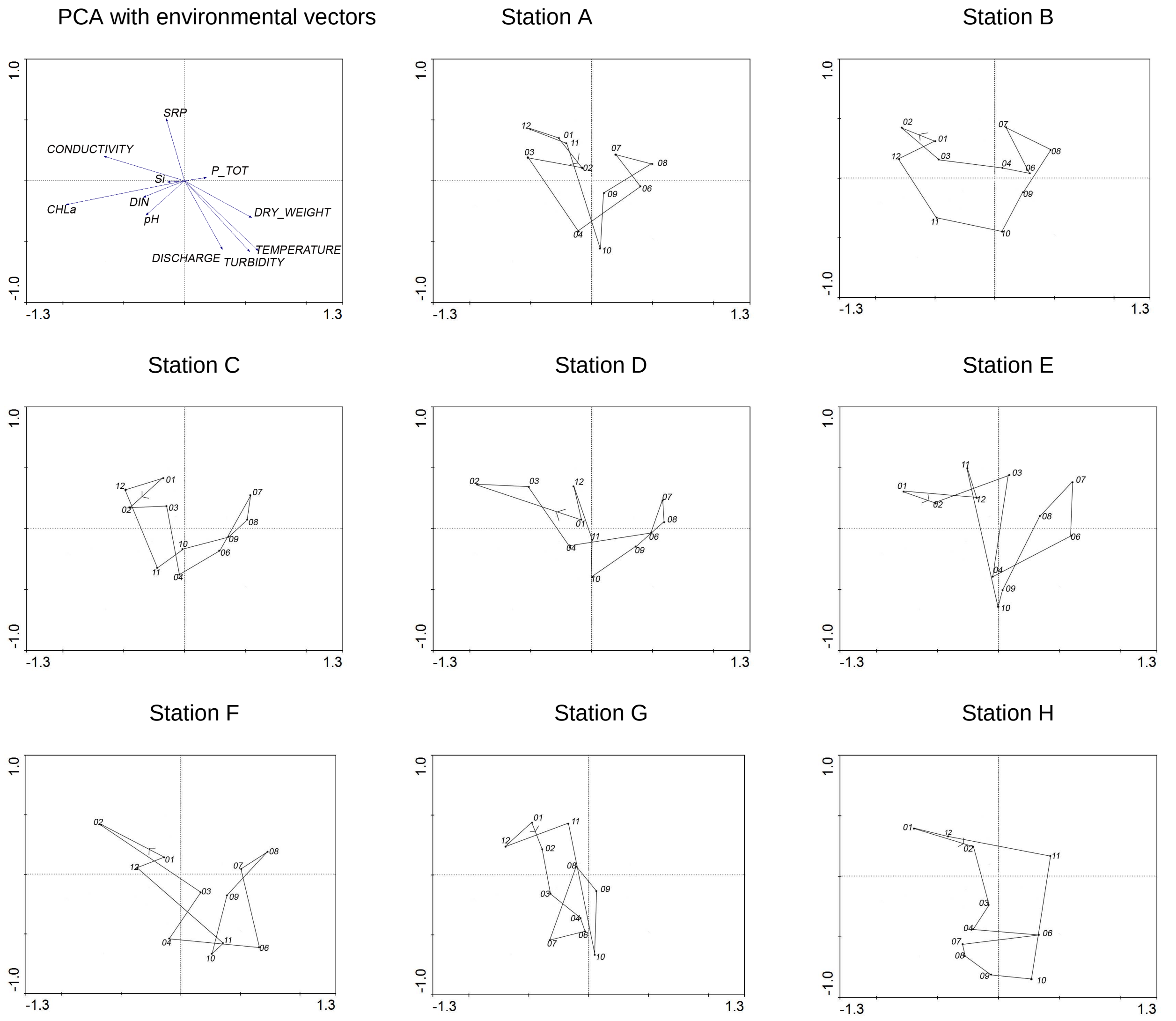


Fig. 4.57- Principal Component Analysis on environmental data plotted for each sampling stations (to avoid sample superimposition)

Overall, analyzing the eight stations, it seems that stations G and H are driven by other variables than the previous sampling points (statA, statB, statC, statD, statE, statF): this is especially true for samples collected from March to September, that seem to be driven by pH, DIN and Chlorophyll *a*. This is particularly evident if analyzing seasonality patterns of the stations along the PCA' s gradients (Fig. 4.58). For samples collected in June and Summer, statG and statH seem to be ruled by different drivers and particularly pH. This could be an effect due to the Avisio stream's basin geology (e.g. porfids) and to its particularly low discharge levels throughout the year (see fig. 4.26). Only in the month of October, in fact, statG displays a slight increase in discharges and this could constrain the diatom community to be ruled by this variable. This, in turn, could influence the diatom community living in statH, giving it similar patterns. For all the stations, October samples (and June samples for the first six stations) which are going through high water discharges are actually driven by this variable. Summer and winter samples, instead, which are characterized by low water levels, seem to be less affected by such variables.

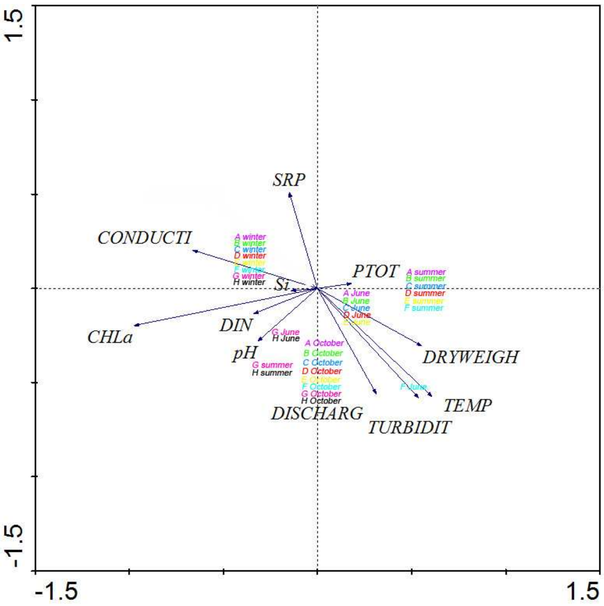


Fig. 4.58- PCA analysis on the seasonality patterns of the stations. Data are resumed from the previous PCA analysis .

Along with these environmental constraints acting on the diatom communities of the stations, spatial patterns exert a control which is more station-specific. It is in fact coupled with the habitat specificity that each diatom shows, regarding its morphology, habitus or modality of adhesion to the substrate. As from the previous chapters' results, in fact, morpho-functional diatom groups have shown different "tastes" for textures, the most evident being PENPED diatoms for coarser substrates and PENLAR diatoms for finer substrates, as shown by PCA and cluster analysis. Indicator Species Analysis has returned higher indicator values of CENPHY as well as CENUNI diatoms for tributaries (statB, statE and statG) even though the former group has been found to be more representative of finer substrates while the latter of coarser ones. PENCOL and CENUNI diatoms have shown higher indicator values in statG (station displaying low water levels) especially in the lithic compartment. PENSMA group has shown similar indicator values among the substrates and within-stations. PENLAR diatoms have returned higher indicator values in the lithic samples in all the stations while PENPED diatoms have in the pelic and psammic samples, with similar numbers in all the stations.

5. DISCUSSION

5.1 DIVERSITY AND DISTRIBUTION OF DIATOMS AND THE ROLE OF ENVIRONMENTAL VARIABLES IN STRUCTURING THE PLANKTONIC DIATOM COMMUNITIES

In the first year of my research, the highest discharges have been recorded between station 1 and 3 and this could be due to the immission of the Adige's major tributaries (Isarco, Noce and Avisio streams) in the mainsteam. In the final station (Boara Pisani), during the summer, discharge has been in several occasions lower than the mean values registered in the northern stations and this effect is due to the withdrawals for agriculture and irrigation purposes in the provinces of Verona, Rovigo e Venezia (ca. 120 $m^3 s^{-1}$ in the "peak" season; Basin Authority of River Adige, 2003). Iob (2008) has recently found that the water volumes in Boara Pisani between May and September are 20% lower than those passing by Trento city (150 km distance). This phenomenon could be a problem in the future and eventually, cause the up-rise of saline waters from the Adriatic Sea (Rossi & Veltri 2007). Along the five sampling stations, temperature has been found to increase southward but it seems that the Adige river bears quite cold waters if compared to the big European rivers like the Po (Rossetti et al., 2008), the Danube (Kiss et al., 1996) and the Meuse (Descy, 1987). In fact, taking into account temperature, River Adige seems more comparable to the northern European rivers such as the Thames (Ruse & Hutchings 1996). The increase in temperature caused a decrease of dissolved oxygen, as expected, which diminishes southward having its lowest value in station 5. Station 4 is an exception to this pattern, probably because of its peculiar situation, characterized by a very low water level: this in turn causes a higher availability of light for the phytobenthos that increases O_2 concentrations therefore increasing also the pH values which are rather high in station 4. The increase of conductivity values registered southward is due to the enrichment of minerals in the lower basin. A dilution effect played by higher discharges in the spring months has been observed in all the five sampling stations. Turbidity has shown a decrement from station 1 to 4, probably given by the high quantities of gross materials coming from the catchment area: in station 5 this effect could have been smothered by the higher water levels passing by this station with a subsequent tumbling and mixing effect of dissolved materials that gets to be resuspended. This patterns is confirmed by dry weight which decreases southward but gets higher in Boara Pisani. Going southward the gradient, phosphorus and nitrogen increase thanks to the prevalent soil use of the area (i.e. agriculture): the use of fertilizers surely increases the concentrations of these two elements in the water column. On the opposite, silica seems to decrease going southward with the exception of Pescantina station and this could be done to the low water levels and greater activity of phytobenthos in this station (see above).

In the five stations sampled, it has been noticed a general increment of species going southward the watercourse (from 93 species in station 1 to 114 in station 5) pointing at a higher biodiversity probably due to allochtonous inputs of the tributaries. This is consistent with the results provided by Jùttner et al. (1996) that found higher species richness in agricultural and/or organically polluted streams rather than undisturbed ones. No correlations have been found between local number of species and environmental characteristics, showing that species richness does not constitute a reliable measure for environmental status in this watercourse as previously found in other rivers (see Heino et al, 2005). On the other hand, an increase in disturbance (computed as an increase in discharges) has not been found to correlate to species richness, as previously pointed by other studies (see among others Wendker, 1992 and Lake, 2000) that associated species richness with disturbance. Essentially, this could be due to the high discharge values that characterise medium and extreme hydrological events in River Adige, therefore putting these events at the far extreme of the disturbance spectrum.

The majority of the recorded species belong to two morpho-functional diatom groups: PENLAR and PENPED. They are having high percentages in all the stations with the exception of station 5 where a higher abundance of CENUNI and CENPHY has been found. PCA and NMDS analysis have shown a direct effect with discharge and its related variables. Torrential conditions in the northern stations, consisting in relatively low water levels and higher water velocity, expose the river bed to a stronger erosion and scraping action by the waterflow so that planktonic diatom community in this site mainly included drifted or tychoplanktonic and meroplanktonic pennate taxa (such as *Diatoma* spp.*, Encyonema* spp.*, Achnanthes* spp.*, Navicula* spp. and *Rhoicosphenia* spp.). On the opposite, in the downstream station (station 5, Boara Pisani) the percentage of centric diatoms (C or CR strategist, *sensu* Reynolds, 2006) like *Melosira* spp.*, Stephanodiscus* spp. and *Cyclotella* spp. was higher, in accordance with higher water levels and more pelagic conditions displayed by this station. This is in accordance with the widespread theories that conceive phytoplankton as being ruled by physical factors such as discharge and temperature, as discovered in the larger European rivers (Meuse River, Thames River, Danube River and Rhine River) (Biggs & Close, 1989; Gosselain et al., 1994; Biggs et al, 1998; Kiss et al., 1998; Bahnwart et al., 1999). Interestingly, the increment of centric taxa in station 5 is due to only a few species (mainly *Stephanodiscus hantzschii, S. parvus, Melosira varians* and *Cyclotella meneghiniana*).

The onset of regulation, below which phytoplankters may be generally controlled by P supply, generally occurs at around 3 µg P l^{-1} (Reynolds, 2006). On the other hand, other species are characterised by possessing higher P requirements: one of these is *Cyclotella*, which has half-saturation growth constant for phosphorus of ≤10 µg P l^{-1} (Wehr & Descy, 1998). Small centric diatoms were abundant in station 5, where SRP never fell below 20 µg P l^{-1}, but constituted only a tiny fraction in the northern stations. Besides hydrological factors, P deficiency especially for this group of pelagic diatoms could not be excluded in the northern reaches of the river. Conversely, TP was always present with large concentrations in both stations. Similarly, silica was always present with non-limiting concentrations. Similarly, minimum DIN concentrations in the stations were from around 15 to 30 times greater than the limits below which phytoplankters may experience problems in obtaining sufficient N to half saturate growth (i.e., 15-30 µg N l^{-1}; Reynolds, 2006). Also silica variations in the southern station were controlled by diatoms: the highest reduction of Si in August coincided with the maximum development of pelagic small centric species during periods of low discharge and nutrient replenishment. However, even in this case, the concentrations (1.1 mg Si l^{-1}) never went below the growth-limiting values encountered in most lacustrine environments (< 0.5-0.1 mg Si l^{-1}; Reynolds, 2006). The lack of a negative relationship between diatoms and Si in the northern stations reflects the absence of a phase of diatom development during summer and, possibly, a major availability of Si coming from the hydrographic basin. Therefore the role of nutrients seems to have a secondary effect on the diatom communities, especially in the case of rivers such as the Adige River which are particularly constrained by hydrological factors (Biggs & Smith, 2002; Leland, 2005) even if it cannot be excluded in “more-natural” watercourses (Bowman et al., 2007). A grazing effect due to zooplankton seems to be unlikely since its abundance in the Adige River is negligible (Salmaso & Braioni, 2008). Some authors have evidenced that a zooplankton effect could be observed in short periods during the vegetative season and with low discharge regimes (Garnier et al. 1995; Gosselain et al., 1998) and these two moments never coincide in this watercourse that has high discharge times in spring and summer months.

It therefore seems that temporal dynamics of planktonic diatoms in the Adige River are strongly controlled by physical factors, mainly water discharge and the variables directly connected to hydrology (turbidity and dry weight and temperature): these factors have a temporal dynamic less or not at all predictable than environmental factors acting cyclically on inertial systems less impacted by hydrological disturbances (e.g., large and deep lakes). This effect is regulated by the relative contribution of the tributaries that undergo a different hydrological regime than the Adige mainsteam and, therefore, allow within-station spatial issue to intervene in the process.

