Universitat de Girona

DISTRIBUTION OF DIATOM COMMUNITIES IN AGRICULTURAL AND MINIG WATERSHEDS OF SOUTHWEST SPAIN

Gemma URREA CLOS

ISBN: 978-84-694-1115-5
Dipòsit legal: GI-48-2011
http://www.tesisenxarxa.net/TDX-0113111-135155/

Universitat de Girona

Institut d'Ecologia Aquàtica

PhD Thesis

DISTRIBUTION OF DIATOM COMMUNITIES IN AGRICULTURAL AND MINING WATERSHEDS OF SOUTHWEST SPAIN

Gemma Urrea–Clos

Girona, 2010

Programa d'Ecologia Fonamental Aplicada

Director: Dr. Sergi Sabater Cortés

Memòria presentada per optar al títol de doctora per la Universitat de Girona

Universitat de Girona

Institut d'Ecologia Aquàtica

El Dr. Sergi Sabater Cortés, catedràtic del Departament de Ciències Ambientals i memebre de l'Institut d'Ecologia Aquàtica de la Universitat de Girona,

CERTIFICA:

Que aquest treball titulat "Distribution of diatom communities in agricultural and mining watersheds of Southwest Spain", per a l'obtenció del títol de Doctora, ha estat realitzat sota la seva direcció.

Dr. Sergi Sabater Cortés
Catedràtic del Departament de Ciències Ambientals
Institut d'Ecologia Aquàtica
Universitat de Girona

La doctoranda:

Gemma Urrea–Clos

Girona, juny 2010

AGRAÏMENTS

Les paraules es queden curtes per expressar tot el que ha suposat arribar fins aquí. Moltes són les persones que d'una manera o una altra s'han vist implicades en aquest projecte, i a totes elles els voldria agrair el temps i l'esforç dedicat.

En primer lloc agrair a en Sergi el fet d'haver confiat en mi d'una manera gairebé incondicional i cega, en tants moments al llarg de tots aquests anys!

Em fa pànic fer una llista, hi ha tanta gent involucrada, que el perill de deixar-me algú és molt gran... espero que ningú se senti ofès.

Moltes gràcies als antics i actuals pobladors del seminari, els fluecos, nacionals i internacionals, els aiguamulleros i els peixòlegs, i tots aquells que esteu disseminats pels passadissos de la casa, zoòlegs, micros, bioquímcs i botànics... gràcies per fer d'aquesta casa un lloc habitable, i sobretot gràcies per fer que cada dia tingui ganes de venir a treballar !!

No puc oblidar-me de la gent de l'associació UdG.doc, malauradament la lluita continua...

Debo mencionar que este estudio e se emmarca en el seno del proyecto "Diseño y explotación de la Red de Control Biológico de la cuenca del río Guadiana" financiado por la Confederación Hidrográfica del Guadiana. Debo agradecer a Ángel Nieva todas las facilidades que nos ha prestado.

Haig de dedicar unes paraules a l'equip humà d'URS, pel inoblidables moments viscuts per *Las Vegas del Guadiana* en els nostres "Gran Hermano" particulars per les Espanyes... i aquí una menció molt especial al Paco i al Virgilio, los niños de los peces... grácias por todo, por estar allí y sobretodo por seguir aquí, al otro lado de la pantalla a pesar de andar boca–abajo!

Vull agrair molt especialment als meus pares, germans, cunyat i nebots el seu recolzament moral tot i no entendre gaire bé què és el que he estat fent tot aquest temps.

Finalment, l’Albert, que ha cregut en mi en tot moment, amb grans dosis de paciència i comprensió. Gràcies pel teu recolzament, per tot el que hem viscut, per l’Isaac, pel que està en camí, i sobretot mil gràcies per tot el que ens queda per viure.

SUMMARY; RESUMEN; RESUM

SUMMARY (ENGLISH)

In many parts of the world freshwater ecosystems are intensely modified and degraded by human activities. Rivers have been altered since historical times, but such modifications have intensified in the last century.

Water extraction and heavily regulated reservoirs are spread disturbances in the Guadiana network. Drainage from agricultural land in river systems results in large inputs of nitrates and phosphates that eutrophy river waters. Inputs of fertilizers and water extraction are especially remarkable in the North–East Guadiana watershed.

The development of tools to assess ecological status of water bodies has been a key issue in the management of the freshwater ecosystems in the last decades in order to protect and restore the biological integrity of ecosystems.

The present study aims to determine distributional patterns of benthic algae communities (with a special attention on diatom microflora) and their causes in Guadiana and associated watersheds in order to establish tools for assessing the conservation status of the water bodies in those watersheds.

CHAPTER II: STUDY AREA

The present study has been developed in the whole Spanish part of Guadiana watershed and less intensively, in several sites of the Tinto, Odiel and Piedras river basins.

The Guadiana is the fourth largest watershed in the Iberian Peninsula. It can be divided in two main regions, the Upper Guadiana watershed with a calcareous substrate, where groundwaters play an important role. The landscape was deeply transformed through extensive agricultural activities, and water extraction is especially remarkable. The Mid and Lower part of the watershed, are characterized by an impermeable substrate. The agricultural practices are so intense that the natural hydrograph is completely altered as a consequence of the river canalization and water abstraction. A total of 244 localities scattered were sampled during winter, spring and autumn of 2005–6.

The Tinto−Odiel system presented part of their courses running over the Iberian Pyrite Belt, and they are seriously affected by acid mine drainage. These waters can be defined as an extreme habitat in terms of their very low mean pH (near 2.5) and high concentration of heavy metals and anions. A total of 18 localities distributed throughout Tinto–Odiel system and Piedras watershed were sampled during winter of 2005.

CHAPTER III: MATERIAL AND METHODS

Environmental monitoring was conducted in every sampling station. Water samples were collected and chemical variables determined. All the analyses followed standard procedures. River habitat was characterized and a riparian quality index was determined.

Diatom collection, preparation and observation followed European standards, and non–diatom algae and cyanobacteria collection and observation were also done.

CHAPTER IV: DIATOM TAXA OF SPECIAL INTEREST FOR THEIR TAXONOMY AND ECOLOGY

A total of 278 diatom taxa were identified. This high diatom richness is a result of the wide geological and hydrodynamic heterogeneity in the Guadiana watershed. The chapter accounts for the taxonomic description, together with the ecological remarks of the most relevant diatom taxa.

CHAPTER V: EPILITHIC DIATOM ASSEMBLAGES AND THEIR RELATIONSHIP TO ENVIRONMENTAL CHARACTERISTICS IN THE GUADIANA WATERSHED

Large–scale patterns of benthic diatom assemblages were analyzed. The distribution patterns of epilithic diatom assemblages were analyzed at different spatial scales: the whole watershed, the upper calcareous subcatchment and the mid–lower siliceous subcatchment.

At the whole watershed scale, two major ecological gradients were revealed. The first one summarized the diatom distribution throughout a nutrient concentration gradient, while the second gradient was related to the geological structure of the watershed. Variance partitioning allowed us to separate the effects of the different sets of environmental parameters involved in each described gradient.

Analyzing the subcatchment gradients with partial CCA allowed us to define specific key factors that affect diatom species composition. Although water chemistry consistently played the most important role in structuring diatom assemblages in the Guadiana, spatial factors such as altitude or geographic location also explained some variation in diatom distribution.

CHAPTER VI: IDENTIFYING REFERENCE BENTHIC DIATOM COMMUNITIES IN THE GUADIANA WATERSHED

Determining the ecological status of river ecosystems should be based on reference (near–natural) conditions and biological communities. Identifying reference conditions is particularly difficult to be achieved in largely disturbed watersheds, such as those of agricultural landscapes. Reference conditions have to be described for every ecotype, and the communities inhabiting them should include the full range of those inhabiting within the ecotype. In this chapter ecotype reference sites and diatom communities characterizing them were described in the Guadiana watershed.

Three different ecotypes were defined: upper watershed calcareous rivers, rivers with siliceous bedrocks, and mainland watercourses. Thirteen key stressors divided in 4 categories (channel alteration; hydrological pressure; pollution pressure; river habitat pressure) were used to select reference stations in each ecotype. Multivariate techniques were used to describe type–specific reference diatom community in each ecotype.

Diatom reference communities in rivers with siliceous bedrocks were dominated by *Encyonema silesiacum* and *Tabellaria flocculosa*. Diatom reference communities in the upper calcareous watershed were composed of species tolerating high conductivities like *Cymbella affinis*, *Navicula tripunctata* and *Diatoma moniliformis*. In spite of the difficulty to define reference stations in the mainland watercourses, some sites were selected under the permissive criterion of minimum impacted sites. Diatoms inhabiting in these situations included species tolerant to high nutrient concentration and mineralized waters like *Nitzschia inconspicua*, *Gomphonema parvulum* and *Navicula veneta*. These taxa defined the best condition (near–to–reference) in an ecotype highly impacted by human activities, and possibly is the potential in large river watercourses in agricultural landscapes.

CHAPTER VII: PATTERNES OF BENTHIC CHLOROPHYLL–*a* IN THE GUADIANA WATERSHED

Benthic algal biomass depends on a number of variables including nutrient availability, the ionic composition of the water, light quality and quantity, water temperature, and grazing pressure. The factors affecting benthic chlorophyll variation were analyzed throughout the Guadiana watershed. Partial Least Square Regressions (PLSR) models were used to determine the main parameters affecting chlorophyll distribution in the different river ecotypes in the watershed. The potential relevance of 24 chemical, physical and physiographical parameters were analyzed at the three ecotypes defined in the watershed, the upper watershed calcareous rivers, rivers with siliceous bedrocks, and mainland watercourses. Nutrients were revealed as the key factor affecting chlorophyll distribution along the three ecotypes. Further, temperature and watershed land uses also played an important role in determining chlorophyll variation.

CHAPTER VIII: COMPARATIVE STUDY OF ALGAL COMMUNITIES IN ACID AND NON–ACID WATERS FROM TINTO–ODIEL SYSTEM AND PIEDRAS RIVER BASINS

This chapter analyzes the distribution patterns of benthic algal assemblages in Tinto−Odiel system and Piedras watershed. The main objective is to assess and compare the algal communities and parameters affecting them both in the zones affected by Acid Mine Drainage (AMD) and in naturally alkaline waters.

A total of 108 benthic diatom taxa and 31 non–diatom taxa were identified. Results showed large differences between algal communities in the two environments: *Pinnularia acoricola*, *P. subcapitata* and *Eunotia exigua* were the most frequent diatom taxa in regions affected by acid mine drainage, while *Klebsormidium* and *Euglena mutabilis* were the most relevant non–diatom taxa. However, in non–acid waters the dominant diatom taxa were *Planothidium frequentissimum*, *Gomphonema angustum*, *Fragilaria capucina* and some species of *Navicula* (*N. viridula*, *N. veneta* or *N. radiosa)*, accompanied by *Oscillatoria* and *Anabaena* as well as by Zygnematales and Desmidiales.

RESUMEN (CASTELLANO)

Los sistemas acuáticos continentales representan uno de los ecosistemas más gravemente amenazados a nivel mundial, como consecuencia del uso intensivo que el hombre hace de ellos. Históricamente los ríos han sido utilizados, regulados y alterados. Dichas alteraciones se han intensificado notablemente en los últimos siglos.

La cuenca del Guadiana no se escapa de dichas presiones antrópicas. Las grandes infraestructuras hiudráulicas destinadas a almacenar agua y la severa extracción de las aguas subterráneas con destino a regadíos, son sólo un ejemplo de los graves problemas que sufre la cuenca. A ellos debe añadirse la escorrentía provinente del la agricultura que introduce al río grandes cantidades de nitratos y fosfatos responsables de la eutrofización de sus aguas. La escorrentía agrícola y la extracción de agua del subsuelo son especialmente destacables en la subcuenca alta Castellano–Manchega, donde la escasez de agua sólo hace que agravar el problema.

Ante dicho panorama, se hace imprescindible el desarrollo de herramientas que permitan evaluar y conocer el estado ecológico de los ecosistemas acuáticos, para poder proponer medidas de gestión destinadas a restaurar la integridad ecológica de los mismos.

El principal objetivo que presenta este estudio es determinar los patrones de distribución de las comunidades algales (con una atención especial para el grupo de las diatomeas) y sus causas en la cuenca del Guadiana y asociadas, con el fin de establecer herramientas que permitan evaluar el estado de conservación de las masas de agua de dichas cuencas.

CAPÍTULO II: ZONA DE ESTUDIO

El estudio se ha desarrollado en la parte española de la cuenca del Guadiana, teniendo en cuanta también, aunque de manera menos intensiva, los ríos Tinto, Odiel y Piedras.

El Guadiana es el cuarto río más caudaloso de la Península Ibérica. Su cuenca puede dividirse en dos grandes zonas, la parte alta del Guadiana, situada en la meseta castellano–manchega, de naturaleza caliza, donde las aguas subterráneas juegan un papel muy importante. En esta zona las actividades agrícolas han transformado totalmente el paisaje, siendo responsables además de una severa extracción de agua del subsuelo, provocando graves problemas de recarga de agua en el acuífero. La parte media y baja de la cuenca, se caracterizan por un sustrato impermeable, que ha permitido el desarrollo de grandes obras hidráulicas, con el fin de garantizar el agua para la agricultura, que han provocado una profunda alteración de la morfología y del cauce fluvial. En el presente estudio se han muestreado 244 localidades entre los años 2005 y 2006, visitándolas en invierno, primavera y otoño.

Gran parte del recorrido de los ríos que conforman el sistema Tinto–Odiel discurre sobre la Banda Pirítica Ibérica y se encuentran severamente afectados por los depósitos ácidos que drenan de las minas. Las aguas afectadas por esta escorrentía ácida suponen un ambiente extremo para la vida, caracterizado por un pH muy bajo (cercano a 2.5) altas concentraciones de metales y aniones en disolución. En total se visitaron 18 estaciones durante el invierno de 2005.

CAPÍTULO III: MATERIAL Y MÉTODOS

En cada visita a los puntos de muestreo se tomaron medidas de diversas variables ambientales a la vez que se recolectaron muestras para la caracterización química de la localidad mediante procedimientos estandarizados. Se realizó la caracterización del hábitat fluvial (IHF) y durante la primavera se determinó el índice de calidad del bosque de ribera (QBR).

La recolección, preparación y observación de las diatomeas se realizó según los protocolos europeos. Se recolectaron también algas no diatomeas y cianobacterias

CAPÍTULO IV: DIATOMEAS CON UNA TAXONOMÍA Y ECOLOGÍA PARTICULAR

Se han identificado un total de 278 taxones de diatomeas que confirman la gran heterogeneidad geológica e hidrodinámica de las cuencas del Guadiana y asociadas. En este capítulo se describen y discuten algunos taxones con particularidades especiales ya sea en su taxonomía o en su ecología.

CAPÍTULO V: COMUNIDADES DE DIATOMEAS EPILÍTICAS Y SU RELACIÓN CON LAS CARACTERÍSTICAS AMBIENTALES DE LA CUENCA DEL GUADIANA

Se han analizado los patrones generales de distribución de diatomeas a lo largo de la cuenca. Dichos patrones se han analizado a diferentes escalas espaciales: a nivel de la cuenca entera, a nivel de la cuenca caliza alta, y a nivel de la cuenca media–baja de sustrato silícico.

A nivel general de cuenca, se han descrito dos grandes gradientes ambientales: el primero describe un gradiente químico de concentración de nutrientes, mientras que el segundo responde a un gradiente de estructuración geológica de la cuenca. Las técnicas multivariantes de partición de la varianza han permitido testar por separado los efectos de cada gradiente sobre la comunidad de diatomeas.

A pesar que la química del agua juega un papel muy importante en la estructuración de dichas comunidades, factores espaciales como la altitud o la localización geográfica también se han revelado como factores clave en la distribución de éstas en todas las zonas de la cuenca.

CAPÍTULO VI: DETERMINACIÓN DE LAS COMUNIDADES DE DIATOMEAS DE REFERENCIA EN LA CUENCA DEL GUADIANA

La determinación del estado ecológico de los ecosistemas acuáticos debe basarse en la comparación del estado de las aguas y de sus comunidades biológicas con las condiciones potenciales de referencia (naturales), pero la identificación de las condiciones de referencia se hace especialmente dificultosa en las grandes cuencas de carácter agrícola. Las condiciones de referencia deben ser descritas para cada ecotipo, y por tanto en cada ecotipo deben describirse las comunidades biológicas de referencia. En este capítulo se han descrito las estaciones de referencia para cada ecotipo fluvial de la cuenca del Guadiana, describiendo las comunidades de diatomeas que los caracterizan.

Se han definido un total de 3 ecotipos: los ríos calizos de la cuenca alta, los ríos silícicos de las cuencas media y baja y finalmente los grandes ríos. Para establecer las estaciones de referencia se han tenido en cuenta 14 estresores ambientales repartidos en 4 categorías (alteraciones en el canal fluvial, presiones hidrológicas, presiones de contaminación y presiones sobre el hábitat fluvial). A partir de técnicas multivariantes se describen las comunidades de diatomeas de referencias propias de cada tipología fluvial.

La comunidad de diatomeas de referencia de los ríos silíceos de la cuenca media y baja del Guadiana está integrada principalmente por *Encyonema silesiacum* y *Tabellaria flocculosa*. La comunidad de referencia de la cuenca caliza alta está dominada por especies que toleran una conductividad alta tales como *Cymbella affinis*, *Navicula tripunctata* y *Diatoma moniliformis*. A pesar de la dificultad en describir estaciones de referencia para los cursos principales de agua, se han seleccionado algunas estaciones bajo criterios muy permisivos, describiéndolas como las localidades menos impactadas. En éstas, la comunidad tipo de diatomeas está integrada sobretodo por especies tolerantes a aguas mineralizadas con una concentración elevada de nutrientes tales como *Nitzschia inconspicua*, *Gomphonema parvulum* y *Navicula veneta*. Estos taxones representan las mejores condiciones de un ecotipo altamente polucionado como efecto de la las actividades humanas. Posiblemente, ésta sea la comunidad potencial en muchos de los grandes ríos de las zonas agrícolas del mundo.

CAPÍTULO VII: PATRONES DE DISTRIBUCIÓN DE LA CLOROFILA–*a* BENTÓNICA EN LA CUENCA DEL GUADIANA

La concentración de productores primarios en los sistemas acuáticos depende de gran número de variables entre las que se cuentan la disponibilidad de nutrientes, la cantidad y calidad de la luz, la temperatura, las características fisicoquímicas del agua o la presión de los ramoneadores.

En este capítulo se analizan los factores que afectan la distribución de la clorofila–*a* bentónica en la cuenca del Guadiana. Para ello se han desarrollado modelos PLSR (regresiones parciales de cuadrados mínimos) en los que se analizan los efectos de 24 parámetros fisicoquímicos y fisiográficos en los 3 ecotipos previamente definidos: la zona caliza alta, la zona silícica media–baja y los grandes cursos fluviales.

Los nutrientes se han revelado como el principal factor que afecta la distribución de la clorofila en los 3 ecotipos, aunque se ha visto que otros factores como la temperatura y los usos del suelo también juegan un papel muy importante en la distribución de la clorofila–*a* bentónica.

CAPÍTULO VIII: ESTUDIO COMPARATIVO DE LAS COMUNIDADES ALGALES QUE POBLAN LAS AGUAS ÁCIDAS Y NO–ÁCIDAS DE LAS CUENCAS DE LOS RÍOS TINTO, ODIEL Y PIEDRAS

En este capítulo se analizan los patrones de distribución de las comunidades algales bentónicas en los ríos Tinto, Odiel y Piedras, sumando un total de 18 estaciones de muestreo visitadas durante el invierno de 2005.

El objetivo principal ha sido evaluar y comparar las comunidades algales y los parámetros que las afectan tanto en zonas influenciadas por el drenaje ácido como en las zonas libres del mismo.

Se han identificado un total de 108 taxones de diatomeas bentónicas y 31 taxones de otras algas. Se observaron grandes diferencias en las poblaciones de productores primarios en ambos tipos de ambientes: *Pinnularia acoricola*, *P. subcapitata* y *Eunotia exigua* fueron las diatomeas más frecuentes en los ambientes afectados por el drenaje ácido, acompañadas por algas como *Klebsormidium* y *Euglena mutabilis*. En las aguas no ácidas las diatomeas dominantes fueron *Planothidium frequentissimum*, *Gomphonema angustum*, *Fragilaria capucina* y algunas especies de *Navicula* (*N. viridula*, *N. veneta* o *N. radiosa*), acompañadas por *Oscillatoria*, *Anabaena*, Zygnematales y Desmidiales.

RESUM (CATALÀ)

Els sistemes aquàtics continental representen un dels ecosistemes més greument amenaçats a nivell mundial, com a conseqüència de l'ús intensiu que l'home en fa. Ja des de temps històrics, els rius han estat utilitzats, regulats i alterats. Aquestes alteracions s'han intensificat enormement en els darrers segles.

La conca del Guadiana no està lliure d'aquestes pressions antròpiques. Les grans infraestructures hidràuliques destinades a garantir les reserves d'aigua i la severa extracció de les aigües subterrànies són només un exemple dels greus problemes que pateix la conca. A tots aquests problemes, cal afegir l'escorrentia provinent de l'agricultura que aporta al riu una gran quantitat de nitrats i fosfats responsables de l'eutrofització de les seves aigües. L'escorrentia de l'agricultura i l'extracció d'aigua del subsòl es fan especialment paleses en la zona alta de la conca, on l'escassetat d'aigua no fa més que agreujar el problema.

Tot això ha generat la necessitat urgent d'avaluar l'estat de conservació d'aquests ecosistemes aquàtics continentals, poder determinar la mesura i la magnitud de les pertorbacions que els estan afectant i així proposar mesures de gestió destinades a restaurar-ne la integritat ecològica.

El principal objectiu que presenta aquest és determinar els patrons de distribució de les comunitats de algals (amb una menció especial en el grup de les diatomees) i de les seves causes en la conca del Guadiana i associades, amb la finalitat d'establir i proposar eines que permetin avaluar l'estat de conservació de les masses d'aigua d'aquestes conques.

CAPÍTOL II: ÀREA D'ESTUDI

El present estudi s'ha desenvolupat en la part espanyola de la conca del riu Guadiana, tenint en compte també, tot i que de manera menys intensiva, les conques dels rius Tinto, Odiel i Piedras.

El Guadiana és el quart riu més cabalós de la Península Ibèrica. La seva conca es pot dividir en tres grans zones, la part alta, situada a la Meseta Castellano–Manxega, caracteritzada per un substrat de natura calcària on les aigües subterrànies juguen un paper molt important. En aquesta zona les activitats agrícoles han transformat totalment el paisatge, provocant a més, una severa extracció de l'aigua del subsòl que ocasiona greus problemes de recàrrega dels aqüífers associats. Les parts mitja i baixa de la conca, es caracteritzen per un substrat de natura impermeable que ha permès el desenvolupament de grans embassaments destinats a garantir l'aigua per l'agricultura, amb la conseqüent alteració de la morfologia i del curs fluvial. En el present estudi s'han mostrejat 244 localitats entre els anys 2005 i 2006, visitant-les durant l'hivern, la primavera i la tardor.

Gran part del recorregut dels cursos d'aigua que conformen el sistema Tinto–Odiel discorre sobre la Banda Pirítica Ibèrica de manera que es troben greument afectats per els dipòsits àcids que drenen de les mines. Les aigües afectades per aquests dipòsits miners suposen un ambient molt extrem per la vida caracteritzat per un pH molt baix (al voltant de 2.5) i elevades concentracions de metalls i anions en dissolució. En total s'han visitat 18 estacions de mostreig durant l'hivern de 2005.

CAPÍTOL III: MATERIAL Y MÈTODES

En cada visita als diferents punts de mostreig es varen prendre mostres de diferents variables ambientals a la vegada que es recol·lectaren mostres per a la caracterització química del punt, mitjançant procediments estandarditzats. Es va fer una caracterització de l'hàbitat fluvial (IHF), i durant la primavera es va determinar l'índex de qualitat del bosc de ribera (QBR).

La recol·lecció, preparació i observació de les algues diatomees es va fer seguint els protocols i normatives europees, Es varen recol·lectar també algues no diatomees i cianobacteris.

CAPÍTOL IV: DIATOMEES AMB UNA TAXONOMIA Y ECOLOGIA PARTICULAR

S'han identificat un total de 278 tàxons de diatomees que confirmen la gran heterogeneïtat geològica i hidrodinàmica de la conca del Guadiana i conques associades. En aquest capítol es descriuen i discuteixen amb profunditat alguns tàxons amb particularitats especials pel que fa a la seva taxonomia i/o ecologia.

CAPÍTOL V: COMUNITATS DE DIATOMEAS EPILÍTIQUES I LA SEVA RELACIÓ AM BLES CARACTERÍSTIQUES AMBIENTALS DE LA CONCA DEL GUADIANA

S'han analitzat els patrons de distribució de les algues diatomees al llarg de la conca. Aquests patrons s'han analitzat a diferents escales espacials: a nivell de la conca sencera, a nivell de la conca calcària alta i a nivell de la conca mitja–baixa silícica.

A nivell general de conca s'han descrit dos grans gradients ambientals: el primer que descriu un gradient químic de concentració de nutrients, mentre que el segon respon a un gradient relacionat amb l'estructura geològica de la conca. Les tècniques multivariants de partició de la variança han permès testar per separat els efectes de cada gradient sobre la comunitat de diatomees.

Malgrat que la química de l'aigua jugui un paper molt important en l'estructuració d'aquestes comunitats, factors relacionats amb l'espai com l'altura o la localització geogràfica també s'han revelat com a factors clau en la distribució d'aquestes en totes les zones de la conca.

CAPÍTOL VI: DETERMINACIÓ DE LES COMUNITATS DE DIATOMEES DE REFERÈNCIA EN LA CONCA DEL GUADIANA

La determinació de l'estat ecològic dels ecosistemes aquàtics s'ha de basar en la comparació de l'estat de les aigües i de les seves comunitats biològiques comparant-les amb les condicions potencials de referència (naturals). La identificació de les condicions de referència però, es fa especialment dificultosa en les grans conques de caràcter agrícola. Les condicions de referència s'han de descriure per a cada ecotipus, per tant es fa palesa la necessitat de descriure les comunitats biològiques de referència per a cada ecotipus. En aquest capítol es descriuen les estacions de referència per a cada ecotipus fluvial de la conca del Guadiana, fent a més una descripció de les comunitats de diatomees que les caracteritzen.

S'han definit un total de 3 ecotipus: els rius calcaris de la conca alta. els rius silícics de les conques mitja–baixa i finalment els cursos principals. Per tal d'establir les estacions de referència s'han tingut en compte un total de 14 estressors ambientals repartits en 4 categories (alteracions del canal fluvial, pressions hidrològiques, pressions de contaminació i pressions sobre l'hàbitat fluvial). Mitjançant tècniques multivariants s'han descrit les comunitats de diatomees de referència pròpies de cada tipologia fluvial.

La comunitat de diatomees de referència dels rius silícics de la conca mitja–baixa del Guadiana està integrada per *Encyonema silesiacum* i *Tabellaria flocculosa*. La comunitat de referència de la conca alta calcària està dominada per espècies tolerants a les altes conductivitats com *Cymbella affinis*, *Navicula tripunctata* i *Diatoma moniliformis*. Malgrat la dificultat de definir estacions de referència en els cursos principals d'aigua, s'han seleccionat, sota criteris molt permissius, algunes estaciones que definim com les menys pol·luïdes. En aquestes, la comunitat de diatomees està integrada per espècies tol·lerants a la mineralització de l'aigua i ales concentracions elevades de nutrients com per exemple *Nitzschia inconspicua*, *Gomphonema parvulum* i *Navicula veneta*. Aquest tàxons representen les millors condicions possibles en un ecotipus altament pol·luït per efecte de les activitats humanes. Possiblement aquesta sigui la comunitat potencial de molts grans rius de les zones agrícoles d'arreu.

CAPÍTOL VII: PATRONS DE DISTRIBUCIÓ DE LA CLOROFIL·LA–*a* BENTÒNICA A LA CONCA DEL GUADIANA

La concentració de productors primaris en els sistemes aquàtics depèn de gran nombre de variables tals com la disponibilitat de nutrients, la quantitat i qualitat de llum, la temperatura, les característiques fisicoquímiques de l'aigua o la pressió dels brostejadors.

En aquest capítol s'analitzen els factors que afecten la distribució de la clorofil·la–*a* bentònica en la conca del Guadiana. A tal efecte s'han desenvolupat models PLSR (regressions parcials de quadrats mínims) en els que s'analitzen els efectes de 24 paràmetres fisicoquímics i fisiogràfics en els 3 ecotipus prèviament definits: la zona alta calcària , la zona silícica mitja–baixa i els cursos principals d'aigua.

Els nutrients s'han revelat com un factor clau en l'ordenació de la clorofil·la–*a* en els tres ecotipus, tot i que altres factors com la temperatura o els usos del sòl també juguen un paper molt important.

CAPÍTOL VIII: ESTUDI COMPARATIU DE LES COMUNITATS ALGALS QUE POBLEN LES AIGÜES ÀCIDES I NO–ÀCIDES DE LES CONQUES DELS RIUS TINTO, ODIEL I PIEDRAS

En aquest capítol s'analitzen els patrons de distribució de les comunitats algals bentòniques dels rius Tinto, Odiel i Piedras, sumant un total de 18 estacions de mostreig que es varen visitar durant l'hivern de 2005.

L'objectiu principal ha estat avaluar i comparar les comunitats algals i els paràmetres que les afecten tant en les zones afectades per el drenatge àcid de les mines, com en les zones lliures d'aquest.

En total s'han identificat 108 tàxons de diatomees bentòniques i 31 tàxons d'altres grups algals. s'han observat grans diferències entre les poblacions de productors primaris en ambdós tipus d'ambients: *Pinnularia acoricola*, *P. subcapitata* i *Eunotia exigua* són les diatomees predominants en els ambients afectats per el drenatge àcid, acompanyades d'algues com *Klebsormidium* i

Euglena mutabilis. En les aigües no–àcides les diatomees dominants són *Planothidium frequentissimum*, *Gomphonema angustum*, *Fragilaria capucina* i algunes espècies del gènere *Navicula* (*N. viridula*, *N. veneta* o *N. radiosa*), acompanyades d'*Oscillatoria*, *Anabaena*, Zygnematals i Desmidials.

CHAPTER I

GENERAL INTRODUCTION

1.1. INTRODUCTION

In many parts of the world freshwater ecosystems are intensely modified and degraded by human activities. Rivers have been altered since historical times, but such modifications have been intensified in the last century. Modifications include river embankments to improve navigation, drainage of wetlands for flood control and agriculture, construction of dams and irrigation channels, and the establishment of inter–basin connections and water transfers. These changes have improved transportation, provided flood control and hydropower, and increased agricultural output by making more land and irrigation water available. Decreased river flows and falling ground water levels were common problems in irrigated areas. While water demand is increasing, pollution from industry, urban areas, and agricultural runoff is limiting the amount of water available for domestic use and food production (Revenga and Kura, 2003). This problem is particularly relevant in Mediterranean regions, where demand for water far exceeds supply.

The development of tools to assess ecological status of water bodies has been a key issue in the management of the freshwater ecosystems in the last decades in order to protect and restore the biological integrity of ecosystems. International laws as the Clean Water Act in the United States or the European Water Framework Directive (WFD) have been introduced in an attempt to address these problems, requiring the protection and restoration of biological integrity as part of water quality standards. The aims of this bioassessment is to help diagnose potential problems causing the deterioration of freshwater ecosystems and to guide objective management plans and corrective actions leading them to a good ecological status.

1.2. THE GUADIANA WATERSHED

Guadiana watershed is a very peculiar basin. It presents a remarkable hydrology and enduring human impacts. Both factors determine large spatial variations in the flow regime, water chemistry, riparian vegetation and biological communities. The hydrology is related to the porosity of its geological substrata and the moderate rainfall in the upper part of its catchment, where groundwater plays an important role in the river flow, and some sections are intermittent (Sabater*, et al.*, 2009). The middle and lower parts of the watershed run on impermeable bedrock and depend on scarce and irregular rainfall. This spatial asymmetry provides the system with shifting environmental characteristics. Water transparency is initially high in the Guadiana headwaters, but suspended solids rapidly increase downriver because of the natural contribution of sediments, agricultural activities or urban inputs. Low water velocity and the overall low flow determine the formation of pools, especially during summer and dry years. Water conductivity is high at the headwaters as well as in some tributaries because of their calcareous substrata, but decreases downriver because of the contribution of poorly mineralised spring sources and of the sedimenting effect of the reservoirs. Nutrients substantially increase downriver, and especially in areas of intensive agriculture.

1.3. TINTO–ODIEL SYSTEM AND PIEDRAS WATERSHEDS

The Tinto and Odiel river systems have their source in the Sierra de Aracena, at an altitude of 900 m a.s.l. Part of their courses run over the Iberian Pyrite Belt (IPB), one of the most extensive sulfide mining regions in the world, and they are seriously affected by acid mine drainage. Even though there is no active mining nowadays, pollution continues to arrive to the watercourses due to the oxidation of mining wastes (Nieto*, et al.*, 2007). These waters can be defined as an extreme habitat in terms of their very low mean pH (near 2.5) and high concentration of heavy metals, especially ferric iron, copper and zinc, as well as some anions such as sulfate (López–Archilla and Amils, 1999). Only extremophilous taxa can survive on these situations of very low pH (López–Archilla*, et al.*, 2001) and high heavy metals deposition (Niyogi*, et al.*, 2002, Gerhardt*, et al.*, 2008). The Tinto and Odiel rivers converge into a common coastal wetland, with marked tidal influence. Around this salt marsh zone there is an intensive agricultural, industrial and urban development (MMA, 2005).

