UNIVERSITAT DE BARCELONA

Ecological factors and diatom diversity at Rivers of the Iberian Mediterranean River Basins: Macro-scale, Meso-scale and Micro-scale

Factores ecológicos y diversidad de diatomeas en los ríos de las cuencas hidrográficas del Mediterráneo ibérico: maro-escala, meso-escala y micro-escala

Andrea M. Burfeid Castellanos

ECOLOGICAL FACTORS AND DIATOM DIVERSITY AT RIVERS OF THE IBERIAN MEDITERRANEAN RIVER BASINS:

MACRO-SCALE, MESO-SCALE AND MICRO-SCALE

Andrea M. Burfeid Castellanos

Tesis Doctoral, 2018

UNIVERSITAT DE BARCELONA

Programa de doctorat de Biodiversitat (H0G01), (HDK04)

Departament de Biologia Evolutiva, Ecologia i Ciències ambientals.

Facultat de Biologia

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FACTORES ECOLÓGICOS Y DIVERSIDAD DE DIATOMEAS EN LOS RÍOS DE LAS CUENCAS HIDROGRÁFICAS DEL MEDITERRÁNO IBÉRICO: MACRO-ESCALA, MESO-ESCALA Y MICROESCALA

Memòria presentada per Andrea Montserrat Burfeid Castellanos per optar al grau de doctora per la Universitat de Barcelona.

Andrea Montserrat Burfeid Castellanos

Director de Tesi/Tutor Jaume Cambra Sánchez

2018

ACKNOWLEDGEMENTS

AGRADECIMIENTOS/DANKSAGUNG

It is said that writing the acknowledgements of a thesis can be difficult. Maybe more so if you want to thank everyone that made it possible but know that they couldn't be able to understand it. So, I hope not to scare anyone with a particularly multilingual thanks to everyone involved. This way can be a good insight into the linguistic chaos that roams my mind...

Primer de tot, voldria agrair la confiança en la meva capacitat científica que va demostrar en Jaume Cambra, el meu tutor i director de tesi, a l'oferir-me un contracte pre-doctoral amb un tema que ja s'anava quallant des del meu Treball Final de Màster. Moltes gràcies pel suport i les moltes idees que varen sortir de les reunions que vàrem tenir durant aquests darrers anys. I gràcies també al teu suport moral d'aquests darrers mesos.

A més, voldria agrair al grup de dins del nostre gran departament. Gràcies a la Núria, pel suport moral i sàvies paraules en moments justos. I a tots els que han estat doctorands d'en Jaume abans de mi, Sandra, Joan i Roser, per la seva feina amb les diatomees de l'Ebre, que m'ha facilitat la meva. Gràcies a en David i l'Albert pel seu ajut inicial amb mapes que han pogut ser utilitzats en aquesta tesi. Gràcies a en Miquel Sala per donar-me accés a les seves mostres de la riera Salada, per apropar-me a les comunitats que m'esperarien. Gràcies als professors que m'han ajudat amb material o treball en altres feines que han aparegut durant el meu període de doctorat. Gràcies a la Mònica, per la seva companyia al transport públic i suport moral quan el necessitava, i a les secretàries, Maria del Mar, per bregar amb els nostres problemes de comandes per ajuts, i l'Ester, pel seu ajut amb la part burocràtica. Voldria demanar-li disculpes a l'Ester Boada per fer-li difícil la neteja en els meus períodes d'horaris estranys de feina i agrair la seva feina feta. Y, finalmente, agradecerle a Estefanía todo en lo que me ha ayudado, aunque no fuera su descripción de trabajo.

I also want to thank Bánk Beszteri for being so kind of making it possible to use the AWI (Alfred-Wegener-Institut, Helmholtz Zentrum für Polar- und Meeresforschung) facilities to study my Ebro samples and using the Slide-Scanner in a busy time. Thanks to this procedure, an exciting new development could be coming soon. Thanks also to his kindness in helping at each bureaucratic step of the way. Ich wollte mich auch bei der Hustedt/Kieselalgen Gruppe vom AWI bedanken. Danke für so viele interessante Gespräche in meinen drei Monaten in Bremerhaven und dafür, mich als eine mehr aufgenommen zu haben. Danke an Mirta (und Tessa), Ute, Lena (und Sam), Sara, Catherine, Barbara, Stefan, Ingrid and many more for making me feel as one more.

Technically, I also wanted to give a big thank you for Marius Bottin, for making his diatSOM package readily available. And to Frédéric Rimet for his diatom life-form database.

Ohne meinem Erasmus-Stipendium am Berliner Botanischen Museum und Botanischen Garten in der Kieselalgen-Forschungsgruppe, hätte ich wahrscheinlich nicht an meinem Diatomeen-studium

verharrt. Danke Regine, Jonas, Nélida, Neela, Wolf-Henning und co., dass Ihr eine etwas verlorene Bachelorstudentin angenommen und so viel gelehrt habt.

Thank you also to the diatom core team, Hannah and Xavi, because "geteiltes leid ist halbes leid", and although working hard, we still had time to compare notes and share the difficulties of PhDs.

Muchísimas gracias a Marina y Nacho, por su apoyo moral en nuestros encuentros, y por hacerme ver (y sentir) que había vida más allá de la tesis doctoral. Gracias también a Marta, por las charlas. Danke, Wolfram, dass ich mir durch Dich fremde Welten vorstellen kann, die mir helfen die Diss zu relativieren. A Santi por tantos consejos… Y a tanta gente más que ayudó a hacerme como soy. Finalmente, pero no menos importante, quería agradecerle a Rafa todo su apoyo, el ver la vida desde un prisma similar (y/o paralelo) y poder hablar de ciencia, ficción, ambos, ninguno y de todo. Gracias por pensar en mis diatomeas aun estando en la Antártida. ¡Cuento con poder seguir colaborando los próximos años!

Gracias a mi Yaya Dorita, que siempre ha creído en mi más que nadie, que escucha mis charlas sobre temas tan diversos como ríos, diatomeas, mitocondrias y genética sin quejarse y con cara de ilusión. Vielen Dank auch an meine Oma, die ich immer präsent habe auch wenn ich nicht immer so oft anrufe, wie es eigentlich notwendig wäre. Gracias a Claudia, mi hermana pequeña que actúa de grande, y que ya hace tanto tiempo que se fue a Alemania. También a ti te tengo conmigo durante este tiempo, aun con la distancia. Y, sobre todo, gracias a mis padres por el apoyo moral, afectivo y efectivo, financiero y de tantas otras cosas que me han dado. No había contado con hacer una tesis doctoral, pero si tenéis esto en la mano, es por vosotros. Por ello, os dedico esta tesis.

Although not particularly scientific, a sampling with dog
was always much more fun. Thank you, Goya. (2003-2017)

Last, but not least, Michael, danke für Deine Hilfe, danke für Sherpa, danke, dass Du mir das Leben so reich und die Gespräche so interessant gemacht hast. Du gehörst zwar auch zum AWI, wie das AWI zu Dir, aber Du hast Dir auch ein eigenes Eckchen von meiner Dissertation verdient, für Deine Unterstützung, Hilfe und dafür, dass Du mein Leben auf den Kopf gestellt hast. Ohne Dich wäre alles anders. Und schlechter. Danke für Alles. Ich freue mich auf alles weitere.

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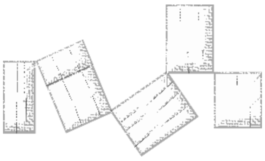
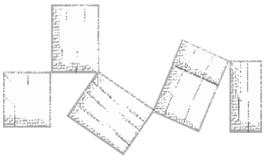


CHAPTER 1:

INTRODUCTION



Chapter 1

INTRODUCTION

1.1 Diatoms

1.1.1 Description of the phylum

Diatoms or *Bacillariophyta* are single-celled, mostly autotrophic organisms. This *phylum* has been found in nearly all kinds of waterbodies (Round et al. 1990) and it is one of the most prolific and species-rich algal groups. It could reach up to 100.000 – 200.000 taxa (Mann and Vanormelingen 2013; Mann and Droop. 1996). They have a self-made silica ($SiO_2\ nH_2O$) cell wall (frustule) divided into an upper epitheca and a lower, smaller hypotheca. Reproduction is mostly asexual (Figure 1a), where cell size decreases with each duplication (Macdonald 1869; Pfitzer 1869). During each division, a new hypotheca grows inside each of the thecae, until the size is too small to continue duplication. Then, the sexual reproduction sets in (Figure 1b-d), and is different depending on whether diatoms are centric, with (multi-)polar symmetry, or pennate (with bilateral symmetry) (Round et al. 1990; Sato 2008). Sexual reproduction culminates in a diatom zygote called auxospore, which can elongate. This gives rise to the initial cell, creating a new biggest cell.

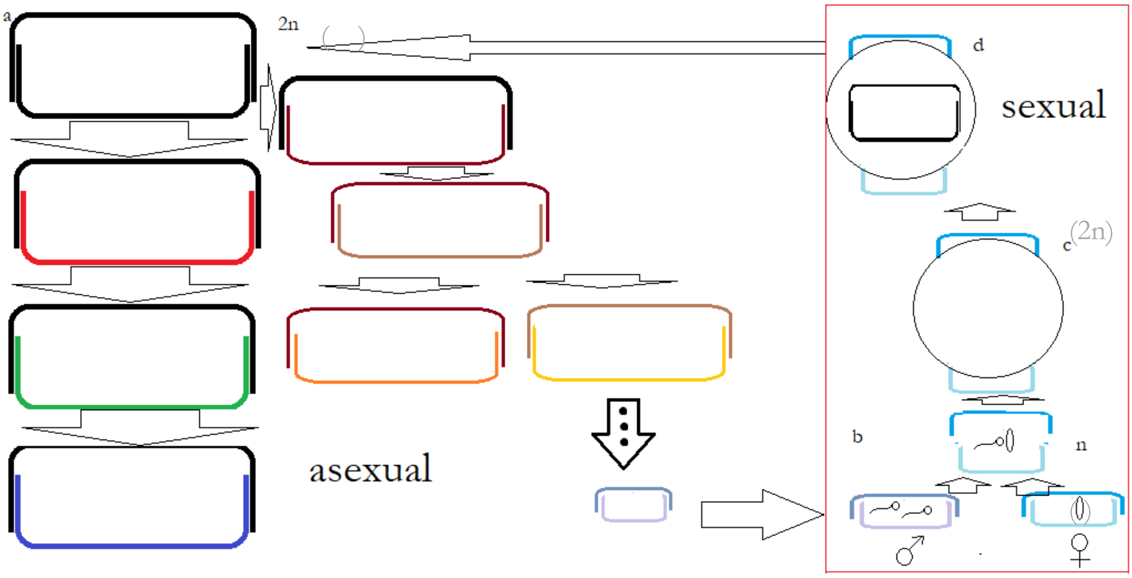


Figure 1 Description of the diatom life cycle of a centric diatom. a) Asexual reproduction after McDonald-Pfitzer (1869) hypothesis in which each theca creates new hypotheca through mitosis. b) Sexual reproduction (red square) follows the fusion of the gametes. It creates a vegetative cell or auxospore (c) that contains the later initial cell (d).

Diatoms are *heterokonts*, yet only gametes of centric diatoms display one flagellum. Pennate forms are known to have autonomic movement whenever a raphe is present. Most of their movement is derived from a mechanism proceeding from the raphe, but some may have movement derived from mucilage extrusion (Round et al. 1990). The raphe is a longitudinal canal located either in the centre (axial, e.g. *Navicula* Bory) or side (eccentric, e.g. *Nitzschia* Hassal) of a valve. It can be present on only one (monoraphid, e.g. *Achnanthidium* Kützing) or both valves (biraphid, e.g. *Navicula* Bory). And for the latter, they can have differing lengths (i. E. brachyraphid diatoms, such as *Eunotia* and *Rhoicosphenia*).

Chloroplasts of diatoms have four membranes and contain chlorophyll a, c1 and c2. Also pigments such as carotenes, fucoxanthin and other xanthinoids are found (Round et al. 1990). These give them their characteristic brown-golden colour. Some potentially heterotrophic or saprophytic diatoms are known (Lewin 1953), although for the most part, diatom photosynthesis is particularly efficient. It produces the by-product of mucilage or extracellular polymeric substances (EPS) in the form of polysaccharides that can increase adhesion to the substrate (Smol and Stoermer 2010). EPS also has an important part in the ecological interaction with bacteria (Bahulikar and Kroth 2008; Windler et al. 2014; Bruckner et al. 2011).

1.1.2 Traits of the diatoms

Diatom frustules have morphological differences as well as very intricate ornamentations on both valve and mantle faces (Figure 2). Traits that can be seen through the optical microscope such as striae, costae, stigmata and other structures (for more information, see Glossary). Some ornamentations, such as cribra and other velae, are visible only when observed under an electron microscope. These traits are the most commonly used in diatom taxonomy, even though the progress in phylogenetic tool development has modified the classical taxonomy to a high degree. Taxonomically, the class *Bacillariophyta* is very complex. Known cladistical systematics used up to date, phenotypic and genotypic, present a paraphyletic subdivision of differently formed diatoms (Williams and Kociolek 2007; Medlin et al. 1996). The division of diatoms of "pennate", bilateral symmetry, and "centric", (multi-)polar, is therefore paraphyletic.

Diatoms can also be subdivided into other subgroups. First, a subdivision into marine and fresh-water diatoms is important for diatom ontogeny. Even a subgroup, able to survive on wet surfaces, is known. The heterogeneity of saline aquatic environments has impeded a clear subdivision of "marine", "freshwater" and "brackish" diatoms, thus this has been changing through time (Clavero 2005). *Bacillariophyta* do not only react to salinity but also to several physical and chemical states of the water. Van Dam *et al.* (1994) created a *taxon* list based on tolerances and optima of ecological values ranging from pH, salinity, nitrogen uptake, dissolved oxygen requirements, to saprobic features, trophic state and moisture.

Diatoms can also be divided depending on whether their habitat is planktonic and benthic. Both kinds are used to ascertain water quality indices following European legislature (Water Framework Directive, Chapter 1.2) for lakes and rivers, respectively (European Commission 2000). In our studies we have focused on the benthic communities, which are attached to a substrate like e.g. on the river bed, since they are the main colonization form of the rivers as lotic systems, and thus the main diatoms used for riparian biomonitoring. Planktonic, or free floating, species tend to be carried away by currents and hence would not be useful in a lotic environment. However, several planktonic diatoms were found at some benthic sites. This can signal the settling of these diatoms, either dead or alive, into the benthos.

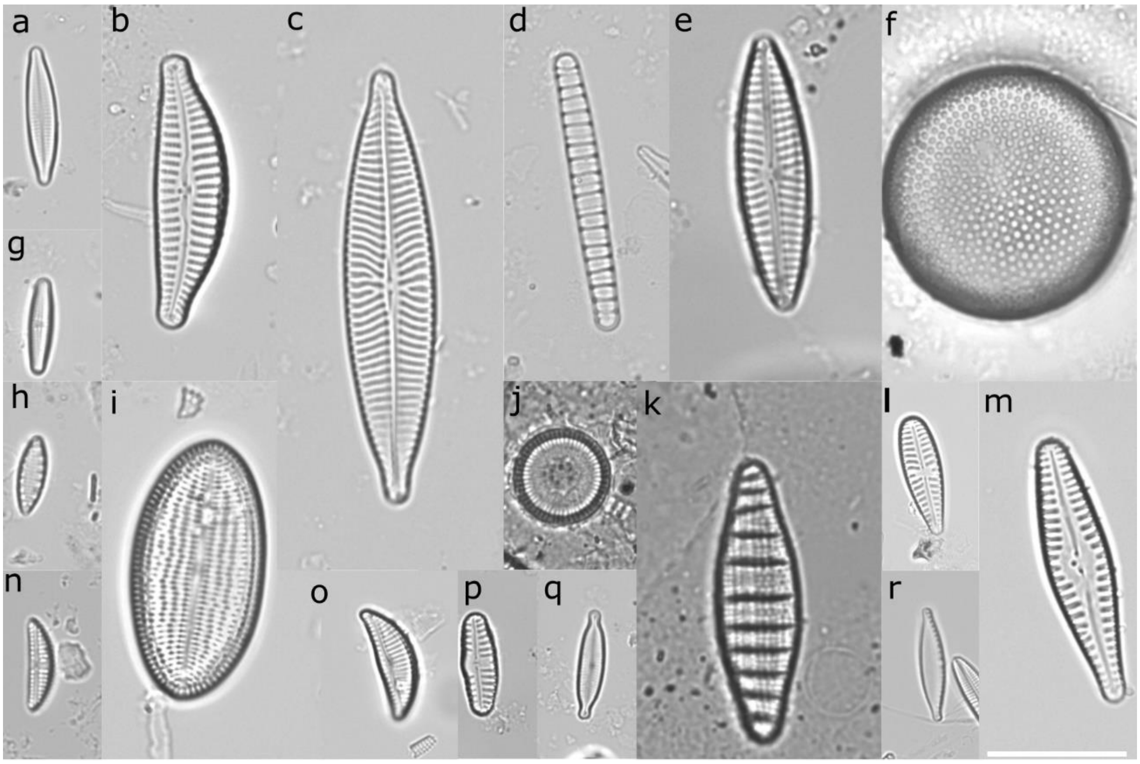


Figure 2 Example of benthic diatoms. Monoraphids: a) *Achnanthidium pyrenaicum* (monoraphid), b) *Cymbella excise* (asymmetric biraphid), c) *Navicula cryptocephala* (symmetric biraphid), d) *Diatoma tenuis* (araphid), *e) Navicula cryptotenella* (biraphid), f) *Aulacoseira granulate* (centric), g) *Achnanthidium minutissimum* (monoraphid), h) *Nitzschia inconspicua* (biraphid), i) *Cocconeis lineata* (monoraphid), j)*Cyclotella cf. ocellate* (centric), k) *Denticula tenuis* (biraphid), l) *Gomphonema olivaceum* (biraphid), m) *Gomphonema stoermeri* (biraphid), n) *Amphora pediculus* (biraphid), o)*Encyonema minusculum* (biraphid)*,* p) *Reimeria sinuate* (biraphid), q) *Encyonopsis minuta* (biraphid), r) *Nitzschia supralitorea* (biraphid). White stripe = 10 μm.

Diatoms can adhere to different substrates. They live on sand (epipsammic), stones (epilithic) and macrophytes (epiphytic), or even animals (epizoic). In a benthic, epilithic diatom sample for biomonitoring, epiphytic diatoms must be ignored or, at least, noted, since communities differ and change bioindicative values. Their valves result from dead cells and hence are not part of the epilithic community. For instance, the *Cocconeis placentula* Ehrenberg complex tends to colonize macrophytes (Cambra and Garcés 2010). Epipsammic diatoms can also be found on stones, either living or dead.

Rimet and Bouchez (2012; Berthon et al. 2011) established a preliminary subdivision of recognizable diatom life forms. They created groups depending on the form and distribution of diatoms, as well as differentiating colonizing, motile and teratological forms. These forms have been increasingly used characterizing water bodies (Riato et al. 2017). The life forms are either properties (whether diatoms are mobile through the entirety of the water column or pioneers) or traits of diatoms' attachment (tubes, rosettes, adnate forms, ribbons, pedunculated). Ecological guilds (Passy 2007a) or groups have been described as high profile (exposition to the environment resulting of the extension from the substrate enabling communities to fight for resources, not resisting mechanical stresses), low profile (small colonies tolerant to external and nutrient stress), motile (moving) and planktonic diatoms.

1.1.3 Diatom physiological response to chemical compounds and physico-chemical traits

Diatoms are organisms with an important impact on the general biogeochemical cycles of e.g. carbon (Brembu et al. 2017; Smetacek 1999), phosphate, nitrate and silica. Due to their overall abundance, their influence on carbon fixation has been deemed to range from 20 – 40 % of total global primary production (Field et al. 1998; Falkowski et al. 1993; Riding 1992; Falkowski et al. 1998). A grave result from diatom biomass is the enormous effect that diatoms, mostly in marine phytoplankton, have on carbon sequestration (Smetacek 1998, 1999).

Diatoms are mostly characterized by their participation in the silica cycle. Thus, the uptake of this mineral is particularly important. Cells obtain silicon from soluble orthosilicic acid, soluble silicate and organosilicon. These compounds are encased in plasmalemma and sequestered into the vacuole during cell formation (Round et al. 1990). Silicate ($SiO_4 \cdot nH_2O$) makes up 10 – 72 % of the cell wall (Schmid et al. 1981). The silicic acid sequestration is achieved through active Silicon Transfer Proteins (Shrestha and Hildebrand 2015; Hildebrand 2003; Reeves and Volcani 1984; Sullivan and Volcani 1973) and thus requires ATP. Most soluble silicate is found at pH below 9. Thus, diatoms are usually not found in particularly alkaline waters. The presence of silicon was related to regulating gene expression (Okita and Volcani 1980). The absence of silicon also paralyzes cell division, inhibits DNA replication and decreases chlorophyll and carotenoid production, reducing photosynthesis and glycolysis and increasing lipid synthesis (Sarthou et al. 2005). An increase in silicate concentration is usually followed by a shift to diatom predominance on the substrate (Round et al. 1990). It increases diatom growth rates, favouring a higher range of intraspecific diatom sizes and thinner frustules (Round et al. 1990).

Also, uptake of other essential functional nutrients depends on silicate presence. As Round et al. (1990) stated: "in waters enriched by run-off from silica-rich sites or on sediments, there is a continual supply of silicic acid and the succession of species must be caused by factors other than limitation by silicate (see also Admiraal 1984)". For example, nitrate and phosphate concentrations, can be the limiting factor. Nitrate tends to be obtained as sodium nitrate. It is transported into the cell actively, as observed in *Phaeodactylum tricornutum* Bohlin (Syrett et al. 1986). In general, nitrogen uptake is linked to the amino pathway and also requires ammonium accessibility (Rhee 1978). Some species are more adept than others for the uptake of nitrogen or phosphate (Suttle and Harrison 1988). Life forms can also determine the ease or difficulty in uptake of nutrients (Burkholder et al. 1990). Limitation in nitrogen or phosphate has different outcomes. Nitrogen limitation drives an increase in cell size for storage (e. g. in marine systems) whilst phosphate limitation tends to decrease cell size, as found in continental waters (Litchman et al. 2008).

Temperature rising can also impact diatom volume, decreasing cell size, though arguably not as the only relevant parameter (Svensson et al. 2014). This trait has no clear, universal effect on carbon and nitrogen uptake of bigger diatoms (Montagnes and Franklin 2001). On the other hand, conductivity affects diatoms to a substantial degree. A stronger ionic gradient increases the probability of cell water depletion from a hypertonic environment, thus some affectation of size may also be due to conductivity (Potapova and Snoeijs 1997). The main counteraction of the cells is the polymerization of mannose to polymannose (Paul 1979), but also other mechanisms are known (Dickinson and Kirst 1987).

Diatoms can be affected by metal pollution. Main examples are germanium, copper and zinc that interfere in the silicate pathway (Azam et al. 1973; Martin-Jezequel et al. 2000; Thamatrakoln and Hildebrand 2008; Round et al. 1990; Jaccard et al. 2009). Mercury, cadmium and lead, inhibit cell division (Gélabert et al. 2007; Morin et al. 2008; Rivkin 1979; Kohušová et al. 2011). They also interfere with cell wall formation, often producing teratological diatoms (Pandey and Bergey 2016). Other heavy metals are targeted by the European priority substance act (European Parliament and Council of the European Union 2001, 2013). Chrome, nickel, selenium and antimony do not affect diatoms up to a concentration of about 1 μM (Round et al. 1990), whilst these concentrations produce fatality in other organisms. Thus, diatoms are mediocre indicators for these metals.

1.1.4 Benthic diatoms as bioindicators (historical review)

Since the 1970s a clear relation of certain diatom taxa with water traits was observed (Lange-Bertalot 1979b; Kobayasi and Mayama 1989). Sensitivity, i.e. whether a specific taxon could endure adverse circumstances, and tolerance, i.e. the range of a substance's concentration within which a taxon may survive and procreate, are the main characteristics studied. Zelinka and Marvan (1961) described a formula based on these aspects of diatom lives:

$$DI = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j}$$

DI is the diatom index, a_j is the abundance of species j, s_j represents the pollution sensitivity of the species and v_j describes its indicator value established empirically (tolerance). Most of the diatom indices are calculated using variations of this formula. The Ebro river basin Authorities usually employ 3 trophic diatom indices. IPS or "Specific Pollution-Sensitivity Index" (Coste 1982) has the highest proportion of diatom taxa for which s_j and v_j have been characterized. For the Spanish diatom flora it has been observed to be a better descriptor than the other indices and therefore is the official diatom index chosen in legislature (Martín et al. 2010; Sabater 2000; Ministerio de Agricultura Alimentación y Medio Ambiente (MAGRAMA) 2013). IBD or "Biological Diatom Index" (Prygiel and Coste 1998; Coste et al. 2009; AFNOR 2007), also based on the Zelinka and Marvan (1961) formula, utilizes a less developed taxon database. But it seems to be highly correlated to IPS (Oscoz et al. 2007), as yearly reports attest (Cambra and Ortíz-Lerín 2005, 2006; Flor-Arnau and Cambra 2007; Flor-Arnau et al. 2008; Cambra et al. 2003; Gomà et al. 2002; Cambra et al. 2012). Finally, the CEE or "European Economic Community Index" (Descy and Coste 1990) compares existing communities within each sampling site, and also has been shown to be correlated, to a lesser degree, to the before mentioned diatom indices. Rarely, TDI or "Trophic Diatom Index" (Kelly and Whitton 1995) has been used. Other parameters such as saprobic input have not been calculated by official authorities. Sládecek index is occasionally calculated in research (Sládeček 1986, 1973).

To calculate these diatom indices, a software was developed by Lecointe et al. (1993). OMNIDIA calculates 18 diatom indices and 33 ecological statistic parameters (Lecointe and Coste 2015). Diatom taxonomy and ecological traits are updated annually.

1.2 European water law : Water Framework Directive

In the year 2000 the Water Framework Directive or WFD (European Commission 2000) was created to homogenize freshwater quality care throughout Europe. One of the main objectives was the expansion of water protection to all water bodies. Another aim was to improve to a specific water quality within a given time and to regulate a basin-based water management. The commission homogenized pollution limits, prices, citizen involvement and a simplified legislation.

To obtain a standard of ecological water quality, it was no longer to be assessed only chemically, it also utilized bioindicators. For receiving a status of "good" quality, a deadline was set ending in 2015, using the "one out, all out" approach: If one indicator had less than "good" quality, the site was regarded as less than good.

To establish the water quality, firstly, undisturbed or "reference" sites were selected and described. Intercalibration exercises were then made to find patterns throughout Europe. Communities of different organisms were compared to check whether they coincided throughout Europe or if they were rare or endemic. After the deadline, in 2016, the policies are tracked in an official website (European Commission and European Environment Agency 2016).

River and lake types were later subdivided using only geomorphological and physical traits. This did not show any correlation to species distribution. WFD established fish, macroinvertebrates, macrophytes, phytoplankton and phytobenthic diatoms as bioindicators. Those appear in all kinds of continental and coastal waters. Thereafter, indices based on them were developed or adapted for most of the countries of the European Union. Better subdivision is still being enforced in 2017 (BOE 2016). Each index or traditional chemical indicator has a specific EQR (ecological quality ratio). As already stated, the final water quality was determined entirely on the worst value.

Later, the Priority Substance Regulation was added (European Parliament and Council of the European Union 2001, 2013) decreasing maximum permissible values of concentration of dangerous products in European Rivers. These regarded heavy metals, pesticides or organochlorides, as well as other substances affecting life. These substances also can bioaccumulate and are monitored at known impact sites, for instance at industrial effluent outlets.

The WFD aimed to "[create] a programme of actions to be implemented by the year 2000 aiming at sustainable management and protection of freshwater resources" (point 3) with one important pitfall: Rivers of an intermittent or temporary regime were not considered. These river types, mostly found in Mediterranean climates, are still exploited, thus, defined as resources, and should be under the legislative umbrella of the WFD. Projects like SMIRES (Science and Management of Intermittent Rivers and Ephemeral Streams) COST Action and Life TRivers try to create a clear management framework for these overlooked waterbodies (more in chapter 1.3.3.1 Micro-scale, Temporary Rivers).

This thesis addresses the effects of water characteristics on diatom taxonomy, morphometry and life forms, their relationship with the bioindicative values and the different scales in the catchments they inhabit.

1.3 Diatom studies in this thesis

In this dissertation, we investigated how diatoms react to their environment in all scales. To do so we not only used typical diatom feature, diatom communities and their resulting bioindication values, but we also studied how other diatom features, such as their three-dimensional adhesion (as life-forms and ecological guilds) and their morphology related to water composition. We have identified diatoms, partly characterized them *in vivo*, and measured the diatoms morphometrically. For each of the three scales (macro-, meso- and micro-scale) we focused on different points. A multi-scalar approach is not unheard of in hydrological (Allan et al. 1997) and entomological studies (Sponseller et al. 2008).

One of the key features of this thesis is the use of 10 years' worth of diatom samples from the Ebro river. A study of these characteristics can potentially help to observe community changes in time and thus increase our fundamental knowledge of diatom community dynamics.

1.3.1 Macro-scale: Diatom taxa communities

Nowadays, standardized diatom communities (or phytosociology) are a mostly forgotten tool (Margalef 1954). However, the subdivision of the river ecotypes, as established by the WFD and enforced by the states of the European Union (Cedex-MMA 2005), does not take organisms into account. This could produce a bias at the EQR establishment. To address this problem, we followed the example of Tison and collaborators (2004) and Tornés et al. (2012) and characterized the Ebro river basin by describing diatom communities, as an addition to the subdivision established for 2005-2006 (Ortíz-Lerín 2012). This way, a more comprehensive division of sampling sites was achieved, providing a deeper understanding of communities in their environment. We used inventories from sampling sites visited more than seven times within ten years (2002-2013).

Diatom inventories from 50 sites of the eight river ecotypes of the Ebro river were used (Table 1Table 1). Physico-chemical traits of the river ecotypes were mostly differentiable by the original subdivision methods (AnnexFigure 1). Because of this, the differentiation was mostly visible in altitude, conductivity, nitrate and water temperature. All of these were traits interrelated with each other: Altitude affected land use (Viviroli and Weingartner 2004) and temperature, which in turn affected nitrate levels due to fertilizer use. Bioindicators were fitted following the Spanish legal framework (Confederación del Ebro 2006).

Table 1 The eight river ecotypes of the Ebro river according to Confederación Hidrográfica del Ebro and their sampling coverage.

River Ecotype	**Sampling Proportion**	**Altitude (m)**
109 - Mineralized Rivers from Low Mediterranean Mountains	6 (12%)	172 – 629
111 - Mediterranean Siliceous Mountain Rivers	5 (10%)	996 – 1630
112 - Mediterranean Calcareous Mountain Rivers	9 (18%)	265 – 833
115 - Continental and Mediterranean Slightly Mineralized Axes	12 (24%)	110 – 484
116 - Continental and Mediterranean Mineralized Axes	2 (4%)	242 – 669
117 - Main Axes in A Mediterranean Environment	3 (6%)	8 – 225
126 - Rivers of Wet Calcareous Mountains	13 (26%)	411 – 1341
127 - High mountain rivers	0 (0%)	> 1300

We investigated whether diatom communities were congruent with the geological-physico-chemical division, since diatom communities usually are dependent partly on conductivity, as well as the concentration of nitrates and silicate tetraoxide. As this was not observed, the expected diatom communities were established using several statistical approaches. The subdivisions were then characterized. "Diatom-ecotypes" were expected to be time resilient, though affected by diatom community dynamics.

1.3.2 Meso-scale: Land use and diatoms

Diatom communities respond to chemical changes in water (Lange-Bertalot 1979a). But these changes are mostly driven by external parameters. Land use is one of them (Quinn et al. 1997). We studied how the changes of land use affected diatom communities in the Segre sub-basin of the Ebro river, taking this area as the meso-scale and defining the influence area of the diatom sampling sites as the catchment 5 km upstream (Burfeid Castellanos and Cambra 2016).

Land uses were extrapolated from overhead aerial orthophotographs. Six main uses were detected: agricultural, dry land, meadow, urban (comprising both industrial and residential cover), waterbodies and wood. All these uses were deemed to differ in effluent characteristics, superficial runoff and water transparency. The characterization, based mostly on Allan (2004), was as follows:

- **Agricultural land**: can have a reduced runoff, due to cultivated fields, that leads to higher transparency. It tends to suffer a transient increase of nutrients (point pollution), because of fertilizer use or animal farming, affecting nutrient concentrations.
- **Dry land**: scarce vegetal coverage can increase runoff and reduce water transparency owing to sediments. No clear correlation to effluents is assumed.
- **Meadow**: reduced runoff due to plant cover. No clear correlation to effluents is assumed, although some increase of nutrients could occur due to animal input.
- **Urban land**: Increase of runoff due to paved streets, and thus higher chance of flash floods. Increase of effluents both in nutrient and dangerous substances (Ministerio de Medio Ambiente y Medio Rural y Marino 2008).
- **Waterbodies**: describing mostly reservoirs, but also takes increase in catchment into account. Can have a filtering effect on nutrients and effluents (Sabater et al. 2011). No effect on water transparency, other than by planktonic growth.
- **Wood**: low runoff due to radicular systems. Nutrients found in mostly low concentrations, which can be increased in autumn because of fall of leaves and subsequent composting.

Both diatom communities and bioindicator values were obtained for each of the 16 sites. The goal was to ascertain, whether and how diatom communities are affected by changes of Land Cover.

1.3.3 Micro-scale: Several approaches

Micro-scale is the most used approach, where diatom communities usually represent the site they were retrieved from, and biogeography or physico-chemical conditioning due to land cover changes are not taken into respect. The taxonomic identification of diatoms for the sites of the Ebro Control Network has already been performed diligently (Ortíz-Lerín 2012; Cambra et al. 2003; Flor-Arnau and Cambra 2007; Cambra and Ortíz-Lerín 2005, 2006; Cambra and Garcés 2010; Cambra, Flor-Arnau, and Burfeid Castellanos 2012). For this thesis chapter we have proceeded to studying additional changes inside diatom communities, such as life forms and morphometry.

1.3.3.1 Life Forms and Ecological Guilds

One disadvantage of species-based biomonitoring is that it is difficult to perform and requires strong skills in diatom taxonomy. Other methods are being developed to ease the determination of water quality through diatoms. Such methods include metabarcoding, obtaining taxon information through the genetic scanning of particular markers (Zimmermann et al. 2011; Zimmermann et al. 2014). Another method is the division of diatoms into the observable life forms and ecological guilds (Pandey et al. 2018; Law et al. 2014; Dunck et al. 2016).

Life forms of benthic diatoms are determined by the adhesion of individual cells to either their substrate or their neighbouring clonal cells (Rimet and Bouchez 2012). Ecological guilds characterize the location inside the periphyton layers (Passy 2007a). A three-dimensional community of diatoms can and will be affected by external influences (Berthon et al. 2011). Thus, more and more studies are made on this trait-based characterization of the diatoms' environment (Riato et al. 2017; B-Béres et al. 2016, 2014). Diatom ecological guilds were also subdivided by biovolume size according to B-Béres et al. (2017): S1 (5-99 μm^3), S2 (100-299 μm^3), S3 (300-599 μm^3), S4 (600-1499 μm^3) and S5 (>1500 μm^3). In this thesis we have tried to obtain a better insight into the water traits that determine both life forms and ecological guilds (AnnexTable 1) by performing colonization experiments and investigating the effects of temporary rivers on those traits which are ecologically moulding.

Ecological guilds were determined by the exposition to their environment or motility of the diatoms as well as diatom colony types (Passy 2007a, 2007b). Passy proposed three groups, thoroughly explained in chapter 6.1: high profile guild (HPG), defined by diatoms extending from the substrate to gain exposition to water, and also big diatom colonies, low profile guild (LPG), with lower, small and less exposed diatom colonies, and motile guild (MG), diatoms with the ability to move inside their microhabitat to the most favourable location. Each of these guilds was also defined correlating to a type of habitat, such as a wide range of tolerance for nutrients, with a high (HPG) or low (LPG) sensitivity to current, or a selection of optimal microhabitat (MG). Later studies established the necessity of adding further life forms and a fourth group to the ecological guild: planktonic diatoms that sediment to the benthic habitat (Berthon et al. 2011; Rimet and Bouchez 2012). The same group later excluded the planktonic group for their characterization of physico-chemical parameters in biomonitoring (Marcel et al. 2017).

Life forms added species characteristics, such as mobility within the water column, teratology and pioneer taxa, as well as 11 life forms and the characterization into either colonial or non-colonial forms (Table 11).

1.3.3.1.1 Water traits and diatom life forms

The experiment was designed to obtain information on how a variation of physico-chemical traits affects diatom life forms. A hypersaline site (Salada stream), an oligotrophic stream (Llobregat headwaters) and an eutrophic stream (Clarà stream) were selected. Artificial substrates, with 9 quarry unglazed tiles, were deposited for one month in 2016 to be colonized. Three of them were left at each sampling site after this colonization period, the other six were divided and put into the other sampling sites for new colonization. After another month, all substrates were extracted. The samples from the Clarà stream had disappeared.

Diatoms were examined *in vivo* to establish taxon identification and the adherence type. Even though life forms and ecological guilds have been both tested and improved (Riato et al. 2017; B-Béres et al. 2017), their physico-chemical characterization is relatively low (Marcel et al. 2017). Other functional groups have also been used and studied in the US (Hausmann et al. 2016). In the present study we compared the Riato-Rimet life forms and B-Béres-Passy ecological guild sizes and how they react to the physico-chemical parameters.

1.3.3.1.2 Temporary Rivers and diatom life forms

One of the most common river types of the Mediterranean Basin is temporary rivers. These rivers are characterized by total or partial loss of surface water (Stubbington et al. 2017). Regulation of intermittent rivers and ephemeral streams (IRES) has been neglected by the Water Framework Directive (WFD, 2000/60/CE, European Commission 2000) until now.

Global change models of Mediterranean climate posit that these types of rivers are deemed to increase (Figure 3 3). Thus, the lack of regularization of these rivers has a potential detrimental effect. In view of this tendency of increasing, studies on the eco- and hydrological effects on all types of water bodies have increased since the late 2000s, e. g. for lakes (Leira and Cantonati 2008) and rivers (Hughes et al. 2009). Currently, an international COST (Cooperation in Science and Technology) Action project named SMIRES (Science and Management of Intermittent Rivers and Ephemeral Streams, project: CA 15113) unites European Scientists working on this subject.

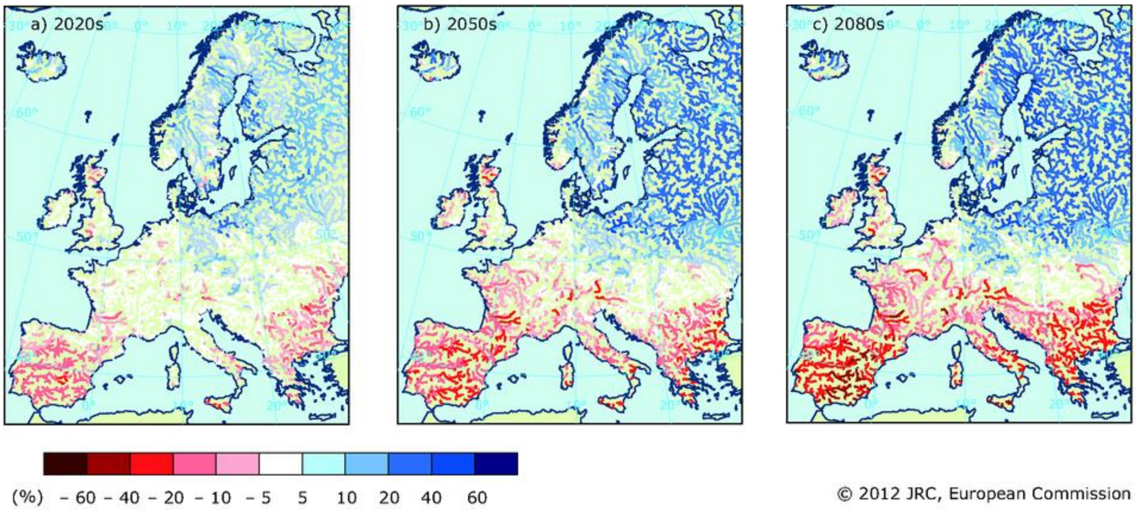


Figure 3 Projections of the change of minimum river flow in Europe facing climate change. Relative change in minimum river flow in a 20 years recurrence interval, modelling the future period on 1961-1990 (SRES A18) (Source: modified from European Environment Agency, JRC, European Commission, 2012).

To create a multidisciplinary tool for temporary river management inside the WFD, the LIFE TRivers project was launched (Project: LIFE13 ENV/ES000341). In this framework, Gallart et al. (2012), building on the MIRAGE project (Mediterranean Intermittent River Management, project: ENV 2007.2.1.2.3), created a toolbox called TREHS in 2016 that divided the river regimes into perennial water bodies, intermittent pools and intermittently dry catchments. The toolbox also regarded the episodic streams, that have not been sampled for diatoms in this instance. Moreover, the sampled water regimes were characterized by their aquatic states (Figure 4). These were hyperrheic (floods), eurheic (normal flow), oligorheic (pools interconnected with water trickle), arheic (disconnected pools), hyporheic (high saturation level of sediment) and edaphic (dry sediment). Even though most rivers had a periodical or temporary variability, some water bodies maintained one aquatic state. This can complicate the biologic/ecological part of the water quality monitoring. The project was granted also to study the affectation of the main riverine indicators, such as macroinvertebrates, macrophytes, fish and diatoms. The aim was to help to the legislation of these potentially indispensable water bodies. To this end, and in the frame of this thesis, diatoms were used as bioindicators, as these rapidly reproducing organisms react to the physico-chemical changes and these in turn can in turn be affected by flow characteristics.

Diatom communities should be able to reflect the loss of connectivity produced by river intermittency due to their rapid generation time. The main aim of this study was to observe whether benthic diatom functional groups and life forms can be used as indicators of flow disruption due to water level fluctuations.

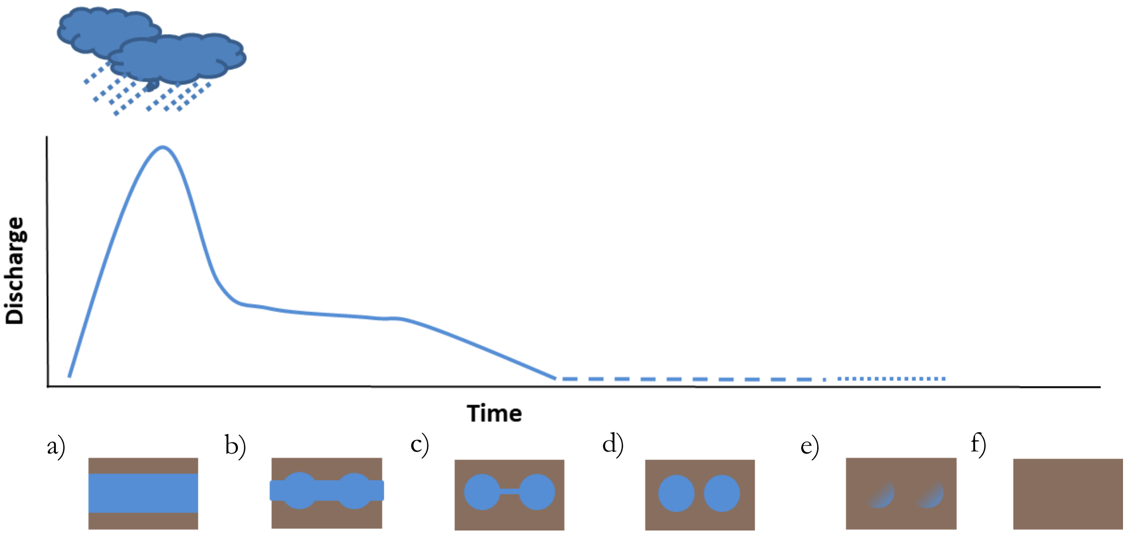


Figure 4 Water discharge and connectivity graph describing aquatic states. Types of discharge: a) hyperrheic, b) eurheic, c) oligorheic, d) arheic, e) hyporheic, f) edaphic. After N. Cid (unpublished).

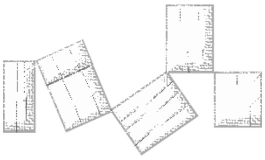
1.3.3.2 Diatom Sizes and Ecological Gradients

Each diatom taxon, as described by taxonomists and biomonitoring agencies, may be found in separate locations with similar physico-chemical traits. Measurements of their specific frustule morphology encompass a range of sizes for each taxon, as described by taxonomists, for instance Dreßler et al. (2015); Jahn et al. (2009); Williams (1985). But until now this variability has not been satisfactorily explored in an environmental context, which is addressed in this thesis chapter. Knowledge of diatom species size distributions are currently mostly based on cultures. Some allometric approaches have been made (Tomas 1979), but these examples are rare. A more systematic approach to observe the allometric effect on the whole community has been started with this work.

Other studies focused on one or more traits of the benthic diatom or phytoplankton morphology. They related morphometry traits mostly to nutrients (Litchman et al. 2008), conductivity (Pandey et al. 2018), pH (Wunsam et al. 2002) and water temperature (Svensson et al. 2014). In additions to these, also traits like concentration of suspended solids (Lavoie et al. 2010) and other indirectly linked environmental parameters such as concentrations of ammonium and dissolved oxygen have been used for our study.

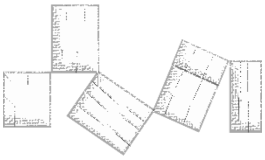
We used a 10-year diatom sample library as an important asset to assess the effect that the environment can have on diatom communities' morphology through time. Technology now permits an automatic and fast capture of sample images. A need for the digitalisation of diatom samples has been a clear but slow endeavour for the big diatom collections: the Hustedt diatom collection (Beszteri and Alfred-Wegener-Institut Helmholtz-Zentrum für Polar und Meeresforschung 2006), or the one at the Natural History Museum London (Natural History Museum 2014), for example. In addition to the automatic digitalisation, new informatic advances have allowed the development of new and more versatile software packages for diatom recognition. Competing laboratories work on software that will finally be able to identify diatoms correctly. Currently, the different programmes are only able to give approximate identifications of taxa (e.g. Bueno et al. 2017; Kloster et al. 2014; Stela Ballester 2016), but machine learning algorithms seem to be on the brink of identifying diatoms.

As the Ebro Basin Biomonitoring Network is an official institution, a reliable diatom database needs to be publicly available. Raw data, both physico-chemical and species inventories are freely accessible (Confederación Hidrográfica del Ebro 2015). An image library of samples of the most characteristic sites would be a useful asset to obtain more information but is not available until now, even though combining the images with physico-chemical data could help to establish the relationship between individual valves and their environment.



CHAPTER 2:

OBJECTIVES



Chapter 2

OBJECTIVES

The main objectives and aims of each of the chapters are:

Macro-scale

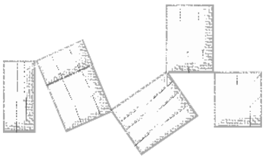
1. Describe diatom communities of the Ebro river basin using river ecotypes suitable for diatoms.
2. Search the relationships between physico-chemical, temporal and diatom communities with the original Ebro river ecotypes.

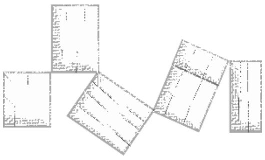
Meso-scale

3. Determine the effects of interannual variation in land use on changes in the diatom communities and physico-chemical composition of the Segre river basin (Ebro tributary).
4. Study how community diversity and bioecological index values are affected by land use and the correlation of all the previous parameters with physico-chemical composition.

Micro.-scale

5. Study changes of diatom characteristics as responses to microhabitat (site) variations.
6. Determine life forms and functional groups of diatoms in brackish-eutrophic water gradients.
7. Compare two methodologies for assessing diatom functional traits for intrinsically differentiated sites.
8. Determine life forms and functional groups in flow gradients in intermittent rivers and ephemeral streams.
9. Extrapolate whether hydrologic regime, aquatic state or both are correlated with life forms.
10. Investigate diatom morphology changes which are a result of spatial and interannual variation of physico-chemical parameters in sites of the Ebro river.





CHAPTER 3:

MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

3.1 Materials and methods

Benthic diatom samples were taken yearly at the Ebro river basin, monthly for the experimental setup and bi-monthly at the temporary river sites (Table 2). For the period investigated in this work, different persons took care of sampling and preparation. The sampling followed the European and Spanish norm (AENOR 2014, 2004) and the Ebro river sampling protocol (Cambra et al. 2005). Thus, diatom communities were scrubbed with either a toothbrush or knife from five randomly collected stones from the river bottom (Kelly et al. 1998). The resulting scrub was put into a mixture of river water and either 4 % formaldehyde (until 2012) or 70 % ethanol. In the laboratory, an aliquot of the samples was digested according to the 30% hydrogen peroxide (110 ºC for 12 h)-hydrochloric acid (1M) protocol and washed with distilled water (Cambra et al. 2005). From the suspension, one drop was dried on a coverslip, which was subsequently mounted on a microscopic slide using Naphrax® (Brunell microscopy) mounting medium.

Table 2 Total number of sampling sites and samplings for each of the studies, including main basin.

Study	Sampling periodicity	Basin	Number of sites	Number of samples
3. Macro-scale	yearly	Ebro	50	356
4. Meso-scale	yearly	Segre (Ebro)	16	60
6.1 Micro-scale I (experiment)	monthly	Llobregat	3	15
6.2 Micro-scale II (temporary rivers)	bi-monthly	Catalan rivers	10	47
	bi-monthly	Ebro	5	25
	bi-monthly	Júcar	9	39
6.3 Micro-scale III (cell size distributions)	yearly	Ebro	8	48

All samples were investigated using a Zeiss Jenaval (Carl Zeiss Jena) microscope using a 100x objective (GF Planochromat PhV HI 100x/ 1,30 ∞/0.17-A) and Differential Interference Contrast (DIC), except for the samples for size measurements that followed a different protocol, explained in Chapter 6.3. Diatoms were identified following the Spanish and European norms (AENOR 2005;

AENOR 2014b). During preparation, diatom frustules usually separate, thus, only valves are used for identification and calculation of diatom biotic indices. For the identification to the lowest taxonomical level possible, general bibliography was used (Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Hofmann et al. 2011; Bey and Ector 2013). If needed, monographs were used to increase the taxonomic resolution (Levkov et al. 2013; Williams 1990, 1985; Romero and Jahn 2013; Trobajo et al. 2013). Nomenclature was updated using Diatombase (Kociolek et al. 2015). To increase statistical significance, a minimum of 400 valves per sample was identified. For the micro-scale experiment 1,000 valves were counted whenever possible. All inventories were standardized to the proportional abundance (percentage of each species on the total count) and transformed [log(x+1)]. The transformation was made because of value disparity, to increase the visibility of rare diatom taxa or life forms.

The resulting diatom inventories were inserted into Omnidia software (Lecointe et al. 1993) to calculate index values. Spanish legislature established the Specific Pollution-sensitivity Index, IPS in its French initials (Coste 1982), to be the standard index for diatom biomonitoring (Ministerio de Agricultura Alimentación y Medio Ambiente (MAGRAMA) 2013; Ministerio de Agricultura 2008). Other indices used were the Biologic Diatom Index, IBD in French (Prygiel et al. 2006; Prygiel et al. 2002) and the CEE index (Descy and Coste 1990). More information regarding diatom indices is given in Chapter 1.1.4.

In the experimental setup, diatoms were observed *in vivo* to gather the main life form of the different taxa (Berthon et al. 2011). The database established by Rimet and Bouchez (2012) was used to translate the taxonomical data into life forms and ecological guilds for the temporary rivers (AnnexTable 1). Ecological guilds were also subdivided in size categories, following B-Béres et al. (2017). The life forms were similarly modified from the database using the Riato et al. (2017) methodology. More information on life forms and ecological guilds is presented in Chapter 1.1.3.1.

Physico-chemical data were obtained from various sources. The Ebro data came from 3-monthly samples taken according to the WFD norm and made available through the website of the authorities (Confederación del Ebro 2015). Llobregat data were obtained in situ and contrasted with the River Authorities data (Agència Catalana de l'Aigua (ACA) 2014). Finally, bi-monthly physico-chemical analyses were made during the sampling efforts of the temporary rivers. The data consisted of chemical (ammonium, oxygen), nutritional (nitrate, nitrite, phosphate, N/P ratio, silica concentrations), conductivity, alkalinity, hardness, temperature and pH. In some studies, the parameter altitude was added, although it also influencing nutrient levels and water temperature.

Statistical analyses were performed using R software (R Development Core Team 2016). The main packages used were "vegan" (Oksanen 2013a, 2014, 2013b) for multivariate analyses, "FactoMineR" (Husson et al. 2015) for PCA, "PerformanceAnalytics" (Peterson et al. 2018) for Spearman and Pearson correlations, "cluster" (Maechler et al. 2015), "MASS" (Venables et al. 2015), and through "labdsv" (Roberts 2015) and "indicspecies" (De Cáceres 2013) for indication values (Dufrêne and Legendre 1997). For the Macro-scale division, the "diatSOM" package was used (Bottin, et al. 2014). The life form studies (Chapter 6.1 and Chapter 6.2) were compared using permanova and anosim calculations (both with"vegan"), and proved using Tukey Honest Significant Distance (HSD) test. Significance (henceforth *p*) was shown with asterisks, which are also re-stated at the necessary tables (0 '***' 0.001 '**' 0.01 '*' 0.05, non-significant values showed no asterisk).

3.2 Studied areas

Rivers with north-eastern river mouths are influenced by Mediterranean or Continental climates. The main studied rivers, Ebro and Llobregat flow into the Mediterranean (Figure 5). The TRivers project Rivers additionally included other Catalan rivers, located in the north east, but also included some rivers going down the shoreline of the Mediterranean, too (1.3.3, Figure 5).

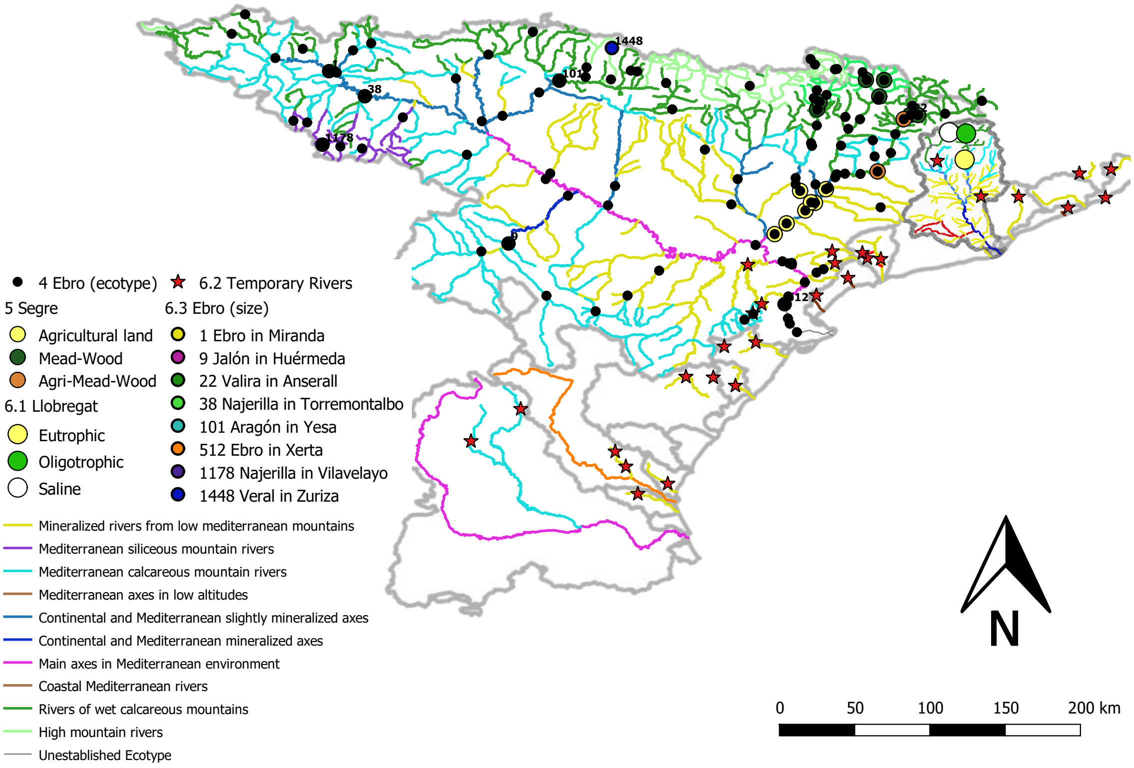


Figure 5 Sites studied in this thesis marked for each of the chapters. Map elaborated with QGIS software.

Following the WFD, Spanish rivers were subdivided into catchments, each with its own authority to bio-monitor and control. Ebro is one of the largest transborder basins in Spain. Spanish Ebro river Authorities have treaties with France and Andorra because of the shared Ebro tributaries. An example for one of such treaties is the Segre basin legislation (Communauté de Communes et al. 2007; Pyrenées-Cerdagne and Communauté de Communes 2008; Préfet des Pyrénées-orientales and Direction Départamentale des Territoires et de la Mer 2014). Other Mediterranean rivers studied in this thesis contain are the Llobregat and other Catalan catchments, managed by the Catalan river authorities (Agència Catalana de l'Aigua, ACA). This administration regulates several river basins, torrents and temporal streams, not yet regularized in the WFD, as do the authorities of the Ebro and the Júcar Rivers (Confederación Hidrográfica del Ebro and C. H. Júcar).

3.2.1 The Ebro river basin

The Ebro river basin (Chapters 4, 5, and 6, Section 3) is the biggest catchment of the Iberian Peninsula flowing west to east, coursing into the Mediterranean (Figure 6). It is more than 85,000 km² wide and exposed to quite variable climates due to its size, wherein the west rainfall is heavier and flows are higher (Barceló et al. 2011). Orography does also vary. The southern riverbank is relatively low in altitude in relation to the northern. The latter is mostly located on the Basque-Cantabrian mountains and the Pyrenees. The eastern river side passes the lower Catalan coastal mountain ridge.

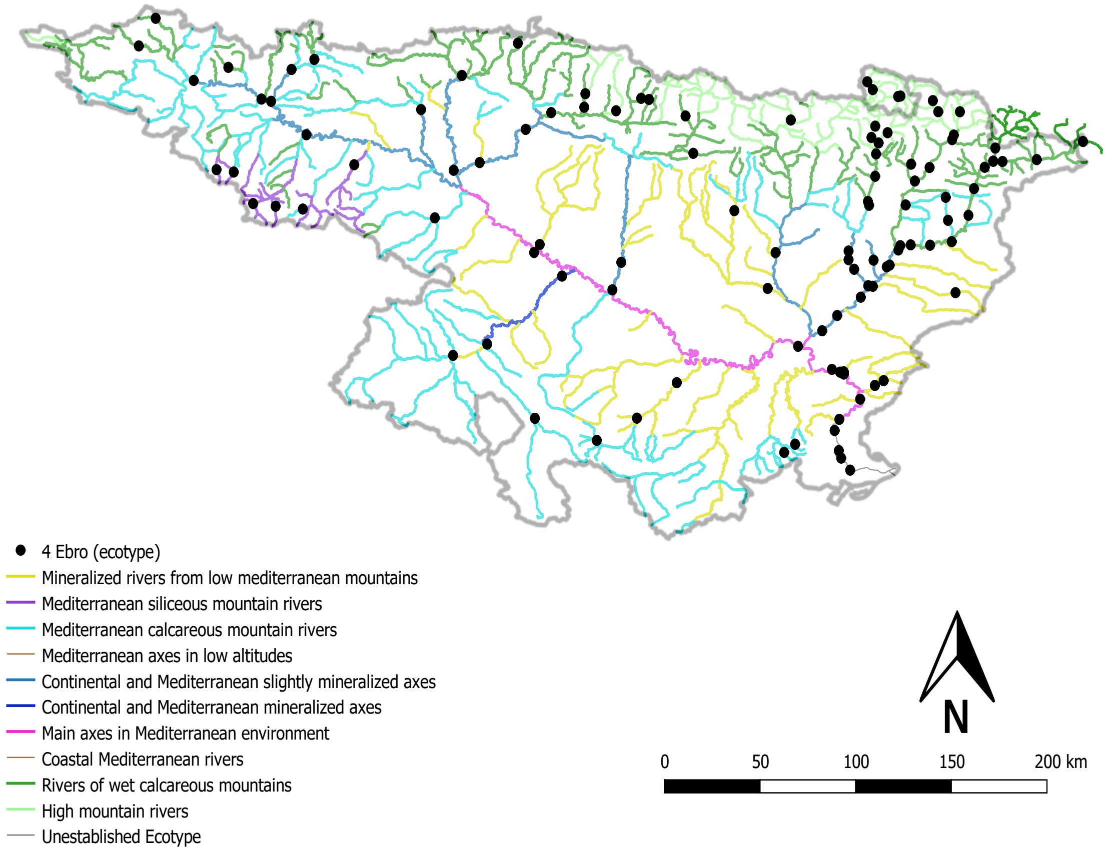


Figure 6 Subdivisions of the Ebro river Basin into Ecoregions. Points = exemplary diatom sampling in 2013.

The Ebro river basin is conformed by 702 rivers and tributaries that conform this basin with a combined length of approximately 12,00 km [www.chebro.es]. Natural rivers predominate, with 70 very modified rivers and two "artificial" ones. There are 102 lakes in the basin. In the delta region there are 16 transitional water bodies and 3 coastal lakes. The average flow in the whole basin is of 14,623 hm^3/year, albeit with substantial interannual variations due to climate.

The Main River Axis is characterized by a high anthropological input due to the location of big cities on its catchment. This implies high industrialization, a potential heavy metal input and priority substance pollution. The population density varies highly throughout the catchment. Big cities alternate with agricultural land or even the Monegros desert (Romaní et al. 2011). Population surpassed three million in 2013.

The whole basin has 125 principal reservoirs (Prats et al. 2011), of capacities over 1 hm^3. These water course changes are not regulated by the WFD. The course of the main axis is also affected by agricultural irrigation canals. These consume an average of 5085 hm^3/year of water. A total 430 hm^3/year is transferred or used for irrigation, drinking water, energy gain, or industry.

In 2005 a report on preliminary evaluation of climate change was made for this basin. An increase of temperature and a reduction of rainfall were foreseen, that would be added to the already erratic effect of the Mediterranean climate (García Vera et al. 2005). A significant reduction of water flow down to 5-50% on the volume measured in the previously studied interval from 1970-2000 were also predicted. This will reduce water quality even further (Aguilera et al. 2015; Bovolo et al. 2011). Thus, water quality needs to be improved to counteract this effect. Rivers of the southern Ebro riverbank are expected to be most strongly affected. The biological study of this basin has lagged in relation to the formal studies.

Big studies were made into flora and fauna. Studies involved creating check-lists and species distribution, based mostly on diatoms and macroinvertebrates, and to a lesser degree fish and macrophytes (Sabater et al. 2011; Oscoz et al. 2007). Nonetheless, further, more physiological understanding of the organisms could improve biomonitoring.

The rivers of the Ebro basin are in two main biogeographical regions, the Euro-Siberian region, located at the higher mountains, and the Mediterranean region, mostly at the river axis. In 2007, the Water Framework Directive established river ecotypes only based on biogeochemical and extrinsic traits (Annex Table 1), later translated for use in Spanish rivers (Ministerio de Agricultura 2008). A previous subdivision of the Ebro Basin had been attempted by Munné and Prat in 1999. They used physical and chemical parameters, cross-referencing them with macroinvertebrate community structures (Munné and Prat 1999).

A new subdivision, including the degree of alteration of the riverbed, was established in 2016 (BOE 2016). In this thesis we have not used this new subdivision, since it follows the previous one, only including a mention when altered.

Bioindicator distribution of the IPS index was calculated for each of the river ecotypes separately. A calculated Ecological Quality Ratio (EQR) was obtained from the respective reference sites to account for the variability between them (Table 3).

Table 3 Comparative ecological state ranks for each ecotype's IPS values established in 2010 (Ministerio de Medio Ambiente y Medio Rural y Marino. 2008, ARM/2656/2008). 109 = mineralized rivers from low Mediterranean mountains, 111 = Mediterranean siliceous mountain rivers, 112 = Mediterranean calcareous mountain rivers, 115 = Continental and Mediterranean slightly mineralized axes, 116 = Continental and Mediterranean mineralized axes, 117 = Main axes in Mediterranean environment, 126 = Rivers of wet calcareous mountains, 127 = High mountain river.

Ecological Scale	109	111	112	115	116	117	126	127
High	>16.8	>16.2	>16	>15.1	>14.2	>11.7	>16.3	>17.4
Good	12.6 – 16.8	12.2 – 16.2	11.9 – 16	11.3 – 15.1	10.6 – 14.2	8.8 – 11.7	12.2 – 16.3	13.1 – 17.3
Moderate	8.4 – 12.5	8.1 – 12.1	8 – 11.8	7.6 – 11.2	7.1 – 10.5	5.9 – 8.7	8.1 – 12.1	8.8 – 13
Poor	4.2 – 8.3	4.1 – 8	3.9 – 7.9	3.8 – 7.5	3.5 – 7	3 – 5.8	4.1 – 8	4.38 – 8.7
Bad	<4.2	<4.1	<3.9	<3.8	<3.5	<3	<4.1	<4.3

3.2.1.1 Diatoms at the Ebro Basin

The diatom river quality control network of this basin was established in 2002, trying to comply with the Water Framework Directive (Gomà et al. 2002). Over time, from control network instauration to 2013, a total of 459 sites have been sampled at least once. A thorough observation of the diatom flora and subdivision of the Ebro river basin (2005 – 2006) appeared in the thesis of Ortiz-Lerín (2012).

An inspection of the total flora and fauna of the Ebro river basin was included into a compendium (Sabater et al. 2011), describing patterns of diatom distribution. Thus, low diversity communities at headwaters were defined by *Achnanthidium minutissimum* (Kützing) Czarnecki and *Achnanthidium pyrenaicum* (Hustedt) Kobayasi. The upper Segre, due to its silicate content, had a quite unique community, with *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *Diatoma mesodon* (Ehrenberg) Kützing, *Encyonema silesiacum* (Bleisch) D. G. Mann, *Hannaea arcus* (Ehrenberg) R. M. Patrick, *Fragilaria capucina* Desmàzieres, *Gomphonema pumilum* (Grunow) E. Reichardt & Lange-Bertalot, *Meridion circulare* (Greville) C. Agardh and *Nitzschia pura* Hustedt.