5.2 DIATOM DISTRIBUTIONS TOWARDS GEOGRAPHICAL AND ENVIRONMENTAL FACTORS

In the second part of my research, thanks to the inclusion of tributaries in the study, I have assessed the role of environmental factors coupled with geographical and spatial issues. The effect of discharge has been found essential to regulate these processes: in statG (Avisio tributary) the low discharges measured throughout the year favoured the recording of high abundances of chlorophyll *a* as proxy for algal colonization. This effect is evident in the PCA analysis (see fig. 4.58) which shows how samples of statG are less chracterised by hydrological variables. This could cause an intense mixing/exchange with the diatom community found in the water column, as proven by the high frequencies of CENPHY diatoms which are typical mero- and tychoplanktonic algae as *Melosira varians*. This effect is confirmed by the water column community analysis which shows a higher number of live diatom cells belonging to colonial groups (CENPHY, CENUNI and PENCOL) confirmed by the high recordings of the same groups in the benthic community. On the opposite, the effect of high discharges in other stations (e.g.statF, statH) is evident in lower abundances of these colonial diatom groups, both in the benthic and phytoplanktonic compartments. The effect of discharge in statE is even more disruptive: the hydropeaking phenomenon (see Bruno et al, 2009a) never leaves a chance for phytobenthic community to establish because of the sudden and violent changes of discharge happening there. Discharge not only has an environmental effect on the diatom communities but it also seems to have a spatial effect. In all the sampling stations, there is a direct link in selecting which diatom population can find its ecological niche. Positive correlations have been found between discharge and PENPED diatoms which can therefore be considered “early colonizers”, able to colonize the substrate before other species. On the opposite, negative correlations found between discharge and PENLAR diatoms point at these diatoms being more abundant in mature biofilms, as pointed by previous studies (e.g. Yallop & Kelly, 2006). This effect has to account for the different “taste” for these two groups for the three substrates: PENPED diatoms have been found to be more abundant in the coarser habitats (e.g. lithon) while PENPLAR diatoms have been found to be more abundant in the finer habitats (such as psammon and pelon). This pattern has been found in all the eight stations and it is not surprising considering that pebbles can offer a more stable environment for early colonizers (e.g. *Achnanthes* spp. and *Encyonema* spp.) to settle while the highly unstable environments of sand and sediments, which are subjected to constant tumbling by the waterflow, can offer part-time habitat stability for mobile taxa such as *Navicula* spp. and *Diatoma* spp. Sand, in particular, offers a three dimensional niche but it is, on the other hand, a highly risky habitat because it exposes diatoms to abrasion risks as confirmed by the high variability of diatoms living in it. Sediments, on the other hand, offer a high amount of nutrients so they could be considered a good environment to dwell on but, on the other hand, they are really unstable and subject to tumbling caused by discharge. It follows that different substrates offer different species composition and that each species has its own “substrate-taste” since each has its own preferences. The scientific community did not have a common thought on this issue: there was still a lot of discordance since many researchers pushed the idea that “substrates lead to different results” (among others Cattaneo et al., 1997; Cox, 1998; Soininen & Heino, 2005; Cetin, 2008) while others thought “substrates lead to similar results” (among others Rott et al., 1998; Kitner & Poulickova, 2003; Cantonati & Spitale, 2009). In the Adige system, substrates have offered different diatom communities and each subject to the game of discharge. This effect has strong implications for water monitoring techniques and should be taken into strong consideration (see the following paragraph). It has been noticed in all the eight stations, that the winter samples collected in the lithic substrate, have higher abundances of CENPHY and PENCOL diatoms and this could point to a different niche availability of this habitat: these two typical colonial groups could be transported as tychoplankton from the water column or could be living as live cells in this habitat where, thanks to low discharge, light could be available for them in this time of the year. Temperature, being covariating with discharge, increases southward but does not seem to affect diatom populations directly: their effect could be mixed and difficult to disentangle. As for nutrients, apart for a general increase southward due to agriculture induced effects cumulating along the mainstream, they seem to be influencing the diatom community when the effect of discharge and its related variables gets weaker. Morpho-Functional Diatom Groups explicited in the first year study (Centis et al., 2010) have proven to be useful in studying the diatom communities of the watercourse and provided an effective synthesis that turned useful in gradients studies.

5.3 POSSIBLE IMPLICATIONS OF THE RESULTS ON THE BIOMONITORING TECHNIQUES AND BIOLOGICAL QUALITY METRICS. WHAT DID WE LEARN THROUGH THE ADIGE RIVER'S DIATOMS

To test the effect of the diatom gradient, a water quality assessment has been performed on the 40 km stretch (from station A to station H) by applying three diatom indices based on phytobenthos: the index EPI-D (which is the only diatom index that has been developed in an Italian watercourse, the River Chienti) , the index ICMi (the comprehensive metrics proposed for the implementation of the WFD) and the IBD index developed in France (for a detailed description of the indexes, see Paragraph 1.3) . These indices have been obtained from the results of the sampling date of June 2009, considered to be the best period for diatom growth and therefore the most appropriate time for water quality assessment (according to Mancini & Sollazzo, 2009).

There is a discrepancy between these three indexes, due to the fact that they have been written in Italian (EPI-D and ICMi) and French (IBD) languages and the water quality assessment do not fit the judgments imposed by the Water Framework Directive 2000/60/CE (Tab. 5.1).

WFD classes	**EPI-D classes**	**ICMi classes**	**IBD classes (calculated on /20)**
High	Ottima	Elevata	16-20 (class 5)
Good	Buona	Buona	12-16 (class 4)
Moderate	Mediocre	Sufficiente	8-12 (class 3)
Poor	Cattiva	Scarsa	4-8 (class 2)
Bad	Pessima	Cattiva	1-4 (class 1)

Tab. 5.1- Water quality classes imposed by the Water Framework Directive, EPI-D Index, ICM index and IBD index.

For this reason, water quality classes have been asserted in the original language in order to avoid confusion.

STATION	SUBSTRATE	Epi-D	ICMi	IBD
A (ADIGE BEFORE ISARCO) MUDDY STATION	**LIT**	1.48 (*Buona* quality)	0.60 (*Sufficiente* quality- A2)	3.5 (class 1)
	PEL	1.65 (*Buona* quality)	0.50 (*Scarsa* quality- A2)	4.5 (class 2)
	PSA	1.50 (*Buona* quality)	0.25 (*Cattiva* quality- A2)	2 (class 1)
B (ISARCO) **PEBBLISH STATION**	**LIT**	1.50 (*Buona* quality)	0.70 (*Buona* quality- A2)	15 (class 4)
	PEL	1.35 (*Buona* quality)	0.68 (*Buona* quality- A2)	14 (class 4)
	PSA	1.43 (*Buona* quality)	0.65 (*Buona* quality- A2)	13 (class 4)
C (ADIGE AFTER ISARCO) **MUDDY STATION**	**LIT**	1.63 (*Buona* quality)	0.61 (*Sufficient* quality- A2)	5 (class 2)
	PEL	1.83 (*Slightly altered* quality)	0.55 (*Sufficiente* quality- A2)	9.5 (class 3)
	PSA	1.80 (*Mediocre* quality)	0.26 (*Cattiva* quality- A2)	1.5 (class 1)
D (ADIGE BEFORE NOCE) **MUDDY STATION**	**LIT**	1.43 (*Buona* quality)	0.65 (*Sufficient* quality- A1)	13 (class 4)
	PEL	1.73 (*Mediocre* quality)	0.60 (*Sufficiente* quality- A1)	12.6 (class 4)
	PSA	1.70 (*Sufficient* quality)	0.30 (*Scarsa* quality- A1)	5.5 (class 2)
E (NOCE) **PEBBLISH STATION**	**LIT**	1.42 (*Buona* quality)	0.75 (*Buona* quality- A1)	15 (class 4)
	PEL	1.50 (*Buona* quality)	0.80 (*Buona* quality- A1)	14.5 (class 4)
	PSA	1.40 (*Buona* quality)	0.77 (*Buona* quality- A1)	14 (class 4)
F (ADIGE AFTER NOCE- BEFORE AVISIO) **MUDDY STATION**	**LIT**	1.50 (*Buona* quality)	0.60 (*Sufficiente* quality- A1)	17 (class 5)
	PEL	1.43 (*Buona* quality)	0.58 (*Sufficiente* quality- A1)	6 (class 2)
	PSA	1.85 (*Mediocre* quality)	0.27 (*Cattiva* quality- A1)	3.5 (class 1)
G (AVISIO) **PARTICULARLY MUDDY STATION**	**LIT**	2.2 (*Cattiva* quality)	0.30 (*Scarsa* quality- A1)	13 (class 4)
	PEL	1.73 (*Mediocre* quality)	0.25 (*Cattiva* quality- A1)	3 (class 1)
	PSA	2.2 (*Cattiva* quality)	0.25 (*Cattiva* quality- A1)	2.5 (class 1)
H (ADIGE AFTER AVISIO) **MUDDY STATION**	**LIT**	1.35 (*Buona* quality)	0.65 (*Sufficiente* quality- A1)	16.5 (class 5)
	PEL	1.73 (*Mediocre* quality)	0.63 (*Sufficiente* quality- A1)	17 (class 5)
	PSA	1.50 (*Buona* quality)	0.28 (*Scarsa* quality- A1)	4.5 (class 2)

Tab. 5.2- Results obtained from the application of the EPI-D and ICMi indeces, on the sampling date of June 2009. Next to each index value there is the corresponded assessment stated by the index . (LIT= lithon; PEL= pelon; PSA= psammon)

As from table 5.2, the results are rather different both among the substrates and between indices in almost each station. The three substrates (lithon, pelon and psammon) show different indices values: in station A, the lithic environment gives a good water quality while the pelic and the psammic ones show a lower quality. The same happens for all the other stations, except for staB and statE. This is in accordance with the hypothesis prompted by several studies that points at different diatom communities inhabiting different substrates (see previous paragraph). These data highlight that biomonitoring is a practice strictly substratum-dependent since diatoms have specific habitat preferences. Stations B and E, instead, have shown similar water quality assessment values between substrates: both the stations are characterized by a typical pebblish texture. Most of the indices point on their protocols to lithic substrate to be sampled but, in many cases, this substrate is not sufficiently (i.e. five pebbles/cobbles are needed to have a reliable sample) or not at all available, as in the case of many lowland rivers (it is also the case of Adige River, in its lowland areas). In those cases, either artificial substrates can be used or (as many Italian Environmental Agencies do) macrophytes or sediments can be sampled: but in this case, resulting diatom communities could exclude some diatom groups (e.g. PENLAR and PENSMA diatoms). The problem could be overcome by a specifically assessment of the operator on which substrate is sampled but this could therefore result in some biases due to this choice/ need. Most probably, it could be necessary to sample all the substrates available in one station (Rothfritz et al, 1997; Reavie et al, 2010) in order to have the highest ecological information.

Indices are based on Weighted Averaging, which is considered to be the most accurate method for quantifying species response to nutrients (Soininen & Niemelä, 2002). Therefore indices have led to several generalized assumptions and in many watercourses have shown different results (e.g. Leira & Sabater, 2005; Fejo et al., 2009) as in this study, where EPI-D and ICMi have shown similar results only in station B and E. These two sampling points have prevalent pebblish textures so again this could point to a higher “reliability” of the lithic substrate in representing an appropriate environment for water quality assessment. On the other hand, in the other stations, the two indices have shown particularly discordant results, leading to the assumption that ICMi is “more severe” in judging water quality, since it is always showing the “worst” results. The highest reliability in showing water quality assessments of this index could be due the embedding effect that this metric proposes: it is in fact the result of an the predictive powers of the indexes IPS and TI (see paragraph 1.3). Only in three stations (C, D and H) the pelic substrate has been overestimated by the ICMi. This result should be kept in mind in this particular moment which is devoted in finding metrics for the implementation of the WFD: the ICMi has been assessed by the GIG in order to effectively measure water quality in State members but it is based on older indices (IPS and TI) that are, in turn, biased by other intrinsic factors (e.g., see introduction).

Looking at within-station values, with the exception of station G, the lithic substrate has always shown “Good quality” with the EPI-D metrics: using the ICMi, instead, the quality is ranging between Good and Sufficient. This could be another evidence of the higher reliability of the lithic substrate but, in turn, would be biased by the fact that pedunculate diatoms (PENPED) are mostly inhabiting this substrate, on the opposite of PENLAR and PENSMA diatoms. Diatoms belonging to PENPED (especially *Encyonema minutum* and *Achnanthidium minutissimum*) have been given an important “sensibility value” in a lot of indices and this could bias the overall judgment of water quality. In the case of station G, which is particularly muddy, this relationship has not been detected but it could be due to the fact that I have found quite difficult to sample proper lithic substrates since many were covered by a veil of mud. The results showed by both the indices point at a very bad water quality in this station.

It is also evident that the psammic substrate is always showing very negative results (e.g. insufficient and scarce), apart from pebblish stations (B and E). This could be due to a contamination of the psammic substrate in the muddy station but could generally mean that this substrate is one of the most appropriate to sample. The different trend shown by pebblish station could again mean that they are more reliable to be sampled and surely that it is easier to discriminate beneath sediments, sand and pebbles in those stations than in the others. Epipsammon is a highly three dimensional habitat with a large surface area available for colonization due to the small size of sand grains: algae can attach everywhere in the sand grain and move around it while on the rocks they can only attach on light surfaces (Krejci & Lowe, 1986). In fact I have noticed a higher species diversity of diatoms in the sandy substrates but, on the other side, this could mean that this habitat is more unstable and therefore needs to be considered differently from the others.

Epipelon, on the other hand, is a very dynamic substrate that plays a role in the transport and accumulation of sediments and associated chemical pollutants (Poulicková et al., 2008) and, in streams, is the major contributor to the autochtonous carbon input and, more generally, in its nutrient release. It follows that this substrate cannot be considered as neutral (Yallop et al., 2009) and this is proved by the characteristics diatom communities found in it (e.g. mostly diatoms belonging to PENLAR group such as *Navicula tripunctata, N. lanceolata, Diatoma vulgaris, D. ehrenbergii, Nitzschia fonticola* and *N. palea*).

Paired-t tests have been used to detect differences in the three types of substrates, for each of the three indexes. Analyses showed that, while the EPI-D index did not show significant differences among substrates (Tab. 5.3a), ICMi and IBD showed significant, different values (Tab. 5.3b,c). In particular, these two indexes have shown significant differences between the lithic and the psammic substrates on one hand, and pelic and psammic substrates on the other hand.

(a)

Substrate1	Substrate2	*p* value	t test value	*p*
LITHON	PELON	0.59	-0.56	n.s.
LITHON	PSAMMON	0.07	-2.04	n.s.
PELON	PSAMMON	0.57	-0.58	n.s.
LITHON,	PELON	PSAMMON		
1.56±0.27	1.62±0.17	1.67±0.27		

(b)

Substrate1	Substrate2	*p* value	t test value	*p*
LITHON	PELON	0.06	2.19	n.s.
LITHON	PSAMMON	0.006	3.82	*p*<0.01
PELON	PSAMMON	0.007	3.73	*p*<0.01
LITHON,	PELON	PSAMMON		
0.61±0.13	0.57±0.16	0.38±0.21		

(c)

Substrate1	Substrate2	*p* value	t test value	*p*
LITHON	PELON	0.30	1.09	n.s.
LITHON	PSAMMON	0.008	3.58	*p*<0.01
PELON	PSAMMON	0.02	2.78	*p*<0.05
LITHON,	PELON	PSAMMON		
12.3±5.2	10.1±5.2	5.8±4.9		

Tab.5.3- Paired t-tests between EPI-D, ICMi and IBD diatom indexes, performed among the sampling substrates of each. The null hypothesis is that means of paired samples are equal. (a) values referred to EPI-D index; (b) values referred to ICMi; (c) values referred to IBD index. For every substrate, average values and standard deviations of the indexes have been reported at the bottom of each table.

From these results, it seems that some indexes are more conservative than others (e.g. EPI-D), showing less individual variation, while others are more substrate-discriminating. Paired t-tests confirmed that lithic and psammic communities on one hand and pelic and psammic ones on the other hand, both using ICMi and IBD indexes, proved to be significantly different. This bias effect can have strong implications for the effective validity of these metrics without a proper sampling substrate assessment. The majority of diatom indexes, in fact, are applied without proper indication of where to sample (i.e. sampling need to be performed according to the available substrate in the sampling station) and, with this study, we have proven that diatom communities are highly influenced by spatial constraints, in particular if lithic and pelic substrates are compared with the psammic ones.