The Piedras river basin is a short stream with very restricted fluvial catchment located between the Guadiana and the Tinto–Odiel system. The final part of this river ends into an extensive and well delimited estuary. Regarding the human occupation, the river is divided in two main zones: the upper part which is of low density and with scarce crops, and the middle–lower part which is more densely populated and includes extensive irrigations (MMA, 2005). In this area, two reservoirs enormously alter the natural fluvial regime.

1.4. USE OF DIATOMS AS BIOINDICATORS

Diatoms have a number of prominent distinctive features that converted them to a useful tool for indication present and past ecological conditions: (i) they account for much of the freshwater biodiversity especially in streams (Pan*, et al.*, 1999), (ii) they present relatively strict preferences for various environmental factors (Soininen, 2007) and quickly react to environmental changes (Rott, 1991) and obviously (iii) they present a siliceous wall that allow reliable taxonomic determination at specific and sub–specific level .

Many efforts have been devoted to the development of efficient tools to measure the ecological status of freshwaters systems based on diatoms. Substantial number of diatom indices have been developed for estimation of water quality in various geographic areas e.g. “Trophic Diatom Indices” (Hoffmann, 1994, Kelly and Whitton, 1995), saprobic indices (Sládecek, 1973), pollution indices (Descy, 1979), the “Generic Diaotm Index” (Coste, 1982), the IDEC for East Canadian (Lavoie*, et al.*, 2006) etc.

The information provided by diatom communities may be understood by two different approaches. First of all diatom indices constitute a way of summarizing the information provided by the autoecological preferences of the taxonomic composition of the diatom community (Sabater and Admiraal, 2005). By contrast, multivariate techniques are powerful methods to assess the important environmental gradients regulating community composition (Soininen, 2002). It becomes necessary to use both techniques to identify major patterns of community structure and to characterize and to predict changes in those patterns in relation to environmental gradients

The taxonomic composition of benthic diatom communities offer suitable indicator species for a variety of situations (Foerster*, et al.*, 2004). Benthic diatom assemblages in rivers are influenced by environmental descriptors not affected by human activities, such as the dominant geology of the river basin (Cantonati, 1998, Tison*, et al.*, 2004, Urrea and Sabater, 2009a), the altitude of the sampling site (Ndiritu*, et al.*, 2006, Rimet*, et al.*, 2007), the distance from the source (Potapova and Charles, 2002), but also by others related to human activities, such as the organic load or nutrient concentration of the water (Leira and Sabater, 2005, Kovács*, et al.*, 2006, Tornés*, et al.*, 2007). Therefore, the accurate characterization of diatom communities may allow the assessment of biological conditions and diagnosing stressors for the aquatic ecosystems (Stevenson*, et al.*, 2008b).

1.5. USE OF CHLOROPHYLL TO PREDICT EUTROPHICATION

Stream biological integrity reveals itself in the condition, abundance, and diversity of its biota, and biological surveys of stream communities have long been used to assess the impacts of human activities on these systems (Hill*, et al.*, 2003). The concentration of primary producers in aquatic systems depends on a number of variables including availability of nutrients, quality and quantity of light, temperature, physico–chemical properties of the water, grazing pressure as well as interactions between these physical, chemical and biological compartments of the system (Hakanson*, et al.*, 2003).

Biomass should be strongly related to nutrient concentrations. However, previous attempts to generate good explanatory power with dissolved nutrient–benthic algal biomass models in rivers has had varied success (Horner and Welch, 1981, Jones*, et al.*, 1984, Aizaki and Sakamoto, 1988, Biggs and Close, 1989, Lohman*, et al.*, 1992, Biggs, 1995, Dodds*, et al.*, 1997, Chételat*, et al.*, 1999). The slow development of robust algal–nutrient models for lotic systems is probably a result of the great complexity of physical and biological interactions that determine biomass at any point at any time (Leland, 1995, Biggs, 1996).

Multiple ecological processes operating at different spatial and temporal scales certainly contribute to this complexity (Stevenson, 1997) and new approaches that recognize scale–dependent constraints are required to reveal patterns and processes at broad scales. (Biggs, 1995) suggested that large regional factors, such as climate, geology, and land use, should be integrated with local processes to study algal–nutrient interaction.

1.6. USE OF ALGAL COMMUNITIES TO DIAGNOSE ACIDITY

The Tinto–Odiel system constitutes an extreme environment for the aquatic life where water pH plays a very important role organizing the development of biological communities. The sites affected by Acid Mine Drainage (AMD) produce both chemical stress (low pH, dissolved heavy metals) as well as physical stress (deposition of metal oxides) on stream biota (Gerhardt*, et al.*, 2008).

Nevertheless, the AMD Tinto–Odiel affected waters presented an unexpected degree of diverse eukaryotic organisms, which are the principal contributors of biomass (representing over 65% of the total biomass) (López–Archilla*, et al.*, 2001, Amaral-Zettler*, et al.*, 2002, Amaral-Zettler*, et al.*, 2003). Green algae, diatoms and euglenoids, as well as ciliates, cercozoans, amoebae, stramenopiles, and fungi have all been detected (Aguilera*, et al.*, 2007b) regarding the eukaryotic community.

Although many papers have been published regarding the AMD affected algal communities in Tinto–Odiel system (López–Archilla*, et al.*, 2001, Amaral-Zettler*, et al.*, 2002, Sabater*, et al.*, 2003a, Aguilera*, et al.*, 2006, Aguilera*, et al.*, 2007a, Aguilera*, et al.*, 2007b) none of them provide information about algal communities in non–affected AMD waters from these watersheds.

This study aims to relate algal composition to differences in pH (and associated stressors) and to analyze changes in species composition in response to physical stress.

1.7. OBJECTIVES

The present study aims to investigate the distributional patterns of benthic algae communities, with a special attention on diatom microflora, in Guadiana and associated watersheds

The main objective is approached by the following specific objectives:

- To describe the diatom taxa composition in the Guadiana, Tinto, Odiel and Piedras watersheds
- To determine the spatial patterns and the ecological determinants regulating diatom community distribution in the Guadiana watershed
- To identify which can be reference conditions and which can be their best indicator diatom taxa and the accompanying benthic algae community in Guadiana watershed
- To explore which ultimate and proximate environmental factors were directly affecting benthic algae biomass distribution
- To investigate how water acidity contributes to the distribution of algal communities in acid and non–acid waters from Tinto, Odiel and Piedras river basins

CHAPTER II

STUDY AREA

The whole Spanish part of Guadiana watershed has been considered in the present work. Less intensively, several sites of the Tinto, Odiel and Piedras river basins have been also studied.

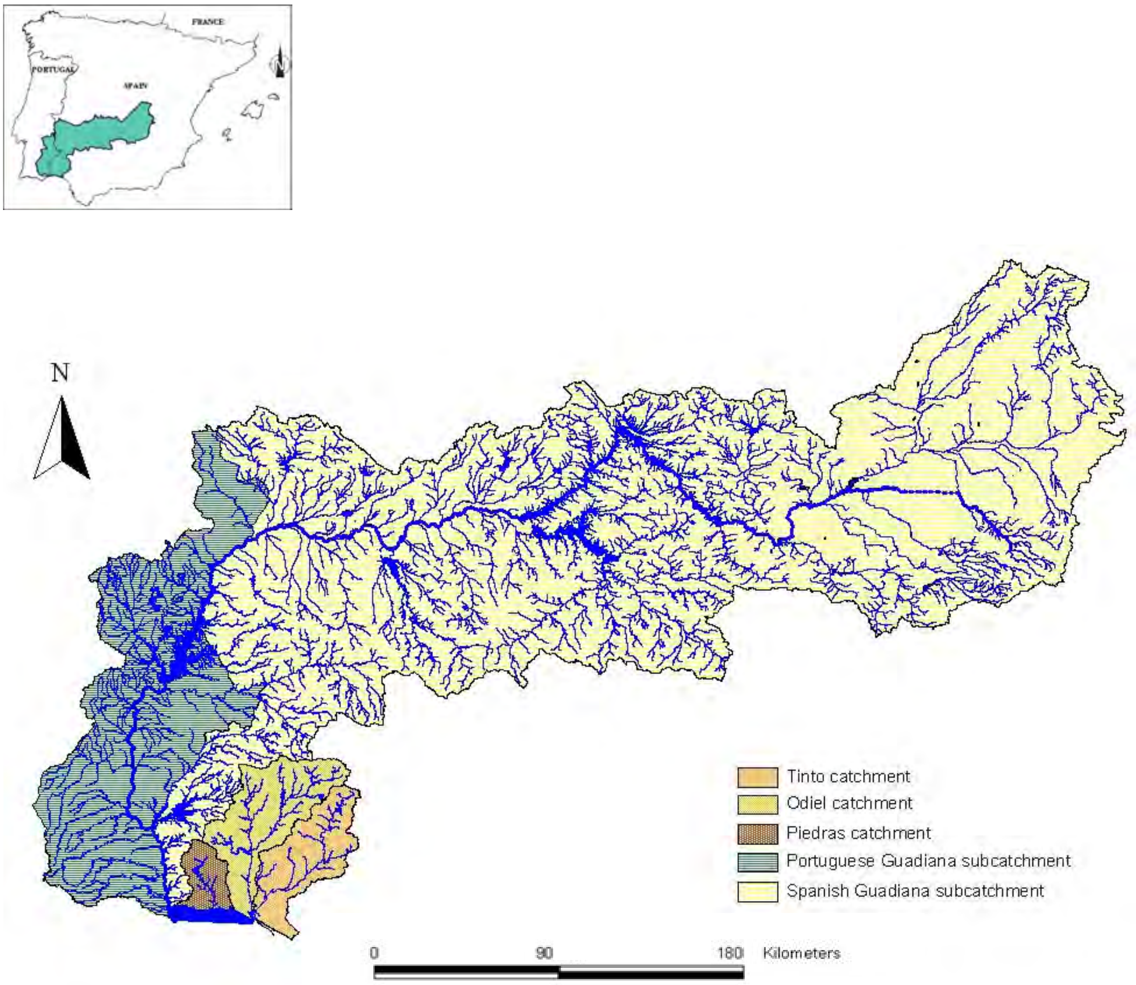


Figure 2.1 Guadiana, Tinto, Odiel and Piedras watersheds

2.1. THE GUADIANA WATERSHED

The Guadiana is the fourth largest watershed in the Iberian Peninsula. It is located in south–central Spain and Portugal and it drains an area of about 67,000 km^2 (83% in Spain and 17% in Portugal). The Guadiana River runs through 550 km in Spain and 260 km in Portugal; part of the river defines the border between the two countries. Most of the catchment lies in the driest area of the Central Spanish Plateau (Meseta). The watershed is therefore non–mountainous, and its water regime is not based on snowfall but on the rainfall patterns. Although the Guadiana is not an overpopulated watershed (28 hab/Km^2), the landscape was deeply transformed through extensive agricultural activities. The Guadiana is mostly an agricultural watershed, since ca 72% of its surface is dedicated to crops mainly based on irrigation, and only a 26% of its drainage area can be considered as natural or semi–natural. Up to 30% of agricultural land is occupied with intensive agriculture in irrigated lands, 18.5% occupied with extensive agriculture (olive groves or fruit trees) and in the lower part of the catchment, vegetables and crops under plastic greenhouses cover extensive areas. The water agricultural demand strongly competes with natural systems for hydraulic resources. About 8.3×10^9 m^3 of water is retained in 86 large reservoirs (>10^6 m^3) and more than 200 smaller ones (<10^6 m^3) for water supply. Agricultural practices can be so intense that the natural hydrograph is completely altered as a consequence of the river canalization and water abstraction. The strong uses in the watershed also imply the degradation of the river habitat and of the riparian forest. Only 3,150 km^2 (5.2% of the basin) is formally reserved and subjected to conservation management strategies.

2.1.1. GEOLOGY AND HYDROLOGY

The Guadiana watershed can be divided into three main regions according to differences in the geological settings and hydrology. Those differences translate into differential soil uses and water resource exploitation.

<u>The Upper Guadiana basin:</u>

It is situated in the north–eastern part (~19,000 km^2) in the central Meseta plateau. This section is flat to undulating, ranging from 550 to 1,000 m a.s.l. This area is made up of Jurassic, Cretaceous and Tertiary limestones. Due to the porosity of its substrata, and the moderate rainfall, surface water infiltrates forming relevant underground aquifers where subterranean waters have a relevant role. This area is drained by rivers that flow quietly from their sources and are closely connected to these aquifers. In this part, the surface waters have a highly irregular flow. These particular substrata cause that a "real" or unique source in the Guadiana cannot be defined, but it is mostly a rather diffuse contribution of several sources. The main tributaries in the right upper part are the Záncara which has an average flow of 2.4 m^3/s (though minimum is 0 m^3/s) and the Gigüela (or Cigüela) with an average water flow of 57.1 mm^3/year (1995–2001), with maxima in February and minima in winter and summer (CHG, 2005). Downstream, when Záncara and Gigüela flow into Alto Guadiana they origin *Las Tablas de Daimiel*, an ensemble of large shallow ponds and swamp areas susceptible of flooding because of the tiny slope of the land. This is an area of 1,712 Ha, which consists in a multitude of small lacustrine openings, spotted by islands, and covered of palustrian vegetation (fens, reeds and rushes). The *Tablas* originally received the contribution of the Gigüela and the discharge from the aquifer 23. The area is naturally submitted to strong fluctuations, flooded after strong rains and dried after low summer flows, but has been strongly affected in the last thirty years because of excess water detraction that affected the phreatic level of the spongy limestone beneath (Sabater*, et al.*, 2009). Today, however, this wetland that used to receive the natural discharge from the Western Mancha aquifer, survive artificially, thanks to the water transfers from the Tagus–Segura Aqueduct and to the artificial pumping of groundwater (Llamas*, et al.*, 2010). The last important tributary on the right margin is the Bañuelos with a very irregular watershed.

On the left margin the Guadiana receives the Córcoles, Alto Guadiana and Azuer, that constitute the first superficial Guadiana waters deriving from the karst formations of the Montiel ranges. These waters form the Ruidera lakes (15 in total), which are structured by the carbonate deposits of the spring waters. The water regime of these ponds is related with the rainfall in the upper mountains, with a reception delay of 4–6 months. These small lakes are interconnected either superficially through small waterfalls or subsuperficially. The first of the series is *La Blanca* (The White, named after the whitish carbonate precipitates in its bottom). The largest lake (*Colgada*) is 100 Ha of surface area, but most others range from less than 12 to 38 Ha. The water depth ranges from 19 m to 8 m. The lower ponds are progressively shallower and covered by macrophytic vegetation. The Peñarroya reservoir collects the waters from Ruidera and later water evaporates and infiltrates in the spongy substrata beneath, and therefore the river wanes for the first time. The outflows from the aquifers formerly originated the “Ojos del Guadiana” (Guadiana’s Eyes), in the La Mancha plain, at 608 m a.s.l., where the river reappeared. This was a collection of springs and emergences that flowed from the topsoil, once considered the “real” Guadiana headwaters, but that were completely vanished more than 30 years ago (Almagro–Costa, 2006).

Finally, in the southern part of La Mancha plain, appears the Jabalón which forms an extended and irregular network with an irregular water flow that usually dries out during summer period.

Figure 2.2. The Upper Guadiana watershed

<u>The Mid Guadiana basin:</u>

Several springs are at the origin of the continuous river network that characterizes most of the remaining part of the watershed. This part (~48,500 km^2) is geographically located in the western central part of the Meseta up to the border between Portugal and Spain. The Mid Guadiana basin, before the rivers turns into the south, is one of the most regulated river basins in Spain.

In this middle part, the first two tributaries on the right margin are the Bullaque and Estena winding north south direction from the Toledo's mountains. Both rivers cross Cabañero's National Park one of the best preserved zone in the whole Guadiana basin. The Estena flows into Cíjara's reservoir, the first of several large and connected reservoirs scattered all over the region (Cíjara, García de Sola, Orellana with 2867 Hm^3 of capacity). The buffering capacity of these huge reservoirs is used for crop irrigation.

On the left margin, the main tributary in this middle part of the watershed is the Zujar, which has the highest water flow in the Guadiana watershed. It hosts La Serena reservoir with 3,200 Hm^3 of capacity.

Guadalupejo, Ruecas and Búrdalo are the following tributaries flowing to Guadiana on the right margin, while Guadámez does on the left margin flowing south – north direction.

The Matachel is the next tributary in the left margin. Flowing from Sierra Morena it presents an extended watershed with a large number of small tributaries. Most of them become dry during summer. The river is regulated by the Alange reservoir that accounts with 825 Hm^3 capacity.

After receiving Matachel's waters, the Guadiana goes through the city of Mérida where it shows an average water flow of 157.4 m^3/s, but the extreme values ranged between 2.2 and 463 m^3/s (Sabater*, et al.*, 2009). Downstream from Mérida, on the right margin the mountain range of Sierra de Sao Mamede and San Pedro origin a wide range of small rivers (Aljucén, Lácara, Guerrero). The Gévora (Xévora in Portuguese) flows between Spain and Portugal becoming a natural border between the two countries. It flows into Guadiana, in Badajoz, where the river turns to the south.

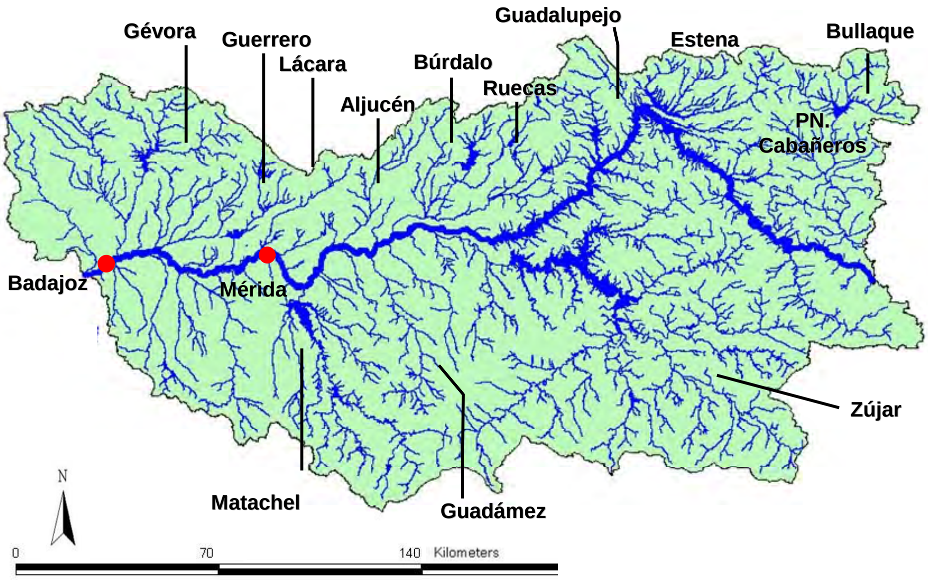


Figure 2.3. The Middle Guadiana watershed

The Lower Guadiana basin:

The lower Guadiana basin is made of carboniferous metamorphic slates rocks. The Portuguese sector is metasedimentary and metavolcanic with alkaline, acidic areas and Cenozoic deposits. In the last kilometers, all these materials yield a broad marsh plain .

The lower Guadiana basin is characterized by a very dense network of small rivers flowing to the Guadiana. After Badajoz, the Guadiana defines the natural border between Spain and Portugal. From the Spanish left margin, it receives waters from Alcarrache and Ardila. In Portuguese territory, the Guadiana hosts the largest artificial lake in Europe, the Alqueva Reservoir (4,150 Hm^3 capacity). Draining from Serra do Caldeirão the Degebre directly flows into the Alqueva reservoir. After the dam, the river turns to the south and gradually lowers from 800 to 200 m a.s.l. On the right margin it receives waters from Odeace and Terges. One hundred km before the mouth, the river narrows from 25 to only 3 meters forming a spectacular waterfall (Pulo do Lobo, 15.5 m high). The rivers flows completely constrained since Mértola where it comes back wider to the mouth. In this part the river receives waters from the tributaries Oeiras and Carreiras.

The last important tributary on the left margin is the Chanza which comes from Sierra de Aracena y Picos de Aroche. After receiving the Chanza waters, the Guadiana becomes again the natural international border till its mouth. In this last lower part, the Guadiana receives a number of short watercourses with an unpredictable hydrological regime: Ribera de la Rochona, Ribera Grande, Ribera de la Golondrina, Arroyo Grande, Arroyo Pedraza from the Spanish part, and Ribeira do Vascão, Odeleite and Beliche from the Portuguese margin. These short watercourses are commonly dry during summer, and some of them also stop flowing during winter. Finally, the Guadiana arrives at Huelva estuary in the Atlantic Ocean, forming a deltaic area where small islands alternate with sandbars. Given the low elevation, tidewater movements can reach Mértola, nearly 70 Km upstream to the mouth.

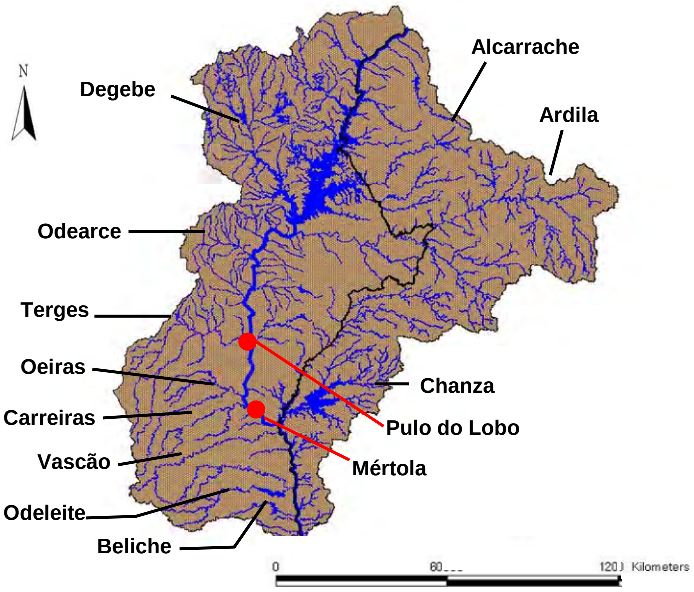


Figure 2.4. The Lower Guadiana watershed

2.1.2. CLIMATE

The general climate of the Guadiana watershed can be classified as Mediterranean with a moderate continental tendency that causes some areas to be extreme in terms of temperature range, and semiarid. The seasonal thermal fluctuations range between less than 5°C to 45°C. The mean annual temperature is 15°C, and the average precipitation is about 450 mm/year. The climate is characterized by cold winters, low rainfall, and an extended summer drought. Low winter temperatures may last for nearly two months, a rather unusual phenomenon in Mediterranean watersheds. Summer temperatures are high, and humidity low. The local climate is therefore characterised by extreme variations both between seasons (annual range of temperatures of ca. 50 ºC) and within the day (20ºC of diel fluctuation, particularly in summer). The watershed is characterized by high intra and interannual discharge variation and severe droughts and floods (Figure 2.5) (Sabater*, et al.*, 2009). Mean annual rainfall is around 400 mm, and falls mostly during winter and spring. Rainfall is nearly absent during summer, this lack of rainfall being more intense towards the eastern part of the watershed.

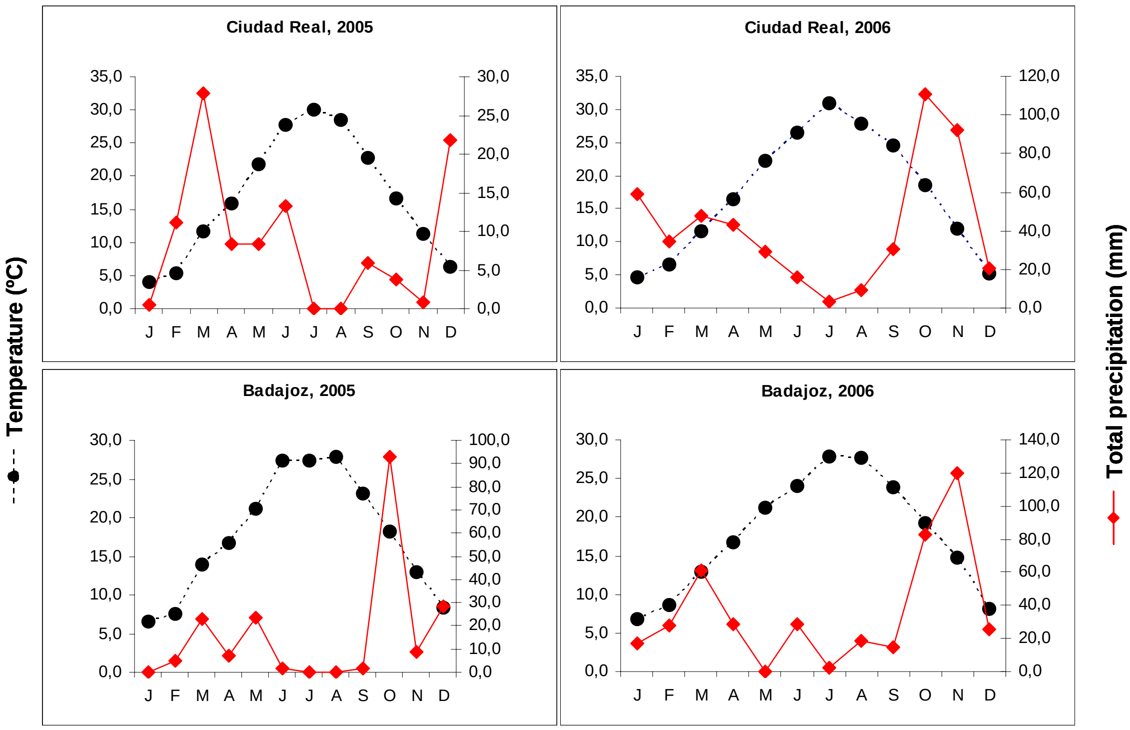


Figure 2.5 Mean mensual temperature and total precipitation in Ciudad Real (Upper watershed) and Badajoz (Middle watershed)

2.1.3. SOIL USES

The Upper Guadiana basin:

The Upper Guadiana constitutes one of the driest river basins in Spain. In this part, UNESCO recognized the collective ecological importance of wetlands in 1980, when it designated the «Mancha Húmeda» Biosphere Reserve (Troya and Bernués, 1990). In a largely arid region, these wetlands provided crucial nesting and feeding grounds for European migrating bird populations and were home to rare animal and plant species. The Tablas de Daimiel National Park (2,000 ha), was designed a Ramsar Site.

This part of the basin is mostly dedicated to agriculture and is the region with the highest groundwater irrigation proportion in the whole Guadiana basin (96%). The main water consuming sector in this region, with about 95% of total water use, are cereal crops. Intensive irrigation has decreased the phreatic level in recent times, overriding the renewal capacity of these waters by 300 Mm^3 per year (Olay*, et al.*, 2004). Intensive pumping by farmers practically exhaust the water flowing to the river and the Tablas, provoking deep conflicts between agriculture and conservation of rivers and groundwater–dependent wetlands.

The Mid Guadiana basin:

The Middle Guadiana Basin is the region with the highest surface irrigation proportion in the whole Guadiana basin (94%) (Aldaya and Llamas, 2008). Most of the crops are irrigated cereals and fresh–tomato. Their irrigation is guaranteed by the buffering capacity of the big reservoirs present in the zone, which is one of the most regulated river basins in Spain.

The Lower Guadiana basin:

This lower part of the catchment is covered by cork–oak forest and Mediterranean shrublands, and has a low human population density (20 inhabitants/km^2). This part of the watershed has short periods of precipitation followed by long dry periods. This extremely irregular water availability regime causes problems of overexploitation and shortage of resources because main agricultural practices in this area are constituted by vegetables and crops growing under plastic greenhouses using both surface and groundwater resources (Aldaya and Llamas, 2008).

2.1.4. SAMPLING LOCALITIES

A total of 244 localities scattered throughout the Spanish part of the Guadiana watershed were sampled during winter, spring and autumn of 2005–6 (Figure 2.6).The summer period was not included in the sampling scheme because many tributaries dried out during this period. The sampling sites were selected according to the previously defined water bodies in the watershed (CHG, 2005). 37 of these sites were situated in the Upper catchment, 174 in the Middle catchment and the remaining 33 in the Lower catchment.