Slower waters at the middle part of the river, were characterized by slower water flow and high mineral contents, as shown by the preponderance of *Amphora pediculus* (Kützing) Grunow ex A. Schmidt and *Cocconeis placentula* Ehrenberg. Finally, with more pollution, communities were described by *Navicula cryptotenella* Lange-Bertalot, *Sellaphora atomoides* (Grunow) Wetzel & Van de Vijver, *Craticula subminuscula* (Manguin) Wetzel & Ector, and *Nitzschia inconspicua* Grunow. In oligotrophic sites with phosphate access, appearance and even mass-formation of *Didymosphenia geminata* (Lyngbye) Mart. Schmidt was observed.

3.2.1.2 Description of exemplary sites

Here we will describe a typical site for each of the river ecotypes established for the Ebro river basin (Ministerio de Medio Ambiente y Medio Rural y Marino 2008; European Commission 2000, Annex II). We have selected sites from each river ecotype that was monitored in the morphology section of chapter 6. Since no site of ecotype 109- mineralized rivers from low Mediterranean mountains was selected, we have taken a reference site. The physicochemical description of the sites is listed in AnnexTable 2.

Ecotype 109 – Mineralized rivers from low Mediterranean mountains (Reference site 1141)

Alcanadre is a tributary to the Aragon river. It can suffer point pollution due to spills and debris and is modified upstream by two diversion dams for irrigation (Confederación Hidrográfica del Ebro and Gobierno de Aragón 2007). Despite of these caveats, both diatom and macroinvertebrate biomonitoring display high water quality (Confederación Hidrográfica del Ebro and Gobierno de Aragón 2007). The river at this site has wide catchments without slopes, moderate salinity (⌀ 371, 297 – 493 µS/cm) and temperature (⌀ 12.8, 0.8 – 24.5 ºC).

Ecotype 111 – Mediterranean siliceous mountain rivers (Referebce site 1178)

Najerilla is a stream of 72.4 km in length with a 1.107 km² wide catchment. Its total elevation gradient is 1,595 m. 8 annual samples were taken in the site of Villavelayo. Its population of over 50 citizens is surrounded by natural and forest land uses. Its water quality has always been good-very good. Nonetheless, urban spills are found in summers (Confederación Hidrográfica del Ebro et al. 2007). The main defining attribute is the soil composition. All sites of this ecotype are on the Iberian ridge.

The water composition was characterized by relatively low conductivity (⌀ 506.8 µS/cm, 272.3 – 658 µS/cm), nitrate and phosphate concentration levels were erratic, mostly low, with a peak in the years 2008 – 2009. The water temperature is moderate (⌀ 11.3 ºC, 2.4 – 16.8 ºC). The most crucial difference is the concentration of Silica (⌀ 6.787 mg/l, 5.9 – 8.53 mg/l) and a tendency to alkalinity (⌀ pH=8.4, 7.05 – 8.7).

Ecotype 112 – Mediterranean calcareous mountain rivers (site 0038)

The Najerilla river ends in the Guatizalema river, a tributary to the Ebro. The lower catchment is warm and wide, and its main land use are viticulture and irrigated land. Its sediment is mostly calcareous and porous. The catchment is 1,107 km² wide and has an average flow of 13.7 m³/s. It has two main alterations given by hydroelectric plants that vary its hydrology (Confederación Hidrográfica del Ebro et al. 2007).

Its waters are very carbonated, with sulphate peaks due to gypsum in the substrate (⌀ 81.3 mg/l, 22.2-129 mg/l). Main conductivity ranges mainly between 200 and 400 µS/cm and can go up to 700 µS/cm, with an increase between 2007 and 2013. Nitrate concentration can reach up to 18 mg/l. Industrial point pollution has been recorded, and thus the protocol for dangerous substances has been activated (European Parliament and Council of the European Union 2001, 2006).

Ecotype 115 – Continental and Mediterranean slightly mineralized river axes (site 0101)

Aragón is a main tributary of the left catchment side of the Ebro. The transversal profile is open and less sloped. It has a catchment size of 2,171 km^2 upstream, and an optimum ecological flow of 4,520 m^3/s. Its quality is good, even though the conductivity tends to increase, due to flow reduction (271 – 361 µS/cm). 28 % of the hydrologic regime are modified due to hydroelectric plants. Nutrient values are below 2 mg/l, no phosphate concentrations were registered, chemical oxygen demand and dissolved oxygen were maintained through time (Confederación Hidrográfica del Ebro et al. 2008).

Ecotype 116 – Continental and Mediterranean mineralized axes (site 0009)

Jalón is a main tributary to the Ebro river on the left side. The slope is reduced and characterized by gypsum sediments. It has an actual flow of 386 hm^3/year, when it should be 396 hm^3/year to maintain ecological function. It can have point pollution due to small townships (under 2,000 inhabitants), diffuse pollution because of dryland agriculture, water extraction and morphological alterations. (Confederación Hidrográfica del Ebro et al. 2007). Conductivity was relatively high (927 – 1869 µS/cm), as are nutrient concentrations (nitrate <2 mg/l, phosphate 0.09 mg/l, nitrites and ammonium were also found), and temperature (4.3 – 19.9 °C) (Confederación Hidrográfica del Ebro 2015).

Ecotype 117- Main axes in Mediterranean environments (site 0512)

The lower Ebro has a catchment of 3,800 km^2 with an average flow of 215 hm^3/year. The site located in Xerta is characterized by its high regulation and depletion. This is due to its use as main water supply for the municipality of Tarragona, bringing water to at least 453,000 inhabitants (Prats et al. 2011). Its riverbed consists of vegetation of holm oak groves (*Quercus ilex* L.) and scrubland, with some vineyards. This site is located at the main river axis after polluting urbanizations. Thus, water quality is not more than mediocre – good.

This river type has just theoretical reference sites, necessary to establish reference diatom communities for the European Ecological Quality Ratio (EQR). Due to being on the main axes of such a populated river, it is highly altered. It is chemically influenced by industry, by a high input of mercury. It may be affected by several industrial spills both in Flix – Ascó , located upstream of the site. A flow volume of 5.4 hm^3/s is diverted to a concession to produce 18,000 kW of electricity

(Prats et al. 2011). It has relatively high conductivity (360 – 1,709 µS/cm), relatively high phosphate concentration (0.05 – 0.51 mg/l) and nitrate levels (1 – 27.83 mg/l) and high temperature (17.6, 7.9 – 26.7 ºC).

Ecotype 126 – Rivers of wet calcareous mountains (site 0022)

Valira is a 44 km long Andorran tributary to the Segre sub-basin of the Ebro. It is characterized by granodiorites and metamorphic sediment formations in a sloped terrain with meadows and conifers as main coverage, followed by low crops. Although this site is found at a high altitude, the physico-chemical composition is defined as bad. A part of the Flow is used for a hydroelectric power. It is located after a wastewater treatment plant (WWTP) and thus, the concentrations of phosphates (0.34 mg/l) are high and dissolved oxygen (6.5 mg/l) is relatively low. Nitrates were relatively high (1.3 – 12.7 mg/l), ammonium was present (0.1 – 2.7 mg/l) and chemical oxygen demand (1.1 – 2 mg/l) and nitrite (0.01 – 0.36 mg/l)are also present (Confederación Hidrográfica del Ebro et al. 2008).

Ecotype 127- High mountain rivers (Reference site 1448)

The Veral River has its headwaters in the Aragonese Pyrenees and has a 47 km long calcareous catchment. The average river flow is of 1.9 m^3/s with a quite undulating pattern (Balcells 1984). The land uses are mainly meadows and natural beech (*Fagus*) woods. The woods reduce probability of point pollution and reduce runoff. Water quality is thus good to very good. Nutrient concentrations were merely traces, see AnnexTable 2 (Confederación del Ebro 2015).

3.2.2 The Llobregat river basin

The Llobregat River (Chapter 6, Section 1, Figure 7) is a 165 km long river placed in a 4,948 km^2 wide catchment with mixed substrate (Sabater et al., 2012). It is a greatly modified catchment (Becker 2014) located in a partially agricultural setting. Many changes were made through the addition of hydroelectric power plants and 3 dams. The first dam , La Baells, is in the main river axis and two in the Cardener tributary (Marcé et al. 2012). It is one of the most populated basins of the Catalan river system, since more than 3 million people occupy it. Most of the demographic density is at the lower course. There, agricultural and industrial pressures affect the river quality to a high degree.

This Basin suffers both anthropological and natural pressures. Some of the headwater streams, such as Saldes and Gavarresa, have a naturally high salt concentration created by halite substrate. It can have up to 20 times the marine salt concentration depending on flow (Badia Guitart 2001; Viladés Ribera 2013).

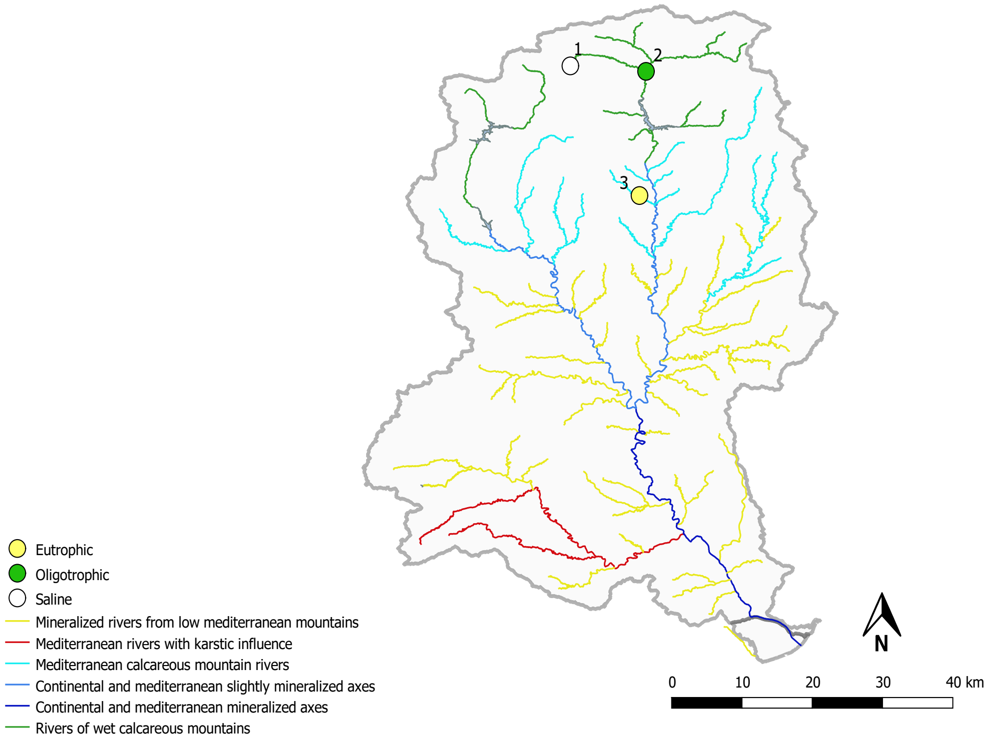


Figure 7 Location of the experimental sites in the Llobregat Basin. 1.- Salada Stream, 2.- Llobregat River and 3.- Clarà Stream.

Rivers of the Catalan catchments have multiple ecoregional divides (Cedex-MMA 2005). The Catalan Water Agency (ACA) has made previous distributions of the rivers in a repeatable pattern (Agència Catalana de l'Aigua 2015). The WFD ecoregional divide of the Spanish North East has 6 river types (Agència Catalana de l'Aigua 2015) which are: wet calcareous mountain (126), Mediterranean calcareous dry mountain (112), Mediterranean mountain rivers with high flow (115), Mediterranean rivers of variable flow (109), Rivers of the low Mediterranean distribution with karstic influences (110) and principal Mediterranean river axes (116).

For the experiment, only two of the regions were used, both Mediterranean calcareous mountain rivers, wet (126) and dry (112). The main characteristic of the first is the relatively low flow (<150 hm^3/s). With low silicon percentage (<10 %), low temperature (<9 ºC) and relatively high annual rainfall (>1000 mm), all of which were determined by the agency (Agència Catalana de l'Aigua et al. 2005). The second has a low flow (<40 hm^3/s). A low silicon percentage (<10 %) and a higher water temperature (10 – 13 ºC). It also has a increased annual rainfall (800 – 1100 mm), as described by Rovira Fernández (2008).

3.2.2.1 Diatoms of the Llobregat Basin

Diatom communities differ along this highly impacted river (Sabater et al., 1987). A pristine headwater stream will present diatoms associated with oligotrophy, for instance, *Hannaea arcus* (Ehrenberg) R. M. Patrick, *Diatoma mesodon* (Ehrenberg) Kützing, *Meridion circulare* (Greville) C.

Agardh and *Encyonema ventricosum* (C. Agardh) Grunow. Downstream, a more impacted river usually displays the *Navicula* Bory *sensu stricto* complex, *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot as well as representatives from the *Nitzschia* Hassal complex.

In extremely polluted sites communities predominated by *Nitzschia palea* (Kützing) W. Smith, *Nitzschia capitellata* Hustedt and *Nitzschia inconspicua* Grunow were observed. When the alteration came from an increased salt concentration *Nitzschia frustulum* (Kützing) Grunow, *Surirella ovata* Brebisson ex Kützing and *Mayamaea atomus* (Kützing) Lange-Bertalot are found. Also, some species of the *Halamphora* genus have been identified in these salinized sites.

3.2.2.2 Description of sites

A selection of three sites of the Llobregat River (Chapter 6, section 1, Figure 7) was made to prepare the experiment. They were intrinsically different to describe the micro-scale in form of microhabitat effect (Hering et al. 2006).

Clarà stream is located on calcareous bioclastic substrate (Figure 8a), in the dry calcareous river type (12), with some mixture of marls and siltstone in its substrate. It lies on agricultural land with livestock, and downstream to a small human settlement. Thus, a seasonal increase of nutrients is repeatedly measured. Water flow can change drastically due to its location downstream of the Casserres reservoir. Average values of nutrient concentrations found since 2007 by the Agència Catalana de l'Aigua (ACA) were 62.75 mg/l nitrates and 1.57 mg/l phosphates.

The Llobregat site is in the higher catchments of the river (Figure 8b). It has a high proportion of woods upstream with only little agricultural activity, and it displays characteristics of median river sites, such as vegetative cover and riffle-and-pool hydrology (Frissell et al. 1986).

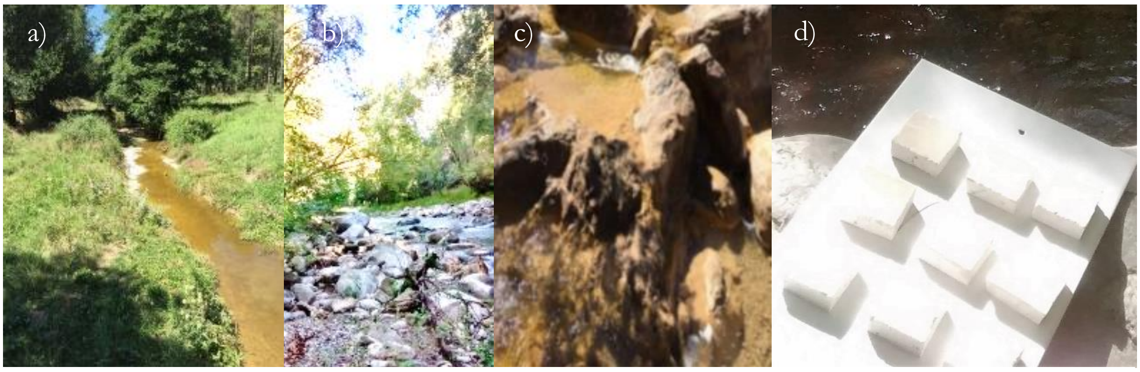


Figure 8 Sampling sites of the Llobregat River a) Clarà Stream, eutrophic, b) Llobregat River over the Baells dam, oligotrophic and c) the halophile Salada Stream. D) example of the experimental setup of unglazed quarry tiles.

Salada stream is hypersaline and has not been included in the river control network (Figure 8c), but has been studied for research purposes (Sala Prat 2014; Torres Roig 2015). Its waters are naturally saline due to halite substrate in the headwaters (Rovira Fernández 2008). It flows into a tributary of the Llobregat river, Saldes, and affects its salinity. Its substrate is naturally silty with gravel that is engulfed in it.

3.2.3 Mediterranean temporary rivers

Temporary rivers (Chapter 6, Section 2, Figure 9) are predominant forms of water bodies in the Mediterranean basin. The climate, characterized by a dry summer period, increases the possibilities of desiccation, more so facing global change (Figure 3, Becker 2014). The TRivers LIFE project was funded, to counteract the lack of legislation, trying to establish how the biomonitoring organisms react to water shortage and variation of current values. Here, physico-chemical, hydrological and ecological parameters were sampled simultaneously to observe the parallel developments of them. Fish, macrophytes, macroinvertebrates and benthic, epilithic diatoms have been the main sampled organisms. To ensure comparability of results, the sampling methods have been homogenized to standard river sampling methods established by the WFD, regardless of connectivity and aquatic state.

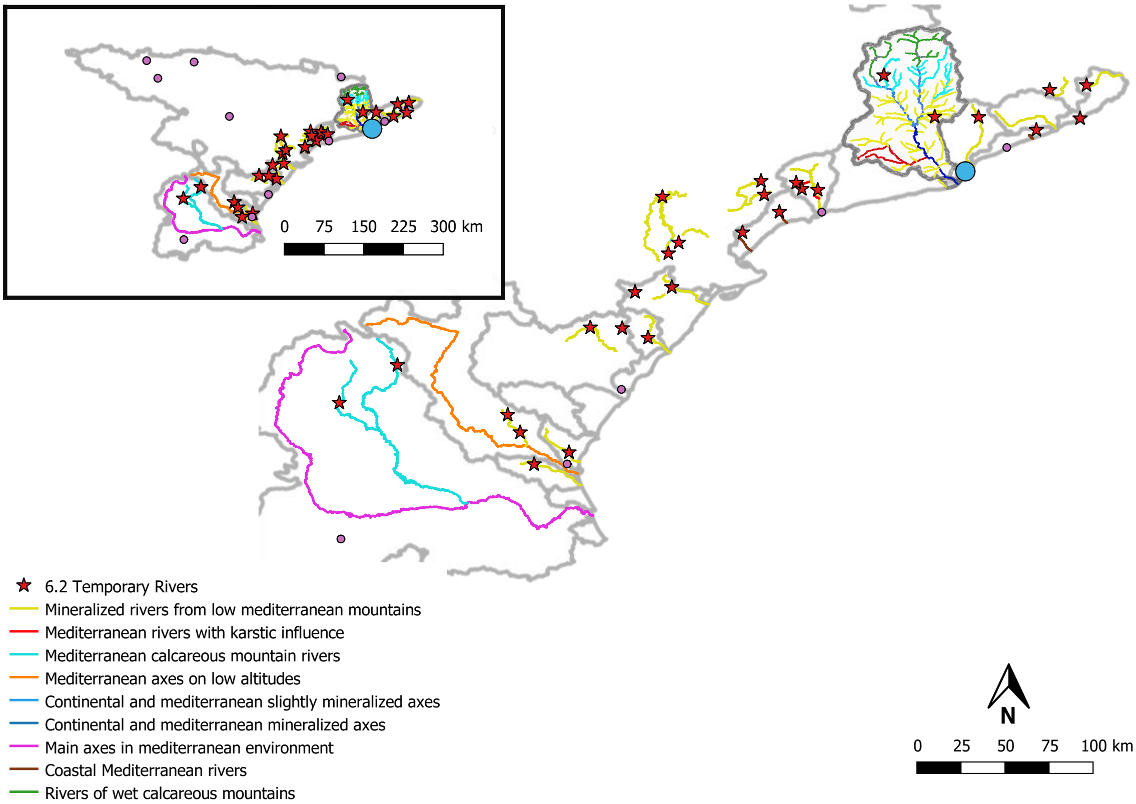


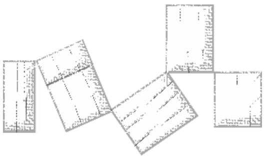
Figure 9 Distribution of temporary rivers. IRES (Intermittent Rivers and Ephemeral Streams) sampled in 2015 for diatoms, macroinvertebrates, fish, hydrological and physico-chemical values. Cities displayed in coloured dots.

A further subdivision of temporary rivers can be seen in Chapter 1 (1.3.1.1 Temporary rivers).

3.2.3.1 Description of sites

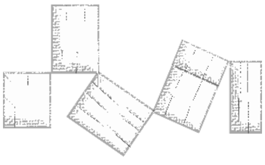
The 24 sites sampled for establishing of ecological characteristics of all the WFD based bioindicators differed on their characteristics (AnnexTable 3). 11 permanent rivers, 4 intermittent dry patches and 8 intermittent pools were sampled bimonthly up to 5 times in 2015. Permanent rivers stayed either in the eurheic state or were reduced to oligorheic waterbodies. Intermittent rivers were sampled from pools as well as streams, following the river sampling methodology of the WFD, although a clear stream flow could be absent.

Rivers were characterized for their state to investigate how diatom life forms were affected by it. The waterbodies from the Catalan Mediterranean Basins were mostly rivers, streams and ravines. Ebro river sites were rivers and headwaters. Water bodies of the Júcar basin were ravines and rivers. Most ephemeral streams were found in the Catalan basins and could not be sampled. Since the diatom sampling was part of a holistic sampling effort, extracting macroinvertebrate, fish and macrophyte samples as well, the lack of water prevented the sampling while using the Water Framework Directive guidelines.



CHAPTER 4:

MACRO-SCALE



Chapter 4

MACRO-SCALE

4. DIATOM COMMUNITIES AT THE EBRO RIVER BASIN

4.1 Introduction

The Water Framework Directive (WFD, European Commission 2000) was started in the year 2000. The freshwater-network of the European Union was meant to be subdivided into similar patches, thus creating a Europe wide network of comparable riverscapes. These "river ecotypes" were to be divided by environmental parameters such as substrate composition and/or mineralization of the water. Ecoregionalization was necessary to adapt several biological indices in different countries for comparability. Each ecotype was adhered to their respective reference sites, where existing, to fit the indices and create a comparable ecological quality ratio (EQR).

Diatoms are specific to several physical and chemical traits. Baas Becking claimed that the dispersal of small organisms, such as diatoms, is cosmopolitan and that it is only filtered by environment (Baas Becking 1959). This is no longer the admitted thesis, and a distinct biogeographical pattern is expected, for instance due to endemisms (Kociolek 2017). Nonetheless, the similarity of tolerance can ease the creation of equivalent communities along similarly composed sites. Many sites, both in Europe and around the globe, have already been characterized by their diatom flora (Tison et al. 2005, 2004; Pan et al. 2000; Rimet 2009, 2012; Pan et al. 1999; Ortíz-Lerín 2012). The goal here is to try to identify standard communities at the Ebro river using diatoms sampled yearly. The samples dated from 2002, the instauration of the control network by the CHE (2006), to the year 2013.

The Ebro river basin, Figure 10, is one of the biggest catchments in Spain. The first regionalizing, made before the WFD, comprehended a total of six river ecotypes according to macroinvertebrate family level distribution (Munné and Prat 1999). These are: high mountain zone, wet mountain, Mediterranean mountain, depression, great rivers and low Ebro river zone. Later, through standardization of the WFD, an eight ecotype system was set in place (Cedex-MMA 2005; CEDEX 2004; CHE, Confederación Hidrográfica del Ebro, and Nogueras Iso 2006, Table 4). Physico-chemical characteristics are depicted in the AnnexFigure 1, and are only partly distinct for each ecotype. Consequently, reference sites were established for each river ecotype. Indicators were then fitted with these reference sites (Ministerio de Medio Ambiente y Medio Rural y Marino 2008), as seen in AnnexTable 4.

Table 4 Ecotype description and number in this chapter.

River ecotypes	Description	Sampling sites (N)
9	Mineralized rivers from low Mediterranean mountains	6
11	Mediterranean siliceous mountain rivers	5
12	Mediterranean calcareous mountain rivers	10
15	Continental and Mediterranean slightly mineralized axes	11
16	Continental and Mediterranean mineralized axes	2
17	Main axes in Mediterranean environment	3
26	Rivers of wet calcareous mountains	12
27	High mountain rivers	1

Following the example of the French studies (Tison et al. 2004, 2005), we wanted to observe differences between the official "hydro-ecoregions" and those that could be inferred from diatom community distribution along the basin, also called "diato-ecoregions" (Rimet 2009). As the original subdivision of the basin followed geological-physico-chemical parameters, we expected to find differences in diatom communities. Temperature, altitude and conductivity affect the diatom communities. Thus, a major subdivision should denote siliceous substrates from calcareous ones. Catchments should also be separated by altitude. A decrease in altitude is often correlated with an increase in phosphate and nitrate concentration levels (Viviroli and Weingartner 2004). This affects trophic levels and determine the abundance of specific diatom taxa (Cabrol et al. 2007; Rimet et al. 2007). Finally, "diato-ecoregions" should be time resilient, although the diatom community dynamics might impede this.

4.2 Materials and methods

The selection of sampling spots of the Ebro Basin Authorities (Confederación del Ebro 2005) database was determined by the frequency of diatom samplings. Only sites with at least 7 samplings (7 years) were used. Another requisite was the existence of at least 3 physico-chemical measures per year. No missing data were accepted. Applying this method, 50 sites were selected (AnnexTable 4, Figure 10). In total, 356 benthic diatom inventories were assessed. The sampling and treatment of diatoms is explained in detail in Chapter 3 (Materials and Methods).

Data were aggregated by location (yearly average) and time. Diatom abundance calculations are also explained in Chapter 3 (Material and Methods). The least abundant diatoms were strengthened by the application of the arcsines on the square root of the proportion. Calculations were made using R software. Packages like "FactoMineR" (Husson et al. 2015), "vegan" (Oksanen 2013b, 2014, 2012), "cluster" (Maechler et al. 2015), "pvclust" (Suzuki and Shimodaira 2015), to produce similarity UPGMA clusters and "CCA" (González et al. 2008) were used. Principal component analysis, CCA and hierarchical clusters (with and without permutations) were calculated using only those parameters shown by BioEnv analysis to eliminate noise.

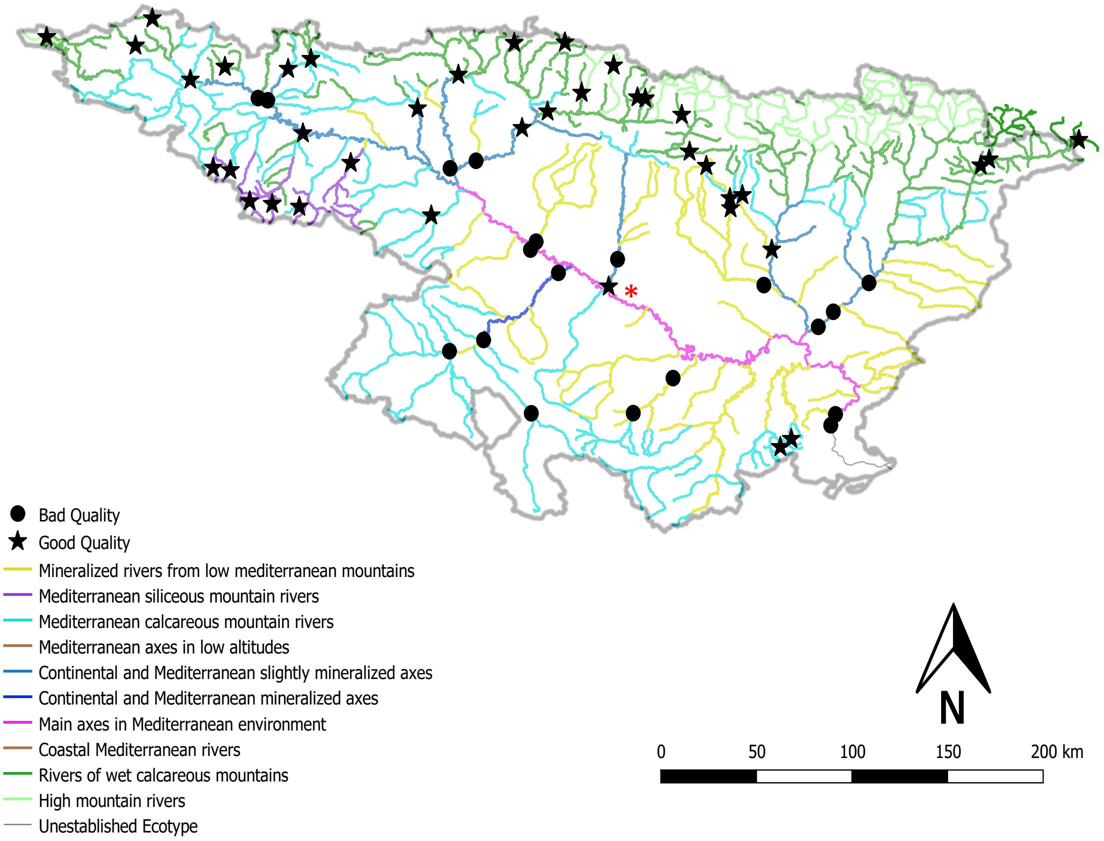


Figure 10 Distribution of the Ebro river basin and location of the sites. Stars mark the eutrophic, circles the oligotrophic sites. The centre oligotrophic site, marked by a red star, may be to the result of a reduction of phosphate concentration levels through an extended wastewater treatment plant.

Indicator values or IndVal (Dufrêne and Legendre 1997) were also calculated, to "*measure the association of a species to a site group*" (De Cáceres 2013). The IndVal analysis was made with the "labdsv" package (Roberts 2015) and, from the site communities, assigned predominantly indicative diatoms for a particular cluster. A diatom community distribution analysis was calculated using the "diatSOM" package (Bottin, Soininen, et al. 2014), employing a distribution algorithm that organizes diatom taxa due to their associations (self-organizing maps). This package was developed to display the similarity of diatom communities in France accentuating diatom tolerance and sensitivity to physico-chemical traits to divide the basins based on diatom taxa (Gosselain et al. 2005; Tison et al. 2005).

This study also looled into the bioindicators, using IPS, IBD and CEE, further described in Chapter 3 (Materials and Methods). They have been used as an additional tool.

Due to the enormity of the macro-scale database, diatoms that appeared either in less than 5 sampling sites or with a total proportion of less than 15% of all the samples were summarized into an artificial category of "other" diatoms. This was done to reduce the background noise of the less numerous diatoms (Busse et al., 1999).

4.3 Results

4.3.1 Physico-chemical subdivision of river ecotypes

A first approach was a physico-chemical value-based clustering (Figure 11). This clustering showed a clear subdivision of mountain and lowland rivers. These rivers were not only classified by their altitude, but also through conductivity and nutrient concentration levels. The 10,000 iteration UPGMA cluster calculation gave a relatively high bootstrap value for the division (>80 %). But the values for a subdivision into 4 subclades were even higher. These were identified as pertaining to siliceous mountains, calcareous mountains, saline lowland and nutrient rich lowland rivers.

The original division into two clades relating to highland and lowland waters gave two very distinct diatom communities, as the IndVal (%) calculation showed (Table 5). In the mountain waters, two taxa were predominant, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi (R^2=0.991***) and *Gomphonema intrincatum var. pumila* Cleve-Euler (R^2=0.809***). With less significance, *Encyonopsis microcephala* (Grunow) Krammer (R^2=0.683**), *Encyonopsis minuta* Krammer & E. Reichardt (R^2=0.675***), *Denticula tenuis* Kützing (R^2=0.577**), *Cocconeis placentula var. pseudolineata* Geitler (R^2=0.568**), *Diatoma tenuis* C. Agardh (R^2=0.548*) and *Gomphonema lateripunctatum* E. Reichardt & Labge-Bertalot (R^2=0.548**). The lowland clade was defined by *Nitzschia inconspicua* Grunow (R^2=0.956***), *Amphora pediculus* Grunow ex. A. Schmidt (R^2=0.906***) and *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot (R^2=0.872***) and many others (for more results, see AnnexTable 5).

The 4-clade subdivision did not have as many indicative species for each of the clusters, and the IndVal (%) values were therefore reduced. Mountain clade a), with a higher siliceous proportion in the substrate had a high incidence of *Encyonema silesiacum* (Bleisch) D. G. Mann (R^2=0.647***) and less of *Reimeria sinuata* (W. Gregory) Kociolek & Stoermer (R^2=0.620*), *Achnanthidium subatomus* (Hustedt) Lange-Bertalot (R^2=0.522*) and *Fragilaria rumpens* (Kützing) G. W. F. Carlson (R^2=0.5*). Calcareous mountain clade b) had significant proportions of *Encyonopsis microcephala* (Grunow) Krammer (R^2=0.702**), *Diatoma tenuis* C. Agardh (R^2=0.648**) and *Gomphonema lateripunctatum* Reichardt & Lange-Bertalot R^2=0.549*). The combined cluster had a similar proportion of the taxa observed at the two-clade-mountain-range diatoms. When the waters were characteristic of lowland pollutant concentrations (saline Lowland a), the diatom communities were composed of *Navicula cari* var. *recens* Lange-Bertalot (R^2=0.631***), *Navicula cryptotenelloides* Lange-Bertalot (R^2=0.625**),

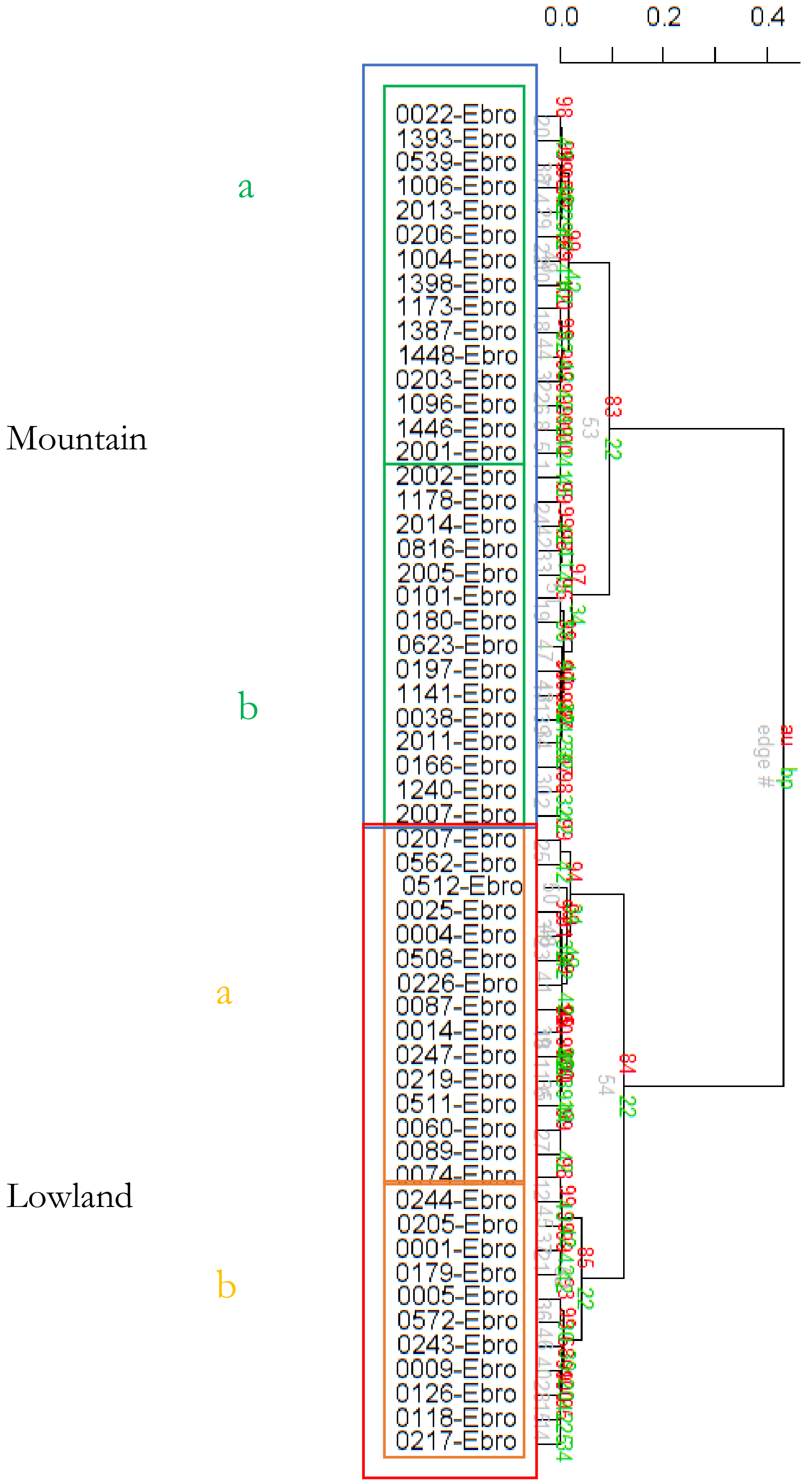


Figure 11 UPGMA clustering (10.000 iterations) of the physico-chemical data from the averaged sites of the Ebro river basin, with subdivision into 2 and 4 clades.

Bacillaria paxillifera (O.F. Müller) T. Marsson (R^2=0.577**), *Pseudostaurosira brevistriata* (Grunow) D. M. Williams & Rounds (R^2=0.577**) and *Fragilaria elliptica* var. *elliptica* Schumann(R^2=0.5*). Nutrient rich lowland (b) clade was determined by *Achnanthidium eutrophilum* (Lange-Bertalot) Lange-Bertalot (R^2=0.606**), *Achnanthidium saprophilum* (Kobayasi & Mayama) Round & Bukhtiyarova (R^2=0.59**), *Diatoma vulgaris* Bory de Saint-Vincent (R^2=0.562*), *Gomphonema olivaceum* (Hornemann) Brébisson (R^2=0.524*) and *Cyclotella ocellata* Pantocsek (R^2=0.509*). Some taxa, such as *Navicula cryptotenella* Lange-Bertalot and *Cymbella affinis* Kützing were correlated with 3 clades. *N. cryptotenella* Lange-Bertalot was found in calcareous mountain, saline lowland rivers and nutrient high lowland rivers. *C. affinis* Kützing was found in both mountain clades and the nutrient rich lowland rivers.

Finally, comparing both approaches, the physico-chemical differentiation as measured in a Permanova calculation, is not significant for the 4-cluster approach (R^2 =-0.006964 n.s.). The 2-cluster subdivision is correlated and significant (R^2 = 0.50411***). The diatom differentiation of both cluster approaches is significant for both but correlate quite poorly.

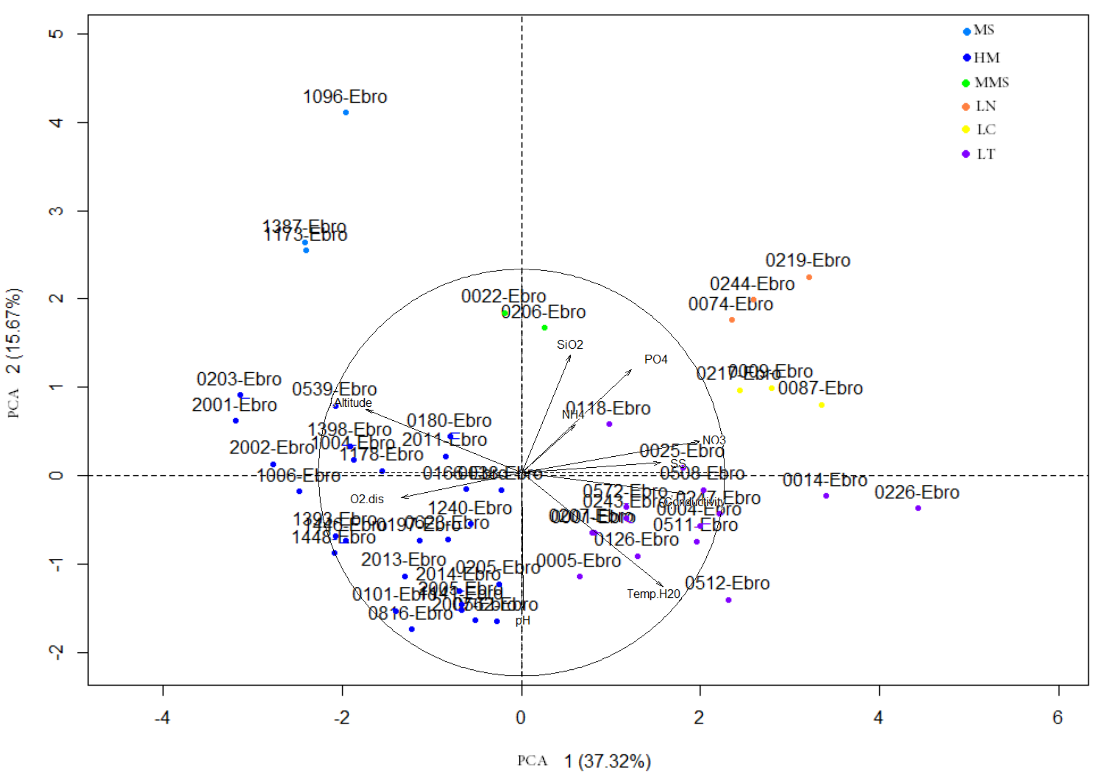


Figure 12 PCA analysis with relatively high variance (PCA 1+PCA 2 = 0.5299 %). Sites and the distribution of physico-chemical traits. Altitude, SiO2 = siliceous acid, PO4 = phosphate, NO3 = Nitrate, Conductivity, TempH2O = water temperature, pH, O2 Dis = dissolved oxygen. MS = Silicic mountain, HM = High mountain, MMS = Medium silicic mountain, LN = nutrientrich lowland, LC = high conductivity lowland, LT = high temperature lowland.

When used in a physico-chemical PCA (Figure 12, variability explained = 52.99%), the subdivisions are reduced into 6 observable groups (AnnexTable 6). High mountain, described by *Achnanthidium minutissimum* (Kützing) Czarnecki, *Encyonopsis microcephala* (Grunow) Krammer and *Cymbella affinis* Kützing are correlated to high altitude and high dissolved oxygen. *Nitzschia inconspicua* Grunow and *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot are mostly correlated to lowlands with high conductivity and high ammonium levels. *Amphora pediculus* (Kützing) Grunow ex A. Schmidt, *Cocconeis euglypta* Ehrenberg, *Nitzschia dissipata* (Kützing) Rabenhorst and *Navicula cryptotenella* Lange-Bertalot defined sites at lower altitudes with high nutrient levels. Sites of high temperature at low altitudes were defined by *Navicula recens* (Lange-Bertalot) Lange-Bertalot. Medium sized silica containing mountains, were identified by *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, *Encyonema minutum* (Hilse) D. G. Mann and *Reimeria sinuate* (W. Gregory) Kociolek & Stoermer. Finally, high siliceous substrate concentration mountain sites were defined by *Diatoma mesodon* (Ehrenberg) Kützing, *Gomphonema intrincatum var. pumila* Cleve-Euler, *Gomphonema rhombicum* Fricke, *Cocconeis placentula var. lineata* (Ehrenberg) van Heurck, *Achnanthidium subatomus* (Hustedt) Lange-Bertalot and *Encyonema silesiacum* (Bleisch) D. G. Mann.

4.3.2 Diatom community clustering vs. official river ecotypes

A preliminary clustering of the diatom Communities was made using Bray-Curtis method and a flexible approach (β=0.75, Figure 13a). It portrayed a well-defined subdivision into 2 clusters, further divisible into 4 articulated clusters. Thereafter, a subdivision of the ecological traits for each cluster was attempted. Only altitude, conductivity, concentrations of phosphate, nitrate and suspended solids, and water temperature were significant in their differentiation (not shown). The three bioindicators showed significant differences. In this instance, the highest rating cluster was the cluster 2, dominated by *Achnanthidium pyrenaicum* (Hustedt) Kobayasi. It also shows the highest distribution and lowest nutrient, conductivity and temperature values.

Iterative clustering (10,000 iterations) using pvclust in a UPGMA approach (Figure 13b) shows a clear subdivision into 2 parts. These pertain to river axis/eutrophic environments and relatively mountainous regions/oligotrophic traits. *P*-values were very high (0.01-0.04). A two-part subdivision of the low riparian area shows the best support through diatom index values (not shown). A higher number of subdivisions could improve extracting both outliers, Urbión in Santa Cruz del Valle (1387) and Zadorra in Vitoria (0079).

Diatom composition through IndVal showed differences between the two-clustered and the five-clustered approach. The principal indicative diatoms of the 5 clusters were *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot and *Nitzschia inconspicua* Grunow. They show lower index values than in the two-clustered approach. Here *Amphora pediculus* (Kützing) Grunow and *Achnanthidium minutissimum* (Kützing) Czarnecki displayed significant index values over 60 %.

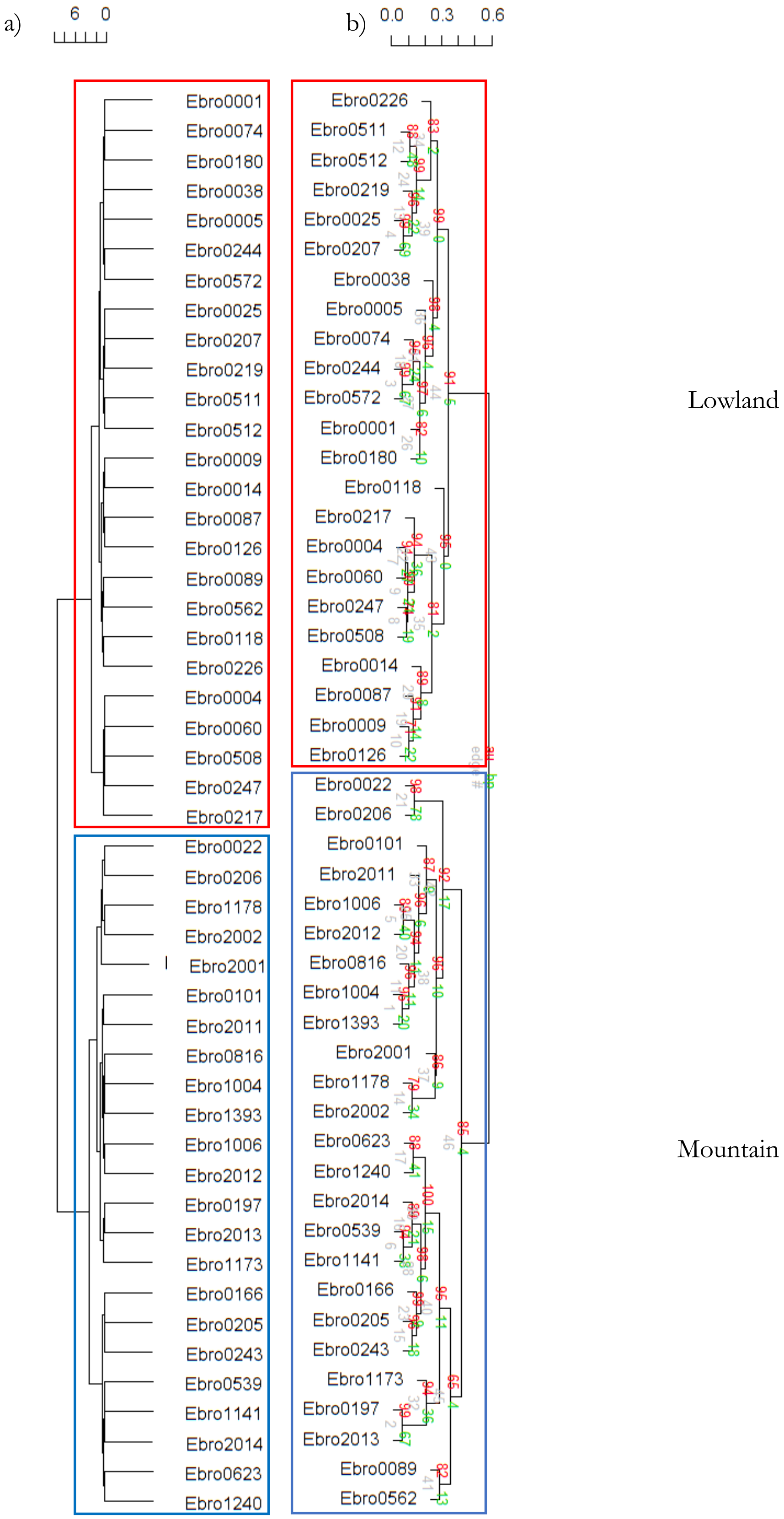


Figure 13 Comparison of locations a) Bray-Curtis flexible linkage clustering using β=0.75 (in this case, contrary to the expected (Legendre and Legendre 2003), a smaller β implies a more conserving display of the sampling sites) b) UPGMA 10.000 iterations. Most sites are maintained in the same clusters, only Gàllego in Zaragoza (0089) changes into the eutrophic waters for the Bray Curtis distribution.

Physico-chemical characterization of two clusters showed a significant difference (AnnexFig. 3). Parameters such as altitude, conductivity, total phosphate concentration, total nitrate concentration, suspended solids concentration and water temperature were differential (AnnexFig. 2), as also observed using ANOVA confirmed by post-Hoc Tukey test. The diatom biologic indices (CEE, IBD and IPS) differed significantly for each of the clusters (not shown). The graphical representation of the River Basin (Figure 10) also showed a subdivision of eutrophic and oligotrophic sites. It coincided mostly with the altitudinal approach.

Table 5 UPGMA clustering with 10.000 iterations of the total clustering of average sites. Division into two clusters of River Axis (A) and Mountain Rivers (B). In comparison to this, the lack of sites 1387 and 0179 results in a marginally higher indicator value. We have used the total database, without regard to the average, since we wanted to extrapolate if the result was applicable to these data.

	Omnidia Code	***IndVal (%)***
Amphora pediculus (Kützing) Grunow Ex A. Schmidt	APED	61***
Nitzschia inconspicua Grunow	NINC	48***
Navicula cryptotenella Lange-Bertalot	NCTE	35***
Achnanthidium minutissimum (Kützing) Czarnecki	ADMI	65***
Achnanthidium pyrenaicum (Hustedt) Kobayasi	ADPY	63***
Gomphonema intrincatum var. pumila Cleve-Euler	GPUM	25***

p-values: 0 '***' 0.001

The CCA analysis portrayed 71 % of the variance (Figure 14) and showed an indistinct clustering of the sites of the established river ecotypes. Low laying sites were somewhat dispersed from the central cluster. Also, diatom communities are compactly adhered. The river ecotypes were mingled with no clear distinction. Finally, the relative position of each of the sites shows a maximal distance of 427 km with an average of 164 km. The maximal distance is of 347 km in the polluted area. The mountainous region has distances up to 427 km. A mantle test showed a weak but significant correlation between diatom taxa and distances (R^2=0.3625**).

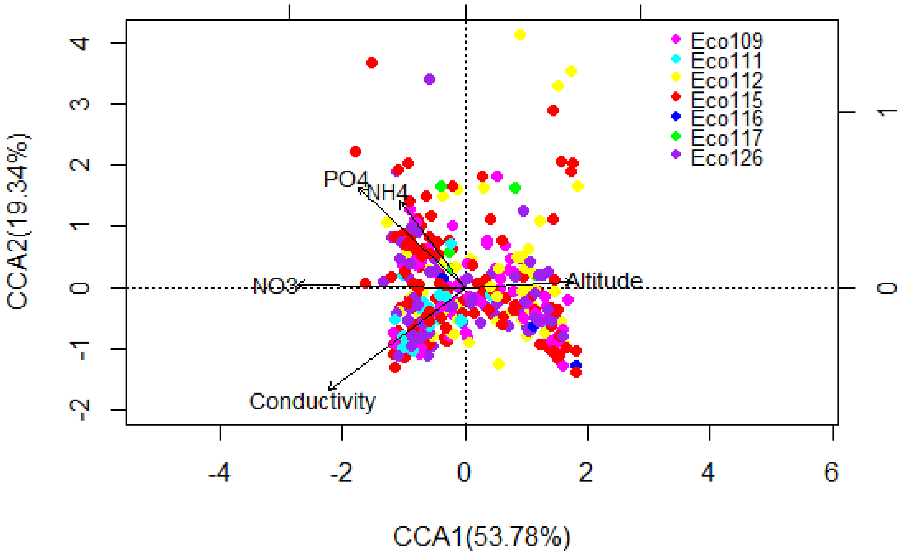


Figure 14 Canonical Correlation Analysis graph showing the distribution of predominant diatom species listed below and sampling sites in relation to the physical-chemical and hydromorphological data. This CCA explains the 70.87% of the variance. Altitude =Altitude, NH4. = ammonium, PO4 = phosphate, NO3 = Nitrate, Conductivity = conductivity. 109= Mineralized rivers from Low Mediterranean Mountains. 111= Mediterranean siliceous mountain rivers. 112=Mediterranean calcareous mountain rivers. 115= Continental and Mediterranean slightly mineralized axes. 116= Continental and Mediterranean mineralized axes. 117= Main axes in Mediterranean environment. 126 = Rivers from wet calcareous mountains.

Using another approach, diatoms abundances were subjected to a self-organizing map (SOM) algorithm to observe which diatoms had the same distribution along both time and space. The diatom taxa were first subdivided into 8 groups to compare the results to the official subdivision of river ecotypes (AnnexFig. 2). Diatom Communities were clustered together using the diatSOM package. The resulting species maps (Figure 15) presents darker hexagons, where the proportion of the taxon was highest. If the shade spreads through more hexagons, the diatom taxon was deemed to be tolerant to different physico-chemical parameters. Thus, in this example (Fig. 15), *Achnanthidium atomoides* Monnier, Lange-Bertalot & Ector (ADAT) had a disjointed distribution.

The physico-chemical data of the 8 diato-ecoregions were portrayed as a boxplot graph (Figure 16). Here the combination of physico-chemical data was divisible into the following river ecotypes: alpine

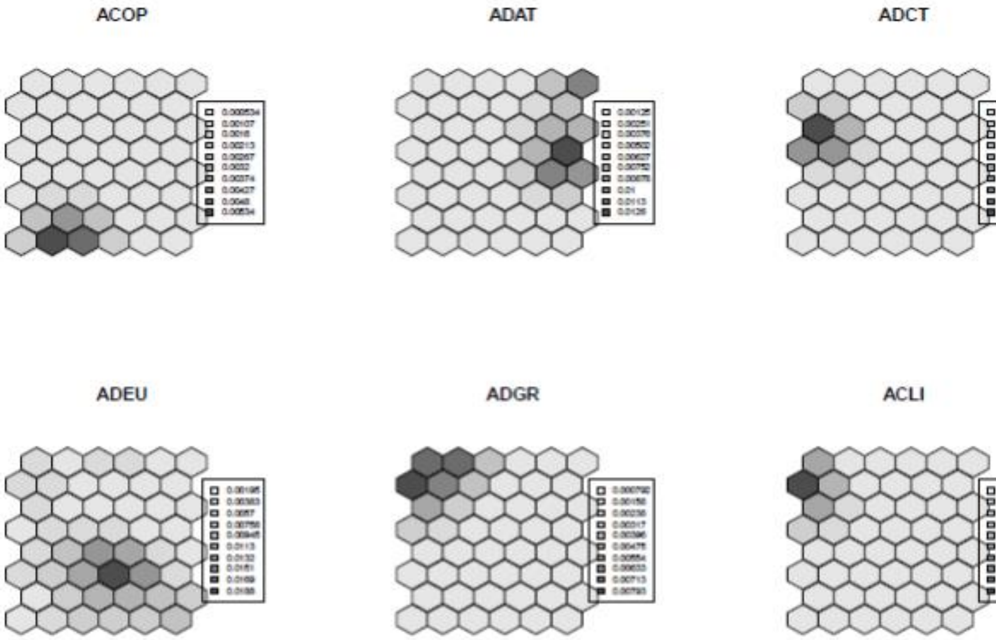


Figure 15 Example of the output of the SOM calculation. Diatoms are distributed along clusters and their dispersion rates. (Diatom species: ACOP = *Amphora copulata*, ADAT = *Achnanthidium atomoides*, ADCT = *Achnanthidium catenatum*, ADEU = *Achnanthidium eutrophilum*, ADGR = *Achnanthidium gracillimum*, ACLI = *Achnanthidium linearioides).*

(1), altered subalpine (2), circumneutral silicate alpine (3), alkaline silicate alpine (4), alkaline lowland (5), low nutrient lowland (6), high nutrient lowland (7) and high conductivity lowland rivers (8). The SOM groups were divided as follows: alpine (1) was indicated by a predominant *Achnanthidium pyrenaicum* (Hustedt) Kobayasi. Altered subalpine (2) had the presence of *Achnanthidium minutissimum* (Kützing) Czarnecki, *Encyonopsis microcephala* (Grunow) Krammer, *Encyonopsis minuta* Krammer & E. Reichardt, *Cymbella excisa* Kützing and *Gomphonema lateripunctatum* Reichardt & Lange-Bertalot. *Gomphonema intricatum* var. *pumila* Cleve-Euler, *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *Encyonema silesiacum* (Bleisch) D. G. Mann, *Reimeria sinuata* (Gregory) Kociolek & Stoermer and *Gomphonema Encyonema silesiacum* (Bleisch) D. G. Mann, *Reimeria sinuata* (Gregory) Kociolek & Stoermer and *Gomphonema rhombicum* Fricke defined the circumneutral silicate mountain (3). The alkaline silicate mountain (4) was defined by *Nitzschia fonticola* Grunow, *Cocconeis lineata* Ehrenberg and *Diatoma vulgaris* Bory de Saint-Vincent. SOM group 5 (alkaline lowland) was predominated by *Nitzschia dissipata* (Kützing) Rabenhorst and *Navicula cryptotenella* Lange-Bertalot. Group 6 (low-nutrient) and 7 (high-nutrient lowland) had only monospecific indicators and were represented by *Cocconeis euglypta* Ehrenberg and *Amphora pediculus* (Kützing) Grunow ex A. Schmidt, respectively. Finally, the high conductivity lowland river group (8) was represented by *Nitzschia inconspicua* Grunow, *N. frustulum* (Kützing) Grunow and *Navicula recens* (Lange-Bertalot) Lange-Bertalot. A rework was attempted, in which the indicator values were extracted for each of the SOM clusters, in order to ascertain their coefficient value and significance, where IBD had the best distinction of SOM sites (AnnexFig. 5).

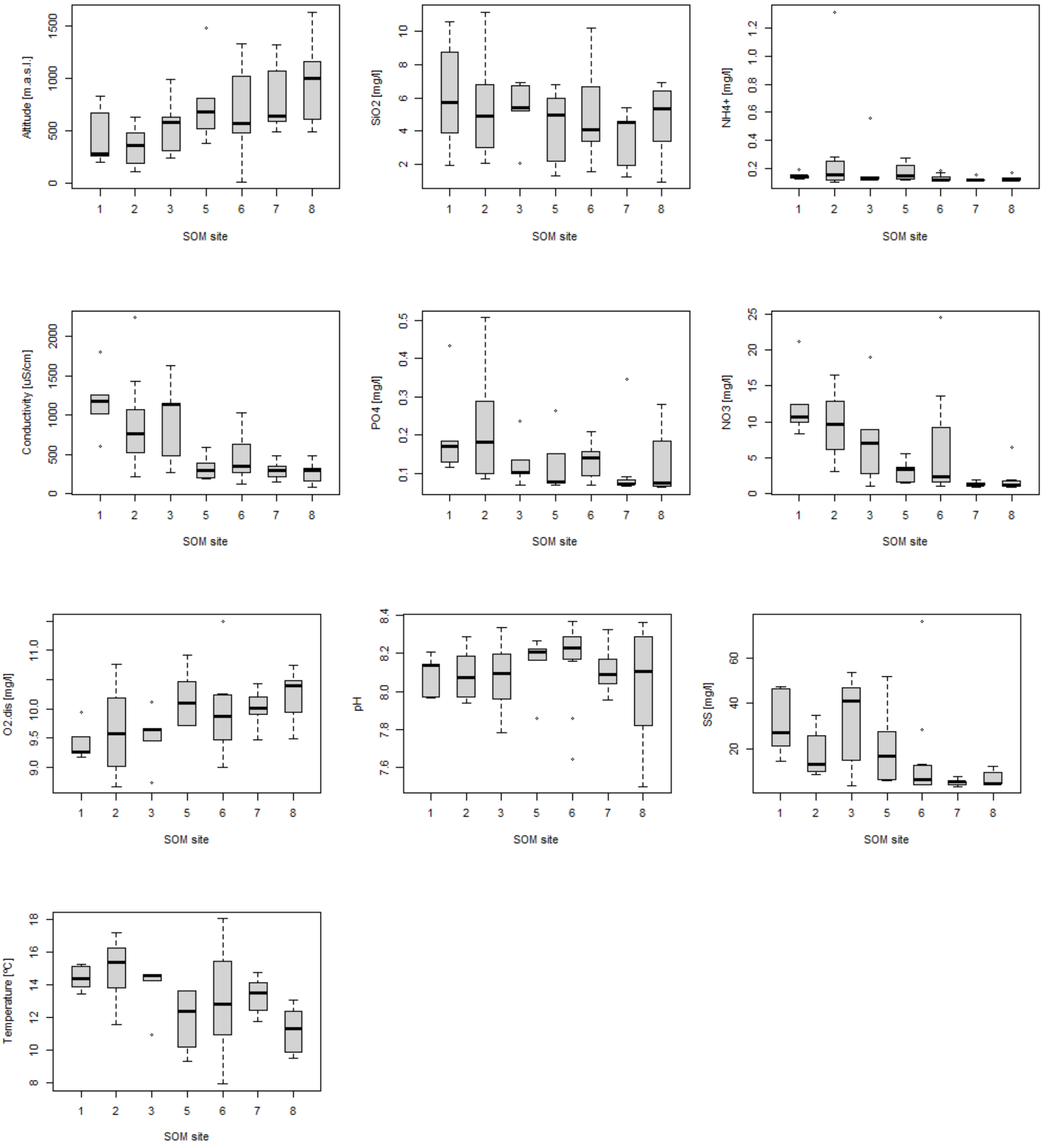


Figure 16 Physico-chemical distribution of the 8 diato-SOM sites are differential in their means and combinations. SiO_2 = Silica concentrion [mg/l], NH_4 = Ammonium concentration [mg/l], PO_4 = phosphate concentration [mg/l], NO_3 = Nitrate concentration [mg/l], O_2 dis = Dissolved oxygen concentration [mg/l], SS = Suspended solids [mg/l].

4.3.3 Yearly comparison of diatom communities

The sampling locations were divided by year and clustered together. The calculation of 10,000 iterations the community similarities (UPGMA method) showed variations in the averaged cluster. The locations did neither adhere nor show a specific temporal clustering. Furthermore, some locations, such as the Jalón River in Grisén clustered in duplets. Nonetheless, they showed high temporal disparities, for instance, communities of 2002 and 2013 clustered together. The total cluster of the UPGMA, 10,000 iterations similarity calculations of theThe resulting total clustering of sites was too cluttered to be shown in this chapter. But it may be accessed in the Annex (AnnexFigure 3).

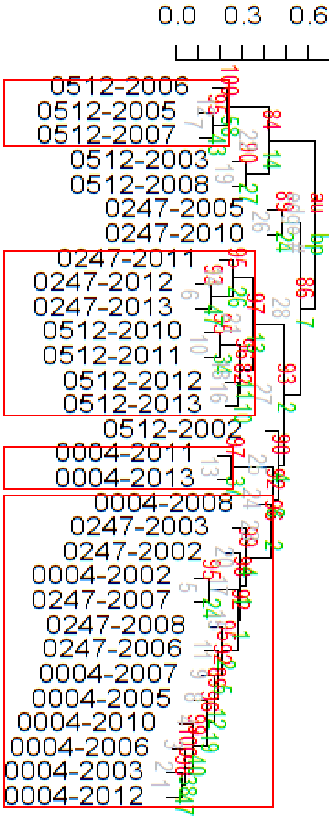


Figure 17 Comparison of the samples that were obtained each year. The clustering for each sample site is not perfect either for location or time differences. Only the Arga in Funes site (0004) has a better cohesion since the samples are clustered into one sub-cluster.

However, to represent the total cluster more visibly, only those sites with ten samplings were used (Figure 17). No pattern is visible since neither years nor locations cluster together. Only the location of the Arga River in Funes shows some cohesion, although communities of both other sites were also introduced into the same cluster. Some years show the predominance of specific diatom taxa. In this instance 2008 had a light majority for *Achnanthidium pyrenaicum* (Hustedt) Kobayasi and 2013 had a predominance of *Cocconeis euglypta* Ehrenberg (Table 6). The most differentiated year community was in 2013. The years are mostly arranged into duplets since 2007-2008 and 2010-2011 cluster together neatly. 2002, 2003, 2005 and 2006 communities had an uneven arrangement, given that 2002 was nearer to 2005 than to 2003.

Table 6 Indicator Values (IndVal) comparison based on the UPGMA clustering in 10.000 iterations according to year. No significantly predominant taxa were found for the years 2002, 2003 and 2011, and are thus not shown in the tablle.

	Omnidia Code	**IndVal (%)**	**Year**
Navicula cryptotenella Lange-Bertalot	NCTE	9*	2002
Cymbella affinis Kützing	CAFF	8**	2002
Nitzschia frustulum (Kützing) Grunow	NIFR	7*	2002
Cocconeis lineata Ehrenberg	CPLI	10**	2003
Encyonopsis microcephala (Grunow) Krammer	ENCM	8*	2007
Cocconeis placentula Ehrenberg	CPLA	6*	2007
Achnanthidium pyrenaicum (Hustedt) Kobayasi	ADPY	10*	2008
Gomphonema intricatum var. pumila Cleve-Euler	GPUM	9**	2008
Nitzschia fonticola (Grunow) Grunow	NFON	6*	2008
Encyonopsis minuta Krammer & E. Reichardt	ECPM	6*	2012
Cocconeis euglypta Ehrenberg	CEUG	12**	2013

p-values: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05

We observed every year in its total composition (AnnexTable 9), comparing the number of sites as calculated for BioEnv, wherein. differences in significance are shown. The years were divided into three groups of four years. The significance in change between subsequent years was ascertained through an MRM (multiple regression on distance matrices, Table 7) approach. Not all of the years showed significant differences in total diatom community composition. For instance, 2006 showed no significance in its arrangement with the years before it.