Therefore, it seems to be evident that substrate specificity is a major issue to be carefully considered especially when assessing water quality, since diatoms do have specific habitat preferences and this, in turn, can influence water monitoring. On the other hand, though, diatom metrics are very useful for water quality assessment, since chemical analysis show only part of the reality (Leira & Sabater, 2005) but still a few problems have to be fixed in order to gain the most using them. In my opinion, a few aspects in the use of diatoms indices should be better evaluated in future invetigations:

- ✓ Some species share the same “sensibility coefficient” : *A. biasolettianum* and *A. minutissimum* (very common species) have the same. In this way, while on one hand a highly skilled operator is not required, we lose a lot of useful ecological data;
- ✓ Many planktonic species are neglected by diatom indices therefore a lot of ecological information is lost especially if excluding certain taxa (e.g. *Cyclotella radiosa*);
- ✓ The metrics are the same for reservoirs, lakes, streams and rivers and this is surely a generalization since these waterbodies have, to many extents, very different features;
- ✓ For certain morphospecies and varieties (e.g. *Cocconeis placentula*) the operators must possess very high skills and this could not always be the case;
- ✓ No updates of the indices are promptly available for the “weights” of the species;
- ✓ The metrics are the same for all the substrates and, as shown below, this is quite a generalisation;
- ✓ Diatom taxonomy is highly evolving and the indices do not always account for that;
- ✓ Most indices do not take in account many frequent taxa such as *Cymbella excisa, Gomphonema innocens, Encyonema ventricosum* and *Achnantidium atomoides*;
- ✓ Indices do not take in account the different seasonality (there should be a difference regarding reference sites in spring when the watercourse is in high flow regime and in fall when the water level is low);
- ✓ Indices do not account for teratological form which points at high pollution levels (Falasco et al, 2009);

- ✓ The majority of indices overweight the allochtonous species such as *Didymosphenia geminata*;
- ✓ Indices do not particularly consider areas which are highly impacted by agriculture, such as lowland basin areas. In my opinion these areas should be carefully treated and assessed separately from natural conditions areas;
- ✓ Indices do not account for morphological pressures: mountain streams surely have less species than valley rivers;
- ✓ Indices do not take into consideration biogeographical differences such as the *A. minutissimum* complex and the species splits;
- ✓ Indices do not consider geological differences beneath the watercourse;
- ✓ Indices do not account for synonyms problems and they could be a source of bias in cases of not specialized operators. Updates should be regularly provided with autoecological studies which should be the starting points for the implementation of diatom indices;
- ✓ Indices sharply discriminate between five quality classes and this process does not take into consideration the consequent “plateau effect” that could bias the process. This effect is unavoidable when sharp cutting is operated;
- ✓ Indices do not particularly weight the not abundant species and neither very abundant species (such as *Achnantidium* spp.) that could be re-weighted.

These problems could be overcome by applying diatom indices bearing in mind that they necessarily have been built without a complete knowledge of the geographical and environmental processes ruling diatom distribution (Potapova & Charles, 2002) which are, in turn, influenced by more localized patterns, as the substrate to be sampled. From Adige River’s case study, environmental and autoecological variables co-work in influencing diatom communities since each substrate has its own preferential diatoms (e.g. PENLAR mostly living in the epipelon and epipsammon while PENPED in the epilithon). The choice of the index to be applied for having a proper water quality assessment should be made considering that spatial variables (e.g. substrates and watershed width) are as important as environmental variables (Potapova & Charles, 2002) for water quality assessment.

5.4. HINTS TOWARDS AN INTEGRATION OF PHYTOPLANKTON AND WATER QUALITY DATA

The Water Framework Directive (2000/60/CE) points at phytoplankton's biomass and taxonomic composition as an important component to be monitored. Several studies have addressed the topic and its outcome in numeric indices: Thunmark (1945) separated oligotrophic and eutrophic waters on the basis of a ratio between Chlorococcocales and Desmidiales. From then onwards, other studies have proposed metrics (Nygaard, 1956; Stockner, 1971) and algal quotients (Catalan, 2003). Rawson (1956) and Kummerlin (1990) have proposed indicator species (mostly diatoms) for trophic states while Reynolds (1984-2002) and Padisák (2003) have proposed characteristics species associations of water habitats. Quantitative indices have been proposed (Hörnström, 1981; Brettum, 1989) and this work is still ongoing thanks some projects (e.g. The Wiser European Project). A lot of this attention, though, has been devoted to lakes since the Directive itself does not directly consider phytoplankton in rivers: this is not surprising considering that the streams owing to the predominance of allochtonous material over autochthonous primary production are naturally heterotrophic systems (Reynolds, 2000). So only a few attempts have been made in this direction, including those by Mischke (Mischke, 2007; Mischke & Behrendt, 2006; Mischke & Behrendt, in prep.) named Phytofluss and by Borics et al (2007).

Both these indices use algal biomass to assess the water quality by means of phytoplankton: only a preliminary version of the Phytofluss needed diatom slides to be done but, since it was a time consuming process mainly done for a few centric species, the authors opted for metrics obtained from the Utermöhl technique (Mischke, pers. comm.). The Phytofluss index has been designed for natural rivers having a real potamoplanktonic community and it is not useful for monitoring heterothrophic rivers where planktonic biomass is missing (Mischke & Behrendt, in prep.). The index does neither apply to heavily modified water bodies. In fact, following the definition of Dodds (2007), eutrophication is the increase in factors that move a river system towards an eutrophic state: so, besides nutrients, also an elongation of the retention time can cause an eutrophication and thus, the hydro-morphological degradation of rivers is not completely separated from its effects by nutrients (Mischke & Behrendt, in prep.). Taking this hydro-morphological effect into account, Phytofluss suggests to assess small and steep rivers, which normally are not plankton sensitive and whose chlorophyll a concentrations surpass 30 µg/L seasonal mean (Mischke & Behrendt, in prep.).

The index proposed by Borics and colleagues (2007) instead, uses the phytoplankton functional groups proposed by Reynolds et al. (2002) and Padisák (2003) in lakes. Lakes and rivers do not fully share the same planktonic communities: rather it seems that these habitats have very specialized algae, especially according to diatoms (Kalff, 2002). For each group, the authors have assigned a value given by the sum of trophic state, turbulence, residence time and a risk factor which ends up in a factor number (F) that indicates the occurrence of this functional group in the riverine phytoplankton and competes for assessing the final index value. Also this index, similarly to Phytofluss, includes Pennales diatom species which are the main constituents of the tycho- and meroplankton to be drifted in the water column (among these, the most frequent taxa are *Navicula* spp., *Diatoma* spp., *Achnanthes* spp. and *Encyonema* spp.). The difference between these two indices, is the definition of pressure scale to be assessed: while the Phytofluss index is calibrated for increasing eutrophication with chlorophyll *a* and total phosphorus (e.g. it shows a very good

correlation with trophic parameters), the Borics et al. index is based on expert functional groups assessment according to different habitats in the river types (in fact, it negatively correlates with chlorophyll *a* and total phosphorus) (Mischke & Behrendt, in prep.) .
It follows that these two phytoplanktonic indices, even though they represent a huge step further in river ecology, they also bear a few "weaknesses" and biases summarized in Tab. 5.4.

PHYTOFLUSS INDEX- (MISCHKE & BEHRENDT, IN PREP.)	**Q INDEX (BORICS ET AL, 2007)**
Calibrated on **increasing eutrophication** along the watercourse	Calibrated on **lakes** functional groups
Local diatom taxa list	Local diatom taxa list
Not useful for heterotrophic rivers	Not useful for heterotrophic rivers
Not useful for highly modified watercourses	Not useful for highly modified watercourses
Does not take in account "Dead Zones" (Reynolds et al, 1991; Reynolds, 2000)	Does not take in account "Dead Zones" (Reynolds et al, 1991; Reynolds, 2000)
Does not account for the most abundant centric species found in rivers (*Stephanodiscus hantzschii*)	Those species that were not mentioned in the Reynold's system (Reynolds et al, 2002) were sorted into groups by expert judgment)

Tab. 5.4- "Weaknesses" of the Phytofluss and Q index

In the case of Adige River, neither of these two indices could be applied with my data: slides have been prepared for each sample in order to get the highest taxonomic resolution so biomass measures have not been taken. Adige River's course is highly modified so phytoplankton does not have sufficient time to accomplish its reproduction along the water column (Salmaso & Zignin, 2010): it is still debatable whether a plankton community can exist in this highly modified watercourses or it is merely the resultant of euplankton and tychoplankton communities. The peculiar hydrological regime of the Adige River strongly regulates its algal community, as shown in this work, and this is the reason why a lot of tycho- and meroplanktonic diatoms have been found in the potamoplankton (Centis et al., 2010). This effect is strongly dependent upon extrinsic factors such as snow melting and air temperatures. On the basis of my observations, in the Adige River, a phytoplanktonic index should take into consideration the reliability of a river station to be "a good phytoplankton bearer": in fact, on the basis of a station's hydrological regime and nutrient characteristics we can judge its consistency.

A phytoplanktonic index should regard these characteristics: it could be meaningless to apply it in the upstream stations since the result would be influenced by a high allochtonous input while it could lead to more meaningful results in the downstream sampling point which bears a true potamoplankton in its waters. A morpho-functional approach seems to be very helpful in this context: the MFDGs discriminate beneath "real" potamoplankton (CENUNI; CENPHY; PENCOL) and mero and tychoplankton (PENPED; PENLAR;

PENSMA) and they could be used in a further implementation and redefinition of a planktonic index concept, therefore continuing with the approach proposed by Borics et al. (2007). This is a very important result since the functional role of species is not yet defined (Harris, 1984) and could constitute an advancement in ecological sciences.

6. CONCLUSIONS

From this study, it seems that spatial patterns and environmental constraints have a coupled effect in driving the diatom communities of the Adige River. Environmental constraints, in particular hydrology related factors, are the main constraining variables in particular in the water column while spatial patterns intervene in a joint action in the benthic communities. Macrohabitat is therefore the main driver in the phytoplanktonic community while microhabitat joins in the phytobenthic community. This process is particularly important in Alpine water systems, such as the Adige River, which is highly subjected to water fluxes. This has strong implications for water quality assessment: diatom indices seem to account for high generalizations and many biases. On the opposite, phytoplanktonic water quality assessment in rivers is still in its infancy and should be developed accounting for the morphological and hydrological patterns of the watercourses.

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APPENDIX I
Diatom species found in the samples

GENRE	SPECIES	SUBS/VARIETY	AUTHOR
Achnanthidium	*biasolettianum*		Grunow Round & Bukhtiyarova 1996
Achnantes	*bioretii*		Germain 1957
Achnantes	*helvetica*		(Hustedt) Lange-Bertalot 1989
Achnantes	*laevis*		Østrup 1910
Planothidium	*lanceolatum*		(Brébisson ex Kützing) Lange-Bertalot 1999
Achnanthidium	*minutissimum*		(Kützing) Czarnecki 1994
Psammothidium	*subatomoides*		(Hustedt) Bukhtiyarova & Round 1996
Amphora	*lybica*		Ehrenberg 1840
Amphora	*pediculus*		(Kützing) Grunow ex A.Schmidt 1875
Aneumastus	*tuscula*		(Ehrenberg) D.G.Mann & A.J.Stickle in Round et al. 1990
Asterionella	*formosa*		Hassall 1850
Aulacoseira	*distans*		(Ehrenberg) Simonsen 1979
Aulacoseira	*granulata*		(Ehrenberg) Simonsen 1979
Aulacoseira	*italica*		(Ehrenberg) Simonsen 1979
Caloneis	*amphisbaena*		(Bory de Saint Vincent) Cleve 1894
Cocconeis	*pediculus*		Ehrenberg 1838
Cocconeis	*placentula*	*euglypta*	(Ehrenberg) Grunow 1884
Cocconeis	*placentula*	*lineata*	(Ehrenberg) van Heurck 1885
Cocconeis	*placentula*	*placentula*	Ehrenberg 1841
Cocconeis	*placentula*	*pseudolineata*	Geitler 1927
Craticula	*ambigua*		(Ehrenberg) Mann 1990 in Round et al
Craticula	*cuspidata*		(Kützing) Mann ex Round et al. (1990)
Cyclostephanos	*dubius*		(Hustedt) Round in Theriot *et al.* 1987
Cyclotella	*atomus*		Hustedt 1937
Cyclotella	*bodanica*		Eulenstein in Grunow 1878
Cyclotella	*ciclopuncta*		Håkansson & Carter 1990
Cyclotella	*distinguenda*		Hustedt 1928
Cyclotella	*meneghiniana*		Kützing 1844
Cycliotella	*ocellata*		Pantocsek 1901
Cyclotella	*planktonica*		Brunnth
Cyclotella	*radiosa*		(Grunow in van Heurck) Lemmermann 1900
Cyclotella	*wuetrichiana*		Pantocsek 1901
Cyclotella	*stelligera*		Cleve & Grunow 1882
Cymatopleura	*solea*		(Brébisson) W.Smith 1851
Cymbella	*affinis*		Kützing 1844
Cymbella	*compacta*		Østrup 1910
Cymbella	*excisa*		Kützing 1844
Cymbella	*helvetica*		Kützing 1844
Cymbella	*lanceolata*		(Ehrenberg) Kirchner (1978)
Cymbella	*sinuata*		W.Gregory 1856
Cymbella	*prostrata*		(Berkeley) Cleve, 1894
Denticula	*tenuis*		Kützing 1844
Diadesmis	*contenta*		(Grunow in van Heurck) Mann ex Round et al 1990
Diatoma	*ehrenbergii*		Kützing 1844

Diatoma	*hyemalis*		(Roth) Heiberg 1863
Diatoma	*mesodon*		(Ehrenberg) Kützing 1844
Diatoma	*moniliformis*		Kützing 1833
Diatoma	*tenuis*		Agardh 1812
Diatoma	*vulgaris*		Bory, 1824
Didymosphenia	*geminata*		(Lyngbye) M.Schmidt in A. Schmidt 1899
Encyonema	*brevicapitatum*		Krammer
Encyonema	*latissimum*		Krammer
Encyonema	*minutum*		(Hilse) Mann in Round, Crawford & Mann 1990
Encyonema	*reichardtii*		(Krammer) Mann 1990
Encyonema	*silesiacum*		(Bleisch in Rabenhorst) Mann(1990)
Encyonema	*ventricosum*		(C.Agardh) Grunow
Eunotia	*arcus*		Ehrenberg
Fragilaria	*arcus*		(Ehrenberg) Cleve 1898
Fragilaria	*capucina*	*austriaca*	(Grunow) Lange-Bertalot 1991
Fragilaria	*capucina*	*capitellata*	(Grunow) Lange-Bertalot 1991
Fragilaria	*capucina*	*capucina*	Desmazières 1825
Fragilaria	*capucina*	*gracilis*	(Oestrup) Hustedt
Fragilaria	*capucina*	*perminuta*	(Grunow) L-B. 1991
Fragilaria	*capucina*	*rumpens*	(Kützing) Lange-Bertalot ex Bukhtiyarova 1995
Fragilaria	*capucina*	*vaucheriae*	(Kützing) Lange-Bertalot 1980
Fragilaria	*capucina*		Desmazières 1825
Fragilaria	*construens*		(Ehrenberg) Grunow 1862
Fragilaria	*crotonensis*		Kitton 1869
Fragilaria	*tenera*		(W.Smith) Lange-Bertalot
Fragilaria	*ulna*		(Nitzsch) Lange-Bertalot 1980
Frustulia	*rhomboides*		(Ehrenberg) De Toni (1891)
Frustulia	*vulgaris*		(Thwaites) De Toni (1891)
Geissleria	*decussis*		(Østrup) Lange-Bertalot & Metzeltin 1996
Gomphonema	*affine*		Kützing
Gomphonema	*angustatum*		(Kützing) Rabenhorst 1864
Gomphonema	*gracile*		Ehrenberg 1838
Gomphonema	*innocens*		Reichardt
Gomphonema	*micropus*		Kützing 1844
Gomphonema	*minutum*		(C.Agardh) C.Agardh 1831
Gomphonema	*olivaceum*		(Lyngbye) Desmazières 1825
Gomphonema	*parvulum*		(Kützing) H.F.Van Heurck 1880
Gomphonema	*pseudoaugur*		Lange-Bertalot
Gomphonema	*pumilum*		(Grunov) Reichardt & Lange-Bertalot 1991
Gomphonema	*truncatum*		Ehrenberg 1832: 88
Gyrosigma	*acuminatum*		(Kützing) Rabenhorst 1864
Gyrosigma	*attenuatum*		(Kützing) Cleve 1894
Hippodonta	*capitata*		(Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski 1996
Luticola	*mutica*		(Kützing) Mann in Round et al. 1990
Melosira	*varians*		Agardh 1827
Meridion	*circulare*		(Greville) C.Agardh 1831
Navicula	*antonii*		Lange-Bertalot
Navicula	*capitatoradiata*		Germain 1981
Navicula	*cincta*		(Ehrenberg) Ralfs in Pritchard(1861)
Navicula	*cryptocephala*		Kützing
Navicula	*cryptotenella*		Lange-Bertalot
Navicula	*gracilis*		Ehrenberg, 1838
Navicula	*gregaria*		Donkin