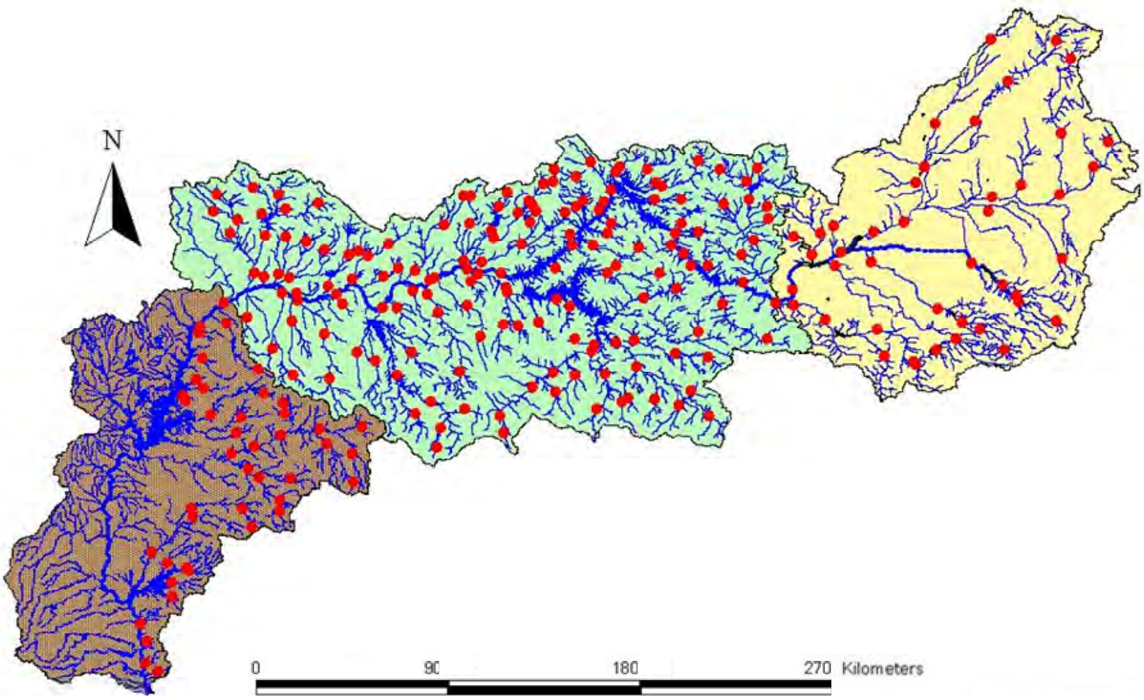


Figure 2.6. Sampling localities in Guadiana watershed

Table 2.1. Code, name and location of the 37 Upper watershed localities. (R=River Aab= downstream; Aar= upstream; Ctra= Road; Rbla=Rambla)

Code	Name	subcatchment	UTM–X	UTM–Y	Altitude
F019	R. Alarconcillo en Ossa de Montiel (Ermita San Pedro)	Alarconcillo	515971	4309629	840
F020	R. Alto Guadiana en Ruidera	Alto Guadiana	509368	4314490	780
F021	R. Pinilla en Ctra. El Bonillo - Viveros	Alto Guadiana	534906	4297509	980
F186	R. Alto Guadiana en el Molino del Ossero (PN Ruidera)	Alto Guadiana	516653	4307186	860
F243	R. Alto Guadiana Aab embalse Peñarroya	Alto Guadiana	495295	4324992	695
F091	R. Azuer Carricosa - Villanueva de los Infantes	Azuer	499889	4296708	815
F092	R. Azuer Aar presa Vallehermosa	Azuer	490568	4296964	760
F093	R. Azuer en Daimiel	Azuer	448274	4325467	620
F298	R. Azuer en ctra. La Solana - San Carlos	Azuer	479383	4303334	750
F302	R. Tortillo en Carrizosa - Vva. Ínfantes	Azuer	499205	4293774	800
F022	R. Bañuelos Aar. Malagón	Bañuelos	424305	4339047	635
F023	R. Bañuelos en Fernán Caballero	Bañuelos	420379	4338790	600
F001	R. Gigüela en Ctra. Torrejoncillodel Rey - N-400	Gigüela	534461	4429254	865
F002	R. Gigüela en Batán de San Pedro	Gigüela	511785	4409945	755
F003	R. Gigüela en Ctra. Villanueva de Alcardete - N-301	Gigüela	496627	4391303	695
F004	R. Gigüela en Ctra. Quero - Villafranca de los Caballeros	Gigüela	473018	4370270	645
F005	R. Gigüela en Villarta de San Juan (Pte Romano)	Gigüela	463213	4343992	615
F007	R. Riansares en Ermita de Riansares	Gigüela	504132	4429881	775
F008	R. Riansares en Ctra. Villa Don Fadrique - Lillo	Gigüela	478052	4390470	675
F009	R. Záncara Palomares del Campo - Abía de la Obispalía	Gigüela	541478	4420661	860
F010	R. Záncara en Ctra. Carrascosa de Haro - N-420	Gigüela	536964	4385476	755
F011	R. Záncara en Ctra. El Provencio - Villarrobledo	Gigüela	536964	4289246	695
F012	R. Záncara en Ctra. Pedro Muñoz - Tomelloso	Gigüela	504737	4355919	670
F014	R. Monreal en Ctra- Las Mesas - Pedro Muñoz	Gigüela	518020	4361702	671
F015	R. Rus en Ctra. Honrubia - Sta. María del Campo Rus	Gigüela	559006	4381961	780
F016	R. Rus en Santuario Virgen del Rus	Gigüela	352815	4289246	755
F017	R. Córcoles en Ctra. Socuéllamos - Villarrobledo	Gigüela	537942	4326705	827
F184	R. Gigüela en Villarrubia de los Ojos	Gigüela	449100	4339115	610
F190	R. Gigüela en salida trasvalse Tajo - Segura	Gigüela	524115	4417769	810
F094	R. Jabalón en Ctra. Montiel - Villanueva de los Infantes	Jabalón	510181	4283782	860
F095	R. Jabalón en Alcubillas	Jabalón	488015	4289032	784
F096	Rbla. Castellar en Ctra. Torrenueva - Valdepeñas	Jabalón	467920	4278129	718
F097	R. Jabalón en Ctra. Moral de Calatrava - Sta. Cruz de Mudela	Jabalón	451055	4293276	650
F098	R. Jabalón en Aldea del Rey	Jabalón	426399	4297844	613
F099	R. Jabalón en EA Puente Morena	Jabalón	411638	4305128	575
F100	Rbla. de Mudela Sta. Cruz de Mudela - Calzada Calatrava	Jabalón	454286	4281401	683
F242	Rbla. Castellar en Ctra. Torrenueva - Valdepeñas	Jabalón	467920	4278129	718

Figure 2.7. Localities in the Upper Guadiana watershed. F002 Gigüela (A); F019 Alto Guadiana (B); F017 Córcoles (Gigüela) (C) F092 Azuer (D)

Table 2.2. Code, name and location of the 174 Middle watershed localities. (R=River; Ctra= Road; Aar= upstream; A=Stream; Aab= downstream; confl=confluence)

Code	Name	subcatchment	UTM–X	UTM–Y	Altitude
F268	R. Albarregas en ctra. embalse de Cornalbo	Albarregas	740281	4316963	275
F046	R. Aljucén en confluencia Barranco de Viñazo	Aljucén	741160	4332713	316
F047	R. Aljucén en Ctra. Mérida - Cáceres	Aljucén	732077	4326495	252
F048	R. Aljucén Aar. confluencia Guadiana	Aljucén	725329	4315225	214
F252	A. Benazaire en Herrea del Duque	Benazaire	326701	4343463	410
F024	R. Bullaque en Retuerta del Bullaque	Bullaque	377515	4369047	730
F025	R. Bullaque Aar Pueblo Nuevo del Bullaque	Bullaque	390713	4354880	635
F026	R. Bullaque en Luciana	Bullaque	388364	4315531	530
F027	A. Bullaquejo en Ctra. a El Robledo	Bullaque	387415	4328665	585
F028	A. Pescados en PN Cabañeros	Bullaque	379302	4352156	634
F029	R. Milagro en El Molinillo	Bullaque	394942	4369541	710
F192	R. Bullaque en Las Casas del Río	Bullaque	393038	4335274	575
F193	A. de los Valles en Cortijos de Arriba	Bullaque	399259	4351419	700
F304	A. Pedralá ctra. Trincheto - Piedralá	Bullaque	400035	4345849	675
F044	R. Búrdalo en confluencia A. Humazo	Búrdalo	257453	4356475	393
F045	R. Búrdalo en Ctra. N-430	Búrdalo	754233	4320604	252
F202	R. Búrdalo en Escurial	Búrdalo	247703	4342457	320
F203	A. de las Muelas Aab embalse de las Muelas	Búrdalo	744490	4322956	310
F142	A. Calamón en camino El Cortijo de la Móra	Calamón	677346	4293871	220
F254	A. Canalijas en Csatiblanco	Canalijas	322954	4353672	375
F266	A. Chaparral en Valdetorres	Chaparral	754146	4311146	240
F274	A. de la Charca	Charca	655567	4287816	200
F207	A. Doña Juana en La Puebla de Don Rodrigo	Doña Juana	354436	4333294	468
F251	A. Encinada en ctra. Bohonal - Villalta de Montes	Encinada	346544	4352289	447
F219	A. Entrin en la Corte de Piedras	Entrin	698794	4294264	224
F031	R. Estena en Navas de Estena (PN Cabañeros)	Estena	367637	4373245	640
F032	R. Estena en Ctra. Anchuras - Horcajo de los Montes	Estena	350561	4360621	485
F033	R. Estenesilla en Ctra. Anchuras - Horcajo de los Montes	Estena	343464	4368632	475
F197	A. Fresnedoso en Minas de Sta. Quiteria	Estena	330696	4370177	445
F241	R. Estomiza en Ctra. Anchuras - Horcajo de los Montes	Estena	347144	4362633	495
F246	A. De los Hilos en emb. La Cabezuela	Estena	475642	4282546	718
F247	A. Sequillo ctra Aldea del Rey - Granátula de Calatrava	Estena	430392	4288621	609
F249	A. Tuno en Casa del Quinto	Estena	388488	4362470	693
F255	A. Puerto Rey en Minas de Sta. Quiteria	Estena	328803	4367833	445
F221	A. Friegamuñoz en Casa Huerta de la Luz	Friegamuñoz	659208	4259144	190
F278	A. de los Cabriles en Cheles	Friegamuñoz	651010	4260079	209
F194	A. Frío en Finca Botín	Frío	371556	4323528	549
F264	A. Grande en Talarrubias	Talarrubias	300689	4322571	352
F042	R. Gargáligas en Casa del Sotillo	Gargáligas	304817	4348609	423
F043	R. Gargáligas en Navalvillar de Pela	Gargáligas	287247	4333594	305
F053	R. Gévora en La Codosera	Gévora	658757	4342260	305
F054	R. Gévora en Carrión	Gévora	669914	4339080	222
F055	R. Gévora en Valdebótoa	Gévora	679099	4318614	80
F056	R. Zapatón en Ctra. Alburquerque - Aliseda	Gévora	688250	4352267	270
F057	R. Zapatón en ctra. Villar del Rey - Alburquerque	Gévora	683628	4332734	180
F058	R. Albarragena en Ctra Alburquerque - Herreruela	Gévora	676385	4354922	330
F059	R. Gavilán en Ctra. Aliseda	Gévora	706981	4349706	304
F205	R. Alcorneo en ctra. La Codosera - San Vicente Alc	Gévora	661289	4348487	300
F206	A. Valdeborrachos en Silvestre	Gévora	666617	4334223	258
F272	A. Palomares ctra. Villar del Rey - Aliseda	Gévora	692187	4345856	200
F291	A. de los Hoyos en Alburquerque	Gévora	681134	4342949	200
F138	R. Guadajira en Solana de los Barros	Guadajira	714298	4288352	230
F139	R. Guadajira en Guadajira	Guadajira	700583	4303109	190
F218	R. Guadajira en Fuente del Maestre	Guadajira	717786	4267540	326
F271	A. Cabrillas en Valdelacalzada	Guadajira	699980	4306701	200
F035	R. Guadalupejo en Guadalupe	Guadalupejo	299529	4368896	570
F036	R. Guadalupejo confl. A. Descortesado	Guadalupejo	313415	4353826	375
F198	R. Silbadillos en Ctra. Guadalupe - Cañamero	Guadalupejo	298945	4363455	585

Code	Name	subcatchment	UTM–X	UTM–Y	Altitude
F199	A. Valdefuentes en Central Nuclear Valdecaballero	Guadalupejo	310223	4351595	405
F297	R. Guadalupejo en Alía	Guadalupejo	310425	4365477	434
F127	R. Guadámez en Retama	Guadámez	255699	4273873	414
F128	R. Guadámez en Ctra. Don Benito - Guareña	Guadámez	240621	4309934	245
F216	R. Guadámez en camino Cortijo Sta. Natalia	Guadámez	245819	4301829	290
F034	R. Guadarranque en Ctra. Puerto San Vicente - Alía	Guadarranque	316778	4372045	462
F072	R. Guadiana en Puente Navarro	Guadiana	434093	4329667	575
F073	R. Guadiana en Puente Alarcos	Guadiana	411530	4312422	584
F074	R. Guadiana en EA Valbuera	Guadiana	403297	4305483	550
F075	R. Guadiana an Luciana	Guadiana	387935	4315596	528
F076	R. Guadiana en La Puebla de Don Rodrigo	Guadiana	360569	4329062	455
F077	R. Guadiana en confluencia Valdehornos	Guadiana	356508	4338779	430
F078	R. Guadiana en presa del Cijara	Guadiana	326197	4359499	365
F080	R. Guadiana Aab. emblase Orellana	Guadiana	274883	4320032	280
F081	R. Guadiana Aar. confluencia Zújar	Guadiana	258963	4323204	255
F082	R. Guadiana Aab. Medellín	Guadiana	241889	4317143	236
F083	R. Guadiana en apeadero Zarza de Alange	Guadiana	740391	4302604	170
F086	R. Guadiana en Azud de Benavides	Guadiana	666444	4299983	162
F187	R. Guadiana en Badén Torremayor	Guadiana	718349	4307676	205
F320	R. Guadiana en Luciana	Guadiana	364508	4323467	528
F050	R. Alcazaba en Casas de San Pedro	Guerrero	710033	4324502	230
F051	R. Guerrero Ctra. La Roca de la Sirena - Villar del Rey	Guerrero	692984	4332771	245
F052	R.Guerrero en Novelda del Guadiana	Guerrero	691092	4314883	185
F204	A. Lorianilla en La Roca de la Sirena	Guerrero	702434	4330898	255
F275	A. Higuera en Cortijo del Ariero	Higuera	655670	4284810	200
F258	A. Horadado Aar confl Guadiana	Horadado	308663	4333140	327
F049	R. Lácara en Ctra. La Neva de Serena - Aljucén	Lácara	723123	4326277	254
F270	R. Lácara en Lácara	Lácara	714333	4311116	208
F140	R. Limonetes en EA Talavera la Real	Limonetes	693024	4306045	170
F141	R. Albuera en Ctra. Albuera - Almendral	Limonetes	689925	4280084	280
F277	R. Salvatierra en camino Nogales - Salvatierra	Limonetes	700451	4267951	750
F129	R. Matachel en Ctra. Campillo de Llerena - Azuaga	Matachel	261374	4249210	560
F130	R. Matachel Valencia de las Torres - Campillo de Llerena	Matachel	241846	4259538	410
F131	R. Matachel en Ctra. Hornachos - Puebla del Prior	Matachel	749131	4271512	318
F132	A. San Juan en vado Dehesa del Cahozo	Matachel	240058	4287745	395
F133	R. Palomillas Puebla de la Reina - Valle de la Serena	Matachel	755250	4282218	360
F134	R. Retín en Ctra. Valencia de la Torres - Usagre	Matachel	758847	4253597	435
F135	A. Valdemedel en Ctra. Villafrnca del B. - Puebla de la R	Matachel	738767	4277380	375
F136	A. Bonhabal en confl. A. del Manantial	Matachel	730125	4280483	365
F217	A. Conejo en Maguilla	Matachel	247079	4247204	520
F269	A. Conejo en ctra. Llerena - Ahillones	Matachel	245086	4237469	600
F143	R. Olivenza en Ctra. Barcarrota - V. de Leganés	Olivenza	683998	4269700	403
F144	R. Olivenza en Ctra. Olivenza - Badajoz	Olivenza	668168	4290237	190
F125	R. Ortigas Quintana de la Serena - Valle de la Serena	Ortigas	265382	4290346	399
F126	R. Ortigas Aar confluencia Guadiana	Ortigas	244234	4317508	250
F301	R. Ortigas en ermita Ntra. Sñra. Antigua	Ortigas	259185	4304553	315
F208	A. Pelochejo en Herrera del Duque	Pelochejo	324738	4338889	438
F276	A. de las Pintas en San Benito de la Contienda	Pintas	660274	4278287	200
F030	A. Bohonal en Bohonal	Rubial	346786	4354481	454
F196	A. Rubial en Horcajo de los Montes	Rubial	359574	4354906	580
F037	R. Ruecas en Cañamero (EA)	Ruecas	295277	4361824	540
F038	R. Ruecas en Ctra. Logrosan - Ex 116	Ruecas	288189	4353847	390
F039	R. Ruecas Aar. del Lavadero	Ruecas	282867	4347614	350
F040	R. Ruecas en Rena (Saica)	Ruecas	257474	4325709	261
F041	R. Alcollarín en Ctra. Zorita - Sta. Cruz de la Serena	Ruecas	260628	4356342	385
F200	A. Pizarroso en Ctra. Zorita - Logrosán	Ruecas	277868	4357948	440
F201	R. Alcollarín en Campolugar	Ruecas	260177	4343166	290
F259	A. Piedrabuena en Casa Navacarrazo	Ruecas	292180	4348865	399
F260	A. Grande en Logrosán	Ruecas	282281	4355621	388
F261	A. Herrera en Zorita	Ruecas	273501	4351725	400
F293	R. Cubilar en EX 116 km. 13	Ruecas	288591	4343788	326
F294	R. Cubilar Aar. Embalse	Ruecas	289658	4351296	399

Code	Name	subcatchment	UTM–X	UTM–Y	Altitude
F295	R. Ruecas en Madrigalejo	Ruecas	271030	4337026	286
F296	R. Gargáligas en Valdivia	Ruecas	265790	4325403	256
F267	A. San Juan en Guareña	San Juan	747485	4302882	257
F195	A. San Marcos en Arroba de los Montes	San Marcos	367085	4339370	557
F220	Ribera de Táliga (Alconchel) en San Benito de la C	Táliga	657733	4274222	177
F101	R. Tirteafuera en Almodovar del Campo	Tirteafuera	400123	428898	649
F102	R. Tirteafuera en Ctra. Abenójar - Saceruela	Tirteafuera	378298	4305651	567
F137	A. Tripero en A. San Serván	Tripero	721327	4303192	225
F250	A. Valdehornos en Navalpino	Valdehornos	359200	4343612	466
F256	A. Valmayor en Casasa de Agua Arriba	Valmayor	317258	4333335	382
F104	R. Zújar en Cuenca	Zújar	276172	4244664	540
F105	R. Zújar en camino Finca el Cintado	Zújar	274373	4252969	528
F106	R. Zújar en Ctra. Belalcázar - Cabeza del Buey	Zújar	311934	4283194	377
F107	R. Zújar en embalse de la Serena	Zújar	318377	4286636	360
F108	R. Zújar en Entreríos (playa)	Zújar	263998	4319360	265
F109	R. Guadalemar en Ctra. Guadalupe - Tamurejo	Zújar	328878	4323561	449
F110	R. Agudo en camino Finca el Guijo	Zújar	349223	4319873	575
F111	R. Esteras en Valdemanco de Esteras	Zújar	341868	4308079	432
F112	A. de la Fuente en Saceruela	Zújar	359691	4312291	553
F113	R. Guadalmez en pista Conquista - Fuencaliente	Zújar	372302	4252806	575
F114	R. Guadalmez en Sta. Eufemia	Zújar	338068	4275943	395
F115	R. Guadalmez en Guadalmez	Zújar	328590	4287425	340
F116	R. Valdeazoques en Lavadero de mineral de Almendrejos	Zújar	352818	4289200	445
F117	R. Valdeazoques en apeadero del Chillón	Zújar	336732	4286574	375
F118	R. Quejigares en Ctra. Abenójar - Fontanosas	Zújar	367334	4292058	525
F119	R. Alcudia en casa Jaralejo	Zújar	356666	4282274	459
F120	R. Guadamora en Ctra. Torrecampo - Conquista	Zújar	358183	4258003	520
F121	A. Sta. María en Ctra. El Guijo - Torrecampo	Zújar	346895	4260892	507
F122	A. Cigüeñuela en Dos Torres	Zújar	334505	4261029	560
F123	R. Guadamatilla en Ctra. El Viso - Hinojosa del Duque	Zújar	324374	4262634	465
F124	R. Guadalefra en Ctra. Castuera - Campanario	Zújar	276041	4295334	345
F183	R. Guadarramilla en Ctra. El viso - Dos Torres	Zújar	331375	4259819	556
F185	R. Zújar en Monterrubio de la Serena	Zújar	289714	4266490	450
F209	A. Patuda en Hinojosa del Duque	Zújar	300358	4264196	485
F210	R. San Juan en Finca Los Claros	Zújar	363634	4264946	570
F211	R. de la Cabra en Bienvenida	Zújar	371693	4280404	605
F212	R. Agudo en Siruela	Zújar	324392	4320183	395
F213	A. Dos Hermanas en embalse de la Serena	Zújar	306956	4304066	388
F214	R. Almonchón en Castuera	Zújar	292608	4296642	425
F215	A. Mejorada en Castuera	Zújar	282752	4295373	340
F240	R. Guadamatilla camino Finca el Mato	Zújar	317337	4283415	363
F262	A. del Bueyu en Cabeza del Buey	Zújar	308827	4289327	474
F263	A. Cebolloso en Fuente del Acebuche	Zújar	301113	4307801	401
F265	A. Molar en La Coronada	Zújar	261149	4315971	277
F290	A. Carneros en Talarrubias	Zújar	314813	4325388	385
F299	R. Zújar en estación de Zújar	Zújar	299357	4273127	411
F300	R. Guadalefra Aar. Confl. Zújar	Zújar	277693	4310786	280
F303	A. Jarrilla en Belalcázar	Zújar	307934	4270130	476
F305	A. Pizarroso aab. Emb. Sierra Brava	Zújar	270629	4339720	295
F306	R. Guadamatilla ctra. Beleacázar - Sta Eufemia	Zújar	320993	4274680	404
F307	R. Alcazabe en Alcazaba	Zújar	703618	4316416	209
F308	A. Valdecondes Aar. A. Coto Calderón	Zújar	728133	4328553	287
F309	R. Zújar Aab. Emb. Zújar	Zújar	277345	4312967	271
F311	R. Guadalemar en Fuenlabrada de los Montes	Zújar	339384	4331750	529

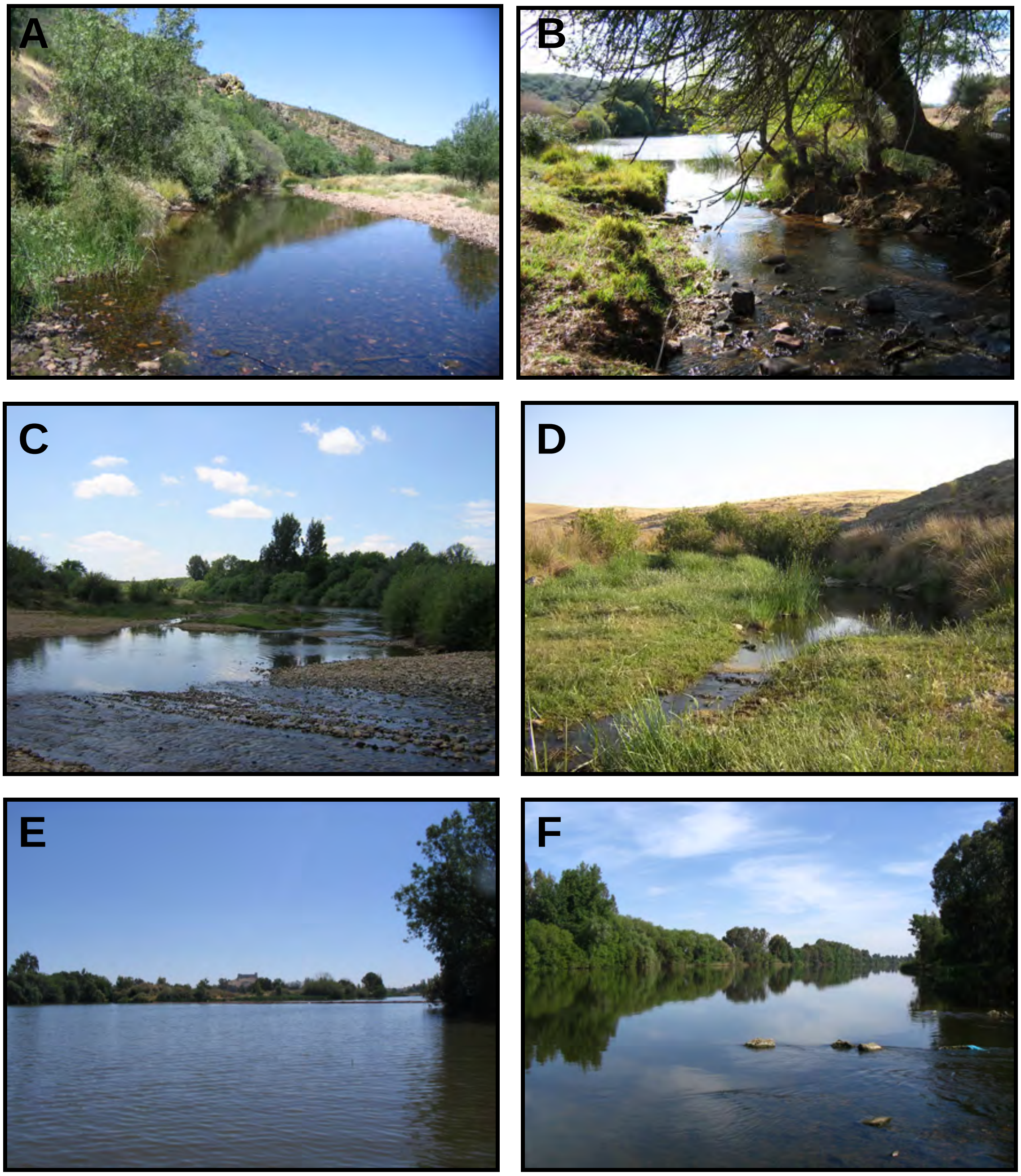


Figure 2.8. Localities in the Middle Guadiana watershed. F032 Estena (A); F111 Zújar (B); F055 Gévora (C); F134 Matachel (D); F082 Guadiana (E); F086 Guadiana (F)

Table 2.3. Code, name and location of the 33 Lower watershed localities. (R=River; Ctra= Road; Aar= upstream; A=Stream)

Code	Name	subcatchment	UTM–X	UTM–Y	Altitude
F145	R. Alcarrache en Ctra. Jerez de los Caballeros - Barcarrota	Alcarrache	687834	4258979	433
F146	R. Alcarroche en Ctra. Villanueva del Fresno - Oliva del Fresno	Alcarrache	663737	4247205	183
F222	R. Godolid en Zahinos	Alcarrache	677486	4246791	280
F149	R. Ardila Aab pantano Valencia del Ventoso	Ardila	718645	4237156	360
F150	R. Ardila en EA Jerez de los Caballeros	Ardila	697049	4239648	258
F151	R. Ardila en ctra. Oliva de la Frontera - Encinasola	Ardila	685147	4233663	211
F153	R. Bodión en Ctra. Fuente de Cantos - Segura de L?	Ardila	730783	4233329	500
F154	R. Atarja en Ctra. Fuente de Cantos- Santos de Maimona	Ardila	734282	4245809	508
F155	R. Múrtigas en La Nava	Ardila	698322	4240439	420
F157	R. Sillo Aar ebmbalse Sillo	Ardila	702720	4219363	425
F223	R. Bodion en Valverde de Burguillos	Ardila	714611	4243733	327
F224	A. Vargas en Valle de Sta. Ana	Ardila	698419	4254583	428
F225	R. Ardila en Calera de Leon	Ardila	732046	4219834	580
F226	A. Tremedera (Frio) en La Nava	Ardila	698173	4209279	347
F227	R. Sillo en Encinasola	Ardila	688178	4219020	258
F281	A. Rubiales en Valle de Sta. Ana	Ardila	698161	4249795	450
F282	R. San Lázaro en Burguillos del Cerro	Ardila	708253	4247041	350
F288	R. Ardila en Frontera Portugal	Ardila	674021	4229744	174
F292	A. Barcía Longa en San Silvestre	Barcía Longa	641506	4138901	100
F158	R. Chanza en EA Aroche	Chanza	680922	4204061	278
F159	R. Chanza Rosal de la Frontera - Barbara de Camino	Chanza	657066	4202072	160
F160	R. Chanza en camino Patmogo-Portugal	Chanza	640152	4180805	87
F161	R. Alcalaboza en Rosal de la Frontera - Barbara de Camino	Chanza	658344	4198200	160
F162	R. Malagón en dehesa Zurita	Chanza	658084	4173706	143
F163	Ribera de Albahacar en Paymogo	Chanza	647693	4176599	141
F164	R. Cobila en Puebla de Guzmán	Chanza	649054	4166873	100
F228	R. Alcalaboza en Cortegana	Chanza	688282	4195956	520
F285	R. Viguera Aab. Embalse del Risco	Chanza	651072	4160894	100
F287	R. Aguas de Miel en Dehesa Zurita	Chanza	657084	4174852	100
F230	A. Grande en Villablanca	Grande	640718	4128533	3
F253	A. Grande en Castiblanco	Grande	320750	4349337	418
F286	R. Grande de la Golondrina ctra. Sanlúcar - San Silvestre	Grande	637150	4147189	100
F231	A. Pedraza en Villablanca	Pedraza	647067	4125321	18

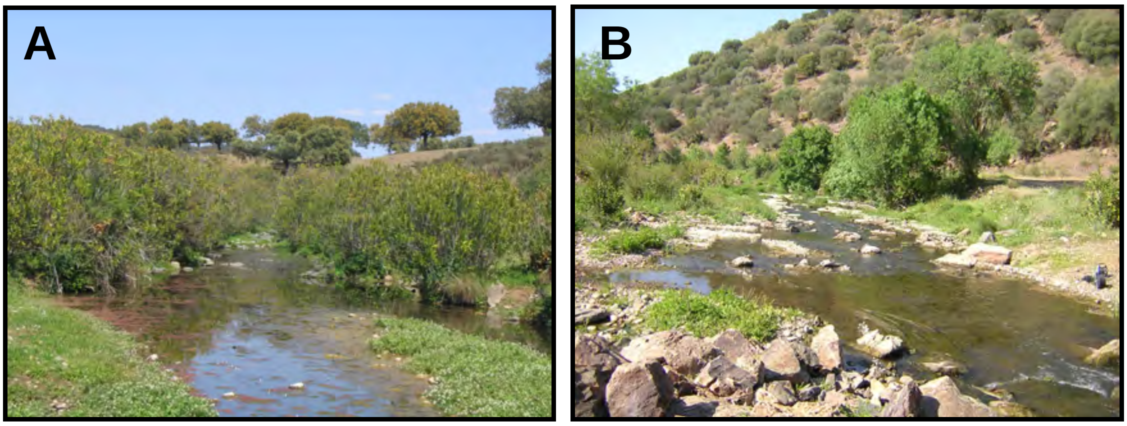


Figure 2.9. Localities in the Low Guadiana watershed. F281 Ardila (A); F146 Alcarrache (B)

2.2. TINTO, ODIEL AND PIEDRAS WATERSHED

Tinto–Odiel and Piedras watersheds have a total extension of 4,847 km^2. They present an extended network draining an area with slight slope. Tinto and Odiel join and drain together, forming the Huelva estuary, an extensive salt marsh which is an important nature reserve. The Piedras river is a short stream with very restricted fluvial catchment.

Part of the Tinto–Odiel courses run over the Iberian Pirite Belt (IPB), and they are seriously affected by acid mine drainage. Even though there is no active mining nowadays, pollution continues to arrive to the watercourses due to the oxidation of mining wastes (Nieto*, et al.*, 2007). These waters can be defined as an extreme habitat in terms of their very low mean pH (near 2.5) and high concentration of heavy metals, especially ferric iron, copper and zinc, as well as some anions such as sulfate (López–Archilla and Amils, 1999). Only extremophilous taxa can survive on these situations of very low pH and high heavy metals deposition (López–Archilla*, et al.*, 2001, Niyogi*, et al.*, 2002, Gerhardt*, et al.*, 2008).

2.2.1. GEOLOGY AND HYDROLOGY

<u>Tinto–Odiel system watershed</u>

The Tinto–Odiel river system has their source in Sierra de Aracena, at an altitude of 900 m a.s.l. Part of their courses run over the Iberian Pyrite Belt (IPB), and they are seriously affected by acid mine drainage.

The Tinto and Odiel rivers converge into a common coastal wetland, with marked tidal influence. Around this salt marsh zone there is an intensive agricultural, industrial and urban development (MMA 2005).

The Tinto receives from the left margin waters from the Jarrama and Corumbel tributaries, whereas Candón, and Rivera de la Nicoba flow from the right margin.

The Odiel flows in a NE–SW direction up to the intersection with the Oraque. From this confluence to the mouth, the river turns in a N–S direction, It receives waters from Arroyo Agrio from the left margin, whereas and the Olivarga, Oraque and Rivera de la Meca waters from the right margin.

Piedras watershed

The Piedras watershed is a small basin (388 Km^2) situated between the Lower Guadiana basin and the Tinto–Odiel system. The short river runs NW–SE direction from the Sierra del Almendro directly to the Atlantic Ocean. It has a total slope of 100 m from its origin to the mouth. In the upper part, it presents a group of small tributaries draining from Sierra del Almendro.

Piedras is a highly regulated watershed with two small reservoirs: the Piedras and Los Machos (59 and 12 hm^3 respectively). Both are used for water supply to agricultural practices in the lower catchment. The first one receives waters from the Chanza reservoir along an inter basin water transfer channel.

The final part of this river ends into an extensive and well delimited estuary.

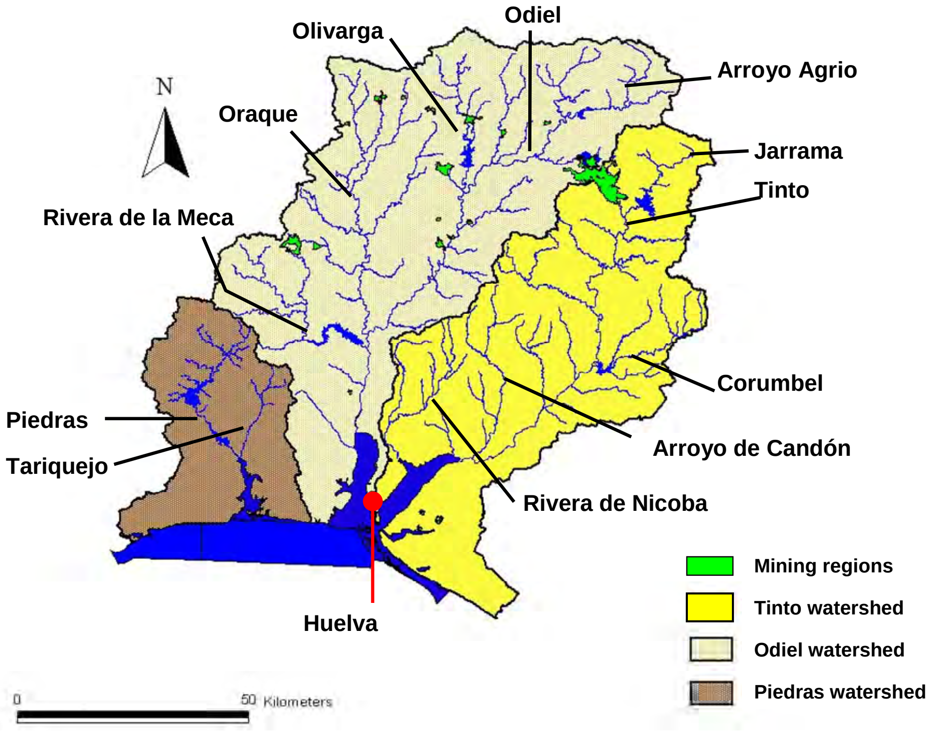


Figure 2.10. The Tinto–Odiel and Piedras watersheds

2.2.2. CLIMATE

Tinto–Odiel and Piedras watersheds are small littoral basins, characterized by the low roughness of their watersheds. The general climate is dry Continental–Mediterranean, with an average precipitation of about 700 mm/year and an average annual temperature of 18–19 ºC. They present mild winters, low rainfall, and an extended summer drought with high temperatures, and low humidity.

Rainfall is irregularly distributed along the year. It is absent during summer, concentrating during October–April period, being maxima during January and February. In some parts of Sierra de Aracena precipitation can achieve 1,200 mm/year while it is only of 400 mm/year near the Ocean (MMA, 2005).

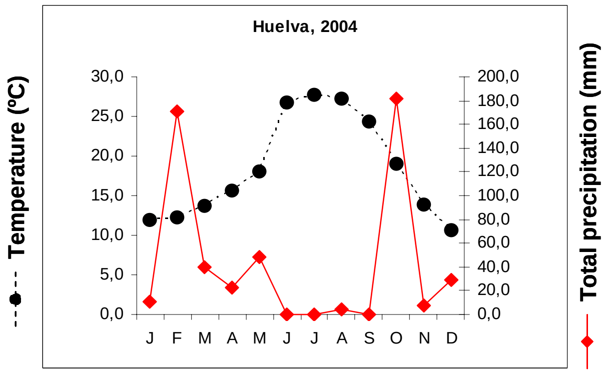


Figure 2.11. Mean mensual temperature and total precipitation in Huelva

2.2.3. SOIL USES

<u>Tinto–Odiel system watershed</u>

The Iberian Pyrite Belt (IPB) is one of the most extensive sulfide mining regions in the world, and it ranges from sw Spain to the Portuguese Atlantic coast. Associated with the complex sedimentary materials in this area, many massive sulphide deposits occur, and they have been explored and mined for more that 5000 years (Nocete*, et al.* 2005). Minas de Río Tinto has been intensively exploited during the Phoenician and Roman periods and again during the 19th century, but about 80 mines have been operative during the last hundred years (Sáez*, et al.* 1999).