Table 7 Multiple Regression of distance Bray-Curtis matrices between successive years, according to BioEnv subdivision. Significance represented with *

	Group	***R^2***	***Group 2***	***R^2***	***Group 3***	***R^2***
2002-2003	*0.243**	*0.073**				
2003-2005	*0.351**	*0.124**				
2005-2006	*0.069*	*0.076*				
2006-2007			*0.397***	*0.232***		
2007-2008			*0.311**	*0.189**		
2010-2011					*0.229***	*0.173****
2011-2012					*0.131*	*0.078*
2012-2013					*0.332****	*0.152****

p-values: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ns (not significant) = “ ”

4.3.4 Comparison of all approaches

BioEnv results showed differences between the significances for each different approach, such as total database, regions, river ecotypes, years and averages. The most representative physico-chemical data for the clusters ranged from two and four. They entailed altitude, ammonium, conductivity, phosphate concentration, nitrate concentration, dissolved oxygen concentration, pH, suspended solids concentration and water temperature (AnnexTable 10 – 14). No phytosociological distribution in the margalefian sense was observed, as the communities did not match the described phytosociological nomenclature. A resulting comparison of the distributions of physicochemical, diatom-based (SOM) and combined distribution as well as the river ecotype division (Figure 18).

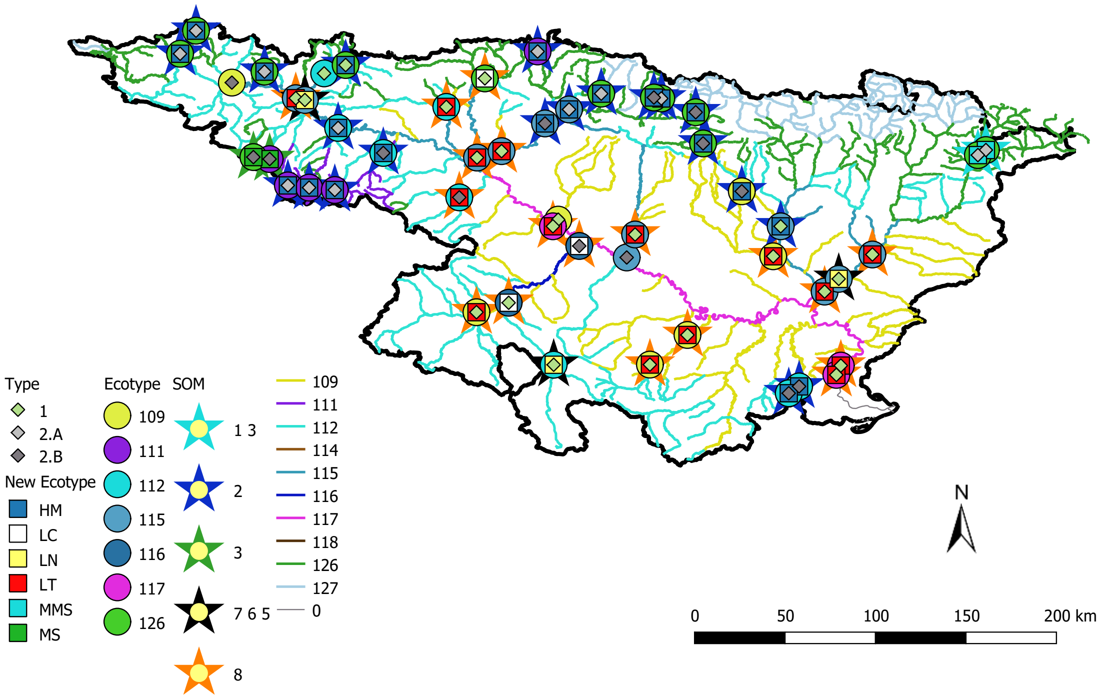


Figure 18 Comparison of physicochemical (Type), diatom-based (SOM) and combined (New Ecotype) distribution of diatom communities compared to the official river Ecotype (Ecotype).

The significance of indicator values (IndVal%) of the clustering divisions of the hierarchical cluster diatom-community-subdivision was relatively high (AnnexTable 10), higher than the official division of reference and impacted sites (AnnexTable 10 – 14). A clear prevalence of the non-official community establishment methods is found both in the hierarchical clustering as in the sites, even more so, when comparing the resulting communities from the subdivision methods and their physico-chemical traits correlations (AnnexTable 10). Here, hierarchical clustering has higher significances than the SOM clustering, but that the official river ecotypes are not correlated equally.

Results of the Canonical Correspondence Analysis (CCA, Figure 14) show, that the sampling locations are aggregated, except for those with significant differences in diatom community structure. Site 0179 was represented by *Sellaphora seminulum* D. G. Mann. Site 1387 had a predominant *Encyonema silesiacum* (Bleisch in Rabh.) D. G. Mann. Both are well differentiated from the other locations. Physico-chemical parameters were also significantly differential. The best subdivision according to indicator values corresponds with the physico-chemical, 2-cluster classification (AnnexTable 5). All found distributions are highly significant. Most are in the range of p <0.001, with percentages ranging between 56 % and 99.1 %. Even using the comparison of every sample/year, the indicative diatoms get to an unequalled 65 % (IndVal %) in the cluster articulated into two groups. They maintain their significance. The division by year gives no clear indicator values. All are distributed below 15 %. The SOM distribution has indication values up to 81 % and strong significance values (AnnexTable 7). The Ebro official river ecotypes were limited descriptors. Only five of the eight river ecotypes were explained significantly by a indicative diatom, with a maximal 35.76 % effectiveness as indicator (AnnexTable 8).

We also compared a subdivision of diatom communities in river ecotypes, physico-chemical traits and diato-ecotypes using a Spearman Rank Correlation of the communities compared to physico-chemical composition (AnnexTable 10). As expected, the physico-chemical distribution was the one that had more significant correlations of all of the approaches. The SOM distribution had a little less significant. The official river ecotypes did not show these correlations.

Finally, the comparison of all classifications (AnnexFig. 9, AnnexTable 11) showed that the physico-chemical and SOM distribution coincided partially, as high mountain clusters from the physico-chemical approach coincided with the altered subalpine rivers of the SOM distribution. A final comparison map is given in AnnexFigure 6.

4.4 Discussion

4.4.1 Physico-chemical subdivision of river ecotypes

Our first approach had been the physico-chemical characterization to find an alternative Ebro subdivision (Munné and Prat 1999). Diatom taxa filtering has been most linked repeatedly to conductivity and nutrient concentration, as well as temperature (Rott et al. 1998). The two-cluster division provided a clear differentiation of these parameters. Even the 4-cluster subdivision can be related to a gradient of these traits. When divided into 4 clusters, the diatom associations point to a clear subdivision of mountain rivers through their substrate composition.

The comparison of both subdivisions points to a more correct 2-cluster division. Physico-chemical and diatom correlation values and Permanova test results showed more significance when divided only into mountain and lowland sites. These results are somewhat surprising, since conductivity and nutrients are usually main drivers of diatom selection (Lange-Bertalot 1979a). But some tolerant diatoms, such as *Cymbella affinis* Kützing, cannot delimit physico-chemical traits satisfactorily (Potapova 2011). Physico-chemical distribution, therefore, was not concise enough to reduce the diatom communities.

4.4.2 Diatom community clustering vs. official river ecotypes

The Baas Becking (1959) hypothesis stated that "everything is everywhere, but the environment selects", at least for the smaller organisms. This has been slowly debunked for diatoms through the application of phylogenetic tools (Kociolek 2017). However, a biogeographical specificity inside a river basin still stands to reason (Williams 1995, 2009). Worldwide it has been repeatedly tried to establish the "diato-ecoregions" described by Tison et al. (2004, 2005), adapting the diatom community occurrence to a more or less similar physico-chemical environmental factors. This also was the basic reason for creating river ecotypes for the implementation of the European Water Framework Directive (WFD, European Commission 2000). Ortiz (2012) also provided a diatom community distribution inside the Ebro Basin, based on the samplings of 2005-2006. In this work we added the temporal scope and found similarities, such as the division of mountain and lowland rivers, with the addition of rarefied sites that have not been looked at in the former study. On the other hand, our results portray a spatial distribution, but only conditionally. The diatom communities have a too substantial temporal variation (Lavoie et al. 2008; Townsend et al. 1997; Singh et al. 2004). This signifies that a relative plasticity of diatom populations would be determinant in diatom distribution. This would in itself describe the diatom response to a physico-chemical change (Leira et al. 2009; Elias et al. 2012; Meador and Goldstein 2003). To reduce the noise given by the temporal variation and to stress spatial differences in our calculations were made on the average of seven to ten years of sampling as suggested by Kelly et al. (2009).

Our first approach using the flexible UPGMA was already clustered at $\beta=0.75$. This might be related to the fact that all the sampling locations were part of a linked river basin. Tornés et al. (2012) used a $\beta=-0.25$ to reduce effect of space, counteracting the influence of unlinked sampling sites from different catchments. Since the results in our study showed that the spatial bias was not high and pertained to the same basin, the iterative clustering chosen was based on UPGMA, since it had better resolution in IndVal values.

Diatom communities for each of the clustering methods varied slightly in their composition (AnnexFig 4), as did the clustering of the locations. Nevertheless, the predominant diatoms were sustained along the clusters. This is shown by the results of the IndVal analyses (AnnexTable 5). The division used in that table, between the *Achnanthidium pyrenaicum* (Hustedt) Kobayasi [D1] and *Achnanthidium minutissimum* (Kützing) Czarnecki [D2] clades gave differences in conductivity and altitude affinity, as well as pH and average temperature (Jüttner et al. 2011; Potapova et al. 2007). Differences in ammonium concentration and conductivity produced changes in predominant diatoms: *Nitzschia inconspicua* Grunow [B2], found with a higher saline content, (Trobajo et al. 2013;

Busse et al., 1999), was differentiated from *Navicula cryptotenella* Lange-Bertalot [B1], a eutraphentic species, (Rott et al. 1998; Winter and Duthie 2000b; Bere and Tundisi 2011; Bolla et al. 2010). Both [A] and [C] were based on outliers and thus not looked into further (not shown). These were the River Zadorra in Vitoria and the Urbión in Santa Cruz del Valle. Both sites have characteristic flora through their physico-chemical characteristics. The first site had an important influx of phosphate and ammonium concentration. The latter site had a particularly low pH and water flow.

The 10,000 times iterated clustering UPGMA method showed a subdivision of the Ebro Basin into mountainous and non-mountainous rivers (or mountain rivers and axis rivers). The former had a lower pollutant incidence and any anthropogenic pollution substance including nutrients, straight polluting effluents, dangerous substances, etc., were reduced (Viviroli and Weingartner 2004; Allan 2004). The pollution was linked to human population density in urban and agricultural areas and was dispersed at the lower heights (Rimet 2009; Allan et al. 1997). This was also confirmed by the former clustering approach, as the significance of chemical and bioindicative values were also high. On the other hand, the outliers of Zadorra in Vitoria and Urbión in Santa Cruz del Valle showed inherently different diatom compositions. These could be due to the location. Zadorra, located after a big city, produced an increment in priority substances. Organochlorides and heavy metals are known to affect diatom communities (Wetzel et al. 2013; Blanco and Bécares 2010; De Jonge et al. 2008), more so due to the low water flow in this site. Urbión had a particularly low pH compared to other sites of even its own official ecotype. This might be related to the low alkalinity measured in this site.

Alkalinity is a very important factor for diatom distribution (Leira and Sabater 2005), affecting the community structure. The spatial dispersion (Figure 10) showed that the subdivision per altitude to be correct for the most part. Mountain rivers have been shown to have characteristic community structures (Rimet et al. 2007). Since the catchment width impedes bigger human settlements, anthropogenic impacts are reduced leaving next to pristine river conditions. The only differential site of the lower river was in the Gállego river and may be due to a wastewater treatment plant (WWTP) put into use in 2009 (Instituto Aragonés de Estadística et al. 2016). Here the elimination of nutrients favoured the appearance of oligotrophy-tolerant communities. The other outlier found at the Cinca River is more difficult to explain. The WWTP of Monzón officially only gets secondary treatment, but a nitrogen reduction tertiary tank may be active since 2006 (Instituto Aragonés de Estadística et al. 2016). Furthermore, the Sosa tributary that crosses the city and flows into the Cinca before the sampling site usually dries up in summer, reducing its polluting effect.

The SOM distribution was a useful tool to classify diatoms. It also displayed a differentiation of the physico-chemical characterization of the environment. Through this method, even though 8 groups were imposed to assimilate the official river ecotypes, 6 clear groups emerged. The diatom taxa in and of themselves displayed clear attachments to differential physico-chemical and local parameters.

These groups could be characterized as medium and high siliceous mountains, calcareous mountains, nutrient-rich lowland rivers, conductivity rich lowland rivers and high temperature lowland rivers.

The species found at each group were aggregated by their tolerances. High mountain rivers were characterized by mostly oligo- to eutraphentic species. The predominant diatom was the cosmopolitan and colonizing *Achnanthidium minutissimum* (Kützing) Czarnecki, known to produce massive blooms in mountain river ranges (Hofmann et al. 2011). Halophile taxa had relatively high correlation with group 1 of the SOM distribution. The nutrient rich lowland rivers had relatively high proportions of eurytrophic and eu- to polytrophic condition tolerant diatoms.

Comparing the three approaches, the hierarchical clustering, Bray Curtis β-clustering and SOM clustering, we need to point out that they give different insights. Clustering algorithms find the similarities in composition and diverge slowly, after comparing the sites or samples for the set interactions. In contrast, diatSOM package requires stipulating how many clusters to force, and distributes diatom communities along a self-imposed grid. The advantage of the SOM calculation is the possibility of comparing directly with already existing subdivisions. It helps to characterize diatom communities, thus, underlining the physico-chemical composition. It also provides a highly visual output, which permits a clearer understanding of the communities. The drawback is, that the output relates clearly either to the sites, or the diatoms. The double output (AnnexFig. 2), such as the distribution of both diatom communities and cluster is possible but falls into the same drawbacks of the clustering methods (lack of clarity). Clusters are may be better at obtaining a more realistic distribution, since it relates to similarity/distance. But the output gets confusing for big databases, such as the complete site-year-database.

4.4.3 Yearly comparison of datom communities

The certainty and noise reduction is lost using the interannual comparison approach for diatom community comparison (Kelly et al. 2009), which is averaging the annual diatom samplings. Therefore, the communities in the cluster were clattered and non-descript. Temporal variation is nonetheless important, since the definition of diatom bioindication depends on diatom plasticity to changing water conditions. These changes are time dependant (Elias et al. 2012; Huang et al. 2012). A major drawback is the uniqueness of the diatom community at any given time (Crossetti, Stenger-Kovács, and Padisák 2013; Vanormelingen et al. 2008; Bayona et al. 2014), which reduces the sampling to a “community snapshot” (Bottin et al. 2014).

Another drawback is the fact that the resulting cluster is nested (AnnexFig. 4). Hence, a subdivision into clear sub-clusters is nearly impossible. Nevertheless, a subdivision into mountainous and axis region can be observed, but some locations such as the River Jalón in Grisén or the Najerilla in Torremontalbo are dispersed along the whole cluster. The special characteristics were due to the high salinity, N/P-ratio and relatively high ammonium concentration of the first site. The second site had a very high dissolved oxygen concentration, as well as big peaks of suspended solids concentration. This translates to a change in diatom predominance. Hence, the bioindicator values range from mediocre to high quality.

Differentiating locations with 10 samplings has shown that the locality-cohesion was not definite. These sites did show extreme ranges of bioindicator values (from bad to good). The one with the lowest range (good to mediocre water quality) showed more cohesion, since the diatom Communities were more homogenous. Those sites with similar values tended to cluster together. As expected, the diatom indicators corresponded to some extend with community structure (Cook 1976; Kelly et al. 2008).

The IndVal % results (Table 6) show that there is no clear indicative species for the years. *Navicula cryptotenella* Lange-Bertalot dominated in 2002, *Encyonopsis microcephala* (Grunow) Krammer and *Achnanthidium pyrenaicum* (Hustedt) Kobayasi in 2007 and 2008 and finally *Encyonopsis minuta* Krammer & E. Reichardt in 2012. The predominant taxa of the *Cocconeis placentula* Ehrenberg complex have not been considered, since they pertain mostly to epiphytic species (Romero and Jahn 2013) that only colonize the epilithic substrates secondarily. The results of these predominant diatoms showed an increase in water quality. Species found since 2007 have been described as oligotraphentic. The first four years had a higher proportion of river axis sites. These were abandoned after time and the last years had a mostly (two thirds) mountainous sampling.

The Multiple Regression Matrices (MRM) study (Table 7) showed significant community changes for each site over time. Therefore, a three-year group approach with different location number was performed. Some years did not show significant differences in diatom communities. This may be related to the physico-chemical stagnancy of some sites. Neither sampling periods 2005 – 2006 nor 2011 – 2012 showed significant differences. This may be related to the disparate diatom communities ("outlaying locations") found in both 2006 and 2012.

4.4.4 Comparison of all approaches

Neither BioEnv calculation showed that a maximum of 9 physico-chemical and geospatial data were not adding noise, thus 7 axes were explaining the diatom communities. The most common predictor was altitude, the only geo-spatial data used. This can be explained by the main division into mountain and axis rivers, which is apparent in average sites subdivision and reference site partition. This parameter is linked, to a lesser extent, also to water temperature. Most important descriptors in other studies (Soininen 2007) have been conductivity, pH, total phosphorous concentration and altitude. These parameters differed in this study. Diatoms at the Ebro sites did not show a preference for pH or phosphate concentration levels. These sites usually have a higher pH (above 8.15) and lower phosphate concentration values (below 0.08 mg/l). The only absent river ecotype was the high mountain ecotype, since the sites had been sampled less than 7 times. Conductivity was not uniformly essential, as it was normally over 400 μS/s (Penalta-Rodríguez and López-Rodríguez 2007; Hlúbiková et al. 2014). Since the high mountain ecotype had the lowest conductivity, its absence increased the mean conductivity to 629.8 μS/cm.

The Canonical Correspondance Analysis (CCA) explains a substantial amount of variance, of the order of 70.25 % (AnnexFig. 5). As expected, conductivity and altitude were juxtaposed (Cantonati et al. 2012). No clear subdivision of the sampling sites appeared at the yearly approach of the CCA, thus not shown. A yearly average approach is pointless, because of the lack of variation produced by the reduction would decrease the explained variability to random levels.

A yearly subdivision into sites is not workable. Thus, the cluster subdivision seems to be the better approach. A subdivision into 2, 4, 5 and 6 groups produced a gradual reduction of the diatom predominance (not shown). Using the successive cluster partitions an important change appeared. The specificity decreased with every added subdivision. The mean site clusters increased the percentage of maximum variance per site, therefore being more specific but decreasing the significance of each of the describing diatoms.

The most striking difference of diatom communities in mountains versus the axis is in physico-chemical composition of the rivers and has been stated. Previous studies provided that river ecotypes should always prevail to further knowledge of diatom adherence (Rimet et al. 2007). The typology of rivers could also be prevalent as a non-negligible source of diatom community variability (Gomà et al. 2005). This was not particularly observed with our data. Altitude prevailed over substrate type. The only observed variability being the one of the silicate substrate which was represented by 5 sites. Ecotype 111 or the not observed 127, did not have enough viable samplings (AnnexTable 9). The calcareous river ecotypes described for 112 and 126 represent most of calcareous river beds. Otherwise, most substrates presented a mixed nature (Bejarano et al. 2010). This explains why a subdivision of further river ecotypes is not observable in our data. No diatom communities were found to adhere to the "margalefian" division of phytosociology (Margalef and Cambra, n.d.; Margalef 1954).

The yearly comparison had such little diatom predominance was expected, since diatoms would change according to environmental characteristics, which change constantly and not necessarily similarly along the sampling sites. Sites do not show any predominance, maybe because most diatom species are relatively ubiquitous, therefore reducing the influence in one site to a relatively low percentage. River ecotypes performed better than expected, since their maximal prevalence was 36 %. Significances were maintained. Since river ecotypes were represented unbalanced, ecotypes with few sites portrayed higher percentage of diatom predominance (see AnnexTable 8). Therefore, river ecotype subdivision is therefore not deemed to be a good indicator of diatom dispersal.

Finally, the subdivision by diatom communities gave the clearest result. It described both, predominant diatoms and physico-chemical differentiation, at a significant level. A subdivided approach may have different advantages. If physico-chemical or diatom based, the significances vary along a two-fold gradient. The more clades are present, the less significance in diatom predominance and the lower the proportion are. Our final advice would be the use of the SOM and physico-chemical clustering since it reduces the effect of the outsiders in composition.

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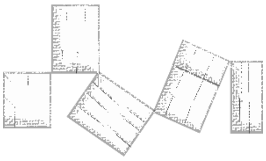
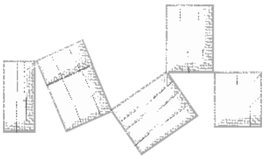


CHAPTER 5:

MESO-SCALE



Chapter 5

MESO-SCALE

5. LAND USE EFFECT ON DIATOM COMMUNITIES

5.1 Introduction

Diatoms are single-celled aquatic organisms which propagate rapidly. This enables them to respond quickly to any physico-chemical changes in water ecosystems (Lange-Bertalot 1979a; Mayama 1994; Prygiel and Coste 1993). This feature makes diatoms useful water bioindicators and several diatom indices have been created to monitor water quality (Prygiel and Coste 1993; Prygiel et al. 2006; Gudmundsdottir et al. 2013; Watanabe et al. 1988). These indices are based on the tolerance of different diatom taxa to varying trophic level and saprobic water pollution. Thus, the Water Framework Directive (WFD, European Commission 2000) utilises benthic, epilithic diatoms in the annual protocols of phytobenthic river-water surveillance.

Different types of land use in a river basin can have a direct or indirect impact on the physico-chemical characteristics of the water in the basin, especially on nutrients and other dissolved substances. For instance, water runoff can cause an increase in suspended sediment and can be reduced by the uptake of nutrients by flora (Pfister et al. 2004). Land Use can change the chemical and physical characteristics of a river and thus affect benthic diatoms (Allan 2004; Dauer et al. 2000). This connection was studied by Biggs (1995), who reported a correlation between periphyton abundance, land use and geology. Moreover, Jüttner et al. (1996), related land use in Nepal with diatom communities.

The Segre river basin is located in northeast Spain and is a tributary to the River Ebro. Several geological substrates and climates, which range from alpine to Mediterranean, are found in this basin. A diatom control network was established in the river in 2002 by the Ebro Water Agency (Confederación Hidrográfica del Ebro, CHE). In tributaries in the studied basin, the climate is mostly dry in summer, which affects the water cycle and may cause a temporary absence of streams (Kennard et al. 2010; Lake 2011, 2003). Regarding river catchment distribution, around the headwaters, natural areas such as meadows and woods tend to be dominant (Gomà et al. 2005; Rimet et al. 2007). In contrast, lower river stretches are mostly surrounded by agricultural land and large urban areas (Barceló et al. 2011; Batalla and Vericat 2011; Romaní et al. 2011). The main objective of this study is: Can we expect each type of land use to generate a characteristic diatom community? Our goal was to study how land use can impact the epilithic diatom communities in Mediterranean rivers, and variations in these communities over time.

5.2 Materials and methods

A total of 61 samples of benthic diatoms were collected at 16 sites in the Segre river basin (Figure 19a). Orthophotographs were accessed via the Catalan National Atlas (ICGC 2015) to classify the different land uses (Figure 19**Fehler! Verweisquelle konnte nicht gefunden werden.**b), taking up to 5 km upstream of each site into account, as we had showed to be reasonable in a previous occasion (Burfeid Castellanos and Cambra 2016). This was performed on a regional scale, since nutrient, sediment and physico-chemical traits are not just local (Allan et al. 1997). Land use was subdivided into 6 identifiable types (Table 8):

Table 8 Land use types and description

Land use	Code	Description
Agricultural	AGRI	Clear agropecuary use
Dry land	DRLD	No identifiable vegetation
Pasture and meadow	MEAD	Low vegetal cover
Urban and industrial use	URBN	Anthropic uses such as urban and industrial cover.
Reservoirs	WATE	Water surface (including river width and reservoirs)
Woodlands	WOOD	Forestall coverage

These recognizable land use classes were polygonised using the QGIS Nødebo 2.16 software (QGIS Development Team 2009). We used an ImageJ plug-in, "RGB measurement" (Abràmoff, Magalhães, and Ram 2004) to render the polygons (Fig. 19b**Fehler! Verweisquelle konnte nicht gefunden werden.**) in order to obtain a percentage of the total land use coverage. A database containing the

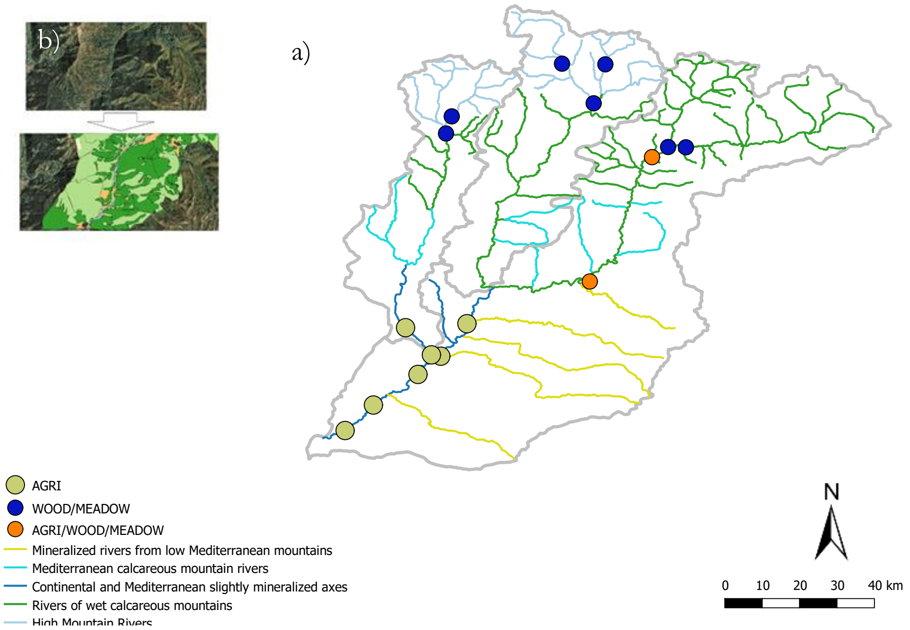


Figure 19 Distribution of the sampling locations (a) and polygonization of the orthophotographs for different kinds of land use (b). (Credits: Confederación Hidrográfica del Ebro, Institut Cartogràfic de la Generalitat de Catalunya).

proportional land cover, physico-chemical values and diatom species was created. This was later subdivided into each of the land uses.

Physico-chemical parameters that affect diatoms most, such as nutritional values (phosphate, nitrate and ammonium concentration), conductivity, suspended solids concentration and temperature were extracted from the Ebro river Authorities website (Confederación Hidrográfica del Ebro 2015). Henceforth, concentration will be implied in the use of concentration of phosphate, nitrate, ammonium, suspended solids and dissolved oxygen.

For the statistical analysis R 3.3.1. was the software used (R Development Core Team 2008). Noise reduction was performed by using average community, chemistry and land use changes (Kelly et al. 1998). To observe changes in time, the datasets were compared using ANOVA and Spearman's (ϱ) correlations. Clustering of sites and land cover was analyzed using the R-package "pvclust" (Suzuki and Shimodaira 2015), principal component analysis (PCA) and canonical correspondence analysis (CCA) related physico-chemical composition to the diatom community using package "vegan" (Oksanen 2014). IndVal % indicator species analysis was performed using the "indicspecies" package (De Cáceres 2013) to observe specificity of diatom taxa relating to land use.

In order to observe changes over time, we used Structural Equation Models both on the whole dataset (n=61), as well as for subsets containing samples from 2002-2007 (n=26) and 2008-2013 (n=35) using the "lavaan" package (Rosseel 2012). After clustering the physico-chemical parameters, the sites studied were aggregated according to land use (Figure 24). Complete database analysis showed good adherence of site over time.

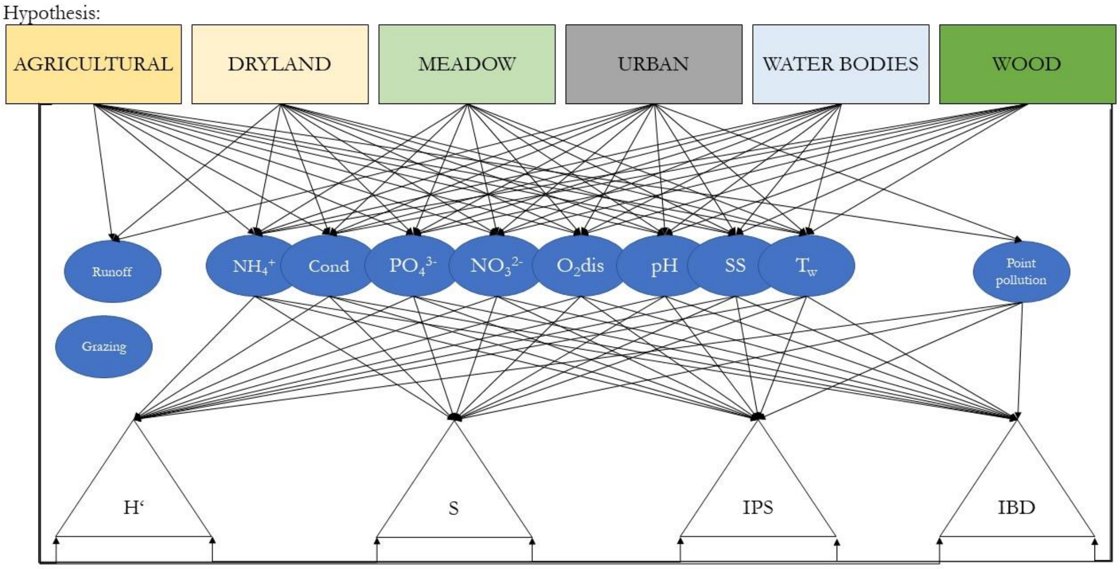


Figure 20 Hypothesis of the Structural Equation Modeling of land use, physico-chemical data and diatom (diversity and bioindicative) indices. All land uses would be connected to diatom indices, not shown for clarity. Unobserved parameters that may condition diatoms were added: runoff, grazing and point pollution. NH_4^+ = ammonium, Cond = Conductivity, PO_4^{3+} = phosphates, NO_3^{2-} = nitrate, O_2dis = dissolved oxygen, SS = suspended solids, T_{H2O} = water temperature. H' = Shannon diversity index, S = Species richness, IPS = Polluosensitivity index, IBD = Biological diatom index.

We based this work on the assumption that land use percentages affect the physico-chemical composition of the river. To do this, we used the parameters established previously to postulate hypothetical links between the parameters. The totality of the land uses, physico-chemical parameters and the Shannon (H') index (Shannon 1948; Shannon and Weaver 1964), Simpson (D) index (Simpson 1949), diatom Richness (S) and diatom biomonitoring index values of IPS and IBD were considered. Based on bibliography a hypothetical network of land use, physico-chemical and diatom parameters was established (Figure 20).

5.3 Results

5.3.1 Land use and physico-chemical traits

Land use could roughly be divided into "anthropic" and "natural". Woodlands and agricultural land at each site had inverse proportions (Figure 21), while meadows were not necessarily linked to agricultural use. Urban land uses were mostly found together with high agricultural use. The main differences for land use typse related to runoff and pollution. Dams and high water type proportions were found randomly at lower altitudes.

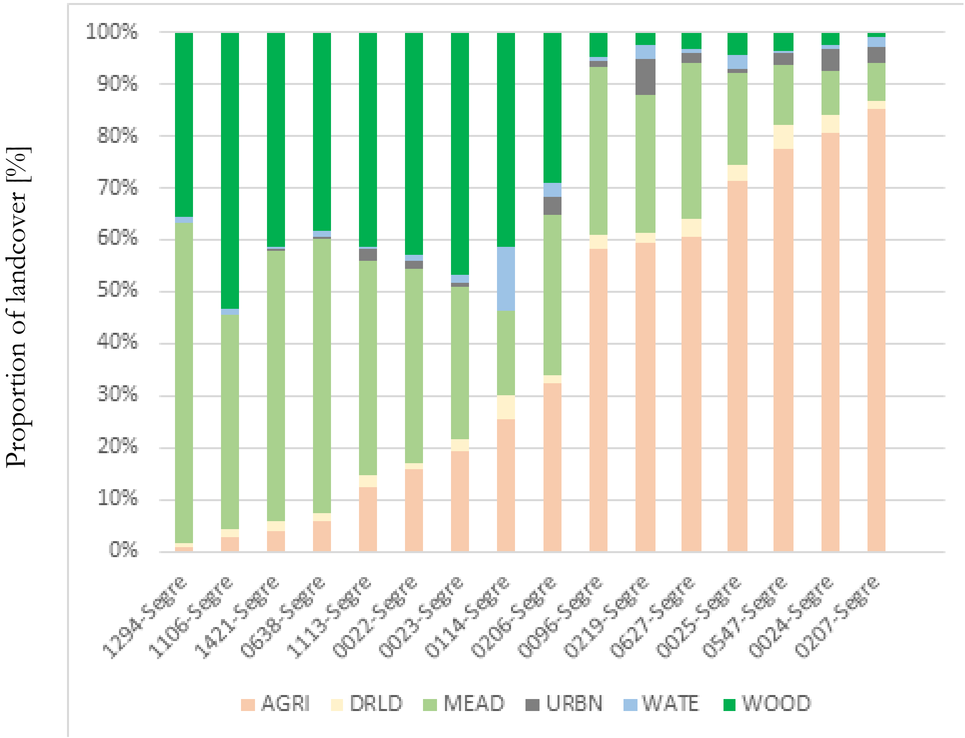


Figure 21 Proportion of different land uses at each site (see Table 8)

The Principal Component Analysis (PCA) of the sites explained 67.7 % of variance on the first two axes of the differentiation of diatom communities in relation to land uses (AnnexFigure 22). The sites were clearly subdivided into two groups: 1) the first axis was related to the natural–anthropogenic sites (50.8 % of the variance); and 2) the second axis represented water types (16.9 % of the variance).

On the natural–anthropogenic gradient, agriculture, meadow and wood were the main drivers. Dryland and urban uses were associated with agriculture. The water-type axis displayed a gradient of diatom types due to the addition of planktonic and motile taxa.

The Canonical Correspondence Analysis (CCA) of physico-chemical parameters of water and land use explained 69.9 % of the variance (Figure 23). The first axis explained 68% of the variance and was related to conductivity, nitrates, and (weakly) temperature. The sites were also clearly divided according to their predominant land use. Sites with predominant agricultural use were mostly clustered. In contrast, those sites with similar proportions of anthropogenic and natural uses were highly differentiated, and had an intermediate physico-chemical composition. Natural land uses were also clustered together with physico-chemical parameters: Meadow presented lower temperature and suspended solid concentrations, while woodlands had low nutrient inputs.

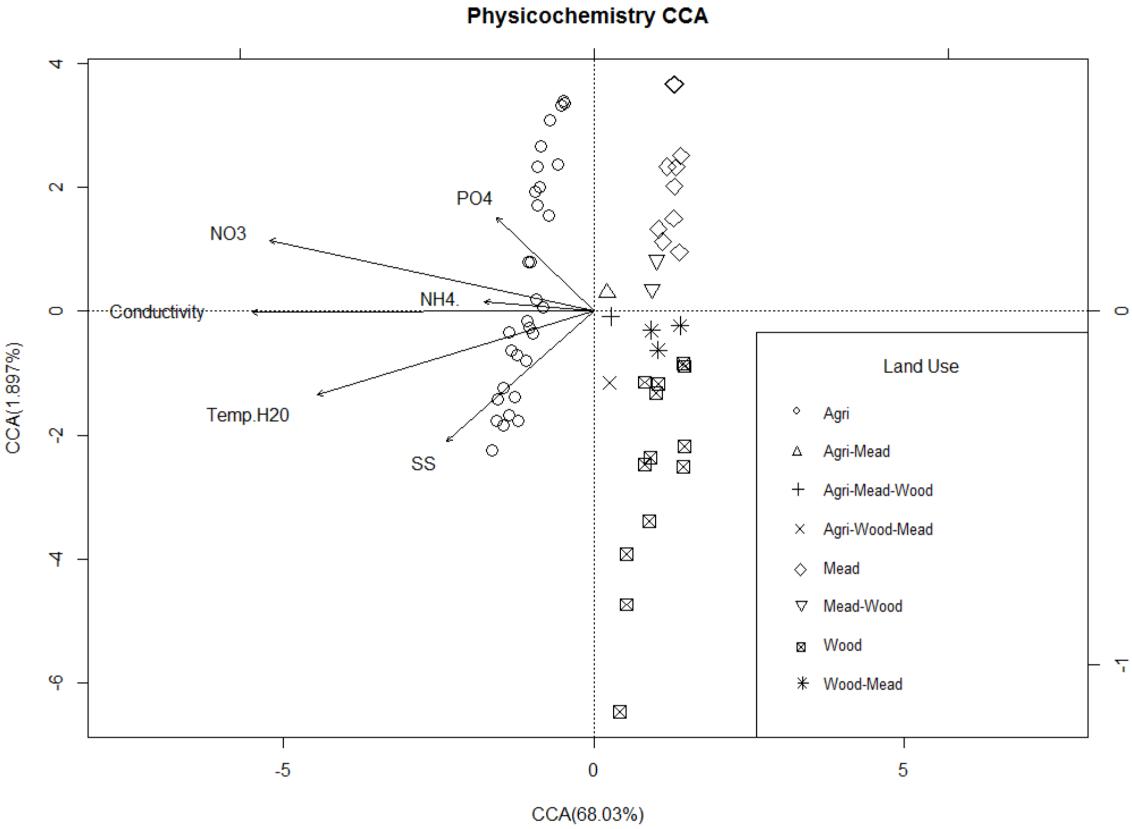


Figure 22 Canonical correspondence analysis (CCA) of physico-chemical parameters and land use. The principal axes represent altitude reduction (x) and nutrient increase (y). Points are sites describing the predominant land uses, as shown in the legend: Agri = agricultural land, Mead = meadow, Wood = woodland. Axes: PO4 = phosphate, NO3 = nitrate, NH4 = ammonium, Temp.H2O = water temperature, Cond = Conductivity, SS = suspended solids.

The ANOVA-test showed that conductivity, nitrate concentration and water temperature were significantly different between clusters. Most parameters, except ammonium and dissolved oxygen, were significantly correlated with the predominant land uses: agricultural land, meadows and woodlands. Moreover, the natural habitats were strongly correlated with a low nitrate concentration (MEAD: ϱ = -0.772***, WOOD: ϱ = -0.723***) and temperature (MEAD: ϱ = -0.664***, WOOD: ϱ = -0.639***). However, only the wood land use was negatively correlated with conductivity (Spearman ϱ = -0.751***). Other land uses were inversely correlated positively to each of these parameters. For instance, agricultural land correlated with conductivity (ϱ = 0.806***), nitrate levels (ϱ = 0.772***) and temperature (ϱ = 0.724***). Dry land, urban land and reservoirs also increased in conductivity, nitrate and temperature. Their significance and correlation was lower, but they showed the same detrimental effect on water quality. Conductivity, in turn, increased (DRLD: ϱ = 0.333*; URBN: ϱ = 0.628***; WATE: ϱ = 0.314*). Nutrients (DRLD: ϱ = 0.279*; URBN: ϱ = 0.618***; WATE: ϱ = -0.321*) and temperature (DRLD: ϱ = 0.348*; URBN: ϱ = 0.395; WATE: ϱ = 0.293*) were also correlated.

5.3.2 Land use, diatom communities and diversity

We identified a total of 177 diatom taxa. The predominant taxa were: *Cocconeis placentula var. euglypta* (Ehrenberg) Grunow*, Achnanthidium pyrenaicum* (Hustedt) H. Kobayasi, *Amphora pediculus* (Kützing) Grunow ex A. Schmidt*, Achnanthidium minutissimum* (Kützing) Czarnecki and *Navicula cryptotenella* Lange-Bertalot.

After clustering the physico-chemical parameters, the sites studied were aggregated according to land use (Figure 24). An analysis of the complete database showed that the sites were adherent through time.

The Spearman Rank correlation was performed on IPS, IBD, and the diversity indices such as Shannon and Species richness, as well as diatom proportion relative to physico-chemical composition of the site (AnnexTable 15). IBD was the index with highest correlation values and significance (ϱ = 0.08*** – -0.7988***). Diatom species richness also was significant, even though the values were lower (ϱ = 0.03*** – 0.46***).

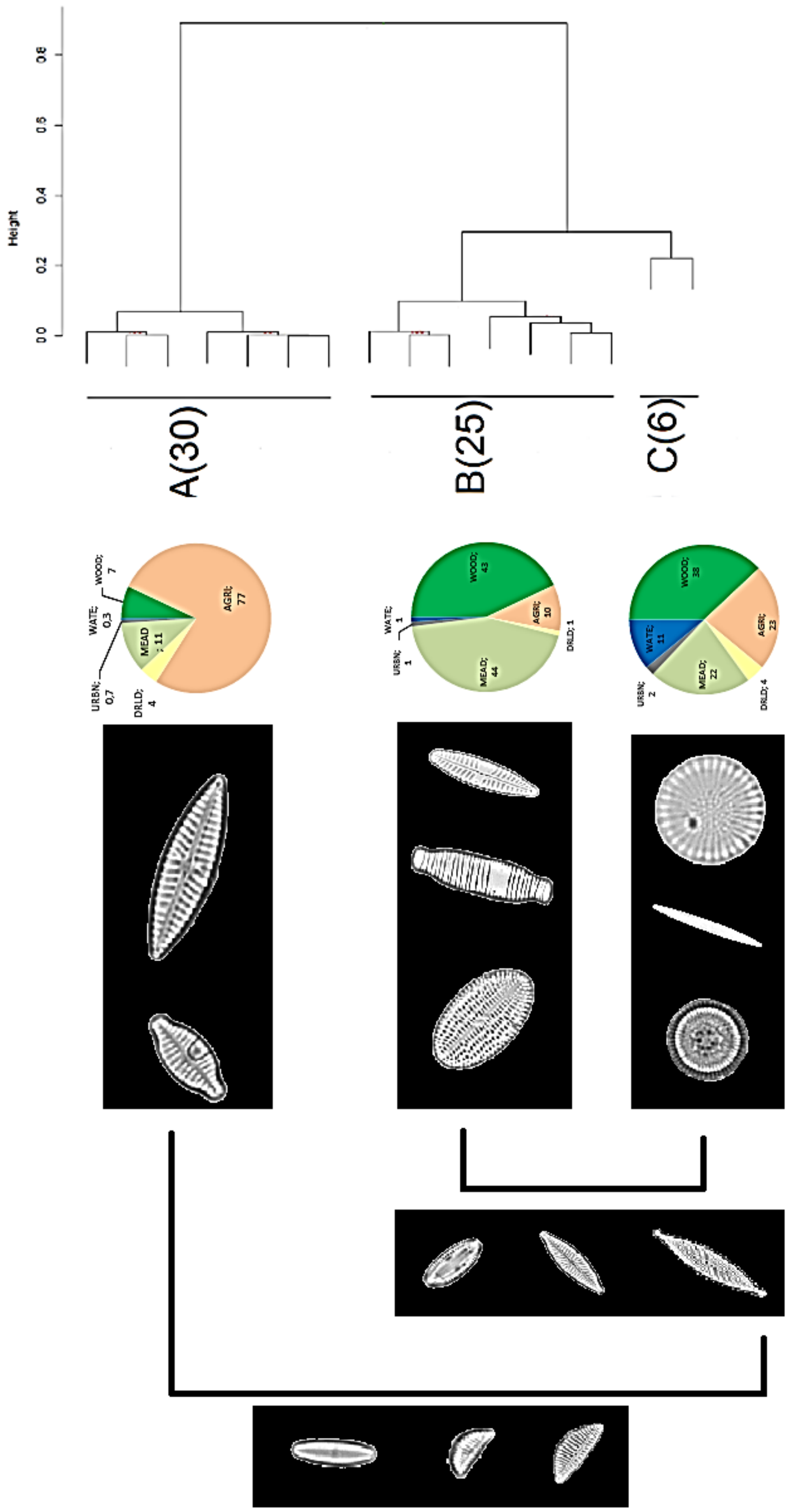


Figure 23 Site distribution, land use and community composition of the clusters of 10,000-iteration hierarchical clustering of the physico-chemical data (UPGMA, average linkage). A is predominantly meadows at low altitude; B is mostly agricultural land; and C is mixed natural uses (meadow and woodland).

The IndVal % analysis classified the indicative diatoms for each cluster as river land use (Table 9). The predominantly agricultural group (A) comprised taxa tolerant to environmental conditions such as *Planothidium rostratum* (Østrup) Lange-Bertalot, *Navicula antonii* Lange-Bertalot and *Staurosirella pinnata* (Ehrenberg) Williams & Round. In contrast, the Mead – Wood sites (B) had sensitive diatoms such as *Cocconeis pseudolineata* (Geitler) Lange-Bertalot, *Diatoma ehrenbergii* Kützing and *Gomphonema tergestinum* (Grunow) Schmidt. The woodland-agriculture-mead group (C) was characterized by usually planktonic species like *Cyclotella ocellata* Pantocsek, *Nitzschia paleacea* (Grunow) Grunow, *Cyclostephanos dubius* (Hustedt) Round and *Stephanodiscus hantzschii* Grunow. Clusters also shared some identifying diatoms: the A and C groups coincided in high frequencies of the tolerant diatoms *Amphora pediculus* (Kützing) Grunow ex A. Schmidt, *Navicula reichardtiana* Lange-Bertalot and *Navicula capitatoradiata* Germain. All diatoms shared between clusters B and C were sensitive to pollution, for example, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, *Encyonema minutum* (Hilse) D. G. Mann and *Encyonema silesiacum* (Bleisch) D. G. Mann.

Table 9 Indicative value analysis of the pvclust clusters. Predominant diatom taxa correlate to land use clusters. A = Agri, B = Mead + Wood, C = Agri + Mead + Wood. Significance of type-describing species calculated through Monte Carlo permutation test with 999 permutations (p<0.001). Average and maximal IndVal proportions are also given. Maximal indicator values are shown in bold. Taxon verification using DiatomBase (Kociolek et al. 2015)

	Omnidia Code	**Cluster**	**R^2**	**Significance class**
Planothidium rostratum (Ostrup) Lange-Bertalot	PROS	A	0.796	**
Navicula antonii *Lange-Bertalot*	NANT	A	0.682	*
Staurosirella pinnata (Ehrenberg) Williams & Round	SPIN	A	0.647	*
Pseudostaurosira brevistriata (Grunow) Williams & Round	PSBR	A	0.632	*
Navicula lanceolata Ehrenberg	NLAN	A	0.601	*
Navicula cryptotenelloides Lange-Bertalot	NCTO	A	0.595	*
Kolbesia gessneri (Hustedt) Aboal	KGES	A	0.548	*
Reimeria uniseriata Sala, Guerrero & Ferrario	RUNI	A	0.584	*
Ellerbeckia arenaria (Moore Ex Ralfs) Crawford	EARE	A	0.516	*
Cocconeis pseudolineata (Geitler) Lange-Bertalot	CPPL	B	0.820	**
Diatoma ehrenbergii Kützing	DEHR	B	0.69	**

Gomphonema tergestinum (Grunow) Fricke	GTER	B	0.632	*
Cyclotella ocellata Pantocsek	COCE	C	0.695	*
Nitzschia paleacea (Grunow) Grunow	NPAE	C	0.663	*
Cyclostephanos dubium (Hustedt) Round	CDUB	C	0.577	**
Stephanodiscus hantzschii Grunow	SHAN	C	0.561	**
Amphora pediculus (Kützing) Grunow Ex Schmidt	APED	A+C	0.962	***
Navicula reichardtiana Lange-Bertalot	NRCH	A+C	0.856	***
Navicula capitatoradiata Germain	NCPR	A+C	0.840	**
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	RABB	A+C	0.840	**
Sellaphora atomoides(Grunow) Wetzel & Ector	EOMI	A+C	0.777	*
Gomphonema minutum (Agardh) Agardh	GMIN	A+C	0.759	*
Craticula subminuscula (Manguin) Wetzel & Ector	ESBM	A+C	0.76	**
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	FSAP	A+C	0.76	*
Navicula cryptocephala var. veneta (Kützing) Rabenhorst	NVEN	A+C	0.728	**
Nitzschia amphibia Grunow	NAMP	A+C	0.711	*
Cyclotella cyclopuncta Hakansson & Carter	CCCP	A+C	0.595	*
Amphora copulata (Kützing) Schoemann & Archibald	ACOP	A+C	0.527	*
Achnanthidium pyrenaicum (Hustedt) Kobayasi	ADPY	B+C	0.941	***
Encyonema minutum (Hilse) Mann	ENMI	B+C	0.922	***
Encyonema silesiacum (Bleisch) Mann	ELSE	B+C	0.918	***
Reimeria sinuata(Gregory) Kociolek & Stoermer	RSIN	B+C	0.856	**
Fragilaria capucina var. vaucheriae (Kützing) Lange-Bertalot	FCVA	B+C	0.831	**
Hannaea arcus (Ehrenberg) Patrick	HARC	B+C	0.803	**

Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM	B+C	0.803	**
Fragilaria capucina subsp. rumpens (Kützing) Lange-Bertalot	FCRU	B+C	0.648	*
"Achnanthidium atomus (Hustedt) Monnier, Lange-Bertalot & Ector"	ADAT	B+C	0.596	*
Gomphoneis minuta (Stone) Kociolek & Stoermer	GSMI	B+C	0.596	*

p-values: 0 '***' 0.001 '**' 0.01 '*' 0.05

The CCA of the diatom communities explained only 9.9 % of the variance: 7.2% on the first axis and 2.7% on the second (Figure 25). Eigenvalues for the first four axes were 0.51221, 0.19409, 0.17312 and 0.0991 respectively. Agriculture, meadows and woodlands were the main drivers for this distribution. Dry land and urban land use had less impact and were strongly associated with agricultural land. Since urban land was not significant in the *p* permutation, it is not depicted in the resulting graph. The juxtaposition of anthropogenic and natural land uses remained. Here, values for both, meadows and woodlands, were inversely proportional to those of agricultural use. There was a good subdivision of site clusters, as bootstrap factors were over 90%.

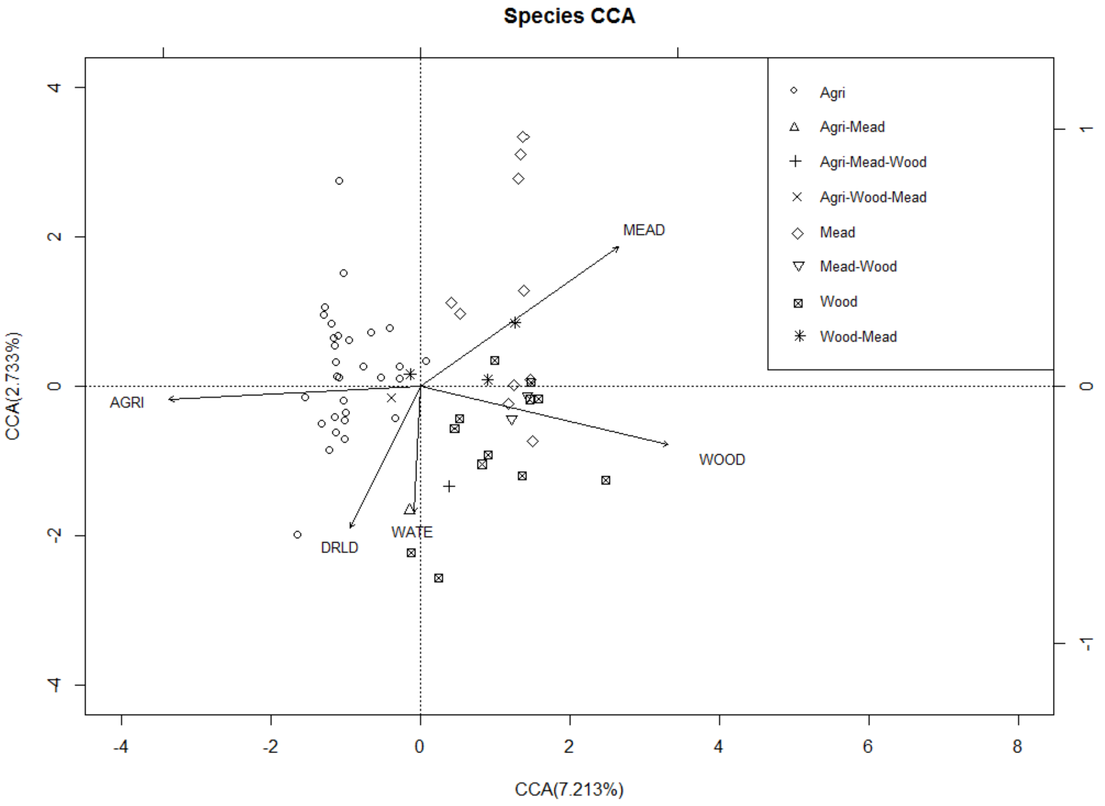


Figure 24 Canonical correspondence analysis (CCA), describing the expected distribution of species at the different sites according to predominant land use: Agri=Agricultural land, Mead = meadow, Wood = woodland. . Total variance explained is 9.9%.

The distribution of diatom communities according to land use composition, as calculated by the IndVal analysis and remaining analyses is summarized in Figure 26.

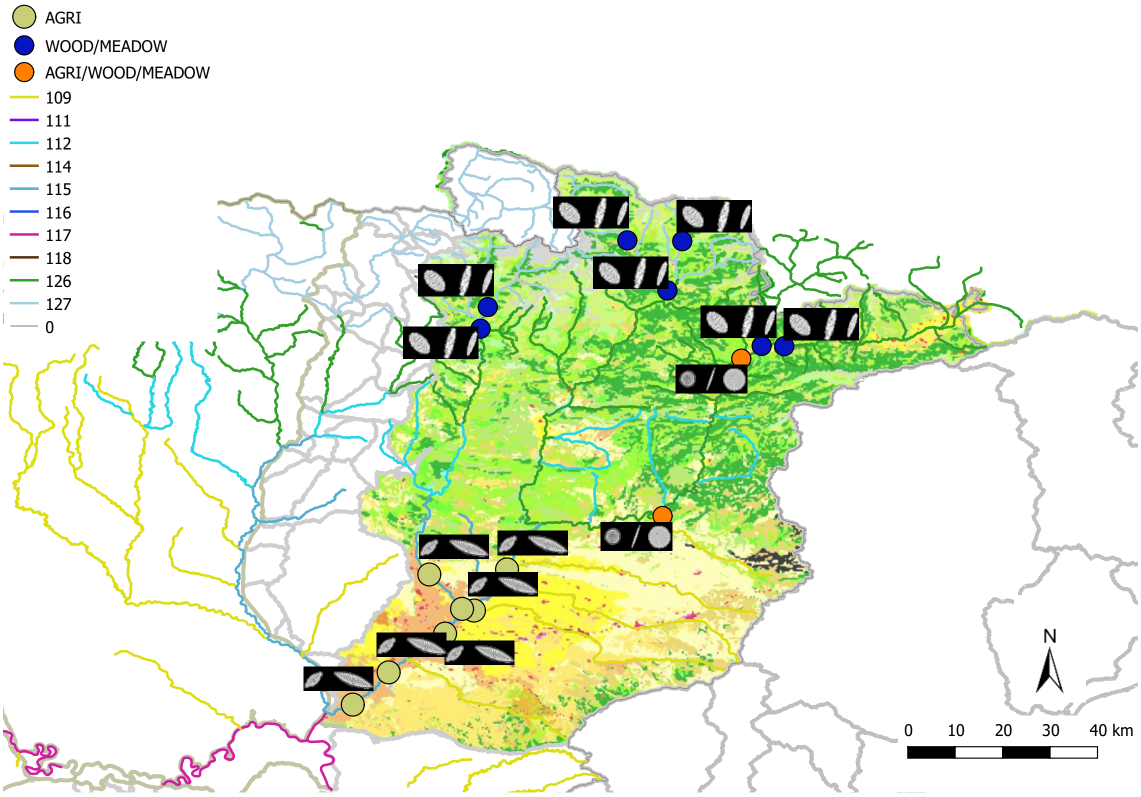


Figure 25 Distribution of the majority diatom communities. AGRI = agricultural cover, MEAD/WOOD = meadow and wood equistasis, AGRI/MEAD/WOOD = agricultural, meadow and wood covers in relative equity. River Ecotypes: 109 = Mineralized rivers from low mediterranean mountains, 111 = Mediterranean siliceous mountain rivers, 112 = Mediterranean calcareous mountain rivers, 114 = Mediterranean axes on low altitudes, 115 = Continental and mediterranean slightly mineralized axes, 116 = Continental and mediterranean mineralized axes, 117 = Main axes in mediterranean environment, 118 = Coastal Mediterranean rivers, 126 = Rivers of wet calcareous mountains, 127 = High mountain rivers, 0 = river ecotype undetermined.

5.3.3 Land uses and diatom indices

Diversity and species richness parameters of the A and C cluster had similar medians (Figure 27a). Group B was not significantly differentiated, but always had a lower value than the other clusters, while increasing the IPS, IBD and CEE biomonitoring values. The Simpson diversity index showed the highest variability, followed by species richness. Only agricultural land and woodlands were correlated with diversity indices. Diatom species richness decreased in woodlands ($r = -0.611^{**}/ \varrho = -0.526^{*}$). Agricultural land use led toe increased diatom diversity index values ($r = 0.49832^{*}$) and richness ($r = 0.6087^{*}/ \varrho = 0.627^{*}$). Meadows did not show a significant correlation with any of the diversity indices.

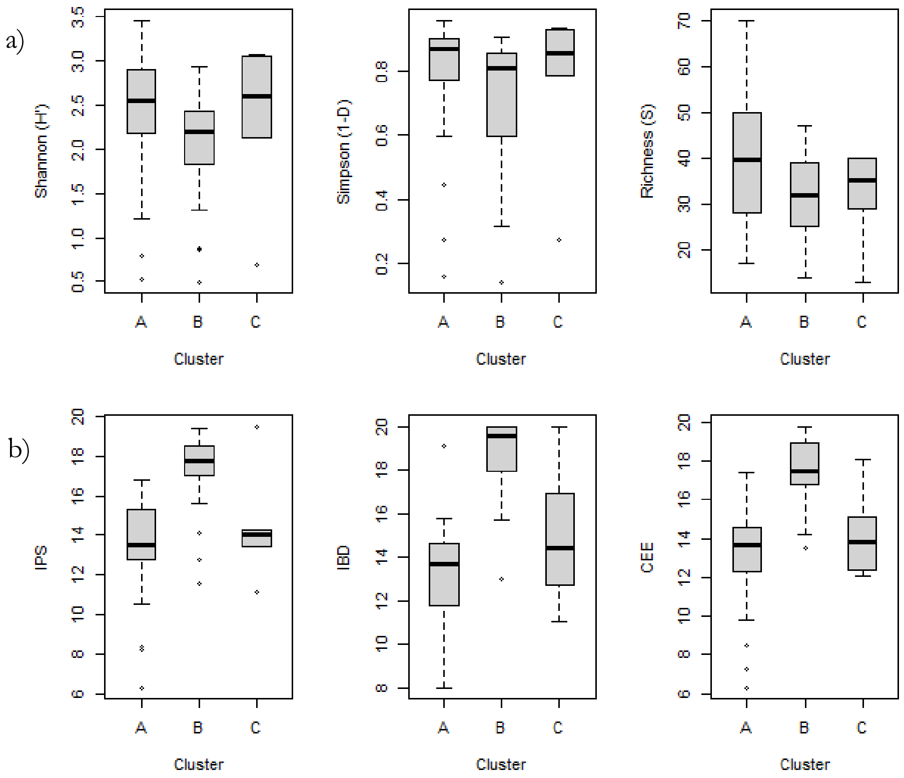


Figure 26 Comparison of diversity index (Shannon and Simpson) and species richness values as well as trophic diatom indices (IPS, IBD and CEE) for each cluster.

Trophic diatom indices were differential and were mostly related on the proportion of agricultural land (Figure 27b). Hence, the most differential was the B cluster (Mead – Wood). The increase of natural land use cover in the C cluster did markedly increase the index values (IBD and CEE) in respect to the A cluster, except for the IPS. Agricultural land proportion decreased index values. The most significant differentiation was found in the IBD trophic index. Neither Pearson calculation of correlation between land use and trophic index was significant for all of the types (Table 10). Nonetheless, water quality seemed to increase with natural uses, as seen through the positive correlation, and decreased significantly for the anthropic uses.

Table 10 Pearson correlations (r) of land use and the trophic diatom indices IPS, IBD and CEE. Maximal value marked in bold.

	AGRI	**MEAD**	**URBN**	**WOOD**
IPS	-0.785***	0.722**	**-0.586***	0.711**
IBD	**-0.920***	**0.801***	-0.570**	**0.882***
CEE	-0.830***	0.751**	-0.577*	0.778***

p-values: -0 '***' 0.001 '**' 0.01 '*' 0.05 .

5.3.4 Mixed approach: Structural Equation Modelling of land use, physico-chemical parameters and diatom indices grouped by communities and years

When observing the whole dataset (61 sites, Figure 28), the χ^2 was of 297.166, with 117 degrees of freedom and highly significant (p < 0.0001). The model was not saturated, and the baseline model showed increased values of the χ^2 statistic, the comparative fit index was 0.784. The only latent variables not significant were waterbodies (standardized Estimate (stdE) = 0.161, p = 0.210), dissolved oxygen (stdE = - 0.128, p = 0.338) and pH (stdE = 0.246, p = 0.093). The correlations of physico-chemical parameters on land use were significant but low (stdE = 0.953, p = 0.002), while diversity was not significantly correlated to neither land use (stdE = 0.542, p = 0.357), nor physico-chemical values (stdE = -0.183, p = 0.752). Bioindicative values were not significant either for land use (stdE = 0.281, p = 0.742) or compositional parameters (stdE = -1.127, p = 0.196). The only significant covariance was found between phosphate concentration levels and IPS values. Land use had the highest variance estimate (stdE = 1) and was significant, while the two dependent parameters (physico-chemical and trophic indices) were not (stdE fq = 0.093, p = 0.175; bioindicator indices = 0.253, p = 0.129). On the other hand, diversity indices had significant and high-ranking values (stdE = 0.862, p = 0.003). The resulting modification of the original hypothesis shown in Figure 28 showed that conductivity, nitrate and suspended solids concentrations were the most affecting parameters. It also underlined the weak correlation of land use with each of the parameters, and the negative

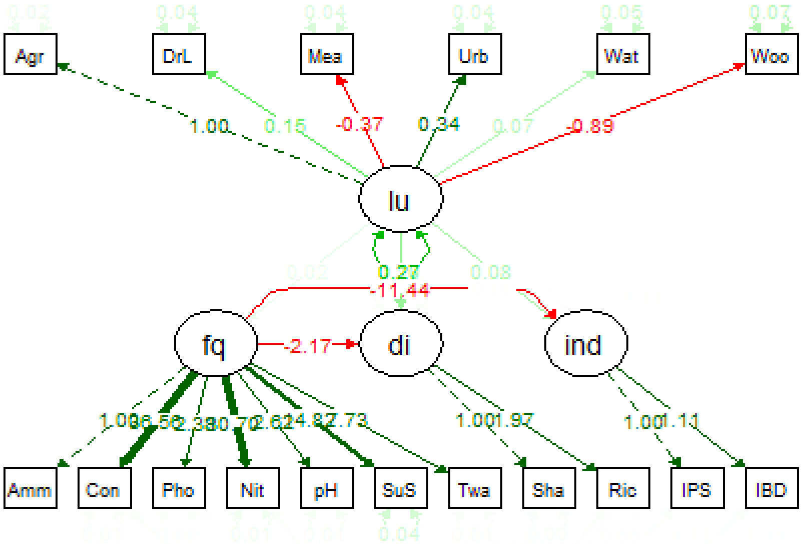


Figure 27 Corrected network of structural equation modelling with covariances and variances of each of the parameters studied. Agr = Agricultural, DrL = Dry Land, Mea = Meadow, Urb = Urban, Wat = Waterbodies, Woo = Wood,. Lu = land use, Fq = physico-chemical parameters, di = diversity indices, ind = trophic diatom indices, Amm = ammonium, Con = conductivity, Pho = phosphate, Nit = Nitrate, pH, SuS = Suspended Solids, Twa = water Temperature, Sha = Shannon Diversity (H') index, Ric = Species Richness (S), IPS = Indice de Polluo-Sensibilité Spécifique, IBD = Indice Biologique des diatomées.

correlation of physico-chemical composition with both, diatom biodiversity index values and trophic index values.

To test, whether the passing of time, and thus modification in use, varied the networks, we divided our samplings into 2 groups of 5 years [timeframe a)2002 – 2007 and timeframe b)2008 – 2013]. The maximum likelihood algorithm needed 411 iterations to converge. In this case, χ^2 increased to a total 609,946 [317,760 for timeframe a) and 334,897 for timeframe b), respectively], with 240 degrees of freedom and a significant difference between the two groups. Networks changed in each period (Figure 29). The second period adhered less to the baseline model than the first, which was related to the general disconnection of the physico-chemical parameters (not shown). The most linked covariances regarded phosphate concentration and species richness (StdE = 2.621***) for both periods, and for the first stage conductivity with both diatom indices (IPS StdE = 4.938 *** and IBD StdE = 1.141***), whilst for the second period conductivity correlated strongest with nitrates concentration and IPS for the second length of time (StdE = 7.137 ***). The regressions in the period of 2002-2007 were significant for land use and diversity values (StdE = 0.298*), physicochemical and diversity values (StdE = -18.35*) and physico-chemical and trophic index values (StdE = -11.841*). All regressions between land use, physico-chemical parameters, diversity measures and indicator values were insignificant for the 2008-2013 samples, apart from physico-chemical parameters and land use (StdE = 0.844***), as well as diversity values and land use (StdE = 1.618*). The most important parameters were conductivity, nitrate and suspended solid concentration values for timelapse a), and nitrate concentration for timelapse b).