Navicula	*lanceolata*	(C. Agardh) Kützing
Navicula	*menisculus*	Schumann 1867
Navicula	*oligotrophenta*	Lange-Bertalot & Hofmann in Lange-Bertalot 1993
Navicula	*placentula*	(Ehrenberg) Kützing 1844
Navicula	*phyllepta*	Kützing
Navicula	*radiosa*	Kützing(1844)
Navicula	*recens*	Lange-Bertalot
Navicula	*reichardtiana*	Lange-Bertalot
Navicula	*reichardtii*	(Grunow) O. Kuntze
Navicula	*reinhardtii*	Grunow in Cleve & J.D.Möller
Navicula	*sp*	Bory de Saint-Vincent, 1822
Navicula	*subhamulata*	Grunow ex Van Heurck 1880
Navicula	*tripunctata*	(Müller) Bory de Saint-Vincent, 1822
Navicula	*trivialis*	Lange-Bertalot 1991
Navicula	*veneta*	Kützing (1844)
Navicula	*viridula*	Ehrenberg
Neidium	*sp*	Pfitzer
Nitschia	*acicularis*	(Kützing) Smith 1853
Nitzschia	*angustata*	(Smith) Grunow in Cleve & Grunow 1880
Nitzschia	*agnita*	Hustedt
Nitzschia	*bacillum*	Hassall, 1845
Nitzschia	*dissipata*	(Kützing) Grunow 1862
Nitzschia	*capitata*	(Smith) Peragallo 1903
Nitzschia	*filiformis*	(Smith) Van Heurck 1896
Nitschia	*fonticola*	(Grunow)
Nitzschia	*frustulum*	(Kützing) Grunov in Cleve & Grunov 1880
Nitzschia	*gracilis*	Hantzsch 1860
Nitzschia	*heufleriana*	Grunow 1862
Nitzschia	*incospicua*	Grunow 1862
Nitzschia	*intermedia*	Hantzsch
Nitschia	*linearis*	Smith 1853
Nitzschia	*microcephala*	Grunow
Nitzschia	*palea*	(Kützing) Smith 1856
Nitzschia	*paleacea*	(Grunow) Grunow in Van Heurck 1881
Nitzschia	*pura*	Hustedt 1954
Nitschia	*recta*	Hantzsch in Rabenhorst 1861-1879
Nitzschia	*sigmoidea*	(Nitzsch) Smith, 1853
Nitzschia	*tubicula*	Grunow in Cleve e Grunow 1880
Nitzschia	*wuellerstorfii*	Lange-Bertalot 1987
Pinnularia	*borealis*	Ehrenberg 1843
Rhoicospenia	*abbreviata*	(Kützing) Grunow 1860
Sellaphora	*pupula*	(Kützing) Mereschkowsy 1902
Stauroneis	*smithii*	Grunow 1860
Stephanodiscus	*hantzschii*	Grunow in Cleve e Grunow 1880
Stephanodiscus	*minutulus*	(Kützing) Cleve & Moller 1878
Stephanodiscus	*parvus*	Stoermer & Håkansson 1984
Stephanodiscus	*sp*	Ehrenberg 1846
Surirella	*angustata*	Kuetzing 1844
Surirella	*brebissoni*	Krammer & Lange-Bertalot 1987
Surirella	*minuta*	Brebisson in Kützing 1849
Tabellaria	*flocculosa*	(Roth) Kützing
Thalassiosira	*pseudonana*	Hasle & Hemdal 1970
Thalassiosira	*weissflogii*	(Grunow) Fryxell & Hasle 1977

APPENDIX II

Correlation matrix of environmental variables in the potamoplanktonic samples

Station1

	DISCHARGE (m3/sec)	**TURBIDITY (NTU)**	**DRY WEIGHT (mg/L)**	**TEMPERATURE (℃)**	**OXI WINKLER (mg/L)**	**pH**	**CONDUCTIVITY (uS/cm)**	**DIN (mg/L)**	**SRP (ug/L)**	**TP (ug/L)**	**Si(mg/L)**
DISCHARGE (m3/sec)	1.000										
TURBIDITY (NTU)	0.464*	1.000									
DRY WEIGHT (mg/L)	0.614	0.473	1.000								
TEMPERATURE (℃)	0.663**	0.432	0.365	1.000							
OXI WINKLER (mg/L)	-0.775	-0.505	-0.364	-0.853	1.000						
pH	-0.275	0.027	-0.003	-0.131	0.394	1.000					
CONDUCTIVITY (uS/cm)	-0.697	-0.389	-0.440	-0.858	0.806	0.358	1.000				
DIN (mg/L)	-0.593	-0.264	-0.385	-0.765	0.533	0.086	0.792	1.000			
SRP (ug/L)	-0.336	-0.010	-0.196	-0.462	0.411	0.333	0.441	0.294	1.000		
T_P (ug/L)	0.255	0.237	0.407	-0.031	-0.053	0.002	-0.185	-0.124	0.550	1.000	
Si (mg/L)	-0.328	-0.360	-0.333	-0.645	0.445	-0.126	0.599	0.545	0.283	-0.024	1.000
ChlA (ug/L)	-0.373	-0.269	-0.152	-0.238	0.273	0.070	0.100	0.146		0.035	-0.259

Station2

	DISCHARGE (m3/sec)	**TURBIDITY (NTU)**	**DRY WEIGHT (mg/L)**	**TEMPERATURE (℃)**	**OXI WINKLER (mg/L)**	**pH**	**CONDUCTIVITY (uS/cm)**	**DIN (mg/L)**	**SRP (ug/L)**	**TP (ug/L)**	**Si(mg/L)**
DISCHARGE (m3/sec)	1.000										
TURBIDITY (NTU)	0.164	1.000									
DRY WEIGHT (mg/L)	0.532	0.788	1.000								
TEMPERATURE (℃)	0.817	0.329	0.455	1.000							
OXI WINKLER (mg/L)	-0.797	-0.396	-0.534	-0.835	1.000						
pH	-0.095	-0.052	-0.195	0.246	0.160	1.000					
CONDUCTIVITY (uS/cm)	-0.885	-0.195	-0.415	-0.888	0.777	-0.157	1.000				
DIN (mg/L)	-0.730	-0.282	-0.383	-0.799	0.664	-0.186	0.900	1.000			
SRP (ug/L)	-0.580	0.042	-0.112	-0.613	0.563	-0.204	0.689	0.685	1.000		
T_P (ug/L)	-0.106	-0.019	0.168	-0.293	0.282	-0.395	0.282	0.404	0.689	1.000	
Si (mg/L)	-0.565	-0.374	-0.409	-0.756	0.609	-0.373	0.751	0.867	0.684	-0.024	1.000
CHLOROPHYLL (ug/L)	-0.612	-0.304	-0.391	-0.685	0.649	-0.069	0.598	0.540	0	0.035	-0.259

Station3

	DISCHARGE (m3/sec)	**TURBIDITY (NTU)**	**DRYWEIGHT (mg/L)**	**TEMPERATURE (℃)**	**OXY WINKLER (mg/L)**	**pH**	**CONDUCTIVITY (uS/cm)**	**DIN (mg/L)**	**SRP (ug/L)**	**TP (ug/L)**	**Si(mg/L)**
DISCHARGE (m3/sec)	1.000										
TURBIDITY (NTU)	0.723	1.000									
DRYWEIGHT (mg/L)	0.464	0.738	1.000								
TEMPERATURE (℃)	0.793	0.650	0.326	1.000							
OXY WINKLER (mg/L)	-0.730	-0.667	-0.351	-0.922	1.000						
pH	-0.454	-0.482	-0.290	-0.152	0.354	1.000					
CONDUCTIVITY (uS/cm)	-0.872	-0.645	-0.368	-0.901	0.809	0.320	1.000				
DIN (mg/L)	-0.753	-0.504	-0.255	-0.824	0.674	0.098	0.925	1.000			
SRP (ug/L)	-0.429	-0.172	-0.002	-0.413	0.272	-0.105	0.534	0.634	1.000		
T_P (ug/L)	0.244	0.512	0.254	0.246	-0.334	-0.335	-0.138	0.081	0.545	1.000	
Si (mg/L)	-0.506	-0.360	-0.164	-0.713	0.536	-0.191	0.729	0.826	0.368	-0.084	1.000
Chla (ug/L)	-0.149	0.010	-0.067	0.040	-0.021	0.475	0.111	0.108	0.218	0.45	-0.234

Station4

	TURBIDITY (NTU)	**DRYWEIGHT (mg/L)**	**TEMPERATURE (℃)**	**OXY WINKLER (mg/L)**	**pH**	**CONDUCTIVITY (uS/cm)**	**DIN (mg/L)**	**P_PO4 (ug/L)**	**T_P (ug/L)**	**Si(mg/L)**
TURBIDITY (NTU)	1.000									
DRYWEIGHT (mg/L)	0.715	1.000								
TEMPERATURE (℃)	0.536	0.314	1.000							
OXY WINKLER (mg/L)	-0.713	-0.597	-0.750	1.000						
pH	-0.601	-0.625	0.096	0.535	1.000					
CONDUCTIVITY (uS/cm)	-0.728	-0.516	-0.797	0.701	0.133	1.000				
DIN (mg/L)	-0.465	-0.278	-0.790	0.445	-0.254	0.885	1.000			
P_PO4 (ug/L)	-0.352	-0.048	-0.406	0.084	-0.274	0.555	0.692	1.000		
T_P (ug/L)	0.497	0.298	0.367	-0.350	-0.271	-0.302	-0.293	-0.249	1.000	
Si (mg/L)	0.073	0.172	-0.408	-0.082	-0.577	0.243	0.554	0.500	-0.519	1.000
Chla (ug/L)	-0.021	0.267	-0.187	0.044	-0.210	0.154	0.084	0.069	0.508	-0.237

Station5

	DISCHARGE (m3/sec)	**TURBIDITY (NTU)**	**DRYWEIGHT (mg/L)**	**TEMPERATURE (℃)**	**OXY WINKLER (mg/L)**	**pH**	**CONDUCTIVITY (uS/cm)**	**DIN (mg/L)**	**P_PO4 (ug/L)**	**TP (ug/L)**	**Si(mg/L)**
DISCHARGE (m3/sec)	1.000										
TURBIDITY (NTU)	0.693	1.000									
DRYWEIGHT (mg/L)	0.175	0.285	1.000								
TEMPERATURE (℃)	0.206	0.569	0.442	1.000							
OXY WINKLER (mg/L)	-0.315	-0.602	-0.368	-0.932	1.000						
pH	-0.458	-0.492	-0.182	-0.391	0.335	1.000					
CONDUCTIVITY (uS/cm)	-0.478	-0.624	-0.328	-0.818	0.766	0.495	1.000				
DIN (mg/L)	-0.159	-0.429	-0.273	-0.805	0.720	0.164	0.877	1.000			
P_PO4 (ug/L)	-0.257	-0.460	-0.075	-0.624	0.529	0.063	0.792	0.874	1.000		
T_P (ug/L)	0.130	0.580	0.295	0.364	-0.298	-0.354	-0.152	-0.081	-0.008	1.000	
Si (mg/L)	0.387	0.006	0.047	-0.476	0.392	-0.066	0.364	0.640	0.455	-0.236	1.000
Chla (ug/L)	-0.369	-0.159	-0.117	0.011	0.062	0.360	0.239	-0.053	0.109	0.039	-0.328

APPENDIX III

Paper published in 2010 in the journal “Hydrobiologia”

Structure of the diatom community of the River Adige (North-Eastern Italy) along an hydrological gradient.

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Abstract

Physical constrains such as water discharge, suspended solids and turbidity act as dominant factors in driving the planktonic diatom assemblages of the River Adige (North-Eastern Italy). Two sampling stations, characterised by different hydromorphological features (Cortina all’Adige and Boara Pisani, with torrential and more potamal characteristics, respectively) were sampled fortnightly following an integrated approach encompassing physical, chemical and biological measurements and aiming at identifying the dominant factors controlling the temporal development of the community. A morpho-functional approach was used to classify the diatom assemblages where Morpho-Functional Diatom Groups (MFDG) were defined for diatom genera, according to their morphology, habitat selection and modality of adhesion to river substrate. In the two sampling points, the algal growth was never limited by nutrients or zooplankton. The irregular development of MFDG was determined by the stochastic hydrological events and changes in variables related to water discharge (suspended solids and light attenuation). Tychoplanktonic, benthic and drifted taxa (such as *Diatoma* spp., *Encyonema* spp., *Navicula* spp. and *Nitzschia* spp.) were dominant in the torrential station (Cortina all’Adige), while the contribution of euplanktonic unicellular centric taxa (such as *Cyclotella* spp., and *Stephanodiscus* spp.) was higher in the potamal station (Boara Pisani).

Key words: diatoms, River Adige, physical forcing, Morpho-Functional Diatom Groups

This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

Like every other potamoplanktonic organism, diatom dynamics in rivers are regulated by hydrological (e.g. water discharge, residence time, turbulence), physical (e.g. water temperature, turbidity), chemical (e.g. mineral content/conductivity, nutrient concentrations), and biological (grazing, competition) factors (e.g. Reynolds & Glaister, 1993; Basu & Pick, 1996, 1997) however, as environmental drivers co-act simultaneously, it is not easy to discriminate which has the most important impact on the river community. Some researchers have concluded that, due to the observed highly significant positive relationship between river phytoplankton abundance and total phosphorus concentration (e.g. Basu & Pick, 1996; Van Nieuwenhuyse & Jones, 1996; Borics et al., 2007), potamoplankton is regulated by nutrient concentrations while other studies indicated hydrology related factors as having greater importance to phytoplankton development in rivers (e.g., Reynolds et al., 1994; Pace et al., 1992). Nevertheless, such hydrological and physical constrains may well be overriding at certain times of the year, which include episodes of high discharge, when plankton is quickly transported seawards, embedded in a medium rendered so turbid by entrained fine particulate matter that net population increase is impossible owing to light deprivation (Reynolds, 2000). It is therefore little wonder that the relatively few types of planktonic organisms that are successful in rivers, as small centric diatoms, are characterised by clearly r-selective properties (i.e. high exploitative ability and opportunistic development; Reynolds, 2000). Ruse & Love (1997) have found a steady and prolonged decline of pennatae diatoms with increasing discharge in the River Thames and a complete unimodal response to discharge for the centric filamentous diatom *Melosira varians*. Laboratory experiments with glass substrata also showed that small cells were dominant at all current velocities and no large taxa were dominant at any current velocity (Wendker, 1992). Experiments performed by Bormans & Condie (1998) indicated that physical and hydrological factors play a key role in the riverine ecosystem and that a proper knowledge of them is crucial for both sampling design and the interpretation of recorded algal densities.

The objective of this contribution is to assess the influence of the main physical and chemical factors on the planktonic diatom community at two stations of the River Adige (North-Eastern Italy), which are characterised by different hydromorphology and seasonal variability. Temporal changes in diatom species composition and relative abundances were assessed by applying multivariate statistical analyses on diatom groups defined on the basis of their morphological and functional characteristics. In fact, classifications based on functional and ecological characters have proven to be a powerful tool in community analysis in respect to previously applied taxonomic grouping (e.g., Reynolds et al., 2002; Salmaso & Padisák, 2007; Padisák et al., 2009).

River Adige is the second longest river in Italy (409 km). Its spring is located in the Eastern Alps, at 1550 m a.s.l. and the mouth is on the Adriatic Sea. More than a half of the catchment area (12.100 km^2) is located in mountainous regions. The northern sampling station is Cortina all' Adige (further on Station 1) which is placed 128 km from the spring. It has torrential characteristics and the height of waters generally ranges values between 0.5 m and 3 m. The southern sampling station, Boara Pisani (Station 2, located 59 km to the mouth), has more potamal characteristics, with a water height generally ranging between 4.5 and 7 m..

Sampling was carried out in both stations every fifteen days, twice a month, from March 2007 to February 2008. The water samples were collected from bridges, at midstream, using a bucket. Temperature was measured immediately after the sample withdrawal, like conductivity and pH. Chlorophyll-*a* concentration was determined spectrophotometrically on acetone extracts. Other determinations in laboratory included water turbidity (NTU, Nephelometric Turbidity Units), dry weight (suspended solids) and nutrients (SRP, Soluble Reactive Phosphorus; TP, Total Phosphorus; DIN, Dissolved Inorganic Nitrogen, NO_3-N+NO_2-N+NH_4-N; Si, Soluble Reactive Silica; A.P.H.A. et al, 1995). Discharges (D_{3d}) were calculated as the average values recorded during the 72 hours before the sampling operations. Further details on field sampling, data collection and analytical procedures are reported in Salmaso & Zignin (this volume).