The upper part of both rivers run over IBP. Mining activities have been present in the zone from the ancient times, and their waters are seriously affected by acid mine drainage.

The upper part of Tinto watershed is rough and human settlements are scarce. In the lower part of the catchment vegetables and crops, mostly under plastic greenhouses, are the dominant soil uses.

The Odiel watershed is poorly humanized along its watershed. In the upper part of the main river, there are numerous evidences of ancient mining activities. Lixiviates coming from this old mines are still affecting Odiel waters.

Around the lower salt marsh zone there is an intensive industrial and urban development (MMA, 2005).

Piedras watershed

Regarding the human occupation, the river is divided in the upper part which is of low density and with scarce crops, and the middle–lower part which is more densely populated and includes extensive irrigations (MMA, 2005). In this area, two reservoirs remarkably alter the natural fluvial regime.

2.2.4. SAMPLING LOCALITIES

A total of 18 sampling stations were visited during winter of 2005 in the Tinto, Odiel and Piedras rivers (Figure 2.12). Nine of these sites were situated in the Tinto catchment, 7 in Oraque catchment and the remaining 2 in the Piedras catchment.

From these 18 stations, six were affected by acid mine drainage, 3 of them situated in the Tinto river basin, and 3 of them were situated in the Odiel river basin.

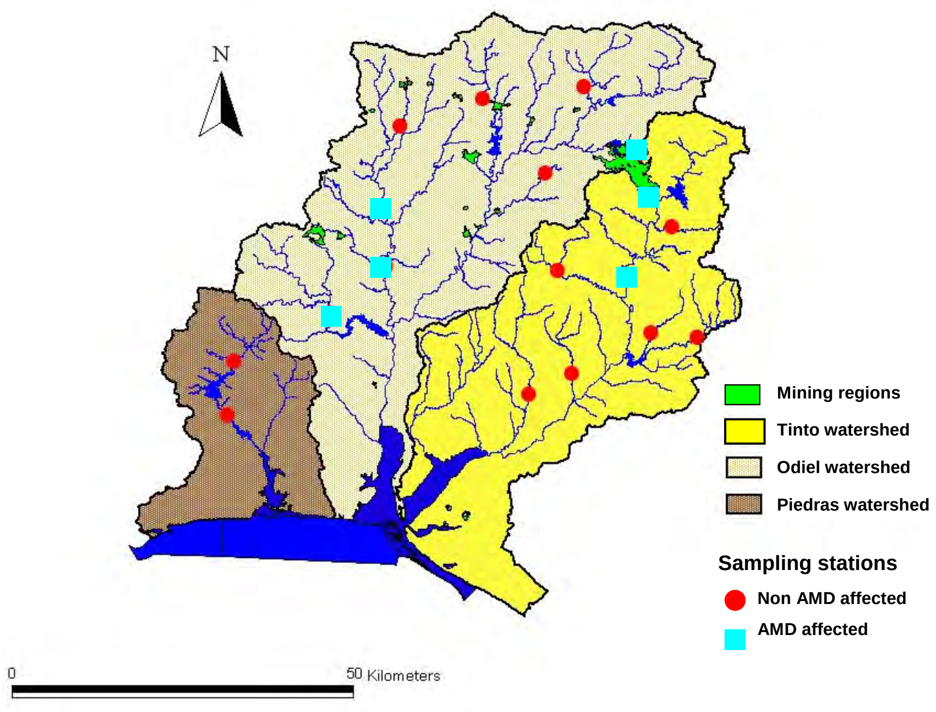


Figure 2.12 Location map of 18 sampling stations in the Tinto–Odiel and Piedras watersheds

Table 2.4. Code, name and location of the 18 Tinto–Odiel and Piedras localities. (# Acid Mine affected localities; R=river; Ctra= road; A=stream; Aab=downstream; Aar= upstream)

Code	Name	Watershed	UTM–X	UTM–Y	Altitude
F176#	R. Meca S. Bartolomé de la Torre - La Nava	Odiel	670049	4150921	67
F177#	R. Oraque en Ctra A-478	Odiel	676794	4167207	80
F178	R. Olivargas Aab embalse Cueva de la Mora	Odiel	690906	4184437	209
F232	Rambla de Sta. Eulalia en Sta. Eulalia	Odiel	705615	4187269	265
F233	Barranco del Fresno en Cerro de Andévalo	Odiel	677802	4178194	155
F234#	R. Oraque en Alosno	Odiel	677919	4155758	35
F235	Rambla del Villar en El Villar	Odiel	700506	4173765	240
F179	R. Piedras Aab embalse Piedras	Piedras	655800	4134700	160
F188	Rambla de los Montes Aar embalse Piedras	Piedras	656254	4143030	80
F168	A. Candón en Candón	Tinto	700509	4140747	35
F169	R. Corumbel en camino Paterma-Berrocal	Tinto	724887	4150713	125
F189	A. Tamujoso Aar embalse Corumbel	Tinto	718252	4151130	105
F237	Rambla de Cañamar en Valverde del camino	Tinto	703950	4160050	300
F238	A. Helechosos en Niebla	Tinto	706672	4143127	49
F239	A. del Gallego en Berrocal	Tinto	718874	4166917	186
F332#	R. Tinto en Berrocal	Tinto	699786	4158712	100
F333#	R. Tinto en Minas Peña de Hierro	Tinto	714818	4178367	350
F334#	R. Tinto Aar. Minas Peña Hierro	Tinto	712574	4171159	–

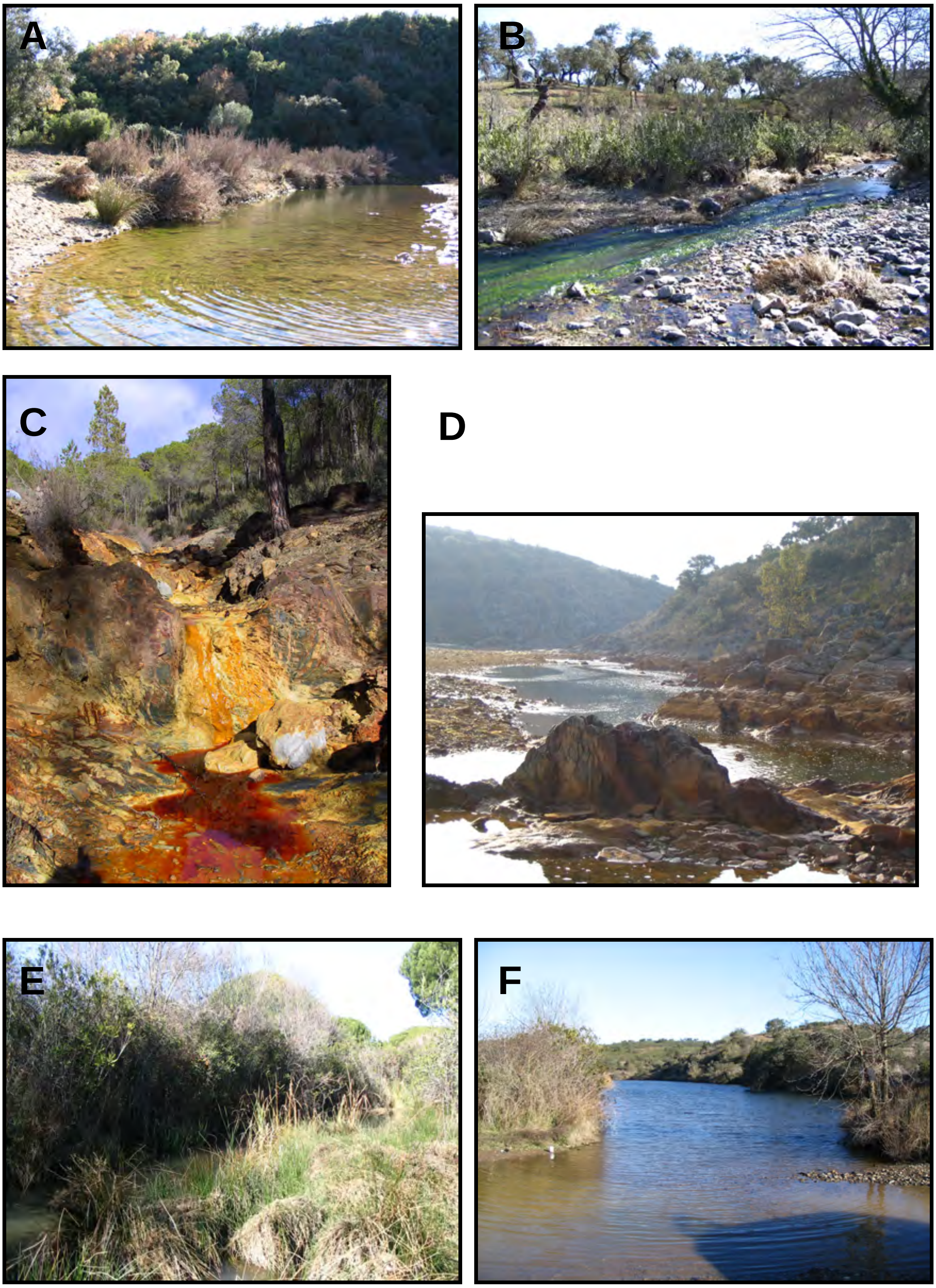


Figure 2.13 Localities non AMD affected in Tinto–Odiel system watershed: F189 Tamujoso in Tinto watershed (A); F232 Santa Eulalia in Odiel watershed (B). Localities AMD affected in Tinto–Odiel system watershed. F334 Tinto (C); F234 Oraque in Odiel watershed (D). Localities in Piedras watershed. F179 Piedras (E); F188 Rambla de los Montes (F)

CHAPTER III

MATERIAL AND METHODS

3.1. ENVIRONMENTAL MONITORING

Several environmental variables were measured in the field. Conductivity, pH, dissolved oxygen concentration and water temperature were measured with WTW MultiLine F/SET–3 P4. River section was derived after measuring total width and partial depth every 10 to 50 cm across a transect. In each partial section, current velocity was measured with a portable current–meter (Neyrflux 80, Neyrtec). Total flow was calculated as the summitry of partial flows of each section (Elosegi and Sabater, 2009). Wet width, wet perimeter, hydraulic radius and maximum depth were also determined from these measurements.

River habitat index (IHF) was determined in the field following Pardo *et al.* 2002 (Pardo*, et al.*, 2002). This index evaluates the relationships between habitat heterogeneity and those physical variables of the stream channel influenced by hydrology and substrata composition. Therefore, this index considers variables such as frequency of riffles, flow velocity, mean depth, and substratum diversity, among others.

Figure 3.1. Flow estimation (left) and river habitat determination (right)

During spring sampling period an index of riparian quality (QBR) was calculated following Munné *et al.* (Munné*, et al.*, 2003). This index was calculated in stream lengths of 100m, and it is the sum of four scores based on respective aspects of riparian quality: 1) total vegetation cover, 2) vegetation cover structure, 3) cover quality and 4) river channel alterations. After completing the analysis, the sum of the four parts gives the final QBR index which oscillates between 0 and 100.

Water for chemical analyses was collected during spring and autumn samplings. Water samples were filtered (Whatman nylon filters, 0.2 μm pore) preserved with chloroform (0.7 mL of chloroform per 100 mL of water) and stored in polyethylene bottles (Murphy and Riley, 1956, Fishman*, et al.*, 1986, Kotlash and Chessman, 1998). The analyzed chemical data included those characterizing the geochemistry of the waters (alkalinity, Ca^{2+}, Cl^{-}, SO_4^{2-}) and those directly affecting the primary producers (NH_4^{+}–N, PO_4^{3-}–P , NO_3^{-}–N). All these analyses were realized following standard procedures (APHA, 1989).

Physiographical variables and some basin characteristics were GIS–derived from the 1993 CORINE Land Cover data. Land use was expressed as the percentage of each of the six land–use types recognized in the watershed (urban, industrial, mining, cultivated land, forested land and water bodies). Drainage area, distance from the source, stream order, dominant geology and geospatial measures (latitude, longitude and altitude) were also obtained from this database.

3.2. DIATOM COLLECTION, PREPARATION AND OBSERVATION

A total of 446 epilithic samples were collected and analyzed, accounting for at least two samples per spring and winter or autumn period. However, the spring samples could not be collected in some cases because the river section was temporarily dry.

Periphyton samples were pooled from rocks or cobbles collected from a 10 m river segment in a well–lit part of the river, avoiding shaded areas and stones covered by filamentous algae. At least 6 rocks or cobbles were collected from a variety of locations within the sample site. A surface area of 1–2 cm^2 from each rock was scraped with a knife and an area delimiter to obtain a mixed sampling area of 10 cm^2 (CEN, 2002). The samples were then preserved with 4% formaldehyde and taken to the laboratory for diatom analysis (Sabater*, et al.*, 2003b, Cambra*, et al.*, 2005).

In the case that hard surfaces were not available, submerged macrophytes were sampled. Some random submerged parts of the macrophytes were cut by using scissors and all the sections were put into a sampling bottle with 4% formaldehyde. Then in the laboratory, the sampling surface was estimated (CEN, 2002).

An aliquot of algal suspension from each sample was prepared by acid oxidation with concentrated sulphuric acid and potassium dichromate. Permanent slides were mounted using Naphrax (r.i. 1.74). Up to 400 valves were counted and identified in each sample with a light microscope using Nomarski differential interference contrast optics at a magnification of 1,000 x (CEN, 2004). They were mainly identified following Krammer and Lange–Bertalot (1985–1991). In case of doubtful taxonomy also the monographs of (Lange–Bertalot, 1993, Krammer, 2000, Lange–Bertalot, 2001, Krammer, 2002) were used.

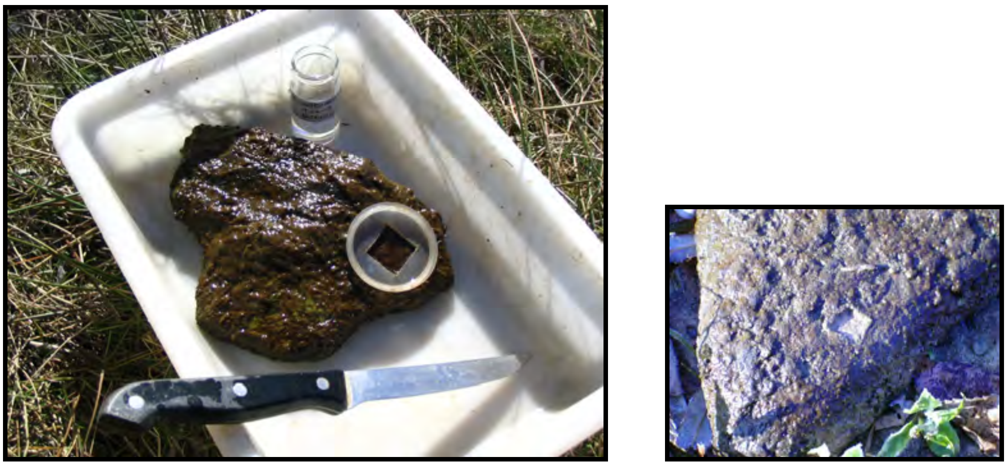


Figure 3.2. Benthic algae collection. Left: knife and plastic square to define the area to collect; Right: area in a rock already scrapped

3.3 NON–DIATOM ALGAE AND CYANOBACTERIA COLLECTION AND OBSERVATION

When macroalgae were present they were collected by using a knife or scissors. The algal sample was placed into a sampling bottle with 4% formaldehyde. Benthic algae and cyanobacteria were sampled from rocks by using a stiff toothbrush. Rocks were collected from a variety of locations within the sample site which fulfill the microhabitat diversity. A mixed sample was obtained and collected into a sampling bottle with 4% formaldehyde (Woelfl and Whitton, 2000).

Samples of non–diatom algae (both macro and benthic) and cyanobacteria were observed for identification in the 18 Tinto–Odiel system and Piedras samples (chapter VIII) and in the 29 selected reference station samples (chapter 6).

Identification was carried out using light microscope equipment at 100x or 400x magnification. The identification mainly followed (Bourrelly, 1957, Von Edith, 1978, Kawecka, 1980, Komárek and Anagnostidis, 1998, 2005).

Figure 3.3. Non–diatom and cyanobacteria collection

3.4 BENTHIC CHLOROPHYLL CONCENTRATION

Up to 3 replicates were collected in each sampling point for chlorophyll–*a* analyses. The collection procedure for a single replicate essentially followed that described for diatom samples in previous 3.1 section, and basically consisted of a pool of x cm^2 scrapped form at least 6 stones. Those stones were mostly collected in the riffle areas of the river. When it was not possible, the biofilm was scrapped from the nearest hard substratum. The samples were then preserved into dark and cold conditions and taken to the laboratory for pigment analysis.

Chlorophyll–*a* content of the periphyton was measured spectrophotometrically (at 430, 665 and 750 nm) after extraction with 90% acetone (Jeffrey and Humphrey, 1975). Estimation of algal biomass was derived from these measurements following

$$Chl_a = 11.4\ (D_{665} - D_{750})\ /\ (LS)$$

were

Chl_a : Chlorophyll–*a* concentration (µg/cm^2)
D_x: absorbance at **x** nm
V: volume of the extract (mL)
L: longitude of the cuvette (cm)
S: total scrapped Surface (cm^2)

This metric was used as an indicator of the trophic state of the system (Sabater, 1988). Thresholds used for the metrics were those proposed by Dodds *et al.*: the oligotrophic–mesotrophic boundary was proposed at 20 mg/m^2 chlorophyll–*a* concentration, and the mesotrophic–eutrophic boundary was fixed at 70 mg/m^2 chlorophyll–*a* concentration (Dodds*, et al.*, 1998a).

The Margalef Index ratio (Margalef, 1964) is the D_{430}/D_{665} ratio of the 'yellow/green' pigments of the plant population and can be used as a proxy of the maturity or successional state within an algal community.

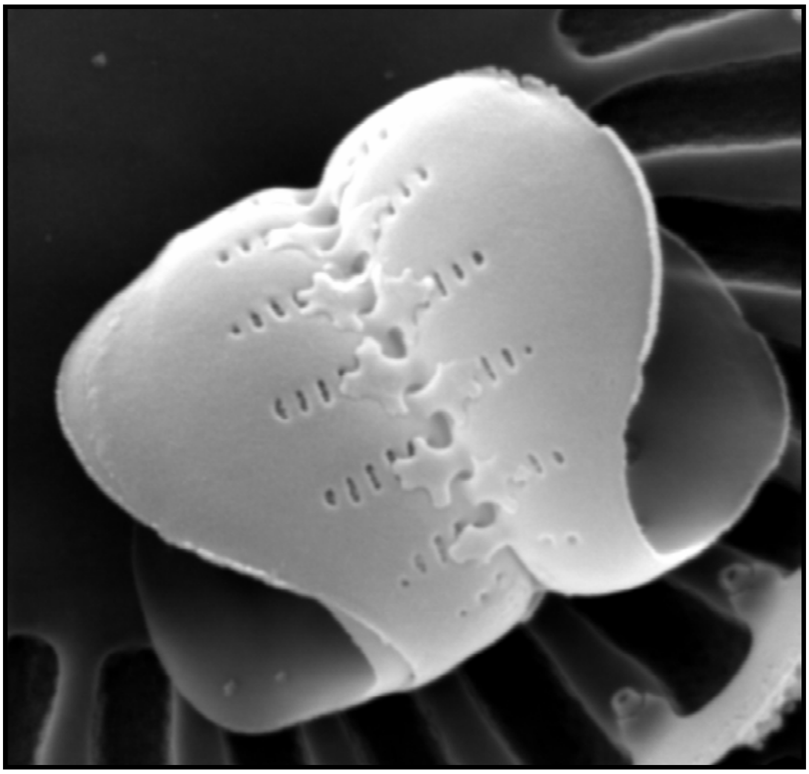
3.5 BIOTIC INDICES

Diatom samples were collected and analyzed in parallel with the chlorophyll–*a* analyses (Urrea and Sabater, 2009a) and were compared to the benthic algal biomass patterns. The IPS (index de pollusensivitée) index (Coste, 1982) and Shannon−Wiener's diversity index were calculated on the diatom data. Diatom richness was determined as the number of taxa encountered in each slide.

Diatom density in relation to the colonized surface area was quantified by means of subsequent dilutions (using appropriate micropipettes) of the digested fraction. Per each sample, drops of suspended diatom material were prepared, and up to 50 fields in each drop were counted. These numbers were later converted into diatom cells per cm^2 and used to estimate the diatom community density (Veraart*, et al.*, 2008).

The macroinvertebrate ASPT (average score per taxa) index (Armitage*, et al.*, 1983) and the macroinvertebrate richness were calculated for macroinvertebrate data also obtained in parallel to the algal data (Red Control, unpublished data).

CHAPTER IV



DIATOM TAXA OF SPECIAL INTEREST FOR THEIR TAXONOMY OR ECOLOGY

4.1. INTRODUCTION

From an historical point of view, papers on diatoms in the studied area are scarce. Some sparse studies have been published, specially those concerning to phytoplanktic diatom species in the estuary part (Rocha*, et al.*, 2002, Domingues*, et al.*, 2005, Domingues and Galvao, 2007, Domingues*, et al.*, 2007, Barbosa*, et al.*, 2010), in the saline lagoon complex of “La Mancha” (Reed, 1996, 1998b, Reed, 1998a) and in the “Lagunas de Ruidera” complex (Bort*, et al.*, 2005, Rojo*, et al.*, 2008).

The only benthic diatom microflora studied in the region, is that of the acidic waters of Rio Tinto (Sabater*, et al.*, 2003a, Aguilera*, et al.*, 2007a, Aguilera*, et al.*, 2007b, Linares-Cuesta*, et al.*, 2007, Oscoz*, et al.*, 2007).

This is without doubt the first important contribution to the benthic diatom microflora of this area.

The aim of this chapter is to present a taxonomic description of some particular taxa found in the area, and a discussion of taxonomical decisions that we have taken.

4.2. SPECIFIC METHODOLOGY

4.2.1. LM PHOTOGRAPHY

Digital photographs were done with an Olympus Color View III–u camera associated to the microscope.

For the analysis of images the analySIS–FIVE software was used.

4.2.2. SEM OBSERVATION

Scanning electron microscope (SEM) was used to confirm the identifications when necessary.

For the SEM observations, the cleaned material (see section 3.2) was gold coated, and samples were observed under a Zeiss DSM 960 SEM.

4.3. DIATOM CHECK–LIST

A total of 278 diatom taxa were identified. This elevated diatom richness confirms the wide geological and hydrodynamic heterogeneity of Guadiana watershed.

Code	Name
acle	*Achnanthes clevei* Grunow
aexg	*Achnanthes exigua* Grunow in Cleve & Grunow
ahun	*Achnanthes hungarica* Grunow in Cleve & Grunow
alvs	*Achnanthes laevis* Oestrup
aobg	*Achnanthes oblongella* Oestrup
apar	*Achnanthes parvula* Kützing
arpt	*Achnanthes rupestoides* Hohn
asat	*Achnanthes subatomoides* (Hustedt) Lange–Bertalot & Archibald
athe	*Achnanthes thermalis* (Rabenhorst) Schoenfeld
abia	*Achnanthidium biasolettianum* (Grunow in Cleve & Grunow) Round & Bukhtiyarova
amin	*Achnanthidium minutissimum* (Kützing) Czarnecki
anmn	*Actinocyclus normanii* (Gregory ex Greville) Hustedt
nmis	*Adlafia minuscula* (Grunow) Lange–Bertalot
nmmu	*Adlafia minuscula* var. *muralis* (Grunow) Lange–Bertalot
apel	*Amphipleura pellucida* Kützing
amdl	*Amphora delicatissima* Krasske
aina	*Amphora inariensis* Krammer
alib	*Amphora libyca* Ehrenberg
ammo	*Amphora montana* Krasske
aova	*Amphora ovalis* (Kützing) Kützing
aped	*Amphora pediculus* (Kützing) Grunow
aven	*Amphora veneta* Kützing
asph	*Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer
augr	*Aulacoseira granulata* (Ehrenberg) Simonsen
bpar	*Bacillaria paradoxa* Gmelin
bgar	*Brachysira garrensis* (Lange-Bertalot & Krammer) Lange-Bertalot
blil	*Brachysira liliana* Lange-Bertalot
bneo	*Brachysira neoexilis* Lange
avit	*Brachysira vitrea* (Grunow) Ross in Hartley
camp	*Caloneis amphisbaena* (Bory) Cleve
cbac	*Caloneis bacillum* (Grunow) Cleve
cmol	*Caloneis molaris* (Grunow) Krammer
csil	*Caloneis silicula* (Ehrenberg) Cleve
cund	*Caloneis undulata* (Gregory) Krammer
cped	*Cocconeis pediculus* Ehrenberg
cpla	*Cocconeis placentula* Ehrenberg
naco	*Craticula accomoda* (Hustedt) Mann
ncam	*Craticula ambigua* (Ehrenberg) Mann
nbdr	*Craticula buderi* (Hustedt) Lange–Bertalot
ncus	*Craticula cuspidata* (Kützing) Mann
nhal	*Craticula halophila* (Grunow ex Van Heurck) Mann

Code	Name
nmlf	*Craticula molestiformis* (Hustedt) Lange–Bertalot
nsmo	*Craticula submolesta* (Hustedt) Lange–Bertalot
fpul	*Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round
cato	*Cyclotella atomus* Hustedt
cbod	*Cyclotella bodanica* Grunow
cdtg	*Cyclotella distinguenda* Hustedt
cglo	*Cyclotella glomerata* Bachmann
cmen	*Cyclotella meneghiniana* Kützing
coce	*Cyclotella ocellata* Pantocsek
cpst	*Cyclotella pseudostelligera* Hustedt
crad	*Cyclotella radiosa* (Grunow) Lemmermann
cros	*Cyclotella rossii* Hakansson
csol	*Cymatopleura solea* (Brébisson) W.Smith
caff	*Cymbella affinis* Kützing
caph	*Cymbella amphicephala* Naegeli
casp	*Cymbella aspera* (Ehrenberg) Cleve
ccis	*Cymbella cistula* (Ehrenberg) Kirchner
ccym	*Cymbella cymbiformis* Agardh
chel	*Cymbella helvetica* Kützing
cinc	*Cymbella incerta* (Grunow) Cleve
clan	*Cymbella lanceolata* (Ehrenberg) Kirchner
cnav	*Cymbella naviculiformis* Auerswald
cprx	*Cymbella proxima* Reimer
cpus	*Cymbella pusilla* Grunow in A. Schmidt & al.
ctum	*Cymbella tumida* (Brébisson) Van Heurck
ctmd	*Cymbella tumidula* Grunow in A.Schmidt & al.
ctgl	*Cymbella turgidula* Grunow in A.Schmidt & al.
dten	*Denticula tenuis* Kützing
dihe	*Diatoma hyemalis* (Roth) Heiberg
dmon	*Diatoma moniliformis* Kützing
dite	*Diatoma tenuis* Agardh
dvul	*Diatoma vulgaris* Bory
dobl	*Diploneis oblongella* (Naegeli) Cleve-Euler
dova	*Diploneis ovalis* (Hill) Cleve
ccae	*Encyonema caespitosum* Kützing
cmes	*Encyonema mesianum* (Cholnoky) D.G. Mann
cmin	*Encyonema minutum* (Hill in Rabenhorst) D.G. Mann
cgra	*Encyonema neogracile* Krammer
cpro	*Encyonema prostratum* (Berkeley) Kützing
csle	*Encyonema silesiacum* (Bleisch in Rabenhorst) D.G. Mann
cces	*Encyonopsis cesatii* (Rabenhorst) Krammer
cmic	*Encyonopsis microcephala* (Grunow) Krammer
eala	*Entomoneis alata* Ehrenberg
nadn	*Eolimna adnata* (Hustedt) Moser Lange–Bertalot & Metzeltin
nmin	*Eolimna minima* (Grunow) Lange–Bertalot
nsbm	*Eolimna subminuscula* (Manguin) Moser Lange–Bertalot & Metzeltin
eadn	*Epithemia adnata* (Kützing) Brébisson
esor	*Epithemia sorex* Kützing
etgr	*Epithemia turgida* var. *granulata* (Ehrenberg) Grunow
afle	*Eucocconeis flexella* (Kützing) Meister
earc	*Eunotia arcus* Ehrenberg

Code	Name
ebil	*Eunotia bilunaris* (Ehrenberg) Mills
eexi	*Eunotia exigua* (Brébisson ex Kützing) Rabenhorst
eimp	*Eunotia implicata* Nörpel, Lange-Bertalot & Alles
emin	*Eunotia minor* Ehrenberg
epec	*Eunotia pectinalis* (Dyllwyn) Rabenhorst
nhel	*Fallacia helensis* (Schulz.) D.G. Mann
npyg	*Fallacia pygmaea* (Kützing) Stickle & Mann
nsbh	*Fallacia subhamulata* (Grunow in V. Heur.) D.G. Mann
nstr	*Fallacia tenera* (Hustedt) Mann in Round
npel	*Fistulifera pelliculosa* (Brébisson) Lange–Bertalot
nsap	*Fistulifera saprophila* (Lange–Bertalot & Bonik) Lange–Bertalot
fbcp	*Fragilaria biceps* (Kützing) Lange–Bertalot
fbid	*Fragilaria bidens* Heiberg
fcme	*Fragilaria capucina* var. *mesolepta* (Rabenhorst) Rabenhorst
fcrp	*Fragilaria capucina* var. *rumpens* (Kützing) Lange–Bertalot. ex Bukhtiyarova
fcva	*Fragilaria capucina* var. *vaucheriae* (Kützing) Lange–Bertalot
fcbi	*Fragilaria construens* f. *binodis* (Ehrenberg) Hustedt
fcve	*Fragilaria construens* f. *venter* (Ehrenberg) Hustedt
fexi	*Fragilaria exigua* Grunow
ffas	*Fragilaria fasciculata* (Agard) Lange–Bertalot
fcgr	*Fragilaria gracilis* Oestrup
fpar	*Fragilaria parasitica* (W.Smith) Grunow
fpsc	*Fragilaria parasitica* var. *subconstricta* Grunow
ften	*Fragilaria tenera* (W.Smith) Lange–Bertalot
fuln	*Fragilaria ulna* (Nitzsch.) Lange–Bertalot
frho	*Frustulia rhomboides* (Ehrenberg) De Toni
fspi	*Frustulia spicula* Amosse
fvul	*Frustulia vulgaris* (Thwaites) De Toni
ndec	*Geissleria decussis* (Oestrup) Lange–Bertalot & Metzeltin
gacu	*Gomphonema acuminatum* Ehrenberg
gaff	*Gomphonema affine* Kützing
gang	*Gomphonema angustatum* (Kützing) Rabenhorst
gant	*Gomphonema angustum* Agardh
gaug	*Gomphonema augur* Ehrenberg
gcla	*Gomphonema clavatum* Ehrenberg
gcle	*Gomphonema clevei* Fricke
ggra	*Gomphonema gracile* Ehrenberg
glat	*Gomphonema lateripunctatum* Reichardt & Lange–Bertalot
gmic	*Gomphonema micropus* Kützing
gmin	*Gomphonema minutum* (Agard) Agardh
goli	*Gomphonema olivaceum* (Hornemann) Brébisson
gpar	*Gomphonema parvulum* (Kützing) Kützing
gpsa	*Gomphonema pseudoaugur* Lange–Bertalot
gpum	*Gomphonema pumilum* (Grunow) Reichardt & Lange–Bertalot
grho	*Gomphonema rhombicum* Fricke
gtru	*Gomphonema truncatum* Ehrenberg
gvib	*Gomphonema vibrio* Ehrenberg
gyac	*Gyrosigma acuminatum* (Kützing) Rabenhorst
gyat	*Gyrosigma attenuatum* (Kützing) Rabenhorst
gnod	*Gyrosigma nodiferum* (Grunow) Reimer
hamp	*Hantzschia amphioxys* (Ehrenberg) Grunow in Cleve & Grunow