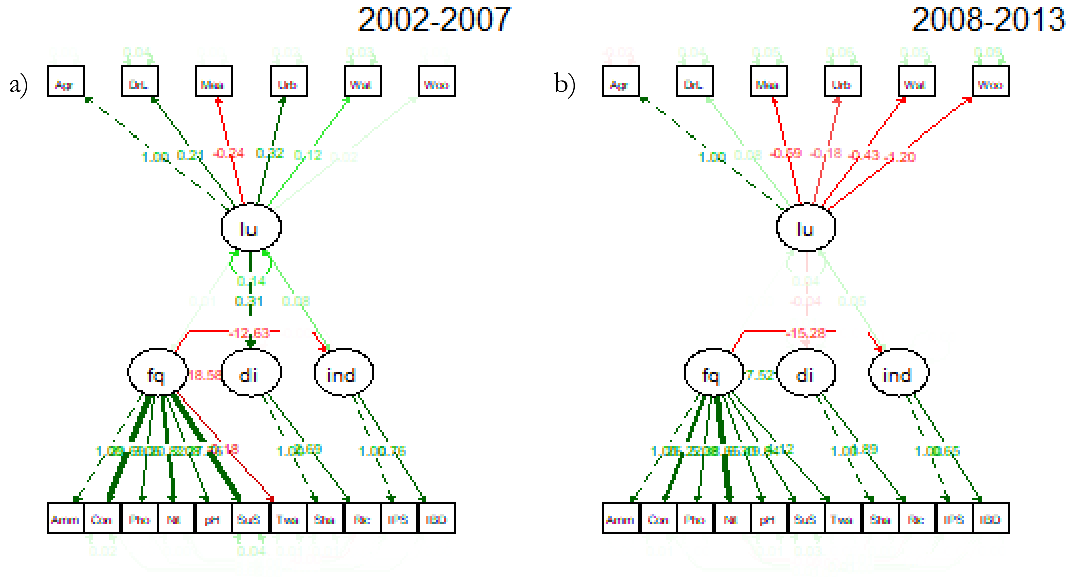


Figure 28 Comparison of the results for the a) 2002-2007 (n=26) and b) 2008-2013 (n=35) year period, changes in importance and connections, can be seen. Compare Fig. 28.

Finally, diatom communities were divided into cluster A) mostly agricultural use, cluster B) mostly a combination of meadow and wood cover. Cluster C), which comprised the sites with near to equal sizes of agricultural use, meadows and woodland, did not comprise enough sites to permit the SEM calculation. Thus, a comparison of the three clusters was not reasonable. The results of the two groupings a) and b) are shown in Figure 30, where the agricultural cover is mostly related to nitrate concentration and mead-wood mostly to conductivity and suspended solids concentration.

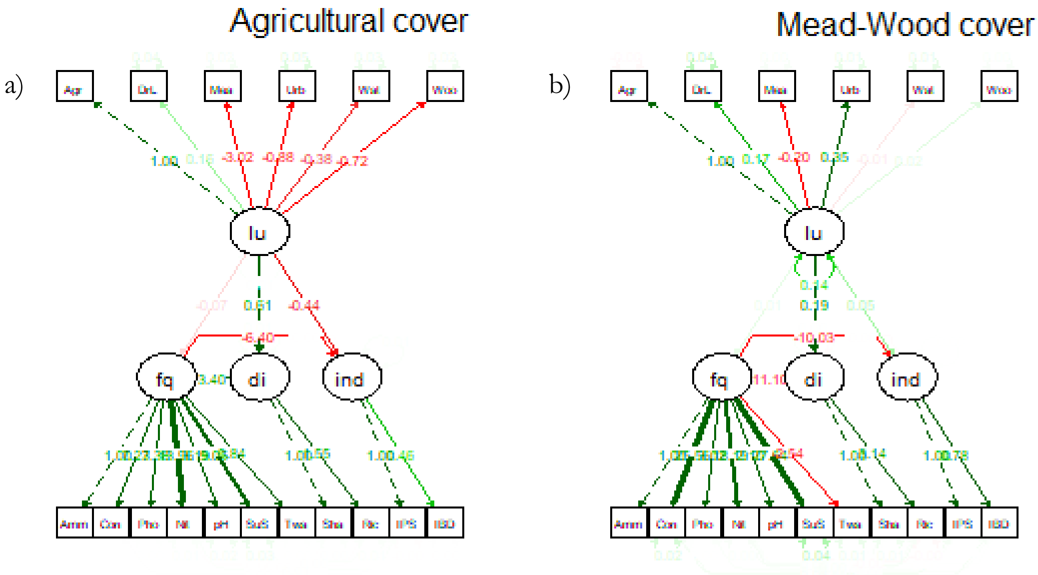


Figure 29 Comparison of diatom communities of cluster A) mostly agricultural land (n=28) and cluster B) mostly a combination of meadows and wood (n=25). Community c) (a combination of Agricultural, meadow and wood cover) was not a big enough group to obtain results (n=8). Compare fig. 28.

5.4 Discussion

5.4.1 Land use and physico-chemical traits

Land use distribution was closely linked to physico-chemical parameters in the Segre river basin. Other studies already correlated land use to changes in water characteristics (Allan 2004; Allan et al. 1997). In our investigation, the sites studied showed differences in the proportions of natural and anthropogenic land use, mostly due to a large altitudinal range.

Each land use category has characteristic effects on water traits. Particularly, anthropogenic land uses increase effluents and runoff. As a whole, agricultural and urban use affect nitrogen levels (Meador and Goldstein 2003). Altitude and temperature are also known to correlate to land use. For instance, higher basin altitude leads to more extensive agriculture and urban colonization in the lowlands (Allan 2004); while low levels of mountain agriculture can contribute to reforestation (MacDonald et al. 2000), thus creating an altitudinal gradient. Due to the annual nature of the sampling, it was not possible to pinpoint the seasonal pollution and runoff peaks expected from agricultural land use (Stevenson et al. 2009). The degree of these two alterations also depends on the crop, since timing, yield and type of fertilizer vary. The lack of plant cover (dry land in our study) increases erosion, but is not necessarily correlated to pollution(Oudin et al. 2008; Sajikumar and Remya 2015).Such sites are too few to have much influence on water composition and diatom community, since they only covered a maximum of 7.4 % of the upstream area: even less than the urban land. Both urban and industrial uses tend to increase runoff, and have also been reported to increase organic matter concentration and dangerous substance pollution (Blanck et al. 2003; Huang et al. 2012; Ramos et al. 1999). This correlates with the occurrence of saprophytic tolerant diatoms and an increase in pollution-tolerant diatoms, such as *Planothidium rostratum* (Ostrup) Lange-Bertalot and *Navicula antonii* Lange-Bertalot, which cause lower diatom index values. Reservoirs may reduce pollutant concentrations through sediment deposition (Morris and Fan 1988). This was not observed in our results, since the Spearman correlation of nutrient concentrations after waterbodies to diatom indices did not alter significantly. Large catchments and reservoirs can increase the incidence of planktonic diatoms in the downstream river (Rimet and Bouchez 2012), which was observed here.

Natural land uses present different characteristics. For instance, meadows often suffer continuous nutrient pollution due to cattle grazing and may present temporal or spatial point pollution, which we could not observe at the Segre river basin, due to the yearly sampling efforts, where meadows displayed the highest IPS values. Woods tend to decrease erosion and runoff (Angradi et al. 2009),while they may increase organic matter concentration and trophic input slightly through fallen leaf degradation. This was observed in our IPS values, since suspended solids concentration increased in predominantly wooded areas. Anthropogenic modifications of the soil tend to increase runoff (Pfister et al. 2004) compared to natural land uses.

Thus, CCA of the land uses and diatom communities mostly described the effects of runoff. Pollution was not represented comparably, since dry land clustered together with anthropogenic uses. Natural land uses clustered together due to the sediment retention caused by vegetation (Fan et al. 2016). These land cover types are usually associated with higher altitudes like alpine meadows, for instance. In such sites, waterbodies were sparse, but they affected lower-lying sites to a greater degree. Our results showed that in most cases, the high proportion of agriculture affects diatom communities strongly.

5.4.2 Land use and diatom communities and diversity

Most of the predominant diatom taxa were cosmopolitan. *Navicula antonii* Lange-Bertalot, *Navicula reichardtiana* Lange-Bertalot and *Navicula cryptocephala* Kützing are common small naviculoids found in eutrophic waters, although they are sensitive to organic pollution (Hofmann et al. 2011). *Navicula lanceolata* Ehrenberg is also deemed to be one of the most common nutrient and organic pollution-tolerant diatoms in Europe (Hofmann, Werum, and Lange-Bertalot 2011). *Achnanthidium pyrenaicum* (Hustedt) Kobayasi is abundant in oligotrophic, fast-flowing streams (Jüttner et al. 2011). *Amphora pediculus (Kützing) Grunow*, found in both agricultural and diverse land uses, is tolerant to trophic pollution. It is one of the most ubiquitous taxa of the genus (Lee and Round 1989; Bey and Ector 2013).

The IndVal results showed important indicator values, surpassing 50%. The A cluster was characterized by trophic-tolerant diatoms: *Planothidium rostratum* (Ostrup) Lange-Bertalot, *Navicula antonii* Lange-Bertalot, *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round and *Pseudostaurosira brevistriata* (Grunow) D. M. Williams & Round are also tolerant to salinity (Bey and Ector 2013). This group was affected by extensive agricultural land use and the resulting nutrient increase (Köck-Schulmeyer et al. 2011; Romaní et al. 2011). Low altitude is linked to greater agricultural exploitation of the land (Allan 2004). In cluster A, agricultural use covered between 50 % and 89 % of the total area. Thus, the presence of diatoms that tolerate mesotrophic and polytrophic conditions was observed, as expected (Winter and Duthie 2000a).

The B cluster had a diatom community that mostly does not tolerate organic pollution - though in some cases, such as *Cocconeis pseudolineata* (Geitler) Lange-Bertalot, they are oligo- to eutraphentic. Both *Diatoma ehrenbergii* Kützing and *Gomphonema tergestinum* (Grunow) Fricke only tolerate up to mesotrophic nutrient conditions (Bey and Ector 2013). This group was found in water associated predominantly with woodland and meadows, and displayed the highest values of the diatom indices.

The third cluster (C) was located at medium altitudes, characterized by both natural and agricultural land uses, and with an increased area covered by water, for instance due to reservoirs. We expected a slightly altered community dominated by planktonic taxa. For the most part, this is the case. *Cyclotella ocellata* Pantocsek, *Cyclostephanos dubius* (Hustedt) Round and *Stephanodiscus hantzschii* Grunow are centric diatoms and thus considered planktonic (Kociolek et al. 2015a). The diatom indices changed according to the proportion of agricultural use.

The A and C groups shared *Amphora pediculus (Kützing) Grunow, Navicula reichardtiana* Lange-Bertalot and *Navicula cryptocephala* Kützing, among other taxa. These taxa tolerate degrees of up to mesosaprobic with high content of dissolved organic matter, they tolerate high nutrient concentrations and can also be tolerant to high salinity. The sites corresponding to both groups (A and C) include important agricultural areas which could be the origin of the pollution.

The B and C group sites had a high proportion of natural land uses. The three most indicative taxa, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, *Encyonema silesiacum* (Bleisch) D. G. Mann and *Encyonema minutum* (Hilse) D. G. Mann are found at oligotrophic sites (Jüttner et al. 2011; Bey and Ector 2013). The other taxa were either alpine or subalpine (Hofmann et al. 2011). This distribution of taxa coincided with the sites. This leads to the conclusion that, the community of diatoms corresponded to relatively unaltered sites.

Diversity indices in diatom communities are linked to nutrients (Marcel et al. 2013). Thus, no clear distinction was expected between sites with predominantly anthropogenic land uses. This was confirmed by the land cover correlation.

Diatom indices display an anthropic gradient. They are therefore expected to correlate to land use (Teittinen et al. 2015; Lainé et al. 2014). All the indices correlated to some land uses, but not all the land uses corresponded to significant variation in the indices. Agriculture and woodland had important and opposing correlations to altitude. Woodland is usually linked to headwaters and oligotrophy (Hlúbiková et al. 2014), as it was in our study. The lack or imbalance of nutrients reduces diversity indices (Watanabe et al. 1988), and thus the B group displayed the least Shannon and Simpson diversity. Algal richness is not linked to land use or water quality (Cuffney et al. 1997), as was observed in our results.

5.4.3 Mixed approach: Structural Equation Modelling of land use, physico-chemical parameters and diatom indices grouped by communities and years

Structural Equation Modelling is a science that has been increasingly used in ecology (Riseng et al. 2004; Passy et al. 2016; Stomp et al. 2011). The complex relationships between different levels of organization in an ecosystem can be described by simple statistical methods such as correlations (Spearman or Pearson) or regressions only limitedly (Grace 2008). Thus, a combination of all can help to form a better idea of all the relationship between all of the parameters.

Given the division of possible problems of the SEM calculation, such as the classification of "1) errors of model specification, 2) problematic data, 3) errors of analysis, and 4) errors of interpretation" (Grace 2008), one could argue that the parameters diversity and indicator values were too sparsely sampled (only 2 of the 5-20 recommended observations). We consider that both of the diatom indices were important, since they are similar enough to give an equivalent result (both based on the same formula (Zelinka and Marvan 1961), but with differences in their index values and degrees of completeness (Coste 1982; Prygiel et al. 2002; Prygiel et al. 2006). Other indices had also been tried but were considered to be too inadequate (e.g. CEE Descy and Coste 1990). The Shannon diversity index was selected for its universal use in all ecological studies. Species richness is a parameter that can be related to multiple water traits, depending on whether the environment is restrictive or nutrient full (Passy 2012).

The main result showed that parameters usually considered as important, such as conductivity, nitrate concentration and suspended solids concentration were the most describing (Rott et al. 1998; Kolada et al. 2016). Nutrients, such as nitrate, are fundamental in the function of diatoms, can filter specific taxa and affect trophic indices. Conductivity is an important filter of diatom species, since the osmotic pressure is difficult to withstand without functional or behavioural adaptations. Suspended solids concentration, a parameter that can also reflect flow velocity and heterotrophic biotic behaviour in the ecosystem, affects photosynthetic potential of the diatoms.

Other studies, such as macroinvertebrate limnology, also established nutrient networks and affectations on the community (Mcgoff et al. 2013; Waite et al. 2010). Diatom communities have been studied mostly regarding phytoplankton (Arhonditsis et al. 2007; Stomp et al. 2011). Phytobenthos studies are scarce (Passy 2012).

Division in periods of time showed that there were differences along time. Maybe a finer model, or a bigger database, could increase the scope, making it possible to study yearly changes (Grace 2008; Maloney and Weller 2011). But we have considered that a) Year-to-year changes in land cover were not important enough to present clear affectation of physico-chemical parameters over the fundamental variability, b) Our experimental setup did not permit the observation of yearly changes both in diatom community and land use. What we have been able to see, is that the weight of physico-chemical parameters changed, leaving suspended solids concentration outside of the importance range. This could be linked to the fact that most of the sites visited in the second period were located at Mediterranean axes that should not present high proportions of tree coverage on the flow (Scanlan et al. 2015).

Finally, community descriptions reiterated a fault in our data, since too few sites were found for the middling river sites (Agri-Mead-Wood coverage), that affected the possibility of making a SEM study of this community. As expected, the mostly agricultural cover increased the importance of nitrate concentration, since here high influxes were found due to fertilisers. Suspended solids were also expected (Meador and Goldstein 2003). The mead-wood mix increases the effect of land use on itself, sharpens the negative effect of physico-chemical parameters on both diversity and bio-indicators and again accentuates conductivity, as well as nitrates and suspended solids concentrations.

This study has tried to show the importance that land use has on diatom communities, diversity and indicator values. Our data have shown that an increase in agricultural land affects nitrate levels that in turn decrease both diversity and biotic indices.

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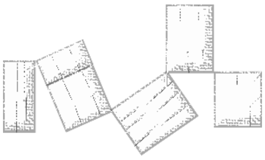

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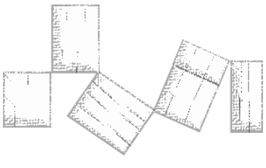
MICRO-SCALE



Chapter 6

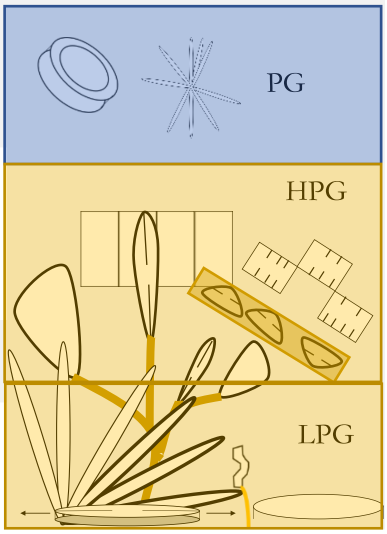
MICRO-SCALE

6.1 FUNCTIONAL GROUPS AND LIFE FORMS OF DIATOMS EXPERIMENT (MICRO-SCALE I)

6.1.1 Introduction

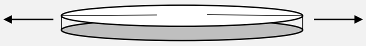
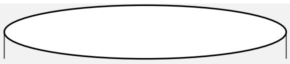
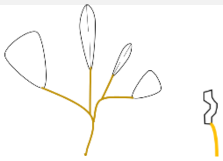
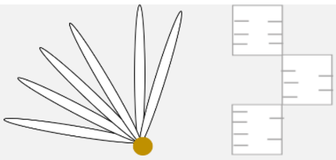
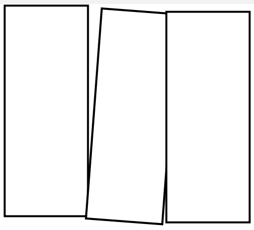
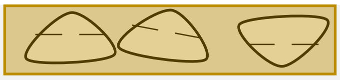
As we stated,correct identification of benthic diatom species is difficult, especially if small diatom identification is dependent of light microscopy (Morales et al. 2001). It also requires strong identification skills. Thus, other methods, such as genetic marker metabarcoding (Zimmermann et al. 2011), and other,reference-based generic and species composition similarity index such as the Diatom Modelling Affinity (DMA) index (Passy and Bode 2004) have been developed. But another method that has been increasingly studied for its simplicity is the division of diatoms into the observable life forms and ecological guilds (Pandey, Sharma, et al. 2018; Law et al. 2014; Dunck et al. 2016; Berthon et al. 2011; Rimet and Bouchez 2012; Marcel et al.2013).

Table 11 Ecological guilds after Passy (2007). Orange squares represent layers of periphton, blue square represents water.

Ecological guilds	Definition	Graphic Representation
High profile guild (HPG)	Protruding diatoms which are exposed to withstand mechanical stress. Better nutrient access.	
Low profile guild (LPG)	Diatoms attached closely to the substrate, exposed to less mechanical stress. Reduced nutrient access.	
Motile guild (MG)	Actively moving diatoms. (signalized by arrows).	
Planktonic guild (PG)	Free floating diatoms that settle on the benthos after dying.	

Ecological guilds, also referred to as functional groups (Table 11), were developed by Passy (2007a, 2007b) and are determined by the extension from the substrate and dimension of profile or motility of the diatoms or diatom colonies. The ecological guilds are explained in more detail in Table 11. Life-forms, as described by Rimet and Bouchez (2012), are based on the communal and individual attachments of valves. The division of these life-forms, explained in chapter 6.1.2 and Table 12, was slightly altered by Riato et al (2017).

Table 12 Subdivision of life forms and a graphic representation for better understanding. Modified from Riato's at al. 2017 life forms based on Rimet and Bouchez (2012). More information about the life forms and the taxa that conform them in AnnexTable 1.

Life Forms	Definition	Graphic Representation
Mobile	Slow or rapid movement within the water column.	
Pioneer	First colonizers after disturbance, r-strategy diatoms.	
Adnate	Attached to substrate by their valve face.	
Pedunculate	Attached to substrate through variable quantities of mucilage (pad, stalk, arbuscular colony).	
Pole attachment / Rosette colony	Colonies attached at the poles. Includes rosette, stellate and zig-zag forms.	
Valve attachment / Ribbon colony	Colonies attached at the valve faces. Includes ribbon and filament forms.	
Mucous tubule	Diatoms inside a mucilage tube within which they can move.	

Even though the application of life forms and ecological guilds have been both tested and improved (Riato et al. 2017; B-Béres et al. 2017), their physico-chemical characterization is relatively scarce (Marcel et al., 2017). Other functional groups have been also used and studied in the US (Hausmann et al. 2016). In the present study we compare the Riato-Rimet life forms and B-Béres-Passy ecological guild sizes and investigate how they react to the physico-chemical parameters.

Diatom life forms and ecological guilds have been observed to change depending on physico-chemical characteristics of the water. Until now, ecological guilds and life-forms had not been characterized according to the nutritional concentrations and conductive levels river of the rivers they inhabit. In the face of a globally changing environment, increased salinization and overall reduction of water quality have been predicted (Orlob et al. 1983). Accordingly, a characterization of these changes by functional groups and life forms could help to create early warning systems without requiring deeper taxonomical knowledge. To do so, we tried to identify which functional group, complete life form and ecological guild (Riato et al. 2017) or sized ecological guilds (B-Béres et al. 2017) would be best representing the physico-chemical changes.

6.1.2 Materials and methods

The Llobregat Basin is located at the north eastern part of the Iberian Peninsula in Catalonia and the river discharges into the Mediterranean sea. It has an area of influence of 4,948 km^2 and an approximate length of 170 km (Sabater and Borrego 2015). It is a highly altered basin, since more than 3 million people live in its catchment (Aguilera et al. 2015). However, alterations do not only originate from anthropic activity. This basin has also halite substrates that can increase the salinity of the river up to 20 times marine conductivity (Badia Guitart 2001). Up to 24 % of the basin are used for agricultural activities, which are responsible for a great proportion of the nutrient concentration (Aguilera et al. 2015).

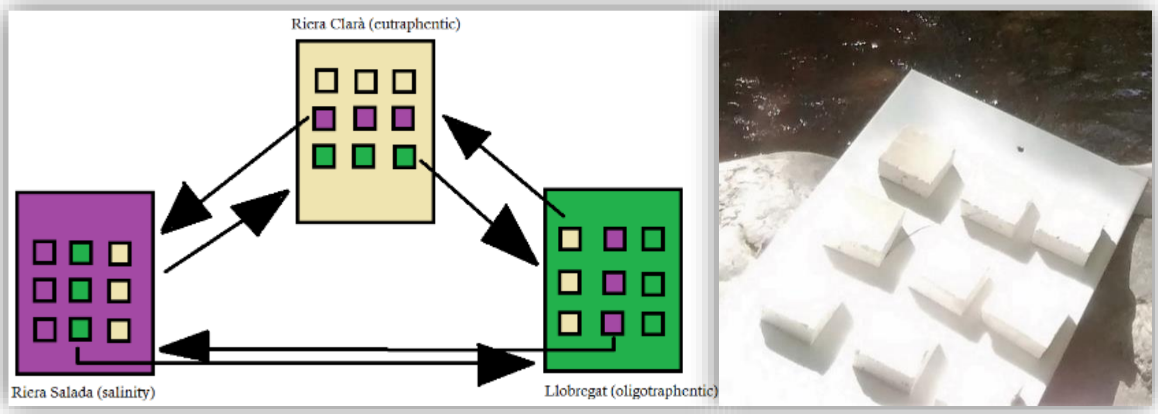


Figure 30 Experimental layout and example of unglazed tiles on glazed tiles.

We wanted to prepare a colonizing experiment to investigate which diatom life forms and ecological guilds occupy which rivers. To prepare the experiment, we installed artificial sample substrate of unglazed quarry tiles of 20 cm^2 of surface area into the river. 9 tiles were mounted on top of a bigger, glazed tile to reduce risk of extrusion (Figure 31). As a control, unattached tiles were added, also to prevent potential vandalism. The artificial substrates were submerged in August 2016 into three rivers of the Llobregat basin. These sites had differing physico-chemical characteristics (Table 13). The Clarà stream was located downriver of agricultural land use and had low conductivity and high nutrient concentrations. The Llobregat river was close to headwaters and had low conductivity and nutrient input. Salada stream also was close to headwaters and, thus, had low nutrient levels. But contrasting Llobregat river, the main characteristic of this site its brackish nature, caused by to halite rich substrates (Torres Roig 2015).

The tiles were immersed for a little bit more than three weeks before being exchanged (Figure 31). Three of the smaller unglazed tiles were then labelled and transferred to each of the other two sites and left to be colonized by diatoms (2) After one month, the tiles were extracted, and diatoms were sampled by scrubbing the unglazed tiles with toothbrushes. Each of the labelled samples were pooled at each site according to treatment. At each step of the experiment, also natural substrates (stones) were sampled, a total of 15. Physico-chemical data were measured in situ (Table 13). Diatoms were examined in vivo to ascertain the predominant life form of the most abundant diatoms.

Table 13 Average values of physico-chemical data (± 1.0 standard deviation) for each site. The data were obtained at the sample sites and from the official register of biomonitoring sites of the Llobregat river taken by the ACA (Agència Catalana de l'Aigua (ACA) 2014)

	Clarà (eutrophic)	**Llobregat (oligotrophic)**	**Salada (brackish)**
Altitude (m.a.s.l.)	476.30	697.50	1111.70
Alkalinity (mg/l)	306.03 (± 18.72)	233.88 (± 85.73)	113.33 (± 11.55)
Conductivity (µS/cm)	749.71 (± 287.74)	452.75 (± 100.47)	11756.67 (± 6656.85)
Hardness (ppm)	411.31 (± 260.53)	240.00 (± 110.23)	1000.00
Nitrates (mg/l)	13.94 (± 6.50)	1.50 (± 3.00)	0.00
Phosphates (mg/l)	4.05 (± 1.57)	0.25 (± 0.35)	N. A.
Salinity (mg/l)	300.67 (± 27.15)	260.00 (± 30.05)	7534.67 (± 4268.35)
Temperature (ºC)	22.33 (± 3.33)	19.00 (± 2.00)	17.83 (± 3.33)

Diatoms were divided into either life forms (Rimet and Bouchez, 2012) or ecological guilds (Passy, 2007a). Ecological guilds have a more ecological foundation, since they relate to the location inside the periphyton (6.1.1 Introduction, Table 11-12).

The sites were compared using Permanova analysis, to determine the significance of the differences of functional group and life form composition in each site. Indicator values of each life form were also measured. A multivariant analysis was performed using non-metric dimensional scaling (NMDS) to establish the relationship between functional groups, life forms and physicochemical composition. Finally, correlations between physicochemical composition and life forms were assessed by Spearman correlation permutation tests. Environmental traits were fitted using the R “envfit” package. Similarity matrices used were Kulczinsky’s distance, that establishes the proportion of forms found in a total database respective to the rest of subgroups (Oksanen 2013a) and Manhattan distance, used while properly standardized to study the absolute distance between two datasets (life forms vs. physico-chemical data).

6.1.3 Results

A total of 202 diatom taxa were identified. The oligotrophic Llobregat site had a richness of 50 to 77 species, the eutrophic Clarà habitat contained 59 to 74 taxa and the brackish Salada site exposed the lowest richness, ranging from 15 to 58 taxa. The most abundant taxon for the oligotrophic site was *Achnanthidium pyrenaicum* (Hustedt) Kobayasi for the first two sampling times, being subsequently substituted by *Amphora pediculus* (Kützing) Grunow ex A. Schmidt. In the eutrophic site, *Craticula subminuscula* (Manguin) Wetzel & Ector and *Nitzschia palea* (Kützing) W. Schmidt were the predominant species. The brackish site was represented by *Diatoma tenuis* C. Agardh in the first two samples, which later were mostly substituted by *Achnanthidium cf. neomicrocephalum* Lange-Bertalot & Staab. Only the sample taken from the substrate initially colonized by eutraphentic diatoms were later overgrown by *Achnanthes thermalis* at the brackish site.

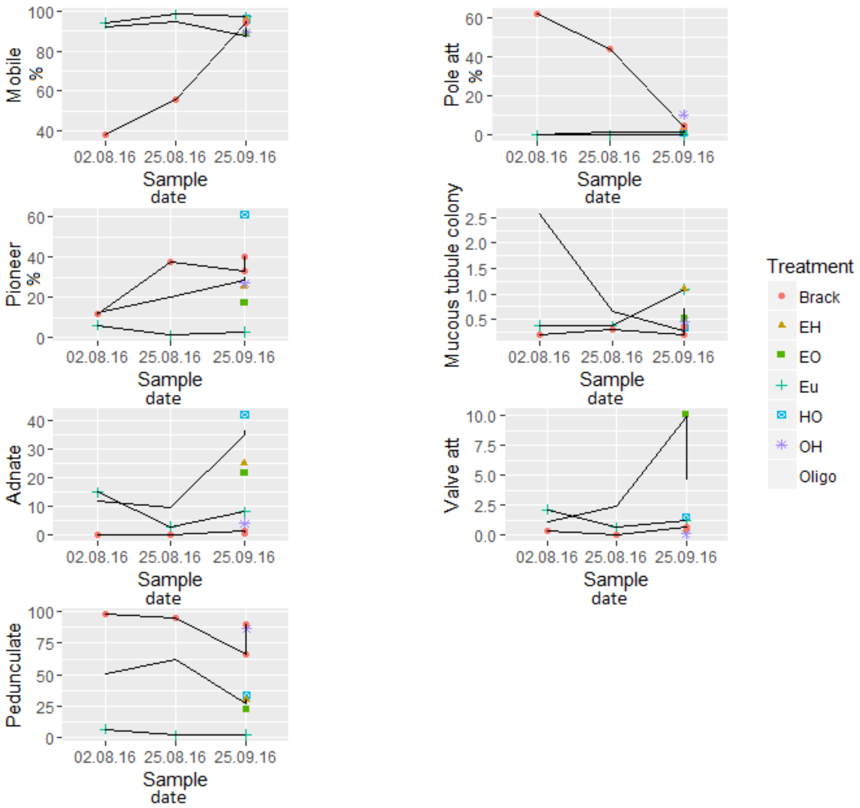


Figure 31 Comparison of the Riato life forms and their relationships throughout time. Brackish = orange dot, eutrophic to brackish (EH) = brown triangle, eutrophic to oligotrophic (EO) = green square, eutrophic (Eu) = turquoise cross, brackish to oligotrophic (HO) = blue square, oligotrophic to brackish (OH) = purple star, oligotrophic (Oligo) = not present.

The most abundant life forms were pedunculated forms (average abundance (aa) = 47 %), motile guild (aa = 37 %) and adnate diatoms (aa = 14 %). The least frequent were planktonic, pole attached colonies and valve attached forms. As for the ecological guilds, the most abundant were the smallest low profile diatoms (aa = 33 %), and motile guild of size 2 (aa = 12 %) and size 3 (aa = 11 %). Ecological guilds had lower frequencies than life forms. The planktonic forms were rarest, except for size 4; size 5 was completely absent. The low profile guild was both more frequent and abundant than the high profile guild.

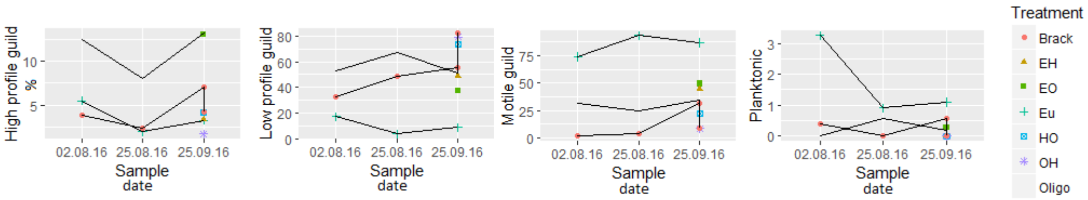


Figure 32Comparison of the Ecological guilds without size division (Riato et al, 2017). Brackish = orange dot, Eutrophic to brackish (EH) = pink triangle, Eutrophic to oligotrophic (EO) = green square, Eutrophic (Eu) = turquoise cross, Brackish to oligotrophic (HO) = blue square, Oligotrophic to brackish (OH) = purple star, Oligotrophic (Oligo) = no point.

Diatom life forms (Figure 32) did not portray similar proportions through time alongexperimental settings. Mobile diatoms, motile guild and planktonic species were mostly found at the eutrophic site. The oligotrophic site had mostly adnate, mucous tubule, valve attachment, high and low profile guild taxa. Finally, the brackish life forms were mostly pioneers, pedunculated and pole attached. From the Riato et al. (2017) ecological guilds (Figure 33), only the high profile guild did have similar patterns, being lowest at each second sample and displayed similar distributions of the eutrophic and oligotrophic sites.

Diatom life forms and ecological guilds were also differential in their relationship with physico-chemical values. The community dissimilarity of the life forms (LF) and ecological guilds (EG) were described best by Kulczynski index, and Manhattan dissimilarities, respectively. Most multidimensional scaling exercises showed that variance was better explained by life forms (Table 14).

Table 14 Comparison of variance explained by life forms and ecological guilds in diverse multivariate analyses against physico-chemical composition.

	PCA	**CA**	**CCA**
Life Forms	0.8622	0.80378	0.7585
Ecological Guilds	0.7765	0.62726	0.51032

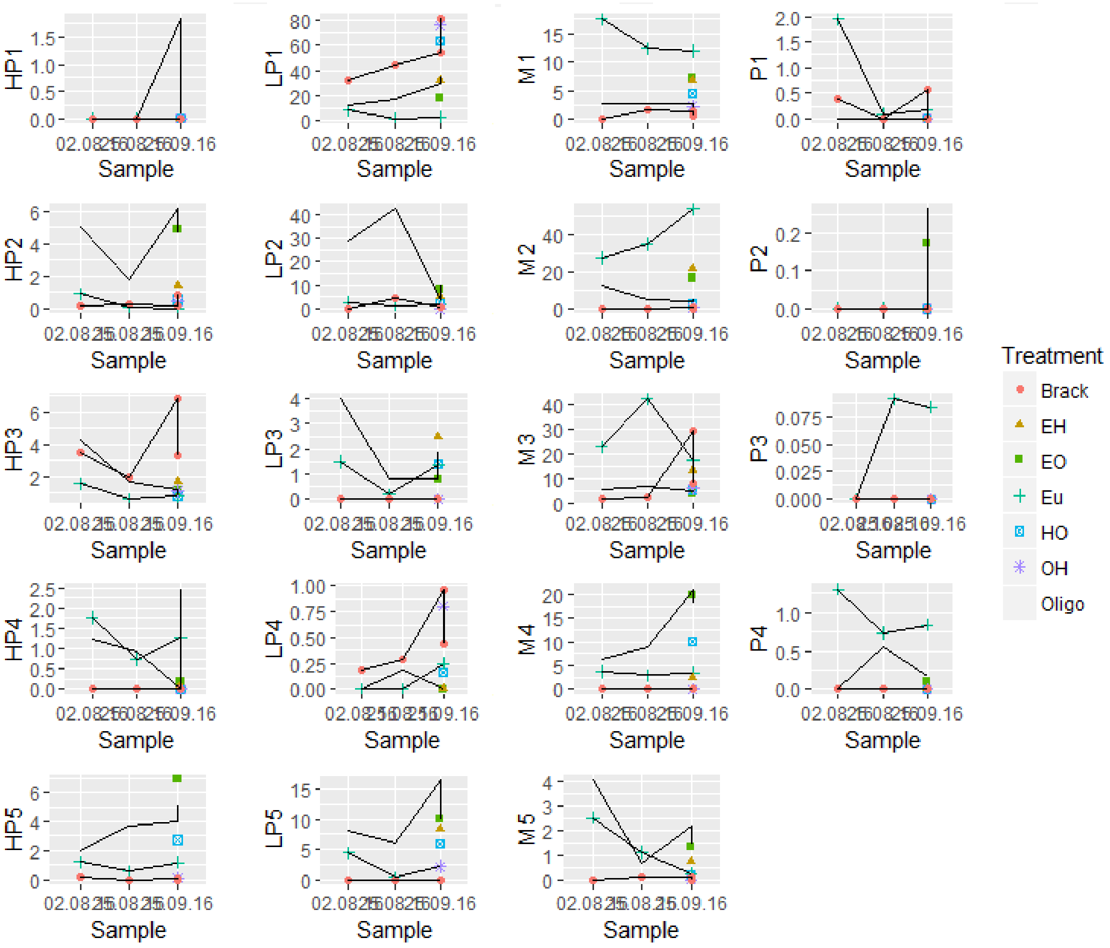


Figure 33 Comparison of ecological guild sizes proportions (%) after B-Béres. The tendencies inside each ecological guild are different depending on the diatom sizes. Ordenate groups: HP = High Profile guild, LP = Low Profile guild, M = Motile guild, P = Planktonic guild. Biovolume size distribution: 5 $\mu m^3 \leq$ **1** $\leq$ 199 $\mu m^3 <$ **2** $\leq$399 $\mu m^3 <$ **3** $\leq$699 $\mu m^3 <$ **4** $\leq$ 1499 $\mu m^3 <$ **5**, Brackish = orange dot, Eutrophic to brackish (EH) = brown triangle, Eutrophic to oligotrophic (EO) = green square, Eutrophic (Eu)=turqoise cross, Brackish to oligotrophic (HO) = blue square, Oligotrophic to brackish (OH) = purple star, Oligotrophic (Oligo) = no point.

When comparing to the B-Béres ecological guild size subdivisions (Figure 34), the percentage of functional groups was higher in general. As expected, each ecological guild size was differently affected by each water site. Smaller motile diatoms were found mostly under eutrophic conditions. Low profile was mostly found in the oligotrophic and brackish sites. In contrast, high profile guild thrived mostly under oligotrophic conditions and only the relatively big high profile diatoms were found at the eutrophic site. Planktonic forms were all also found in the Clarà stream.

Only pedunculated forms and the low profile guild diatoms from the Riato approach were positively and significantly correlated under the Spearman method to the IPS diatom index (Ped ϱ = 0.51*, LPG ϱ = 0.8***), for the B-Béres approach only the size 2 high profile guild (HP2 ϱ = 0.51*) was positively correlated to the index. Motile guild and plankton were significantly negatively correlated to IPS (MG ϱ = -0.6*, P ϱ = -0.53*). Only medium motile, size 1 and size 3 planktonic taxa were significantly correlated to IPS (M3 ϱ = -0.44*, P1 ϱ = -0.61*, P3 ϱ = -0.57*). The original distribution of life forms is displayed in Figure 35.

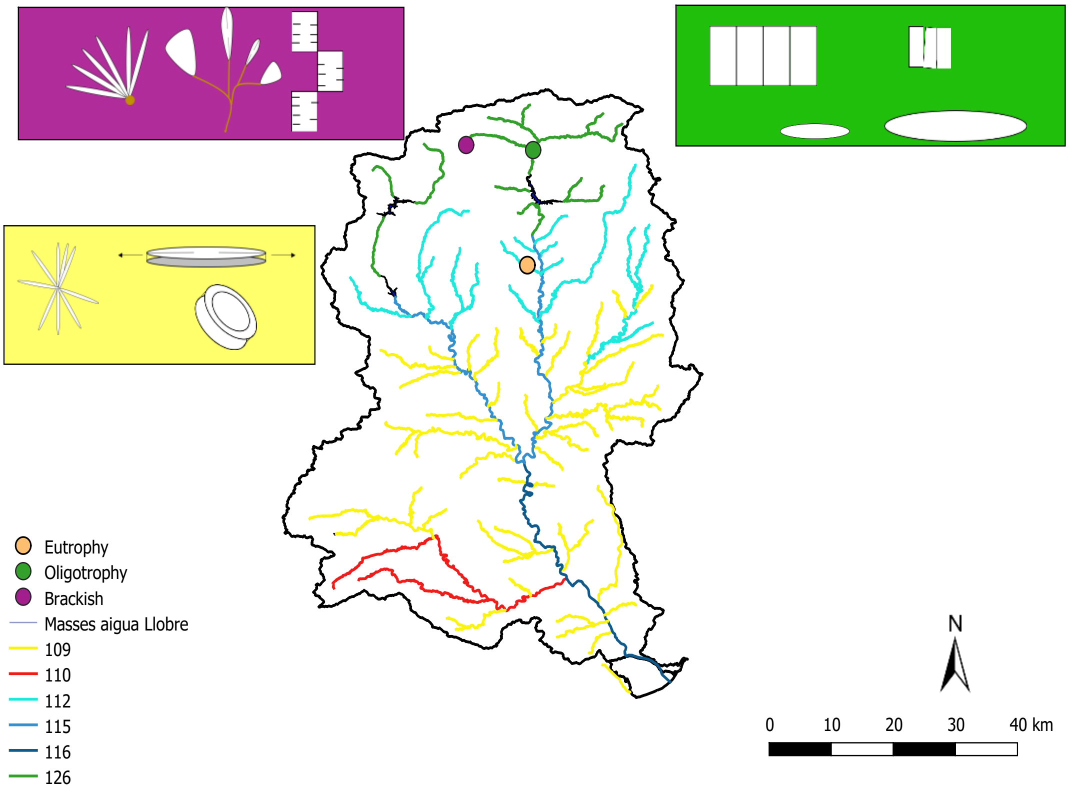


Figure 34 Summary figure of Riato Life forms at each site

The non-metric dimensional scaling of life forms and ecological guilds showed different powers in differentiating sites (Figure 36). When fitting the environmental traits, the results showed that the life form division of diatom taxa had higher and more significant correlations with environmental conditions. For instance, altitude (r^2 = 0.8488***), NO_3 (r^2 = 0.8616**) and conductivity (r^2 = 0.8364***), the highest correlated physico-chemical parameters for life forms, were lower for ecological guild sizes: altitude (r^2 = 0.7272***), NO_3 (r^2 = 0.6546**), conductivity (r^2 = 0.8151***).

Table 15 Diatom functional groups and life forms that act as indicator forms of each site.

	Functional Groups	INDVAL [%]	Significance
Brackish	Pole attachment	0.879	***
	Pedunculate	0.681	***
	Pioneer	0.649	*
	Low profile, size 1	0.671	***
Eutrophic	Planktonic	0.861	**
	Motile Guild	0.655	*
	Planktonic, size 4	0.908	**
	Planktonic, size 1	0.879	*
	Planktonic, size 3	0.816	*
	High Profile Guild, Size 4	0.786	*
	Motile Guild, size 2	0.751	**
	Motile Guild, size 1	0.727	**
	Motile Guild, size 3	0.677	**
Oligotrophic	Valve attachment	0.770	*
	Adnate	0.706	*
	High Profile Guild	0.668	**
	Low Profile Guild	0.625	*
	High Profile Guild, size 2	0.828	**
	High Profile Guild, size 4	0.826	**
	Motile Guild, size 4	0.778	**
	Low Profile Guild, size 5	0.766	*
	Low Profile Guild, size 2	0.750	*

p-values: 0 '***' 0.001 '**' 0.01 '*' 0.05

The IndVal analysis confirmed the suitability of the Riato approach (Table 15, Figure 36a). Brackish waters were defined by pole attached diatoms, pedunculate and pioneering taxa. The eutrophic site had mostly planktonic and motile guild taxa. The oligotrophic site, on the other hand, had taxa attached by the valve, adnate diatoms and both high and low profile guild representatives. The B-Beres approach clearly differentiated the nutrient gradient. Nonetheless, it was not significant for a brackish distribution of ecological guild sizes, only affecting for the low profile small diatoms (as *Achnanthes* Bory and *Achnanthidium* Kützing representatives). The eutrophic Clarà stream was defined by medium sized planktonic and small motile taxa. The oligotrophic Llobregat headwaters were

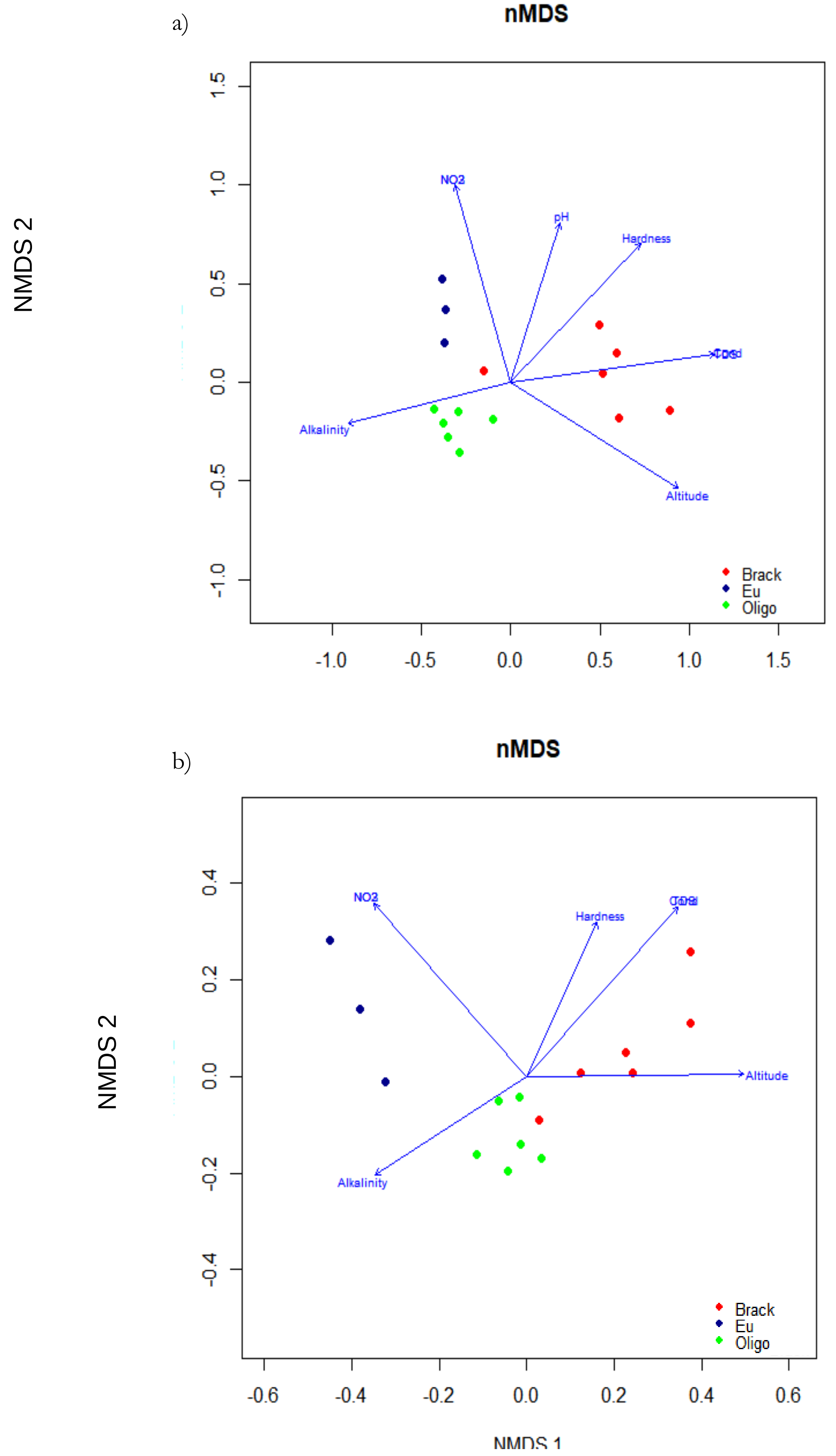


Figure 35 Comparison of non-metric multidimensional scaling (NMDS) of a) life forms and b) subdivided ecological guilds.Hardness, Cond = conductivity, TDS = Total dissolved solids, Altitude, Alkalinity and NO3 = nitrate (NO_3). Brack = brackish, Salada stream, Eu = eutrophic, Clarà stream, Oligo = oligotrophic, Llobregat headwaters.

represented by high profile guild, medium to large motile guild and large low profile guild diatoms.

We not only compared sites, but also investigated ifchanges, tile colonization or resilience of original diatoms when being translocated could affect similarities. This was assessed by a Permanova analysis. Only the sites parameter was significant for both approaches (R^2 (life form) = 0.72943***, R^2 (ecological guild) = 0.6775***). The ecological guild was also significant for resilient diatom guilds (R^2 (ecological guild) = 0.1225*).

Spearman correlation rank calculations were made to correlate functional groups and life forms with physico-chemical parameters at the studied sites (Table 16). Nitrate concentration and conductivity were both the most significantly correlated parameters. The correlation matrix was difficult to interpret, since each life form or ecological guild reacted differently to each physico-chemical stimulus. The ecological guild seemed to have more significant relationships, although, in proportion, more life forms had significant correlations.

Table 16 Spearman correlation permutation test relating physico-chemical data to functional traits and ecological guilds (sensu Riato et al 2017). Significance of type-describing species calculated through Monte Carlo permutation test with 999 permutations ($p<0.001$). Average and maximal IndVal proportions are also described. The life forms and ecological guilds that are missing had no significant correlations to physico-chemical traits. Bold describes significant correlations.

Life form / Ecological guild	Altitude **(m.a.s.l.)**	SS **(mg/l)**	NO_3 **(mg/l)**	Hardness **(PPM)**	Alkalinity **(PPM)**	Conductivity **(µS/cm)**	pH
Pioneer	**0.5867***	0.0073	**-0.6944****	0.1877	-0.3460	0.0073	0.028
Adnate	-0.4255	**-0.817*****	-0.0772	**-0.6588****	0.5072	**-0.8173*****	-0.32
Pedunculate	**0.8396*****	**0.5734***	**-0.6944****	0.4312	**-0.6566****	**0.5734***	0.19
Pole att.	**0.9324*****	**0.6121***	**-0.6950****	**0.6374***	**0.6907****	**0.6121***	0.022
Valve att.	**-0.6326***	**-0.87*****	0.0772	**-0.7826****	**0.7687*****	**-0.8737*****	**-0.54***
HPG	-0.1898	**-0.6553****	-0.3472	**-0.6508****	0.3480	**-0.6553****	-0.36
LPG	0.4945	-0.0892	**-0.6944****	0.0120	-0.3735	-0.0892	-0.082
MG	**-0.7246****	-0.4004	**0.6944****	-0.3075	0.4581	-0.4004	-0.011
PLANKT	**-0.6295***	-0.1062	**0.7171****	-0.1567	**0.5259***	-0.1062	0.55

p-values: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ns (not significant) = “ ”

Ecological guild sizes varied along the sites and differential physico-chemical composition (Table 17). In average, correlation values were reduced in the size-divided approach, but increased for individual subgroups, such as HP5 and TDS (ϱ = 0.9***). Some ecological guild sizes were either not present, or not significant for any of the measured physicochemical values, and thus are not shown.

Table 17 Spearman correlation permutation test relating physico-chemical data to ecological guild sizes (sensu B-Béres et al. 2017). Results without significant correlations to physico-chemcial data are not shown. HP = high profile guild, LP = low profile guild, M = motile guild, P = planktonic guild, the following number gives the size class:: 5 $\mu m^3 \leq$ **1** $\leq$ 199 $\mu m^3 <$ **2** $\leq$399 $\mu m^3 <$ **3** $\leq$699 $\mu m^3 <$ **4** $\leq$ 1499 $\mu m^3 <$ **5.**

	Altitude	TDS	NO_3	Hardness	Alkalinity	Conductivity	pH
HP2	-0.0690	**-0.5988***	-0.4629	**-0.5989***	0.1848	**-0.5988***	-0.328
HP3	**0.6441****	**0.5552***	-0.4629	0.2396	**-0.6920****	**0.5552***	-0.397
HP4	**-0.7213****	-0.4259	**0.5286***	**-0.5977***	**0.5388***	-0.4259	0.224
HP5	**-0.6233***	**-0.9*****	0.0	**-0.818*****	**0.8050*****	**-0.9024*****	**-0.7****
LP1	**0.7936*****	0.2894	**-0.6944****	0.4552	**-0.6154***	0.2894	0.343
LP2	-0.3048	**-0.5497***	-0.1929	**-0.6548****	0.4168	**-0.5497***	**-0.6***
LP3	**-0.5153***	**-0.5783***	0.1571	**-0.7116****	0.3904	**-0.5783***	-0.180
LP4	**0.5759***	**0.6024***	-0.2033	**0.5387***	**-0.5678***	**0.6024***	0.451
LP5	0.3946	**-0.81*****	-0.1557	**0.6970****	0.4960	**-0.8063*****	-0.409
M1	**-0.794*****	-0.4314	**0.6944****	-0.4352	**0.5701***	-0.4314	-0.175
M2	**-0.794*****	-0.4459	**0.6944****	-0.4512	**0.5210***	-0.4459	-0.084
M3	-0.3220	0.1201	**0.6172***	0.1557	-0.0865	0.1201	0.425
M4	**-0.6267***	**-0.95*****	0.0	**-0.822*****	**0.7677*****	**-0.9532*****	**-0.63***
M5	**-0.6752****	**-0.6686****	0.2710	**-0.7293****	**0.5623***	**-0.6686****	-0.381
P1	-0.2602	0.3532	**0.6430***	0.1807	0.0632	0.3532	0.326
P3	**-0.5832***	-0.0431	**0.7825*****	0.0742	0.3489	-0.0431	0.044
P4	**-0.861*****	-0.4140	**0.754*****	-0.4249	**0.7845*****	-0.4140	-0.366

p-values: 0 '***' 0.001 '**' 0.01 '*' 0.05 ns = " "

6.1.4 Discussion

Diatom species richness depends mostly on the accessibility of nutrients and the absence of biotic and pollution stresses (Biggs and Smith 2002; Teittinen et al. 2015). Given the relatively low number of samples, the high taxon richness of the entire experiment might be a result of the diversity of physico-chemical parameters and biotic stresses, which should generate unique diatom communities (Virtanen and Soininen 2016; Göthe et al. 2013). The low richness at the brackish site can be related to the high salinity, that can select some diatoms because of the osmotic stress (Cohen 2010). Also, high current and low nutrient concentration could affect this site, increasing stresses and additionally reducing the diatom taxa that are able to colonize the substrates. We expected the oligotrophic site to have less richness than we observed, since nutrient stress could favour a low diversity through massive colonization by one or two taxa (Leira et al. 2009) by favouring them whilst impeding other taxa. The decrease of species richness at the eutrophic Clarà stream at the third sampling could be the result of a flooding event originating from the Casserres reservoir located upstream. This hypothesis also is supported by the fact that the artificial substrates had disappeared at the third sampling and the increased presence of *Procambarus clarkii* Girard, because this species is known to increase in shallow waters with low flow velocities (Klose and Cooper 2012; Kerby et al. 2005).

Verification of the main form of the predominant diatoms was a prerequisite for differentiating the life forms (Berthon et al. 2011). This was particularly important for the modified form of *Diatoma tenuis* C. Agardh that was found in the brackish Salada stream. Since this site was located at the highest site-elevation with fastest current, the planktonic forms would have been dragged downstream (Lange et al. 2016). This shows the importance of a preliminary observation of the untreated diatoms to assess the life forms of the predominant taxa, to establish a "expert criterion" as it was already demanded by Berthon et al. (2011).

The predominant life forms, motile, pedunculated and adnate, were mostly shared with the wetland-based Riato et al. study (2017). Only the adnate life form was anecdotal at the original study, which could be due to the fact that the samples were taken from different substrates and under different hydrologic regimes. When observing the ecological guild sizes, our results contradict the observations of B-Béres et al. (2017), since in our study the motile forms are usually more predominant than the small low profile guilds. It could have an effect of the relative altitude of the sampled sites. Since B-Béres presented a quite complete appraisal of more than 700 sites in Hungary, the more diverse database could add valuable information of lower laying and differently impacted sites.

Dissimilarity matrices showed high differences between the two functional group approaches. Life forms were explained by Kulczinsky's distance, comparing subsets and ecological guilds had Manhattan distance depends on the absolute distance between ecological guilds vs. physico-chemical data. The better explanation of physico-chemical variance by the life forms dataset indicates a better description of sites, at least in the frame of this study.

Using the Riato approach, mobile diatoms predominated, since at least minimal motility either within the water column or upon the substrate was found in most diatom taxa. Since motility is an allencompassing term (Tang et al. 2006), it did not show a significant Spearman correlation to any of the physico-chemical parameters. Neither did mucilage tubule dwelling diatoms, nor those pertaining to small high profile guild or planktonic guild in its second smallest form.

The pioneering diatoms showed increased abundance not only in the oligotrophic site (Llobregat), but also at the brackish site (Salada stream). Oligotraphentic pioneering diatoms were represented by the increase of *Achnanthidium minutissimum* (Kützing) Czarnecki through time. This species is the paradigm of colonizing diatoms (Rimet et al. 2009; Hofmann et al. 2011). On the other hand, the brackish site can have a high proportion of pioneers, because of its challenging environment. Changing salinity and conductivity can influence diatom survival (Snoeijs 1994; Porter-Goff, Frost, and Xenopoulos 2013), thus favouring pioneering diatoms.

Considering the life forms, adnate forms were mostly found in oligotrophic sites, which coincides with oligo-mesotraphentic diatoms such as the *Cocconeis placentula* Ehrenberg group (Romero and Jahn 2013), being the predominant diatoms. These were negatively correlated with conductivity and hardness. The pedunculated forms showed the highest correlation of life forms with diatom indices and were mostly in the *Achnanthidium* Kützing, *Cymbella* Agardh and *Gomphonema* Ehrenberg genera. These taxa have mostly a positive correlation with altitude and correlate negatively with both nitrate concentration and alkalinity. They were found mostly in the eutrophic site, followed by the oligotraphentic habitat, which may be a result of elevated phosphate concentration levels.

The pole attached diatoms were mostly found at the brackish sites, though a declining gradient was present through time. These diatoms, predominated by *Diatoma tenuis* C. Agardh, attached to the substrate either by mucilage or through spines. Their abundance correlated positively to altitude, conductivity and negatively to nitrate concentration. The attachment by mucilage observed in this habitat can be related to the combined effects of high conductivity and low nutrient concentration, which has shown to increase mucilage production (Najdek et al. 2005). Valve attached forms were predominantly *Melosira varians* C. Agardh and *Staurosirella pinnata* (Ehrenberg) Williams & Round. Abundance of these diatoms correlated negatively with conductivity and positively with alkalinity, and were usually found in lower and partially degraded habitats (Morales 2010; Potapova 2009). Diatom colonies in mucilage tubes, most predominantly in the *Encyonema* Kützing genus, were not correlated to any physico-chemical traits. This could be due to the very physico-chemically diverse habitats of these taxa. *Berkeleya rutilans* (Trentepohl ex. Roth) Grunow, for instance, is a very halophile diatom, while the *Encyonema* genus is mostly related to the meso- to oligotrophic river sites (Bey and Ector 2013).

The ecological guilds from the Riato approach differed from the size mediated B-Béres approach. For instance, the medium sized high profile guild diatoms (HP3) displayed distributions along the physico-chemical spectrum which were juxtaposed to that of the other high profile guilds. Since the

Gomphonema species, like *Gomphonema parvulum* f. *saprophilum* Lange-Bertalot & Reichardt, *G. minutum* Fusey, *G. parvulum* (Kützing) Kützing, *G. parvulum var. exilissima* Grunow in Van Heurck ,which were the most abundant for this subgroup because they are mostly tolerant to nutrient variation (Krammer and Lange-Bertalot 1986; Abarca et al. 2014). The low profile guild exhibited a similar pattern. Here only one size fraction(LP2) was sensitive to all pollution. LP3 was tolerant to nutrient variations, as most of the taxa found in this group had wide tolerance ranges (order *cymbellales* such as *Cymbella excisa* Kützing, *Encyonopsis microcephala* (Grunow) Krammer, *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot, and some more). Size fractions LP1 and LP4 were tolerant to hardness and conductivity, and comprised taxa as *Achnanthidium thermale* Rabenhorst, highly correlated to salinity (Hindakova 2009), as well as *Amphora montana* Krasske (Stepanek and Kociolek, 2011). Both motile guild and planktonic forms correlated similarly with physico-chemical traits. Some size fractions diverged minimally, such as M3 (a wide range of nutrient tolerant *Navicula* Bory de Saint-Vincent s.l.) and P1 (highly tolerant small *Cyclotella* (Kützing) Brébisson taxa), but the main distribution was similar.

To sum up, the brackish waters were characterized by pole attached and pedunculate forms, followed by small low profile dwellers. The eutrophic site had mostly planktonic and motile forms, in all shapes and forms. The oligotrophic site can be described by how valves attached to substrate and to each other and adnate forms, as well as by specimens belonging to the high profile guild. Comparing both approaches, ecological guilds and life forms reacted differently to either physico-chemical or locational differences.

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6.2 DIATOM LIFE FORMS IN TEMPORARY RIVERS (MICRO-SCALE II)

6.2.1 Introduction

Current prediction models on global climate change (EEA 2012, see Figure 3 in 1.3.3) predict an increase in temporary rivers, which are characterized by a total or partial loss of surface water (Stubbington et al. 2017), particularly in the already affected Mediterranean climate (García Vera et al. 2005). Several studies have been made to characterize temporary water bodies, e. g. lakes: (Leira and Cantonati 2008), rivers: (Hughes et al. 2009), depressional wetlands (Riato et al. 2017). Nevertheless, the European legislature has yet to regulate these potential affectors of the Water Framework Directive (European Commission 2000). To remedy this, the LIFE TRivers project was launched (LIFE13 ENV/ES000341) to create a multidisciplinary tool for temporary river management inside the WFD.

This project resulted in toolboxes for the division of river regimes into perennial rivers, intermittent pools and intermittently dry catchments (Gallart et al. 2012, 2016). Also, the sampled water regimes were characterized by their aquatic states: hyperrheic, eurheic, oligorheic, arheic, hyporheic and edaphic (more information given in Chapter 1.3.3.3). Even though most rivers had a periodical or temporary variability, some water bodies maintained one aquatic state. This can potentially complicate the biologic/ecological part of the water quality monitoring.

As we showed in preceding chapters, benthic diatoms are one of the priority biomonitoring organisms of connected riparian systems. Due to their fast generation time through asexual reproduction, their reactivity is high. Thus, diatom communities should be able to reflect the loss of connectivity produced by river intermittency.

As discussed in chapter 6.1, the division of diatoms into functional groups and life forms (Margalef 1978; Berthon et al. 2011; Rimet and Bouchez 2011; Dunck et al. 2016; Marcel et al. 2017; Pandey et al. 2018; Law et al. 2014; Riato et al. 2017; B-Béres et al. 2017, 2014, 2016, Passy 2007b, 2007a), can potentially increase ecological information and might also reduce need for taxonomic investigation Life forms have been used to characterize temporary waters before (Riato et al. 2017), thus a similar methodology has been followed to increase comparability. Here we investigated life forms (Chapter 6.1.2, Table 12) in a modified form of the Rimet and Bouchez (2012) database, and used ecological guilds (Chapter 6.1.2, Table 11) not subdivided by size.

6.2.2 Materials and Methods

Diatom samples were taken from 24 sites of the eastern Spanish basins flowing into the Mediterranean Sea (Figure 9, see Chapter 3.3.3). These sites were visited up to five times in 2015. The temporary rivers have been characterized according to their aquatic state (Gallart et al. 2012). The sampled rivers were divided into aquatic states (AnnexTable 16) and water regimes (AnnexTable 17).

Diatom life forms and ecological guilds were obtained using the Passy (2007) and Rimet and Bouchez (2012) subdivision and modified into fewer categories, as used in Riato et al. (2017), see AnnexTable 1. For instance, arbuscular, pad and stalk forms were joined into the pedunculate category, while ribbon and rosette described those colonial associations that were attached similarly (either by valve face or poles).

Life forms were subjected to non-metric multidimensional scaling (NMDS) and ANOSIM calculations to establish the differentiation of aquatic states, river types or both. Spearman's rank correlation coefficients and distribution of life form percentages were also calculated.

6.2.3 Results

A total of 408 taxa was identified, 90 % to species level and below. The 22 visited arheic river sites displayed 250 taxa, eurheic (35) had 304 taxa, the hyporheic river had only 55 taxa, and oligorheic sites (56) had 354 identified taxa.

The highest abundances of life forms was given by the mobile forms (97.54%), Rosette life forms (65.12 %) and the motile guild (68.32 %). *Achnanthidium minutissimum* (Kützing) Czarnecki and *Achnanthidium pyrenaicum* (Hustedt) Kobayashi contributed 26.5 % and 9.26 % of the mobile diatoms respectively. The rosette colony life forms was formed by 80 % of *Ulnaria* (Kützing) Compère, 12 % *Meridion circulare* (Greville) C. Agardh and 5 % *Diatoma moniliformis* (Kützing) D. M. Williams. The motile guild was composed by 14.10 % of *Nitzschia frustulum* (Kützing) Grunow and 6 % of *Epithemia sorex* Kützing.

The spatial distribution of the sites, based on the diatom life forms against the physico-chemical data, were displayed as three NMDS graphs (Figure 37a – c). These showed the relative inadequacy of aquatic states as differentiation factors. In comparison, there seems to be a slight improvement on the differentiation of the three river types. Given the high proportion of perennial and intermittent pool water, the directionality was observable even though a high clustering was still present. ANOSIM showed no significant differences between aquatic states, while both river types and an amalgamation of both had little significance (River type: R= 0.05671*, combination: R=0.07697*).

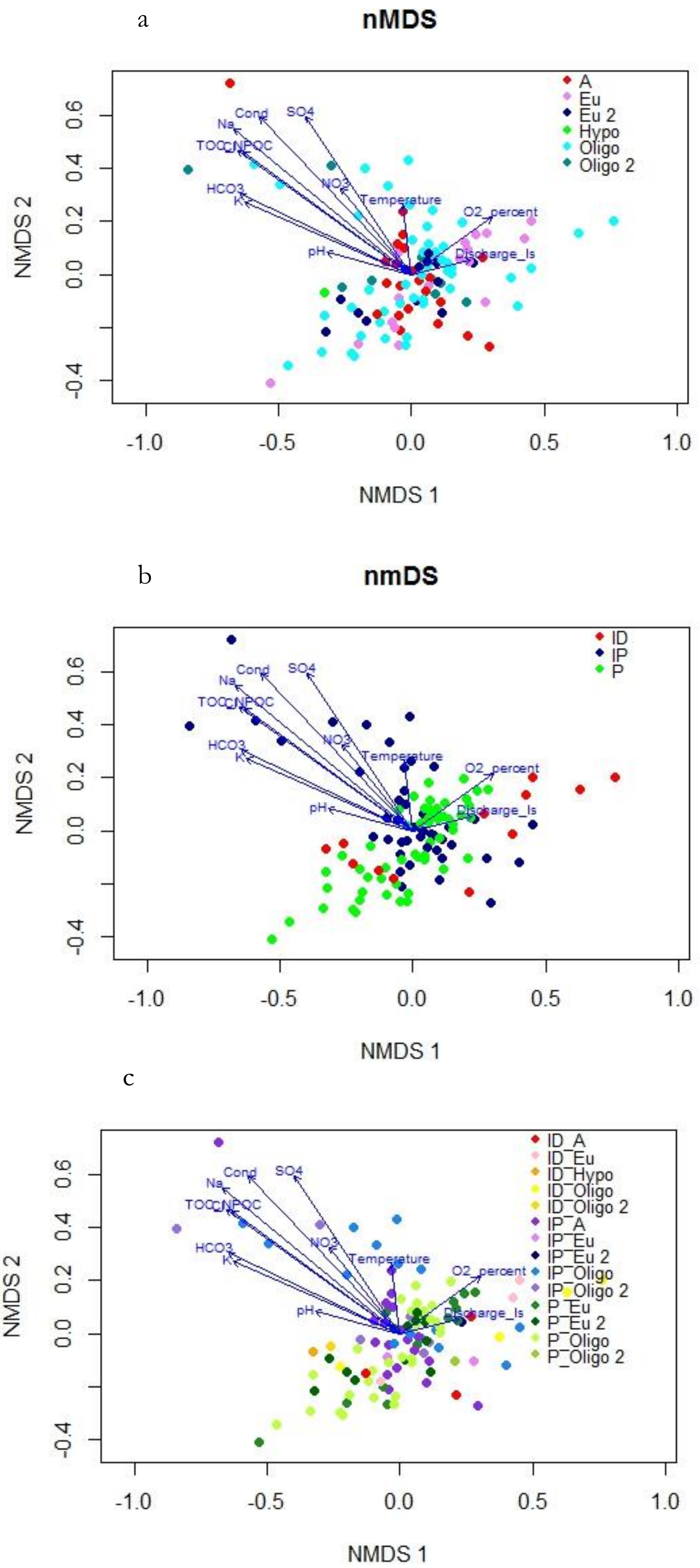

Figure 36 Comparison of aquatic states (a), river types (b) and both combined (c) as differentiators of the sites. No great differentiation powers are observed.