Water samples for diatom analyses were concentrated by sedimentation (1:50) and then cleaned in 30% hydrogen peroxide and 37% hydrochloric acid (Kelly et al., 1998). Cleaned diatom frustules were permanently mounted in Naphrax® resin. On each slide 400 valves were counted (European Committee for Standardization, 2004) under a light microscope at 1000 magnification. Taxa were identified following the more recent monographs of the series Süßwasserflora von Mitteleuropa, established by A. Pascher (Gustav Fisher Verlag, and Elsevier, Spectrum Akademischer Verlag) and the most updated literature.

The ordination of diatom relative abundances was carried out by Non Metric Dimensional Scaling (NMDS) (Kruskal & Wish, 1978) applied to Bray and Curtis dissimilarity indices (Podani, 2000) computed on species percentages, after an arc-sinus transformation to reduce the weight of the most abundant taxa. The same normalisation procedure was performed also for Principal Components Analysis (PCA) on relative abundances of Morpho-Functional Diatom Groups. Statistical analyses were carried out with SYSTAT™ 10.2 and CANOCO™ 4.5 packages.

Seasonal diatom variations were analysed considering Morpho- Functional Diatom Groups (MFDG). The criteria adopted to discriminate the groups include morphology and, partly, habitat selection and modality of adhesion to river substrate (Fig.1). The first division separates the two Bacillariphyceae orders (Centrales and Pennales). Within Centrales, the unicellular euplanktonic diatoms (CENUNI) are separated from the filamentous diatoms that can be planktonic or benthic, unattached to any substratum (namely the CENPHY) (Barber & Hawart, 1981). A similar splitting has been applied to the Pennales: PENPED comprehends taxa being attached with stalks better adapted to high current velocities and displaying tychoplanktonic status (Sabater, 2009). PENCOL encompasses colonial diatom taxa. Further subdivisions were based on size ending in two heterogeneous groups mostly benthic or tychoplanktonic (Barber & Hawarth, 1981) (PENLAR and PENSMA).

Water discharge and turbidity in the two stations from March 2007 to February 2008 are reported in Fig. 2. In the northern station, monthly discharge values ranged between around 50 and 200 m^3 sec^{-1}. Water turbidity varied between 4 and 34 NTU, with the exception of the peak occurred in early August of 318 NTU (Fig. 2a). The southern station showed higher discharge values (67–231 m^3 sec^{-1}), while turbidity values were between 3 and 37 NTU, with the exception of a higher peak recorded in late June (Fig. 2b). Water temperatures ranged between 0.4 ℃ and 15 C° in station 1 and between 2.8 ℃ and 22.4 ℃ in station 2. Conductivity, suspended solids and pH ranged around 173-303 µS cm^{-1}, 3-43 mg l^{-1} and 7.9-8.5 (Station 1), and 208-358 µS cm^{-1}, 2-44 mg l^{-1} and 7.8-8.5 (Station 2).

DIN and silica showed similar concentrations in the two sampling stations, with values always higher than 0.5 mg N l^{-1} and 1 mg Si l^{-1}. SRP showed higher values in the southern station (21-65 □g l^{-1}) than in the northern one (2-26 □g l^{-1}). In the latter station, SRP concentrations showed values below 5 µg l^{-1} in the second half of June and between September and the first half of November. In both stations, TP concentrations were always above 20 µg l^{-1} (Salmaso & Zignin, this volume).

Chlorophyll-*a* concentrations ranged between values below 0.5 µg l^{-1} and 5.7 µg l^{-1} (Station 1) and 6.9 µg l^{-1}(Station 2). In the northern station, the dominant species were mostly represented by tychoplanktonic and drifted taxa such as *Diatoma vulgaris* and *D. ehrenbergii*, *Encyonema silesiacum* and *E. minutum*, *Navicula lanceolata* and *N. tripunctata*. In the downstream station there was a neatly higher abundance of small centric taxa such as *Cyclotella meneghiniana*, *Stephanodiscus hantzschii*, *S. parvus* and *Melosira varians*.

The different structure of the diatom community is well reflected by the results of NMDS and PCA ordinations (Fig. 3 and 4). The two NMDS configurations are the result of a single NMDS analysis but, to avoid superimposition of different samples, the results are presented separately for each station. The chronological order of the diatom samples in the two stations followed very different paths. Samples were characterised by pronounced, but not directional, seasonal development. Both the coordinates of the first and second axes of the NMDS configuration showed no significant correlations with the environmental variables (p>0.1, n=46). By converse, when considered separately, the configurations of the two stations showed clear and significant correlations with a few physical variables. The first axis of Station 1 was positively linked (p<0.05, n=23) with D$_{3d}$, dry weight and turbidity, while the second axis was negatively correlated with D$_{3d}$ and turbidity. The correlation of the NMDS configuration with the physical variables in the Station 2 was apparent only along the second axis, which showed a negative and significant (p<0.05, n=23) relationship with D$_{3d}$, dry weight and turbidity.

The different biological characteristics of the two river stretches were further confirmed by the results of Principal Components Analysis (PCA) based on MFDG, which showed a stronger presence of tychoplanktonic, drifted and benthic taxa in the northern station and a higher abundance of euplanktonic taxa in the southern station. The hydrological regimes of the two stations seemed to play a crucial role in selecting different functional groups. Torrential conditions in the northern station, consisting in relatively low water levels and higher water velocity, expose the river bed to a stronger erosion and scraping action by the waterflow so that planktonic diatom community in this site mainly included drifted or tychoplanktonic and meroplanktonic pennate taxa (as shown by PCA on Morpho-Functional Diatom Groups, Fig. 4). In the downstream station, on the opposite, the percentage of centric diatoms (C or CR strategist, *sensu* Reynolds, 2006) was higher (Fig. 4), in accordance with higher water levels and more pelagic conditions displayed by the station and consistent with the results provided by previous investigations (Salmaso & Braioni, 2008).

According to Roeder (1977), the statement that benthic diatom communities are the source of the riverine phytoplankton may be too simplistic further Reynolds & Glaister (1993) argued that the distinction between algae, which obligately grow on surfaces and those which lead a planktonic existence, is not so simple because some species are not necessarily restricted to either habitat. Typical examples may be represented by *Aulacoseira* and *Melosira* spp. that we have found in the samples. From the perspective of further development of the MFDG cluster (e.g., considering splitting based on pelagic and benthic life-styles; Fig. 1), more detailed information are needed on the autoecology of the single taxa. In this context, further research on River Adige will be aimed at studying the connection between the benthic and pelagic river habitats.

In the two sampling stations, the algal growth was never limited by nutrients. The concentrations TP and DIN were always above limiting values (cf. Reynolds, 2006). Similarly, silica was always present with non-limiting concentrations. Considering a few cases of very low concentrations of available SRP, P deficiency for diatoms having higher P requirements (such as the small centric species, Wehr & Descy, 1998) could not be excluded in the northern station. However, taking also into account the very low abundances of zooplankton in River Adige (Salmaso & Braioni, 2008), temporal diatom dynamics were strongly controlled by physical factors, mainly water discharge and the variables directly connected to hydrology (light absorption). These factors have a temporal dynamic less or not at all predictable than environmental factors acting cyclically on inertial systems less impacted by hydrological disturbances (e.g., large and deep lakes). This is reflected also on the irregular seasonal variability of diatoms and confirms the results presented in other studies (e.g., Dokulil, 1994), where combination of discharge, suspended particle concentration and temperature revealed to regulate algal growth rates and hence biomass levels.

Acknowledgements

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“Legends for figures”

Fig.1 – Morpho-Functional Diatom Groups (MFDG). CENUNI (Centrales, unicells), CENPHY (Centrales, filaments), PENPED (Pennales with stalk), PENCOL (Pennales with colonial habits), PENSMA (Pennales smaller than 30 µm), PENLAR (Pennales larger than 30 µm

Fig.2 - Water discharge (D_{3d}) and turbidity (NTU) from March 2007 to February 2008 at (a) Cortina all’Adige and (b) Boara Pisani.

Fig.3 - Ordination of diatom samples by Nonmetric Multidimensional Scaling on Morpho Functional Diatom Groups (MFDG); stress=0.22. (a) Cortina all’ Adige, (b) Boara Pisani; the Arabic numbers indicate the month of sampling, from March 2007 to February 2008

Fig.4 - PCA analysis on Morpho-Functional Diatom Groups (MFDG) for Cortina all’Adige (station1- marked with ●) and Boara Pisani (station2- marked with △) from March, 19th to February, 18th.

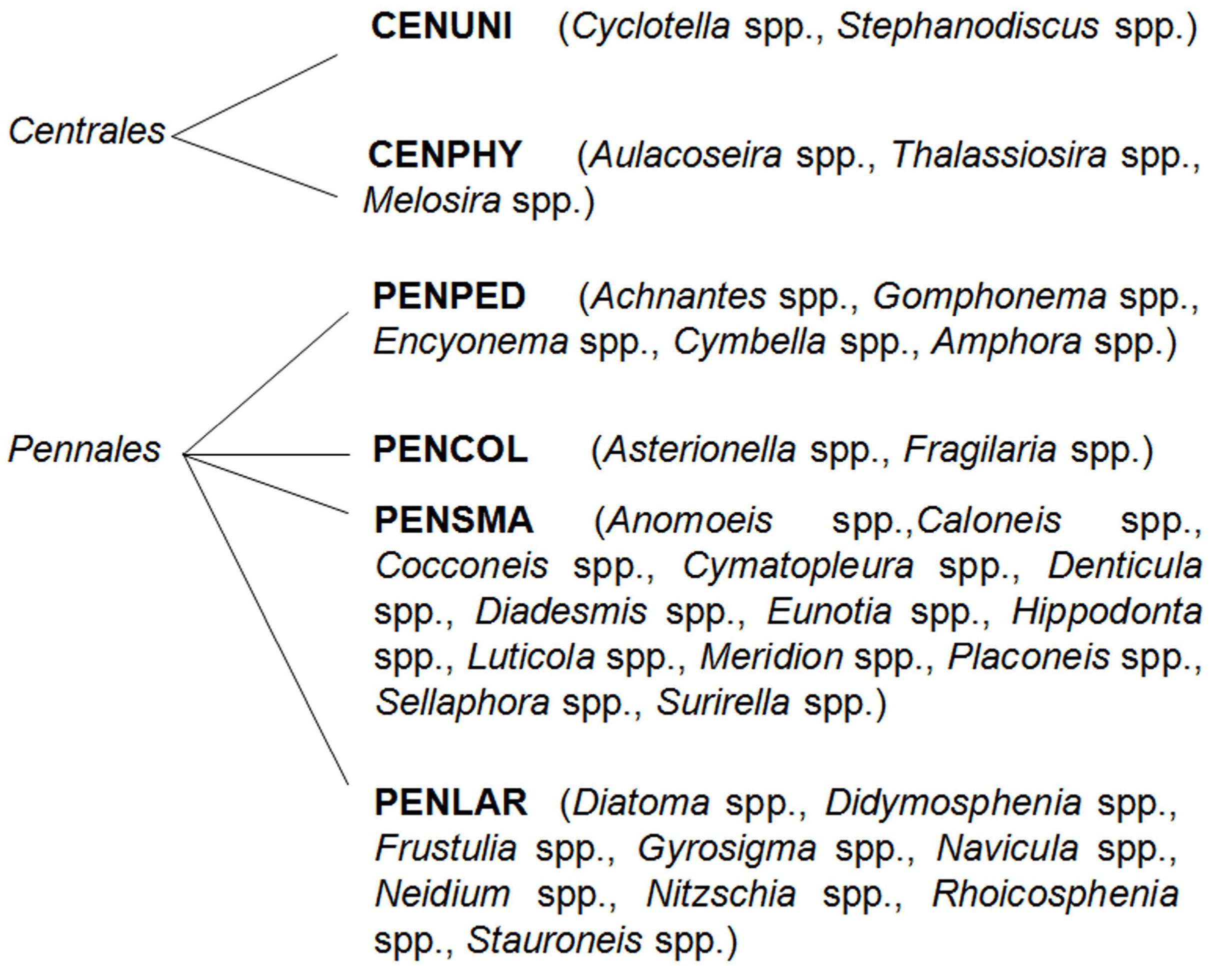
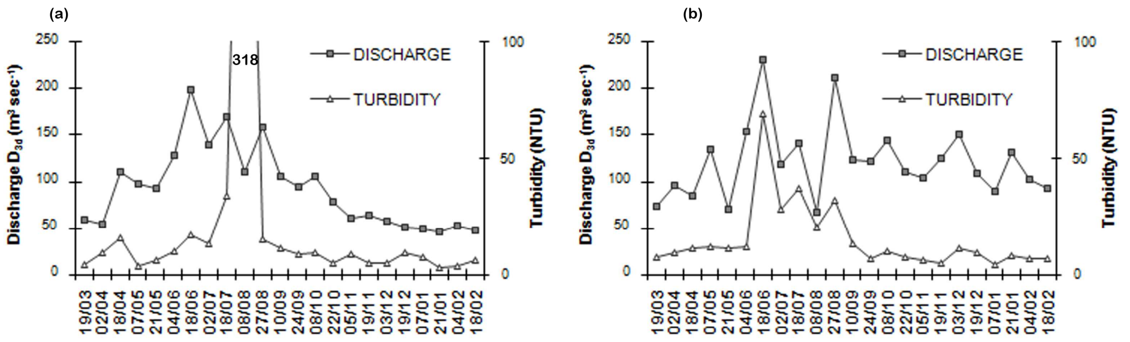

Centrales
CENUNI (*Cyclotella* spp., *Stephanodiscus* spp.)
CENPHY (*Aulacoseira* spp., *Thalassiosira* spp., *Melosira* spp.)
Pennales
PENPED (*Achnantes* spp., *Gomphonema* spp., *Encyonema* spp., *Cymbella* spp., *Amphora* spp.)
PENCOL (*Asterionella* spp., *Fragilaria* spp.)
PENSMA (*Anomoeis* spp.,*Caloneis* spp., *Cocconeis* spp., *Cymatopleura* spp., *Denticula* spp., *Diadesmis* spp., *Eunotia* spp., *Hippodonta* spp., *Luticola* spp., *Meridion* spp., *Placoneis* spp., *Sellaphora* spp., *Surirella* spp.)
PENLAR (*Diatoma* spp., *Didymosphenia* spp., *Frustulia* spp., *Gyrosigma* spp., *Navicula* spp., *Neidium* spp., *Nitzschia* spp., *Rhoicosphenia* spp., *Stauroneis* spp.)