Code	Name
ncap	*Hippodonta capitata* (Ehrenberg) Lange–Bertalot Metzeltin & Witkowski
nchu	*Hippodonta hungarica* (Grunow) Lange–Bertalot Metzeltin & Witkowski
ncoh	*Luticola cohnii* (Hilse) D.G. Mann
ngoe	*Luticola goeppertiana* (Bleisch in Rabenhorst) D.G. Mann
nmut	*Luticola mutica* (Kützing) D.G. Mann
msmi	*Mastogloia smithii* Thwaites
nagr	*Mayamaea agrestis* (Hustedt) Lange–Bertalot
nato	*Mayamaea atomus* (Kützing) Lange–Bertalot
nape	*Mayamaea atomus* var. *permitis* (Hustedt) Lange–Bertalot
naex	*Mayamaea excelsa* (Krasske) Lange–Bertalot
mvar	*Melosira varians* Agardh
mcir	*Meridion circulare* (Greville) Agardh
nant	*Navicula antonii* Lange–Bertalot
ncpr	*Navicula capitatoradiata* Germain
ncin	*Navicula cincta* (Ehrenberg) Ralfs in Pritchard
ncof	*Navicula confervacea* (Kützing) Grunow
ncry	*Navicula cryptocephala* Kützing
ncte	*Navicula cryptotenella* Lange–Bertalot
nexi	*Navicula exilis* Kützing
ngre	*Navicula gregaria* Donkin
nhus	*Navicula hustedtii* Krasske
nkot	*Navicula kotschyi* Grunow
nlan	*Navicula lanceolata* (Agardh) Ehrenberg
nlen	*Navicula lenzii* Hustedt
nmen	*Navicula menisculus* Schumann
npnu	*Navicula perminuta* Grunow in Van Heurck
nphy	*Navicula phyllepta* Kützing
npla	*Navicula placentula* (Ehrenberg) Kützing
npsl	*Navicula pseudolanceolata* Lange–Bertalot
nrad	*Navicula radiosa* Kützing
nrcs	*Navicula recens* (Lange–Bertalot) Lange–Bertalot
nrch	*Navicula reichardtiana* Lange–Bertalot
nrhy	*Navicula rhynchocephala* Kützing
nros	*Navicula rostellata* Kützing
nsal	*Navicula salinarum* Grunow in Cleve & Grunow
nshr	*Navicula schroeteri* Meister
sesp1	*Sellaphora* sp1
nsmt	*Navicula submitis* Hustedt
nten	*Navicula tenelloides* Hustedt
ntpt	*Navicula tripunctata* (O.F.Muller) Bory
ntrv	*Navicula trivialis* Lange–Bertalot
nvda	*Navicula vandamii* Schoeman & Archibald
nven	*Navicula veneta* Kützing
nvir	*Navicula viridula* (Kützing) Ehrenberg
nvro	*Navicula viridula* var. *rostellata* (Kützing) Cleve
neam	*Neidium ampliatum* (Ehrenberg) Krammer
nedu	*Neidium dubium* (Ehrenberg) Cleve
nzcd	*Nitzschia acicularioides* Hustedt
naci	*Nitzschia acicularis* (Kützing) W.M.Smith
namp	*Nitzschia amphibia* Grunow
namh	*Nitzschia amphibioides* Hustedt

Code	Name
naur	*Nitzschia aurariae* Cholnoky
nbcl	*Nitzschia bacillum* Hustedt
nbre	*Nitzschia brevissima* Grunow
ncpl	*Nitzschia capitellata* Hustedt in A.Schmidt & al.
ncom	*Nitzschia communis* Rabenhorst
nico	*Nitzschia commutata* Grunow in Cleve & Grunow
ncps	*Nitzschia compressa* (Bailey–Watts) Boyer
ndis	*Nitzschia dissipata* (Kützing) Grunow
ndub	*Nitzschia dubia* W.M.Smith
nfas	*Nitzschia fasciculata* Grunow
nfil	*Nitzschia filiformis* (W.M.Smith) Van Heurck
nfon	*Nitzschia fonticola* Grunow in Cleve & Moller
nifr	*Nitzschia frustulum* (Kützing) Grunow
nges	*Nitzschia gessneri* Hustedt
nigf	*Nitzschia graciliformis* Lange–Bertalot & Simonsen
nigr	*Nitzschia gracilis* Hantzsch
nheu	*Nitzschia heufleriana* Grunow
nhyb	*Nitzschia hybrida* Grunow
ninc	*Nitzschia inconspicua* Grunow
nlin	*Nitzschia linearis* (Agard) W. Smith
nlsu	*Nitzschia linearis* var. *subtilis* (Grunow) Hustedt
nzlt	*Nitzschia linearis* var. *tenuis* (W.Smith) Grunow
nmic	*Nitzschia microcephala* Grunow in Cleve & Moller
nimd	*Nitzschia modesta* Hustedt
nnan	*Nitzschia nana* Grunow in Van Heurck
nobt	*Nitzschia obtusa* W. Smith
npal	*Nitzschia palea* (Kützing) W. Smith
npei	*Nitzschia peisonis* Pantocsek
nipu	*Nitzschia pusilla* (Kützing) Grunow
nrec	*Nitzschia recta* Hantzsch in Rabenhorst
niro	*Nitzschia rostellata* Hustedt
nsig	*Nitzschia sigma* (Kützing) Smith
nsio	*Nitzschia sigmoidea* (Nitzsch) W. Smith
nsin	*Nitzschia sinuata* (Thwaites) Grunow
nsit	*Nitzschia sinuata* var. *tabellaria* Grunow
nsoc	*Nitzschia sociabilis* Hustedt
niso	*Nitzschia solita* Hustedt
nter	*Nitzschia terrestris* (Petersen) Hustedt
ntry	*Nitzschia tryblionella* Hantzsch
numb	*Nitzschia umbonata* (Ehrenberg) Lange–Bertalot
paco	*Pinnularia acoricola* Hustedt
pbre	*Pinnularia brebisonii* (Kützing) Rabenhorst
pdvg	*Pinnularia divergentissima* (Grunow) Cleve
plun	*Pinnularia lundii* Hustedt
pneo	*Pinnularia neomajor* Kramer
psca	*Pinnularia subcapitata* Gregory
pvif	*Pinnularia viridiformis* Krammer
adel	*Planothidium delicatulum* (Kützing) Round & Bukhtiyarova
alfr	*Planothidium frequentissimum* (Lange–Bertalot) Round & Bukhtiyarova
plev	*Pleurosira laevis* (Ehrenberg) Compere
nzco	*Psammodictyon constricta* (Gregory) D.G. Mann

Code	Name
fbre	*Pseudostaurosira brevistriata* (Grunowin Van Heurck) Williams & Round
rsin	*Reimeria sinuata* (Gregory) Kociolek & Stoermer
runi	*Reimeria uniseriata* Guerrero & Ferrario
rabb	*Rhoicosphenia abbreviata* (Agard) Lange–Bertalot
rgib	*Rhopalodia gibba* (Ehrenberg) O.Muller
rgmi	*Rhopalodia gibba* var. *minuta* Krammer
nbac	*Sellaphora bacillum* (Ehrenberg) D.G.Mann
npup	*Sellaphora pupula* (Kützing) Mereschkowksy
nsem	*Sellaphora seminulum* (Grunow) Mann
stan	*Stauroneis anceps* Ehrenberg
spho	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg
ssmi	*Stauroneis smithii* Grunow
sund	*Stauroneis undata* Hustedt
fcon	*Staurosira construens* Ehrenberg
fell	*Staurosira elliptica* (Schumann) Williams & Round
fpin	*Staurosirella pinnata* (Ehrenberg) Williams & Round
shan	*Stephanodiscus hantzschii* Grunow in Cleve & Grunow
sneo	*Stephanodiscus neoastraea* Hakansson & Hickel
snia	*Stephanodiscus niagarae* Ehrenberg
sang	*Surirella angusta* Kützing
sbre	*Surirella brébissonii* Krammer & Lange–Bertalot
sbku	*Surirella brébissonii* var. *kuetzingii* Krammer & Lange–Bertalot
sovi	*Surirella ovalis* Brébisson
sspl	*Surirella splendida* (Ehrenberg) Kützing
tflo	*Tabellaria flocculosa* (Roth) Kützing
tbal	*Thalassiosira baltica* (Grunow) Ostenfeld
tpsn	*Thalassiosira pseudonana* Hasle & Heimdal
twei	*Thalassiosira weissflogii* (Grunow) Fryxell & Hasle
nian	*Tryblionella angustata* W. Smith
nica	*Tryblionella calida* (Grunow in Cleve & Grunow) D.G. Mann
ncoa	*Tryblionella coarctata* (Grunow in Cleve & Grunow) D.G. Mann
nihu	*Tryblionella hungarica* (Grunow) D.G. Mann
nlev	*Tryblionella levidensis* W. Smith

4.4. LIST OF TAXA WITH PARTICULAR TAXONOMY AND ECOLOGY

1. *Amphora montana* Krasske [ammo]
2. *Brachysira garrensis* (Lange–Bertalot & Krammer) Lange–Bertalot [bgar]
3. *Brachysira liliana* Lange–Bertalot [blil]
4. *Caloneis amphisbaena* (Bory) Cleve [camp]
5. *Cocconeis placentula* Ehrenberg [cpla] *sensu lato*
6. *Craticula buderi* (Hustedt) Lange–Bertalot [nbdr]
7. *Craticula* cf. *ambigua* (Ehrenberg) Mann [ncam]

8. *Cyclotella* cf. *distinguenda* Hustedt [cdtg]
9. *Cymbella cistula* (Ehrenberg) Kirchner [ccis]
10. *Diatoma tenuis* species complex
11. *Entomoneis alata* Ehrenberg [eala]
12. *Eucocconeis flexella* (Kützing) Meister [afle]
13. *Eunotia arcus* Ehrenberg [earc]
14. *Eunotia bilunaris* (Ehrenberg) Mills [ebil]
15. *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst [eexi]
16. *Eunotia implicata* Nörpel, Lange–Bertalot & Alles [eimp]
17. *Eunotia minor* (Kutzing) Grunow in Van Heurck [emin]
18. *Eunotia pectinalis* (Dyllwyn) Rabenhorst [epec]
19. *Gyrosigma attenuatum* (Kützing) Rabenhorst [gyat]
20. *Lemnicola hungarica* (Grunow) Round & Basson [ahun]
21. *Nitzschia dissipata* (Kützing) Grunow [ndis] *sensu lato*
22. *Nitzschia heufleriana* Grunow [nheu]
23. *Nitzschia inconspicua* and *Nitzschia frustulum* complex
24. *Pinnularia accoricola* Hustedt [paco]
25. *Pinnularia brebisonii* (Kützing) Rabenhorst [pbre]
26. *Pinnularia divergentissima* (Grunow) Cleve [pdvg]
27. *Pinnularia lundii* Hustedt [plun]
28. *Pinnularia neomajor* Kramer [pneo]
29. *Pinnularia subcapitata* Gregory [psca]
30. *Pinnularia viridiformis* Krammer [pvif]
31. *Reimeria sinuata* and *Reimeria uniseriata* complex
32. *Sellaphora* sp.1 [sesp1]
33. *Stephanodiscus hantzschii* Grunow in Cleve & Grunow [shan]
34. *Tabellaria flocculosa* (Roth) Kützing [tflo]

Abbreviations used in this chapter were:

Size range of the specimens studied

minimum–maximum and mean values (in brackets)

n_{in}=number of individuals measured

L=length

W=width

Distribution and ecology

n_{loc}= number of localities

cond= conductivity (µS/cm)

temp= Temperature (ºC)

O2= Oxygen (mg/L)

pH= pH

arti= % Artificial Land Use

cult= % Cultivated Land Use

natu= % Natural Land Use

NH4= Ammonia (mg/L)

Cl= Chloride (mg/L)

SO4= Sulfate (mg/L)

PO4= Soluble Reactive Phosphorus (mg/L)

NO3= Nitrate (mg/L)

Ca= Calcium (mg/L)

alka= Alkalinity (mg $CaCO_3$/L)

MAX= Maximum

MIN= Minimum

MEAN= Mean value

SD= Standard deviation

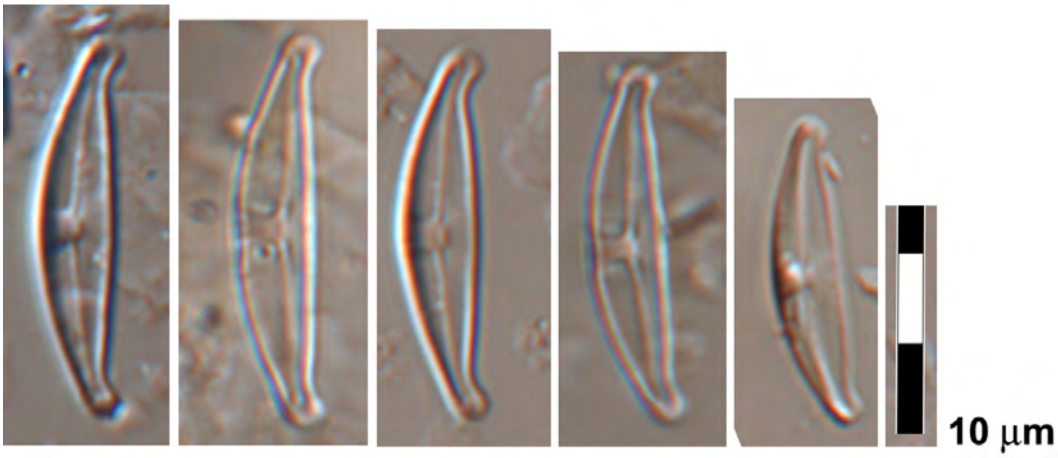
Amphora montana Krasske [ammo]

Taxonomical remarks:

All the specimens of *Amphora* with lunate to subcapitated shape, dorsal margin deeper than ventral margin, and a central dorsal margin with a conspicuous hyaline area, have been included under the nomination *A. montana*.

.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=9)	14.0 – 17.7 (16.3 ± 1.2)	3.7 – 3.9 (3.5 ± 0.2)	–



General distribution and ecology:

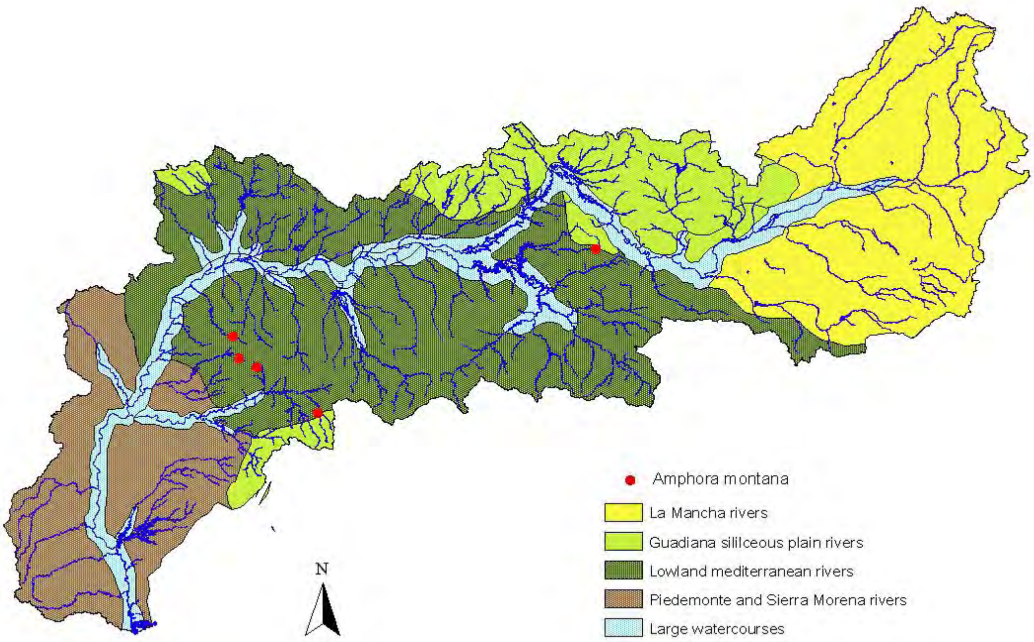
A. montana is a cosmopolitan, thermal and subaerial taxon (Krammer and Lange–Bertalot, 1985) inhabiting alkaline waters (Van Dam*, et al.*, 1994).

It has been described both in calcareous and siliceous waters from Pyrenees (Margalef, 1948), Duero basin (Álvarez–Blanco, 2009) and eutrophic localities in the north east of the Peninsula (Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n_{loc}=7	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	593.0	24.7	11.5	10.6	2.9	96.1	88.2	0.3	39.1	30.0	0.1	0.9	40.9	184.0
MIN	32.1	3.2	5.7	7.2	0.0	11.8	3.9	0.0	15.6	7.7	0.0	0.4	9.7	35.0
MEAN	376.9	15.2	8.5	8.6	0.4	61.1	38.5	0.2	28.7	21.3	0.1	0.6	22.3	99.6
SD	197.3	9.5	1.8	1.2	1.1	28.7	28.4	0.1	9.0	11.9	0.0	0.2	11.5	53.7

This taxon appeared in upper streams. Its maximum abundance was associated to oligotrophic with mid−conductivity waters during spring.

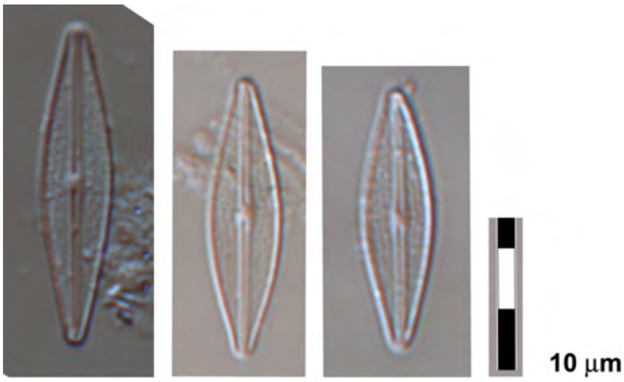


Brachysira garrensis (Lange–Bertalot & Krammer) Lange–Bertalot [bgar]

Taxonomical remarks:

I have included under *B. garrensis* name all samll *Brachysira* with narrow and pointed ends.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=6)	15.8 – 21.9 (18.8 ± 2.3)	4.6 – 4.8 (4.7 ± 0.1)	–



General distribution and ecology:

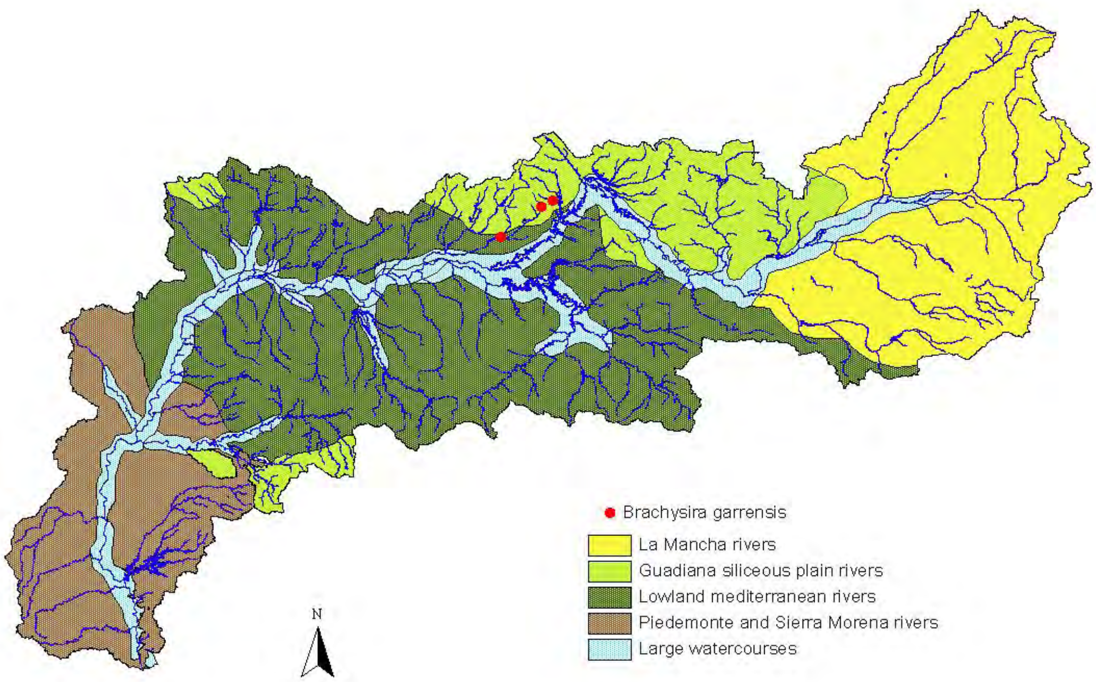
It is a taxon typically found siliceuous waters near acidophilous taxa. It has been described in shallow lakes of Belgium (Denys, 2009and Irish ecoregion (Chen, 2008) and in Italian alpine lakes (Tolotti, 2001).

It has been cited ocasionally in some rivers in the north-esastern Penisnula (Tornés, 2009), and in some parts of the Ebro river (Cambra, 2005).

Distribution and ecology in the Guadiana watershed:

n_{loc}=3	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	752.0	9.2	13.0	8.5	0.3	49.8	99.8	0.0	9.0	9.0	0.0	7.8	3.3	10.0
MIN	40.0	6.6	9.6	8.0	0.0	0.3	49.9	0.0	6.3	8.0	0.0	0.8	2.1	5.5
MEAN	279.7	7.5	11.2	8.2	0.1	21.0	78.9	0.0	7.7	8.5	0.0	4.3	2.7	7.8
SD	409.1	1.5	1.7	0.2	0.2	25.7	25.9	0.0	1.9	0.7	0.0	5.0	0.8	3.2

This taxon appeared in central part of small rivers of the siliceous watershed.

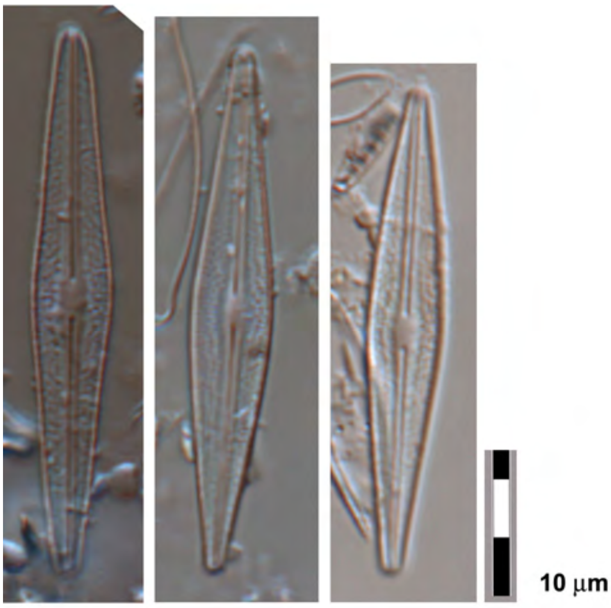


Brachysira liliana Lange–Bertalot [blil]

Taxonomical remarks:

B. liliana includes all the long *Brachysira* forms, with distant central pores of the raphe branche.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=5)	29.8 – 38.3 (34.9 ± 3.3)	5.1– 5.8 (5.4 ± 0.3)	–



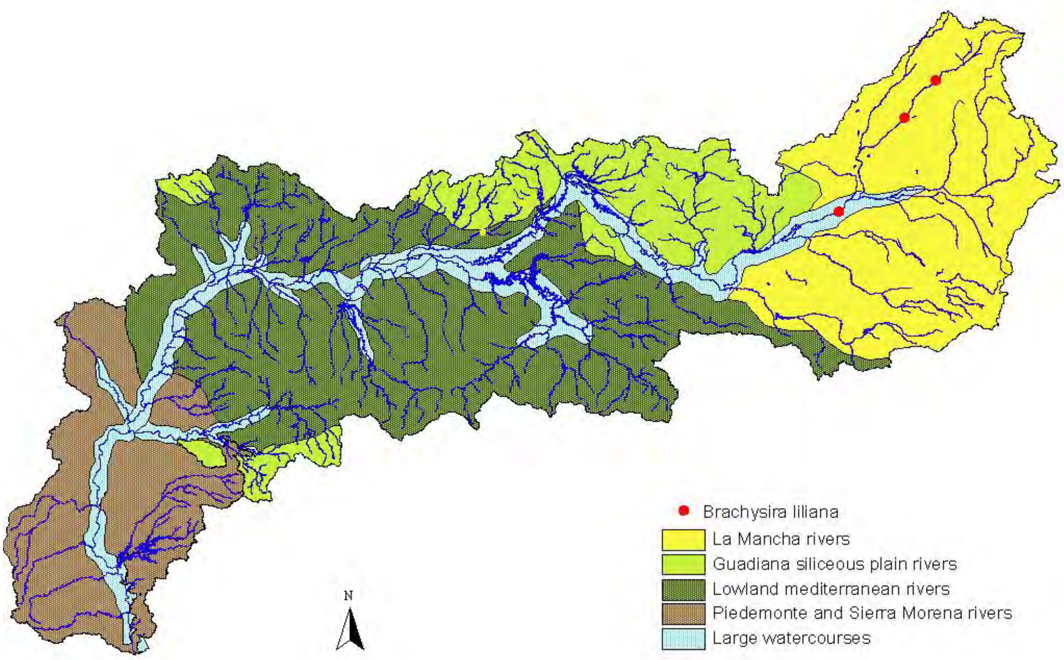
General distribution and ecology:

This taxon was associated to karstic waters in central and north German (Schaumburg, 2007) and it has not been cited previously in the Iberian Peninisula.

Distribution and ecology in the Guadiana watershed:

n_{loc}=3	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2560.0	15.1	13.3	8.2	1.2	88.0	20.9	0.8	121.8	1499.1	0.1	17.9	453.4	198.8
MIN	2070.0	6.3	6.9	7.9	0.0	79.1	10.9	0.0	18.0	984.0	0.0	15.2	400.0	143.0
MEAN	2266.7	11.1	10.1	8.0	0.4	82.3	17.3	0.3	53.4	1211.0	0.0	16.3	430.1	168.6
SD	258.9	4.5	3.2	0.2	0.6	5.0	5.6	0.4	59.2	262.9	0.0	1.4	27.3	28.2

This taxon apperared in sites with high conductivity (≈2,000 µS/cm) and high sulfate content (≈1,000 mg/L).



Caloneis amphisbaena (Bory) Cleve [camp]

Taxonomical remarks:

The measurements of our specimens coincide with the size range described by Krammer and Lange–Bertalot (1985). *C. amphisbaena* is characterized by frustules of 20–80 µm in length, 20–30 µm in width and 15–18 dorsal striae in 10 µm in the middle of the valve. Only observed large size specimens were observed in the Guadiana.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=4)	69.3 – 74.2 (72.0 ± 2.3)	24.2 – 25.7 (24.9 ± 0.7)	15 – 18 (16 ± 1)

General distribution and ecology:

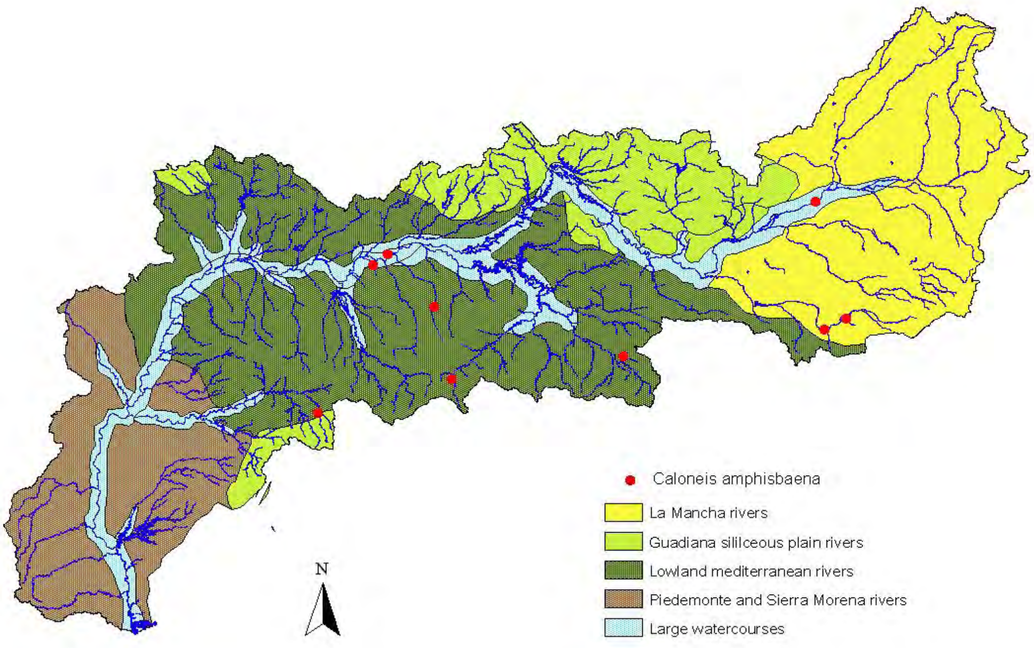
It is a widespread and epipelic taxon inhabiting waters from moderate to high electrolyte content, not common in rivers (Krammer and Lange–Bertalot, 1985).

It has been found in low concentrations in mesotrophic waters from the north–east of Iberian Peninsula running over calcareous and siliceous substrate (Tomàs, 1988, Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n_{loc}=9	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2560.0	21.8	11.5	8.2	1.4	95.3	89.7	0.8	121.8	1499.1	1.0	34.8	453.4	311.0
MIN	60.0	6.3	3.6	7.7	0.0	10.3	4.3	0.0	35.3	30.0	0.0	0.4	27.8	91.6
MEAN	1027.2	14.8	7.2	8.0	0.5	71.8	27.7	0.3	82.0	326.2	0.3	10.6	139.9	214.4
SD	823.8	6.1	2.6	0.2	0.6	25.2	25.4	0.3	38.4	586.6	0.4	13.1	158.3	72.2

This taxon appeared widespread thorough the watershed in sites with low flow (0–0.31 m^3/s), both in winter and spring. It has been reported in low densities (<5%), showing no preferences for substrata.



Cocconeis placentula Ehrenberg [cpla] *sensu lato*

Taxonomic and Ecological comment:

This is a very widely–distributed diatom, found in almost all freshwaters with circumneutral or alkaline pH, with the exception of the most oligotrophic sites. It also extends to brackish water.

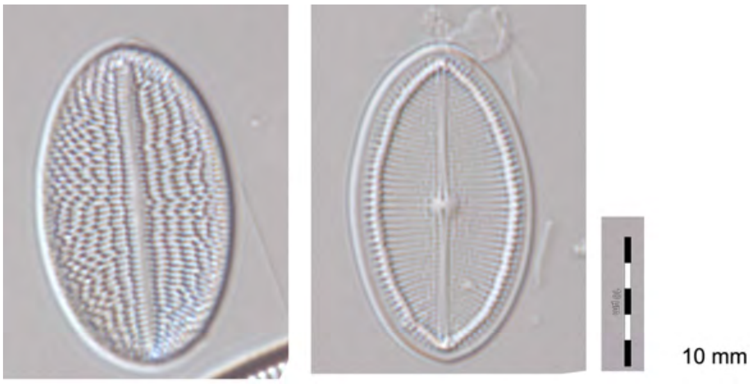
C. placentula is very common in benthic habitats, where it attaches to rocks, macrophytes and algae. It is a fast–growing, pioneer species able to quickly colonize bare substrata. The prostrate growth form and the firm attachment via mucus secreted by the raphe valve means that *C. placentula* is relatively resistant to scour and to selective grazers (Antonie and Benson-Evans, 1983). It is tolerant of moderate but not severe, organic pollution (Van Dam*, et al.*, 1994).

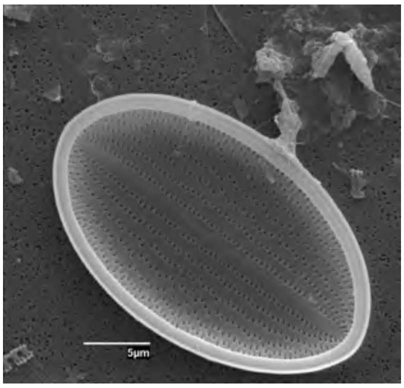
At least eight varieties have been described: *C. placentula* Ehrenberg var. *placentula*, *C. placentula* var. *pseudolineata* Geitler, *C. placentula* var. *euglypta* (Ehrenberg) Grunow, *C. placentula* var. *klinoraphis* Geitler, *C. placentula* var. *lineata* (Ehrenberg) Van Heurck, *C. placentula* var. *rouxii* (Heribaud & Brunow in Heribaud) Cleve, *C. placentula* var. *tenuistriata* Geitler and *C. placentula* var. *intermedia* (Heribaud & Peragallo) Cleve-Euler.

The raphe valve is very characteristic of the species, but is difficult to assign to variety in the absence of a corresponding rapheless valve (Geitler, 1982). Prygiel*, et al.* (2002) noted misidentification of *C. placentula* varieties to be a major source of variability in an intercalibration exercise performed in France. The varieties do not appear to have distinct preferences with respect to the most common "pollution" variables, although there is some evidence that var. *euglypta* is slightly more tolerant of organic pollution than the other varieties, e.g. Jones (1978) used the relative abundance of *Cocconeis placentula* var. *euglypta* to estimate the nitrate content of running waters.