No clear tendencies are observable for each of the life forms (AnnexFig 6 – 7). Since we investigated a total of 24 sites, we subdivided them according to basin authorities for better clarity. A strong variability of the abundance of some life forms, such as rosette colonies, impeded a closer differentiation of the results. Finally, river types marked a clear affectation of those sites afflicted by intermittent dry spells. The resulting life forms reacted differently to other river types.

Life forms were highly correlated with physico-chemical characteristics of the river, following the environmental fitting algorithm results. Most significant were conductivity (R^2 = 0.5461***), carbonate (R^2 = 0.4103***), chlorine (R^2 = 0.5218***) and sodium concentration (R^2 = 0.6027***). Least significant were temperature (R^2 = 0.0556*), pH (R^2 = 0.0875**) and nitrate concentration (R^2 = 0.1420**). The differentiation was measured using ANOSIM of life forms against aquatic state and did not show significant differences. But river type was significant (R = 0.2076**), as well as the addition of both river type and aquatic state (R = 0.1957**).

Intermittently dry streams were correlated with the motile guild (62.4 %**), intermittent pools had both high profile and planktonic guilds (71.8 %* and 69.2 %**, respectively). Finally, perennial lotic systems were indicated by valve attached colonies and low profile guild (80.9 %**, 68.6 %*). Furthermore, river types were at least partially describable by their physico-chemical parameters. Indication value calculations showed that the concentration of sodium (81 %***), chloride, potassium, sulphite, nitrate, magnesium, calcium,TOC/NPOC, conductivity, hardness, temperature, alkalinity and pH (59 %***) were significantly indicative of intermittent pools, while discharge (76 % *) was indicative of perennial streams.

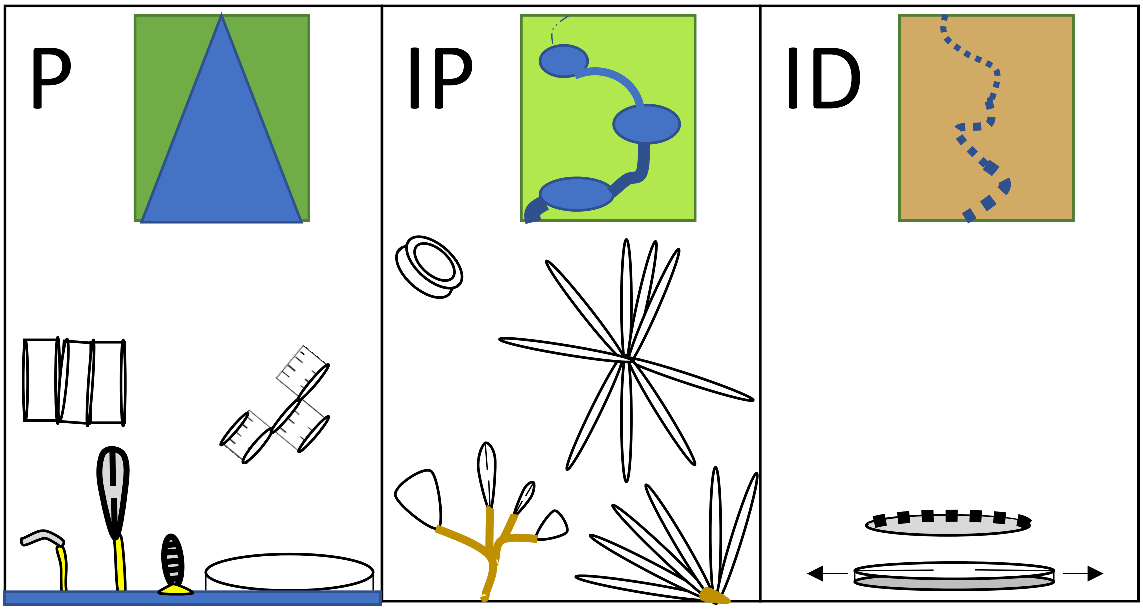

Figure 37 Comparison of diatom life form composition of different river regimes according to their IndVal values. P (perennial rivers): ribbon colonies, low profile guild. IP (intermittent pools): planktonic, high profile guild. ID (intermittently dry): motile guild.

The rosette colonies were positively correlated with oxygen dissolution percentages (Table 18). But negative correlations dominate. Sodium concentration and Total Organic Carbon were the parameters with highest negative correlation. All saline parameters showed negative effects on rosette colonies and even pH had a negative effect. Pioneer diatoms are also negatively correlated with saline parameters. The less correlated diatom life form was mobile forms which were only related to dissolved oxygen. The only significant correlation with discharge was of the low-profile guild. Asummary of the results that can be consulted in Figure 38.

6.2.4 Discussion

One could argue that the sampling method was inadequate, since it is only useful for flowing waters (Kelly et al. 1998). The problem lies with those rivers that do not have a consistent flow, or only exist through groundwater addition when arheic or hyporheic. In our study, for instance, the San Miguel River only was found in its arheic form. If a subterranean water exchange takes place, these rivers should reflect the physico-chemical state of the basin. Thus, we judge the use of benthic diatoms to be justified. For future studies, however, we encourage an adaptation. Planktonic or epiphytic diatoms could be used for arheic and, to some extent, oligorheic conditions. When the river is mostly edaphic, further thought should be given to the significance of the study. New developments on edaphic diatom sampling described by Antonelli et al (2017) would argue for the sampling of the soil as a diagnostic tool for basin quality.

The taxonomy found in the temporary rivers was quite diverse. The most diverse genus was *Nitzschia* Hassal (54 species), which is usually related to altered habitats and mostly eutraphentic. The second was *Navicula* Bory, with 36 species, also mostly eutraphentic. The third was the first usually oligotraphentic genus, *Gomphonema* Ehrenberg, 31 species. Even though these genera were the most diverse, *Achnanthidium* Kützing was the most abundant. It also comprises the flagship cosmopolitan and pioneer species (Hofmann et al. 2011). *Achnanthidium minutissimum* (Kützing) Czarnecki, was in a 94% of the total and predominant in 10% of them.

The current taxonomic biomonitoring technique, identifying diatoms under the optical microscope, has some disadvantages due to possible misidentification, for instance of small taxa (Morales 2001; Blanco et al. 2004). The identification effort can be reduced by using the functional group and life form investigation which is based on the genus level (Rimet and Bouchez 2012). Thus, more and more efforts have been invested into the study of these functional groups and life forms, be it the ecological guilds (B-Béres et al. 2017) or a combination with life forms (Riato et al. 2017). Though some authors have pointed out the potential loss of information working at the genus level (Passy 2007b; Lange et al. 2011), the life form approach in this case explained variability better than the species-based methods. Riato and collaborators had similar results that they justified with the homogeneity of their predominant taxa, pertaining to the same genus. In our case, even though the

Table 18 Spearman rank calculations with columns: Dls = Discharge, Con = Conductivity, O2 = dissolved oxygen percentage, Alk = alkalinity, Ca = Calcium, Cl = chlorine, Har = Hardness, Mg = Magnesium, NO3 = Nitrate, TOC/NPOC = Total Organic Carbon/Non-purgeable Organic Carbon, pH, K = potassium, Na = sodium, SO2 = sulphite. Rows: Mob = mobile, Pio = pioneer, Tub = tube, Ros = Rosette, Rib = Ribbon, Ped = Pedunculate, Adn = Adnate; HP = High Profile Guild, LP = Low Profile Guild, Mot = Motile Guild, Pla = Planktonic. . (Legendre and Legendre 2003; Passy 2007a).

	Dls	*Con*	*O2*	*Alk*	Ca^{2+}	Cl^-	*Har*	Mg^{2+}	*NO3*	*TOC*	*pH*	K^+	Na^+	SO^{2-}
Mob	0.11	-0.05	-0.19 *	0.16	-0.00	0.02	-0.02	-0.04	0.10	0.07	-0.11	0.05	0.02	-0.06
Pio	-0.04	-0.35 ***	0.15	-0.36 ***	-0.24 *	-0.39 ***	-0.27 **	-0.29 *	-0.08	-0.36 ***	-0.29 **	0.38 ***	-0.41 ***	-0.31 **
Tub	-0.08	-0.16	0.10	-0.22 *	-0.17	-0.17	-0.12	-0.04	-0.11	-0.17	0.12	-0.23 *	-0.21 *	-0.09
Ros	0.13	-0.45 ***	0.30 **	-0.45 ***	-0.23 *	-0.57 ***	-0.25 *	-0.24 *	-0.14	**-0.54 *****	-0.32 **	**-0.56 ****	**-0.65 *****	-0.28 **
Rib	0.15	-0.18	0.02	-0.10	-0.19 *	-0.12	-0.17	-0.07	-0.04	-0.10	-0.06	-0.10	-0.06	-0.13
Ped	-0.05	0.18	-0.19 *	0.12	-0.05	0.29 **	-0.03	0.01	0.12	0.20 *	0.07	0.27 **	0.34 ***	-0.05
Adn	-0.11	-0.29 **	0.15	-0.23 *	-0.15	-0.29 **	-0.16	-0.18	-0.10	-0.25 *	-0.20 *	-0.28 **	-0.32 ***	-0.20 *
HP	0.14	-0.24 *	0.22 *	-0.27 **	-0.14	-0.29 **	-0.13	-0.10	-0.28 **	-0.28 **	-0.13	-0.34 ***	-0.27 **	-0.06
LP	0.30 **	-0.09	0.25 *	-0.05	0.07	-0.15	0.11	0.15	-0.06	-0.19 *	0.00	-0.11	-0.16	0.14
Mot	-0.18	0.41 ***	-0.23 *	0.48 ***	0.23 *	**0.53 ****	0.28 **	0.30 **	0.04	**0.50 *****	0.42 ***	0.49 ***	**0.56 *****	0.31 ***
Pla	0.11	-0.42 ***	0.12	-0.45 ***	-0.30 **	**-0.52 *****	-0.35 ***	-0.35 ***	-0.11	**-0.50 *****	-0.39 ***	-0.47 ***	**-0.54 *****	-0.38 ***

p-values displayed as: 0 '***' 0.001 '**' 0.01 '*' 0.05 ns " ".

predominant taxa are more diverse, the main life forms are shared and relate to physico-chemical parameters. The IndVal calculations (Legendre and Legendre 2003) showed that mostly the ecological guilds (Passy 2007a) were descriptive of the river types.

Mobile taxa, i.e. those which can move along each axis, were negatively correlated to oxygen levels. Since this life form is mostly related to lentic environments, it was found preferentially in arheic river sites. Pioneers, such as the predominant *A. minutissimum* (Kützing) Czarnecki, were negatively correlated to each saline parameter, ions and pH, but reacted positively to a sodium increase. This particular species has been linked to lakes and rivers with circumneutral to alkaline and sodium rich waters (Van Dam et al. 1994; Andrén and Jarlman 2008).

Life forms did also differentiate physico-chemical parameters. The abundance of diatoms living within mucilage tubes declined in the presence of alkalinity, potassium and sodium concentration increase, which is understandable since these chemicals have been used in laboratory conditions to eliminate extracellular polysaccharides (Bahulikar and Kroth 2008).

Rosette forms, or colonies attached by valve pole, were negatively correlated to all ions, pH and salinity markers. Only the oxygen percentage positively correlates with this life form, which shows that these diatoms are mostly found in flowing rivers (Rimet and Bouchez 2012).

Ribbon colonies were unspecific in their relationship to the physico-chemical characteristics, except for a negative correlation to calcium. This suggests the need of further subdividing this group, since the use of life forms should rely on their descriptiveness. Further efforts should be made in this instance. Ribbon colonies were also descriptive of the perennial river types, which varied in their composition, thus explaining the lack of clear physico-chemical correlations, and indicating the need of permanent flow for the formation of this form.

Pedunculate diatoms were more significant in defining their sites. These diatoms were positively correlated with the saline ions (chlorine, sodium and potassium) and total organic carbon and non-purgeable organic carbon (TOC/NPOC), while being negatively correlated to oxygen percentage. This could be pointing to their predominance in (relatively) disconnected aquatic states, from oligorheic down to arheic conditions.

Adnate diatoms correlated negatively to increased salinity and ion concentrations, as well as to pH and TOC/NPOC. One example for this group is *Cocconeis* Ehrenberg, which is known to be an indicator of good water quality and only partially tolerant to salinity (Bey and Ector 2013).

The ecological guilds were more definitive than life forms, since each of them was related to at least three physico-chemical traits. This is why some studies are addressing to fine tuning this categorization as a biomonitoring tool (B-Béres et al. 2017). The Rimet and Bouchez inclusion of the planktonic guild has been shown to be a clearer divisor of physico-chemical traits than the Passy provision (Riato et al. 2017). Thus, it was used in this work.

The high-profile guild was defined by its protruding stature which increases sensitivity against high water current and provides a supposed tolerance to nutrients (Passy 2007a). Our data showed positive correlation to oxygen dissolution and negative to the other factors, including nitrate concentration. That this guild provided an indicator value for intermittent pools was not surprising, since the reduction of flow would facilitate the growth of high colonies.

The low-profile guild is determined by the low laying diatom forms and can overcome mechanical strain from the current flow, which was also observed here through the positive correlation with the discharge volume. This guild is usually found at headwater locations, and thus can be related to good or high quality waters (Passy 2007a). In this case, the physico-chemical data correlated negatively to TOC and positively dissolved oxygen concentration increase. The results do not point clearly to a nutrient state. The low-profile guild was also related to perennial streams, which also shows no clear relationship with nutrient content.

Motile guild diatoms are capable of autonomous movement and thus can relocate to other microhabitats to improve their conditions (Passy 2007a). These diatoms are usually linked to main rivers or lowland streams, and thus are tolerant to anthropogenic impacts. It is not surprising to find this guild to be positively correlated to each parameter, except for oxygen dissolution. This can again point to a tendency to be found in intermittently dry streams, where oxygen levels may be reduced through increased incidence of heterotrophs.

Finally, the planktonic guild was added by Rimet and Bouchez and later confirmed (B-Béres et al. 2017). It is based on that diatoms found on benthic substrate, where these species usually are of planktonic origin and sediment after dying. This guild may not necessarily be linked to any nutrient proportion. As expected, the planktonic forms were correlated with intermittent pools, which is due to the change in hydrological regime.

6.2.5 References

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6.3 DIATOM SIZES AND ECOLOGICAL GRADIENTS (MICRO-SCALE III)

6.3.1 Introduction

Bioindication depends on taxonomy (Lange-Bertalot 1979a, 1979b; Kobayasi and Mayama 1989). Each taxon, as described by taxonomists and biomonitoring agencies, may be found in different locations with similar physico-chemical traits and is characterized by its specific frustule morphology and dimensions. These encompass a broad range of sizes for each taxon, as described by taxonomists (for instance Dreßler et al. 2015; Jahn et al. 2009; Williams 1985). But this variability has not yet been satisfactorily explored in an environmental context. Taxa (Jüttner et al. 2011) and diatom communities (Snoeijs et al. 2002) are important but have scarcely been studied in this context. Some allometric approaches have been made (Tomas 1979), but these examples are rare and negligible. A more systematic approach to observe the allometric effect on the whole community is still a necessary step to take, which we started in this work.

Size variability is one of the main mechanisms for organisms to react to their environment. Diatoms have been used for environmental biomonitoring of soil (Antonelli et al. 2017) and freshwater systems (Lange-Bertalot 1979b). But a methodical approach to using size and allometry parameters for this purpose has not been attempted until now, probably due to the expenditure required. By automatization of microscopy and diatom measurement software (e. g. SHERPA, Kloster et al. 2017), an efficient tool of diatom community characterization has been developed, which we utilized for this study, Others methods addressing this have been also developed by Stela Ballester (2016) and Bueno et al. (2017). We wanted to take advantage of this and our large diatom sample bank to see how the different diatom parameters link to physico-chemical characteristics of the rivers they inhabit.

Based on the differenced of diatom sampling sites regarding environmental traits, geographic distance and quality of substrates, we posit that:

1. Diatom valve area should be differential for each site (Finkel et al. 2007).
2. Diatom surface to volume ratio (S/V henceforth) should be highest for oligotrophic and lowest for eutrophic sites. S/V should be increased for nutrient absorption when these are limiting (Roselli and Basset 2015).
3. Diatom width (diameter along the transapical axis) should be maintained more or less due to its clonal maintenance (Cox et al. 2012; Mann 1984).
4. Surface and volume can also be influenced by nutrient variation. Limitation of either nitrogen or phosphorus in the Redfield ratio (16:1) can affect them (Litchman et al. 2008).

6.3.2 Materials and methods

The Ebro river basin is located on the northeast of the Iberian Peninsula. Eight sampling sites (AnnexTable 16) were selected randomly from the 459 sample sites of the Ebro river bioindication network (Confederación del Ebro 2015). The requisite was a minimum of three samples being available, and for the sites to be mostly differential in their characterization as per their subtypes (Munné and Prat 2004). Two samples were obtained from the main river, two in direct tributaries, and the remaining from sub-tributaries (Figure 39). The diatom samples were selected mostly from each Water Framework Directive subdivision, see AnnexTable 16, River types (Cedex-MMA 2005; Confederación del Ebro 1998, 2006).

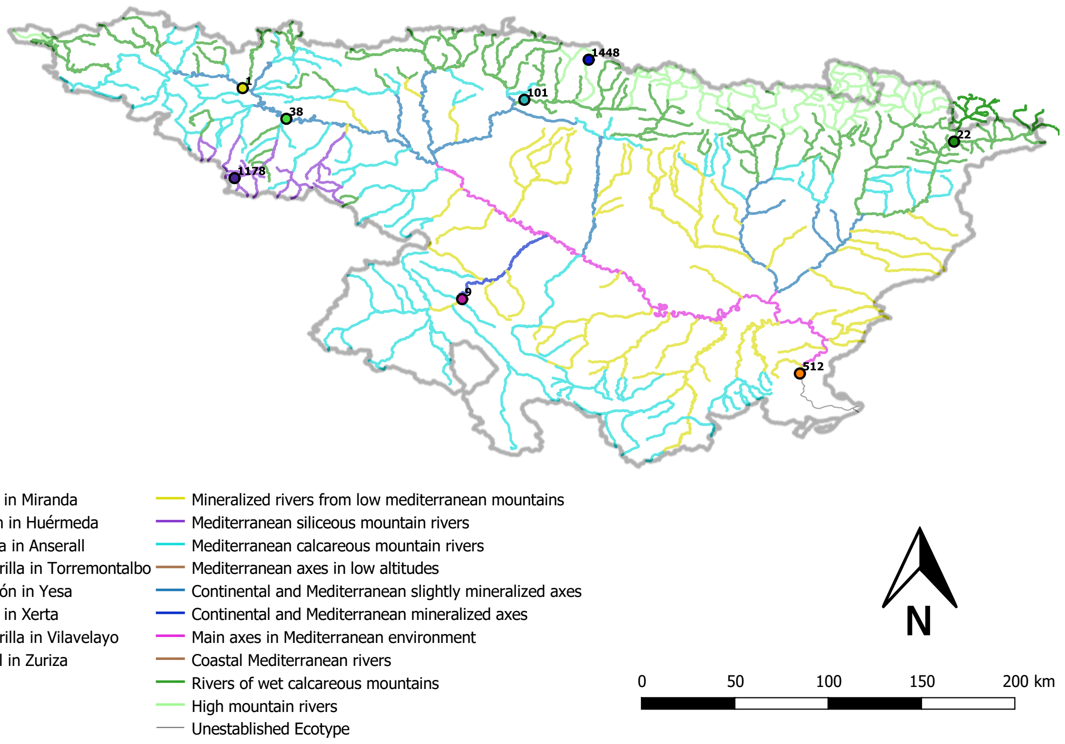

Figure 38 Distribution of the diatom sampling sites. Modified from: Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente.

Slides of 48 samples from 8 sites from the Ebro river basin were scanned by a Metafer 4 system attached to a Zeiss Axio M2 Imager (AnnexFig. 7). Depending on diatom density, a slide area represented by a total of 2,500-4,000 pictures was imaged at 63x magnification.

Diatoms were selected from these images using the SHERPA software (Kloster et al. 2014), a diatom form recognizing and measuring software (AnnexFig. 8-9). To exclude debris, a preselection of results was done using area and perimeter minima of 14 μm^2 and 21.6 μm respectively. These had been established during a first run selection of smallest diatoms. The diatoms were identified both in valvar and pleural disposition, where possible.

SHERPA's output was the automatic measurement of, amongst other values, valve area, perimeter, apical length and transapical width, which are basic features for taxonomic identification. The success rate of SHERPA correctly identifying an object as diatom valve depended on the existence of the blueprint of the valve form (AppendixFig. 10). For not yet recognizable diatom geometries, such as the "2 cylinder and elliptic prism" form of *Encyonopsis* Krammer (Sun and Liu 2003), new blueprints were developed. Some diatoms did not adhere to the forms, for example because of a teratology. In those cases, the form could be drawn manually. Diatoms were identified (following methodologies of Chapter 3) and annotated using software developed by Kloster (unpublished), called SHERPA-ANT. Redundant results were removed using an R-Script created by Kloster (Kloster et al. 2017). The resulting imaged inventories comprised 290 – 800 diatoms.

Since diatom frustules separate in preparation, a direct volume calculation and 3D measurement is not feasible. Thus, the mean diatom pervalvar breadth of identifiable pleural (girdle-view) images was used to calculate the frustular dimensions for each taxon following preceding studies (Snoeijs et al. 2002). From there, diatom taxa were divided into sizes according to formulae proposed by Hillebrand et al (1999) and amended by Sun and Liu (2003). Diatoms were defined as elliptic prisms, prism parallelograms, cymbelloid and gomphonemoid cells. Each category had its own formula to estimate frustule surface, volume and, from there, S/V, which was supposed to explain ecological relationships the best. Each size was assigned to its quartiles within the size range to be able to observe changes in multivariate analysis (AnnexTable 17).

The measurements obtained by SHERPA were compared to the physico-chemical characteristics of the sampling sites, as available from the Ebro Basin website (Confederación del Ebro 2015) for the sampling year. The physico-chemical traits selected were those usually correlated to diatom size, as for instance water temperature, nutrient concentrations (phosphate, total phosphorus, nitrate, total nitrogen, N:P ratio, silicon dioxide/tetroxide, and N:P:Si ratio), conductivity, pH and suspended solid concentration. We also included other factors that could hypothetically influence size, such as dissolved oxygen concentration and water flow (AnnexTable 18).

The statistical analyses were performed using R software 3.4.3 (R Development Core Team 2008, 2016) and RStudio 1.1.414 (RStudio Team 2015) . Global comparisons of samples were made using AMOVA ("ade4" package) on log-normal distributions and violin plots (Wickham 2009), as well as mean and confidence interval plots (Warnes et al. 2015, 2016). All measurements were correlated to physico-chemical parameters using Spearman rank correlation. Length and width for each site and year were regressed using general linear models. The relation of size distribution to environmental factors was studied using canonical correspondence (CCA) analysis (Oksanen 2013a).

6.3.3 Results

p-values: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ns = “ ”

A total of 324 diatom taxa were identified, including a 25% of occurred only once. The smallest valve areas pertained to *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot, the largest cells were Amphora ovalis (Kützing) Kützing. The shortest cells (apical axis) belonged to *Cyclotella atomus var. gracilis* Genkal & Kiss, the longest to *Tabularia fasciculata* (C. Agardh) D. M. Williams & Round. Regarding the transapical width, the smallest cells pertained to *F. saprophila*, while the largest were the centric *Actinocyclus normannii* (Gregory ex. Grève) Hustedt. The frustule’s S/V ratio was smallest for *Nitzschia intermedia* Hantzsch ex Cleve & Grunow, the biggest for *Encyonema minutum* (Hilse) D. G. Mann. The most abundant diatom taxa comprised *Cocconeis euglypta* Ehrenberg, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, *Navicula cryptotenella* Lange-Bertalot, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cocconeis lineata* Ehrenberg. Also *A. minutissimum* and *N. cryptotenella* Lange-Bertalot appeared to be frequent and found in each site.

Table 19 Spearman correlation of geometric valve features and physico-chemical data. “Morphometric” data (Area, Length, Width, L/W) was extracted from a 22.872 images database. “Ecological” data (Surface, Volume, S/V ratio) was extracted from 10.642 images.

	Area	**Length**	**Width**	**L/W**	**Surface**	**Volume**	**S/V Ratio**
Ammonium (mg/l)	0.076***	0.006	**0.11*** **	-0.06***	0.0099	-0.04***	0.048***
Conductivity (µs/cm)	0.19***	0.096***	**0.23*** **	-0.14***	-0.1***	0.088***	**-0.31*** **
Phosphate (mg/l)	0.11***	0.011	**0.16*** **	**-0.2*** **	-0.1***	0.039***	**-0.3*** **
Phosphorus (mg/l)	0.12***	0.054***	**0.14*** **	-0.09***	0.024*	0.09***	**-0.3*** **
Nitrate (mg/l)	0.17***	0.003***	**0.27*** **	**-0.3*** **	-0.04***	0.12***	**-0.4*** **
Dissolved Oxygen (mg/l)	-0.04***	0.018*	-0.04***	**0.1*** **	0.13***	**0.15*** **	-0.09***
pH	-0.04***	0.031***	-0.12***	**0.15*** **	-0.019.	-0.07***	**0.20*** **
Water Temperature (ºC)	**0.24*** **	0.20***	0.19***	-0.013.	0.073*	0.16***	**-0.3*** **
Flow (m^3/s)	0.064***	0.064***	0.041***	0.028**	**0.10*** **	0.055***	0.049***
Suspended Solids (mg/l)	-0.08***	-0.05***	-0.06***	0.058***	-0.1***	-0.021*	-0.04***
Total Nitrogen (mg/l)	-0.0084	0.0068	-0.04***	0.042***	0.079**	-0.034**	**0.28*** **
N:P Ratio	-0.15***	-0.1***	**-0.2*** **	0.19***	**0.15*** **	0.057***	0.13***
Silicon Dioxide (mg/l)	0.088***	-0.00059	0.16***	**-0.2*** **	0.13***	0.27***	**-0.71*** **
IPS	**-0.3*** **	-0.22***	**-0.2*** **	0.01	0.05***	-0.1***	**0.38*** **

The bulk of diatom measurements were dependent on the sampling site, AMOVA highly significant on the site (R2 = 0.41615***), but not so on sampling time (R2 = 0.01701 n.s.). Effect of variations on the spatial as well as on the temporal scale was significant (R2 = 0.13546*). The only morphometric features having significant Spearman and environmental fitting correlations to each physico-chemical parameter were valve width and S/V ratio (Table 18, Table 19). Valve area was positively correlated with water temperature and conductivity, length weakly with water temperature. Width correlated positively with nitrate concentration and conductivity and L/W negatively with nitrate, phosphate and silicate concentrations. The Redfield N:P:Si ratio was not always maintained. Surface correlated positively with the N:P ratio and Volume with silicate concentration. The strongest significant ecological parameter was silicate concentration in correlation to S/V ratio (ϱ = -0.71 ***). Both width and S/V ratio have been selected to closely observe them in their environment, along its time series (see below).

Depending on the imaging plane, depicting either the valvar oder the pleural view and consequent lack of frustule measurements, diatom and physico-chemical data were divided into “morphometric” or direct (n = 22.877) measurements and “ecological” data where not the valve faces, but the pervalve axis was measured because they were oriented in pleural view. (n = 10.642). A multiple linear regression scenario of the measurements with highest correlation values (Width, L/W, S/V) presented low outcomes for their R^2 fit values (W = 0.09249***, L/W = 0.07545***, S/V = 0.1887***). All physico-chemical parameters were significant for the calculation of S/V. Not so for width (neither phosphate nor nitrate concentrations) or L/W ratio (neither phosphate concentration, nor flow).

6.3.3.1 Width distribution

The distribution of valve width for each site, regardless of the sampling period, is displayed in Figure 40. Sites with higher IPS index values (oligotrophic) comprised narrower valves than those with lower index values. The widest cells were found at the most polluted main river sites. Meanwhile, the narrowest range was found at the dry calcareous mountain (E0101 and E1448). The saline site had peaks at two differen sizes. A linear regression of the length-width relationship of each site showed linear correlation without significance. This was probably because of the log-normality not being continuously maintained, since residuals were neither homoscedastic nor unbiased. The same happened for a selection of size comparison of *Achnanthidium minutissimum* (Kützing) Czarnecki, it maintained the same bias and heteroscedasticity, undoing its log-normality (AnnexFig 20-22).

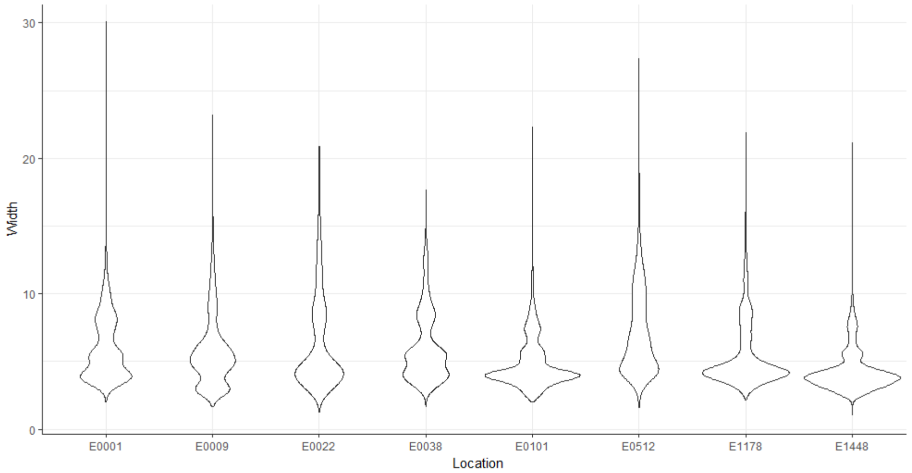

Figure 39 Violin plots of the valve width for each location. Oligotrophic comprise more narrow valves, with distributions being strongly skewed towards higher values.

For some differential sites, valve width means showed similar patterns through time (Figure 41, compared simultaneously in AnnexFig. 23). Diatoms from the Ebro river (in Miranda de Ebro E0001 and Xerta E0512) had comparable tendencies over time. Although these were lagging one year in the upper river (E0001), with the mean width value shifting within a ± 1.5 µm range. In the Aragón river, which is an Ebro tributary, (Aragón in Yesa E0101 and Veral in Zuriza E1448), the mean width value range differed only by 1 – 1.2 µm, and the distribution since 2007 showed similar trends, only softened in the high mountain site. Both sites found in calcareous substrate (Valira in Anserall E0022 and Najerilla in Torremontalbo E0038), were also similar from 2007 on. Our results showed that higher altitude produced higher variation in diatom width (± 2 µm in E0022 instead of ± 1.5 µm in E0038). The effect of altitude is proportional, so narrower cells are found at higher altitude.

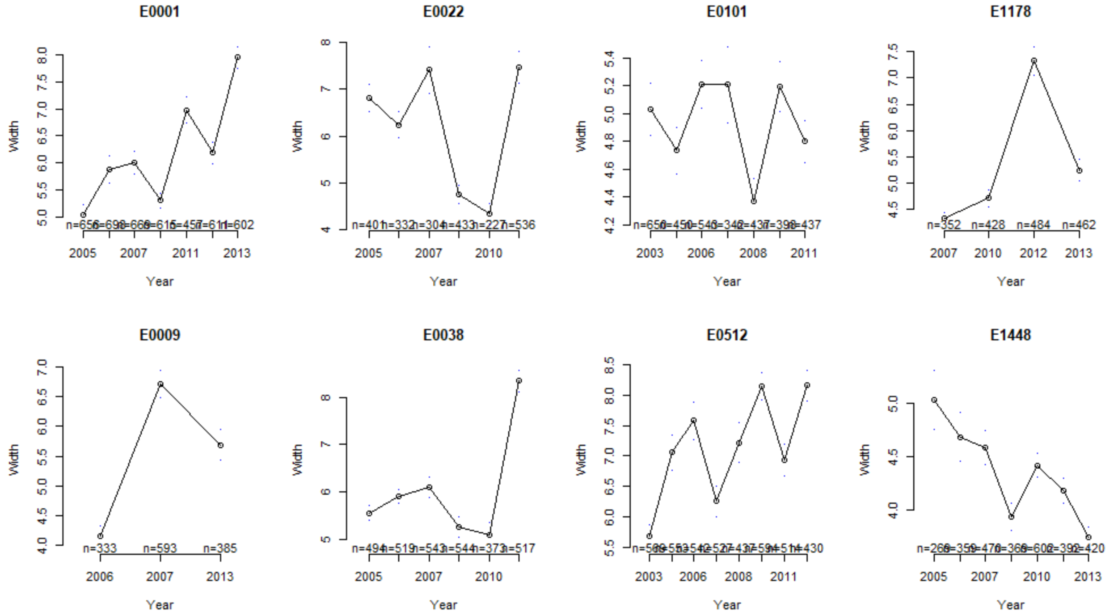

Figure 40 Comparison of the diatom width means and confidence intervals for each site. There is a pattern present for diatoms found in the same basin-sub basin (E0001 – E0512 and E0101 – E1448). Also, diatoms of similar substrate (E0022 – E0038). Some samples are significantly differentiated inside of the site.

When comparing these similar sites further, those located at the river axis were related to mediocre water quality (IPS: 5.3 – 14.5). The unconnected calcareous sites (E0022 and E0038) had mostly good (IPS: 9.5 – 18.3) and the higher sections (E0101 and E1448) had very good water quality values (IPS: 16.3 – 18.7). Thus, there seems to be a link between water quality and width size range. Moreover, with lower quality, the mean width range was higher (i.e. from 3-4 µm for good water quality to ± 2.5 µm the lower water quality gets.

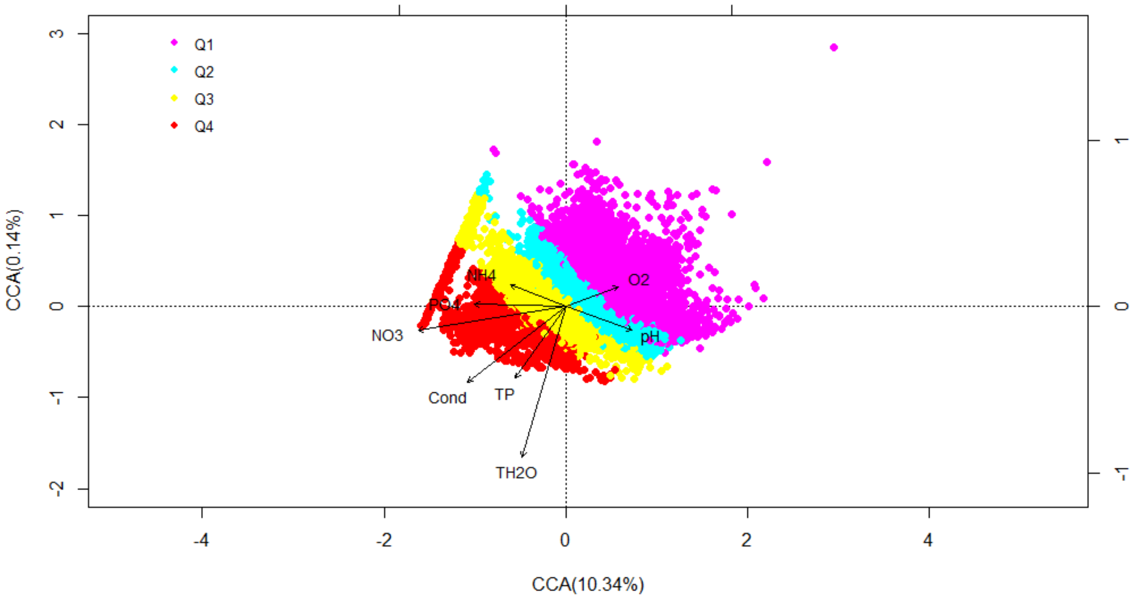

Figure 41 Canonical correspondence analysis comparing quartiles of diatom width and with their distribution along the physico-chemical axes. 10.48 % of the variance is explained. Quartiles of width: (Log (x+1) values): Q1 = 0.024 –0.5929, Q2 = 0.5929 – 0.6907, Q3 = 0.6907 – 0.8655, Q4 = 0.8655 – 1.4781. NH4 = Ammonium, Cond = Conductivity, NO3 = Nitrate, PO4 = Phosphate, TN = Total Nitrate, TP = Total Phosphorus, O2 = dissolved oxygen, pH, TH2O = water temperature.

The canonical correspondence analysis (CCA, Figure 42) explained only 10.48% of the variance (eigenvalues axis 1 = 1.392×10^{-3}, axis 2 = $1,77 \times 10^{-5}$). Narrower diatoms seemed to converge with higher dissolved oxygen. On the other hand, wider diatoms were found with high nutrient concentrations, salinity and water temperature.

The mean widths of the predominant diatom taxa reacted differently. Within the taxonomically established width range, the individual transapical width range was variable. When investigated separately, *Achnanthidium minutissimum* (Kützing) Czarnecki *sensu lato*, the most abundant and frequent taxon, did reflect the similar distribution of the width averages as seen in figure 41 (not shown). Other, less frequent taxa had different width distributions for each sampling site. The similarly frequent but less abundant *Navicula cryptotenella* Lange-Bertalot did not show a similar the same width variation over time.

6.3.3.2 S/V distribution

S/V ratio of diatoms exhibited no clear pattern (Figure 43). The range was highest in the high mountain sites (calcareous and mixed). The siliceous mountain site (E1178) had a smaller range of S/V ratios. The saline site (E0009) had not a continuous distribution, but clearly distinct focal peaks.

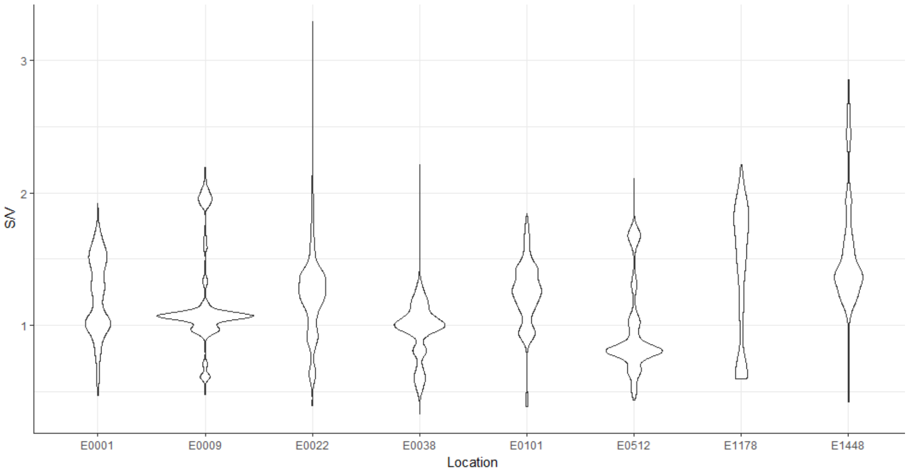

Figure 42 Violin plots depicting the S/V ratio of the diatoms at each site. More pronounced peaks of S/V values are found at the altered sites.

The comparison of the total S/V means (Figure 44) showed that different sites reacted differently over time. Nevertheless, two pairs of sites exposed similar patterns. The distribution for the two high

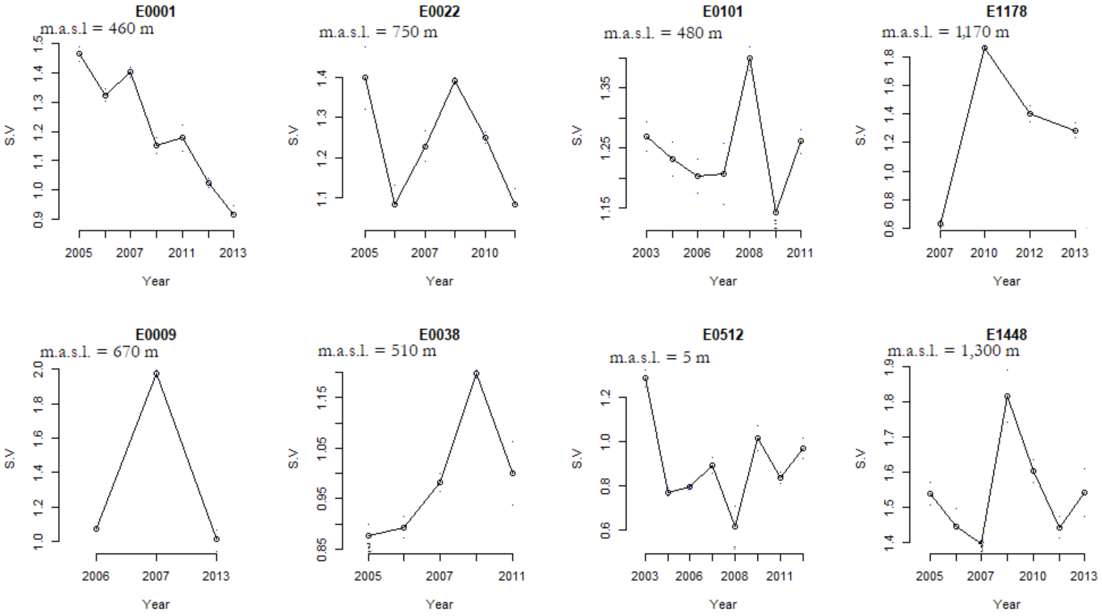

Figure 43 Comparison of the diatom S/V means and confidence intervals for each site.

water quality sites (E0101 and E1448) were similar, as well as for the good quality sites (E0022 and E0038). For the other sites, no pattern is discernible.

The S/V ratio value range at each site was very variable and seems not to be related to altitude or water characteristics. In the similarly shaped mean plots, the ratio ranges on a lower level than for the lower altitude. The lowest laying sites, at the Ebro main river (E0001 and E0512), had no longer a clear connection and similarity.

In the canonical correspondence analysis of the S/V ratio (Figure 44 45), eigenvalues were small, thus explained little of the 14.503 % of variance (ev1=0.0010739 and ev2=0.0000213). The lowest quartile was highly correlated with nutrients, conductivity and water temperature, whilst the highest qartile was positively correlated with pH. Neither ammonium nor dissolved oxygen concentration appeared to affect the S/V ratio significantly.

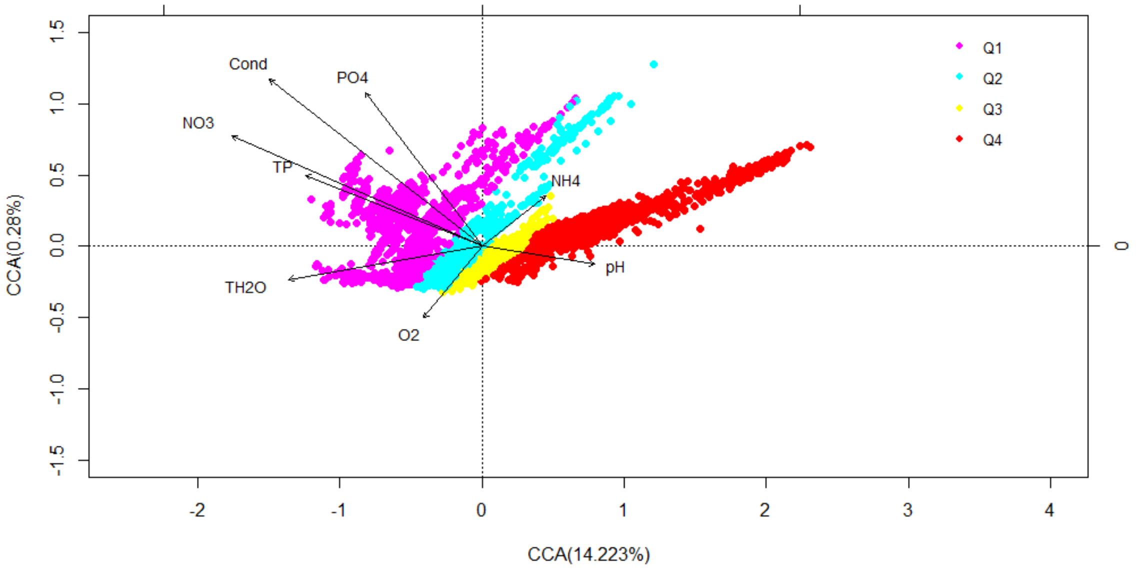

Figure 44 Canonical correlation Analysis showing the S/V ratio quartiles relating to the physico-chemical characteristics. Display of quartile distribution. (Variance explained = 14.503%). Quartiles of S/V: (Log (x+1) values): Q1 = 08348 – 0.9963, Q2 = 0.9963 – 1.0389, Q3 = 1.0389 – 1.0774, Q4 = 1.0774 – 1.4032.

6.3.4 Discussion

Many diatom metrics have been explored in previous studies. Finkel's lab preferred the valve area as a more comparable parameter (Finkel et al. 2009), since in biomonitoring one usually finds and identifies diatoms oriented in a position where the valve area can be measured directly. Diatom length (apical axis) has also been used (Pandey and Bergey 2016; Pandey et al. 2018). But length seems to be affected by heavy metals, due to their physiological effects. As far as we know, neither perimeter nor width have been linked to physico-chemical parameters of the water prior to this studt. Perimeter has only been measured in centric diatoms, to count striae/10 µm (Dreßler and Hübener 2006).

Biovolume is by far the most assessed parameter, due to its established relationship with external parameters. But no unified approach has been reached yet. For general plankton, a distance-matrices-from-images approach was tried (Moberg and Sosik 2012), but was not applicable for our data. Neither was the use of biomass values through cell density (Litchman et al. 2008). A manual approach for measuring mean girdle breadth (pleural view) was assessed by Snoeijs and collaborators (2002). We found this to be the best approach for our calculations. The total frustule surface has mostly been considered in addition to volume, in order to calculate the S/V ratio, usually linked to external changes (Snoeijs et al. 2002; Roselli and Basset 2015; Nielsen and Sand-Jensen 1990; Law et al. 2014).

We selected valve width and surface-to-volume ratio due to the significance of their Spearman correlation values. Width is a morphological parameter that has been widely overlooked. This could be due to the expected clonal maintenance of width of a particular culture (Cox et al. 2012; Mann 1984). But, owing to the sexual reproduction mechanism of pennate diatoms, width may be variable in the production of the initial cell in a low nutrient environment through the establishment of the virgae, extrusions from the raphe, and the later formations of valves during mitosis (Round et al. 1990). Because "*transapical ribs appear on both the primary central band and secondary arms, and cross extensions join to form the sieve plate areas.*" (Chiappino and Volcani 1977), width could be more variable than expected when the medium is fluctuant. Thus, the medium could determine the maximal and/or minimal valve width for the whole community. We hypothesize that this might be related with the N:P:Si ratio of the sites. This could explain why similar rivers had parallel distributions.

The apparent delay between the mean width distribution in both the main river sites (Ebro at Miranda de Ebro and in Xerta) brings up new questions upon the cause of this trend. The site closer to headwaters is the one where signals lag those of the downstream site. Neither taxon composition nor width distribution were identical. The mean valve width varied by ± 1.5 μm for both sites, but was higher at the lesser altitude site. The other pairs of sites exhibiting similar patterns were synchronous from the year 2007 onwards. Our data do not suggest any physico-chemical parameter driving this pattern. Silicate dioxide concentration might influence the valve width, but we have not enough measurements of them to establish them as the main driver.

The mechanism of valve formation in raphid diatoms starts with building the raphe canal. After that the virgae or transapical elements develop (Round et al. 1990). Since silicic acid transfer at low $Si(OH)_4$ levels is mediated by active silicon transporters (SiTs) that depend on ATP (Thamatrakoln and Hildebrand 2008; Volcani 1978), low nutrient sites without much silicic acid concentration, e.g. Veral in Zuriza (1448) and Aragón in Yesa (0101), could affect valve formation. Another study states that all diatom silicate metabolisms seem to be regulated by purine monomers like e.g. ATP and GTP (Aline et al. 1984). One of the sites, Najerilla in Villavelayo (E1178), was on a silicate mountain. As Round et al (1990) stated: "*In waters enriched by run-off from silica-rich sites or on sediments, there is a continual supply of silicic acid and the succession of species must be caused by factors other than limitation by silicate (see also*

Admiraal 1984)". For the location stated above, these factors could be the variability in other nutrients, like the limiting phosphorus. We find it also necessary to state that the N:P:Si ratio was very erratic whenever we were able to calculate it, where often the necessary silicon dioxide measurements lacked. But, whenever measured, silicon dioxide consistently surpassed the Redfield ratio (N:P:Si, 16:1:20). This can be important for the future, since the Redfield ratio is probably going to be affected by the variation of anthropogenic nutrient input (Garnier et al. 2010).

It is necessary to repeat that most of our findings are based on whole community data. Diatoms are selected by external factors and have characteristic morphology traits. But, specific taxa reflect the communal response, such as *Achnanthidium minutissimum* (Kützing) Czarnecki. This diatom, as well as being part of a big and poorly understood diatom complex (Potapova and Hamilton 2007; Wojtal et al. 2011), is characterized by its colonizing attitude (Rimet et al. 2009). This type of diatoms is a good colonizor owing to the increase of sexual and asexual reproduction. C (colonizing) strategy for fitness could be the driver for a similar reaction of species valve width as for the whole community (Law et al. 2014). Thus, we can observe how natural selection affects this diatom taxon as well as communities.

What about S/V distribution? Silicon oxide was inversely correlated with the S/V ratio. It was the most important water characteristic as far as diatom measurements were concerned. S/V ratio was highest in the most oligotrophic sites, including those having high conductivity values. This may reflect the need of a higher surface-to-volume ratio to increase the surface for capturing important nutrients (Marcel et al. 2017). Our surface and volume data are somewhat artificial, since they are estimated using the species' average cell pervalvar axis. Nevertheless, we consider them a good approximation to the actual values.

S/V ratio has long been established to be highly related to nutrients. Comparisons of diatom cell volumes in marine or freshwater environments showed that nitrogen limitation in the former drives increase in volumes. Smaller cells are found for limitations in phosphorus accessibility (Litchman et al. 2008). This in turn implies that the S/V ratio is higher for freshwater diatoms. Another parameter that has been found to be influential for size variations is pH (Wunsam et al. 2002). *Achnanthidium minutissimum* (Kützing) Czarnecki, the C type species, is also characterized by a high S/V ratio (Law et al. 2014).

Effects of salinity have also been observed in previous studies (Roselli and Basset 2015; Svensson et al. 2014). In our findings, conductivity does not have such an impact on morphological traits as nutrients do. This may be because diatoms that survive in saline environments usually produce metabolites that deter the effect of osmotic pressure (Fujii et al. 1995). In other studies, morphological aspects of the cells have been affected (Paasche et al.1975). As we have seen in the figures 40 and 43, width and S/V have multiple peaks. The focus seems to be more on species or genera, in this case, which build these osmotically effective metabolites. The saline sites showed an increased abundance of the *Nitzschia* Hassal genus. Thus, as genera display high ranges of size variation (Rond et al. 1990), the sizes were very variable here.

Length variations have been related to alterations caused by dangerous substances and heavy metals (Morin and Coste 2006; Pandey and Bergey 2016). We have not investigated these chemical parameters, since the available data are semi-quantitative and not exact. Nevertheless, our physico-chemical data show some correlation of cell length with temperature. Other morphological parameters as valve area, frustule surface and volume were less correlated with physico-chemical traits. Valve area is affected negatively by nutrients in phytoplankton (Finkel et al. 2009), a finding which was mirrored in our data. Cell surface was the only morphological trait to be significantly correlated with flow. In headwaters, mechanical stress is added to low phosphate concentration. Because of this, an increase in cell surface, to ease nutrient uptake (Denny 1993), stands to reason.

Our study suggests that diatom morphology at the Ebro river basin is affected by its physico-chemical environment. We also posit that the previously overlooked valve width is a good candidate for describing water quality. Other studies such as Lavoie et al. (2010) have not found a clear relationship between biovolume and sites. We conclude that more and larger datasets on global freshwaters could provide more information on how diatom morphometrics could be important descriptors of water quality.

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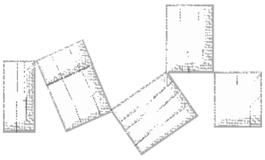
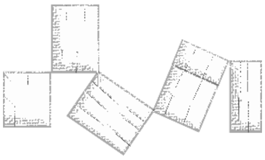


DISCUSSION



Chapter 7

DISCUSSION

Diatoms are affected by all aspects of their surroundings (Oda et al. 2014; Lange-Bertalot 1979b; Jüttner et al. 2011). That is why a multi-scalar comparison was undertook in this thesis, to characterize general diatom responses. The scalar subdivisions that we have attempted were: whole basin, sub-basin and local site. This thesis has tried to elucidate benthic diatom community aggregations, effects of land use and specific site reactions. It also compared diatom water quality indication methods, such as the diatom community, bioindicators, life forms and equivalent parallel indication methods, as well as diatom morphology. This is the first general, multiscale view into the characteristics of diatoms of the Iberian Mediterranean Rivers.

It was important for us to study how variations of environmental conditions on the spatial as well as on the temporal scale affected the diatoms of the Ebro river. Utilizing our comprehensive sample library, a totality of 10 years sampling at 458 sites, can facilitate creating basic knowledge in diatom assemblage time variability. The comparison of the different diatom features can add information on whether and how these features link to the external water traits. To support this knowledge, the macro-scale, meso-scale diatom taxa and the micro-scale, for example by the life forms of the Llobregat river in the experimental setup were studied. Land uses affect the physico-chemical conditions found at the surrounding sites, where the most affecting would be the 5 km upstream catchment (Burfeid Castellanos and Cambra 2016). On the other hand, the Llobregat catchment was particularly suited for an experimental setup, since, although being a relatively small basin, it contains a vast variety of water types. Additionally, intermittent rivers and ephemeral streams (IRES) were investigated, where previously such studies were mostly based on diatom taxonomy and bioindication suitability (Delgado et al. 2012; Novais et al. 2014; Burfeid Castellanos et al. 2017).

The WFD subdivision of river ecotypes (Ministerio de Medio Ambiente y Medio Rural y Marino 2008) is not useful for diatom communities (Tison et al. 2005, 2004, 2007; Bottin et al. 2014). This affects the ecological quality ratios of the so-called ecotypes. Diatom communities would reflect the changes in physico-chemical gradients better than a subdivision (Elias et al. 2012). The utilization of physico-chemical distribution and diatom aggregation distribution has not yet been tried, although the communities have been used to establish the typology of of French rivers (Tison et al. 2005, 2004; Rimet 2009), confirming that the WFD subdivision is not ecologically relevant for diatoms (Bottin, et al. 2014).

The meso-scale, or sub-basin, is a scale that received only little investigation. If such a study was performed, it usually regarded a whole-basin (Alvarez-Blanco et al. 2011). We deemed this smaller scale more appropriate for the land-use study, in order to use the orthophotographs of the Catalan

Institute for Cartography and Geology (ICGC 2014) as a better source of clear land use differentiation due to its lower altitude. The link of diatoms to land use hase been investigated only scarcely (Hlúbiková et al. 2007; Bere and Tundisi 2011; Tolkkinen et al. 2016; Teittinen et al. 2015), even though there is a clear effect on water chemistry due to land use (Allan 2004). Our study shows that there seems to be a dependency between specific land uses and diatom communities, and even between land uses and the diatom indices.

Micro-scale is the most studied scale of this thesis, at least regarding to the taxon communities (Lange-Bertalot 1979a; Kalyoncu et al. 2009; Riato et al. 2017; Lainé et al. 2014). However, the life-form and size distribution of diatoms in relationship to the physico-chemical traits have not been studied to the same degree.

Importance of the life forms has increased recently with the necessity of systematic water quality assessment as an alternative to longsome taxonomic studies or in preparation before the separated diatom metabarcoding (Rimet and Bouchez 2012). Diatom life forms are not always the same, and thus a first look on the fresh sample to establish the most important life forms found is necessary (Berthon et al. 2014). Ecological guilds (also known as functional groups) and their sizes (B-Béres et al. 2017) were better to pinpoint specific physico-chemical composition of the water. On the other hand, the traditional life forms, slightly modified (Riato et al. 2017), were better to select between the three experimental rivers that we worked with at the Llobregat basin.

Following the physico-chemical characterization, we applied the second method for the temporary rivers, to better discern between trophy and salinity gradients of the sites, as well as comparing it to other temporal water studies (Riato et al. 2017). Life forms are important traits that lock diatoms to the ecology they are living in. Both the survival tactic of the single cell or community, as well as its location inside the periphyton (ecological guild) has to do with the habitat surrounding it (Lange et al. 2011). Thus, life forms can give us additional information about the general structure of the ecology of the river site and have a very important micro-scalar involvement.

Diatom size studies have mostly reduced them to either transapical length (Pandey and Bergey 2016), biovolume (Montagnes and Franklin 2001), or surface area for comparability (Finkel et al. 2007). The biovolume is still one of the preferred morphometric features to study (Reavie et al. 2010; Ulanova and Snoeijs 2006; Morin et al. 2007), although it is not always connected to specific physico-chemical data (Lavoie et al. 2010). In our results, both diatom width and surface-to-volume ratio are the most important parameters, closer to the physico-chemical composition of the site than had been expected. S/V ratio is a common morphometric trait to be observed, although there has not been much effort to investigate it, probably due to the lack of a clear and reliable method of calculating it. The morphology formulae (Hillebrand et al. 1999; Sun and Liu 2003) have facilitated it enormously, but the current incapability of observing the whole individual cell structure other than with distance maps (Moberg and Sosik 2012), the biovolume is still not well resolved.

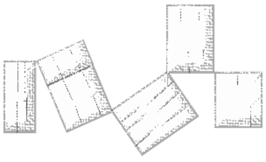
Width has been for the most part overlooked, probably due to the fact that experimental and taxonomical work with clone cultures have established a kind of homeostasis in generations of diatoms (Cox et al. 2012; Mann 1984). This could be due to the stasis of the medium in which diatom clone cultures are kept, which does not reflect reality and randomness of chemical composition in a river site. Abrupt changes in nutrient concentration could affect the valve's lateral growth, since it is supposed to follow the raphe formation, which would determine diatom length (Round et al. 1990) and affect growth due to ATP shortage which limits the silica deposition (Sullivan and Volcani 1973; Reeves and Volcani 1984; Coombs et al. 1967). The morphometric study only made sense in a micro-scale environment. Since diatoms can be affected by the physico-chemical traits of the waters they live in, only a study comparing the effects on different sites and in time was a possibility.

The fact that diatom sizes seem to react to substrate and climatic influences brings us back to the macro-scale. A substrate seems to affect not necessarily the species composition but can create clear delimitations on diatom width and S/V ratio.

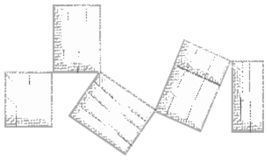
The importance of the Ebro study lays within its big temporal scale. Usually, longest term studies are known for marine phytoplankton and paleoecological studies (Reid et al. 2007; Wasell and Håkansson 1992; De Laender et al. 2012), studies on the effect of priority substances (Morin et al. 2009; Rimet 2012), o bioindicators (Virtanen and Soininen 2016). But studies on current samples have observed 2 to 5 years, paleoreconstruction and marine studies from 20 to thousands of years. A longer period of sampling has permitted us to investigate temporal patterns and gather more information on single sample site diatom communities and their characteristics.

Diatom taxonomy, functional groups, life forms and morphometric studies have not yet been investigated together to this degree. Life forms seem to be an easy way to obtaining information about the composition of the waters they inhabit (B-Béres et al. 2016; Passy and Larson 2011) and the ecology thereof (Lange et al. 2011). On the other hand, diatom morphometry is also linked to compositional aspects of the water (Olenici et al. 2017; Reavie et al. 2010; Busse 2002), but cannot be sufficient for the identification of specific taxa (Blanco et al. 2017) nor necessarily reflect physico-chemical composition (Lavoie et al. 2010). Diatom taxonomy has been the long-wearing tool for bioindication. It is being replaced by other methods, such as metabarcoding (Zimmermann et al. 2014; Zimmermann 2014). But community distribution and diatom biogeography can still be seen as a source of interesting ecological aspects of diatom communities (Mann and Vanormelingen 2013; Williams and Reid 2006; Potapova and Charles 2002; Rimet 2009).

To sum up, this thesis has tried to provide new insights into diatom behaviour in its taxonomic, ecologically functional and morphometric sense. We think that it highlights the potential importance of functional traits and diatom morphometry in the water quality monitoring. It also hints to complex ecological relationships with the diatoms' environment.



CONCLUSIONS



Chapter 8

CONCLUSIONS

1. Diatom communities did not correlate with established river ecotypes.

2. Physicochemical similarity clustering underlined the division due to altitude (mountain-lowland). The mountain cluster could be subdivided by substrate and the lowland by physico-chemical composition.

3. Diatom communities aggregated through clustering methods are more natural than SOM maps, which can be forced to ensure comparability. But SOM maps still have significant correlations with physico-chemical composition, whilst being a very visual description of diatom community aggregations.

4. The yearly communities' variation was not statistically relevant enough to be considered. A site-based mean community increased significance and reduced noise.

5. Combined diatom and physico-chemical clustering (SOM and PCA, respectively) have reduced the Ebro Basin to 6 "new" or alternative, ecologically sounder river ecotypes for diatom communities: 1) High mountain, 2) Lowland with conductivity, 3) Lowland with nutrients, 4) Lowland with elevated temperature, 5) Medium high silicate Mountain and 6) Mountain with siliceous substrate.

6. Land uses affect diatom communities, which differ according to physico-chemical parameters that are in turn affected by land use.

7. The diatom communities corresponded with proportions of land cover. Three main diatom communities were found in the Segre basin.

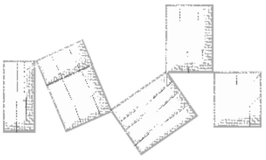
8. The IBD index can be linked to predominant land cover proportions, mostly by woods, meadows, agriculture and urban uses.
9. In an ecological-structural sense, physico-chemical composition is positively correlated to land use and negatively correlated to both community biodiversity and diatom bio-indicative values.
10. Life forms and ecological guilds are descriptive of physico-chemical distributions and hydrological alterations.

11. Diatom life forms can be used as identifiers and key elements of the sampled sites and they reflect the combination of all the physico-chemical traits of the sites (broad-brush approach).

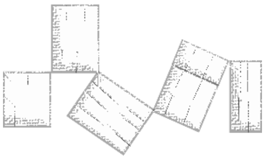
12. Ecological guilds (subdivided or not) can be found to be better descriptors for each physico-chemical trait, being able to fine-tune diatom characteristics (fine-comb approach). High

conductivity is linked to the high profile guild, and high nitrate concentrations leads to an increase of mobile and planktonic forms, whilst decreasing low profile guild diatoms.

13. Salinity was related to an increase in taxa with mucilage, eutrophic conditions increased mobile diatoms and oligotrophic waters increased adnate diatom species.
14. The un-subdivided ecological guild had more correlation to the physico-chemical values than life forms.
15. Water regimes were correlated to diatom life forms to a higher degree than aquatic states or a combination of both.
16. The planktonic guild was especially helpful, since the change to a lentic waterbody decreased flow, thus permitting the appearance of planktonic species and related life forms.
17. Physico-chemical composition of the site affects diatom morphology, mostly for valve width and frustule surface-to-volume parameters. In oligotrophic waters, Width decreased and S/V ratio tended to increase.



CHAPTER 9. RESUMEN DE LA TESIS



Chapter 9

RESUMEN DE LA TESIS

9.1 Introducción

Las diatomeas son unos organismos microscópicos con una pared celular de composición silícea que se desarrollan en sistemas acuáticos (Round et al. 1990). Estos organismos tienen una clara afinidad con su medio (Lange-Bertalot 1979b) y una elevada tasa de reproducción asexual (Round et al. 1990) y por ello han sido usados como bioindicadores de la calidad del agua, mayoritariamente en sistemas acuáticos terrestres (Kobayasi and Mayama 1989). Su compleja forma de reproducción asexual, en la cual se produce una valva menor (hipoteca) dentro de la mayor de las dos valvas (epiteca) del frústulo, implica una reducción paulatina del tamaño celular dentro de sus poblaciones (Drebes 1977; Macdonald 1869; Pfitzer 1869). Las diatomeas han colonizado prácticamente todos los hábitats con un nivel de humedad suficiente, acuáticos o terrestres (Antonelli et al. 2017), y gracias a las enormes comunidades planctónicas marinas pueden llegar a producir un 20-40% del oxígeno terrestre (Field et al. 1998; Falkowski, Greene, and Kolber 1993; Riding 1992; Falkowski, Barber, and Smetacek 1998). En esta tesis nos hemos ceñido a investigar aspectos poco estudiados de las diatomeas bentónicas en sistemas lóticos.

Los ríos forman ecosistemas altamente complejos e importantes. Su complejidad radica en la conectividad original que han tenido históricamente las cuencas hidrográficas. Debido a que, en general, los asentamientos humanos han estado unidos a la presencia de masas de agua, esta conectividad ha cambiado a lo largo de la historia. Los seres humanos han modificado los cursos por medio de canalizaciones y transvases, y han impedido la conexión por medio de embalses. Además, han influido en la contaminación de este medio.