Fig. 1


(a)
(b)
Discharge D$_{3d}$ (m^3 sec^{-1})
Turbidity (NTU)
DISCHARGE
TURBIDITY
318
0
50
100
150
200
250
19/03
02/04
18/04
07/05
21/05
04/06
18/06
02/07
18/07
08/08
27/08
10/09
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04/02
18/02

Fig. 2

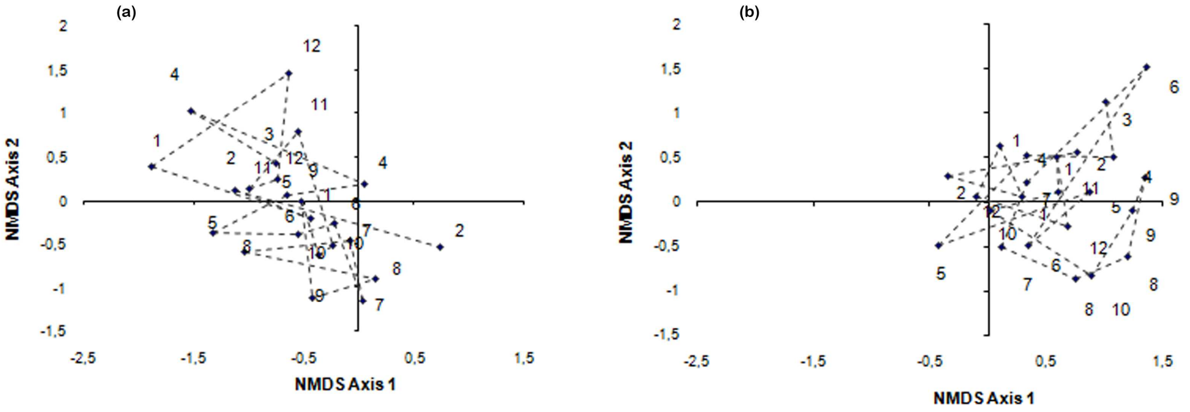
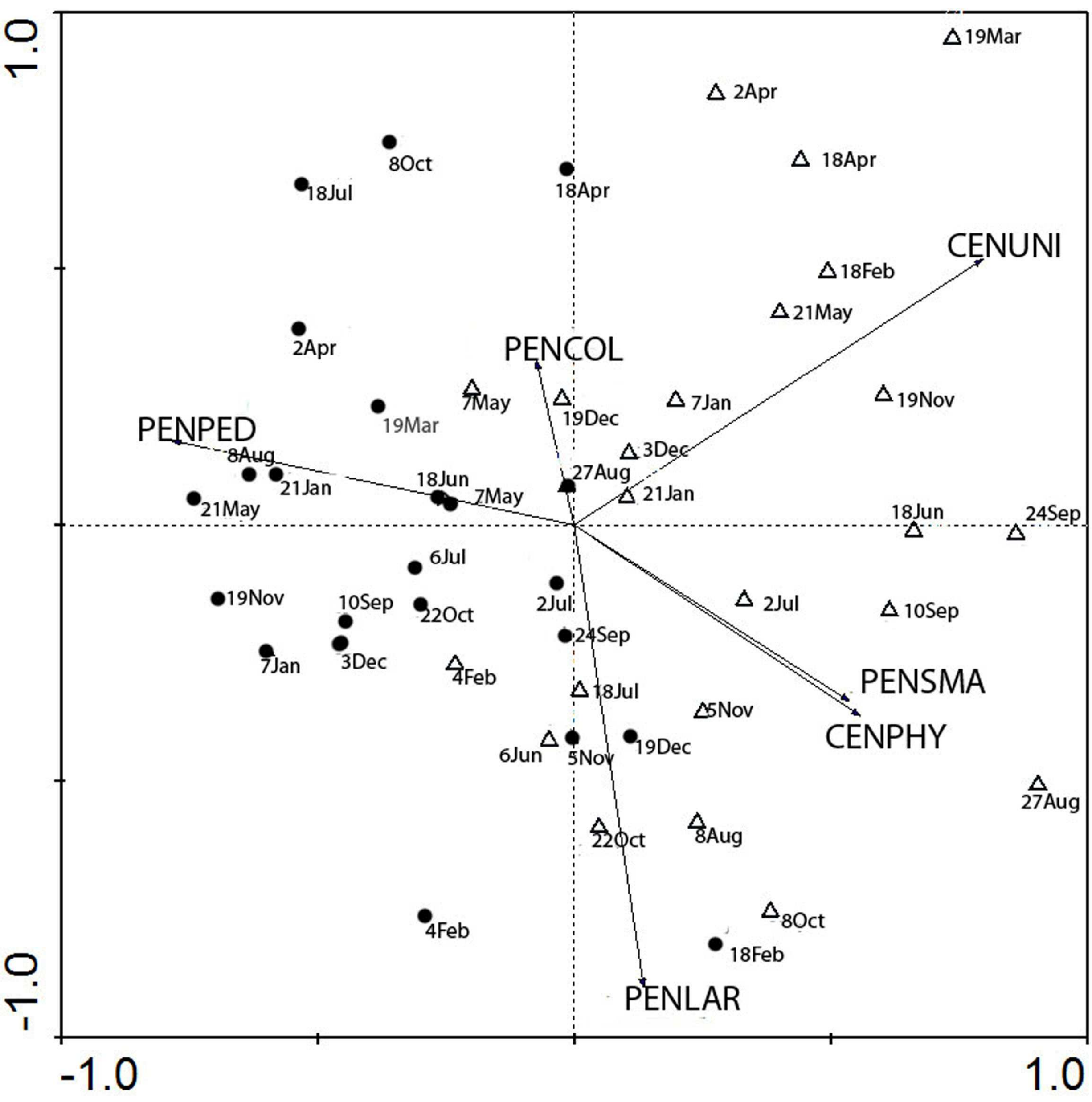

(a)
(b)
NMDS Axis 2
NMDS Axis 1
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2,5
-1,5
-0,5
0,5
1,5

Fig. 3


1.0
-1.0
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1.0
CENUNI
PENCOL
PENPED
PENSMA
CENPHY
PENLAR
19Mar
2Apr
18Apr
8Oct
18Jul
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18Feb
21May
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2Jul
19Nov
10Sep
22Oct
2Jul
10Sep
24Sep
7Jan
3Dec
4Feb
18Jul
5Nov
6Jun
5Nov
19Dec
27Aug
22Oct
8Aug
8Oct
4Feb
18Feb

Fig. 4

APPENDIX IV

Submitted paper to “Annales de Limnologie- International Journal of Limnology”

Running title: Diatom community structure along substrate gradients

Spatial patterns and environmental drivers of benthic diatom communities: a case study from an Alpine river system

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Abstract

Diatom periphyton assemblages were investigated along a 40 km stretch of the Adige river which is a typical Alpine watercourse (South-Eastern Alps, Italy). The mainstream and other three main tributaries in the northern catchment were sampled. Samplings and measurements were carried out once a month for one hydrological year. Benthic samples were collected from the main substrata (stones, sand and sediments). Both physical and chemical variables and diatom communities, showed higher within-site variability. On a seasonal basis, water discharge was the major driver in this system. Its effect had profound consequences on the selection of diatom assemblages. High discharges decreased the abundances of colonial taxa. This effect was even exacerbated in the case of high water fluctuations, as in the case of hydropeaking phenomena whose tumbling effects were particularly disruptive for the diatom communities. Moreover, the study demonstrated significant interactions between hydrological variables and the sampled substrate. Adnate diatoms were mostly found in the coarser substrates (e.g., pebbles and cobbles), while large motile diatoms were mostly identified in finer substrata, like sand and sediments. Owing to their high concentrations, nutrients had a secondary effect in this Alpine river system The results obtained by the application of three diatom metrics showed differences especially between lithic and pelic vs psammic diatoms, highlighting the need to perform both spatial and environmental calibrations.

Keyword: Diatoms, discharge, substrates, river, environmental drivers

Introduction

An important goal for community ecology is to identify major patterns of community structure and to characterize and predict changes in those patterns in relation to environmental gradients. These goals can be achieved through spatially-extensive sampling that could allow ecologists to assess the relative importance of various environmental factors, often effective at different, yet partly overlapping, spatial and temporal scales. Basic knowledge of environment-community relations is a fundamental step in community studies. The physical and chemical environment acts like a filter and provides a template for biological communities (Townsend and Hildrew, 1994; Cereghino *et al*., 2002; Soininen, 2004). However, environment-community relations should be interpreted taking into account relevant spatial scales and it becomes important to identify relative roles of local, in-stream variables and large-scale spatial factors.

Benthic stream diatom communities have traditionally been considered as being regulated more by local environmental conditions than by broad-scale climatic, vegetation and geological factors (Pan *et al.*, 1999; 2000; Kitner and Poulickova, 2003; Yallop *et al.*, 2009). However, some researchers point to an underestimation of spatial patterns in the study of diatom distribution (Mann and Droop, 1996; Kociolek and Spaulding, 2000; Jesus *et al.*, 2009; Reavie *et al.*, 2010). Studies identifying spatial community patterns and most significant environmental factors contributing to phytobenthos assemblages have been performed in North and South America (e.g., Solari and Claps, 1996; Reavie and Smol, 1998; Rott *et al.*, 1998; Pan *et al.*, 2000; Licurso and Gómez, 2002; Griffith *et al.*, 2002; Potapova and Charles, 2002), New Zealand (Biggs, 1990) and Europe (Pipp and Rott, 1994; Loncin *et al.*, 1998; Soininen, 2004; Cambra *et al.*, 2007). The majority of these studies have looked at the driving force imposed by environmental drivers and spatial constraints such as substrate variability (e.g., sampling of different geological textures) to diatom communities. Substrate-specificity could be an issue since specific diatoms give a signal that is representative of the diatom community living there (Yallop *et al.*, 2009). More specifically, rocks or stones have been considered to act as inert substrate (Eminson and Moss, 1980) but other substrates including sediments may potentially provide nutrients and therefore cannot be considered as neutral (Blindow, 1987). Nevertheless, little is known about factors driving diatom species diversity and geographic distribution. Studies focusing on the mechanisms generating species diversity are needed (Vanormelingen *et al.*, 2008; Larned, 2010) since force fitting and ecological generalization of species' preferences have severe consequences both on ecological and management sides (e.g., Water Framework Directive, 2000/60/CE). In fact, bioassessing with diatoms has long been performed with techniques that rely on the sensitivity of these algal groups and by relating changes in the composition of the diatom community to environmental factors.

Diatom indices surely constitute a way of summarizing the information provided by the autoecological preferences of single taxa units and adding these effects to obtain an unique water quality assessment based on the sampled diatom community. The majority of indices are calculated according to the formula designed by Zelinka and Marvan (1961), which accounts for the concept of weighted averaging where the ecological niche is considered in an unique dimension - even if it actually develop in an hyper-volume being influenced by a variety of environmental, spatial and biotic factors. Many indices have been developed nowadays according to each country (among others: EPI-D in Italy- Dell'Uomo, 2004; IBD in France- Prygiel and Coste, 2000) but lately there has been a push towards a common approach so that intercalibration exercises lead to adopt common metrics like ICM index (Mancini and Sollazzo, 2009). The question whether diatom indices are actually able to reflect effectively diatom communities' dynamics is still under debate and even if we have a fair knowledge of these metrics still a lot of work has to be done (Porter *et al.*, 2008) at least for the most sensitive species. Nowadays, diatom indices have been applied regardless of where or under which hydrological regime they have been sampled but this could lead to biases and miss-management.

The general objective of this contribution is to understand the effects of spatial patterns and environmental drivers on a typical Alpine river system and to assess their interplay as determinants of diatom communities. More specifically, the main objectives addressed in this study are: i) to test the hypothesis that in this Alpine system the major drivers controlling temporal dynamics are hydrological variables; ii) to test the influences of the different substrates' textures in selecting the different diatom communities living in each; iii) to verify if differences in ecological communities are effectively reflected by diatom water quality metrics.

Materials and methods

Study site

The River Adige is the second longest river in Italy, after the Po River. It originates in the Eastern Alps at 1,550 m a.s.l., and flows into the Adriatic Sea. The river is 409-km long and the hydrographic basin extends to Albaredo (SE of Verona), but the major tributaries are located between the provinces of Bolzano and Trento (Rivers Isarco, Noce, Avisio and Fersina) (Fig. 1). The River Adige has a typical Alpine flow regime, with high flow and river flooding occurring in spring and summer, in connection with the thawing of snow and ice, and low water period occurring in late autumn and winter (Kristensen and Hansen, 1994). The mountain zone of the hydrographic basin contains more than 30 dams with an overall storage of 570×10^6 m^3.

Samplings and measurements in the field

We have conducted our investigation in an area of ca. 40 km length, including the Adige river mainstream and its three main northern tributaries (Isarco, Noce and Avisio streams) for a total of eight stations (stations from A to H; Fig. 1). The first group of stations (A-C) include the Isarco stream and the River Adige before and after the entry of Isarco. The second group of sampling stations (D-H) includes, besides the Noce and Avisio streams, three stations along the River Adige, before and after the entry of these two tributaries. Stations B and E (streams Isarco and Noce) display a typical coarse bed dominated by pebbles and cobbles; the other stations have instead a finer texture, especially station G (Avisio stream) whose river bed is composed by muddy sediments.

Stations were chosen if they allowed an easy entrance in the reach and having care in avoiding shaded areas that could affect diatoms physiological processes. Attention was paid to find areas that could have been submerged for weeks therefore avoiding shallower areas without proper diatom communities. Stations were sampled once a month, from March 2009 to February 2010 with the exception of the month of May, when high discharges did not let a safe sampling of the watercourses. Water samples were collected using a rinsed plastic bucket. Water temperature was measured directly on the field with a Testo 926 thermometer.

Diatoms were sampled in the main three habitats (epilithon, epipsammon and epipelon). For epilithon, five pebbles/cobbles were randomly selected and removed from the stream and placed in a pan for processing onshore. They were scraped into another pan with a knife to remove most of the algae and then with a toothbrush to remove more tightly attached individuals. The subsampling bottle was then filled with this material, recording the total volume of the sample. Epipsammon was sampled through shaking a small quantity of the sand substrate (obtained directly on the station) in a bottle containing water so that the sand would quickly fall to the bottom of the bottle after agitation and the suspended algae could be poured off the top into another 100 ml plastic container (Lowe and LaLiberte, 2007). When the substrate was coarser, a swirl-and-pout technique was used to remove algae by repeatedly adding small amounts of water to the sample, swirling it to tumble the sediments and thereby scouring algae from the fine substrate. Afterwards, the suspended algae were gently poured from the sample to a white pan (this step was repeated 5-10 times or until the poured water appeared relatively clean) and then to the subsampling bottle (Stevenson and Rollins, 2006). Epipelic sample was collected with a pipette having extreme care to avoid the penetration of the sediments too deeply or with a Petri dish and spatula from at least five representative locations of the station (Stevenson and Rollins, 2006).

Laboratory analyses

Total phosphorus (TP) was measured on unfiltered samples. Soluble reactive phosphorus (SRP), nitrate (NO_3-N), nitrite (NO_2-N) and ammonium (NH_4-N) were determined on filtered samples. Chemical analyses were carried out following the standard methods described by APHA, (1995). After removing of larger particles with a 200 µm mesh plankton net, seston dry weight (total suspended solids, 105 ℃) and ash-free seston dry weight (550 ℃) were determined by filtering water samples on previously combusted (550 ℃) and weighed Whatman GF-C filters (A.P.H.A., 1995). Water turbidity (NTU, Nephelometric Turbidity Units) was estimated by a turbidimeter Hach 2100N. Chlorophyll-*a* (Chl-*a*) was determined by spectrophotometry after filtration on Whatman GF-C glass-fiber filters, disruption of the filters with a grinder and 24 h extraction in 90% acetone. Phytoplankton analysis was carried out on sub samples preserved in acetic Lugol's solution.

Water samples for diatom analyses were concentrated by sedimentation (1:50) and then cleaned in 30% hydrogen peroxide and 37% hydrochloric acid (Kelly *et al.,* 1998). Cleaned diatom frustules were permanently mounted in Naphrax® resin. On each slide 400 valves were counted (EN, 14407, 2004) under a Leica light microscope at 1000 magnification. Taxa were identified following the more recent monographs of the series Süßwasserflora von Mitteleuropa, established by A. Pascher (Gustav Fisher Verlag, and Elsevier, Spectrum Akademischer Verlag) and the most updated literature.

Data analysis

Seasonal diatom variations were analysed considering Morpho-Functional Diatom Groups (MFDG) elicited specifically for river diatom communities by Centis *et al*., (2010). Following these classification, six diatom groups were appointed using a morphological criteria supported by habitat selection and modality of adhesion to river substrate of each taxon. Centric diatoms were assigned to two groups, separating unicellular euplanktonic diatoms (CENUNI group) from filamentous diatoms that can be planktonic or benthic, unattached to any substratum (namely the CENPHY group). Other groups separated diatom taxa being attached with stalks better adapted to high current velocities and displaying tychoplanktonic status (PENPED groups) from colonial diatom taxa (PENCOL group). Further subdivisions were based on cell size ending in two heterogeneous groups mostly benthic or tychoplanktonic (PENLAR and PENSMA).

Two Principal Components Analysis (PCA) based separately on environmental variables and MFDGs were used to summarize variations among sites and to elicit environmental and biotic gradients. Before the analysis, environmental variables were linearized by logarithmic transformation, while diatom relative abundances were transformed using an arc-sinus transformation to reduce the weight of the most abundant taxa (Sokal and Rohlf, 1995).