Due to the uncertainties in the taxonomic position of the varieties, the specimens in this study have been considered as *Cocconeis placentula sensu lato*.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=14)	10.1 – 79.2 (32.2 ± 12.3)	8.2 – 25.7 (14.9 ± 7.7)	20 – 23 (22 ± 1)





Distribution and ecology in the Guadiana watershed:

n_{loc}=33	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2300.0	25.3	11.4	9.5	1.6	92.9	86.5	2.7	87.7	1090.0	3.4	20.6	498.0	319.0
MIN	457.0	5.5	4.4	6.8	0.0	13.5	6.8	0.0	2.0	7.7	0.0	0.2	4.2	24.1
MEAN	578.0	16.5	9.6	8.2	0.4	55.1	44.5	0.2	45.3	201.4	0.6	2.7	86.1	164.4
SD	258.2	6.1	3.6	0.7	0.5	19.5	19.7	0.5	31.0	354.3	0.9	5.3	134.3	88.5

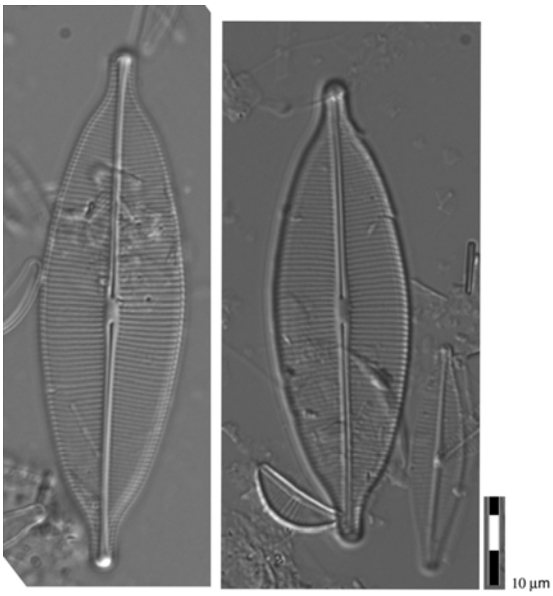
This is a widespread taxon along Guadiana watershed. *C. placentula* is present in high conductivity (>500 µS/cm) and moderate to high nutrient concentration waters, with moderately–high alkalinities (≈100 mg $CaCO_3$/L). Its ecology is referred only to those samples in which *C. placentula* presented abundances higher than 10%.

Craticula cf. ***ambigua*** (Ehrenberg) Mann [ncam]
Syn. *Navicula cuspidata* Kützing var. *ambigua* (Ehrenberg) Cleve

Taxonomical remarks:

The measurements of our specimens coincide to the size range described by Lange–Bertalot (2001): frustules of 49–74 µm in length and 15–19 µm in width. However the individuals encountered in this study possessed higher density of dorsal striae (15 –17 striae/10 µm) than those described in the literature (14 striae/10 µm).

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=4)	53.7 – 60.3 (57.6 ± 2.8)	16.4 – 17.2 (16.7 ± 0.4)	15 – 17 (16 ± 1)



General distribution and ecology:

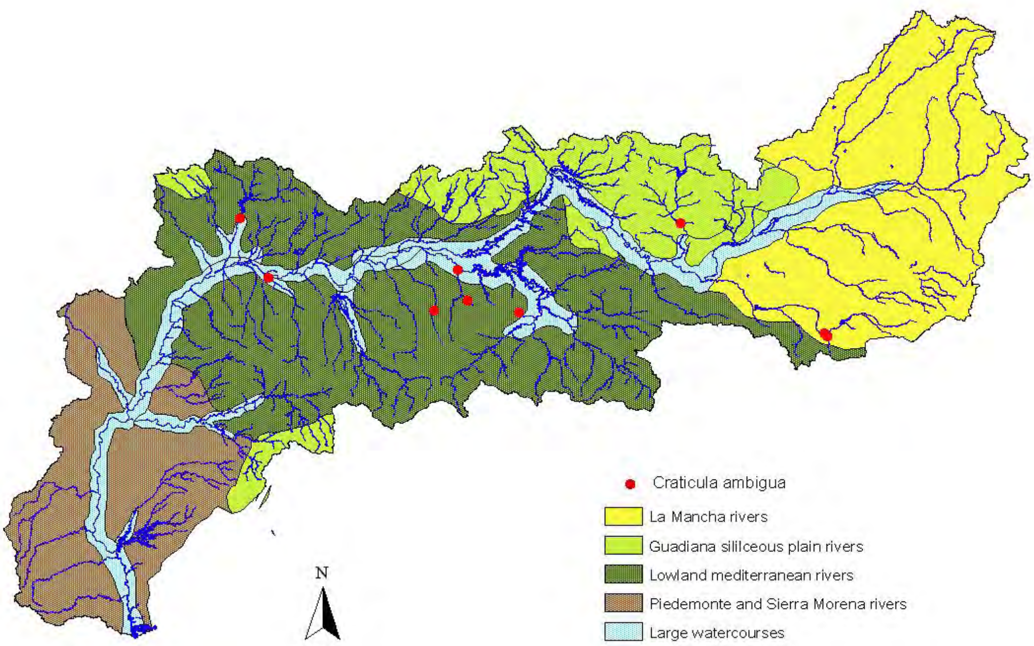
C. ambigua is a cosmopolitan taxon common in Europe, usually described as epipelic in moderate to very electrolyte–rich eutrophic waters (Lenoir and Coste, 1996, Lange–Bertalot, 2001).

It has been reported in some parts of the Llobregat river (N–E Spain), running over calcareous bedrock and supporting high nutrient levels (Tornés, 2009) and in Duero basin (Blanco*, et al.*, 2008)

Distribution and ecology in the Guadiana watershed:

n_{loc}=9	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	1940	26.9	12.6	8.6	2.6	85.2	83.1	1.1	119.0	80.0	4.3	39.6	62.3	297.0
MIN	317	8.8	5.9	7.5	0.0	16.9	13.2	0.1	38.5	20.0	0.3	0.4	25.3	103.0
MEAN	884.6	18.4	8.5	7.9	0.8	64.7	34.6	0.5	69.2	38.0	1.8	11.0	41.4	188.4
SD	567.1	7.2	2.5	0.3	0.8	22.5	22.9	0.4	31.4	24.9	1.8	16.4	16.1	77.3

In the Guadiana has been found with low abundances (<1%) in the middle and lower parts of the rivers, associated to cultural land uses, supporting high nutrient concentrations.



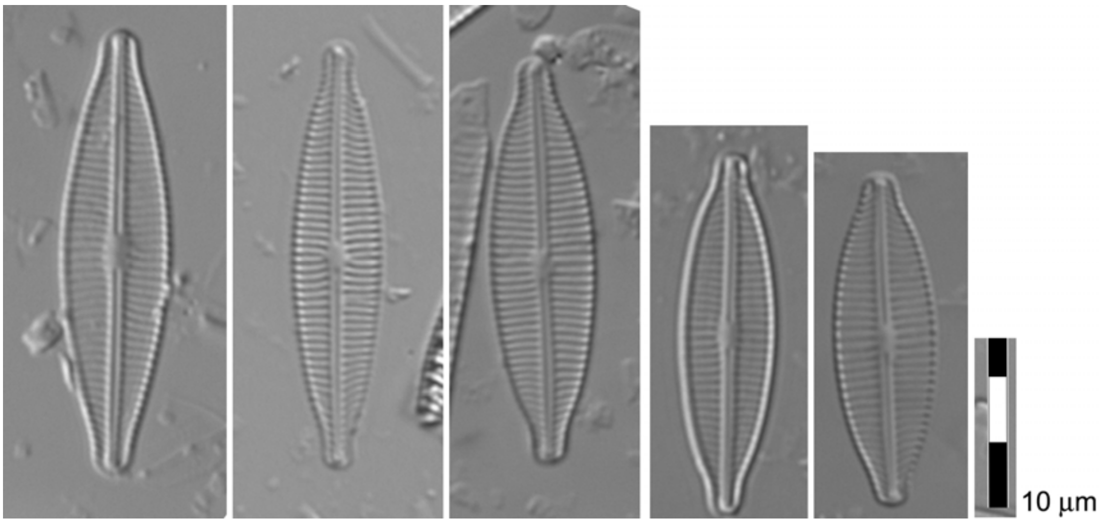
Craticula buderi (Hustedt) Lange–Bertalot [nbdr]
Syn. *Navicula buderi* Hustedt

Taxonomical remarks:

Craticula buderi was described as belonging to a species complex composed by *C. buderi*, *C. pseudohalophila* and *C. halophila* (Round, *et al.*, 1990).

I have adopted the criteria proposed by Lange–Bertalot (2001) who described under *C. buderi* the smallest forms (usually *C. buderi* and *C. pseudohalophila*) with elliptical shape and rostrate to subcapitate ends, whereas *C. halophila* was reserved to higher forms with roundish ends, usually associated to high electrolyte content waters.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=14)	20.5 – 27.7 (25.4 ± 2.0)	5.7 – 7.2 (6.4 ± 0.4)	17 – 23 (20 ± 2)



General distribution and ecology:

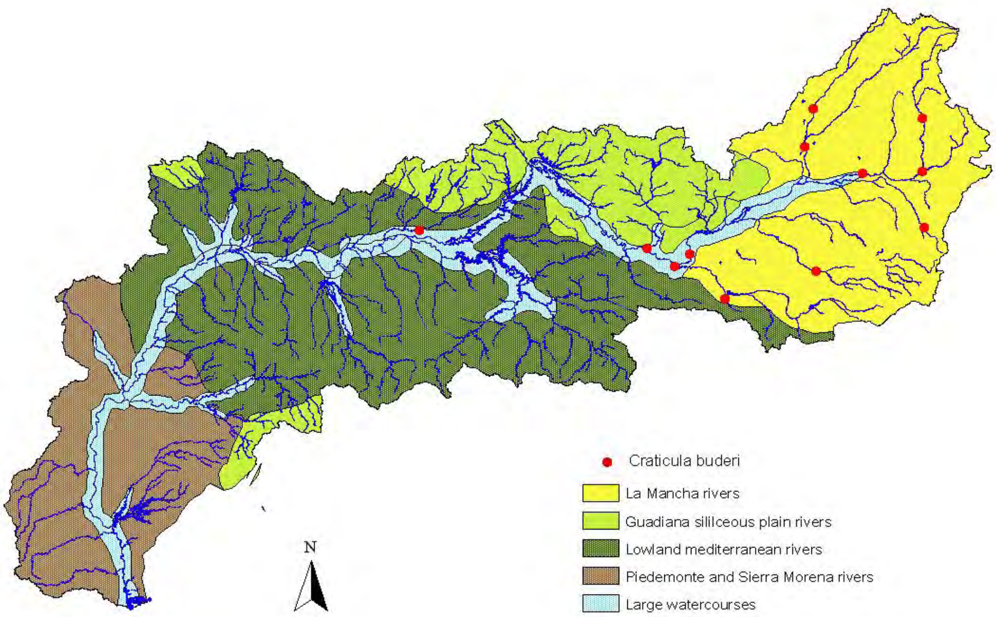
It is a cosmopolitan taxon often found in salines, associated with *C. halophila*, though it is most common in freshwaters with high electrolyte content, e.g. chalk–rich springs. It has a very broad trophic tolerance (Lange–Bertalot, 2001).

In the Iberian Peninsula it has been reported in low abundances in some parts of the main Ter river (Sabater, 1987) and in the main Ebro river (Gomà*, et al.*, 2003).

Distribution and ecology in the Guadiana watershed:

n_{loc}=16	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	3230.0	22.6	13.3	8.4	1.5	94.2	50.3	12.5	170.0	1410.0	6.7	41.5	504.0	541.0
MIN	249.0	3.1	4.7	7.2	0.0	49.7	5.3	0.0	20.7	9.0	0.0	0.4	16.1	60.1
MEAN	1570.8	14.1	8.4	7.8	0.7	79.6	19.7	1.9	75.4	454.1	1.1	9.6	192.5	237.6
SD	1001.1	5.7	3.0	0.3	0.4	13.3	13.5	3.5	51.6	518.2	1.9	12.8	182.7	134.6

The distribution of this taxon was restricted to high conductivity waters (≈1,000 µS/cm) both in the calcareous upper part of the watershed, and in the middle and lower part of the river, associated to cultural practices with high nutrient content. Its highest abundance was reported in the calcareous upper watershed in tributaries with high sulfate (>500 mg/L) and calcium (>200 mg/L) content.

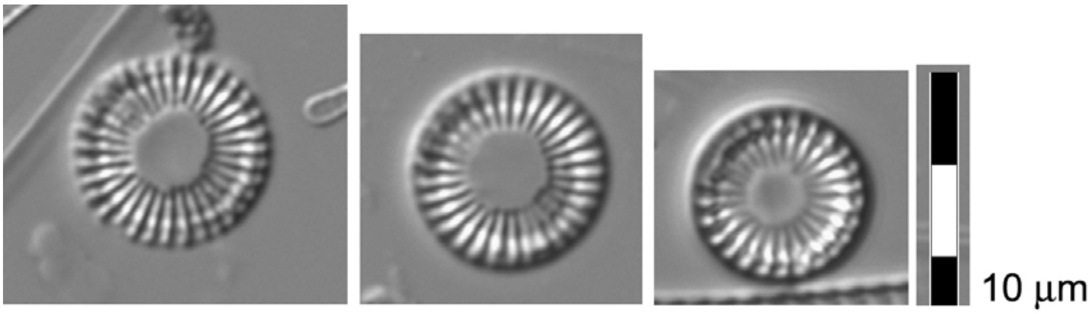


Cyclotella cf. ***distinguenda*** Hustedt [cdtg]

Taxonomical remarks:

The diameter and general aspect of our specimens coincide to the size range described in the literature (6–35 µm with 12–14 striae/10 µm), however the Guadiana specimens are more densely striated: 15–24 striae/10 µm in front of 12–14 striae/10 µm described by Hakanson (1989) and Krammer and Lange–Bertalot (1991a).

	Diameter (µm)	**Marginal striae in 10 µm**
LM (n_{in}=7)	5.5 – 12.9 (7.8 ± 2.5)	15 –24 (20 ± 3)



General distribution and ecology:

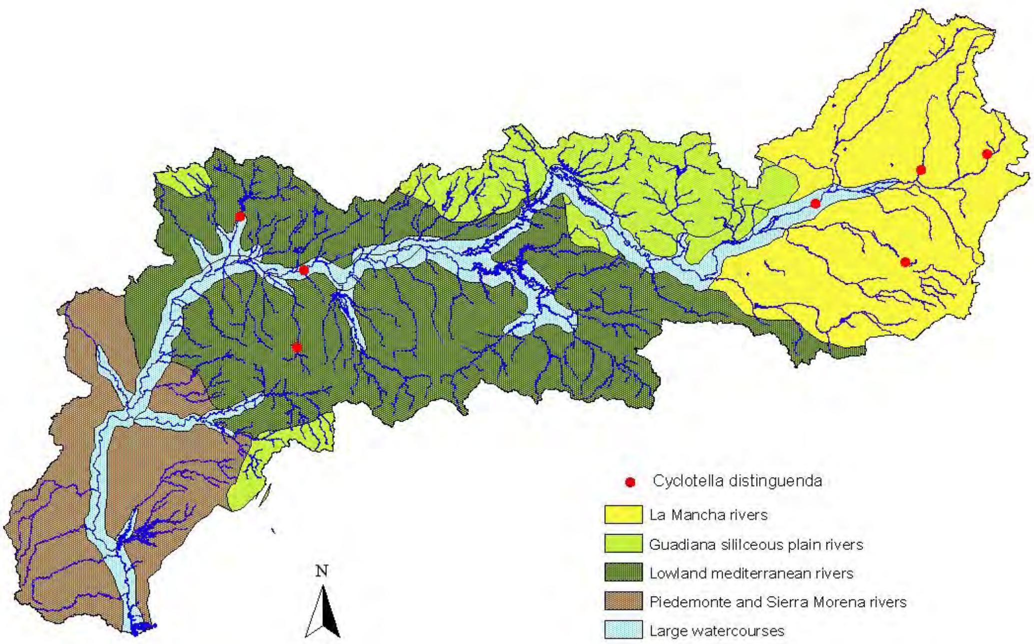
C. distinguenda is a cosmopolitan taxon inhabiting waters with high electrolyte content.

It has been reported in karstic lakes with high sulfate concentration in La Mancha (Rodrigo*, et al.*, 2000) and in some saline lagoons in the south of Spain (Ubierna and Sánchez-Castillo, 1992). In the north east of the Peninsula it has been reported in calcareous rivers with slow flowing waters (Gomà*, et al.*, 2005, Cantoral–Uriza and Aboal, 2008, Tornés, 2009) as well as in some siliceous rivers (López–Rodríguez and Penalta, 2004).

Distribution and ecology in the Guadiana watershed:

n_{loc}=9	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	3050.0	20.0	13.3	9.5	1.6	88.0	47.4	3.9	121.8	1499.1	2.3	44.0	453.4	273.0
MIN	229.0	4.0	2.7	7.8	0.2	52.3	10.9	0.0	20.6	30.0	0.0	0.4	15.3	78.0
MEAN	1352.3	10.6	8.4	8.3	0.9	72.5	26.5	0.8	64.4	504.6	0.7	22.8	173.0	188.2
SD	1026.8	6.3	3.7	0.5	0.6	12.6	12.5	1.5	40.5	583.9	1.0	17.2	169.4	65.4

This taxon appeared widespread throughout the watershed. Its maximum abundance was related to cultivated lands with high conductivity (>700 µS/cm) and high calcium content (>90 mg/L).

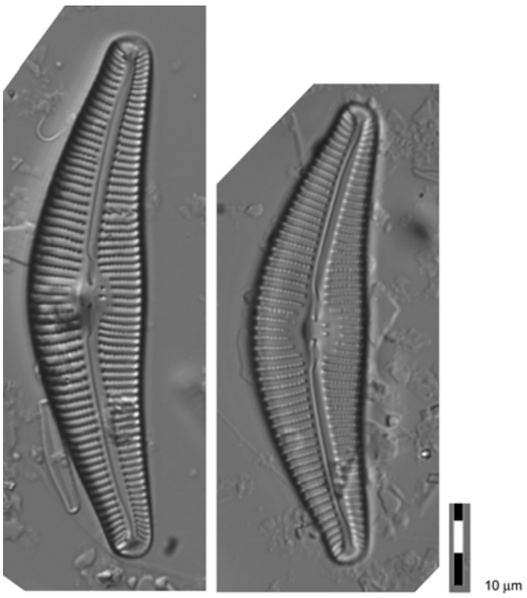


Cymbella cistula (Ehrenberg) Kirchner [ccis]

Taxonomical remarks:

Cymbella cistula is presently a complex including at least 12 different taxa from Krammer (2000). Nevertheless, I used the classical taxonomy and grouped under *C. cistula* name all the forms that coincide with the general description of the taxon based on Cleve (1894): “Large forms with distinctly central area dilated on the dorsal and ventral side of the central nodule, commonly less than 21 puncta/10 µm and more than 1 stigma”.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**	**Ventral striae in 10 µm**	**Puncta in 10 µm**
LM (n_{in}=6)	53.6 – 66.3 (60.4 ± 4.9)	14.2 – 15.4 (14.8 ± 0.5)	7 – 10 (9 ± 1)	12 – 15 (13 ± 1)	16 – 24 (20 ± 3)



General distribution and ecology:

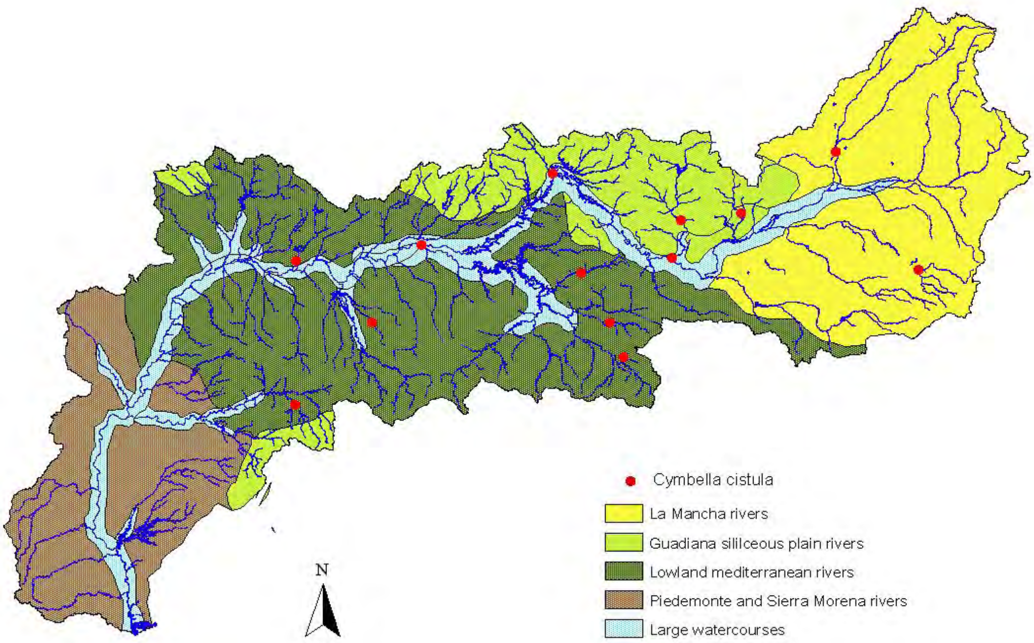
It has been found in small numbers across a wide range of European water bodies, typically with moderately high electrolyte content (Krammer and Lange–Bertalot, 1985, Kelly*, et al.*, 2005).

It has been described in calcareous waters from the NE of the Iberian Peninsula (Sabater, 1987, Tomàs, 1988, Cambra, 1992b, 1992a, Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n_{loc}=15	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2370.0	26.5	10.9	9.5	2.9	89.3	89.7	2.1	144.0	516.0	1.4	41.5	144.8	257.0
MIN	32.1	6.0	6.1	7.0	0.0	10.3	9.5	0.0	4.8	4.6	0.0	0.2	3.8	15.0
MEAN	509.5	16.0	9.2	8.1	0.7	58.1	41.3	0.3	40.0	103.0	0.2	7.8	48.0	120.5
SD	603.4	7.4	1.3	0.6	0.8	17.8	18.0	0.6	39.7	153.3	0.4	12.3	46.4	92.9

This taxon appeared widespread throughout the watershed, but its maximum abundance was reported on calcareous waters with high electrolyte content (>25 mg Cl/L).



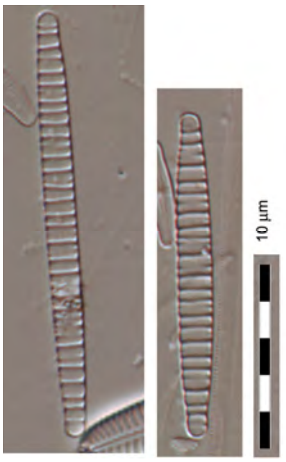
Diatoma tenuis Agardh [dten] and **Diatoma moniliformis** Kützing [dmon] species complex

Taxonomic remarks:

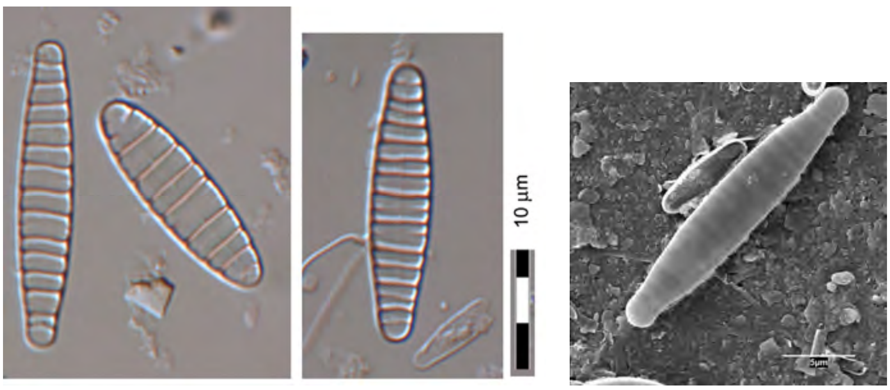
In this work I used the distinction applied by Prygiel and Coste (2000). *Diatoma tenuis* presents narrow valves with linear (occasionally linear–lanceolate) outline and distinctly swollen (occasionally rounded) ends, with 6–10 costae in 10 µm. *Diatoma moniliformis* presents valves elliptic, elliptic–lanceolate to linear with rounded, occasionally slightly rostrate ends, with 7–12 costae in 10 µm.

dten	**L** (µm)	**W** (µm)	**Costae in 10 µm**
LM (n_{in}=9)	24.1 – 55.9 (38.5 ± 10.7)	2.6 – 4.0 (3.2 ± 0.4)	6 – 10 (8 ± 2)

dmon	**L** (µm)	**W** (µm)	**Costae in 10 µm**
LM (n_{in}=8)	20.3 – 25.4 (22.5 ± 2.0)	2.8 – 5.8 (4.7 ± 0.9)	8 – 11 (9 ± 1)



Diatoma tenuis



Diatoma moniliformis

General distribution and ecology:

Diatoma tenuis is present in oligotrophic stream headwaters (Sabater*, et al.*, 2003b). It appeared in upper calcareous springs from the Pyrenees (Sabater and Roca, 1992) and in lower mesotrophic courses of Finnish waters (Soininen, 2002).

Diatoma moniliformis is present in eutrophic and electrolyte–rich waters (Soininen*, et al.*, 2004, Urrea and Sabater, 2009b) and it is abundant in halophilous and brackish waters (Potapova and Charles, 2003).

Distribution and ecology in the Guadiana watershed:

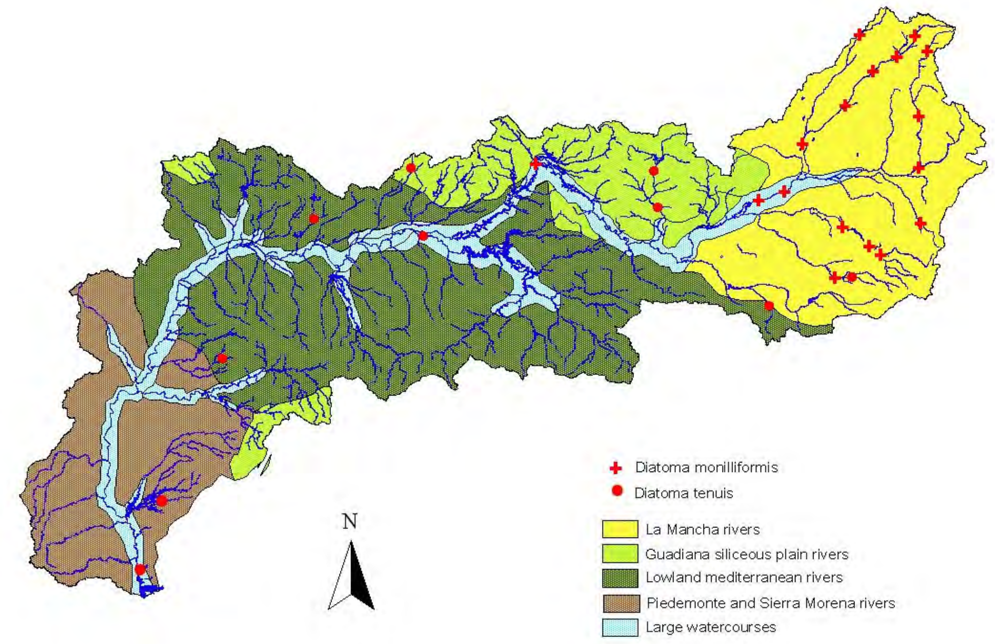
dten

n_{loc}=11	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2131.0	23.9	15.1	9.1	1.2	84.9	89.2	0.5	143.0	1090.0	0.9	20.6	434.0	265.0
MIN	219.0	3.8	7.0	7.4	0.0	10.8	14.0	0.0	24.1	10.0	0.0	0.1	14.6	57.3
MEAN	719.9	15.9	11.7	7.9	0.6	54.4	45.0	0.2	42.9	226.0	0.1	5.8	97.8	135.0
SD	668.6	5.8	2.7	0.5	0.5	22.2	22.6	0.1	36.3	371.0	0.3	6.3	134.5	81.4

dmon

n_{loc}=20	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2960.0	15.4	13.3	8.6	1.4	89.3	49.3	0.8	121.8	1499.1	0.7	41.5	498.0	331.0
MIN	103.0	2.7	5.8	8.0	0.0	50.7	9.5	0.0	11.0	60.0	0.0	4.6	70.4	150.0
MEAN	1671.8	7.3	10.0	8.2	0.6	74.1	25.3	0.1	36.3	609.6	0.1	17.3	257.8	214.8
SD	843.5	3.7	2.0	0.2	0.5	11.9	12.0	0.2	32.3	512.7	0.2	9.4	165.5	50.8

In Guadiana watershed, most specimens considered as *D. moniliformis* were found exclusively in the upper watershed with calcareous substrate. The specimens considered as *D. tenuis* were confined to middle and low watersheds with siliceous bedrocks.

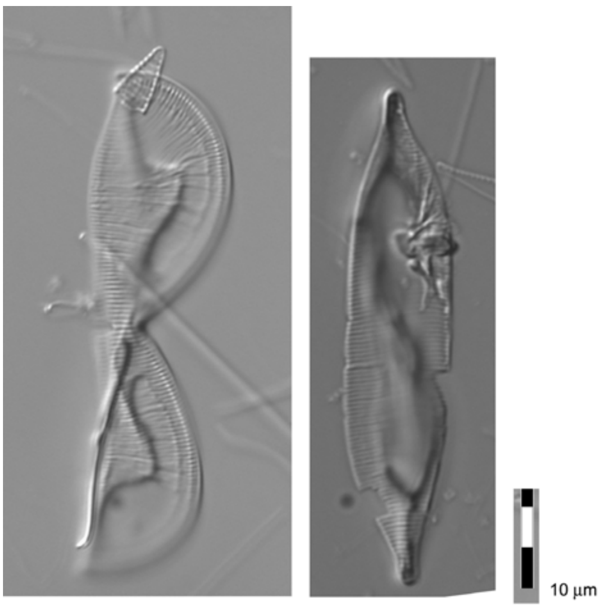


Entomoneis cf. ***alata*** (W.Smith) Reimer [eala]

Taxonomical remarks:

The general aspect and the size range of our specimens coincide both with *Entomoneis alata* and *Entomoneis paludosa* described in the literature. The only criterion to differentiate them is the striae density: 15–17 striae in 10 µm in *E. alata*, 19–23 in *E. paludosa*, according to Krammer and Lange–Bertalot (1985). Since our specimens presented 18 striae in 10 µm and striation could not be solved under SEM, because the low abundance did not allow to analyse the ultrastructure of our individuals, and the similar ecology of the two species in question, the individuals in this study have been considered as *E. alata sensu lato*.

	L (µm)	**W** (µm)	**Marginal striae in 10 µm**
LM (n_{in}=12)	44.3 – 56.5 (50.2 ± 3.7)	27.1 – 34.6 (30.8 ± 2.3)	16–22 (18 ± 2)



General distribution and ecology:

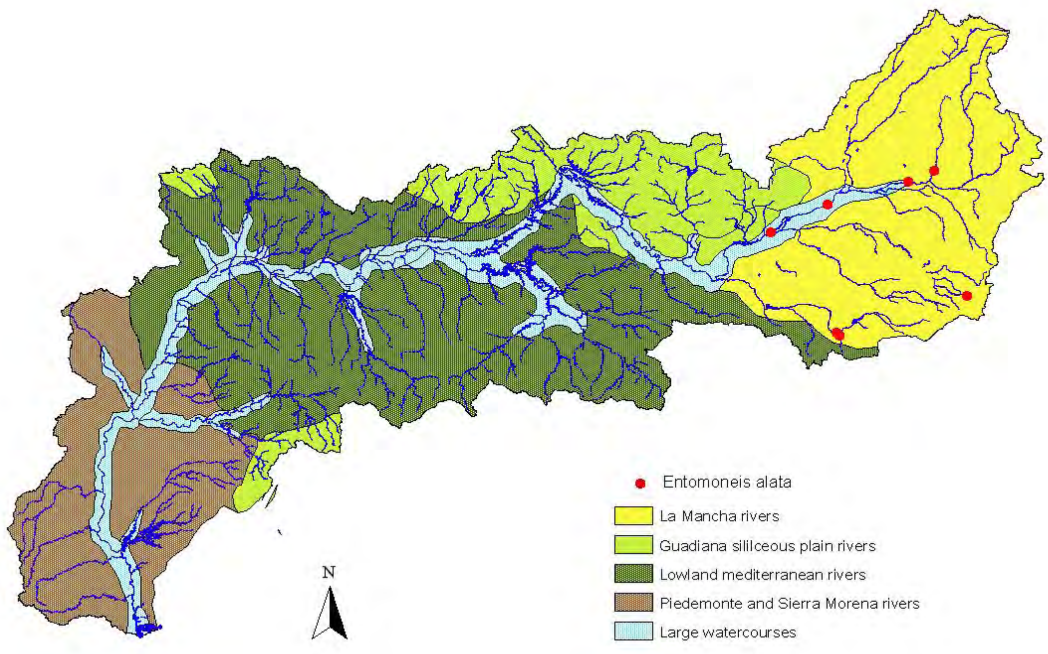
It's a cosmopolitan and widespread taxon inhabiting saline waters with high electrolyte content (Krammer and Lange–Bertalot, 1985).

This taxon was described in some saline ponds of Ebro Delta (Comín, 1984, Gómez, 1998), other littoral lagoons in Mediterranean coast (Aboal, 1987, Tomàs, 1988, Ubierna and Sánchez–Castillo, 1992, Cambra, 1993, Rieradevall and Cambra, 1994, Trobajo, 2003) and in brackish waters of Guadiamar river (Sabater, 2000).