Es por esto que en el año 2000 desde la comisión europea se estableció la Directiva Marco del Agua (DMA, (Parlamento Europeo and Consejo de la Unión Europea 2000, 2007)) para homogeneizar la biomonitorización y conservación del agua a lo largo de Europa. Entre sus objetivos se pretendía conseguir que el año 2015 los ríos europeos pasaran a tener una calidad de agua buena o muy buena. Para ello se usarían no solo los datos fisicoquímicos que se habían usado hasta el momento, sino que se complementaría con inventarios de especies de diferentes *phylums*. Ya anteriormente se había observado que existían diferencias en la composición de especies de organismos acuáticos que vivían en aguas limpias o contaminadas (Lange-Bertalot 1979b; Wilhm and Dorris 1968; Pan et al. 1999; Karr 1981). Diatomeas y macroinvertebrados, por ejemplo, demostraban una alta afinidad con óptimos reducidos y fueron usados para crear índices bioindicadores (Descy and Coste 1990; Gaufin 1973). Así pues, la DMA estableció que diatomeas, macroinvertebrados, peces y macrófitos (plantas superiores, musgos y algas macroscópicas) se usarían para obtener datos de la calidad ecológica del agua estableciendose redes de control. Tanto las diatomeas como los macroinvertebrados han demostrado tener una mayor sensibilidad para diferentes contaminantes.

Para incrementar la comparabilidad paneuropea, se organizaron redes de control biológico en cada cuenca hidrográfica. En 2008 se presentó una subdivisión de los ríos europeos basada en rasgos geológicos y físico-químicos (Ministerio de Agricultura 2008). A partir de estas subdivisiones y sus puntos de referencia se calcularon ratios de calidad ecológica (EQR) para cada una, según puntos de referencia reales o a criterio de experto. Estos EQR permiten comparar todos los bioindicadores de cada ecorregión entre sí.

La Confederación Hidrográfica del Ebro (CHE) estableció la red de control de diatomeas el año 2002 en la cuenca del Ebro. Desde entonces se ha llevado a cabo un muestreo de diatomeas anual. Aparte de ello, se han hecho sendos muestreos a diferentes intervalos para los datos fisicoquímicos, los macroinvertebrados, los peces, el fitoplancton y los macrófitos.

Esta tesis presentará, entre otros, los resultados obtenidos de 10 años de muestreo de diatomeas bentónicas en la cuenca hidrográfica del Ebro. Estos datos nos ayudarán a observar los efectos a nivel de cuenca hidrográfica (macro-escala), subcuenca (meso-escala) y punto de muestreo (microescala). El estudio experimental se produjo en la cuenca del Llobregat, no ligada a la cuenca hidrográfica del Ebro y regulada por el organismo oficial de la Agencia Catalana del Agua (ACA). Otro capítulo no ligado exclusivamente a la cuenca del Ebro fue la participación en el proyecto LIFE TRivers (LIFE13/ENV/ES/000341). El objetivo principal de este proyecto era recabar información para permitir la regulación legislativa de ríos temporales. Estos son ríos que pierden parte o la totalidad de su agua superficial a lo largo del año (Stubbington et al. 2017), hasta ahora obviados por la Directiva Marco del Agua. Los puntos de muestreo pertenecían no solo a las jurisdicciones de ACA y CHE, sino que también contenían puntos de la demarcación de la Confederación Hidrográfica del Júcar (CHJ).

Las diatomeas bentónicas se estudiaron no solo en su faceta taxonómica-ecológica (macro-escala y meso-escala) sino también respecto a rasgos característicos de los taxones por medio de sus formas de vida o sus funciones vitales (micro-escala). Como formas de vida entendemos el tipo de adhesión al sustrato y a la adherencia entre células clónicas. Los tipos de formas vitales se presentan en la tabla 9.1. Las funciones vitales se derivan de las "ecological guilds" descritas por Passy (2007a). Estas distribuyen las diatomeas en estratos del perifiton situadas a diferentes alturas. Por ello son influenciadas según dos gradientes: la tolerancia física y la tolerancia química. También estas se describen en la tabla 9.1. Las diatomeas móviles no sufren estas presiones ya que solo se localizan en puntos en los que las eluden y pueden optimizar sus microhábitats por medio de su locomoción. Finalmente, el estrato planctónico se añadió en el trabajo de Rimet y Bouchez (2012). Ahí razonaron que las especies típicamente planctónicas que se pueden observar entre estratos bentónicos sólo podían provenir de comunidades planctónicas previas que sedimentaron por acción de peso o muerte de la célula.

Aparte de las formas vitales, con el auge de metodología informática de medida e identificación de taxones de diatomeas, el estudio morfométrico de las diatomeas es un fenómeno que está empezando a generarse (Kloster et al. 2017; Spaulding et al. 2012; Bueno et al. 2017; Pedraza et al. 2017). En la observación de la calidad del agua y la taxonomía estas herramientas aún no han sido usadas en demasía. Por ello el uso de las muestras del Ebro podían aportar no solo conocimiento respecto a datos fisicoquímicos, sino también en el caso de la distribución de morfometría a lo largo del tiempo.

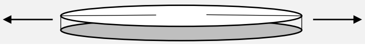
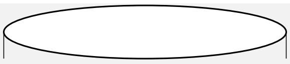
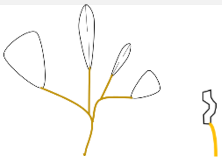
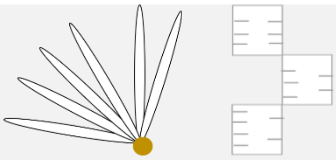
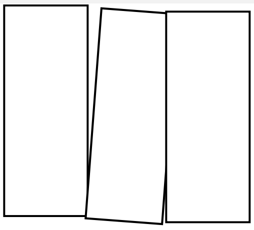
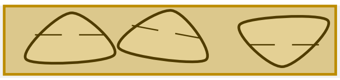
9.2 Material y métodos

Todos los muestreos de diatomeas siguieron el mismo procedimiento. Diversos técnicos muestrearon las diatomeas siguiendo los estándares legales (AENOR 2004, 2014) raspando piedras de más de 20 x 20 cm de área de colonización sin presencia de macrófitos con cepillos de dientes o un cuchillo. Las muestras se fijaron añadiendo formaldehido 4 % o etanol absoluto. El procedimiento de quemado de las diatomeas se hizo con peróxido de hidrógeno 33 % a 110ºC durante 12h seguido del añadido ácido clorhídrico (Cambra, Ector, and Sabater 2005). Estos procesos se siguieron para eliminar materia orgánica y excesos de carbonato cálcico, respectivamente. Posteriormente se pasó a hacer 3 lavados con agua destilada para eliminar los restos abrasivos. La muestra se diluyó para preparar los cubreobjetos que se pusieron a secar en un lugar a resguardo del polvo. Al día siguiente, las muestras se montaron sobre portaobjetos con una gota de la resina Naphrax®.

Las muestras se observaron en un microscopio Zeiss Jenaval con contraste de fases a 100x (menos en el caso de las muestras fotografiadas para el capítulo 6.3, Microescala III), objetivo GF Planochromat PhV HI 100x/ 1,30 ∞/0.17-A. Los primeros años, hasta el 2003, se contaron un mínimo de 300 valvas. En años posteriores se contó un mínimo de 400 valvas. En el experimento del Llobregat se contó un mínimo de 800 valvas. Las diatomeas se identificaron usando bibliografía especializada de diatomeas bentónicas (Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Hofmann et al. 2011; Bey and Ector 2013) o específicas según necesidad, p. ej. (Krammer 2002; Williams 1985).

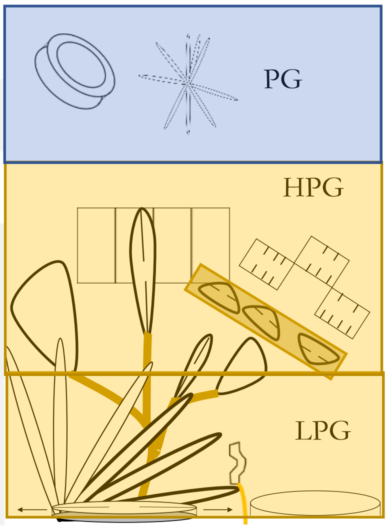
Las formas de vida de las diatomeas se extrajeron usando la base de datos generada por Rimet y Bouchez (2012) y modificada por Riato y colaboradores (2017). Esta modificación dividía las formas de vida en móviles, cualquier movimiento a lo largo de la columna de agua, y pioneras, las primeras colonizadoras. En cuanto a las formas de adhesión o gregarismo, las subdivisiones fueron los tubos mucilaginosos, tubos en los que las diatomeas se mueven, pedúnculos que se adhieren al substrato, formas adnatas, que se adhieren al substrato por la valva, y la adhesión de colonias, por la valva o por los polos (para más información ver Tabla 9.1).

Tabla 9. 1 Descripción de las formas de vida de Rimet y Bouchez (2012) modificadas por Riato y colaboradores (2017). Más información sobre los taxones que los conforman en AnnexTable 19.

Formas De Vida	**Definición**	**Graphic Representation (ABC)**
Móvil	Movimiento rápido o lento a lo largo de la columna de agua.	
Pioneras	Primeras colonizadoras después de una perturbación, diatomeas de estrategia r.	
Adnata	Adhesión al sustrato por la superficie valvar.	
Pedunculada	Adhesión al sustrato por medio de una cantidad variable del mucílago (cojinete, tallo o colonia arbuscular).	
Adhesión Polar / Colonia En Roseta	Colonias adheridas por los polos. Puede incluir rosetas, estrellas y formas en zigzag.	
Adhesión Valvar / Colonia En Cinta	Colonias adheridas por la superficie valvar. Puede incluir Cintas y formas en filamentos.	
Túbulo Mucilaginoso	Diatomeas dentro de túbulo mucilaginoso en el que se pueden mover.	

Se le agregó la división de Passy (2007a) modificada por Rimet y Bouchez (2012) en grupos funcionales de perfil bajo, diatomeas de poco volumen que soportan el estrés mecánico pero no el trófico, el perfil alto, de tamaño grande, sensibles al estrés hídrico y tolerantes a grandes gradientes tróficos. Las diatomeas de grupo funcional móvil son las que muestran una movilidad horizontal reducida. Las planctónicas se encuentran entre los grupos funcionales, porque Rimet y Bouchez (2012) argumentaron que diatomeas de zonas de régimen léntico podrían sedimentar en zonas lóticas y ser muestreadas a la par con las especies generalmente bentónicas. Este subgrupo fue comprobado y corroborado como una adición útil por B-Béres et al. (2017). En el caso de la microescala experimental del río Llobregat, previamente al tratamiento de diatomeas, una parte de la muestra se observó bajo el microscopio sin tratar previamente para comprobar las formas de vida en las que se encontraban los taxones mayoritarios.

Table 21 Grupos funcionales (Ecological Guilds) según la descripción de Passy (2007a)

Formas De Vida	**Definición**	**Graphic Representation (ABC)**
		GRAPHIC REPRESENTATION (ABC)
Fracción De Perfil Alto	Diatomeas altas que no aguantan estrés mecánico, pero soportan altas concentraciones de nutrientes.	
Fracción De Perfil Bajo	Low ranging diatoms that can survive high mechanical stress.	
Fracción Móvil	Diatomeas con velocidad de movimiento lateral variable.	
Fracción Planctónica	Diatomeas del plancton que sedimentan.	

9.2.1 Macro-escala: La cuenca del Ebro

En esta parte de la tesis se ha intentado tipificar las comunidades de diatomeas encontradas en los puntos de la cuenca del Ebro muestreados al menos 7 veces. Esto dejó un total de 50 puntos con 356 muestras.

La clasificación oficial de ecorregiones o ecotipos fluviales, ya mencionada, fue creada usando datos fisicoquímicos y geológicos (CEDEX 2004). En estudios anteriores ya se había establecido una desconexión entre las agrupaciones teóricas y las reales. Los datos fisicoquímicos se obtuvieron desde la base de datos pública del Ebro (Confederación Hidrográfica del Ebro 2015), y se usaron concentraciones de nutrientes (nitratos, fosfatos, silicatos, amonio), datos de salinidad (conductividad), respecto a la química del agua (pH, alcalinidad, oxígeno disuelto) y físicos del agua (temperatura del agua y sólidos en suspensión).

Para el análisis se usaron herramientas estadísticas como los mapas auto-organizadores (SOM), el análisis multivariante o la aproximación cladística. Los estudios estadísticos que se hicieron fueron mayoritariamente jerárquicos (clúster) sobre físico-química, las comunidades de diatomeas y la distribución ecotípica oficial.

9.2.2 Meso-escala: La subcuenca del Segre (Cuenca del Ebro)

Las diatomeas del Segre se estudiaron con el fin de conocer el efecto de los usos del suelo. Para ello se seleccionaron 16 puntos y se tomaron 61 muestras. Los usos del suelo que se describieron fueron, acorde a CORINE 2006 (CLC 2006) y la división de Allan (2004): agrícola, secano, pasto, urbano, aguas continentales y boscoso. Los usos del suelo se observaron usando ortofotos extraídas del instituto de cartografía de la Generalitat de Catalunya (ICGC 2014). Desde allí se dibujaron polígonos sobre los usos del suelo reconocibles descritos anteriormente. La herramienta utilizada para ello fue el programa QGIS 2.16 Nødebo (QGIS Development Team 2009).

Inicialmente se generó un gradiente de usos mayoritarios basados en los usos más extendidos: agrícola, pastos y bosques. La proporción de bosques estaba claramente yuxtapuesta a la prevalencia de agricultura. En menor medida, los pastos también mostraban correlación con la altitud. Otros usos del suelo, secano, aguas continentales y usos urbanos eran minoritarios y, por ello, su efecto no se pudo observar de forma más que intuida, con un aumento de especies y datos fisicoquímicos acordes a lo esperado. Los resultados esperados eran:

- **Uso agrícola**: aumenta la polución puntual por medio de nutrientes debido al uso de abonos. Afecta a la erosión del sustrato aumentándolo en caso de cultivos herbáceos y mixto en caso de cultivos arbóreos, ya que la distribución simétrica no permite el correcto retenimiento de substratos intermedios. (Allan 2004)
- **Pastos/Praderas**: puede tener picos de polución de nutrientes por efecto de las deposiciones de ganado que pueda encontrarse en ellas. Por regla general no afectarán significativamente.
- **Bosques naturales**: Puede presentar polución por materia orgánica en caso de sustrato arbóreo caduco. La retención de sustrato contra la erosión es máxima.
- **Urbano**: Puede presentar polución por nutrientes, sustancias prioritarias, materia orgánica y metales pesados. Retención de sedimentos nula por pavimentación. Contiene: Industrial: Similar al uso urbano, con un mayor peso de las sustancias prioritarias y metales pesados. De nuevo, retención nula de sedimentos.
- **Aguas continentales**: se tienen en cuenta tanto embalses como incremento de la superficie acuática. Afecta a la composición de diatomeas, ya que propicia formas planctónicas.
- **Suelo sin cobertura vegetal**: Erosionable y sin particulares efectos sobre la contaminación del río.

Los datos fisicoquímicos de cada punto de muestreo se obtuvieron de la base de datos pública de la Confederación Hidrográfica del Ebro (2015). Los datos fisicoquímicos observados fueron los mismos que en el capítulo de macro-escala. Se incluye una modelización de la estructura ecológica.

9.2.3 Microescala

9.2.3.1 Microescala I: Grupos funcionales de diatomeas en el Llobregat

A modo de experimento se planteó un intercambio de comunidades de diatomeas para observar los cambios que se dan en el fenotipo de las formas de vida, en vistas a posibles afectaciones por efectos antrópicos. Para ello, se seleccionaron tres localidades, una de alta salinidad natural, la Riera Salada cerca de Saldes, otra bajo la influencia de fuerte actividad agrícola en la Riera Clarà cerca de Casserres y, finalmente, con niveles bajos de salinidad y eutrofia, el caso de las aguas altas del Llobregat. El procedimiento seguido fue el siguiente: se preparó una baldosa grande con nueve baldosines de travertino de al menos 20 cm^2 para cada localidad. Estas se sumergieron el 2 de agosto de 2016 en cada uno de los puntos seleccionados a la par de obtener las comunidades de diatomeas presentes en el sustrato natural. Tras tres semanas se subdividieron y tres baldosines se dejaron en la localidad y los seis restantes se dividieron y la mitad se sumergió en cada uno de los otros puntos (Figura 45). También en este caso se tomaron muestras del sustrato natural.

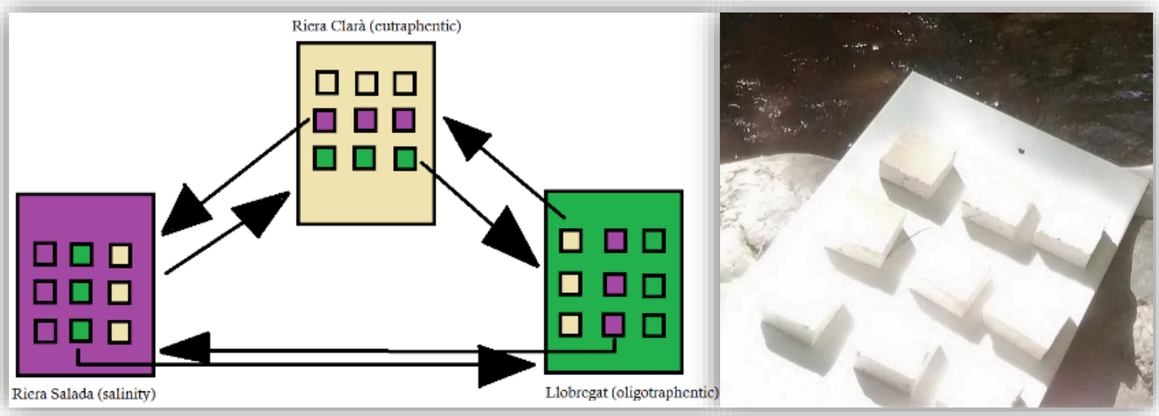


Figura 45 Diseño experimental de los baldosines intercambiados.

Tras un mes de colonización, los baldosines fueron recuperados el 24 de septiembre de 2016. En la localidad eutrófica se habían perdido todos y se procedió a obtener las comunidades desde sustratos naturales (piedras del tamaño adecuado sin colonización por macrófitos). Las muestras se observaron tanto en “vivo”, fijadas con alcohol, como tratadas. Se utilizaron los resultados para comparar la efectividad de formas de vida respecto a los grupos funcionales divididos por tamaños, comparando entre la modalidad usada por Riato y colaboradores en aguas temporales (Riato et al. 2017) y la que se desarrolló para los ríos húngaros (B-Béres et al. 2017). Los datos fisicoquímicos se obtuvieron “in situ” y se comprobaron con los datos de la Agencia Catalana del Agua (ACA 2014).

9.3.2.2 Microescala II: Formas de vida de las diatomeas en los ríos temporales

El clima Mediterráneo se caracteriza por un periodo de estiaje seco e inviernos húmedos. Es por ello por lo que se producen altas fluctuaciones en el caudal y eso permite la predominancia de ríos y masas de agua temporales que se pueden considerar intermitentes o efímeras.

La definición de ríos intermitentes se basa en la pérdida total o parcial de agua superficial durante una parte del año (Stubbington et al. 2017). Los ríos se dividieron según su temporalidad interanual, dividiéndolos en ríos perennes, pozas intermitentes y ríos intermitentemente secos(Gallart et al. 2016). En el caso de los ríos efímeros, la excepción es la existencia de agua superficial. Existe una subdivisión de los caudales de agua (Gallart et al. 2012) que definen la conectividad y van ligados a diversas problemáticas. Así pues, el estado acuático hiperreico es el caudal excesivo, las inundaciones, con los efectos económicos (nocivos) y ecológicos (benignos) que conllevan. El estado eurreico es el caudal (ecológico) estándar. Oligorreico define el estado en el que pozas aisladas están conectadas por un reguero de agua de bajo volumen. El estado arreico se caracteriza por la desconexión de dichas pozas. Finalmente, los estados típicamente efímeros, son el hiporreico, caracterizado por una alta saturación del sedimento, y el edáfico, de menor humedad.

El problema principal de estas masas de agua es que, debido al desconocimiento de las repercusiones ecológicas de la variación de caudales, hasta ahora no habían sido legisladas, por mucho que sean usadas para consumo agrícola y urbano. Para contrarrestarlo se presentó en el año 2014 el proyecto LIFE TRivers, cuyo objetivo principal es permitir la regularización de las aguas temporales dentro de la Directiva Marco del Agua (DMA).

Para hacerlo, en el año 2015 se tomaron muestras de físico-química, macrófitos, peces, macroinvertebrados y diatomeas bentónicas en 5 muestreos de los 24 puntos seleccionados. Se muestrearon en meses alternos para comprobar la reacción de cada uno de estos parámetros ante el cambio de estado acuático.

En el marco de este estudio se ha partido de la comparabilidad, con lo que todas las muestras se tomaron usando el protocolo adaptado de ríos, para las diatomeas bentónicas. Se tomaron un total de 111 muestras de 24 puntos de muestreo repartidos entre las cuencas catalanas, cuencas hidrográficas del Ebro y las del Júcar.

El objetivo principal era observar si las formas de vida de las diatomeas eran más influenciadas por los estados acuáticos, los regímenes del río (perenne, pozas intermitentes y sequías intermitentes) o una conjugación de ambos.

9.2.3.3 Microescala III: Morfometría de diatomeas y gradientes ecológicos

Varios estudios han revelado la correlación inversa entre el tamaño valvar de las diatomeas y la temperatura. Hasta ahora, en investigaciones que trataban de correlacionar aspectos morfométricos de las diatomeas con gradientes de salinidad no han tenido éxito (Snoeijs, Busse, and Potapova 2002).

Pero, hasta el momento, no se han estudiado las tendencias a lo largo del tiempo ni en localidades intrínsecamente diferentes. Por ello, hemos querido usar parte de nuestra colección de diatomeas para caracterizar las comunidades del río Ebro.

Para ello, partimos de 48 muestras del río Ebro, seleccionadas aleatoriamente de las muestras tomadas en 10 años durante el periodo de 2003 a 2013 (Tabla 20). Estas muestras se fotografiaron automáticamente por medio de un microscopio óptico de barrido automático. Las fotografías luego se midieron usando el programa informático creado por el grupo de investigación del instituto de estudios polares y marinos Alfred Wegener de Bremerhaven, en el que se hizo la estancia trimestral. El programa usado es ShERPA (Kloster, Kauer, and Beszteri 2014). Con la ayuda de este software se reconocieron formas de diatomeas y se midieron los tamaños de las especies en cada una de las muestras.

La metodología de captación de datos se basa en una modificación del protocolo creado por Kloster y colaboradores (Kloster et al. 2017). Primero, se escanearon los portaobjetos automáticamente utilizando sistema Metafer 10.3.4 a 63 aumentos con aceite de inmersión con un microscopio acoplado (Zeiss AxioImager.Z2). Las imágenes apiladas de los campos de visión individuales pasan a ser analizadas con ShERPA. Es este programa el que mide los parámetros de largo, ancho, relación de largo/ancho, superficie y contorno de cada valva. El programa añade también la posible identificación de taxón, que no siempre será acertada, pero habrá sido calculada por medio de algoritmos de similitud. El programa basa sus identificaciones en la existencia de patrones identificados de la morfología externa de la valva, que es comparada con la morfología encontrada en la muestra. Un análisis Fourier (Rosenfeld and Thurston 1971) compara los patrones con la imagen tomada, y ordena las imágenes según un gradiente de parecido, dependiendo de hasta 5 métodos de segmentación.

Hemos estudiado también parámetros que se consideran más importantes por su correlación con el medio ambiente como la superficie del frústulo, su volumen celular y la relación de ambos. Para calcularlos, tomamos las fotografías pleurales identificables por especie y calculamos la media (Snoeijs, Busse, and Potapova 2002). A través de la media y las medidas automáticas, se calcularon las morfometrías de las especies usando las fórmulas establecidas en estudios de fitoplancton previos (Hillebrand et al. 1999; Sun and Liu 2003).

De nuevo usamos los datos fisicoquímicos de la confederación hidrográfica del Ebro (Confederación del Ebro 2015) para compararlo a los datos morfológicos. Se utilizaron datos de características relacionadas previamente con morfometría. Es el caso de los nutrientes (fosfatos, fósforo y nitrógeno totales, nitratos, relación N:P, sílice), conductividad, pH, temperatura del agua y sólidos en suspensión. Añadimos además los parámetros que podrían afectar a las diatomeas, como el caudal, el amonio, oxígeno disuelto.

9.3 Resultados

9.3.1 Macro-escala: La cuenca del Ebro

En este estudio hemos corroborado que las comunidades de diatomeas no se han distribuido acorde a la subdivisión oficial, creada a partir de datos geológicos y fisicoquímicos. Pero sí se ha observado que hay una cierta relación de “comunidades típicas” dependiendo de rasgos de la físico-química general. Tanto la clasificación físico-química como la diatomológica han dado resultados similares y de buena definición.

Para que los resultados fueran comparables, se establecieron 8 grupos de diatomeas para el cálculo de la la distribución SOM (self-organizing maps, mapas de autoorganización). De estas 8 agrupaciones, 5 eran ocupadas por una sola especie indicadora (método IndVal), aunque la definición, que en gran medida coincidía con las comunidades integradoras de las agrupaciones, sí se mantenía. Así pues, las comunidades resultantes se podrían definir por sus tolerancias físico-químicas. El grupo I estaba caracterizado por especies mayoritariamente alpinas. El grupo II estaba formado por puntos localizados en alturas subalpinas y alteradas. El tercer grupo se caracterizaba por ser circumneutral, silicatado y alpino. El último punto alpino era silicatado y alcalino. En la caracterización de los puntos de menor altitud, se encontraron diatomeas de plano bajo alcalino, el de baja concentración de nutrientes, el de alta concentración de nutrientes y los puntos de alta conductividad. Las diatomeas predominantes variaban a lo largo de los 8 grupos, como se puede observar en la imagen resumen Figura 18.

9.3.2 Meso-escala: La subcuenca del Segre (Cuenca del Ebro)

Los resultados parecen indicar que las diatomeas sí tienen una relación clara con los usos de suelo circundantes. Los usos más influyentes fueron los usos agrarios, boscosos y prados, ya que a su vez eran los que ocupaban una mayor extensión. Los embalses también afectaron a la flora. Dentro de la subcuenca se caracterizaron 3 comunidades particulares, dependiendo de la proporción de usos del suelo. Las diatomeas de puntos de mayor proporción de agricultura eran diatomeas tolerantes a la concentración alta de nutrientes (*Planothidium dubium* (Grunow) Round & Bukhtiyarova y *Navicula cryptotenella* Lange-Bertalot). En el caso de un recubrimiento predominante de usos no alterados (boscosos y en forma de prado), las especies tendían a ser mayoritariamente mesotróficas (*Cocconeis pseudolineata* Ehrenberg, *Diatoma ehrenbergii* Kützing, *Gomphonema tergestinum* (Grunow) Schmidt. El resultado final se puede observar en la Figure 26.

9.3.3 Microescala

9.3.3.1 Microescala I: Grupos funcionales de diatomeas en el Llobregat

Después del tratamiento de las muestras, las comunidades de las diferentes localidades demostraron ser altamente diversificadas. En el caso de las formas vitales, el cambio por fecha de extracción demostró ser menor que el cambio por físico-química del agua. Las formas de vida demostraron reflejar mejor los puntos de muestreo, mientras que los grupos funcionales subdivididos por tamaños permitían la delimitación de características físico-químicas.

El agua de la riera Salada produjo una prevalencia de diatomeas de adhesión polar y pedunculadas. Las diatomeas de la riera eutrófica eran mayoritariamente móviles pequeñas y medianas, y tenían las máximas proporciones medidas de especies planctónicas, debido a que por la amplitud del cauce aumentaban las balsas de baja velocidad. El río Llobregat, por otro lado, estaba caracterizado por unas formas de vida adnatas y adhesión valvar.

Tras el cambio, las formas pioneras colonizaban los baldosines insertados en la riera salada. Los travertinos originarios de la riera Clarà mantuvieron una proporción alta de formas móviles, de igual manera que los taxones se habían mantenido en gran medida. Las formas de diatomeas oligotróficas se perdieron en la localidad salina. La localización en oligotrofia propició el aumento de diatomeas de perfil bajo, sobre todo de tamaño pequeño. Una imagen resumen se puede ver en Figure 35.

9.3.3.2 Microescala II: Formas de vida de las diatomeas y los ríos temporales

Los resultados demostraron que los régimenes se correspondían mejor con las formas de vida que los estados acuáticos y la combinación de ambos. Después se pasó a la caracterización de estos utilizando diferenciación ANOSIM y el cálculo multidimensional NMDS. Los tipos fluviales se definían predominantemente por parámetros fisicoquímicos como el sodio, la conductividad, el cloro y el carbonato.

Los tipos fluviales se podían identificar por medio de los grupos funcionales. Así pues, los ríos intermitentemente secos (ID) tenían una correlación significativa con las diatomeas móviles, cosa que se puede relacionar con la existencia de diatomeas sub-aéreas. Por otro lado, los puntos localizados

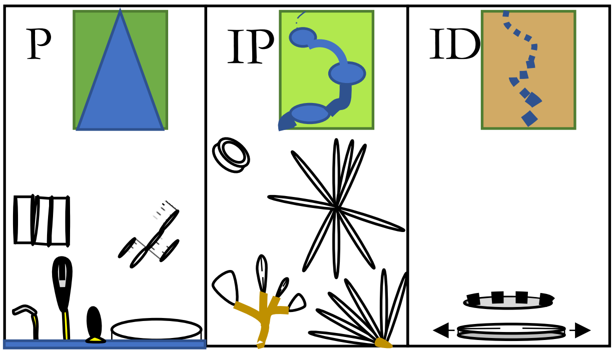


Figura 46 Imagen resumen de formas de vida que se encuentran en ríos perennes (P), pozas intermitentes (IP) y ríos intermitentemente secos (ID).

en las masas de agua de pozas intermitentes estaban relacionados con las diatomeas de perfil alto y las diatomeas planctónicas (Figura 47).

9.3.3.3 Microescala III: Morfometría de diatomeas y gradientes ecológicos

Nuestros resultados en la comparación morfométrica según los puntos de muestreo demostraron ser diferenciales (estudios no paramétricos como Kruskal Wallis y tests Dunn postHoc). El efecto más llamativo fue la correlación clara que existe entre la físico-química y la anchura transapical además de la ya esperada relación superficie volumen (S/V). Tanto anchura como S/V media seguían distribuciones similares a lo largo del tiempo si el sustrato de los puntos era parecido. La distribución de proporciones de anchura también permitía corroborar estado trófico, ya que la restrictiva oligotrofia aumentaba la proporción de células de una medida, en este caso pequeña, relacionada con la predominancia de especies de *Achnanthidium* Kützing. Cuanto más eutrófico el ambiente, mayor la homogeneidad dentro de la distribución de proporciones de tamaño. Las regresiones lineales sobre los valores log-normales señalaban claras diferenciaciones tanto en tiempo como en espacio (AnnexFig. 12-19), aunque los residuales señalaban posibles sesgos y heteroscedasticidades. En algunos taxones suficientemente amplios, como en el caso del complejo de *Achnanthidium minutissimum* (Kützing) Czarnecki, se observan tendencias similares a las que se pueden ver en la totalidad de las comunidades.

Aparte de esto, el biovolumen calculado de los taxones se comparó con la división hecha por Rimet y Bouchez. En el caso de *Gomphonema* Ehrenberg se constató una reducción de volumen respecto a lo establecido. Por otro lado, géneros como *Encyonopsis* Krammer, *Navicula* Bory de Saint-Vincent y *Nitzschia* Hassal variaban de manera aleatoria.

9.4 Discusión

Esta tesis se planteó como una observación multiescalar de cómo las diatomeas se distribuyen y son seleccionadas en el medio fluvial. Para ello usamos no tan solo la extensiva colección de muestras recogida en el marco del establecimiento de la red de inter-calibración básica y de referencia de diatomeas (Cambra and Ortíz-Lerín 2005, 2006; Flor-Arnau and Cambra 2007; Flor-Arnau et al. 2008; Cambra, Flor-Arnau, and Burfeid Castellanos 2012), sino que se añadieron muestras experimentales tomadas en la cuenca del Llobregat y las muestras pertenecientes al proyecto LIFE TRivers. Una ventaja crucial de los capítulos y secciones relacionadas con el Ebro es la observación de comunidades de diatomeas a través del tiempo. Esto permite vislumbrar patrones comunes y acercarnos a la comprensión de la biología de las diatomeas, al menos en este tipo de ríos. Esta forma de estudio es más conocida para investigaciones de plancton marino y sedimento (Reid et al. 2007; Wasell and Håkansson 1992; De Laender et al. 2012), estudios de sustancias peligrosas (Morin et al. 2009; Rimet 2012), o los bioindicadores (Virtanen and Soininen 2016). Los estudios de muestras vivas y aguas continentales comprendían de 2 a 5 años, los marinos y sedimentos podían contener desde 20 a miles de años.

Los diferentes capítulos han aportado diversos conocimientos para cada una de las escalas estudiadas. Así pues, la macro-escala ha permitido desarrollar una división en “diato-ecorregiones” equiparable a las que se generaron en Francia o en los ríos catalanes (Tison et al. 2004, 2005; Tornés, Leira, and Sabater 2012; Tornés 2009) añadiendo un prisma temporal a la división de Ortiz-Lerín (2012). Por otro lado, la división físico-química de la cuenca también ha aportado información y se puede correlacionar con otras combinaciones de taxones de diatomeas. El resultado confirma la hipótesis de Kelly y colaboradores que una observación promedio de cada punto de muestreo permite reducir el ruido estadístico generado por taxones minoritarios permitiendo unos resultados de mayor significación (Kelly et al. 1998).

Por otro lado, a una escala menor, el uso del suelo también afecta a la composición del medio acuático y, por tanto, a las diatomeas (Tolkkinen et al. 2016). Los usos del suelo agrícola, boscoso y de prados fueron los que mayor influencia tenían sobre las comunidades de diatomeas. Además de afectar a la calidad del agua. Esto se debía al hecho de ser los usos más mayoritarios de todos los cauces estudiados. La relación de la proporción del uso del suelo antrópico (agrícola, urbano e industrial) y las comunidades bentónicas ya fue marcado en otros estudios (Pan et al. 1996). Una correlación total de los usos del suelo y los valores resultantes de los bioindicadores podría permitir una modelización aproximada de calidad del agua y usos del suelo. En este estudio únicamente mantuvieron la significación los usos de bosque, prado, agrícola y urbano, que son los que más pueden influir en la físico-química del agua.

A continuación, pondremos el foco sobre la menor escala. El caso de la microescala es el más observado en los estudios generales de taxonomía (Lange-Bertalot 1979a; Kalyoncu et al. 2009; Riato et al. 2017; Lainé et al. 2014). Pero los casos de formas vitales y morfometría no han sido estudiados con la misma dedicación.

Las formas vitales y grupos funcionales se han estudiado para humedales temporales (Riato et al. 2017) y ríos generales de Hungría (B-Béres et al. 2014, 2016, 2017). Pero la observación de cambios experimentales no ha sido tan numerosa, y subrayados por el experimento funcional de Lange y colaboradores (2011). Por ello, una investigación de intercambio de sustratos y el estudio de afectación por cambios de régimen de caudal de agua permitieron indagar algo más en definir el significado de estas características.

El experimento del río Llobregat, si bien fue mermado por la pérdida de los baldosines localizados en el punto eutrófico (Riera Clarà), pudo darnos información sobre la relación de las formas vitales y la físico-química. La mayor afinidad que se encontró fue entre la riera Salada y especies de adhesión polar (representadas predominantemente por *Diatoma tenuis* C. Agardh), formas pedunculadas y los grupos funcionales de perfil bajo de menor tamaño (diversas especies de *Achnanthidium* Kützing, altamente correlacionado con la existencia de especies de *Nitzschia inconspicua* Grunow). Todas las especies fueron relacionadas en estudios anteriores con diversos grados de salinidad (Kelly et al. 2014;

Williams 2012; Hindáková 2009). Con una menor significación, las formas de vida que se correlacionan más significativamente con el punto eutrófico punto eran las planctónicas (debido a la existencia de piletas naturales y el pantano de Casserres, que las propicia), cosa que coincide con las expectativas (Rimet and Bouchez 2012). Otros grupos funcionales pertenecían además a los tres tamaños menores de diatomeas móviles y el tamaño 4 de especies planctónicas. Este fenómeno de cambio a especies móviles en aguas eutróficas refleja también el gradiente altitudinal y el régimen de río en los que este tipo de formas suelen prosperar (Sabater et al. 2011). Finalmente, en las aguas oligotróficas las formas más características pertenecen al grupo funcional de perfil alto, incluyendo la división por tamaños. Dentro del periliton, este estrato se ve más afectado por la presión mecánica que la nutricional (Passy 2007a).

La comparación de las formas de vida y los grupos funcionales se llevó a cabo para intentar establecer cuál de las dos estrategias era la más adecuada para la definición de la físico-química de las localidades (Riato et al. 2017; B-Béres et al. 2017). Se escogió este planteamiento, ya que al ser tan diversificados los puntos de muestreo, se podría extrapolar la validez de estos métodos para la identificación en un gradiente de salinidad y de eutrofia. Los resultados señalaron que las formas de vida de Riato y colaboradores eran más adecuadas para definir el punto de muestreo. Todos los resultados de los cálculos multidimensionales mostraron una mayor proporción de variabilidad explicada por las formas de vida. Por el contrario, para definir cada parámetro fisicoquímico, los tamaños de los grupos funcionales pueden tener un mayor impacto, definiendo del 42% a 63%, contra los 36% a 55% de las formas de vida.

En los ríos temporales, se intentó observar si cambiaban según el estado de caudal de agua. Usamos el planteamiento Riato y colaboradores, para poder compararlo debido a su pertenencia a las masas de agua temporales. Los resultados han mostrado que la subdivisión de río en perenne, secos intermitentes y pozas intermitentes era un mejor separador de las formas que los estados del agua (arreico, eurreico, oligorreico, edáfico) o combinación de ambos. Los grupos funcionales estaban más significativamente correlacionados con la composición físico-química de los puntos. Las especies móviles se correlacionan proporcionalmente con los valores de salinidad, conductividad y el carbón orgánico total, mientras los taxones planctónicos invertían esta relación.

En el caso de las comunidades de diatomeas, la desconexión que sucede en ríos temporales provoca un claro perjuicio ya únicamente para su supervivencia y el muestreo. La metodología de muestreo de diatomeas bentónicas estándar, recogida en Kelly y colaboradores (1998), requiere un flujo y corriente de agua para el establecimiento de la calidad del agua. Así pues, la pérdida de flujo dada a partir del estado arreico dificulta dos características principales: que las piedras de muestreo no se vean colonizadas por macrófitos, que alteran el resultado debido a la aparición de diatomeas epífitas, y que evitan la renovación del agua, con lo que la calidad del agua medida será, únicamente, la de la poza. Pasa pues de un sistema lotico a un sistema lentico. Estos sistemas están regulados de forma diferente en la DMA. Los sistemas lóticos están estandarizados a favor de las diatomeas bentónicas, mientras que los sistemas lacustres serán diagnosticados a partir del fitoplancton. El estado edáfico, por mucho que actualmente no esté regulado, podría ser muestreado usando el protocolo creado por Antonelli et al. (2017) para la obtención de diatomeas sedimentarias.

La morfología ha sido estudiada en diferentes maneras: largo celular, relación superficie/volumen, biovolumen (Joux-Arab, Berthet, and Robert 2000; Snoeijs, Busse, and Potapova 2002; Reavie et al. 2010)… Pero la anchura de la valva hasta ahora no se había tenido en cuenta, probablemente debido a los estudios experimentales en los que se determinaba que la anchura se mantenía bastante estable desde la célula inicial (Cox, Willis, and Bentley 2012; Mann 1984). Esto puede relacionarse con la importancia del sustrato, que en el caso de ser cultivos se mantiene estático y no sufre las alteraciones que una masa de agua natural puede sufrir.

Nuestra hipótesis es que el crecimiento lateral secundario (Round et al. 1990; Volcani 1978) permite la variabilidad de anchura, que será seleccionada por el medio. La anchura valvar también se modifica en especies colonizadoras como *Achnanthidium minutissimum* (Kützing) Czarnecki, debido a su característica necesidad de colonizar todo tipo de ambientes (Rimet et al. 2009), por lo que se ven afectadas por la selección natural. Ya se esperaba la correlación físico-química con la relación superficie-volumen, dado al aumento la necesidad de la célula de captar nutrientes en un ambiente limitante (Roselli and Basset 2015). Las demás morfometrías también demostraron alguna correlación con la composición del agua, pero en un grado menor. Las áreas valvares estaban relacionadas con nutrientes, cosa que coincide con estudios anteriores (Finkel et al. 2009). La longitud apical sólo estaba relacionada con fosfatos y oxígeno disuelto. Estudios anteriores habían establecido que la longitud está afectada por sustancias peligrosas (European Parliament and Council of the European Union 2006) y los metales pesados. No hemos podido corroborarlo debido a no tener datos cuantitativos al respecto para todos los puntos de muestreo. Superficie y volumen obtuvieron valores reducidos. La superficie se relacionaba con fosfatos y caudal, ya que ambos están relacionados entre sí. Las cabeceras de ríos suelen presentar niveles de fosfatos bajos y caudales altos (Rimet et al. 2007; Viviroli and Weingartner 2004), por lo que la relación S/V deberá ser menor para captar los nutrientes (Roselli and Basset 2015; Law et al. 2014). Por ello, la superficie debería aumentar. Nuestros resultados muestran que, además de la relación superficie/volumen, que necesita la generación de un protocolo estándar para su establecimiento, la anchura valvar es un importante parámetro relacionado con la físico-química del punto de muestreo. Parece haber también una alta afinidad con el sustrato en el que se encuentra, cosa que puede depender de la concentración de sílice disponible para las células. La variabilidad de tamaños dentro de los géneros puede estar relacionada a que son taxones con amplias tolerancias (Elias et al. 2012; Round et al. 1990), que por tanto podrían ser modificados por la variabilidad del medio.

Así pues, la taxonomía, grupos funcionales y morfometrías de las diatomeas hasta ahora no se habían investigado en un trabajo conjunto. Las formas vitales parecen ser una forma fácil de obtener información sobre la composición de aguas que habitan (B-Béres et al. 2016; Passy and Larson 2011) y la ecología en este hábitat (Lange et al. 2011). Por otro lado, la morfometría también ha sido relacionada con los aspectos composicionales del agua (Olenici et al. 2017; Reavie et al. 2010; Busse 2002), aunque no sean suficientes para la identificación de taxones específicos (Blanco, Borrego-Ramos, and Olenici 2017). La taxonomía es la herramienta históricamente más usada para la bioindicación. Se está observando un cambio a otros métodos, que incluyen el metabarcoding (Zimmermann et al. 2014; Zimmermann 2014). Aun así, la biogeografía taxonómica puede ser una fuente de interesantes aspectos ecológicos concernientes a las comunidades de diatomeas (Mann and Vanormelingen 2013; Williams and Reid 2006; Potapova and Charles 2002; Rimet 2009). Más aún, ya que hasta el momento se han observado las características abióticas y no las relaciones bióticas que pueden existir dentro de los ecosistemas.

En resumen, esta tesis ha procurado proveer una observación de las reacciones taxonómicas, funcionales y morfométricas de las diatomeas. Creemos que los resultados subrayan la importancia potencial de los grupos funcionales y morfometría de diatomeas en el análisis de la calidad de agua. Además de ello, se adivinan indicios de la complejidad de las relaciones ecológicas que envuelven a las diatomeas en su entorno.

9.5 Conclusiones

1. La aplicación del método clúster de similitud al estudio de la físico-química del agua en la cuenca del Ebro puso de manifiesto una división altitudinal (montaña-eje principal del río): El clúster de montaña se subdividió según el sustrato. El clúster del eje principal del río se subdividió según la composición físico-química del agua.

2. Las comunidades de diatomeas no se agruparon siguiendo los ecotipos fluviales establecidos en base a los criterios de la DMA.

3. Los métodos clúster de similitud ofrecen resultados más fiables de las comunidades de diatomeas que los mapas SOM, ya que estos se prestan a subjetividades, pues con el fin de aumentar su comparabilidad se puede llegar a forzar un número de comunidades determinado. Este método, no obstante, ofrece correlaciones significativas con la composición físico-química del agua y es altamente visual en la descripción de una cuenca fluvial, por lo que es recomendable su uso.

4. A lo largo del tiempo estudiado (entre 7 y 10 años, según el caso) no se observó una variación anual estadísticamente relevante de las comunidades de diatomeas. La comunidad promedio de un punto de muestreo contribuyó a aumentar la significación del estudio de las comunidades principales y redujo la relevancia de las especies secundarias.

5. Al combinar los resultados de las comunidades de diatomeas agrupadas según las variables físico-químicas (PCA y SOM, respectivamente), el río Ebro se pudo clasificar en 6 ecotipos alternativos: 1) Alta montaña, 2) Eje principal con conductividad elevada, 3) Eje principal eutrófico, 4) Eje principal con temperatura elevada, 5) Media montaña con elevada concentración de silicatos y 6) Alta montaña con concentración elevada de silicatos.

6. Las comunidades de diatomeas se vieron afectadas tanto por los usos del suelo como por los impactos de estos en las variables físico-químicas del agua.

7. Dada la correspondencia entre la proporción de los diferentes usos del suelo y los tipos de diatomeas, se encontraron tres comunidades mayoritarias de estas, según predominaran los usos agrícolas, bosques y pastos.

8. El índice trófico de diatomeas IBD refleja los principales usos del suelo reconocidos en la cuenca del río Segre: bosques, pastos, agricultura y usos urbanos.

9. En un sentido ecológico-estructural, la composición físico-química está directamente correlacionada con los usos del suelo e inversamente correlacionada con los valores de los índices diatomológicos de calidad del agua, tanto a nivel de biodiversidad de diatomeas, como ecológicos.

10. Las comunidades de diatomeas y sus características de la morfología celular están estrechamente relacionadas con la composición físico-química del agua.

11. La forma en las que las diatomeas se adhieren entre sí o al sustrato (formas de vida) sirvió para identificar rasgos distintivos de los puntos de muestreo, dado que manifiesta una combinación de todas las variables físico-químicas del agua.

12. La ubicación de los grupos de diatomeas dentro del perifiton (grupos funcionales o "*ecological guilds*"), también se relaciona con cada parámetro físico-químico y caracteriza de forma más precisa estos parámetros. Por ejemplo, la conductividad se correlaciona significativamente con la fracción de perfil alto y el aumento de concentración de nitrato conlleva un declive de la fracción de perfil bajo, pero incrementa tanto la fracción móvil como la planctónica.

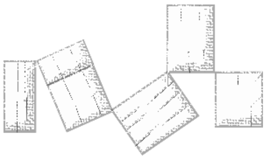
13. La salinidad se relaciona con un aumento de la excreción de mucílago, las condiciones eutróficas favorecen a las diatomeas móviles y las aguas oligotróficas aumentan la cantidad de diatomeas adnatas (diatomeas adheridas al sustrato por su valva).

14. Independientemente de su tamaño, los grupos funcionales demostraron tener más correlación con los parámetros físico-químicos que las formas de vida.

15. Las formas de vida se correlacionan más significativamente con los regímenes hidrológicos que con los estados acuáticos transitorios o con una combinación de ambos.

16. La presencia del grupo de diatomeas planctónicas describe de forma significativa el cambio de régimen fluvial de lótico a léntico (lacustre). La reducción de caudal permite la aparición de especies planctónicas y formas de vida relacionadas con dicha vida planctónica.

17. La composición físico-química afecta a la morfología de las diatomeas, sobre todo incide sobre la anchura valvar (que se reduce en ríos oligotróficos) y también en la relación superficie/volumen (que tiende a aumentar en condiciones de oligotrofia).



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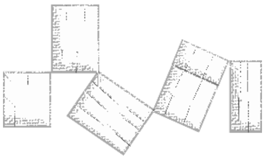

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GLOSSARY

Adnate. Diatoms that firmly attach to a substrate by one valve or girdle, without mucilage. Examples of this would be Cocconeis Ehrenberg and Eunotia Ehrenberg.

Alveolus. Stria formed as an elongated chamber. With external areolae and internal slit.

Apical axis. Longest axis of a pennate diatom that passes through the apices.

Araphid. Pennate diatoms that do not present a raphe. Non-systematic descriptor. Examples: Diatoma Bory, Fragilaria Lyngbye.

Arbuscular colony. Long and branched mucilage stem that houses several diatom cells. E. g. Gomphonema Ehrenberg.

Areolae. Pores of the diatom valve forming a stria.

Auxospore. Resulting cell, usually linked to sexual reproduction. It is a "special cell that expands before producing a frustule" (Spaulding, Lubinski, and Potapova 2010).

Benthos. Organisms found at the bottom sediments of waterbodies. The WFD describes phytobenthos (bottom flora) as an indicator for water quality.

Biraphid. Pennate diatoms that present a raphe on both valves. E. g. Navicula Bory, Nitzschia Hassall.

Centric diatom. Diatoms with radial symmetry. Unofficial descriptor. Usually juxtaposed to Pennate diatoms.

CORINE Land Cover. CoORdination of INformation of the Environment, established by the European Environmental Agency (EEA). A multi-year approach of LANDSAT and SPOT geographical image comparison to observe changes in land uses. Sites are subdivided into four basic groups: artificial soils, agricultural lands, forest soils and water bodies. Further subdivision is explained below.

Costa. Siliceous transapical thickening that increases stability of the valve.

Cribrum. Plate of silica that covers areolae. Only observable under Electronic Microscope. (Type of velum)

Diato-ecoregion. Cartographical representation of the results of a self-organizing map result.

Eutraphentic. Diatoms that are found in nutrient rich (eutrophic) sites.

Filament colony. Centric individuals juxtaposed by their valves, attached either with spines, granules or polysaccharides to create long chains.

Frustule. Diatom silicon oxide case surrounding the whole cell. It is divided into two halves (valves) that can be separated by girdle-bands.

Functional group. Used as a synonym for ecological guilds.

High profile guild. Generally big diatoms that form colonies and are tolerant to nutrient pollution but sensitive to physical disturbances such as turbulences. Sensitivity to pesticide pollution is reported.

Invasive diatom. Alien diatoms that are found to be adapted to a new habitat, as to be able to be more than rare, even producing blooms. Example: *Didymosphenia geminata* (Lyngbye) Schmidt making blooms in oligotrophic waters around Europe.

Land use. Uses of the soil do affect run-off effects and pollution

Lentic system. Lacustrine environments. Aquatic systems with more than one directionality (vertical-horizontal).

Life form. In these studies, this expression is used to portray the relationship of diatoms with their environment. Whether and how they attach to substrate or to each other. Functional groups.

Lotic system. River, headwaters or connected stream with one-directional movement.

Low profile guild. Small colonial diatoms that are tolerant to physical disturbances but sensitive to nutrient pollution.

Macro-scale. Scale of the whole basin, subdivisible into ecotypes/ecoregions

Meso-scale. Scale of a sub-basin, tributary catchment or stream and the influence of its affectation on diatom species and communities.

Micro-scale. Scale of a Microhabitat system.

Monoraphid. Pennate diatoms that present a raphe only on one of their valves. *E. g. Achnanthidium* Kützing, *Cocconeis* Ehrenberg.

Motile guild. Fast moving species that were hypothesized to be found at high turbulence and nutrient pollution levels.

Mucilage. Polysaccharide produced by diatoms due to their excessive effectivity in photosynthesis. Usually fastened by a symbiosis with microorganisms.

Mucilage pad. Adhesion on the valve face through mucilaginous extrusions of the diatom. *E. g. Achnanthidium* Kützing.

Mucilage stalk. A short mucilage stem, usually attached by one apex of the diatom. *E. g. Achnanthidium* Kützing.

Mucus tubule. Diatom colony form that engulfs single cells in a tube of their own extruded mucilage.

Oligotraphentic. Diatoms that are found in sites with low nutrient concentrations (oligotrophic).

Pedunculate. A long mucilage stalk.

Pennate diatom. Unofficial descriptive of diatoms that have bilateral symmetry. Usually juxtaposed to centric diatoms. They can be araphid, monoraphid or biraphid.

Plankton. Organisms floating or drifting in the water column, usually of a lake or a sea.

Planktonic guild. Diatoms from the plankton that settle when dead and can be found in the diatom benthos.

Raphe. Canal located in one or more valves of the diatoms, usually useful for locomotive purposes of the cell.

Ribbon colony. Valve-face adhesion of pennate diatoms. Linkage by spine or mucilage excretions.

Rimoportula. Silicic extrusion into the valve as an extension of the stigma.

Rosette colony. Stalked mucilage adhesion to the substrate, clones adhere to stalk forming fan or rosette. *E. g. Ulnaria* (Kützing) Compère.

Striae. Row of pores or stripes of diatoms, usually pennate. They are important diatom recognition traits, usually counted for 10 µm of cell.

Stellate colony. Adhesion of valves by mucilage or spine-based aggregation of the apices. Example: *Asterionella formosa* Hassall.

Stigma. Small slit located somewhere on valve mantle or valve. Can be extended through a rimoportula.

Stria. Areolae or Alveolae that form thin bands that are focusable under an optic microscope.

Sherpa. Image processing software (SHapE Recognizing, Processing and Analysing software) programmed to recognize diatom forms and to measure each valve for Area, Perimeter, Length and Width.

Teratology. Malformation of diatom cells due to physiological disruptions created by pollutants or disruptions in generation.

Theca. Half of the frustule, including valve and mantle. Forms an approximate petri-dish closure where the lid is the epitheca and the smaller container is called hypotheca.

Transapical axis. Transverse axis, shortest axis of the pennate valve.

Valve. Face of the theca, can have an aperture in form of a raphe and/or a rimoportula.

Valvocopula. Also known as girdle band, is adhered to the valve.

Velum. Silica construction covering the areola. Can be either cribrum or volum.

Virga. Interstria, silicic protrusion located between striae, the last structure before vimins to form when creating the new valve.

Volum. Silicate structure in the form of a projection that covers the areola.

WFD (Water Framework Directive). Directive 2000/60/EC of the European Parliament establishing a framework for Community action in the field of water policy.

Zig-zag colony. Barely attached diatom chains that create a zig zag distribution due to being adhered only by the apices. Example: *Diatoma* Bory.

ACRONYMS

ACA = Agència Catalana de l'Aigua (Catalan water agency)

CHE = Confederación Hidrográfica del Ebro (Ebro river water Authorities)

CHJ = Confederación Hidrográfica del Júcar (Júcar river water Authorities)

CORINE Land Cover. CoORdination of INformation of the Environment

EQR = Ecological Quality Ratio

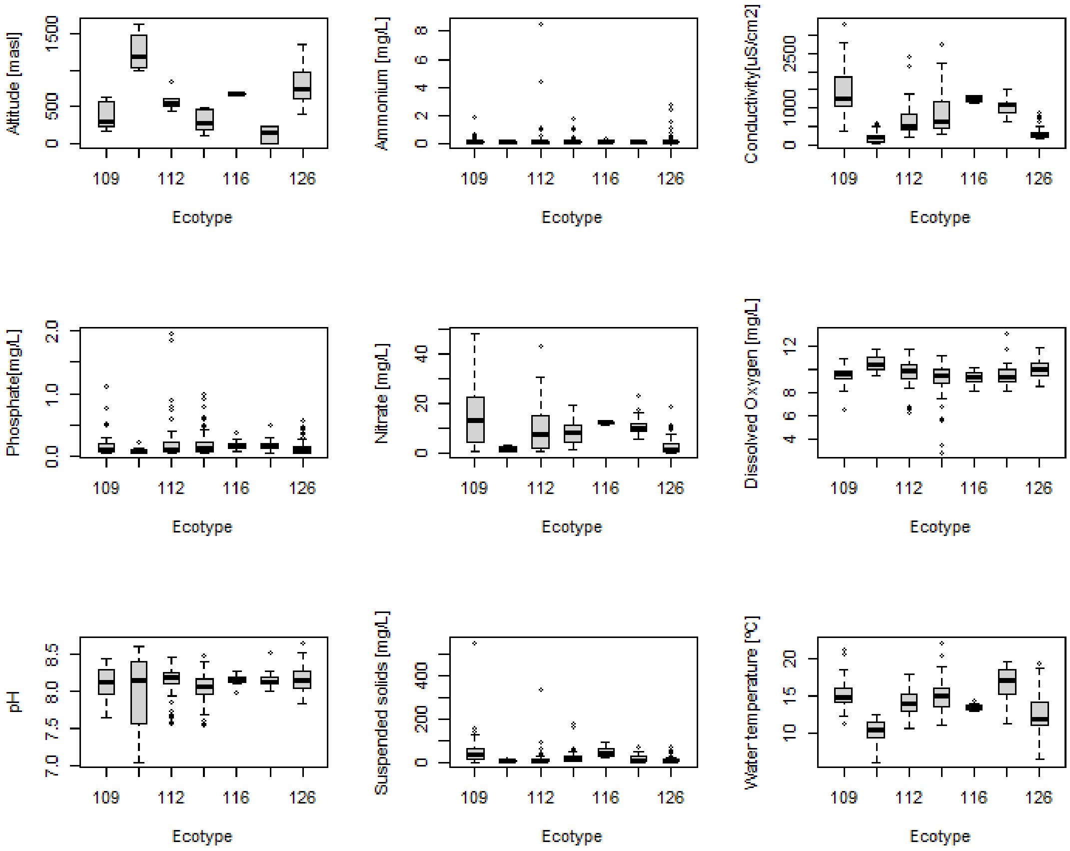
SHERPA = Shape Recognition, Processing and Analysis (software by Kloster et al. 2013)

WFD = Water Framework Directive

ANNEX

ANNEX

Annex chapter 3: materials and methods



AnnexFigure 1 Comparison of water composition at each official ecotype. Significant differences mostly in altitude, conductivity, nitrates and water temperature. The three last characteristics are dependent on altitude. (Chapters 4, 5 and 6.3)

AnnexTable 1 Diatom life forms and ecological guilds described by Passy (2007) and Rimet & Bouchez (2012), after the table of Riato et al (2017) with values from this study. Grey shade = Llobregat/ White = Temporary Rivers and Llobregat.

Functional Groups	**Description**	
Life Forms		
Mobile	Moving free, either in the water column or along the substrate.	*Achnanthes, Achnanthidium, Adlafia, Amphipleura, Amphora, Bacillaria, Brachysira, Caloneis, Cavinula, Chamaepinnularia, Cocconeis, Craticula, Cymbella, Cymbopleura, Delicata, Denticula, Diadesmis, Diploneis, Encyonema, Encyonopsis, Epithemia, Eucocconeis, Eunotia, Fallacia, Frustulia, Geissleria, Gomphocymbella, Gomphonema, Gomphosphenia, Gyrosigma, Halamphora, Hippodonta, Humidophila, Karayevia, Luticola, Mastogloia, Mayamaea, Microcostatus, Navicula, Naviculadicta, Neidiomorpha, Neidium, Nitzschia, Nupella, Parlibellus, Pinnularia, Planothidium, Platessa, Reimeria, Rhopalodia, Rossithidium, Sellaphora, Stauroneis, Surirella, Tryblionella.*
Pioneer	Faster colonizers than other species.	*Achnanthes minuscula, Achnanthidium affine, A. minutissimum var. jackii, A. minutissimum, A. rivulare, A. straubianum, Amphora inariensis, A. pediculus.*
Mucous Tubule	Inhabit a mucilage tube and able to move within.	*Amphipleura pellucida, Berkeleya rutilans, Delicata delicatula, Encyonema caespitosum, E. lange-bertalotii, E. minutum, E. prostratum, E. reichardtii, E. silesiacum, E. ventricosum, E. vulgare, Frustulia crassinerva, F. marginata, F. viridescens, F. vulgaris, Parlibellus crucicula (?)*
Rosette Colony	Attach to the ground via a mucilage stalk and form fan-shaped colonies.	*Diatoma moniliformis, D. moniliformis subsp. ovalis, D. problemática, D. tenuis, D. vulgaris, Meridion circulare, Ulnaria acus, U. bíceps, U. capitata, U. danica, U. ulna.*
Ribbon Colony	Valve face attachment with either spines or mucilage as adhesive.	*Achnanthidium catenatum, Aulacoseira granulata, Diadesmis confervacea, Ellerbeckia arenaria, Eunotioforma genuflexella, Eunotia implicata, E. minor, E. soleirolii, Fragilaria amphicephala, F. austriaca, Fragilaria brevistriata, F. capucina, F. crotonensis, F. famelica, F. gracilis, F. mesolepta, F. pararumpens, F. perminuta, F. radians, F. recapitellata, F. capucina subsp. rumpens, F. capucina var. vaucheriae, Fragilariforma bicapitata, F. virescens, Humidophila contenta, Melosira varians, Punctastriata mimetica, Pseudostaurosira parasitica, Stauroforma cf. exiguiformis, Fragilaria cf. construens, S. venter, Staurosirella oldenburgiana, S. pinnata.*
Pedunculate	Mucilage attachment with upright growth.	*Achnanthes, Achnanthidium, Cymbella, Diatoma, Didymosphenia Encyonopsis, Eucocconeis, Fragilaria, Gomphocymbella, Gomphonema, Gomphosphenia, Karayevia, Meridion, Platessa, Pseudostaurosira, Punctastriata, Reimeria, Rhoicosphenia, Reimeria Rossithidium, Stauroforma, Staurosira, Staurosirella, Tabularia, Ulnaria.*
Adnate	Valve-face attached to the substrate.	*Achnanthidium trinodis, Amphora, Cocconeis, Diploneis, Halamphora, Plonthidium, Platessa, Reimeria, Rhopalodia.*
Pole Attachment	Colonies attached at each pole.	*Diatoma ehrenbergii, D. hiemalis, D. tenuis, D. vulgaris, Ulnaria acus, U. ulna.*
Valve Attachment	Colonies attached at the valve face.	*Fragilaria brevistriata, F. capucina, F. austriaca, F. radians, F. famelica, F. gracilis, F. perminuta, Fragilaria sp., F. recapitellata, F. capucina var. vaucheriae, Melosira varians, Staurosira construens var. exigua, Staurosirella pinnata.*

Ecological Guilds			
High Profile Guild	Species of tall stature (erect, filamentous, branched, chain forming…)		*Achnanthidium catenatum, Amphipleura pellucida, Cymbella, Delicata, Diadesmis, Diatoma, Diploneis elliptica, D. krammeri, Ellerbeckia, Encyonema, Eucocconeis, Eunotia, Fragilaria, Fragilariforma, Frustulia, Gomphocymbella, Gomphonema, Gomphosphenia, Humidophila, Melosira, Parlibellus, Pseudostaurosira, Punctastriata, Stauroforma, Staurosira, Staurosirella, Tabularia, Ulnaria.*
Low Profile Guild	Species of short stature (prostrate, adnate, solitary centrics, slow-moving…)		*Achnanthes, Achnanthidium, Amphora, Brachysira, Cocconeis, Cymbella, Cymbopleura, Diploneis, Encyonopsis, Eucocconeis, Halamphora, Karayevia, Meridion, Planothidium, Platessa, Reimeria, Rhoicosphenia, Rossithidium.*
Motile Guild	Fast-moving species.		*Adlafia, Anomoneis, Bacillaria, Caloneis, Cavinula, Chamaepinnularia, Craticula, Cymatopleura, Denticula, Entomoneis, Epithemia, Fallacia, Fistulifera, Geissleria, Gyrosigma, Hantzschia, Hippodonta, Luticola, Mastogloia, Mayamaea, Microcostatus, Navicula, Naviculadicta, Neidiomorpha, Neidium, Nitzschia, Nupela, Pinnularia, Rhopalodia, Sellaphora, Simonsenia, Stauroneis, Surirella, Tryblionella.*
Planktonic	Solitary or colonial centrics, pennates.		*Actinocyclus, Aulacoseira, Cyclostephanos, Cyclotella, Diatoma tenuis, Fragilaria, Nitzschia acicularis, Ulnaria acus*
High Profile Guild	High reaching diatoms that cannot withstand mechanical stress.	1	*Fragilaria perminuta*
		2	*Berkeleya rutilans, Encyonema minutum, E. reichardtii, E. ventricosum, Fragilaria capucina, F. austriaca, F. radians, F. famelica, Fragilaria sp., F. recapitellata, Fragilaria capucina var. vaucheriae, Gomphonema pumilum var. elegans, G. pumilum, Fragilaria brevistriata, Staurosira exigua, Staurosirella pinnata.*
		3	*Gomphonema exilissimum, G. minutum, G. olivaceum var. calcareum, G. olivaceum, Gomphonema sp., G. parvulum, G. parvulum f. saprophilum.*
		4	*Encyonema caespitosum, E. lange-bertalotii, E. silesiaca, Gomphonema angustatum, G. clavatum, G. italicum, G. tergestinum.*
		5	*Amphipleura pellucida, Brebissonia lanceolata, Cymbella helvetica, C. tumida, Diatoma ehrenbergii, Didymosphenia geminata, Diatoma hyemalis, D. vulgaris, Melosira varians, Ulnaria ulna.*
Low Profile Guild	Low ranging diatoms that can survive high mechanical stress.	1	*Achnanthidium, Cymbella, Encyonopsis*
		2	*Achnanthidium, Brachysira vitrea, Cymbella laevis, Planothidium*

			frequentissimum, P. delicatulum, P. dubium, P. rostratum
		3	*Cymbella excisa, Encyonopsis microcephala, Planothidium hauckianum, P. lanceolatum, Rhoicosphenia abbreviata, Reimeria sinuata, R. uniseriata*
		4	*Halamphora montana, Cymbopleura sp., Cymbopleura sp. 2*
		5	*Amphora copulata, A. hungarica (?), A. minutissima, A. ovalis, Cocconeis placentula var. euglypta, C. euglyptoides, Cocconeis sp. Cymbopleura subaequalis*
Motile Guild	Diatoms with more or less rapid movement.	1	*Adlafia minuscula var. muralis, Fistulifera saprophila, Mayamaea, Niztschia, Navicula, Simonsenia, Sellaphora.*
		2	*Adlafia bryophila, Craticula, Fallacia, Mayamaea atomus, Nitzscia, Navicula*
		3	*Denticula tenuis, Hantzschia amphioxys, Hippodonta capitata, Nitzschia, Navicula, Staurosira.*
		4	*Cymbella lancettula, Craticula molestiformis, Luticola goeppertiana, Navicula, Surirella angustata, S. brebissonii var. kuetzingii, Sellaphora pupula, Tryblionella apiculata*
		5	*Bacillaria paxillifera, Craticula ambigua, C. cuspidata, Cymbopleura elliptica, Cymatopleura solea var. apiculata, C. solea, Gyrosigma attenuatum, G. obtusatum, Navicula, Pinnularia microstauron, Surirella brebissonii, S. linearis var. helvetica.*
Planktonic	Diatoms from the plankton that settle on benthos.	1	*Cyclotella atomus, C. pseudostelligera, Fragilaria gracilis.*
		2	*Fragilaria tenuissima, Nitzschia acicularis.*
		3	*Cyclotella ocellata, Ulnaria acus.*
		4	*Cyclotella distinguenda, Cyclotella meneghiniana, Cyclostephanos dubius.*

Taxa verified in DiatomBase (Kociolek et al. 2015)

Studied sites:

AnnexTable 2 Comparison of physico-chemical data for each descriptive location and year.