We have calculated three indexes: EPI-D (Dell'Uomo, 2004), ICMi (Mancini and Sollazzo, 2009) and IBD (Prygiel and Coste, 2000). Paired t-tests were performed to assess differences between the three sampled substrates (Sokal & Rohlf, 1995). Analyses were carried out using SYSTAT™ 10.2, and CANOCO™ 4.5 packages.

Results

Chemical environmental variables of each sampling station are shown in Table 1. Conductivity and pH showed higher variations among stations: pH values were always higher in the tributaries (stations B, E, G) than in the mainstream. By converse, conductivity showed higher values in the Adige river's stations. Nutrients, showed high within-site variation. Higher SRP values were recorded in the tributaries, on the opposite of what was recorded for total phosphorus. Higher DIN concentrations were recorded in the southern stations, as well as in the case of silica.

Physical environmental variables showed high within-site variation (Fig. 2 a-d). The highest mean water discharges were recorded in the final station (stat. H) (100 m^3 sec^{-1}) and the lowest in the Avisio stream (stat. G) (30 m^3 sec^{-1}) (Fig. 2a). The low discharges measured in the Avisio stream were measured along with high abundances of silica and chlorophyll-*a*, which in this sampling point is at its maximum (mean year abundance of 5.53 mg L^{-1}). Many variables were collinear with water discharge. Highest turbidity values were recorded in the final station (stat. H, 5.8 NTU) while the lowest in the Avisio stream (stat. G, 5.4 NTU) (Fig. 2b). In the different stations, correlations between discharge and turbidity ranged between 0.65 ($p<0.05$) and 0.95 ($p<0.01$). Also dry weight showed its minimum and maximum records in these sampling points (6.0 mg L^{-1} in station H and 3.1 mg L^{-1} in station G) (Fig. 2c). As expected, dry weight showed a positive correlation with water discharge ($0.60 \leq r \leq 0.90$, $p \leq 0.05$). The lowest recordings of these three variables were observed in the winter months (from November to February) when the discharge was at its seasonal minimum.

Temperature showed a different pattern: the highest mean values were recorded in stations G and H, (8.7℃ and 8.4 ℃, respectively) while the lowest in station C (6.9 ℃) (Fig. 2d). Stations G and H should be considered separately from the previous ones (stat. A-F).

With the exception of oxygen, which showed low variations among sites (see Tab. 1), all the recorded environmental variables were used in the ordination of samples performed with PCA. The result of this analysis has been presented separately for each sampling point to avoid samples superimposition, but they are the outcome of a unique PCA analysis (Fig. 3). The first two PCA axes were both significant ($p<0.01$; Monte Carlo permutation test, 999 random permutations), explaining 97% of the total variation. Discharge, turbidity, dry weight, water temperature, SRP and conductivity were the most significant contributors to the configuration. In all the stations, winter samples (December-February) were characterized by higher values of conductivity and SRP, and this was paralleled by the lower discharges measured in that period of the year. In early summer (June), the driving effect of hydrological variables became evident in all the stations but the Avisio stream (station G) where the effect was, as expected, not relevant due to the lowest discharge values recorded in this reach (see figure 2a). As expected, the configurations of samples in the different stations did not follow clear and regular cycles. Rather, the main differences were driven by the temporal variations in water discharge and associated variables directly linked to water fluxes. These results were summarized according to their seasonality along the PCA gradients (Fig. 4). The strong driving effect played by hydrological variables in separating the samples was rather apparent, highlighting the minor impact of hydrology in the winter months.

The seasonality of the different hydrological and environmental variables had an important impact on the control of diatom communities. Forty-four genera and 146 species of diatoms were collected from the study system. Fifteen genera were represented by a single species; another 12 genera had < 5 species. We have noticed a general increase of centric taxa (belonging to CENPHY and CENUNI groups) and PENCOL diatoms when water level was low, while pennate taxa such as PENPED, PENLAR and PENSMA increased during high water levels. More specifically, positive and negative correlations were found, respectively, between discharge and PENPED diatoms 0.60≤ ($0.59 \le r \le 0.90$, p at least ≤ 0.06) and between discharge and PENLAR diatoms ($-0.50 \le r \le -0.91$, p at least ≤ 0.1). A paradigmatic example was represented by the Noce stream (station E), where hydropeaking events (cf. Salmaso *et al.*, 2010) did not allow phytobenthic community to establish completely because of the sudden and violent changes and the tumbling effects due to discharges. On the opposite, the lower discharges measured in station G allowed higher frequencies of CENPHY diatoms to dwell.

Along with environmental constraints acting on the diatom communities of the stations, spatial patterns exerted a control which was more station-specific. It is in fact coupled with the habitat specificity that each diatom shows, regarding its morphology, habitus or modality of adhesion to the substrate. To avoid samples superimposition in the configuration, the result of the PCA analysis based on MFDGs has been reported according to the three "sub- basins" composing the system (Figs. 5a-c). Diatom types collected from the different substrates showed different "tastes" for textures (lithon, psammon and pelon). In particular, in all the eight stations, a gradient on the first PCA axis represented by PENPED and PENLAR diatoms clearly separated lithon samples from the pelon and psammon samples. PENPED diatoms (whose most abundant taxa are *Achnanthes* spp. Grunow in Cleve and Grunow 1880- and *Encyonema* spp. Kützing, 1833) are typical adnate taxa that live abridged to the substratum thanks to the mucilage stalk while PENLAR such as *Navicula* spp. (Bory de Saint-Vincent, 1822) and *Diatoma* spp. (Bory de Saint-Vincent, 1822), on the opposite, live free from the bottom and are easily transported drifted by the water current. PENPED diatoms were more abundant in the coarser substrate (lithon) while PENLAR diatoms were more easily found in the finer substrates (Fig. 5). Along the second axis, PENCOL, CENPHY and CENUNI diatoms shared a common taste for lithic substrates and these samples were found especially during low water periods (from December to February), when the system showed its lowest discharge.

The application of three widely used diatom indices (EPI-D; IBD and ICMi) was aimed to test the performance of these metrics in the different substrates. These metrics were chosen for geographical (the first two) and management reasons (the ICMi is the outcome of a two-years European calibration exercise). The application of these indices to the dataset of River Adige highlighted discordant water quality estimations when results were tested (paired-t tests) for differences in the three types of substrates. Analyses showed that, while the EPI-D index did not show significant differences among substrates, ICMi and IBD showed significant, different values (Table 2). In particular, these two indexes have shown significant differences between the lithic and the psammic substrates on one hand, and pelic and psammic substrates on the other hand.

Discussion

Water discharge was the most effective variable regulating the algal communities of the River Adige system, both phytoplanktonic (Salmaso and Zignin, 2010) and benthic ones (Centis *et al.*, 2010). This variable influenced all the other hydrological parameters, in particular turbidity and dry weight. This effect was evident in the PCA analysis (Fig. 4) which showed the higher importance of these variables in the temporal pattern of river samples compared with other variables (e.g. chemical ones). Periods of high water levels negatively affected the abundances of certain colonial diatom taxa, such as PENCOL and CENUNI. In these systems, the disruptive effect on the phytobenthos community became particularly apparent when discharges assumed intermittent frequency due to the functioning of the large hydroelectric impoundements present in the river catchment (e.g., Noce stream). Positive correlations between discharge and PENPED diatoms could lead to consider these diatoms as "early colonizers", able to settle in the substrate before other species, as also pointed out by several authors (e.g. Stevenson and Peterson, 1991; Kralj *et al*., 2006). This is not surprising, since mucilage stalks allow these taxa to have a better grip to the river bottom. By converse, besides typical euplanktonic taxa (small unicellular centric diatoms), lower discharges allowed higher frequencies of typical mero- and tychoplanktonic algae (e.g., CENPHY diatoms), that typically dwell in low discharge waters such as the lakes' phytoplankton. Negative correlations between discharge and PENLAR diatoms suggest an easier dwelling of these algae when water regimes are low so that they do not get tumbled or drifted away. This, in turn, allows higher frequencies of such taxa in more mature biofilms, as pointed by previous studies (e.g. Yallop and Kelly, 2006).

This environmental effect has to account for the different "taste" of diatoms for the three sampling substrates: PENPED diatoms were more abundant in the coarser habitats (e.g. lithon) while PENLAR diatoms were more abundant in the finer habitats (such as psammon and pelon). This pattern was found in all the eight stations and it is not surprising considering that pebbles can offer a more stable environment for early colonizers to settle while the highly unstable environments of sand and sediments, which are subjected to constant tumbling by the waterflow, can offer part-time habitat stability for mobile taxa. Sand, in particular, offers a three dimensional habitat but it is, on the other hand, a highly risky habitat because it exposes diatoms to abrasion risks as confirmed by the high variability of diatoms living in it. Sediments, on the other hand, offer a high amount of nutrients so they could constitute a good environment to dwell on. Nevertheless, they are really unstable and subject to tumbling. It follows that different substrates offer different species composition and that each species has its own "substrate-taste".

It leads that in a chemically- homogeneous system like the one we have studied, the only variables that could influence spatial and temporal patterns acting on diatom communities are the kind of sampling substrate and the hydrological related variables (e.g. discharge, turbidity, dry weight and temperature). It follows that the other environmental variables (e.g., pH, conductivity and SRP) are less important or can be considered as less impacting drivers being that their effect are at least "masked" by hydrological effects.

These results have profound implications on the water management side. The application of three diatom indices on lithic, pelic and psammic substrates provided different results both between and among them. Nevertheless, some indexes are more conservative than others (e.g., EPI-D), showing less individual variation, while others are more substrate-discriminating. Paired t-tests confirmed that lithic and psammic communities on one hand and pelic and psammic ones on the other hand, both using ICMi and IBD indexes, proved to be significantly different. This bias effect can have strong implications for the effective validity of these metrics without a proper sampling substrate assessment. In this study, diatom assemblages were highly influenced by spatial constraints, in particular when lithic and pelic substrates were compared with the psammic ones. Researchers have different beliefs on this issue, since many thought that sampling different substrates could lead to different results (among the others, Cattaneo *et al.,* 1997; Cox, 1988; Soininen and Heino, 2005; Cetin, 2008) while others believed that sampling different substrates could lead to similar results (among others Rott *et al.*, 1998; Kitner and Poulickova, 2003). The whole process is strictly influenced by hydrological variables that affect the benthic diatom communities living in lotic system, with different effects according to the water regime they have been collected from and to substrate composition and patterns. More specifically, in the high flushed Adige system, substrates – in particular lithic and pelic vs. psammic – seem to control the selection of different diatom communities each differently impacted by discharge variability. Nevertheless, it is the psammic substrate that shows the highest variability among the three, as from Tab.2.

In conclusion, benthic diatom communities exhibit strong dependence on substrate type, yet environmental factors, especially hydrological ones, are equally impacting in this system and act coupled with spatial factors in explaining diatom distributions. Their effect has strong implications for the water management and need to be taken into strong consideration.

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Tab. 1- Average values and range (minimum and maximum values are reported in brackets) of the chemical environmental parameters recorded in each stations (n= 11). DIN: NO_3-N + NO_2-N

	pH	CONDUCTIVITY (µS cm^{-1})	OXYGEN (mg L^{-1})	DIN (mg L^{-1})	SRP (mg L^{-1})	TP (mg L^{-1})	SILICA (mg L^{-1})
ST_A	7.83	260.54	11.51	0.66	8.14	24.09	5.26
	(7.69-7.94)	(205-345)	(10.01-13.60)	(0.40-0.90)	(2-12)	(14-52)	(0.67-6.72)
ST_B	8.11	242.27	12.43	0.70	12.78	25.27	4.55
	(7.94-8.36)	(186-366)	(10.56-14.54)	(0.43-0.95)	(6-21)	(15-41)	(3.8-5.43)
ST_C	8.09	243.63	12.75	0.71	10.80	33.18	4.52
	(7.89-8.32)	(186-373)	(11.52-14.28)	(0.42-1.00)	(4-21)	(14-41)	(3.91-5.45)
ST_D	7.98	257.09	12.11	0.71	13.17	42.90	5.13
	(7.86-8.15)	(204-341)	(10.75-13.35)	(0.41-1.09)	(5-21.10)	(19-94)	(4.44-6.36)
ST_E	8.14	235.36	12	0.69	15.77	42.63	4.24
	(7.87-8.60)	(139-344)	(10.75-12.85)	(0.12-1.08)	(7.8-29)	(18-94)	(3.25-5.85)
ST_F	8.08	273.18	12.42	0.75	12	38.27	5.23
	(7.93-8.43)	(203-413)	(10.67-14.95)	(0.43-1.08)	(5-19.80)	(17-69)	(4.48-6.65)
ST_G	8.51	293.18	12.44	0.84	13.10	24.36	5.33
	(7.94-9.02)	(203-411)	(10.57-13.83)	(0.18-3.39)	(0.8-25)	(13-32)	(2.42-7.60)
ST_H	8.49	289.91	12.28	0.94	13.96	36.72	5.27
	(8.22-8.78)	(207-416)	(10.26-13.84)	(0.20-3.39)	(5-28)	(14-60)	(3.20-6.93)

Tab.2. Paired t-tests between EPI-D, ICMi and IBD diatom indexes, performed among the sampling substrates of each. The null hypothesis is that means of paired samples are equal. (a) values referred to EPI-D index; (b) values referred to ICMi; (c) values referred to IBD index. For every substrate, average values and standard deviations of the indexes have been reported at the bottom of each table.

(c)

Substrate1	Substrate2	*p* value	t test value	*p*
LITHON	PELON	0.59	-0.56	n.s.
LITHON	PSAMMON	0.07	-2.04	n.s.
PELON	PSAMMON	0.57	-0.58	n.s.
LITHON,	PELON	PSAMMON		
1.56±0.27	1.62±0.17	1.67±0.27		

(d)

Substrate1	Substrate2	*p* value	t test value	*p*
LITHON	PELON	0.06	2.19	n.s.
LITHON	PSAMMON	0.006	3.82	$p<0.01$
PELON	PSAMMON	0.007	3.73	$p<0.01$
LITHON,	PELON	PSAMMON		
0.61±0.13	0.57±0.16	0.38±0.21		

(c)

Substrate1	Substrate2	*p* value	t test value	*p*
LITHON	PELON	0.30	1.09	n.s.
LITHON	PSAMMON	0.008	3.58	$p<0.01$
PELON	PSAMMON	0.02	2.78	$p<0.05$
LITHON,	PELON	PSAMMON		
12.3±5.2	10.1±5.2	5.8±4.9		

Figure legends

Fig.1. Map of the sampling area. Dots indicate the sampling stations, identified by a progressive alphabetical letter

Fig.2. Range of the hydrological parameters recorded for each sub-basin (stations A,B,C for the Isarco; stations D,E,F for the Noce; stations G,H for the Avisio). Boxplots report the median and 25% and 75% percentiles; the variability between the samples is indicated by the distance between the whishers while outliers (*) are large or low values. (a) Discharge (m^3 sec^{-1}) plots; (b) Turbidity (NTU) plots; (c) Dry weight (mg L^{-1}) plots; (d) Temperature (°C) plots

Fig.3. Principal Components Analysis based on environmental data. Numbers indicate the sampling month of the year. The first two axes explain 96.6% of total variance.

Fig.4. Principal Components Analysis based on environmental data highlighting seasonal patterns (cf. Fig. 3).

Fig. 5. Principal Components Analysis based on MFDGs. Crosses (x) refer to samples collected in the LITHON; empty circles (o) refer to samples collected in the PELON, Plusses (+) to samples collected in the PSAMMON. (a) refers to stations A, B, C; (b) refers to stations D, E, F; (c) refers to stations F, G, H (station F has been repeated since it belongs to both sub-basins). The first two axes explain 92.6% of total variance.