Distribution and ecology in the Guadiana watershed:

n_{loc}=7	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	4140.0	15.1	13.3	8.6	1.4	88.0	27.0	0.8	270.2	1889.1	0.1	42.0	516.8	313.0
MIN	925.0	3.1	6.9	7.6	0.0	73.0	10.9	0.0	7.2	13.9	0.0	1.5	108.6	198.8
MEAN	2467.9	8.4	10.6	8.2	0.7	82.9	16.4	0.2	118.3	1094.1	0.0	23.5	369.2	233.8
SD	1068.4	4.2	2.7	0.3	0.5	5.6	5.9	0.3	95.5	712.8	0.0	15.4	165.2	46.3

The distribution of this taxon along Guadiana watershed was restricted to calcareous substratum. Its maximum abundance was reported in the Gigüela tributary, where sulfate and chloride concentrations were high.

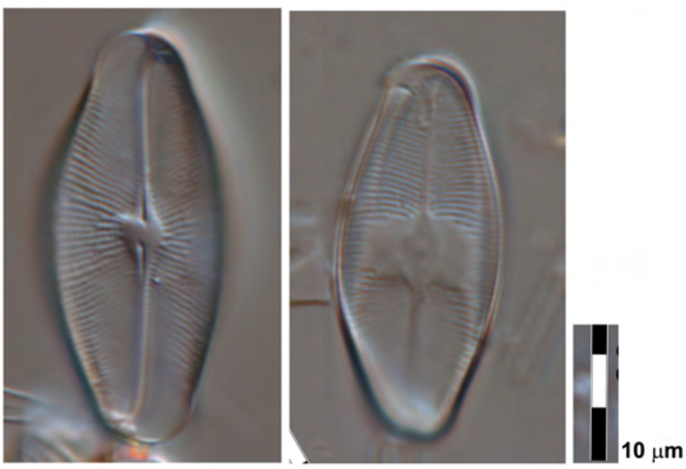


Eucocconeis flexella (Kützing) Meister [afle]
Syn. *Achnanthes flexella* (Kützing) Grunow

Taxonomical remarks:

The measurements of our specimens coincide with the size range described by Krammer and Lange–Bertalot (1991b). *E. flexella* is characterized by frustules of 14–82 µm in length, 7–26 µm in width. They are heterovalvar, and are bent about the median transapical plane and also twisted about the apical axis. The raphe valve is concave, with 18–28 radiate striae in 10 µm in the middle of the valve. The rapheless valve has striae that are straight or only slightly radiate at the centre, becoming radiate towards the ends. The central area is larger than that for the raphe valve, extending almost to the edge of the mantle.

	L (µm)	**W** (µm)	**Striae in 10 µm**
LM (n_{in}=8)	27.8 – 32.2 (30.2 ± 1.4)	12.4 – 15.0 (13.8 ± 0.8)	19 – 23 (21 ± 1)



General distribution and ecology:

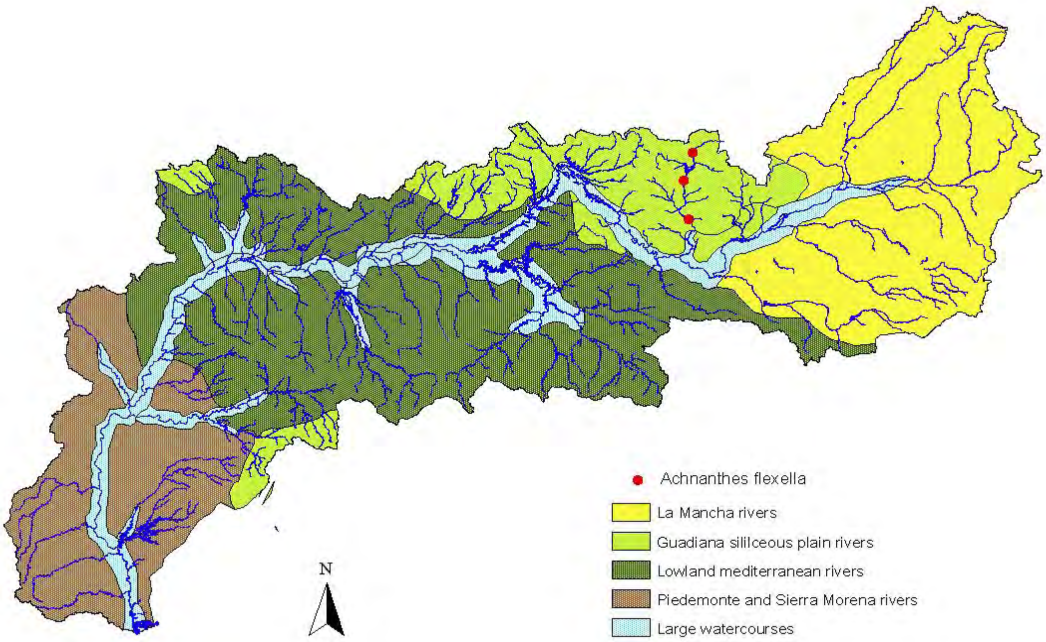
E. flexella has a cosmopolitan distribution, in slighlty alkaline or alkaline waters around the world (Germain, 1981), usually associated with oligotrophic environments (Dell'Uomo, 2004).

It was found in current and oligotrophic waters at high and mid altitude mountain rivers of the Iberian Peninsula (Margalef, 1950a, 1950b, 1954, Aboal, 1987, Cambra and Perera, 1988, Sabater and Roca, 1992, Linares-Cuesta*, et al.*, 2007) and occasionally in low altitudes near the coast (Margalef, 1951, Tomàs, 1988).

Distribution and ecology in the Guadiana watershed:

n_{loc}=5	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	492.0	17.6	11.2	8.3	1.1	57.5	49.5	0.0	78.2	70.0	0.0	2.6	46.0	42.0
MIN	186.0	4.1	9.5	7.1	0.0	49.5	42.5	0.0	20.4	19.2	0.0	1.3	15.3	37.0
MEAN	352.6	10.9	10.7	8.0	0.5	53.7	45.8	0.0	40.2	36.4	0.0	2.1	26.6	39.8
SD	146.7	6.3	0.8	0.5	0.5	4.0	3.5	0.0	32.9	29.1	0.0	0.7	16.9	2.6

This taxon was restricted to the Bullaque tributary, in the north eastern part of the watershed. It was present in waters with low nutrient content and moderate conductivity (≈500 µS/cm). It was more abundant during spring sampling period.



Eunotia Ehrenberg **genus**

Eunotia is essentially a freshwater genus frequently associated with acidic waters (Alles*, et al.*, 1991). *Eunotia* is a large genus that comprises around 200 species, with a world–wide distribution (Ortiz and Cambra, 2007).

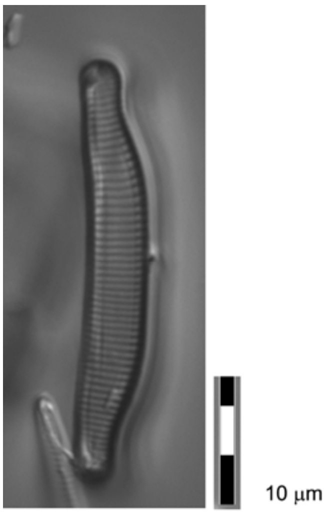
Despite its abundance in freshwater habitats, literature concerning *Eunotia* species is scant. There are few studies about the species's valve structure and there are unsolved taxonomical problems because of resemblance between different taxa (Mayama and Kobayasi, 1991, Mayama, 1992, Gaul*, et al.*, 1993, Mayama, 1997).

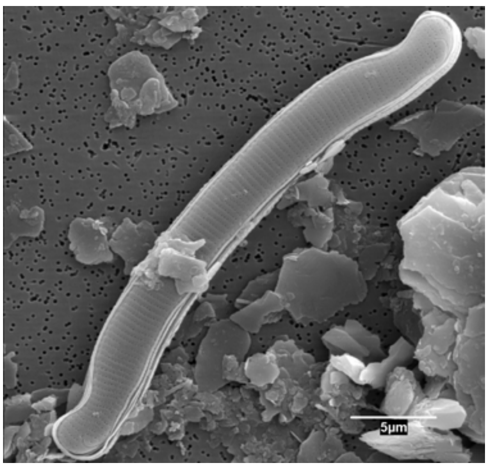
The aim of this section is to present the description of *Eunotia* species found in Guadiana watershed which have been classified mainly following Krammer and Lange–Bertalot (1991a).

Eunotia arcus Ehrenberg [earc]

<u>Taxonomical features:</u>

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=13)	8.6 − 34.2 (17.8 ± 7.6)	2.3 − 5.5 (3.6 ± 1.0)	14 − 17 (16 ± 1)





General distribution and ecology:

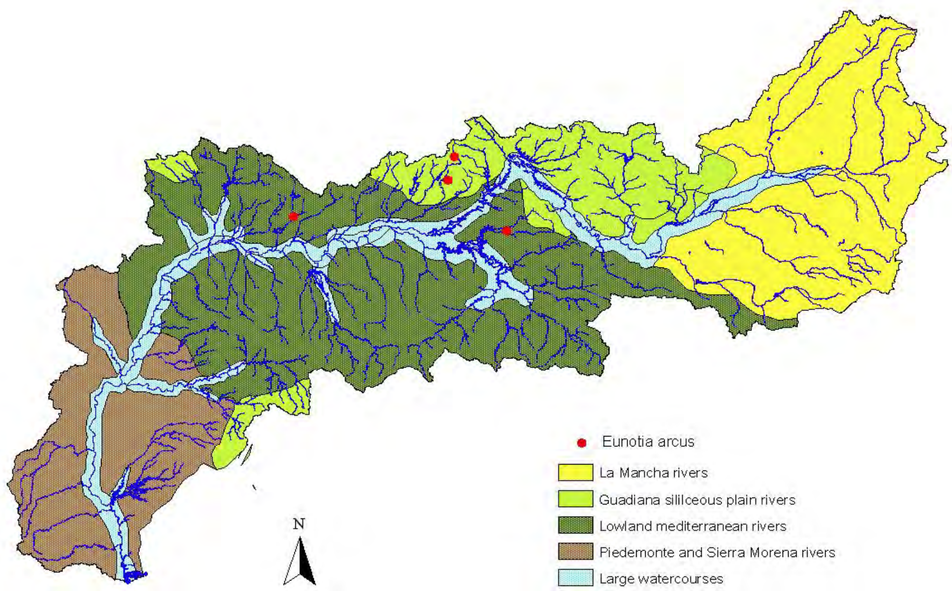
E. arcus is generally considered an acidobiontic taxon (Van Dam*, et al.*, 1994, Hill*, et al.*, 2003) capable of surviving in highly acidic environments (DeNicola, 2000). In addition Margalef (1956b) defines it like one of the few species of the genus able to develop on calcareous substratum.

It is widely distributed in running waters (Margalef, 1944, 1954, 1955, 1956a, Aboal and Llimona, 1984, Aboal, 1987, 1989b, Cambra and Gomà, 1997, Sabater, 2000) and in some karstic ponds (Tomàs, 1988, Cambra, 1991a, Ubierna and Sánchez-Castillo, 1992) along the Iberian Mediterranean Coast. Moreover, it was also reported in some siliceous springs (Massanell, 1966, Sabater and Roca, 1992) and lakes (Cambra, 1991b) of the Pyrenees.

Distribution and ecology in the Guadiana watershed:

n_{loc}=5	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	195.0	25.1	9.9	8.3	2.6	69.5	81.8	0.3	41.8	30.0	0.2	1.2	12.7	53.3
MIN	41.0	3.8	5.8	5.9	0.0	15.5	30.5	0.1	9.0	9.0	0.0	0.4	3.2	15.0
MEAN	153.8	18.2	7.9	7.4	0.6	43.3	56.1	0.1	21.4	19.5	0.1	0.6	8.1	32.2
SD	64.3	8.4	1.5	1.0	1.2	23.4	22.7	0.1	15.7	12.1	0.1	0.4	4.2	20.1

This taxon is distributed in headwaters of siliceous bedrock rivers. Its maximum abundance was associated to oligotrophic waters with slightly acid pH (≈ 6).

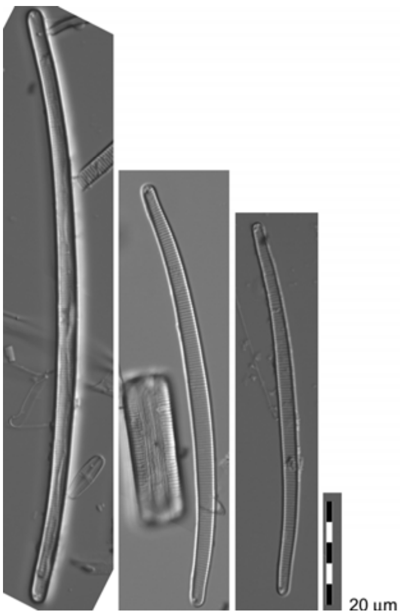


Eunotia bilunaris (Ehrenberg) Mills complex [ebil]

<u>Taxonomical features:</u>

Despite some varieties of this taxon have been described, I have grouped under *E. bilunaris* all the forms that coincide with the general description of the taxon based on Krammer and Lange–Bertalot (1991a): lunate appearence with small terminal nodules and indistinct raphe.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=12)	55.6 − 117.5 (77.8 ± 18.2)	2.8 − 4.1 (3.5 ± 0.4)	16 − 18 (17 ± 1)



<u>General distribution and ecology:</u>

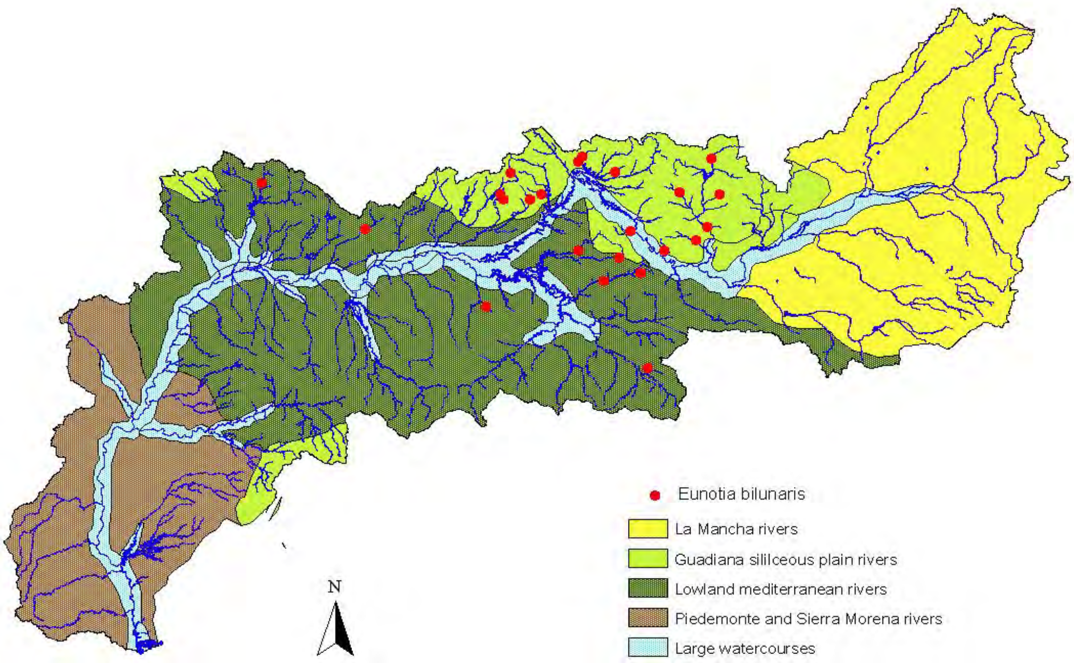
This taxon is widely distributed in waters with a low mineral content; commonly found in acid waters, but may occur in slightly alkaline waters (Patrick & Reimer, 1966). It is frequently found as epiphytic on filamentous algae (Krammer and Lange–Bertalot, 1991a).

This taxon is widely distributed along the siliceous waters of Iberian Peninsula (Aboal, 1989a, Aboal*, et al.*, 1998, Ortiz and Cambra, 2007, Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n_{loc}=24	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	492.0	26.8	12.4	9.4	2.6	77.3	100.0	0.3	96.0	30.0	0.4	7.8	25.3	103.0
MIN	38.0	4.6	6.7	5.9	0.0	0.0	22.7	0.0	6.3	2.5	0.0	0.4	2.1	5.5
MEAN	188.8	17.1	9.9	7.8	0.1	24.4	75.4	0.1	30.1	16.4	0.1	1.0	8.6	33.5
SD	141.0	7.7	1.7	0.7	0.5	22.2	22.2	0.1	27.1	9.8	0.1	1.7	6.9	28.3

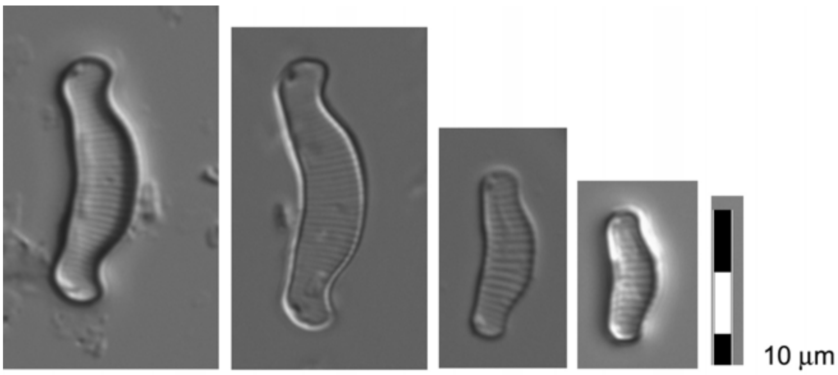
The distribution of this taxon in Guadiana watershed was restricted to the middle watershed, being its maximum abundance associated to oligotrophic and low mineralized headwaters during spring period, both in 2005 and 2006.

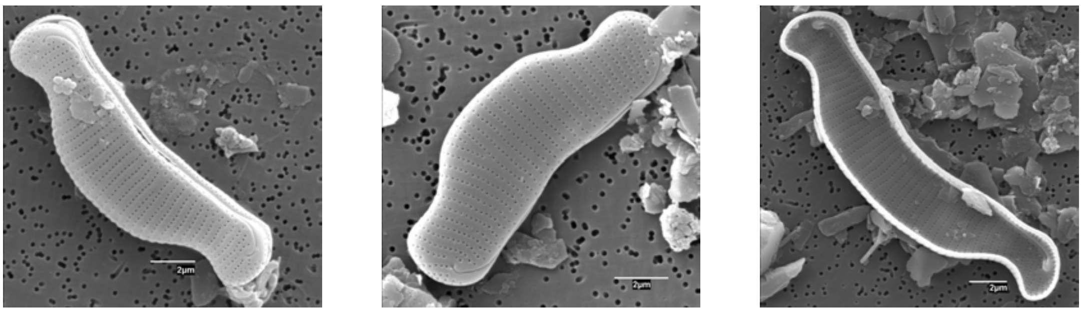


Eunotia exigua (Brébisson ex Kützing) Rabenhorst [eexi]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=11)	9.6 − 18.1 (16.3 ± 2.6)	3.1 − 4.3 (3.7 ± 0.4)	19 − 25 (23 ± 1)





General distribution and ecology:

E. exigua is generally considered an acidobiontic taxon (Van Dam*, et al.*, 1994) and is one of the most widespread species reported from North America, Asia and Europe in lakes and streams receiving acid mine drainage (Lackey, 1938, Negoro, 1985, Lessmann*, et al.*, 2000). This taxon is extremely common in oligotrophic, poorly–buffered sites and acidic habitats. *E. exigua* can thrive at pH levels down to 2.2 (DeNicola, 2000) and is one of the few species capable to survive in acidic mine drainage where high levels of acidity are accompanied by high concentrations of nutrients and heavy metals (Whitton and Diaz, 1981).

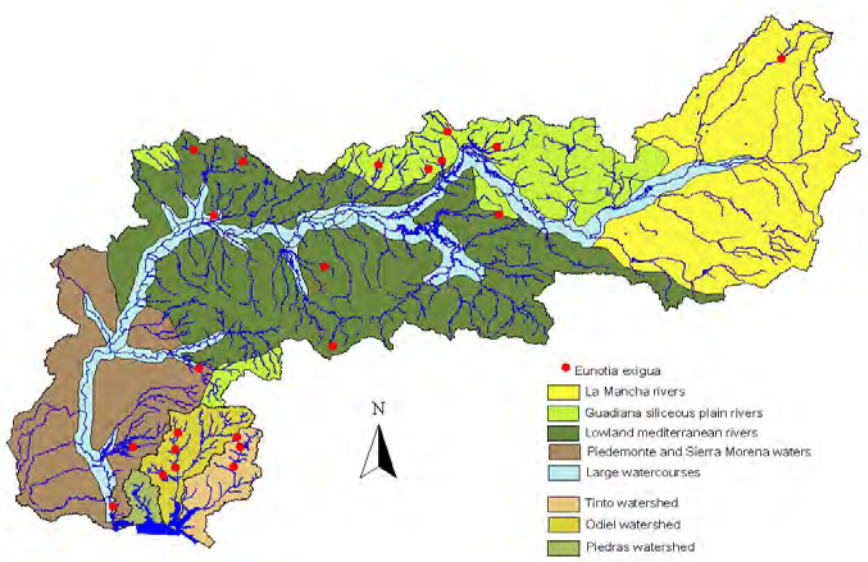
It has been found in some oligotrophic waters along the Iberian Peninsula (Margalef, 1955, 1956b, Varela*, et al.*, 1991, Almeida and Gil, 2001, López-Rodríguez and Penalta, 2004, Ortiz and Cambra, 2007, Álvarez–Blanco, 2009, Tornés, 2009), and it has also been cited in acid mine drainage waters from Rio Tinto (Urrea and Sabater, 2009b).

Distribution and ecology in the Guadiana watershed:

n_{loc}=17	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2470.0	9.2	12.8	9.3	0.9	92.8	99.8	0.5	84.8	30.0	0.1	0.4	67.7	351.0
MIN	40.0	4.1	6.2	3.1	0.0	0.3	7.2	0.0	2.5	30.0	0.1	0.4	3.5	15.0
MEAN	889.5	6.8	11.5	6.7	0.1	40.8	59.1	0.2	30.4	30.0	0.1	0.4	31.8	175.8
SD	955.5	1.7	2.5	2.5	0.3	25.8	25.8	0.2	37.1	0.0	0.0	0.0	31.3	148.1

This taxon was associated to AMD affected waters, both in Tinto–Odiel system, and also in Cobica tributary (Chanza sub–watershed, in lower Guadiana basin). All this AMD affected localities, presented high conductivity (>1,500 µS/cm) and extremely low pH (≈ 3).

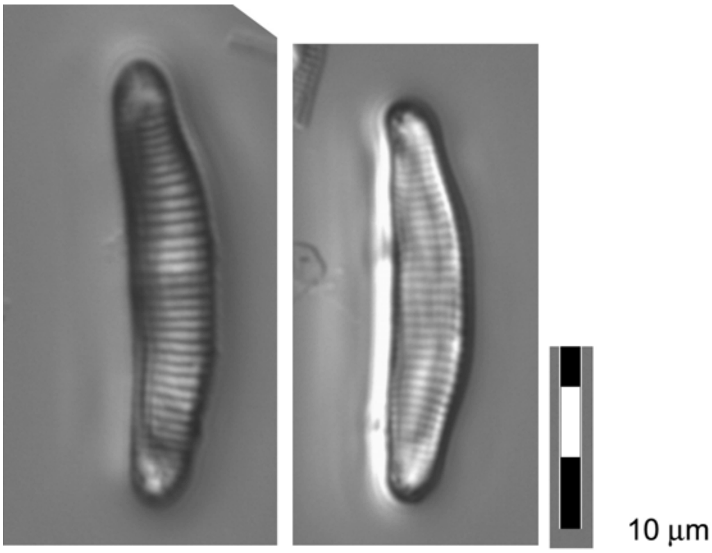
It has been collected with low abundances (<1 %) in oligotrophic headwaters in middle Guadiana watershed.



Eunotia implicata Nörpel, Lange-Bertalot & Alles [eimp]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=3)	20.7 − 22.7 (21.7 ± 1.4)	3.3 − 4.0 (3.7 ± 0.2)	15 − 17 (16 ± 1)



General distribution and ecology:

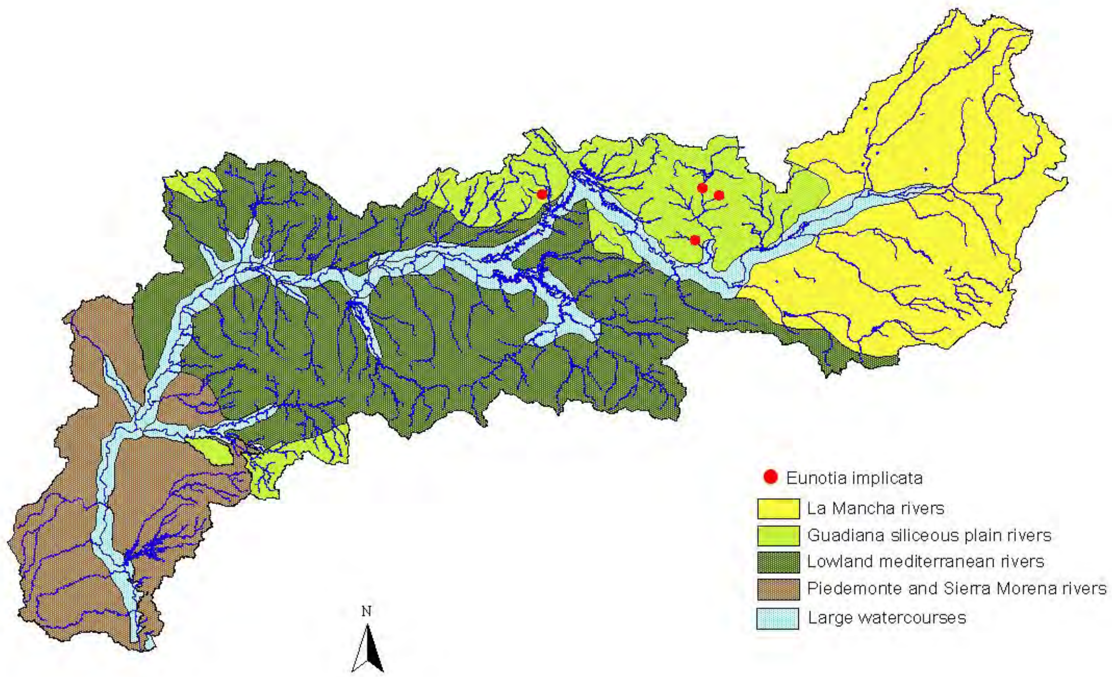
E. implicata has been considered acidophilus taxa mainly occurring at pH < 7 (Van Damm *et al*., 1994). It is frequent in headwaters of silicoeuos springs in alpine waters from northern Italy (Robinson, 2009) and it has also been described in some acidophilous waters from Poland (Rakowska, 2010).

It is one of the most abundant taxa from the *Eunotia* genus present in in rivers and streams of Northern Spain characterized by their low conductivities (Ortiz and Cambra, 2007). It has aslo been cited in lagoons of the northern Peninsula (Leira, 1997).

Distribution and ecology in the Guadiana watershed:

n_{loc}=4	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	205.0	21.6	23.4	7.3	1.1	49.7	88.5	0.1	22.0	20.0	0.1	10.3	19.0	83.0
MIN	47.0	4.1	9.6	6.1	0.0	11.5	49.3	0.0	6.3	2.5	0.0	0.4	3.3	5.5
MEAN	149.2	13.6	14.0	6.8	0.4	30.7	68.9	0.0	15.4	12.1	0.0	4.4	13.0	36.5
SD	70.3	7.7	5.9	0.4	0.6	18.7	19.2	0.0	6.5	7.4	0.0	4.4	6.8	30.1

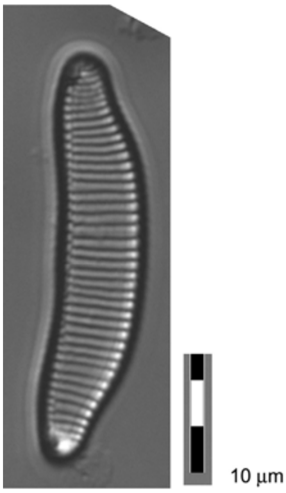
In the study area this taxon was distributed in siliceous rivers occupying upper part of the stretches.



Eunotia minor (Kutzing) Grunow in Van Heurck [emin]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=5)	33.8 − 48.1 (34.2 ± 3.8)	5.2 − 8.0 (9.5 ± 1.2)	9 − 15 (11 ± 2)



General distribution and ecology:

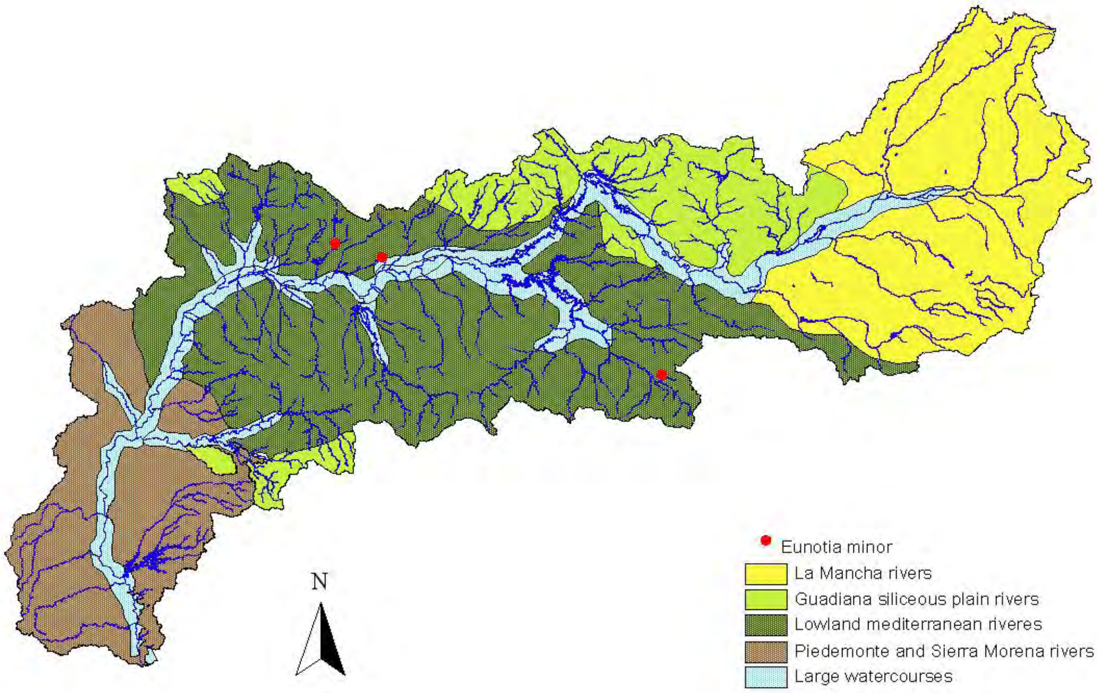
It is a frequently epilithic or epiphytic taxa inhabiting circumneutral waters, particularly springs and small streams. Its optimal abundance is around pH 7 (Alles *et al*., 1991).

It is one of the most abundant taxa from the *Eunotia* genus present in in rivers and streams of Northern Spain characterized by their low conductivities (Ortiz and Cambra, 2007).

Distribution and ecology in the Guadiana watershed:

n_{loc}=3	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	219.0	21.6	12.6	9.1	0.0	73.3	89.7	0.4	29.0	30.0	0.1	0.4	15.1	60.7
MIN	67.0	6.3	6.4	7.0	0.0	10.3	26.7	0.2	24.3	30.0	0.1	0.4	14.6	58.7
MEAN	162.8	13.8	9.0	8.1	0.0	51.2	48.8	0.3	26.7	30.0	0.1	0.4	14.9	59.7
SD	71.5	7.6	2.6	0.9	0.0	29.8	29.8	0.2	3.3	0.0	0.0	0.0	0.4	1.4

In the study area this taxon was distributed in siliceous rivers occupying upper part of the stretches.