	ECO	**YEAR**	**NH_4^+ [mg/L]**	**COND [µS/cm]**	**PO_4^{3-} [mg/L]**	**NO_3^{2-} [mg/L]**	**O_2 [mg/L]**	**pH**	**SS [mg/L]**	**T H_2O [ºC]**
1141	109	2007	0.1	422.5 [397-448]	0.1	1.1 [0.5-1.7]	10.9 [9.2-12.6]	8.4 [8.3-8.5]	2.45 [2-2.9]	11.15 [3-19.3]
		2008	0.1	362 [339-396]	0,1	0.9 [0.5-5.3]	10.2 [8.9-11.3]	8.4 [8.3-8.4]	2	13.2 [4.4-18.9]
		2010	0.13 [0.13-0.14]	351 [335-395]	0.05	1.4 [1.1-1.7]	10.3 [8.8-11.8]	8.3 [7.6-8.5]	7 [5-9]	11.95 [5-18.9]
		2011	0.13	364 [331-403]	0.05	1.3 [1-2.4]	10.8 [8.9-11.6]	8.3 [7.9-8.5]	5	11.9 [8.4-17.7]
		2012	0.13	389.5 [297-475]	0.05	1 [1-4]	10 [7.6-13.4]	8.3 [8-8.6]	5	15.6 [0.8-24.5]
		2013	0.13	357 [326-493]	0.05	1.2 [1-1.7]	10.45 [9.9-11]	8.35 [8-8.5]	5	12.15 [8.4-15.9]
1178	111	2007	0.1 [0.1-0.2]	620 [272.3-647]	0.1 [0.05-0.1]	3.5 [1-3.6]	11.2 [10.1-12.7]	8.4 [7.05-8.7]	2.45 [2-2.9]	9.5 [2.4-13.5]
		2010	0.13	547 [384-558]	0.095 [0.06-0.13]	2.45 [2-2.9]	10.4 [9.1-10.5]	8.4 [8.3-8.5]	5.5 [5-6]	11.1 [8.6-16.8]
		2012	0.13	562 [480-658]	0.075 [0.05-0.3]	2.8 [2.1-4.2]	10 [9.1-11]	8.5 [8.28-8.6]	5	11.8 [8-15.9]
		2013	0.13	466 [400-578]	0.08 [0.06-0.1]	2.65 [2.3-3]	9.93 [9.4-10.8]	8.4 [8.1-8.6]	5	11 [7.3-13.5]
0038	112	2005	0.115 [0.1-0.13]	445 [189-564]	0.135 [0.05-0.19]	6.35 [5.8-7.8]	11.3 [8-14.2]	8.15 [7.9-8.5]	14 [5-27]	11.7 [5.1-21.5]
		2006	0.1	435 [266-511]	0.175 [0.1-0.25]	7.35 [6.1-8.8]	10.6 [9-13.6]	8.2 [8.1-9.1]	7.5 [1-35]	14.4 [6.5-21.3]
		2007	0.1	388 [272-479]	0.14 [0.1-0.18]	6 [1.7-6.9]	11.35 [8.4-12.8]	8.2 [7.74-8.6]	6 [1-84]	11.55 [5.2-15.8]
		2008	0.1 [0.05-0.1]	433 [228-477]	0.17 [0.17-0.28]	6.8 [2.7-6.96]	10.25 [8.8-12.3]	8.1 [7.85-8.3]	5 [2-26]	14.3 [9.6-16.98]
		2010	0.13	367 [342-388]	0.07	3.35 [2.9-3.8]	9.92 [9.3-10.7]	8.25 [8.2-8.3]	5.5 [5-6]	16 [12.6-19.1]
		2011	0.13	351 [305-493]	0.13 [0.08-0.17]	3.55 [2.6-4.5]	9.47 [9.2-12.6]	8.4 [8.08-8.6]	5	16.2 [7-19.7]
0101	115	2003	0.13	305 [280-340]	0.05	2.1 [2-2.2]	11.4 [2-12.8]	8.4 [8.3-8.6]	7 [3-17]	10.9 [5.8-22.2]
		2005	0.115 [0.1-0.13]	313 [275-322]	0.075 [0.05-0.1]	1.35 [1.2-1.5]	11.525 [8.2-13.6]	8.26 [8.1-8.41]	6 [1-12]	14.2 [5.3-20.5]
		2006	0.1	309.5 [271-361]	0.1	1.75 [1-2.5]	9.8 [8.2-12.6]	8.31 [8.1-8.52]	7 [1-65]	13.7 [4.5-20.6]
		2007	0.1	311.5 [297-344]	0.1	2.09 [1.7-2.7]	10.465 [9.1-12.1]	8.45 [8.3-8.88]	7 [1-16]	11.75 [7.3-19.83]
		2008	0.1 [0.05-0.1]	328 [314-336]	0.1 [0.07-0.1]	1.5 [1.24-1.9]	11.3 [8.7-12.6]	8.3 [7.97-8.4]	19.5 [4-53]	13.4 [5-14.5]
		2010	0.13	293 [280-305]	0.05	1.45 [1.3-1.6]	10.3 [9.9-11.5]	8.3 [7.96-8.5]	6.5 [5-8]	13.8 [10.8-16.2]
		2011	0.13	310 [278-315]	0.05	1.5 [1.1-1.7]	10.35 [8.85-13.1]	8.4 [8.16-8.6]	5 [5-6]	15.5 [5.2-23]
0009	116	2006	0.1	1344.5 [927-1869]	0.145 [0.1-0.19]	12.65 [8-17.3]	8.35 [6.4-10.5]	8.11 [7.9-8.3]	25.5 [3-317]	13.4 [4.3-19.9]
		2007	0.15 [0.1-0.36]	1390.5 [1038-1638]	0.115 [0.09-0.13]	13.15 [2.3-15.9]	8.3 [5-10.2]	8.1 [7.41-8.2]	11 [1-149]	13.2 [7-19.7]
		2013	0.32	1297	0.05	14.6	10.8	8.05	11	9.15

			[0.17-0.55]	[1140-1548]	[0.05-0.06]	[12.1-15.4]	[9.9-11.8]	[7.75-8.2]	[9-78]	[6.9-16]
0512	117	2003	0.13 [0.13-0.2]	740 [360-1070]	0.105 [0.05-0.29]	9.25 [8.2-11.5]	9.75 [7.4-11.7]	8.2 [8.1-8.5]	4.5 [3-21]	16.3 [7.5-26.8]
		2005	0.1 [0.1-0.14]	1170 [785-1437]	0.18 [0.09-0.48]	9.5 [7-11.4]	9.4 [5.4-14.9]	8.18 [7.7-8.4]	4 [1-5]	16.2 [9.6-25.8]
		2006	0.1 [0.022-0.1]	1057.5 [748-1546]	0.18 [0.1-0.35]	9.55 [5.7-14.9]	9.05 [7.1-10.8]	8.1 [7.99-8.28]	4 [1-19]	21.45 [9.8-26.6]
		2007	0.1 [0.019-0.22]	1104 [622-1709]	0.12 [0.1-0.51]	10.5 [1-18.2]	8.8 [7.3-10.8]	8.2 [7.75-8.4]	6 [1-14]	20.5 [10.3-25.5]
		2008	0.1 [0.024-0.1]	1205 [610-1447]	0.14 [0.05-0.25]	10 [6.4-27.83]	8.32 [5.57-10.8]	8.1 [7.54-8.3]	2.5 [2-8]	20.1 [9.9-26.4]
		2010	0.13 [0.014-0.13]	846 [430-1351]	0.14 [0.05-0.27]	9.65 [5.5-13.33]	8.96 [7.7-11.7]	8.2 [7.6-8.5]	5 [5-9]	18.7 [10.9-25.8]
		2011	0.218 [0.067-0.306]	1049 [770-1550]	0.165 [0.09-0.28]	7.75 [1.49-14.77]	9.3 [7.4-10.33]	8.2 [7.9-8.49]	5	17.6 [9.8-26.7]
		2012	0.13 [0.028-0.16]	1052 [825-1560]	0.08 [0.05-0.19]	7.7 [1.11-11.6]	8.7 [7.4-11.4]	8.1 [7.7-8.3]	5 [5-9]	19.8 [7.9-26.42]
		2013	0.13 [0.048-0.18]	720 [530-1389]	0.07 [0.05-0.13]	7.85 [1.57-12.8]	9.48 [7.56-12.2]	8.2 [7.92-8.39]	5 [5-33]	15 [8.7-25.2]
0022	126	2005	0.35 [0.1-2.7]	200 [105-290]	0.405 [0.28-0.77]	4.75 [2.9-12.7]	11.05 [7.2-13.8]	7.9 [7.76-8.6]	12 [3-210]	10.25 [3.2-19.7]
		2006	0.1 [0.1-1.64]	197 [90-303]	0.33 [0.1-1.04]	3.75 [1.7-5.6]	9.25 [6.5-12.6]	8.275 [7.81-8.74]	4.5 [1-151]	9.5 [2-20.7]
		2007	0.1	196 [94-263.9]	0.25 [0.13-0.78]	4 [2-7.52]	8.8 [6.85-11.3]	8.2 [7.5-8.8]	3 [2-42]	9.5 [4-20.6]
		2008	0.1	209 [118-231]	0.155 [0.1-0.31]	3.4 [2.3-4.3]	8.7 [6.9-11.1]	8 [7.4-8.4]	4 [2-10]	6.7 [1.7-15.5]
		2010	0.13	204 [88-256]	0.14 [0.05-0.23]	2.2 [1.3-3.1]	8.9 [7.8-9.34]	8 [7.9-8.1]	18.5 [6-31]	9.5 [5.8-14.6]
		2011	0.13	205 [117-240]	0.1 [0.05-0.2]	4 [1.7-4.2]	9.475 [7.8-9.2]	8.55 [8.3-9.2]	5 [5-8]	12 [5.8-16.5]
1448	127	2005	0	213	12.55			8.16		6
		2006								
		2007	0.1	242 [234-270]	0.7 [0.05-0.1]	0.375 [0.05-0.7]	8.8 [8.74-12.4]	8.6 [8.4-9.28]	1.5 [1-2]	15 [1.3-20.09]
		2008	0.1 [0.05-0.1]	237.5 [216-271]	0.1 [0.03-0.1]	0.6 [0.42-0.8]	9.35 [7.05-11.3]	8.4 [8.16-8.6]	2 [2-3]	8.65 [3.5-26.38]
		2010	0.13	239 [237-249]	0.05	1	8.7 [8.5-11.3]	8.4 [8.18-8.5]	5	18.1 [13.4-20]
		2012	0.13	253 [237-268]	0.05 [0.05-0.1]	1	10.1 [8-11.1]	8.5 [8.4-8.55]	5	6 [5.7-11.9]
		2013	0.13	199.5 [176-223]	0.05	1	9.78 [7.9-11.7]	8.115 [7.73-8.5]	5	12.3 [8.8-15.8]

AnnexTable 3 Sampled sites of the TRivers LIFE Project. River Authorities: ACA = Catalan Water Agency (Agència Catalana de l'Aigua), CHE = Ebro River Authorities (Confederación Hidrográfica del Ebro) and CHJ = Júcar River Authority (Confederación Hidrográfica del Jícar.

CODE	RIVER	BASIN	RIVER AUTHORITY
1.1	Onyar Headwaters	Ter	ACA
1.2	Tossa Stream	-	ACA
1.3	Pineda Stream	-	ACA
1.4	Mura Stream & Talamanca Stream	-	ACA
1.5	Aigua d'Ora Basin	Cardener/Llobregat	ACA
1.6	Brugent River	Francolí	ACA
1.7	Puig Stream	Francolí	ACA
1.10	Daró River Headwaters	“Ter” canalized	ACA
1.11	Glorieta River Headwaters	Francolí	ACA
1.12	Congost River	Besós	ACA
2.1	Matarraña River	Ebro	CHE
2.2	Algars River	Matarraña	CHE
2.3	Canaletes River	Ebro	CHE
2.4	Montsant Headwaters	Siurana	CHE
2.5	Siurana River	Ebro	CHE
3.1	Poyo Watercourse	-	CHJ
3.2	Carraixet Ravine	-	CHJ
3.3	Roig Wtc. (Cañuelo Ravine) Artaj	-	CHJ
3.4	Guadazaón Headwaters: Ayo. Prado Olmeda	Contreras	CHJ
3.5	Cabriel River Headwaters	Júcar	CHJ
3.6	Monleón River Headwaters	Viuda Stream	CHJ

3.7	San Miguel River	-	CHJ
3.8	Sérvol Headwaters	-	CHJ
3.9	Sénia	-	CHJ
3.10	Castellana Watercourse: Wtc. Roig – Turia River	-	CHJ
3.11	Wtc. de la Viuda: Segarra Ravine Headwaters	Mallars	CHJ

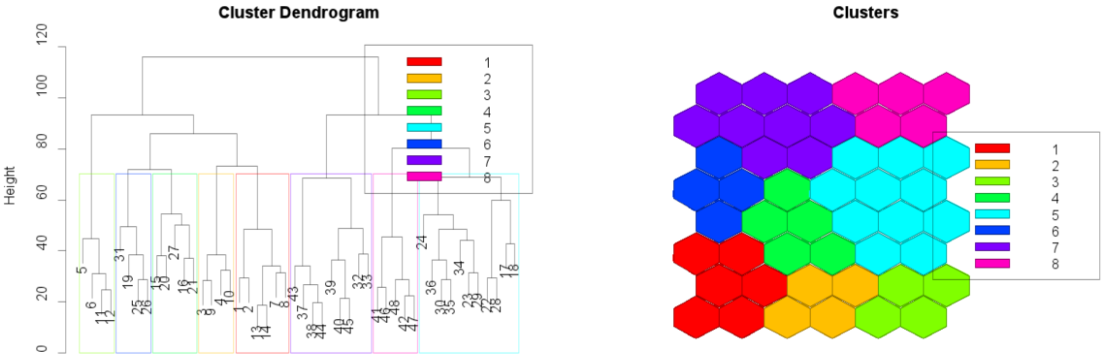
Annex chapter 4: Diatom-Ecoregions

AnnexTable 4 Sampling sites as extracted from each sub-basin, location, code, original ecotype and subtype.

Watershed and River	Site Location (Utm)	Code	N	Original Type	Hierarchical Cluster Diatoms	Som Diatoms	Hierarchical Cluster Pq
Alcandre	Ontiñena	0226	8	109	1	7	SL
	Puente a las Cellas	1141	6	109	2.2	8	CM
Algas	Mas de Bañetes	0623	7	112	2.2	8	CM
Alhama	Venta de Baños de Fitero	0243	7	112	2.2	8	LN
Aragón	Caparroso	0005	7	115	1	8	LN
	Cáseda	0205	6	115	2.2	8	LN
	Yesa	0101	8	115	2.1	8	CM
Arba	Luesia	0060	7	109	1	1	LS
Arga	Funes	0004	10	115	1	1	LS
	Ororbia	0217	8	126	1	2	LN
Aurín	Isín	0539	7	126	2.2	8	SM
Cinca	Monzón	0562	6	115	1	8	LS
Ebro	Benifallet	0511	7	117	1	6	LS
	Gallur	0508	7	117	1	1	LS
	Miranda de Ebro	0001	8	115	1	6	LN
	Xerta	0512	10	117	1	6	LS
Ega	Arinzano	0572	7	112	1	3	LN
Erro	Sorogaín	1393	6	126	2.1	8	SM
Escá	Burgui	0816	6	126	2.1	8	CM
Estarrón	Aínsa	2012	6	126	2.1		
Gállego	Villanueva	0247	10	115	1	6	LS

	Zaragoza	0089	7	115	2.2	2	LS
Guarga	Ordovés	2014	6	126	2.2	8	MC
Jalón	Ateca	0126	9	109	1	1	LN
	Grisén	0087	9	116	2.2	1	LS
	Huérmeda	0009	7	116	1	1	LN
Jerea	Palazuelos de Cuesta Urría	0166	8	109	2.2	8	CM
Jiloca	Luca de Jiloca	0244	7	112	1	3	LN
Leza	Ribafrecha	0197	7	112	2.2	8	CM
Martín	Hijar	0014	8	109	1	1	LS
	Oliete	0118	7	109	1	8	LN
Matarraña	Beceite, Parrizal	1240	6	112	2.2	8	CM
Mayor	Villoslada de Cameros	2002	6	111	2.1	7	SM
Najerilla	Torremontalbo	0038	8	112	2.1	7	CM
	Villavelayo	1178	6	111	2.1	7	CM
Nela	Puentedey	1004	6	126	2.1	7	SM
Omecillo	Corro	2011	6	126	2.1	7	CM
Osia	Jasa	2013	6	126	2.2	8	SM
Segre	Pla de Sant Tirs	0206	7	126	2.1	7	SM
	Serós	0025	8	115	1	6	LS
	Torres de Segre	0219	7	115	1	6	LS
	Vilanova de la Barca	0207	7	115	1	1	LS
	Fresneda de la Sierra	1173	6	111	2.2	8	SM
Tirón	El Vado	1006	6	126	2.1	7	SM
Urbión	Santa Cruz del Valle	1387	6	111	2.2	7	SM

	Viniegra de Abajo	2001	6	111	2.1	7	SM
Valira	Anserall	0022	8	126	2.1	7	SM
Veral	Arce/Miranda de Ebro	0074	8	115	1	3	LN
Zadorra	Vitoria, Trespuentes	0179	7	112	1	6	LN
	Mendivil, Durana	0180	7	126	1	6	CM



AnnexFigure 2 Output of cluster and diatom community distribution output of the diatSOM package divided into 8 communities.

AnnexTable 5 UPGMA clustering with 10.000 iterations of the total clustering indicator value of diatom taxa. Division into five clusters divided into River Axis (A/A+B) and Mountain Rivers (B/C+D) with different physical chemical compositions to account for the differences in diatom community. As in the previous subdivision into two, the extraction of the outliers the indicator value is marginally higher in its significance. SM=Siliceous mountain, M = Mountain, L=Lowland, CM=Calcareous Mountain, SL=saline lowland, NL= nutrient lowland

	2 Clusters		**4 Clusters**	
	Cluster	**IndVal(%)**	**Cluster**	**INDVAL(%)**
Achnanthidium pyrenaicum (Hustedt) Kobayasi	M	99.1***	SM+CM	**99.1*** **
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	M	80.9***	SM+CM	**80.8*** **
Cymbella affinis Kützing			SM+CM+NM	**73.3** **
Encyonopsis microcephala (Grunow) Krammer	M	68.3**	CM	**70.2** **
Encyonopsis minuta Krammer & Reichardt	M	67.5***	SM+CM	**67.6*** **
Encyonema silesiacum (Bleisch) Mann			SM	**64.7*** **
Reimeria sinuata (Gregory) Kociolek & Stoermer			SM	**62***
Denticula tenuis Kützing	M	57.7**	SM+CM	**57.7** **
Cocconeis pseudolineata (Geitler) Lange-Bertalot	M	56.8**	SM+CM	**56.7***
Diatoma tenuis Agardh	M	54.8*	CM	**64.8** **
Gomphonema lateripunctatum Reichardt & Lange-Bertalot	M	54.8**	CM	**54.9***
Achnanthidium subatomus (Hustedt) Lange-Bertalot			SM	**52.2***
Fragilaria rumpens (Kützing) Carlsson			SM	**50***
Nitzschia inconspicua Grunow	L	95.6***	SL+NL	**95.7*** **
Amphora pediculus (Kützing) Grunow Ex. A Schmidt	L	90.6***	SL+NL	**90.2*** **
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	L	87.2***	SL+NL	**87.1*** **
Navicula cryptotenella Lange-Bertalot	L	83.8**	CM+SL+NL	**87.5*** **
Nitzschia dissipata (Kützing) Rabenhorst	L	78.6***	SL+NL	**78.3*** **
Nitzschia frustulum (Kützing) Grunow	L	78***	SL+NL	**78*** **
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	L	77.1***	SL+NL	**77.2** **
Nitzschia palea (Kützing) Smith	L	67.7**	SL+NL	**68***

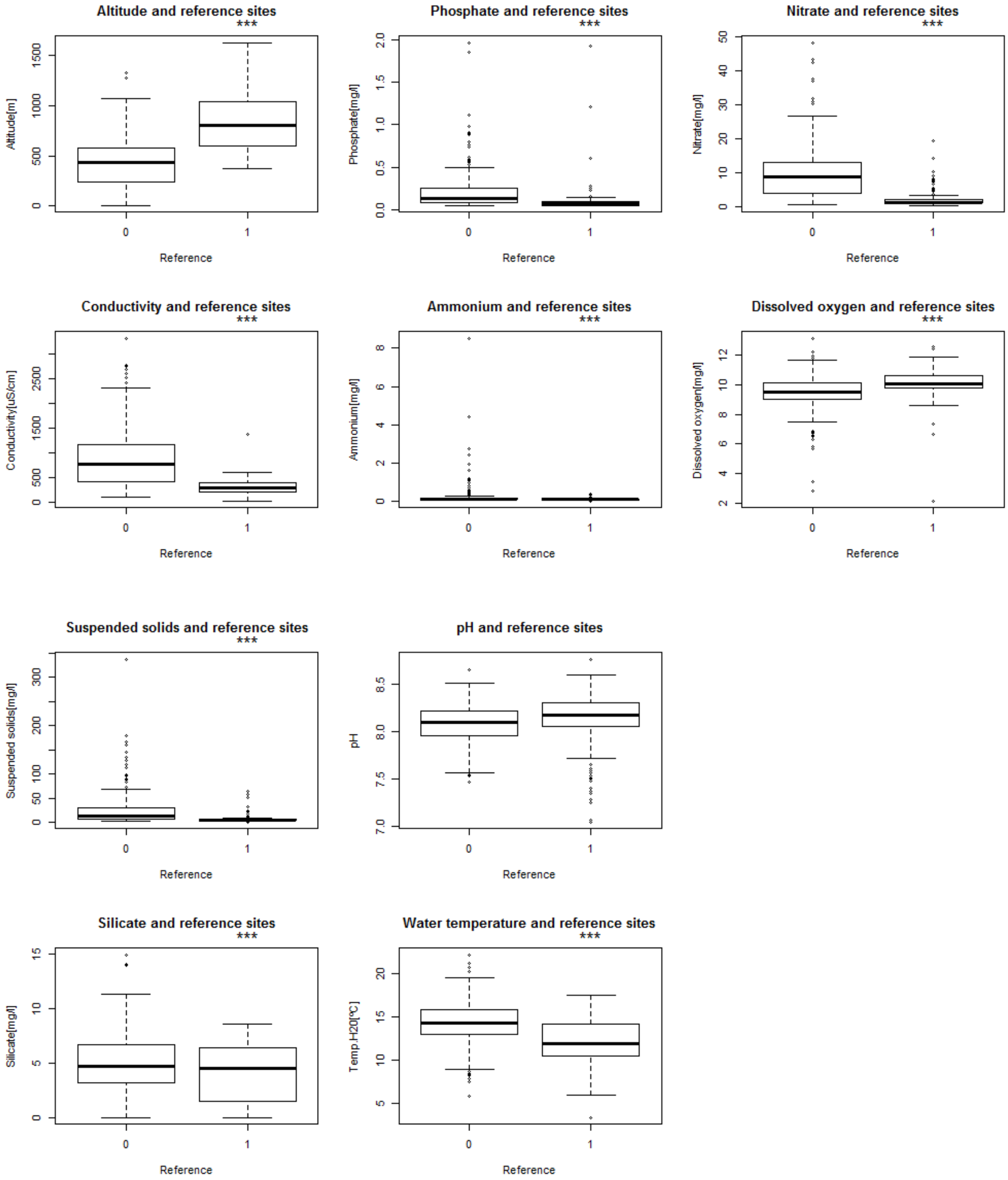
Navicula cryptotenelloides Lange-Bertalot			SL	**62.5****
Navicula tripunctata (Müller) Bory	L	62.4*		
Achnanthidium saprophilum (Kobayashi & Mayama) Round & Bukhtiyarova			NL	**59****
Navicula lanceolata Ehrenberg	L	59**	SL+NL	**59****
Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot	L	58.8**	NL	**60.6****
Bacillaria paxilifera (Müller) Marsson			SL	**57.7****
Fragilaria brevistriata (Grunow) Williams & Round			SL	**57.7****
Diatoma vulgaris Bory			NL	**56.2***
Gomphonema parvulum (Kützing) Kützing	L	55.7**	SL+NL	**53.9***
Navicula antonii Lange-Bertalot	L	54.3**	SL+NL	**54.2***
Nitzschia amphibia Grunow	L	53.1**	SL+NL	**53***
Gomphonema olivaceum (Hornemann) Brébisson			NL	**52.4***
Navicula recens (Lange-Bertalot) Lange-Bertalot	L	51.1**	SL	**63.1*****
Cyclotella ocellata Pantocsek			NL	**50.9***
Pseudostaurosira elliptica (Schumann) Edlund, Morales & Spaulding			SL	**50***

Significance level: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1

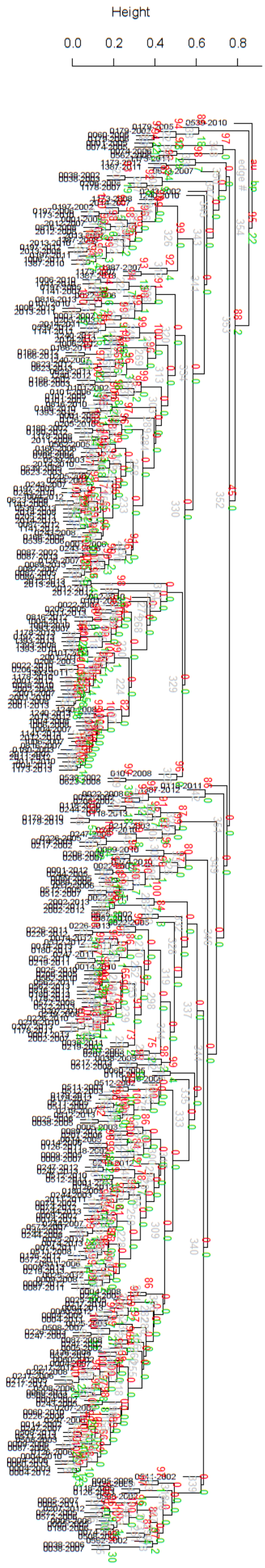
AnnexTable 6 UPGMA clustering of 10.000 iterations of the total clustering of average sites. Division into six clusters: High ammonium axis (A), High conductivity axis (B1) and Medium conductivity axis (B2) found at the river axis, Low pH oligotrophic mountain, High oligotrophic mountain and Low oligotrophic mountain.

	Omnidia Code	**Indval**	**Group**	**Total Proportion(%)**						**P. Val**
				A	B1	B2	C	D1	D2	
Planothidium frequentissimum	PLFR	58***	A	58	0	0	0	0	0	0,001
Nitzschia amphibia	NAMP	49***	A	49	0	0	0	0	0	0,001
Sellaphora seminulum	SSEM	41***	A	41	0	0	0	0	0	0,001
Fistulifera saprophila	FSAP	36**	A	36	0	0	0	0	0	0,004
Eolimna minima	EOMI	20*	A	20	0	0	5	0	0	0,032
Navicula cryptotenella	NCTE	30*	B1	0	30	9	0	0	0	0,01
Amphora pediculus	APED	29**	B1	9	29	23	0	0	0	0,007
Nitzschia dissipata	NDIS	22*	B1	0	22	6	0	0	0	0,033
Nitzschia inconspicua	NINC	61***	B2	0	6	61	0	0	0	0,001
Rhoicosphenia abbreviata	RABB	27**	B2	0	0	27	0	0	0	0,009
Nitzschia frustulum	NIFR	22*	B2	0	0	22	0	0	0	0,013
Encyonema silesiacum	ESLE	63***	C	0	0	0	63	0	0	0,001
Diatoma mesodon	DMES	46***	C	0	0	0	46	0	0	0,001
Achnanthidium subatomus	ADSU	44***	C	0	0	0	44	0	0	0,001
Gomphonema pumilum	GPUM	44***	C	0	0	0	44	0	0	0,001
Gomphonema rhombicum	GRHO	27***	C	0	0	0	27	0	0	0,001
Encyonema mnutum	ENMI	19*	C	0	0	0	19	0	0	0,03
Ulnaria ulna	UULN	13*	C	0	0	0	13	0	0	0,034
Achnanthidium pyrenaicum	ADPY	55***	D1	0	0	0	9	55	8	0,001
Achnanthidium minutum	ADMI	44***	D2	0	0	0	0	15	44	0,001
Gomphonema lateripunctatum	GLAT	13*	D2	0	0	0	0	0	13	0,05

Significance level: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



AnnexFigure 3 Significant changes in the two-cluster diatom-community based division. Significant differences marked with ***.



AnnexFigure 4 Total yearly comparison of diatom assemblages through time.

AnnexTable 7 Indval for new ecotypes and SOM distribution. AM=High Mountain, LC=Lowland with conductivity, LN=Lowland with nutrients, LT=Lowland with high temperature, MMS=Medium mountain rivers with silicon, MS=Mountain with silicon.

Species	**Code**	**Group**	**SOM Div**	**Group Max**	**SOM Max**
Achnanthidium pyrenaicum (Hustedt) Kobayasi	ADPY	MMS	1	35,5101 ***	83.2***
Achnanthidium minutissimum (Kützing) Czarnecki	AMIN	HM	2	35,624 ***	73.7***
Encyonopsis minuta Krammer & Reichardt	ECPM	HM	2	12,4841 *	45**
Encyonopsis microcephala (Grunow) Krammer	ENCM	HM	2	14,9107 *	43.6**
Cymbella excisa Kützing	CAEX	HM	2	14,245 *	39.3*
Gomphonema lateripunctatum Reichardt & Lange-Bertalot	GLAT	HM	2	10,7143 *	36.3*
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM	MS	3	32,4906 ***	68.6***
Achnanthidium subatomus (Hustedt) Lange-Bertalot	ADSU	MS	3	20,5989 ***	50.4***
Encyonema silesiacum (Bleisch) Mann	ESLE	MS	3	20,0335 ***	46.6**
Reimeria sinuata (Gregory) Kociolek & Stoermer	RSIN	MMS	3	25,5842 ***	42.4**
Gomphonema rhombicum Schmidt	GRHO	MS	3	22,9234 ***	41.7**
Encyonema minutum (Hilse) Mann	ENMI	MMS	3	32,7356 ***	38.1**
Planothidium lanceolatum (Brébisson Ex Kützing) Lange-Bertalot	PTLA	MS	3	14,2857 ***	34**
Diatoma mesodon (Ehrenberg) Kützing	DMES	MS	3	33,3333 ***	28.9*
Cocconeis pseudolineata (Geitler) Lange-Bertalot	CPPL	MS	3	15,1615 **	28.9*
Cocconeis placentula var. lineata Ehrenberg	CPLI	MS	4	20,7502 ***	59.7**
Fragilaria rumpens (Kützing) Carlson	FCRU	MS	4	18,7081 ***	28.6*
Nitzschia dissipata (Kützing) Grunow	NDIS	LN	5	20,8484 **	66.2***
Navicula cryptotenella Lange-Bertalot	NCTE	LN	5	18,5152 *	60.3***
Discostella pseudostelligera (Hustedt) Houk & Klee	CPST	LN	5	10,9872 *	39.1*
Cocconeis placentula var. euglypta Ehrenberg	CEUG	LN	6	21,3994 **	80.3***
Amphora pediculus (Kützing) Grunow Ex. Schmidt	APED	LN	7	30,0079 ***	76.7***
Nitzschia inconspicua Grunow	NINC	LC	8	46,9231 ***	81.9***

Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS	LT	8	12,5538 *	38.5*

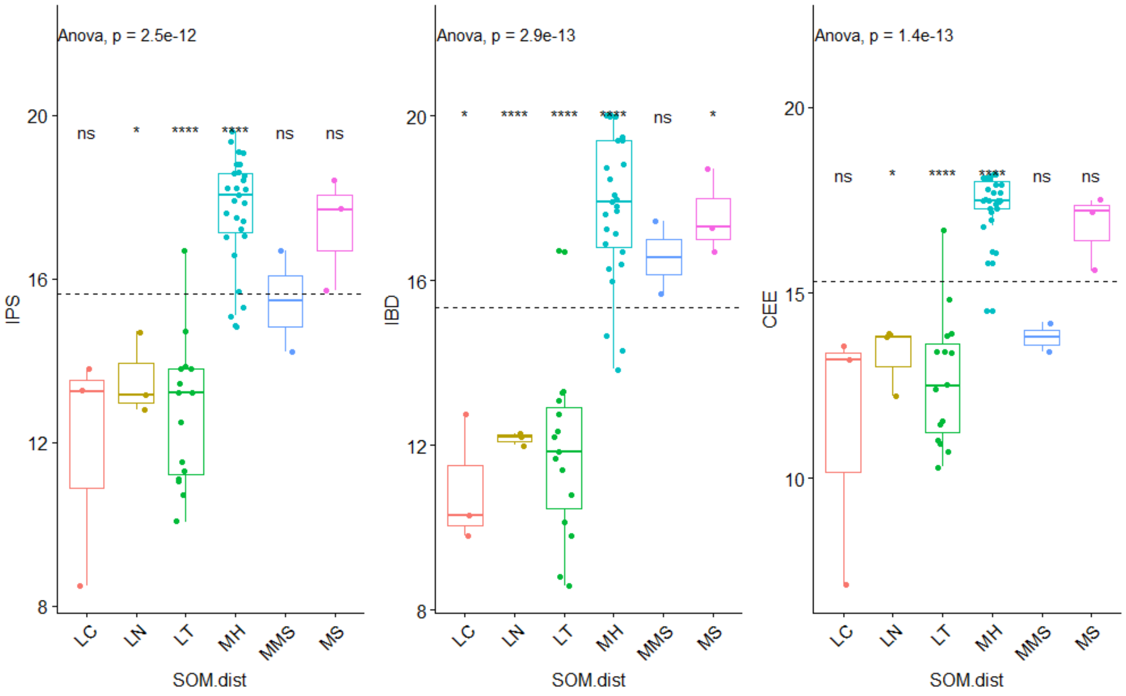
Significance level: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

AnnexTable 8 UPGMA comparison of 10.000 iteration distribution of the official ecotypes established by the Ebro River Authorities. The high river mountains were not defined by indicative species. Neither were 109=Mineralized mountains of low Mediterranean rivers and Continental. 111= Mediterranean siliceous mountain rivers, 112 = Mediterranean calcareous mountain rivers, 116=Mediterranean and Continental mineralized river axes, 117=Main river axis in Mediterranean environment, 126= Rivers of wet calcareous mountains.

	Omnidia Code	**Indval**	**Group**	**Total Proportion%**						
				109	111	112	115	116	117	126
Achnanthidium pyrenaicum	ADPY	35***	111	0	35	5	0	0	0	24
Gomphonema pumilum	GPUM	27**	111	0	27	0	0	0	0	5
Diatoma mesodon	DMES	20**	111	0	20	0	0	0	0	0
Achnanthidium subatomus	ADSU	14**	111	0	14	0	0	0	0	0
Cocconeis placentula var. lineata	CPLI	12*	111	0	12	0	0	0	0	0
Cocconeis placentula var. pseudolineata	CPPL	11*	111	0	11	0	0	0	0	0
Encyonema silesiacum	ESLE	10*	111	0	10	0	0	0	0	0
Gomphonema rhombicum	GRHO	9*	111	0	9	0	0	0	0	0
Delicata delicatula	DDEL	9*	112	0	0	9	0	0	0	0
Rhoicosphenia abbreviata	RABB	35**	116	9	0	0	0	35	0	0
Nitzschia inconspicua	NINC	32***	116	15	0	0	7	32	13	0
Amphora pediculus	APED	31***	116	8	0	5	10	31	18	0
Nitzschia dissipata	NDIS	22**	116	0	0	0	5	22	0	0
Gomphonema olivaceum var. olivaceum	GOLI	20**	116	0	0	0	0	20	0	0
Diatoma moniliformis	DMON	20**	116	0	0	0	0	20	0	0
Navicula antonii	NANT	36***	117	0	0	0	0	0	36	0
Nitzschia frustulum	NIFR	14*	117	5	0	0	0	0	14	0
Nitzschia palea	NPAL	13*	117	0	0	0	0	0	13	0
Encyonopsis subminuta	ESBM	12*	117	0	0	0	0	0	12	0

Nitzschia amphora	NAMP	10*	117	0	0	5	0	0	10	0
Achnanthidium minutissimum	ADMI	20*	126	7	0	15	6	5	0	20

Significance level: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1



AnnexFigure 5 Comparison of bioindicative values.

AnnexTable 9 Spearman Rank Correlation of diatom distribution methods and physicochemical traits.

Chemical	**Ecotype (8 groups)**	**8 clusters**	**SOM (8 GROUPS)**
CONDUCTIVITY (µs/CM)	-0.2773*	-0.6166 ***	-0.6334 ***
Phosphate (mg/l)	0.0918	-0.7403 ***	-0.423***
Nitrate (mg/l)	-0.0992	-0.7873 ***	-0.6931 ***
Dissolved oxygen (mg/l)	0.014	0.4539 ***	0.4018 ***
Ph	-0.191	0.3469*	0.1368
Water Temperature (ºc)	-0.133	-0.3351*	-0.4686 ***

*Significance level: 0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1*

AnnexTable 10 Significance of physicochemical traits on a two-cluster subdivision (hierarchical clustering).

Data Set	**# Sites**	**# Variables**	**Alt**	**Nh4**	**Cond**	**Po4**	**No3**	**O2dis**	**Ph**	**Ss**	**Water Temp**
Regions (Total)	352	5	X	X	X	X	X				
River Axis	188	5		X	X	X				X	X
Mountain Rivers	168	8	X	X	X	X		X	X	X	X

AnnexTable 11 Significances of physicochemical traits for impacted and reference sites (official subdivision)

Data Set	**# Sites**	**# Variables**	**Alt**	**Nh4**	**Cond**	**Po4**	**No3**	**O2dis**	**Ph**	**Ss**	**Water Temp**
Reference	102	3	X						X		X
Impacted	105	4	X	X						X	X

AnnexTable 12 Significance of physicochemical traits for temporal communities

Data Set	# Sites	# Variables	Alt	Nh4	Cond	Po4	No3	O2dis	Ph	Ss	Water Temp
2002	16	3	X		X			X			
2003	16	3	X	X	X						
2005	16	4	X	X			X	X			X
2006	16	4	X			X			X	X	
2007	10	3	X					X			X
2008	10	4				X	X		X		X
2010	12	4	X			X	X	X			
2011	12	4	X	X					X		X
2012	12	5	X		X	X			X		X
2013	12	4	X	X	X		X				

AnnexTable 13 Averaged assemblages of the different approaches.

Data Set	# Sites	# Variables	Alt	Nh4	Cond	Po4	No3	O2dis	Ph	Ss	Water Temp
Average Years	10	4	X	X			X		X		
Average Sites	50	7	X	X	X	X	X		X		X
Averaged Data	120	6	X	X	X		X		X		X
Ecotype	115	4	X	X			X				X

AnnexTable 14 Significance of physicochemical traits for each of the official ecotypes.

Data Set	# Sites	# Variables	Alt	Nh4	Cond	Po4	No3	O2dis	Ph	Ss	Water Temp
109	**16**	**4**	**X**	**X**		**X**					**X**
111	**18**	**1**	**X**								
112	**16**	**3**	**X**	**X**		**X**					
115	**16**	**3**	**X**					**X**		**X**	
116	**16**	**1**					**X**				
117	**17**	**2**						**X**			**X**
126	**16**	**4**	**X**		**X**	**X**					**X**

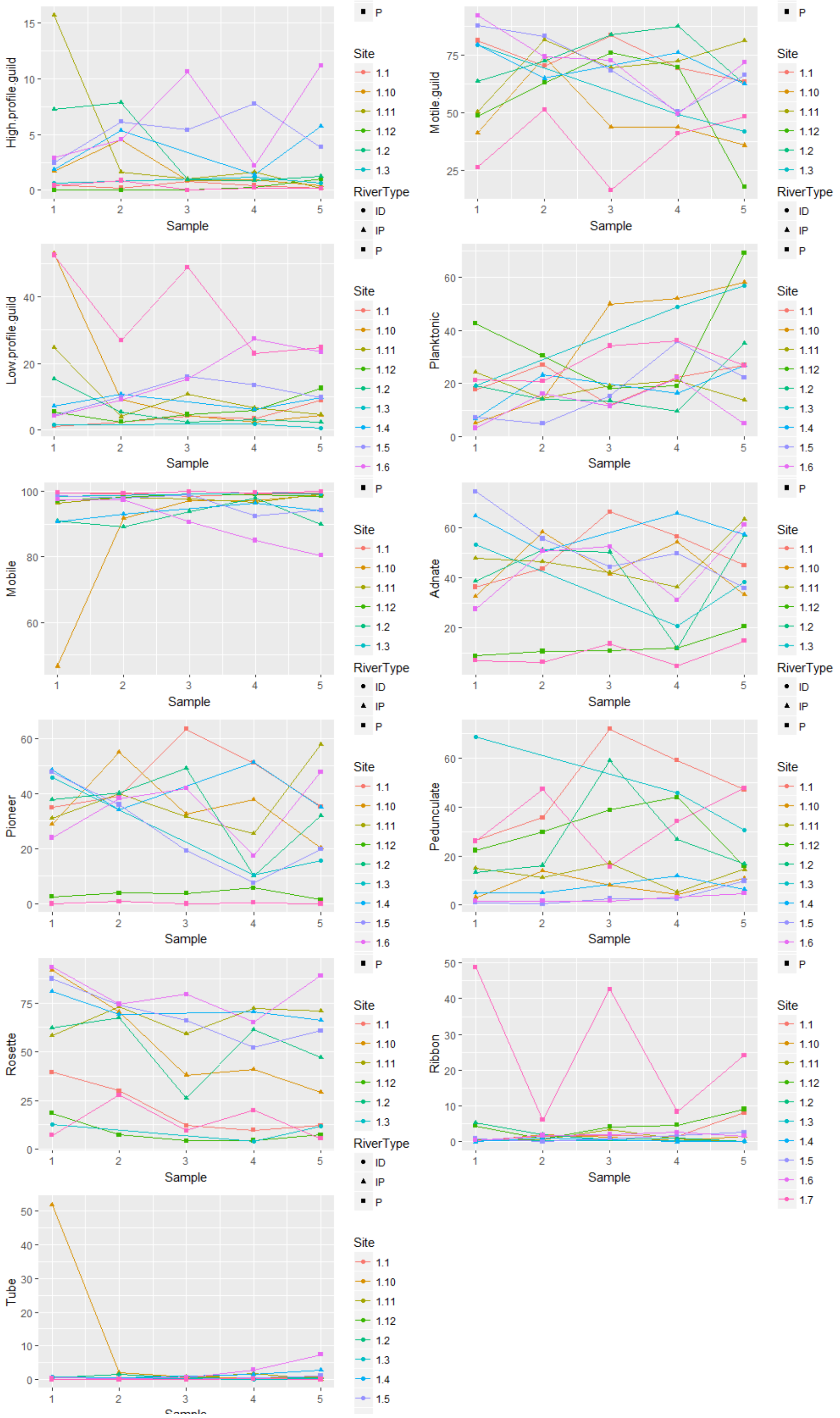
Annex chapter 5: MESOSCALE

AnnexTable 15 Spearman Rank correlation of Diatom proportion, bioindication and diversity with physicochemical parameters (retrieved from CHEbro web) Significance level: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

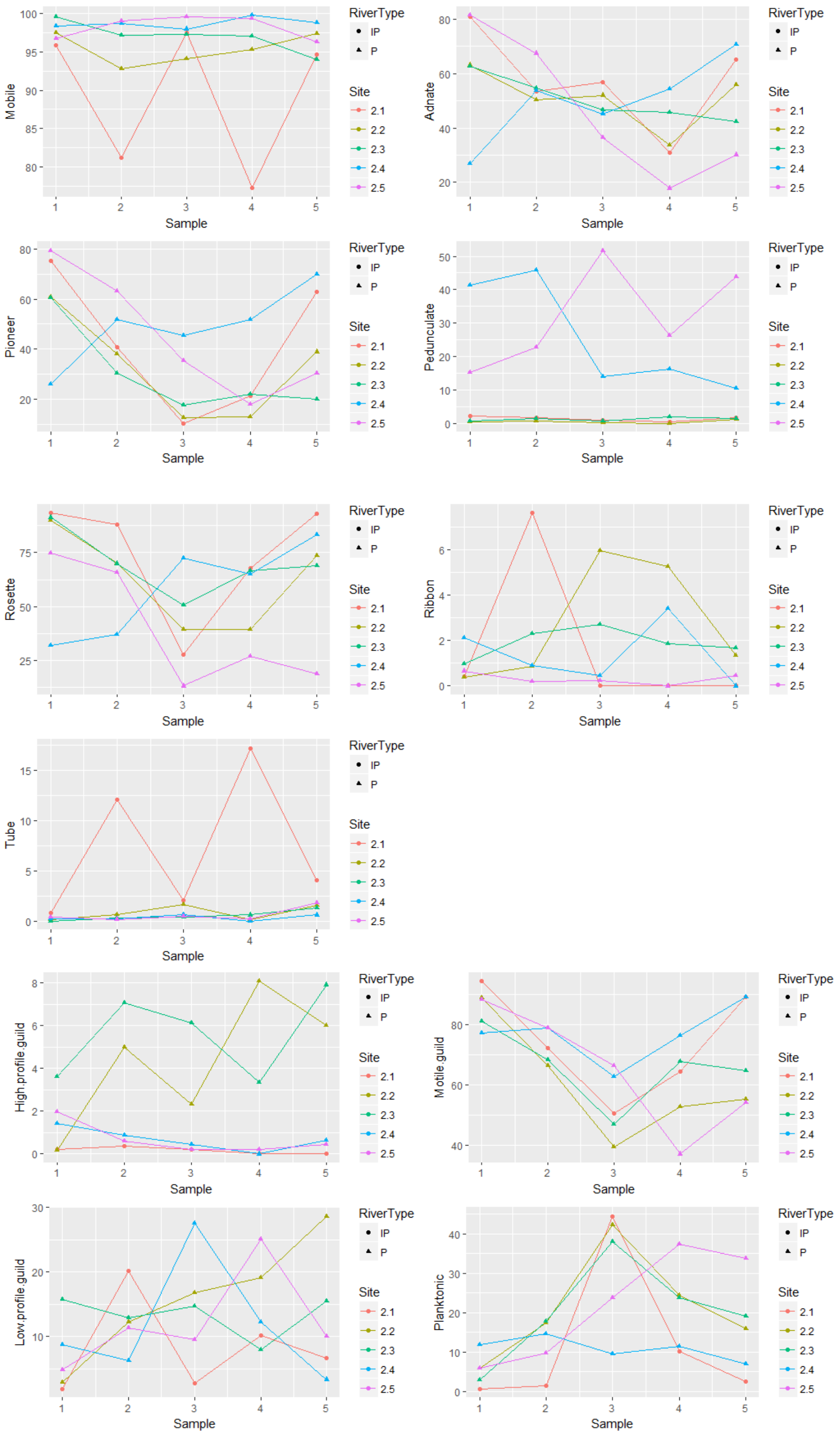
Chemical	IPS	IBD	Proportion	Shannon H'	Richness S
Conductivity (µs/cm)	-0.714 ***	-0.7411 ***	-0.0125	0.4172 ***	0.4245 ***
Phosphate (mg/l)	-0.6827 ***	-0.5227 ***	-0.0178	0.417 ***	0.4519 ***
Nitrate (mg/l)	-0.7841 ***	-0.7988 ***	-0.006	0.4635 ***	0.4738 ***
Dissolved Oxygen (mg/l)	0.2312 ***	0.0868 ***	0.0444 *	-0.224 ***	-0.3668 ***
Ph	-0.042	-0.1201 ***	0.0898 ***	-0.0367	-0.2409 ***
Water Temperature (ºC)	-0.572 ***	-0.6356 ***	-0.0173	0.2936 ***	0.3024 ***

Annex chapter 6: MICROSCALE

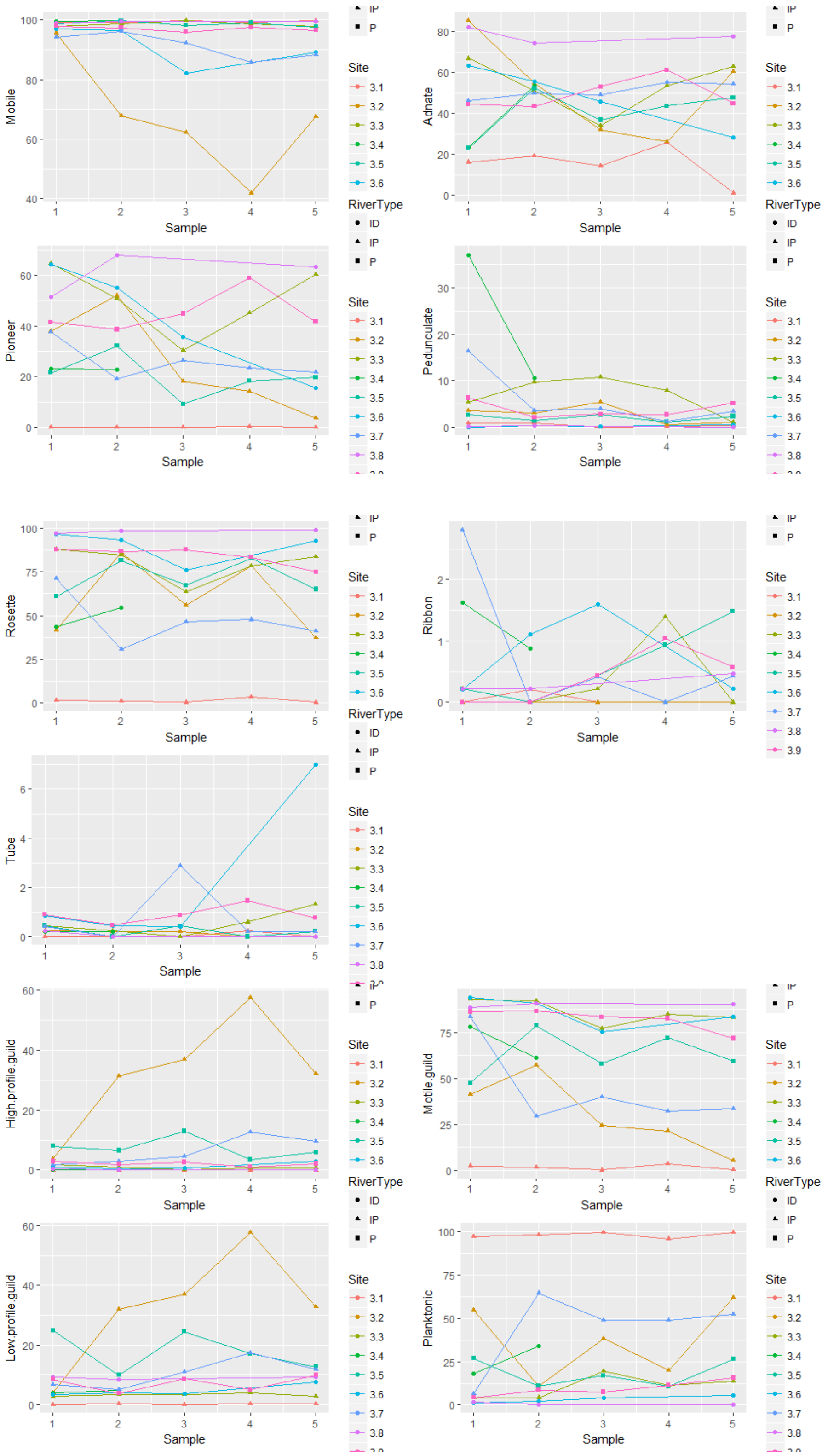
Section 6.2: Temporary Rivers



AnnexFigure 6 Total distribution of ecological guilds (top) and life forms (Riato et al 2017) in the Catalan Rivers. Temporary rivers shown as perennial (P, ▲), Intermittently Dry (ID, ■) and Intermittent Pools (IP, ●).



AnnexFigure 7 Total distribution of ecological guilds (top) and life forms (Riato et al 2017) in the Rivers at the Ebro river basin. Temporary rivers shown as perennial (P, ▲), Intermittently Dry (ID, ■) and Intermittent Pools (IP, ●).



AnnexFigure 7 Diatom life forms and ecological guilds of the Júcar Basin. Temporary rivers shown as perennial (P, ▲), Intermittently Dry (ID, ■) and Intermittent Pools (IP, ●).

Section 6.3: Diatom size

AnnexTable 16 Comparison of the sampling locations, sample dates and their belonging ecotype description.

Sample site	River type	M. A. S. L.	2003	2005	2006	2007	2008	2010	2011	2012	2013
Ebro in miranda de ebro 0001	**Continental and mediterranean slightly mineralized axes**	**458.4**		**1**	**1**	**1**	**1**		**1**	**1**	**1**
Jalón in huérmeda 0009	**Continental and mediterranean mineralized axes**	**523.4**			**1**	**1**					**1**
Valira in anserall 0022	**Rivers of wet calcareous mountains**	**683.8**		**1**	**1**	**1**	**1**	**1**	**1**		
Najerilla in torremontalbo 0038	**Mediterranean calcareous mountain rivers**	**422**		**1**	**1**	**1**	**1**	**1**	**1**		
Aragón in yesa 0101	**Continental and mediterranean slightly mineralized axes**	**423.8**	**1**	**1**	**1**	**1**	**1**	**1**	**1**		
Ebro in xerta 0512	**Main axes in mediterranean environment**	**5**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	
Najerilla in villavelayo 1178	**Mediterranean siliceous mountain rivers**	**982.1**				**1**		**1**		**1**	**1**
Veral in zuriza 1448	**High mountain rivers**	**1219.4**		**1**	**1**	**1**	**1**	**1**	**1**		**1**

River type extracted from CHE 1998, (Cedex-MMA 2005), BOE 1/2016, M. a. s. l. = metres above sea level.

AnnexTable 17 Quartile division of log(x+1) measurements.

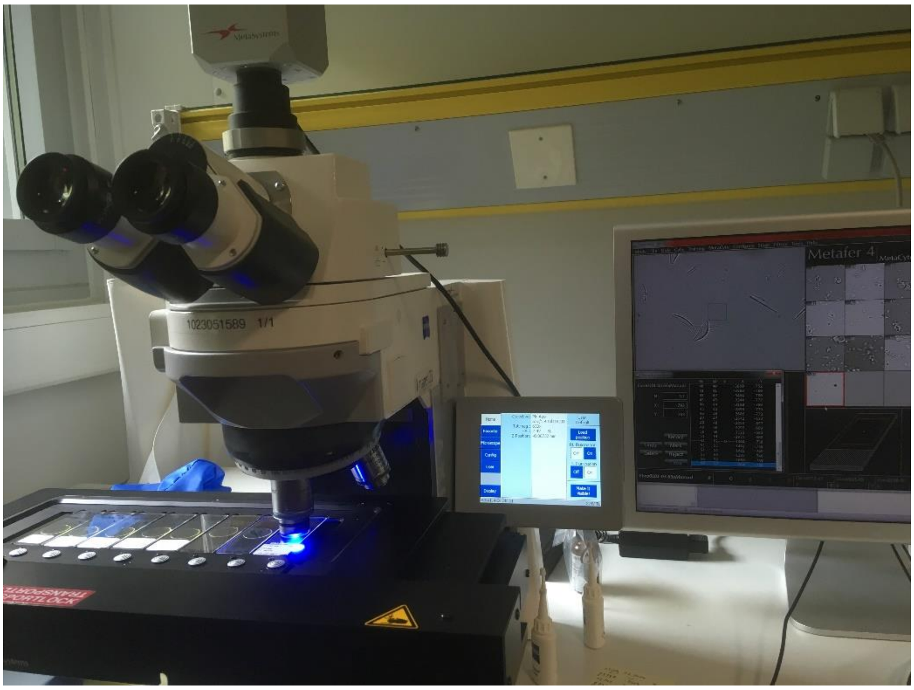
	Q1	**Q2**	**Q3**	**Q4**	**Mean**
Area	0.5684-1.6151	1.6151-1.8013	1.8013-2.0196	2.0196-2.9723	1.8194
Perimeter	1.0282-1.4885	1.4885-1.5861	1.5861-1.7017	1.7017-2.3929	1.595
Length	0.5885-1.1064	1.1064-1.208	1.208-1.3272	1.3272-2.0539	1.2152
Width	0.024-0.5929	0.5929-0.6907	0.6907-0.8655	0.8655-1.4781	0.7275
L/W	1.2E-7-0.3681	0.3681-0.6962	0.6962-1.844	1.844-6.2374	1.1107
Breadth	0.2154-0.4566	0.4566-0.5342	0.5342-0.596	0.596-1.054	0.5464
Surface	1.0667-2.0663	2.0663-2.2394	2.2394-2.4259	2.4259-3.3826	2.248
Volume	1.013-1.9689	1.9689-2.1609	2.1609-2.4102	2.4102-3.7298	2.1797
S/V	08348-0.9963	0.9963-1.0389	1.0389-1.0774	1.0774-1.4032	1.0392

AnnexTable 18 Physicochemical mean parameters and standard deviations per year.

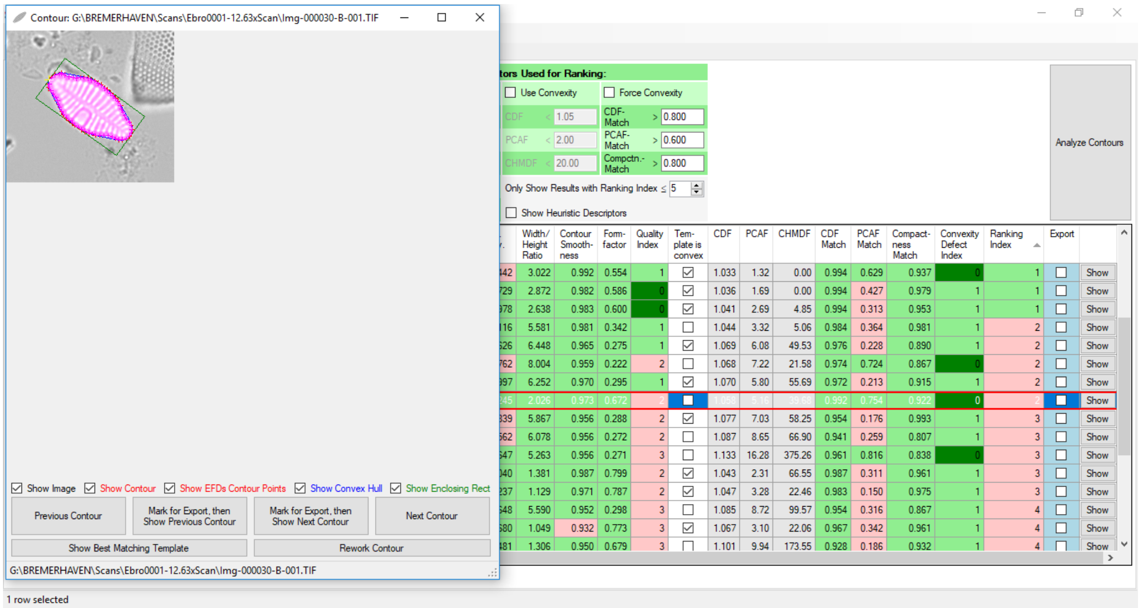
		NH4^{+} (mg/l)	**COND [µS/cm]**	**PO$_4^{3+}$ (mg/l)**	**TP (mg/l)**	**NO$_3^{2+}$ (mg/l)**	**TN KJ (mg/l)**	**N/P**	**O$_2$ (mg/l)**	**pH**	**SS (mg/)]**	**T$_{H2O}$ (ºC)**	**Q (m^3/s)**
2003	AY	0.13	305 ±22.125	<0.05	<0.05	2.1 ±0.141	2.1 ±0.141	45	11.4 ±1.75	8.4 ±0.1	7	10.2 ±5.355	10.25 ±108.5
	EX	0.13 ±0.021	740 ±210.948	0.105 ±0.066	0.105 ±0.066	9.25 ±1.087	1 ±0.05	9.524	9.75 ±1.52	8.2 ±0.127	4.5	16.3 ±6.595	
2005	ME	0.13 ±0.102	443 ±76.662	0.1 ±0.02	0.038 ±0.033	10.3 ±5.471	10.3 ±5.471	271	8.5 ±2.124	8.095 ±0.171	7	11.9 ±7.028	28.52 ±43.223
	VA	0.72 ±0.949	200 ±58.577	0.405 ±0.173	0.18 ±0.067	4.75 ±3.12	1	5.556	11.05 ±2.022	7.9 ±0.26397	12	10.25 ±5.522	3.65 ±2.524
	NT	0.13 ±0.017	426 ±112.656	0.135 ±0.073	0.135 ±0.073	6.35 ±0.88	1	7.407	10.9 ±0.881	8.2 ±0.296	14	16.45 ±5.63	2.9 ±1.95
	AY	0.115 ±0.021	313 ±18.06	0.075 ±0.035	0.075 ±0.035	1.35 ±0.212	1.35 ±0.212	18	11.525 ±1.752	8.26 ±0.094	6	14.2 ±6.3	5.2 ±1.5
	EX	0.1 ±0.017	1170 ±246.251	0.18 ±0.103	0.18 ±0.103	9.4 ±3.537	1	5.556	9.5 ±1.593	8.18 ±0.203	4	16.2 ±6.466	
	VZ	0.05	213	0.05	0.05	0.7			12.55	8.16		6	
2006	ME	0.1 ±0.087	513 ±125.557	0.1	0.033 ±0.005	3.96 ±6.253			8.45 ±1.667	8.1 ±0.142	6.5	18.6 ±5.497	29.59 ±12.188
	JH	0.1	1344.5 ±267.622	0.145 ±0.064	0.145 ±0.064	12.65 ±6.576			8.35 ±1.43	8.11 ±0.119	25.5	13.4 ±5.521	5.645 ±2.573
	VA	0.1 ±0.445	197 ±61.586	0.33 ±0.252	0.131 ±0.114	3.75 ±1.159	1 ±0.548	7.634	9.25 ±1.719	8.275 ±0.263	4.5	9.5 ±5.434	0.575 ±5.741

	NT	0.1	435 ±90.837	0.175 ±0.061	0.175 ±0.061	7.35 ±1.111	1	5.714	10.6 ±1.283	8.2 ±0.273	7.5	14.4 ±4.83	2.9 ±1.988
	AY	0.1	309.5 ±32.414	0.1	0.1	1.75 ±1.06			9.8 ±1.44	8.31 ±0.114	7	13.7 ±4.901	5 ±29.877
	EX	0.1 ±0.021	1057 ±248.223	0.18 ±0.075	0.18 ±0.075	9.55 ±3.358	1	5.556	9.05 ±1.308	8.1 ±0.09	4	21.45 ±5.923	
	VZ	0.1	227	0.05	0.05	0.7			8.5	8.4		5.9	
2007	ME	0.1 ±0.113	533 ±60.129	0.1 ±0.001	0.044 ±0.017	8.2 ±3.671	1	22.727	9.8 ±2.122	8.15 ±0.202	4	16.45 ±5.215	42.27 ±292.744
	JH	0.15 ±0.123	1390.5 ±167.46	0.115 ±0.021	0.063 ±0.023	13.15 ±6.048	1	15.873	8.3 ±1.533	8.1 ±0.268	11	13.2 ±4.472	5.47 ±2.382
	VA	0.1 ±0.001	196 ±59.27	0.25 ±0.2	0.102 ±0.047	4 ±1.992	1	9.804	8.8 ±1.26	8.2 ±0.437	3	9.5 ±5.139	1.015 ±7.077
	NT	0.1	388 ±109.132	0.14 ±0.029	0.0595 ±0.005	6 ±2.382	1	16.807	11.35 ±1.521	8.2 ±0.246	6	11.55 ±3.763	8.96 ±8.835
	AY	0.1	311 ±15.501	0.1 ±0.025	0.033	2.09 ±0.441	1	30.303	10.465 ±1.139	8.45 ±0.174	7	11.75 ±4.917	5.55 ±54.105
	EX	0.1 ±0.047	1104 ±321.744	0.12 ±0.111	0.0495 ±0.002	10.5 ±3.84	1	20.202	8.8 ±1.16	8.2 ±0.159	6	20.5 ±5.873	
	NV	0.1 ±0.058	620 ±208.975	0.1 ±0.029	0.037 ±0.006	3.5 ±1.473	1	27.027	11.2 ±1.293	8.4 ±0.879	2.45	9.5 ±5.622	
	VZ	0.1	242 ±18.903	0.05 ±0.029	0.033	0.375 ±0.263	1	30.303	8.8 ±2.096	8.6 ±0.461	1.5	12.13 ±9.718	
2008	ME	0.1 ±0.153	590 ±114.815	0.1 ±0.033	0.039 ±0.02	7.2 ±4.21	1	25.641	9 ±1.902	7.95 ±0.156	5	14.75 ±5.845	40.43 ±68.553
	VA	0.1 ±0.165	209 ±40.771	0.155 ±0.101	0.07 ±0.046	3.4 ±0.954	1	14.286	8.7 ±1.431	8 ±0.275	4	6.7 ±4.429	2.215 ±8.212
	NT	0.1 ±0.022	433 ±91.485	0.17 ±0.049	0.0575 ±0.002	6.8 ±1.926	1	17.391	10.25 ±1.24	8.1 ±0.169	5	14.3 ±2.934	4.04 ±11.89
	AY	0.1 ±0.022	328 ±10.354	0.1 ±0.013	0.033	1.5 ±0.236	1	30.303	11.3 ±1.589	8.3 ±0.175	19.5	13.4 ±3.928	4.1 ±0.739
	EX	0.1 ±0.024	1205 ±283.856	0.14 ±0.045	0.054 ±0.005	10 ±5.167	1	7.143	8.32 ±1.374	8.1 ±0.202	2.5	20.1 ±5.963	
	VZ	0.1 ±0.025	237.5 ±22.811	0.1 ±0.035	0.033	0.6 ±0.175	1	30.303	9.35 ±1.776	8.4 ±0.18	2	8.65 ±10.367	
2010	VA	0.13	204 ±86.008	0.14 ±0.127	0.095 ±0.007	2.2 ±1.273	1	7.143	8.9 ±0.793	8 ±0.1	18.5	9.5 ±4.419	20.155 ±21.871
	NT	0.13	367 ±48.667	0.07	0.09	3.35 ±0.636	2.5	27.778	9.93 ±0.512	8.25 ±0.058	5.5	16 ±3.453	16.15 ±13.302
	AY	0.13	293 ±12.503	0.05	0.095 ±0.007	1.45 ±0.212	2.5	26.316	10.3 ±0.833	8.3 ±0.273	6.5	13.8 ±2.706	33.1 ±40.87
	EX	0.13 ±0.046	846 ±275.423	0.14 ±0.072	0.09	9.65 ±2.434	1.75 ±0.866	19.444	8.96 ±1.406	8.2 ±0.21	5	18.7 ±4.972	
	NV	0.13	547 ±97.439	0.095 ±0.0495	0.09	2.45 ±0.636	1	11.111	10.4 ±0.775	8.4 ±0.141	5.5	11.1 ±4.203	
	VZ	0.13	239 ±6.439	0.05	0.09	1	2.5	27.778	8.7 ±1.562	8.4 ±0.164	5	18.1 ±3.398	
2011	ME	0.13 ±0.053	478.5 ±131.894	0.05	0.09 ±0.018	2.6 ±2.763	1.62 ±0.729	18	7.25 ±1.688	7.95 ±0.276	5	17.9 ±5.196	22.39 ±73.242
	VA	0.13	205 ±52.519	0.1 ±0.076	0.09	4 ±1.389	1	11.111	9.475 ±1.53	8.55 ±0.387	5	12 ±4.586	0.52 ±7,137
	NT	0.13	351 ±71.468	0.13 ±0.038	0.09	3.55 ±0.785	1.4 ±0.777	15.555	9.47 ±1.215	8.4 ±0.159	5	16.2 ±4.407	13.11 ±4.986

	AY	0.13	310 ±17.086	0.05	0.09 ±0.035	1.5 ±0.306	1.75 ±1.061	19.444	10.35 ±1.776	8.4 ±0.198	5	15.5 ±7.685	4.2 ±19.131
	EX	0.13 ±0.053	1049 ±258.912	0.165 ±0.059	0.09 ±0.006	7.75 ±3.051	1 ±0.716	11.111	9.3 ±1.342	8.2 ±0.203	5	17.6 ±6.106	
	VZ	0.13	227 ±39.582	0.05 ±0.085	0.09 ±0.0369	1	1 ±0.75	11.111	8.64 ±1.595	8.5 ±0.413	5	15.3 ±9.118	
2012	ME	0.14 ±0.025	412 ±83.128	0.05 ±0.03	0.05 ±0.116	1.5 ±1.049	1	20	7.4 ±2.014	7.95 ±0.25	6	18.35 ±6.85	28.03 ±46.152
	EX	0.13 ±0.035	1052 ±231.249	0.08 ±0.054	0.07 ±0.11	7.7 ±3.195	1 ±0.035	14.286	8.7 ±1.622	8.1 ±0.159	5	19.8 ±6.675	
	NV	0.13	562 ±71.931	0.075 ±0.0925	0.05 ±0.025	2.8 ±0.914	1	20	10 ±0.683	8.5 ±0.121	5	11.8 ±2.937	
2013	ME	0.13 ±0.01	449.5 ±77.225	0.05 ±0.045	0.05 ±0.06	5.7 ±3.252	1 ±0.05	20	9.9 ±1.559	8.05 ±0.297	5	11.7 ±5.348	49.665 ±110.374
	JH	0.32 ±0.191	1297 ±172.543	0.05 ±0.006	0.05	14.6 ±1.721	1.1 ±0.141	22	10.8 ±1.522	8.05 ±0.225	11	9.15 ±4.318	5.19 ±2.379
	NV	0.13	466 ±89.985	0.08 ±0.028	0.08 ±0.042	2.65 ±0.495	1	12.5	9.93 ±0.707	8.4 ±0.252	5	11 ±3.119	
	VZ	0.13	199.5 ±33.234	0.05	0.05	1	1	20	9.78 ±2.715	8.115 ±0.544	5	12.3 ±4.95	



AnnexFigure 8 Zeiss Axio M2 Microscope attached to Metafer system as an automatic slidescanner.