Fig.1

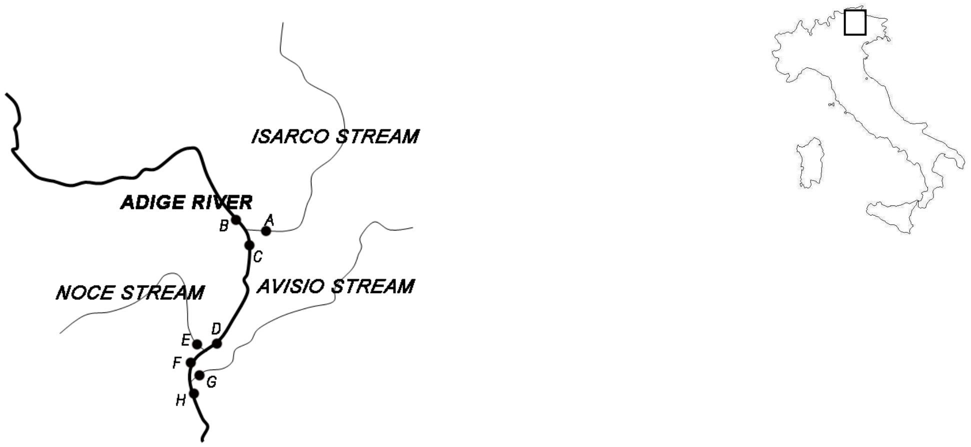

ISARCO STREAM
ADIGE RIVER
A
B
C
NOCE STREAM
AVISIO STREAM
D
E
F
G
H

Fig. 2

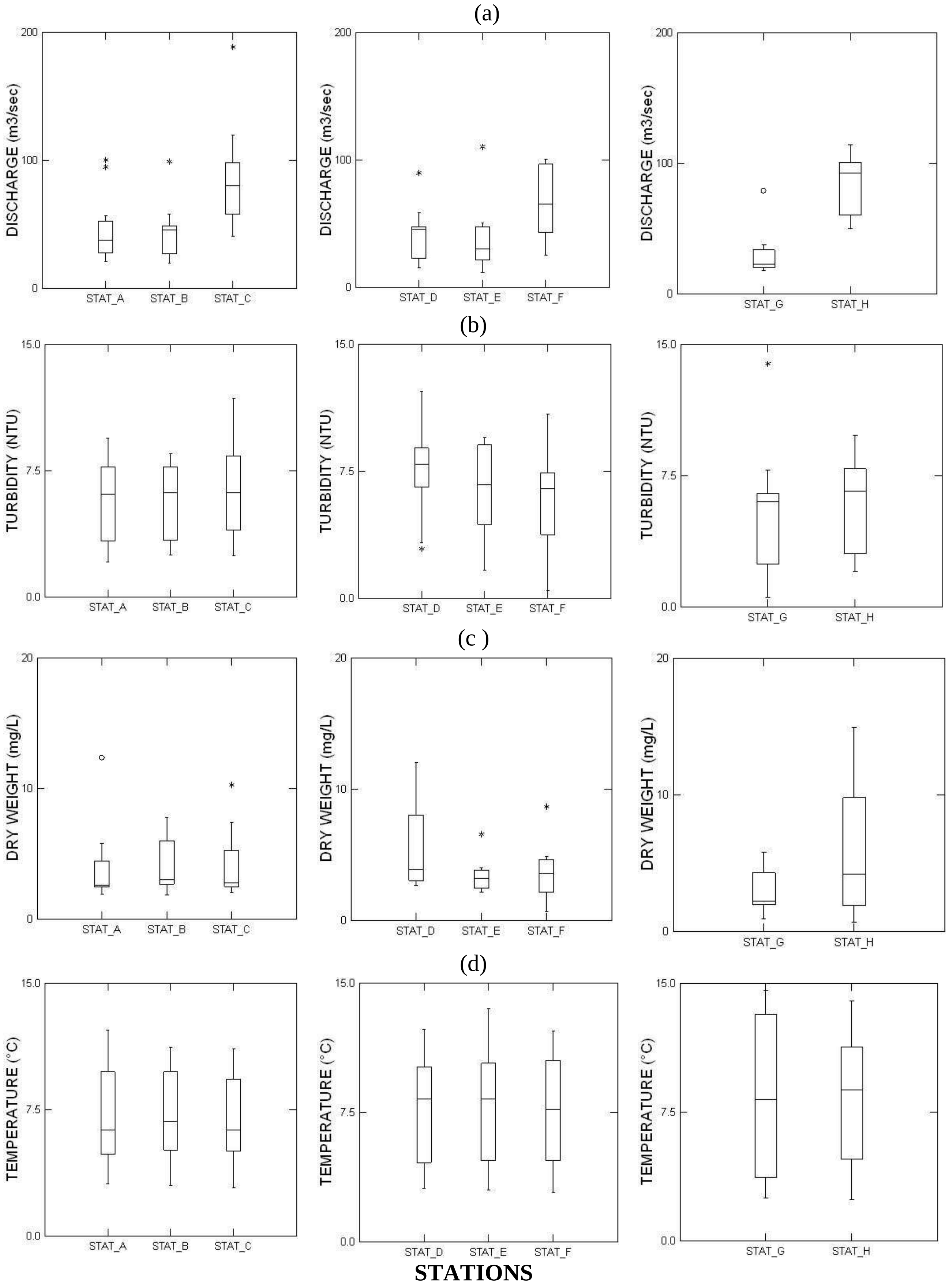

(a)
DISCHARGE (m3/sec)
0
100
200
STAT_A
STAT_B
STAT_C
STAT_D
STAT_E
STAT_F
STAT_G
STAT_H
(b)
TURBIDITY (NTU)
0.0
7.5
15.0
(c)
DRY WEIGHT (mg/L)
0
10
20
(d)
TEMPERATURE (°C)
0.0
7.5
15.0
STATIONS

Fig. 3

PCA with environmental vectors

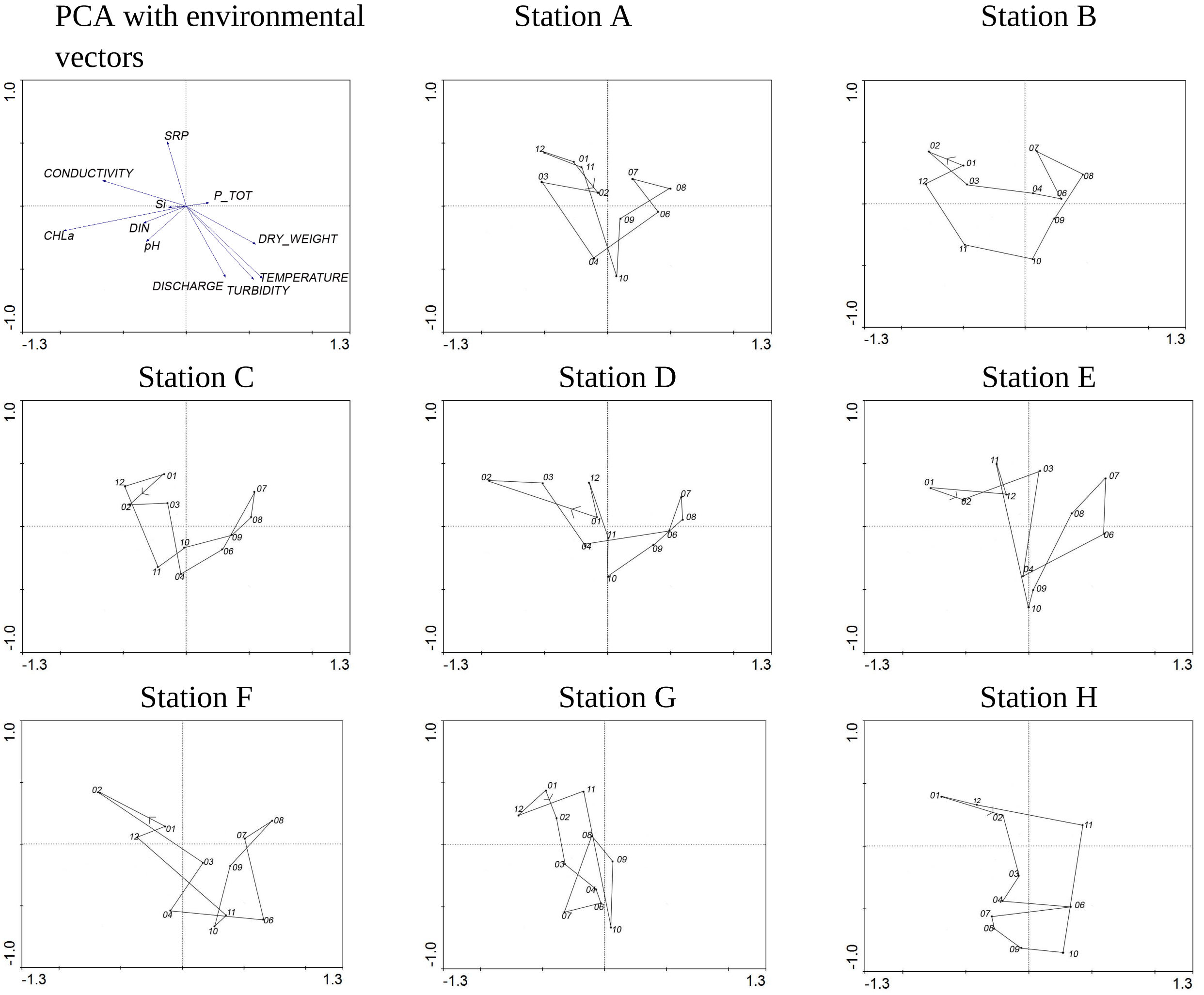


Fig. 4

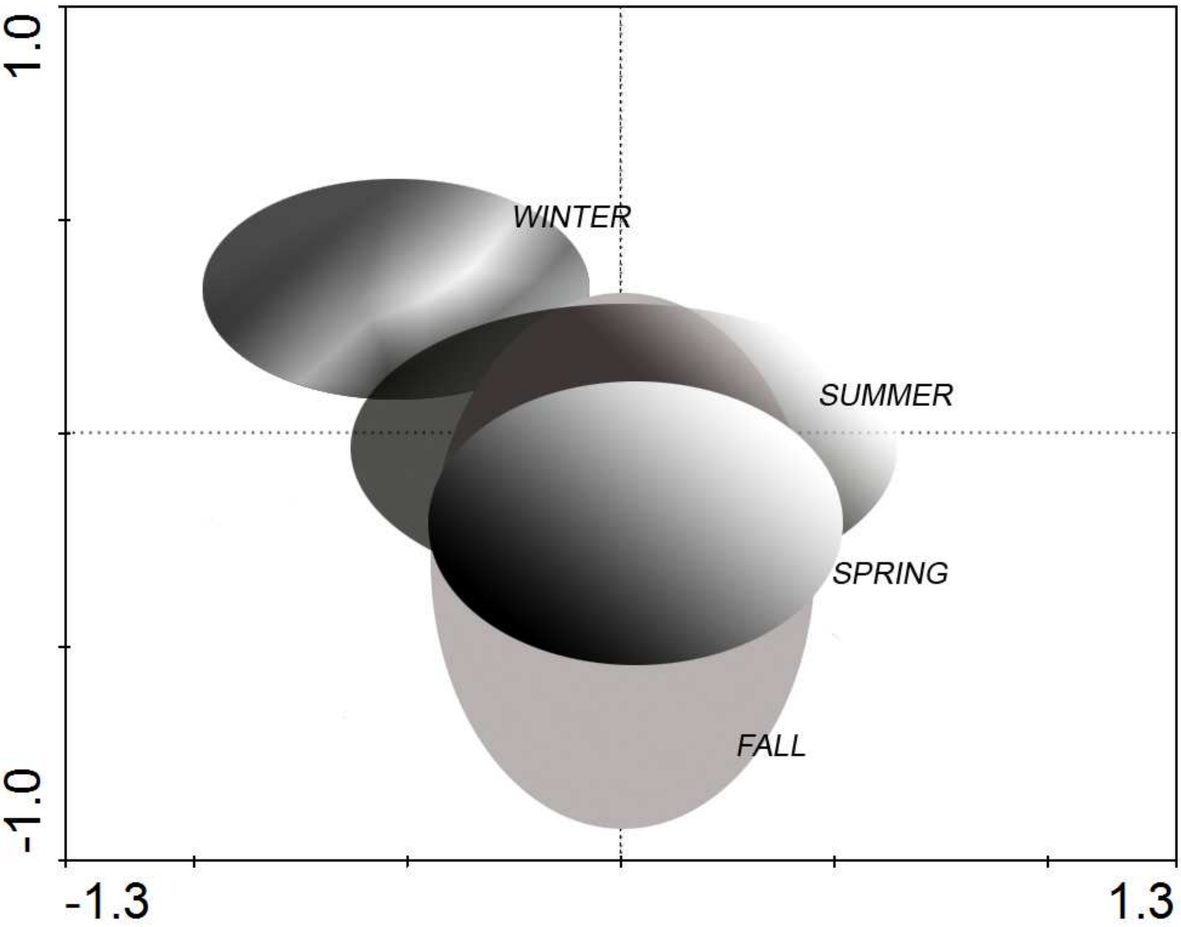
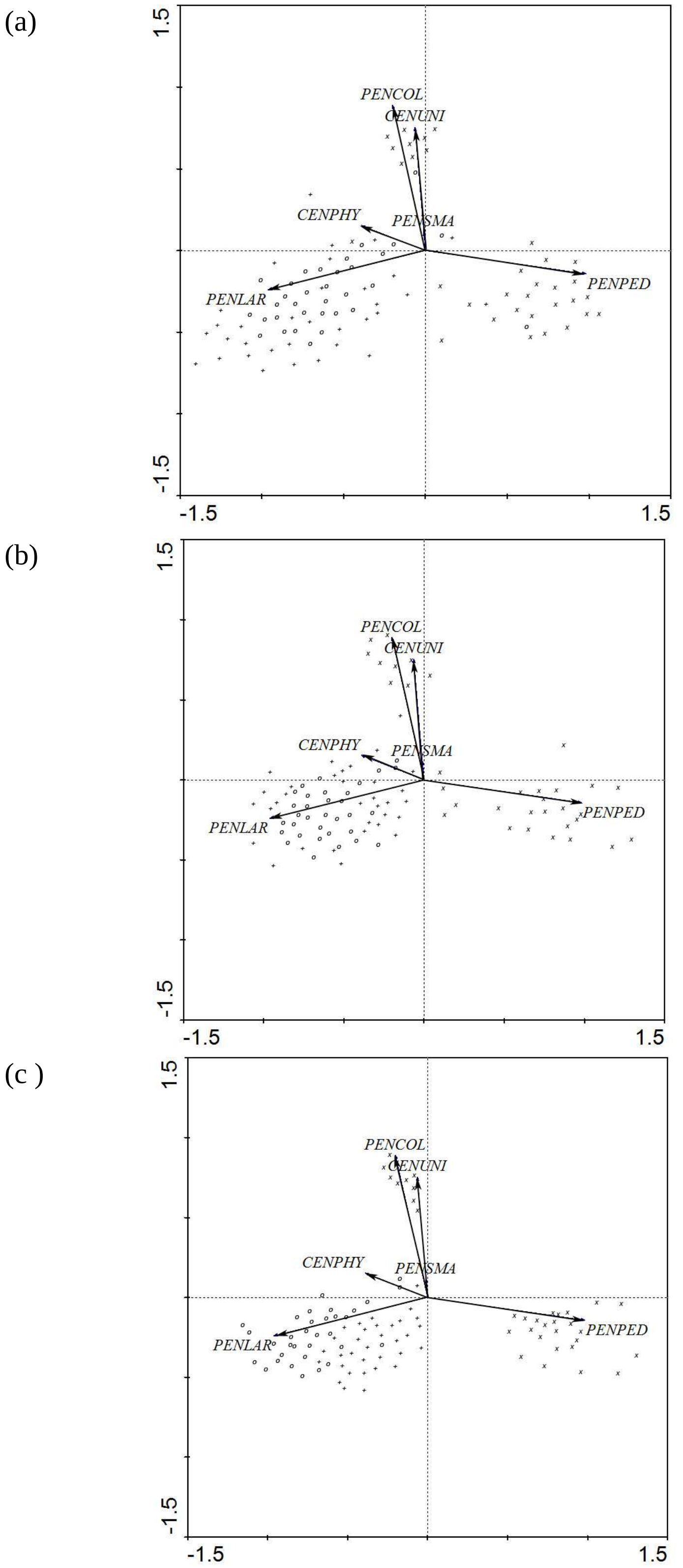


Fig. 5



APPENDIX V

Pictures of some diatom species found in the samples