Contour: G:\BREMERHAVEN\Scans\Ebro0001-12.63xScan\Img-000030-B-001.TIF

☑ Show Image ☑ Show Contour ☑ Show EFDs Contour Points ☑ Show Convex Hull ☑ Show Enclosing Rect

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tors Used for Ranking:

☐ Use Convexity ☐ Force Convexity

CDF < 1.05 CDF-Match > 0.800

PCAF < 2.00 PCAF-Match > 0.600

CHMDF < 20.00 Compctn.-Match > 0.800

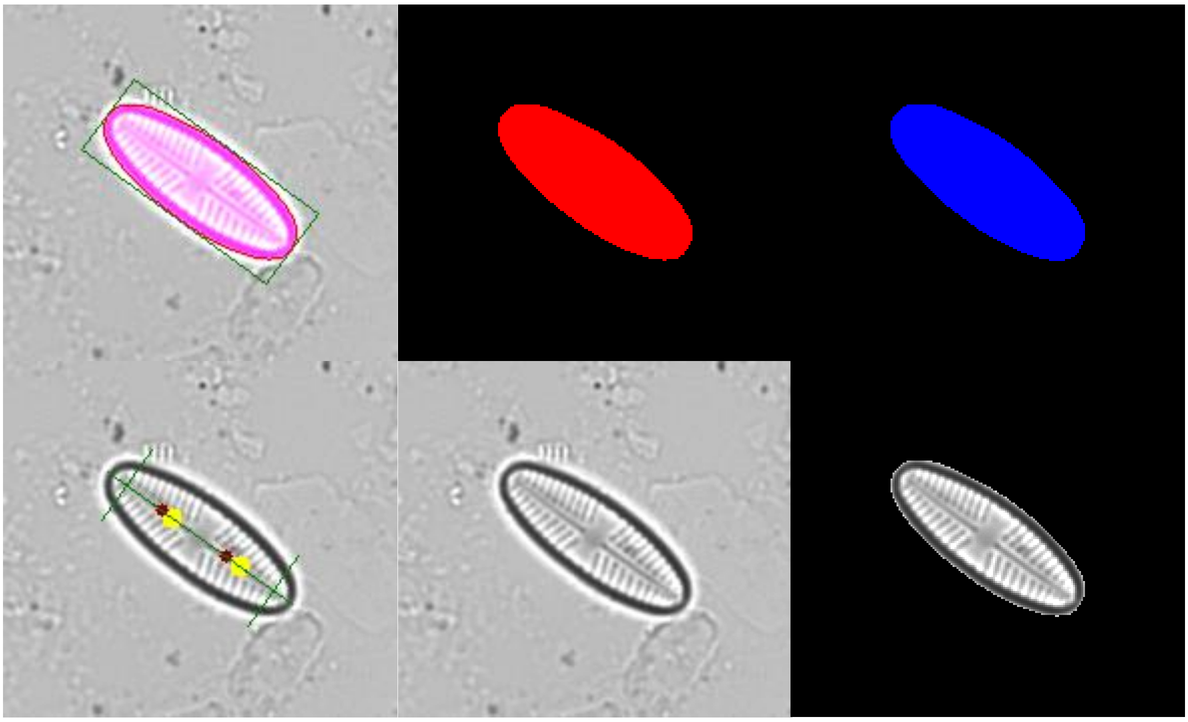
Only Show Results with Ranking Index ≤ 5

☐ Show Heuristic Descriptors

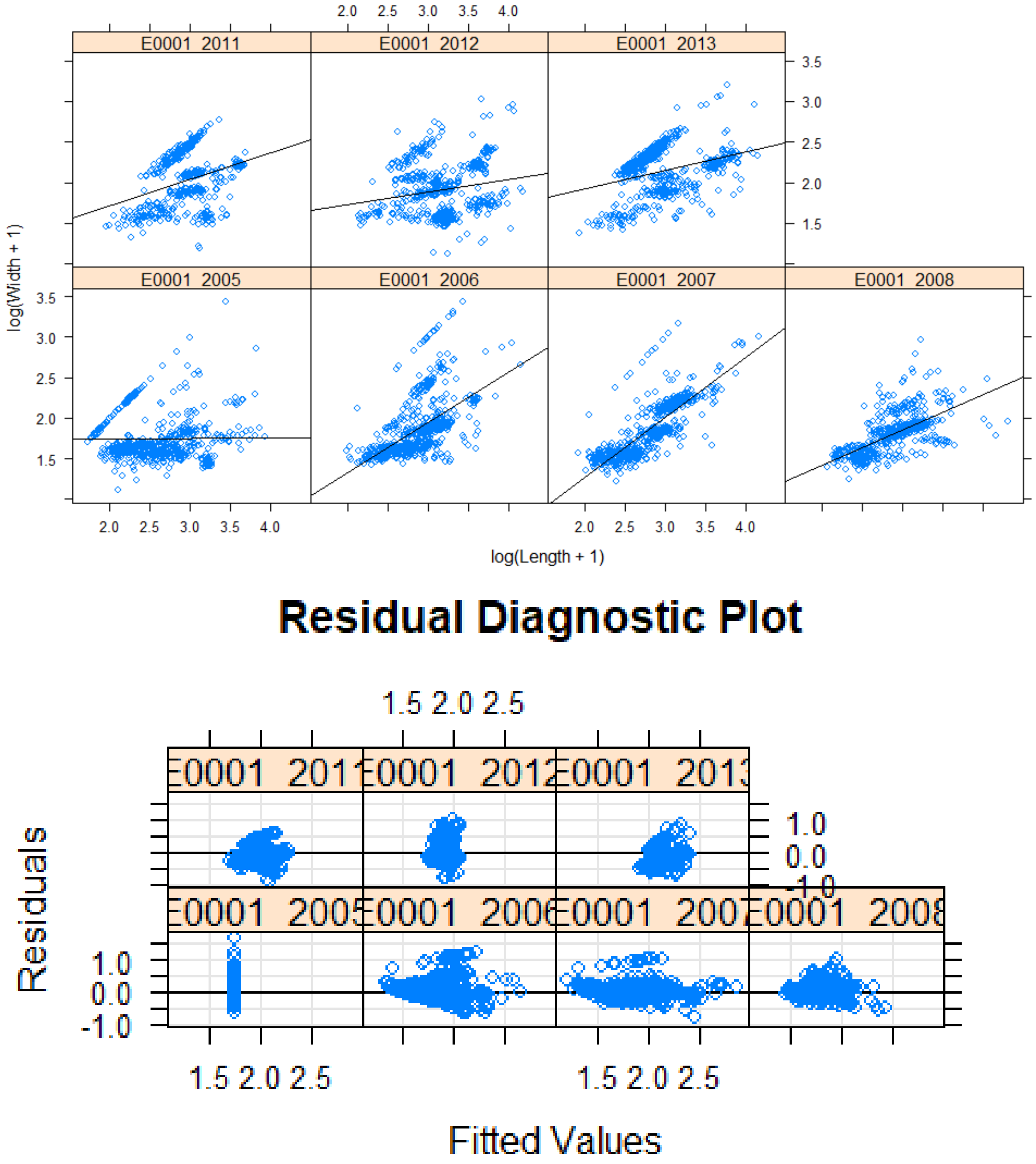
	Width/ Height Ratio	Contour Smooth-ness	Form-factor	Quality Index	Tem-plate is convex	CDF	PCAF	CHMDF	CDF Match	PCAF Match	Compact-ness Match	Convexity Defect Index	Ranking Index	Export	
42	3.022	0.992	0.554	1	☑	1.033	1.32	0.00	0.994	0.629	0.937	0	1	☐	Show
729	2.872	0.982	0.586	0	☑	1.036	1.69	0.00	0.994	0.427	0.979	1	1	☐	Show
978	2.638	0.983	0.600	0	☑	1.041	2.69	4.85	0.994	0.313	0.953	1	1	☐	Show
16	5.581	0.981	0.342	1	☐	1.044	3.32	5.06	0.984	0.364	0.981	1	2	☐	Show
26	6.448	0.965	0.275	1	☑	1.069	6.08	49.53	0.976	0.228	0.890	1	2	☐	Show
762	8.004	0.959	0.222	2	☐	1.068	7.22	21.58	0.974	0.724	0.867	0	2	☐	Show
97	6.252	0.970	0.295	1	☑	1.070	5.80	55.69	0.972	0.213	0.915	1	2	☐	Show
245	2.026	0.973	0.672	2	☐	1.058	5.16	39.68	0.992	0.754	0.922	0	2	☐	Show
339	5.867	0.956	0.288	2	☑	1.077	7.03	58.25	0.954	0.176	0.993	1	3	☐	Show
62	6.078	0.956	0.272	2	☐	1.087	8.65	66.90	0.941	0.259	0.807	1	3	☐	Show
47	5.263	0.956	0.271	3	☐	1.133	16.28	375.26	0.961	0.816	0.838	0	3	☐	Show
40	1.381	0.987	0.799	2	☑	1.043	2.31	66.55	0.987	0.311	0.961	1	3	☐	Show
237	1.129	0.971	0.787	2	☑	1.047	3.28	22.46	0.983	0.150	0.975	1	3	☐	Show
48	5.590	0.952	0.298	3	☐	1.085	8.72	99.57	0.954	0.316	0.867	1	4	☐	Show
80	1.049	0.932	0.773	3	☑	1.067	3.10	22.06	0.967	0.342	0.961	1	4	☐	Show
481	1.306	0.950	0.679	3	☐	1.101	9.94	173.55	0.928	0.186	0.932	1	4	☐	Show

1 row selected

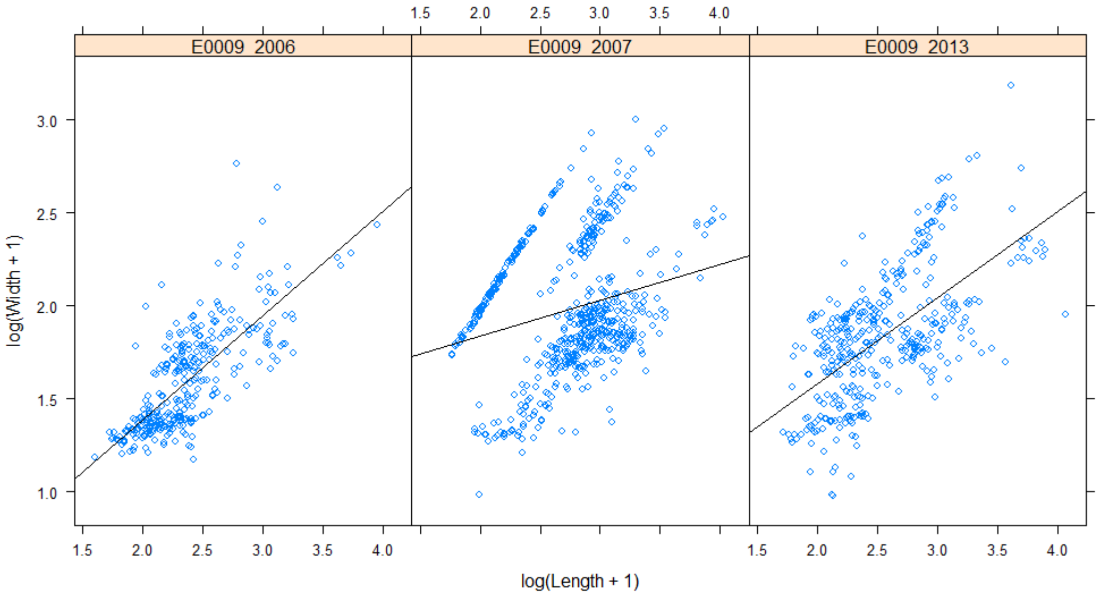
AnnexFigure 9 Example of Sherpa Output

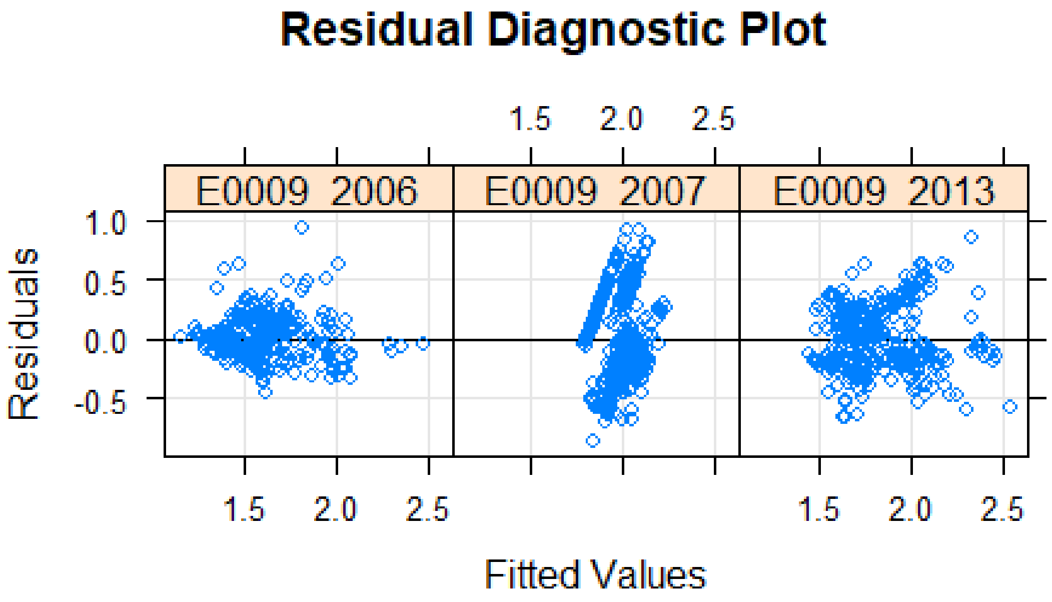


AnnexFigure 10 Example of the Sherpa output and measurement of the diatom *Sellaphora nigri/ Eolimna minima*.

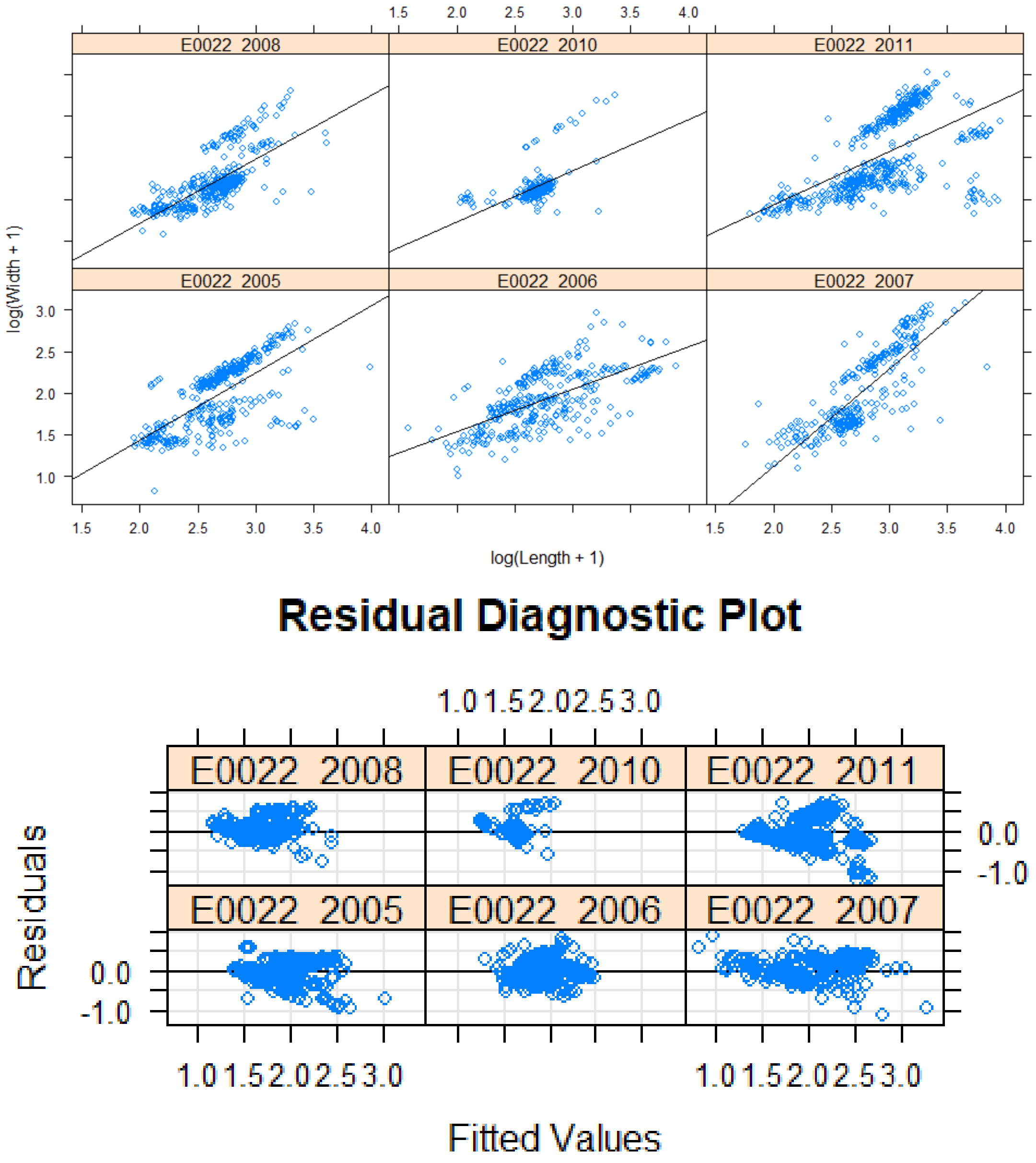


AnnexFigure 11 General Linear model of Width over Length (log(x+1)) of the Ebro in Miranda site. Heterosedasticity and bias.

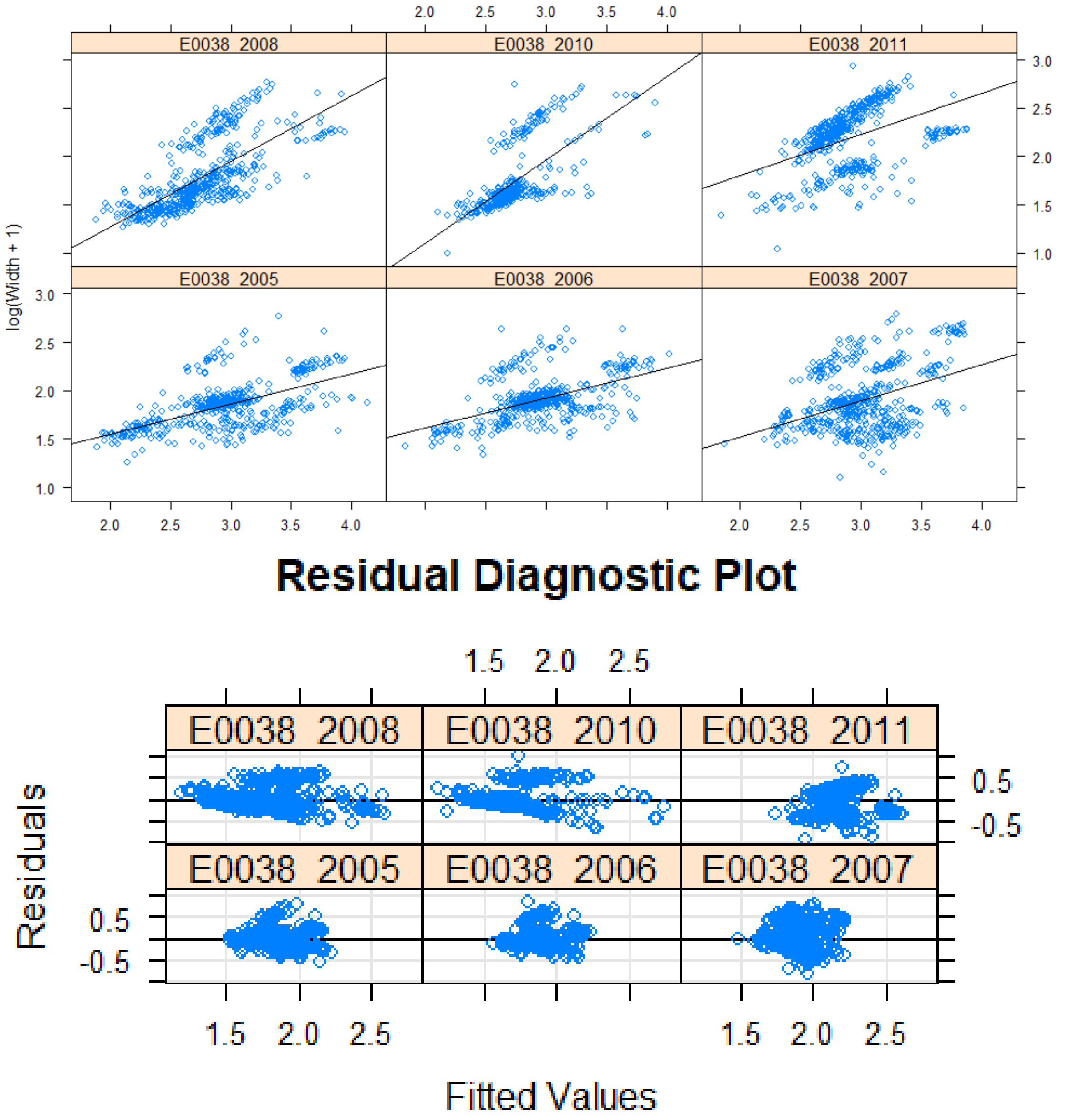




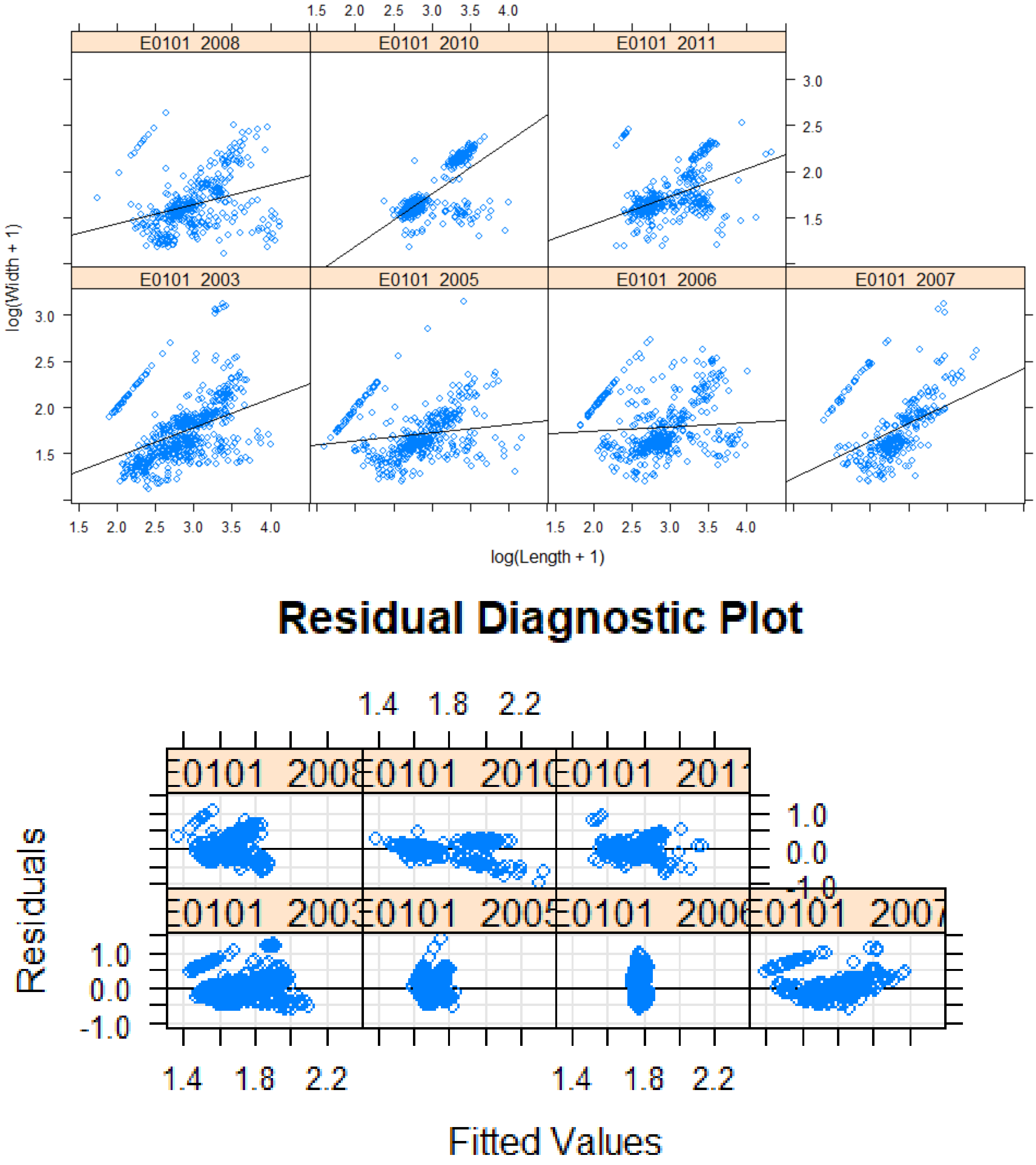
AnnexFigure 12 General Linear model of Width over Length (log(x+1)) of the Jalón in Huérmeda site. Heterosedasticity and bias.



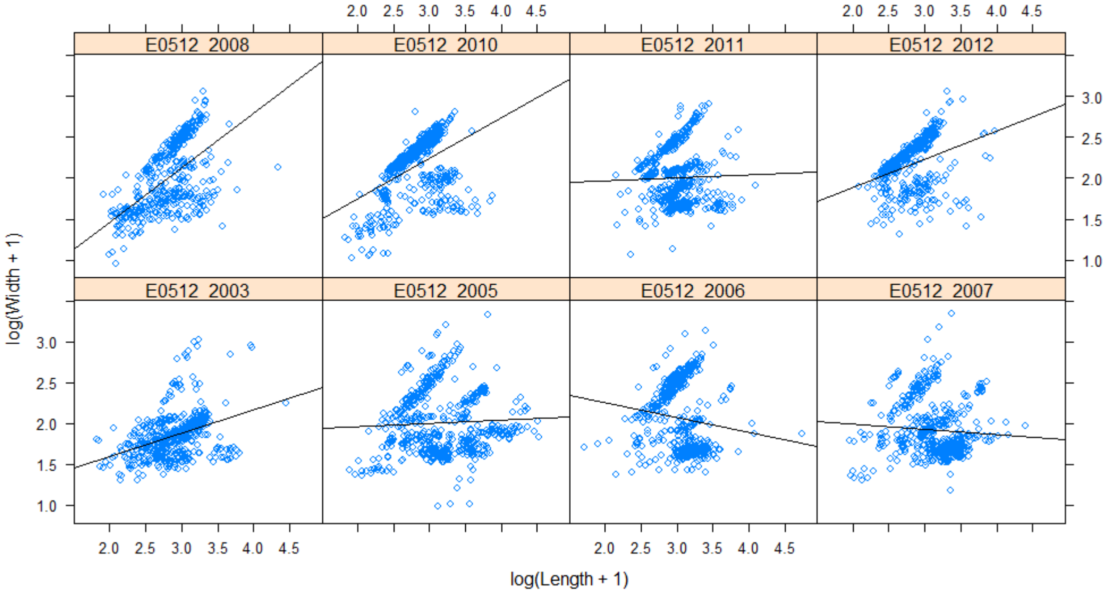
AnnexFigure 13 General Linear model of Width over Length (log(x+1)) of the Valira in Anserall site. Heterosedasticity and bias.

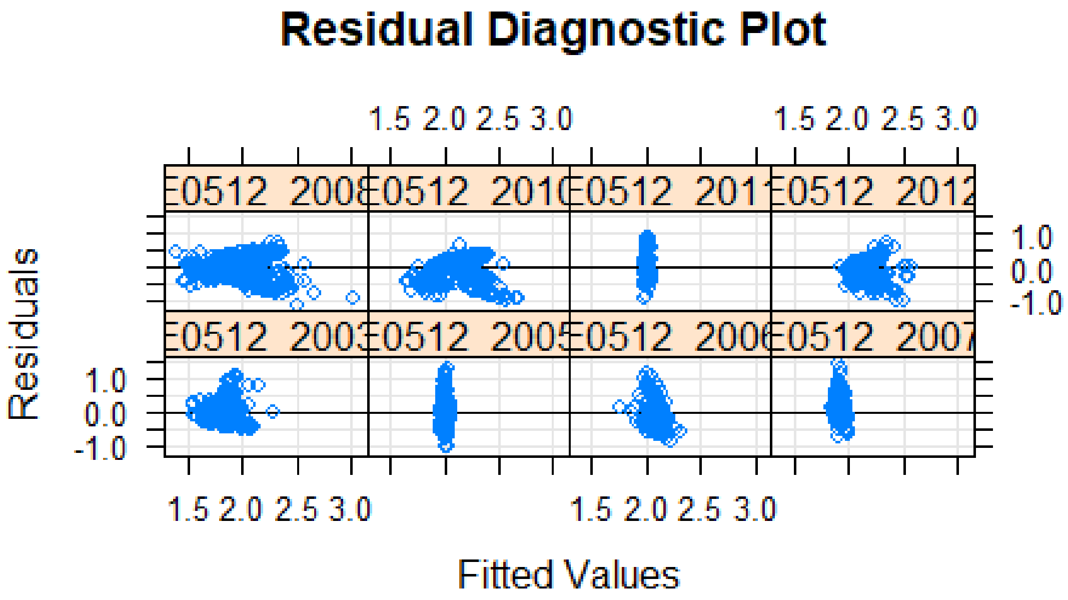


AnnexFigure 14 General Linear model of Width over Length (log(x+1)) of the Najerilla in Torremontalbo site. Heterosedasticity and bias.

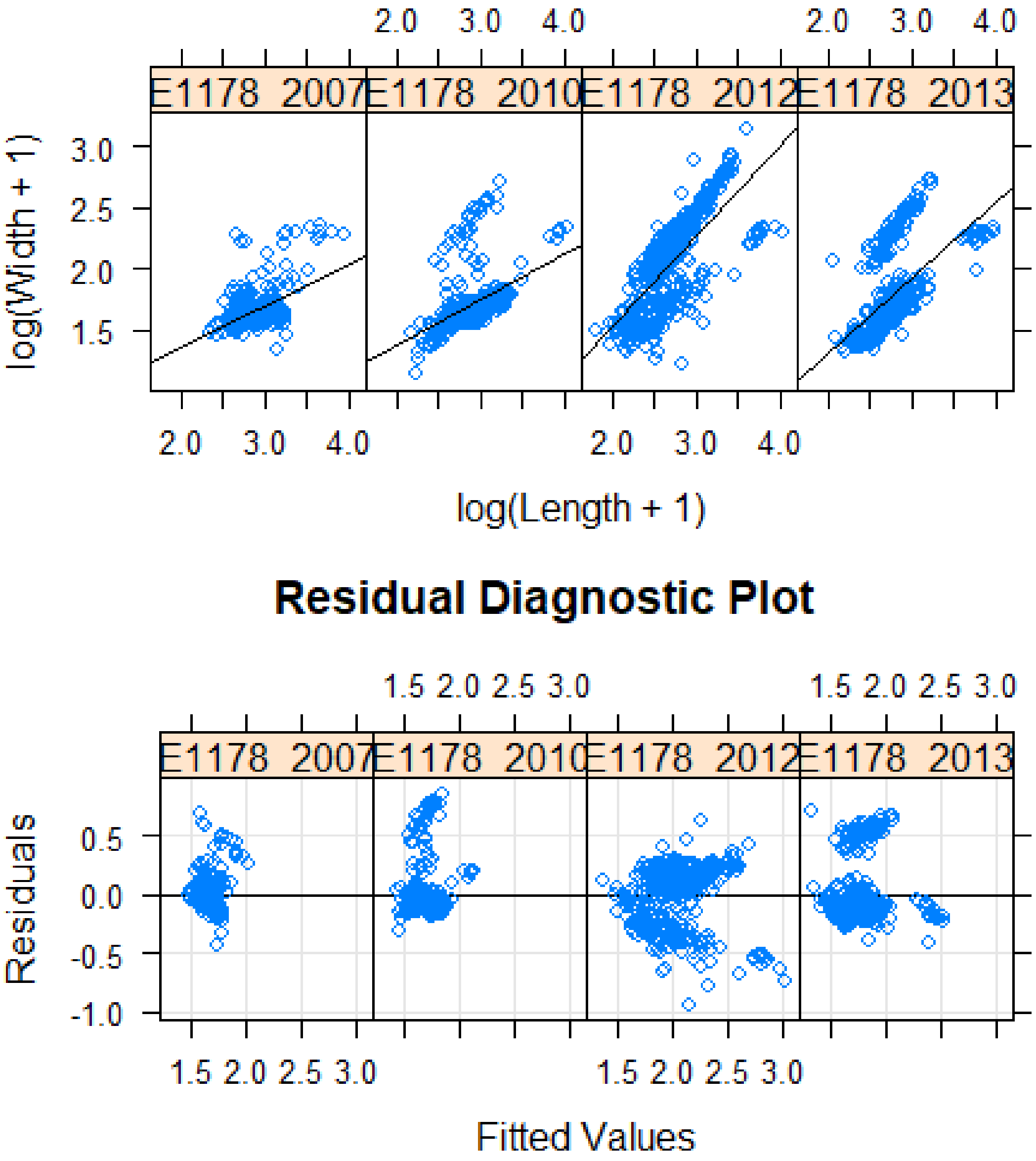


AnnexFigure 15 General Linear model of Width over Length (log(x+1)) of the Aragón in Yesa site. Heterosedasticity and bias.

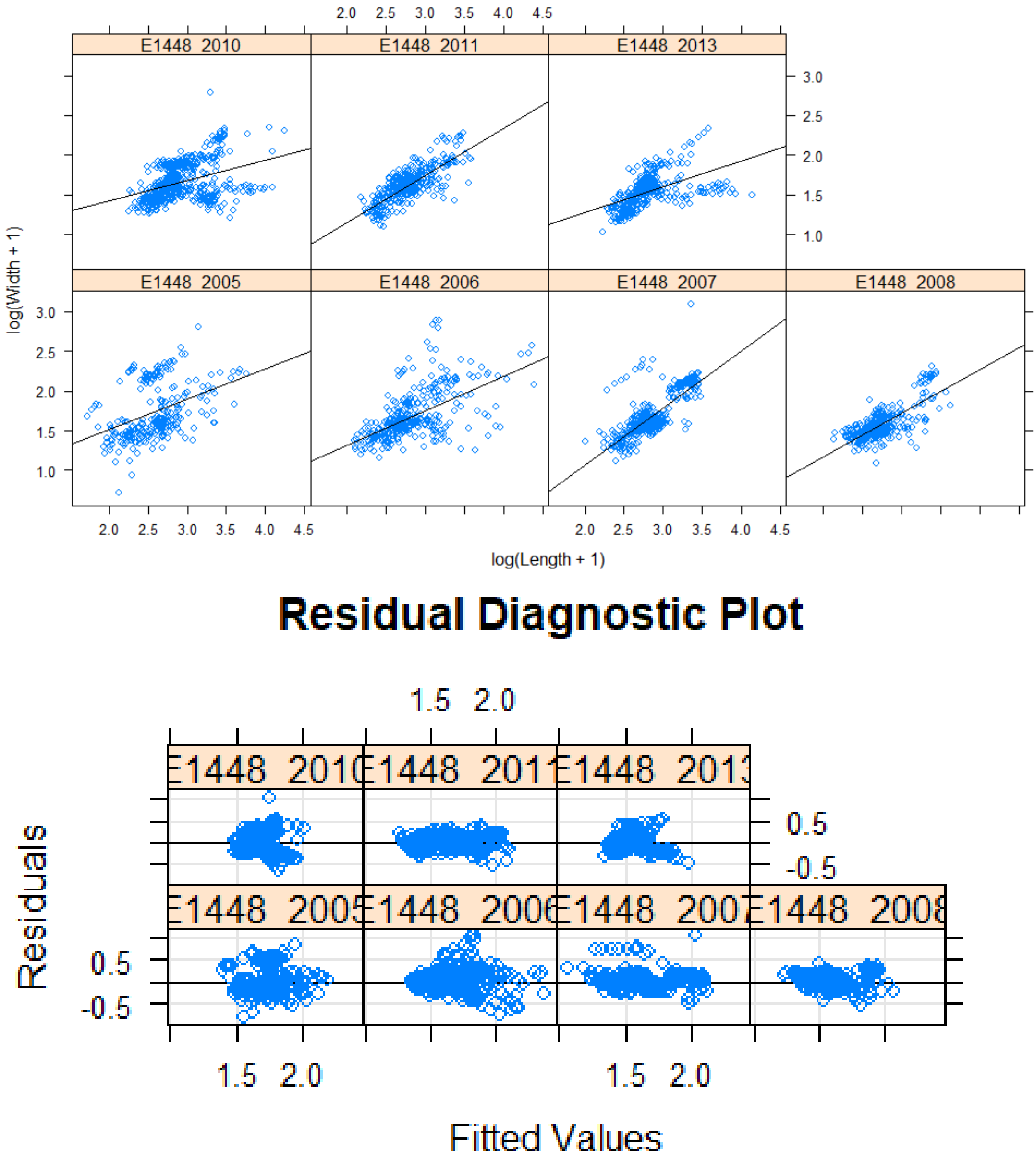




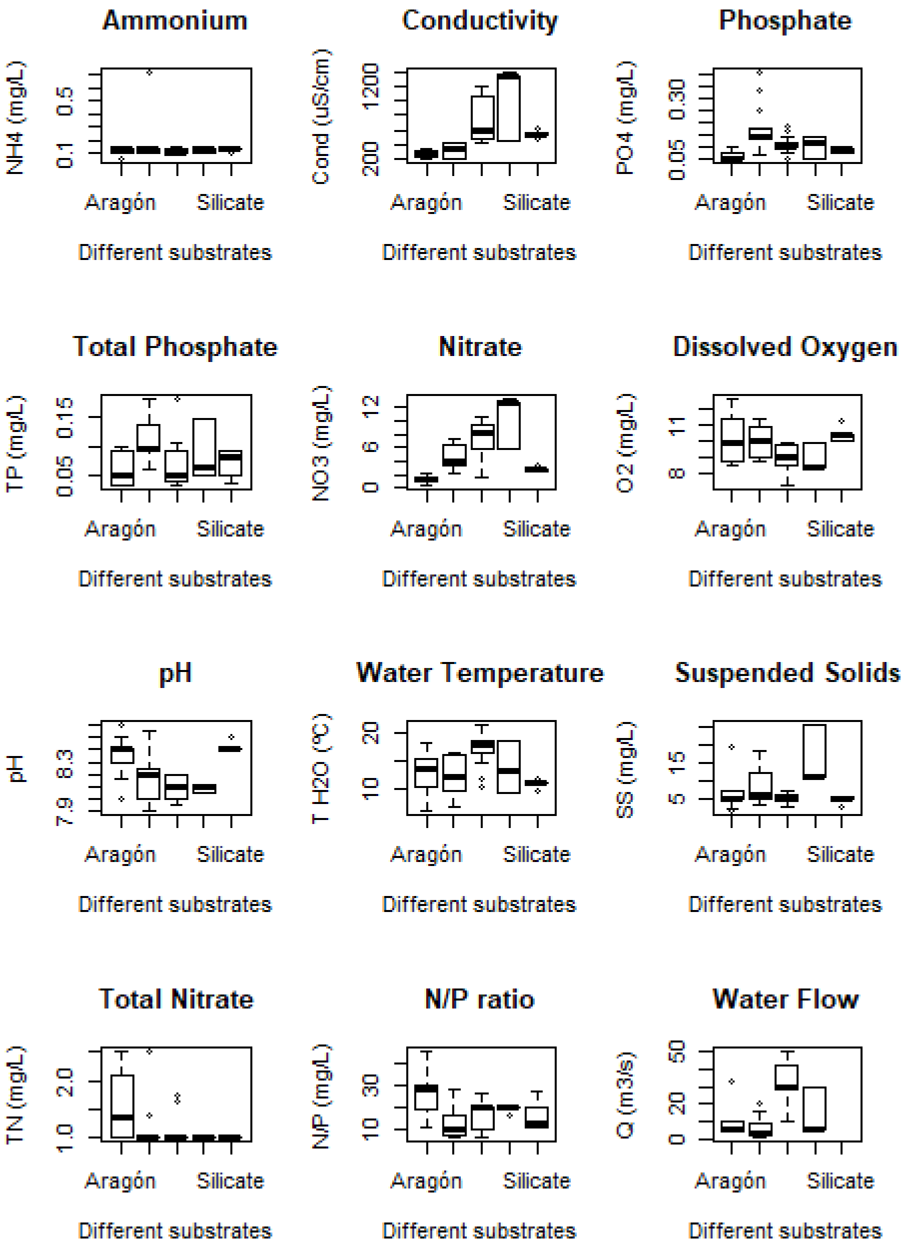
AnnexFigure 16 General Linear model of Width over Length (log(x+1)) of the Ebro in Xerta site. Heterosedasticity and bias.



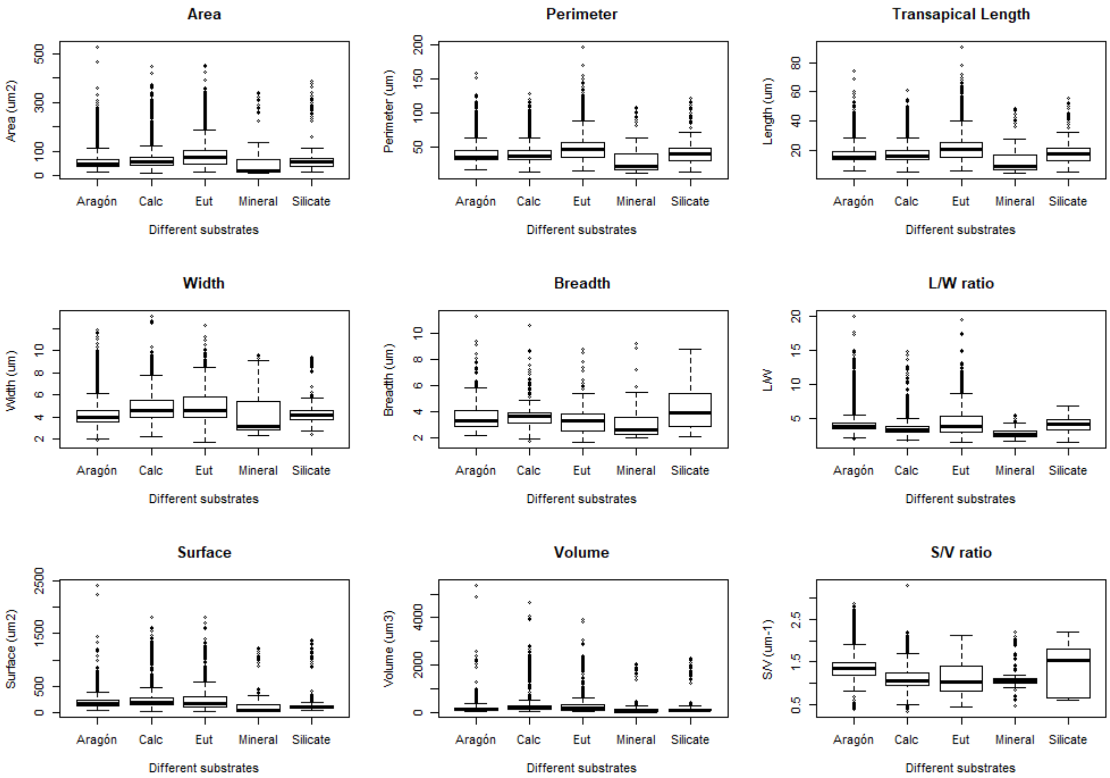
AnnexFigure 17 General Linear model of Width over Length (log(x+1)) of the Najerilla in Villavelayo site. Heterosedasticity and bias.



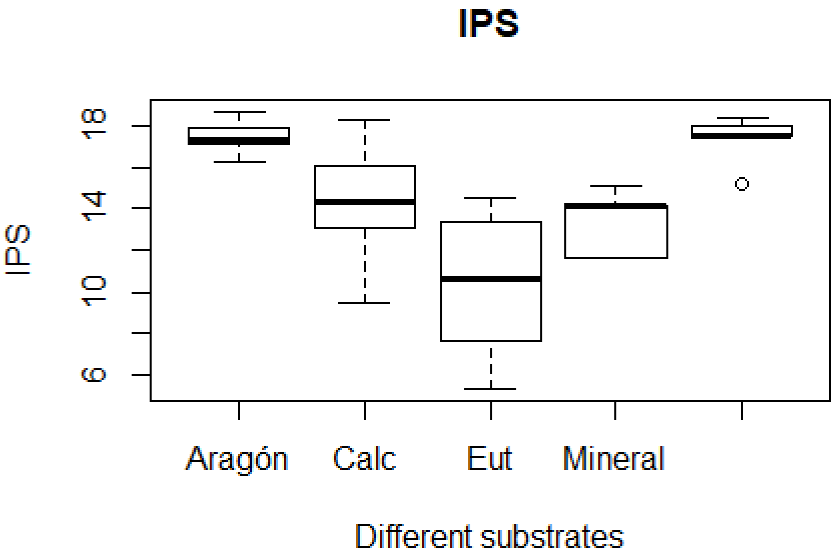
AnnexFigure 18 General Linear model of Width over Length (log(x+1)) of the Veral in Zuriza site. Heterosedasticity and bias.



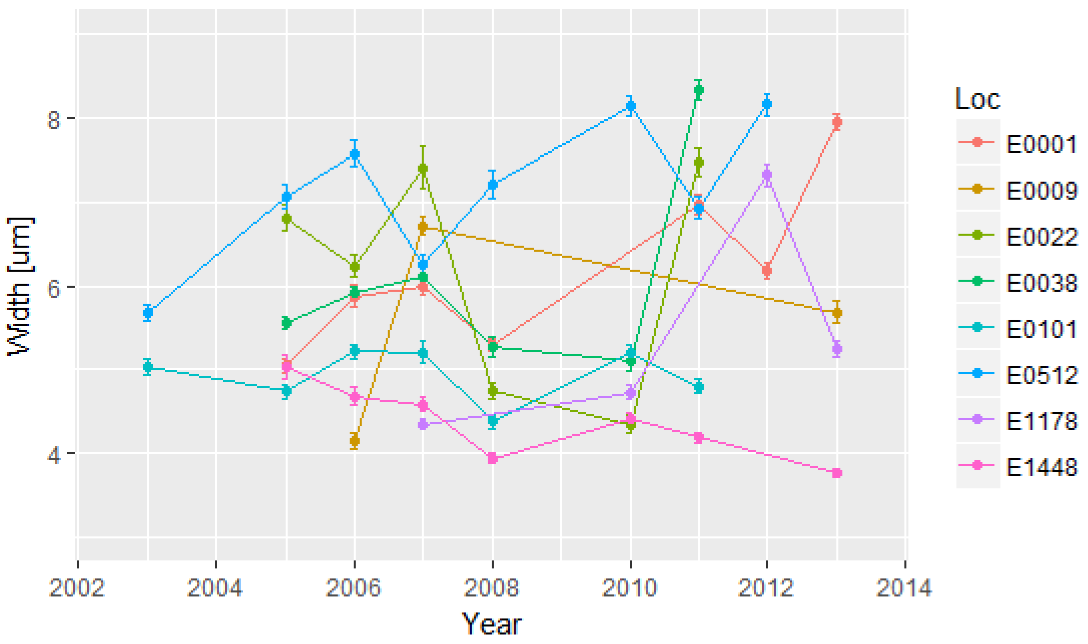
AnnexFigure 19 Comparison of physicochemical composition of similar clusters



AnnexFigure 20 Comparison of diatom sizes for each cluster.



AnnexFigure 21 Clear division of substrates and IPS values



AnnexFigure 22 Width means and confidence interval of the sites. Note similarities between E0022 and E0038, E0101 and E1448, and E0001 and E0512.

Annex chapter 9: Resumen de la tesis

AnnexTable 19 Definición de las formas de vida y grupos funcionales de las diatomeas. Se sigue la subdivisión de Riato y colaboradores 2017 y B-Béres et al 2017 (Riato et al. 2017; B-Béres et al. 2017)

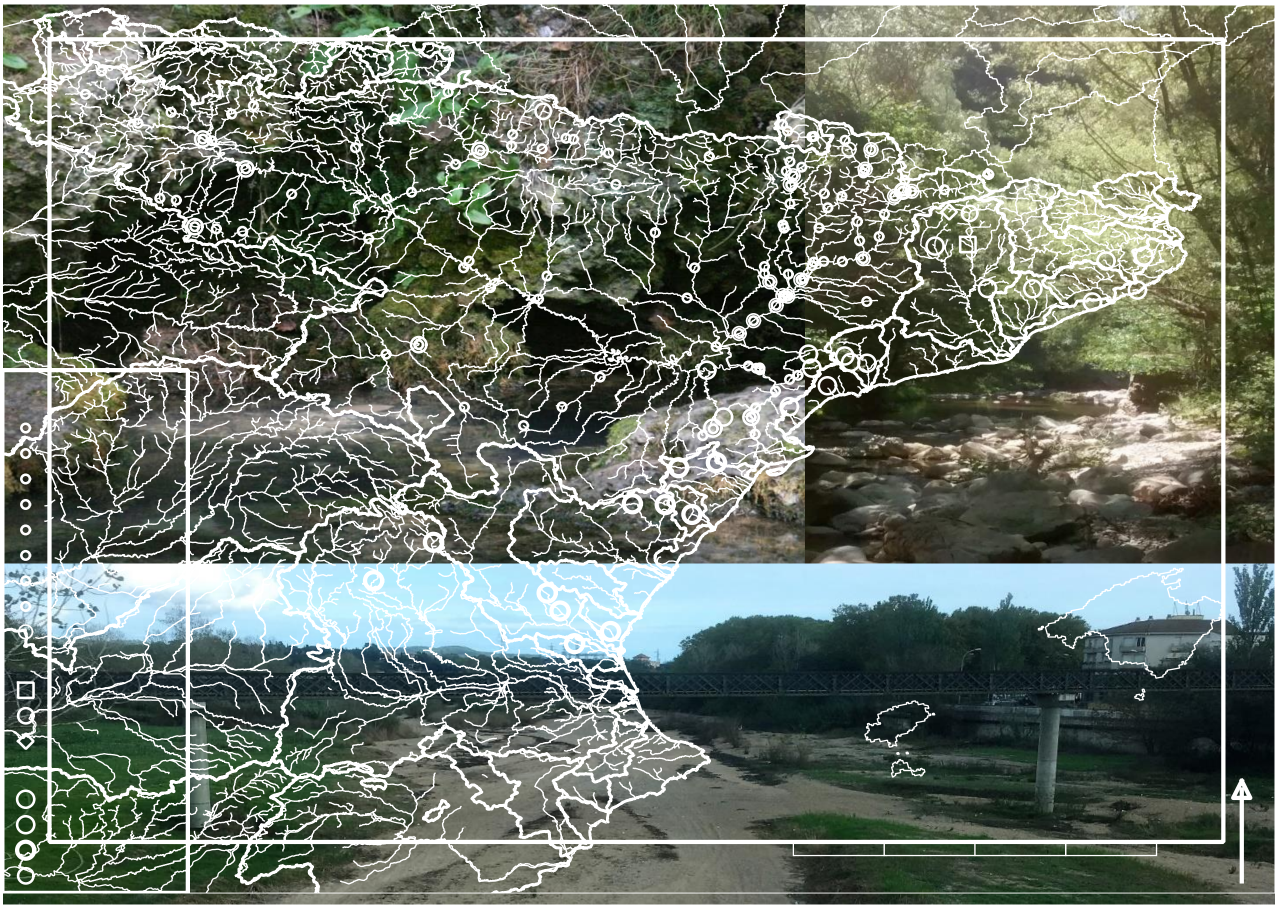
Forma De Vida	Descripción	Géneros
Móviles	Diatomeas capaces de moverse en cualquier dirección en la columna de agua.	*Achnanthes, Achnanthidium, Adlafia, Amphipleura, Amphora, Aneumastus, Anomoeneis, Bacillaria, Berkeleya, Biremis, Brachysira, Caloneis, Campylodiscus, Catenula, Cavinula, Chamaepinnularia, Cocconeis, Craticula, Cymatopleura, Cymbella, Cymbopleura, Delicata, Denticula, Diadesmis, Diploneis, Encyonema, Encyonopsis, Entomoneis, Epithemia, Eucocconeis, Eunotia, Fallacia, Fistulifera, Frustulia, Geissleria, Gomphoneis, Gomphonema, Gomphosphenia, Gyrosigma, Hantzschia, Haslea, Hippodonta, Karayevia, Kobayasiella, Kolbesia, Lemnicola, Luticola, Mastogloia, Mayamaea, Microcostatus, Navicula, Naviculadicta, Neidium, Nitzschia, Nupela, Parlibellus, Pinnularia, Placoneis, Planothidium, Platesssa, Psammothidium, Pulchella, Reimeria, Rhopalodia, Rossithidium, Sellaphora, Simonsenia, Stauroneis, Stenopterobia, Surirella, Tryblionella.*
Pioneras	Primeras colonizadoras de substrato.	*Achnanthes, Achnanthidium, Amphora.*

Tubos Mucilaginosos	Tubos extruidos por las células que permiten el movimiento de las diatomeas individuales en su interior.	*Amphipleura, Berkeleya, Cymbella, Delicata, Encyonema, Frustulia, Muelleria, Parlibellus.*
Adhesiones Valvares	Colonias de células adheridas con mucílago o estructuras silícicas valva con valva. Forman cintas y filamentos.	*Achnanthidium, Aulacoseira, Diadesmis, Ellerbeckia, Eunotia, Fragilaria, Melosira, Pseudostaurosira, Skeletonema, Stauroforma, Staurosira, Staurosirella, Synedra, Synedrella.*
Adhesiones Polares	Colonias de células adheridas con mucílago o estructuras silícicas con los polos. Forman rosetas, formas estrelladas y cintas en zig-zag.	*Asterionella, Diatoma, Fragilaria, Meridion, Opephora, Pleurosira, Staurosira, Tabellaria, Ulnaria.*
Pedúnculos	Formas de adhesión al sustrato de células individuales o colonias que forman estructuras más o menos complejas. Tallos, colonias arbusculares, pedúnculos.	*Achnanthes, Achnanthidium, Ctenophora, Cymbella, Diatoma, Didymosphenia, Encyonopsis, Eucocconeis, Fragilaria, Gomphoneis, Gomphonema, Gomphosphenia, Karayevia, Kobayasiella, Lemnicola, Licmophora, Meridion, Opephora, Platessa, Psammothidium, Pseudostaurosira, Reimeria, Rhoicosphenia, Rossithidium, Stauroforma, Staurosira, Staurosirella, Synedra, Synedrella, Tabularia, Ulnaria.*
Adnatas	Adhesión al sustrato por la valva.	*Achnanthes, Achnanthidium, Amphora, Cocconeis, Diploneis, Fragilaria, Kobayasiella, Kolbesia, Planothidium, Platessa, Psammothidium, Reimeria, Rhopalodia.*
Perfil Alto	Diatomeas de estructuras altas con dificultad de mantenerse bajo flujo alto y que acepta grados variables de eutrofia.	1 *Achnanthidium catenatum, Berkeleya fennica, Cymbella perpusilla, Diadesmis pirenaica, Eunotia exigua, Fragilaria oldenburgiana, Fragilaria perminuta, Gomphonema pseudobohemicum, Gomphonema pseudotenellum, Gomphosphenia, Pseudostaurosira parasítica var. Subconstruens, Staurosira berolinensis, Staurosira elliptica, Synedrella subconstricta.*
		2 *Amphipleura rutilans, Berkeleya rutilans, Delicata delicatula, Diadesmis, Encyonema, Eunotia, Fragilaria, Gomphonema, Pseudostaurosira, Stauroforma, Staurosira, Staurosirella.*
		3 *Cymbella, Delicata, Diadesmis, Diatoma moniliformis, Encyonema, Eunotia, Fragilaria, Frustulia creuzburgensis, Gomphoneis calcifuga, Gomphonema, Parlibellus bennikei, Staurosira, Tabularia fasciculata.*
		4 *Ctenophora pulchella, Cymbella caespitosa, Diatoma anceps, D. Mesodon, D. Problematica, Encyonema, Eunotia, Fragilaria martyi, Frustulia spicula, Gomphonema, Gomphosphenia lingulatiformis, Muelleria gibbula, Opephora mutabilis, Parlibellus protracta,*

			Staurosira leptostauron, Staurosirella dubia, S. martyi, Tabellaria flocculosa, T. ventricosa.
		5	*Achnanthes, Amphipleura, Berkeleya scopulorum, Cymbella, Cymbopleura, Diatoma, Didymosphenia, Diploneis, Ellerbeckia, Encyonema prostratum, E. triangulum, Eucocconeis flexella, Eunotia, Fragilaria, Frustulia, Gomphoneis, Gomphonema, Licmophora gracilis var. Gracilis, Melosira, Parlibellus crucicula, Pleurosira laevis, Synedra fasciculata, Ulnaria bíceps, Ulnaria capitata, Ulnaria ulna.*
Perfil Bajo	Diatomeas de estructuras bajas y movimiento lento. Les permite mantenerse en flujo alto, pero son sensibles ante cambios tróficos.	1	*Achnanthes, Achnanthidium, Amphora pediculus, Brachysira microcephala, Cocconeis neothumensis, Encyonopsis, Karayevia amoena, Planothidium, Platessa, Psammothidium, Rossithidium pusilllum.*
		2	*Achhnanthes, Achnanthidium, Amphora inariensis Brachysira, Cocconeis neodiminuta, Cyclostephanos, Cymbella, Diploneis, Encyonema minutum, Encyonopsis falaisensis, Eucocconeis diluviana, Karayevia, Kobayasiella, Kolbeisa, Nupela lapidosa, Planothidium, Platessa, Psammothidium, Rossithidium.*
		3	*Achnanthidium bioretii, A. daonense, A. helveticum, Amphora aequalis, A. oligotraphenta, Biremis motschii, Cocconeis disculus, C. pseudothumensis, Cymbella, Encyonopsis microcephala, Eucocconeis laevis, Karayevia, Kolbesia kolbei, Lemnicola hungarica, Meridion circulare var. Constrictum, Planothidium, Psammothidium, Reimeria, Rhoicosphenia.*
		4	*Amphora montana, Amphora tenerrima, Cocconeis placentula complex, Cymbella, Cymbopleura, Diploneis, Encyonopsis cesatii, Fragilaria arcus, Karayevia laterostrata, Meridion circulare var. circulare, Planothidium oestrupii.*
		5	*Amphora, Cocconeis, Cymbopleura subcuspidata.*
Perfil Móvil	Diatomeas de movimiento horizontal rápido.	1	*Adlafia, Chamaepinnularia, Craticula, Denticula, Fallacia, Fistulifera, Hoppodonta, Mayamaea, Microcostatus, Navicula, Naviculadicta, Nitzschia, Nupela, Sellaphora, Simonsenia, Stauroneis.*
		2	*Adlafia bryophila, Coloneis fontinalis, Cavinula, Craticula, Fallacia, Geissleria, Hippodonta, Luticola, Mayamaea, Microcostatus, Navicula, Naviculadicta, Neidium, Nitzschia, Nupela, Pinnularia Pulchella, Sellaphora hustedtii, Stauroneis, Tryblionella debilis.*
		3	*Caloneis, Cavinula, Craticula accomoda, Denticula, Entomoneis pseudoduplex, Fallacia helensis, Geissleria, Hantzschia amphioxys,*

			Hippodonta, Luticola, Navicula, Naviculadicta, Neidium, Nitzschia, Pinnularia, Sellaphora mutatoides, Stauroneis, Surirella terrícola.
		4	*Caloneis, Craticula halophila, Fallacia, Hantzschia abundans, Haslea spicula, Luticola, Mastogloia, Navicula, Neidium, Nitzschia, Pinnularia, Placoneis, Sellaphora, Stauroneis, Steopterobia delicatissima, Surirella, Tryblionella.*
		5	*Aneumastus, Anomoeneis, Bacillaria, Caloneis, Campylodiscus hibernicus, Craticula, Cymatopleura, Entomoneis, Epithemia, Gyrosigma, Navicula, Neidium, Nitzschia, Pinnularia, Placoneis placentula f. rostrata, Rhopalodia, Sellaphora, Stauroneis phoeniteceron, Surirella, Tryblionella.*
Perfil Planctónico	Diatomeas que flotan en ambientes lénticos y sedimentan en lóticos sobre las bentónicas y son muestreadas con ellas.	1	*Aulacoseira subborealis, Catenula, Cyclotella, Discostella, Fragilaria gracilis, Nitzschia, Stephanodiscus vestibulis, Thalassiosira pseudonana.*
		2	*Aulacoseira, Cyclostephanos invisitatus, Cyclotella, Fragilaria, Nitzschia, Skeletonema potamos, Stephanodiscus minutulus, S. parvus, Thalassiosira visurgis.*
		3	*Asterionella Formosa, Aulacoseira, Cyclotella, Fragilaria, Stephanodiscus, Thalassiosira, Ulnaria.*
		4	*Aulacoseira, Cyclostephanos dubius, Cyclotella, Diatoma elongatum, D. tenuis, Discostella, Puncticulata, Stephanodiscus alpinus.*
		5	*Actinocyclus normanii, Arachnoidiscus ornatus, Auñacoseira, Cyclotella, Praetriceratium incospicuum, Puncticulata bodanica, Stephanodiscus neoastraea, Thalassiosira.*

Tamaños de EG: *0 μm ≤1<100μm≤2<300 μm≤3<600 μm≤4<1500 μm≤5*



Benthic diatoms, silicate plated, aquatic, unicellular, photoautotrophic organisms dwelling on substrate, have been used in bioindication because they are good indicators of ecological water state. In this thesis, we have used a multi-scalar approach to observe how diatoms from Spanish Mediterranean rivers are affected by the characteristics of the habitats they live in. These organisms were observed under a taxonomic-indicative, functional-grouping and morphometric prism. The structure of this thesis takes a gradual decrease in work scale

Andrea M. Burfeid Castellanos