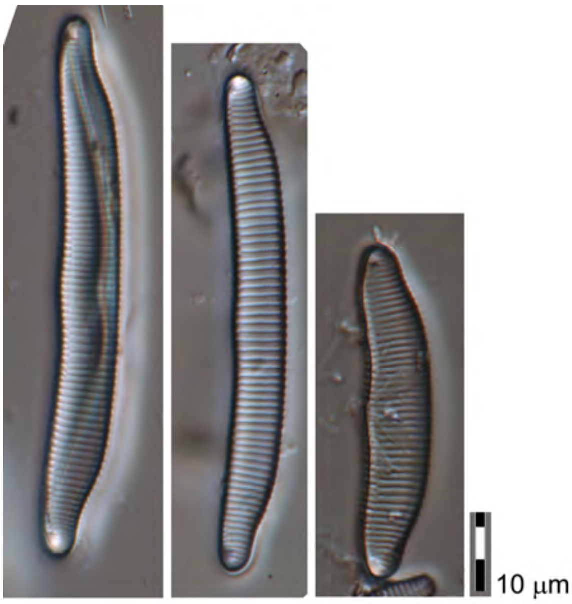


Eunotia pectinalis (Dyllwyn) Rabenhorst [epec]

Taxonomical features:

This is a rather variable taxon whose morphological variability is well documented (Geitler, 1932, Steinman and Sheath, 1984). The varieties which are recognized represent the extremes of a series of variations from the typical form. In the present work the forms rectangularly shaped and those with undulations on the dorsal margin are included under *E. pectinalis*.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=4)	36.3 − 80.7 (49.8 ± 17.8)	5.0 − 7.4 (6.0 ± 0.9)	9 − 15 (14 ± 1)



General distribution and ecology:

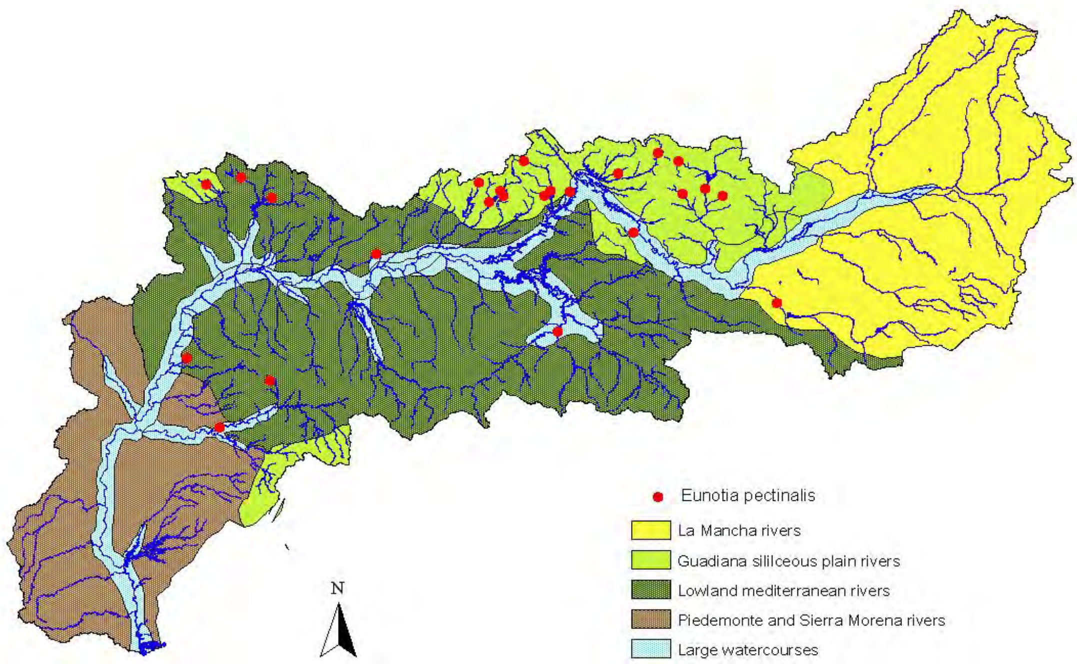
This is an acidophilous taxon (Van Dam*, et al.*, 1994) with a great morphological variability (Geitler, 1932, Steinman and Sheath, 1984), capable of living in highly acidic environments (pH 2.8–3.4) (DeNicola, 2000).

It has been described in the northwest waters of the Iberian Peninsula running on siliceous substrate (Margalef, 1954, 1955, 1956a, Tomàs, 1988, Noguerol-Seoane, 1990, 1994, Ortiz and Cambra, 2007), but it has also been reported in high mountain calcareous springs (Tomàs and Sabater, 1985, Sabater and Roca, 1990).

Distribution and ecology in the Guadiana watershed:

n_{loc}=30	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	1354.0	28.2	13.0	10.6	1.1	96.1	100.0	0.8	111.0	273.0	0.3	10.8	104.0	489.0
MIN	39.0	3.5	5.7	6.4	0.0	0.0	3.9	0.0	2.0	9.0	0.0	0.2	2.7	15.0
MEAN	252.7	16.2	9.5	7.9	0.2	42.4	57.5	0.2	22.7	31.1	0.1	1.2	15.6	70.4
SD	286.2	7.0	2.8	0.9	0.3	27.1	27.2	0.2	23.7	54.9	0.1	2.3	21.7	102.3

This taxon was distributed along siliceous rivers occupying upper and middle part of the stretches with moderate conductivity (≈ 200 µS/cm) and low nutrient content.



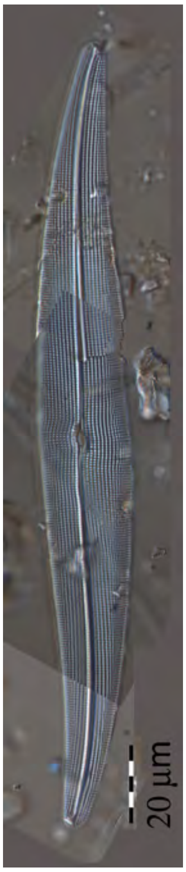
Gyrosigma attenuatum (Kützing) Rabenhorst [gyat]

Taxonomical remarks:

The general aspect of the *G. attenuatum* specimens in the Guadiana coincides with the description given by Krammer and Lange–Bertalot (1985): “Valves linear at the centre, apices sigmoidly deflected to round apices. Central fissures oppositely deflected and polar fissures curving over apices. Transverse striae parallel, crossed by more widely spaced and less distinct longitudinal striae”.

Here included are the large *Gyrosigma* forms (>200 µm in length) despite the Guadiana specimens were larger than those described in the literature (140–250 µm in length, 23–26 µm in width with 14–16 striae in 10 µm in the central part of the valve).

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=5)	235.3 − 275.0 (254.9 ± 19.9)	24.7 − 31.1 (28.1 ± 3.2)	14 − 16 (15 ± 1)



General distribution and ecology:

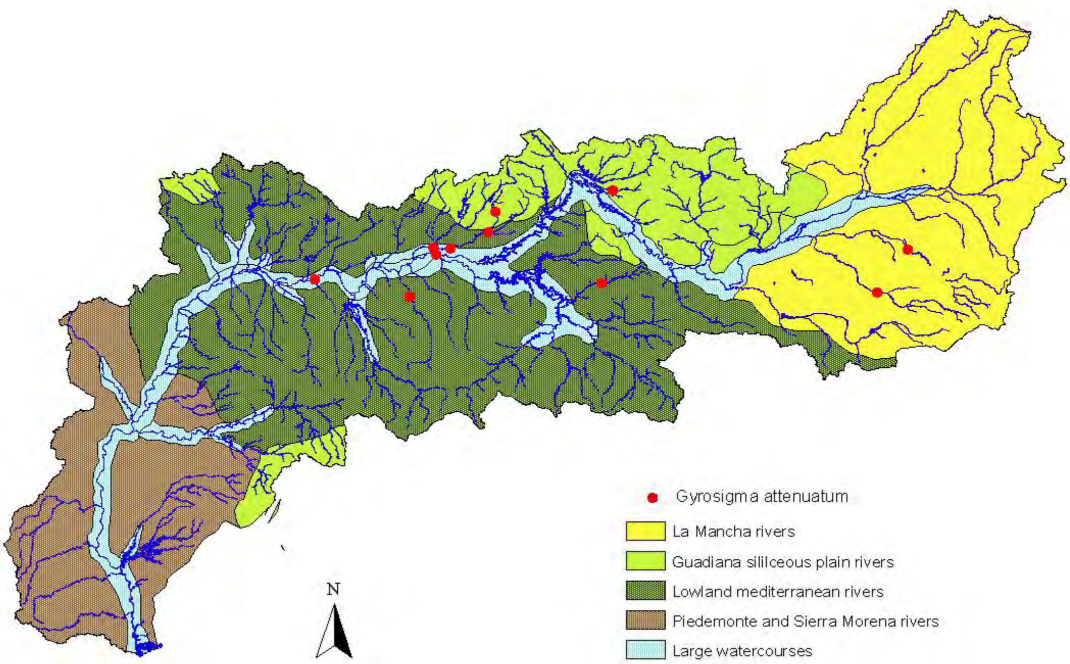
G. attenuatum is a widespread taxon inhabiting waters from moderate to high electrolyte content along Europe (Krammer and Lange–Bertalot, 1985).

It has been described in some freshwaters from all over the Iberian Peninsula (Margalef, 1950a, 1954, 1958, Tomàs and Sabater, 1985, Sabater, 1987, Tomàs, 1988, Cambra, 1992b).

Distribution and ecology in the Guadiana watershed:

n_{loc}=13	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	1430.0	25.3	13.1	8.9	1.5	72.4	72.0	0.4	126.0	445.0	0.2	9.8	190.0	194.0
MIN	64.2	7.3	1.4	7.3	0.0	27.7	27.2	0.0	4.8	9.0	0.0	0.2	4.1	15.0
MEAN	591.4	19.2	8.9	8.2	0.5	58.3	41.2	0.1	47.4	85.3	0.1	2.2	47.4	106.1
SD	432.9	5.1	3.3	0.5	0.5	13.3	13.5	0.1	34.5	134.1	0.1	3.0	51.5	56.7

Its distribution along Guadiana watershed is both in siliceous and calcareous waters, but always with very low densities (<5 %). Its maximum abundance was associated to high conductivities (>500µS/cm) and moderate sulfate content (>30 mg/L).



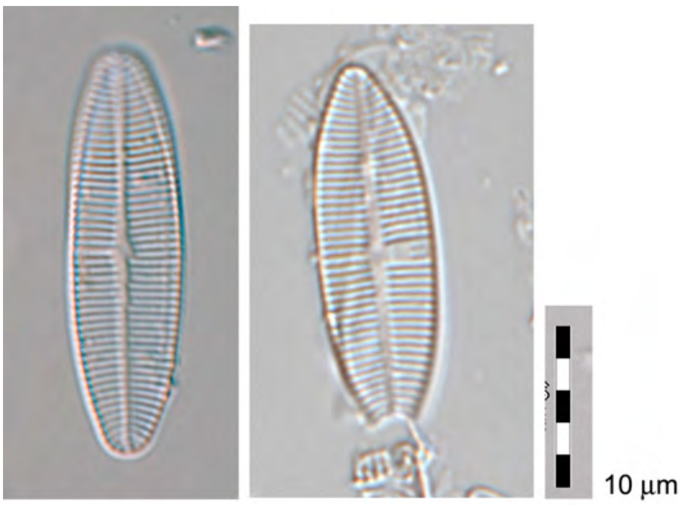
Lemnicola hungarica (Grunow) Round & Basson [ahun]
Syn. *Achnanthes hungarica* Grunow in Cleve et Grunow

Taxonomical remarks:

Round and Basson (Round and Basson, 1997) erected a new genus for this species. The general description was: "linear–elliptical to linear–lanceolate valves with wedge–shaped to broadly rounded ends. The raphe valve has a narrow, linear axial area and a thickened central stauros (often asymmetric) that reaches to the edge of the valve. The rapheless valve is similar but the central stauros is reduced or absent".

In the Guadiana only large specimens with linear–lanceolate and roundish ends have been observed.

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=5)	27.3 − 30.5 (28.9 ± 2.2)	8.6 − 9.0 (8.8 ± 0.3)	13 − 14 (13 ± 1)



General distribution and ecology:

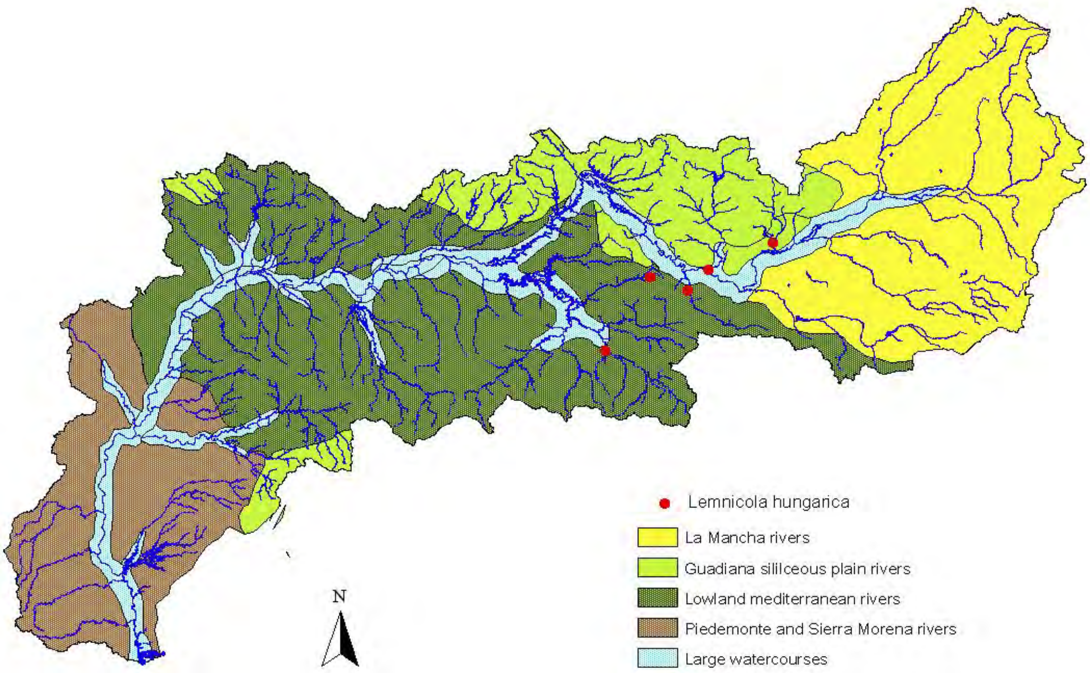
Lemnicola hungarica is a common and widespread diatom occurring all over the World. Sometimes it is very abundant in biofilms formed on various submersed objects in freshwaters (Buczkó, 2007) specially associated to macrophytes like *Lemna* (Hustedt, 1985).

This taxon is commonly related with high conductivities due to high calcium content in Portuguese waters (Almeida and Gil, 2001). It has been described in some mineralized waters from all over the Peninsula (Margalef, 1954, Sabater, 1987, Tomàs, 1988, Álvarez–Blanco, 2009, Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n_{loc}=7	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	1604	24.3	11.8	9.4	1.4	94.5	71.9	0.8	228.0	114.0	2.3	11.8	103.0	452.0
MIN	304	18.2	7.9	6.9	0.0	28.1	4.7	0.0	22.6	10.0	0.0	0.4	21.8	82.1
MEAN	847.3	20.5	9.7	8.0	0.6	65.5	33.9	0.2	95.7	37.7	0.9	2.4	48.4	191.2
SD	471.6	2.5	1.9	0.8	0.6	21.9	22.0	0.3	84.4	35.9	1.0	4.2	28.2	138.6

This taxon appeared in some eutrophic sites situated in the middle area of the watershed associated to cultural land uses. It appeared in low densities (<5 %) during spring and autumn, both in 2005 and 2006. It has been collected attached to submerged hard substrata.



Nitzschia dissipata (Kützing) Grunow [ndis] *sensu lato*

Taxonomical remarks:

This name is used in the broad sense of Krammer & Lange–Bertalot (1988). Varieties (e.g. var. *media*) are sometimes recognized within *N. dissipata* and some of these probably deserve species status. In spite of that, I decided to include all forms to *N. dissipata sensu lato*.

	L (µm)	**W** (µm)	**Dorsal fibulae in 10 µm**
LM (n_{in}=12)	16.7 − 31.8 (24.7 ± 5.4)	3.3 − 5.2 (4.2 ± 0.6)	7 − 11 (9 ± 1)

General distribution and ecology:

N. dissipata is a cosmopolitan taxon occurring in numerous freshwater systems inhabiting in well oxygenated and non polluted waters (Coste and Richard, 1980). It has been observed in numerous freshwater systems along Iberian Peninsula (Margalef, 1945, 1954, 1956a, Vicente*, et al.*, 1984, Tomàs and Sabater, 1985, Sabater, 1987, Sabater*, et al.*, 1987, Tomàs, 1988, Ubierna and Sánchez-Castillo, 1992, Varela, 1992, Cambra and Gomà, 1997, Aboal*, et al.*, 1998, Álvarez–Blanco, 2009)

Distribution and ecology in the Guadiana watershed:

n_{loc}=52	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2980.0	23.0	13.4	9.5	2.9	96.5	81.9	6.5	171.0	1499.1	0.8	44.0	453.4	332.0
MIN	49.0	2.2	1.10	6.7	0.0	15.5	3.5	0.0	3.7	2.6	0.0	0.1	3.3	15.8
MEAN	989.8	10.2	9.3	8.3	0.7	67.1	32.2	0.5	49.0	185.7	0.1	11.6	96.5	176.8
SD	811.3	6.2	3.0	0.5	0.7	18.2	18.2	1.3	39.0	335.7	0.2	13.7	97.9	89.2

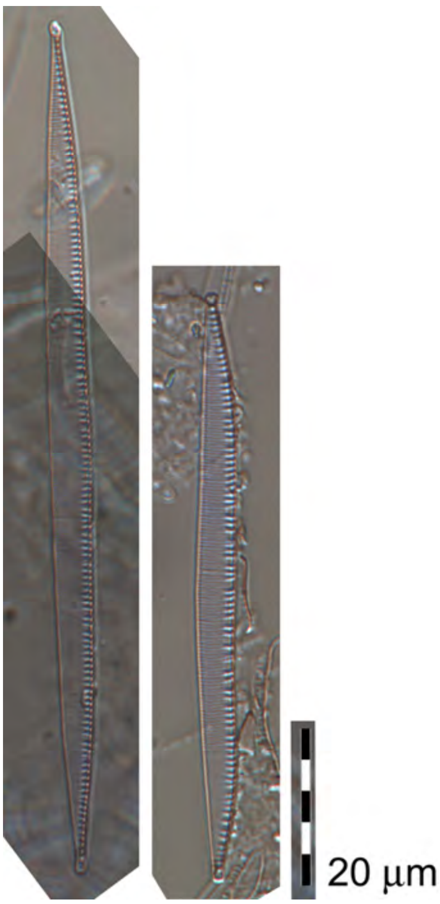
N. dissipata was a widespread taxon along the watershed. It was indifferent to substratum type and its maximum abundance was under a large variety of situations.

Nitzschia cf. ***heufleriana*** Grunow [nheu]

Taxonomical remarks:

The general description of this taxon is that proposed by Krammer and Lange–Bertalot (1988): "Isopolar frustules with bilateral symmetry, linear to linear–lanceolate. Poles are subcapitate or capitate in strict valve view, but appearing asymmetrical and cuneate or rounded in oblique or 'girdle' views. Transverse striae are easily visible in the light microscope. Central pair of fibulae not more widely separated than the others". In the Guadiana the number of fibulae and striae was slightly higher than those described in the literature: 10–11 (14) fibulae/10 µm and 20–25 striae in 10 µm.

	L (µm)	**W** (µm)	**Dorsal fibulae in 10 µm**	**Dorsal striae in 10 µm**
LM (n_{in}=5)	75.2 − 119.2 (92.4 ± 20.7)	4.8 − 6.0 (5.3 ± 0.5)	11 − 14 (12 ± 1)	25 − 27 (26 ± 1)



General distribution and ecology:

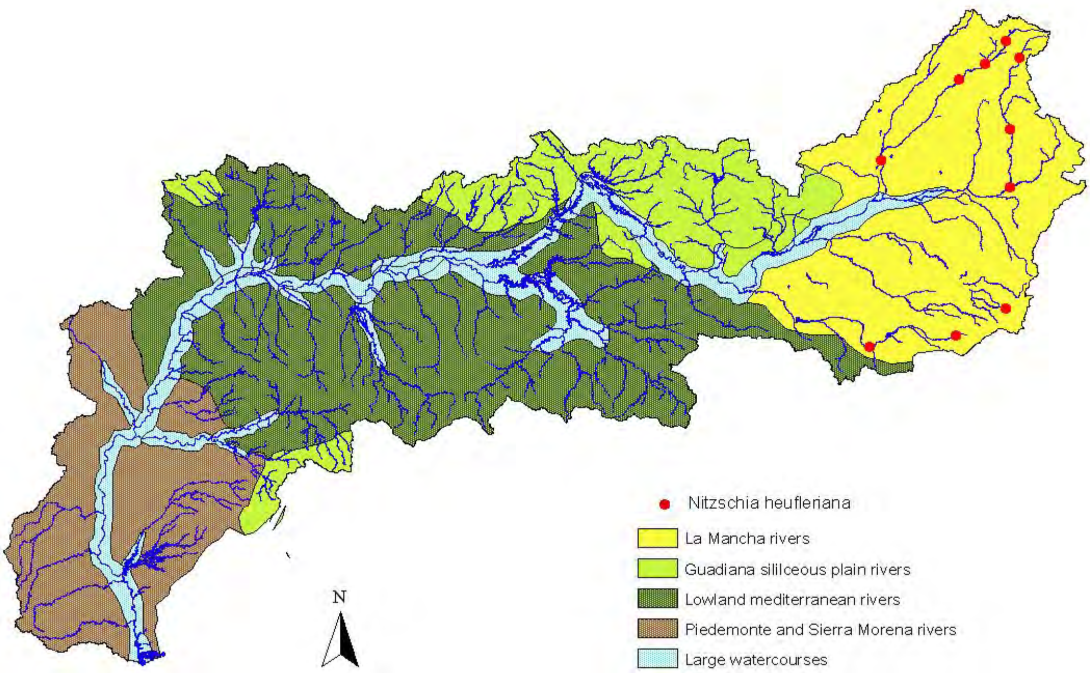
Nitzschia heufleriana has been described in European and North–American waterbodies in waters with low to medium electrolyte content (Krammer and Lange–Bertalot, 1988).

It was described in large number of calcareous freshwaters from the North East of the Iberian Peninsula (Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n_{loc}=11	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2370.0	17.7	11.8	8.5	1.2	89.3	88.8	6.5	115.0	411.9	0.1	41.5	185.0	313.0
MIN	649.0	2.7	5.8	8.0	0.0	10.8	9.5	0.0	7.2	13.9	0.0	0.4	33.3	92.7
MEAN	1761.4	7.8	9.2	8.2	0.4	70.4	29.2	1.2	36.8	216.1	0.1	19.4	127.9	225.6
SD	628.0	4.7	1.9	0.1	0.4	22.6	22.7	2.6	39.5	160.0	0.0	16.2	54.5	81.6

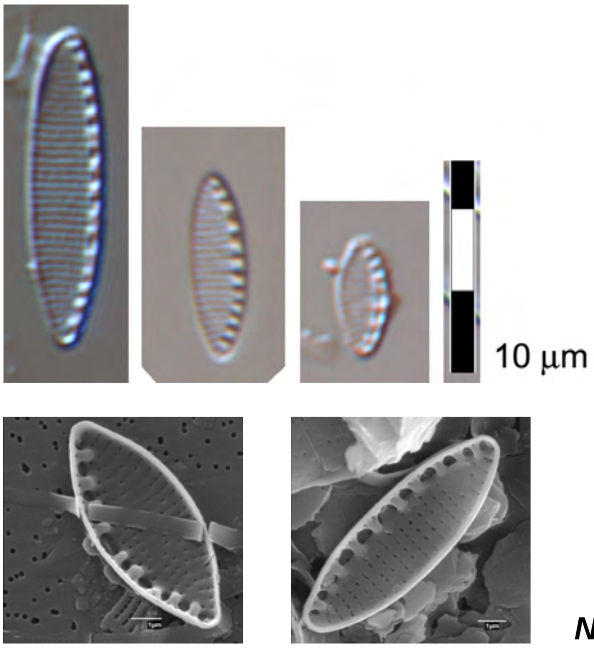
The distribution of this taxon in Guadiana watershed was restricted to calcareous waters with high conductivities. Its maximum abundance was associated to high sulfate content waters in Gigüela tributary (≈2000 µS/cm of conductivity and ≈200 mg SO_4 /L).



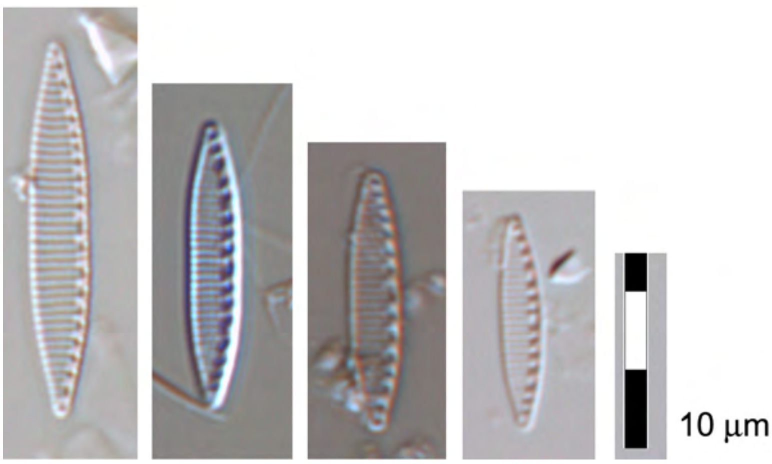
Nitzschia inconspicua Grunow [ninc] and **Nitzschia frustulum** (Kützing) Grunow [nifr]

<u>Taxonomical remarks:</u>

Although several works (Wendker, 1990, Lange–Bertalot, 1993, Trobajo*, et al.*, 2004) suggest the conspecificity of *N. frustulum* and *N. inconspicua*, the two were separated in the present work by following classical taxonomy: the short and roundish shape has been identified as *N. inconspicua* and the longer and thinner shape as *N. frustulum.*



Nitzschia inconspiqua



Nitzschia frustulum

Distribution in Guadiana watershed:

Although both taxa shared the same ecological ratio along Guadiana watershed, *N. inconspicua* was more abundant and preferred highly mineralized and more eutrophic waters than *N. frustulum*.

Distribution and ecology in the Guadiana watershed:

ninc > 20%

n_{loc} =39	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2960.0	29.6	12.8	9.1	2.1	96.5	97.5	6.9	228.0	516.0	4.1	29.4	78.0	452.0
MIN	45.6	2.3	2.7	6.8	0.0	2.5	3.5	0.0	9.0	9.0	0.0	0.2	5.1	15.0
MEAN	764.0	14.5	8.5	8.2	0.5	54.2	45.3	0.9	71.0	55.7	1.0	3.3	40.3	180.0
SD	660.6	8.3	3.5	0.5	0.7	27.3	27.5	2.0	60.2	106.0	1.2	6.4	23.4	128.1

nifr > 20%

n_{loc} =12	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	1109.0	25.3	11.7	9.2	1.3	84.6	40.4	6.9	94.5	31.4	0.9	0.4	69.6	407.0
MIN	107.0	7.6	4.3	7.9	0.0	59.6	14.8	0.0	9.0	9.0	0.0	0.1	5.8	33.1
MEAN	704.6	14.6	9.0	8.7	0.5	70.0	29.5	2.3	46.8	23.5	0.3	0.3	34.4	184.7
SD	437.5	8.6	4.1	0.5	0.5	10.8	11.0	4.0	43.6	12.5	0.5	0.2	32.4	196.7

Pinnularia **Ehrenberg genus**

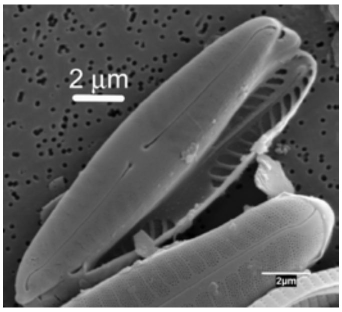
The genus *Pinnularia* is very frequent in algal communities of acidic environments (DeNicola, 2000). The identification of *Pinnularia* species is mainly based on light microscope descriptions and this leads to extensive inconsistencies in the infra–generic taxonomy (Ciniglia*, et al.*, 2007).

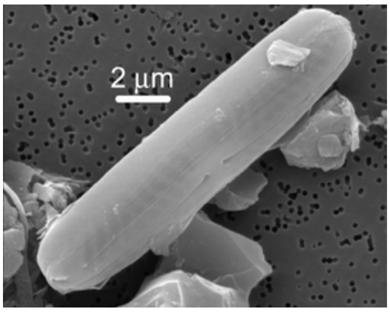
The aim of this section is to present the description of *Pinnularia* species found in Guadiana and related watersheds. The species have been classified according to Krammer and Lange–Bertalot (1985).

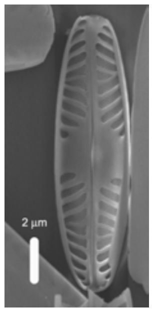
Pinnularia acoricola Hustedt [paco]

<u>Taxonomical features:</u>

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=11)	45.2 − 86.3 (63.0 ± 15.0)	15.2 − 18.5 (16.1 ± 1.0)	4 − 5 (4 ± 1)







<u>General distribution and ecology:</u>

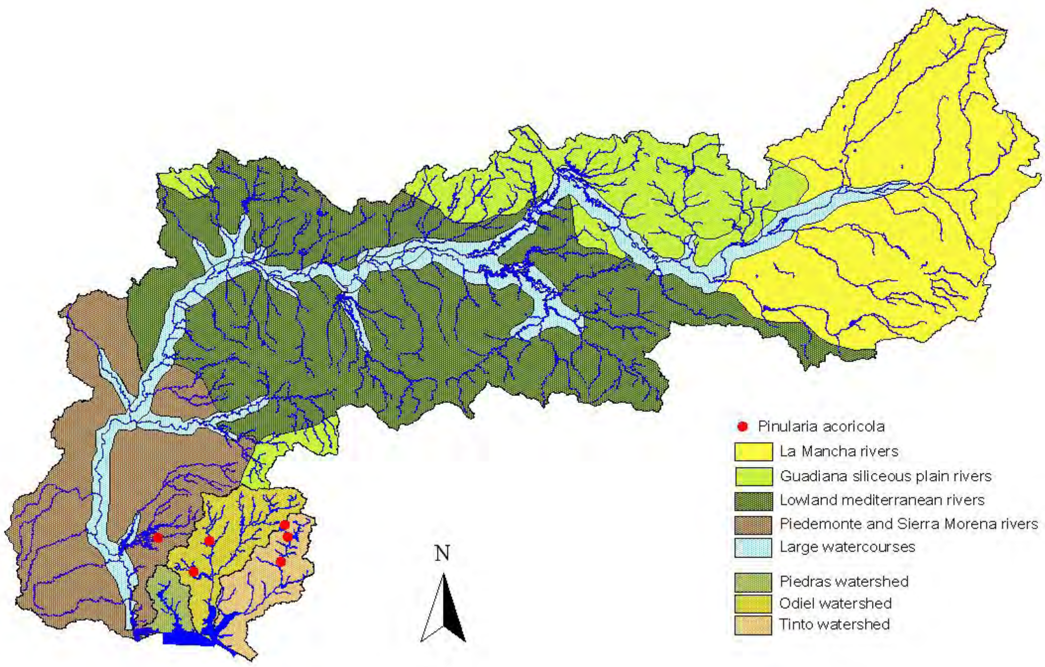
This taxon is associated to acidic streams around the world (DeNicola, 2000). It was described by Hustedt (1985) as common in sulphur springs in Java at pH around 3.0. It has also been reported in New Zealand hot springs at a pH less than 1 (Cassie and Cooper, 1989). Negoro (1985) found it to be widespread in a variety of highly acidic habitats in Japan from pH 2.0–4.0. Watanabe and Asai (1995) cite the occurrence of this taxon at pH values as low as 1.1 from a variety of naturally acidic sites.

P. acoricola has also been described as one of the few species capable to survive in acidic mine drainage where high levels of acidity are accompanied by high concentrations of nutrients and heavy metals (Whitton and Diaz, 1981). It has been reported from a variety of acid mine drainage sites in the Iberian Peninsula (Sabater*, et al.*, 2003a, de la Pena and Barreiro, 2008, Luís*, et al.*, 2008, Urrea and Sabater, 2009b).

Distribution and ecology in the Guadiana watershed:

n_{loc} =6	cond	temp	O2	pH	arti	cult	natu
MAX	2470.0	26.6	13.7	3.6	52.5	36.2	90.1
MIN	661.5	5.1	1.4	2.2	0.9	0.0	47.5
MEAN	1775.5	11.0	10.4	3.1	9.6	18.6	71.6
SD	641.8	7.9	4.6	0.5	21.0	14.8	16.3

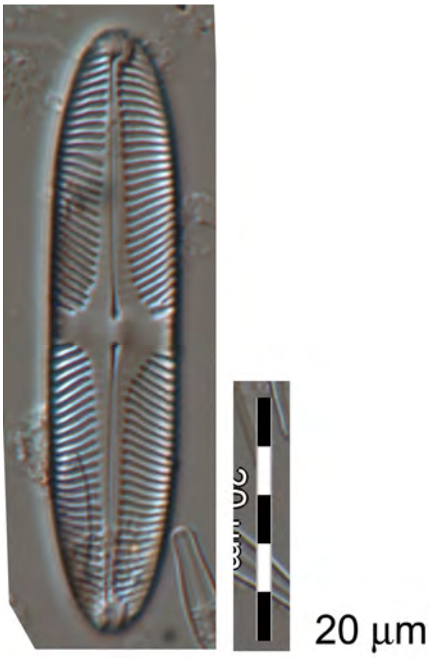
This taxon appeared exclusively in AMD affected waters, both in Tinto–Odiel system, and also in the Cobica tributary (Chanza subwatershed, in the lower Guadiana basin). They presented high conductivity waters (>1,000 µS/cm) and extremely low pH (≈3).



Pinnularia brebissonii (Kützing) Rabenhost [pbre]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=3)	49.6 − 51.2 (50.6 ± 0.9)	10.5 − 11.0 (10.7 ± 0.2)	13 − 14 (14 ± 1)



General distribution and ecology:

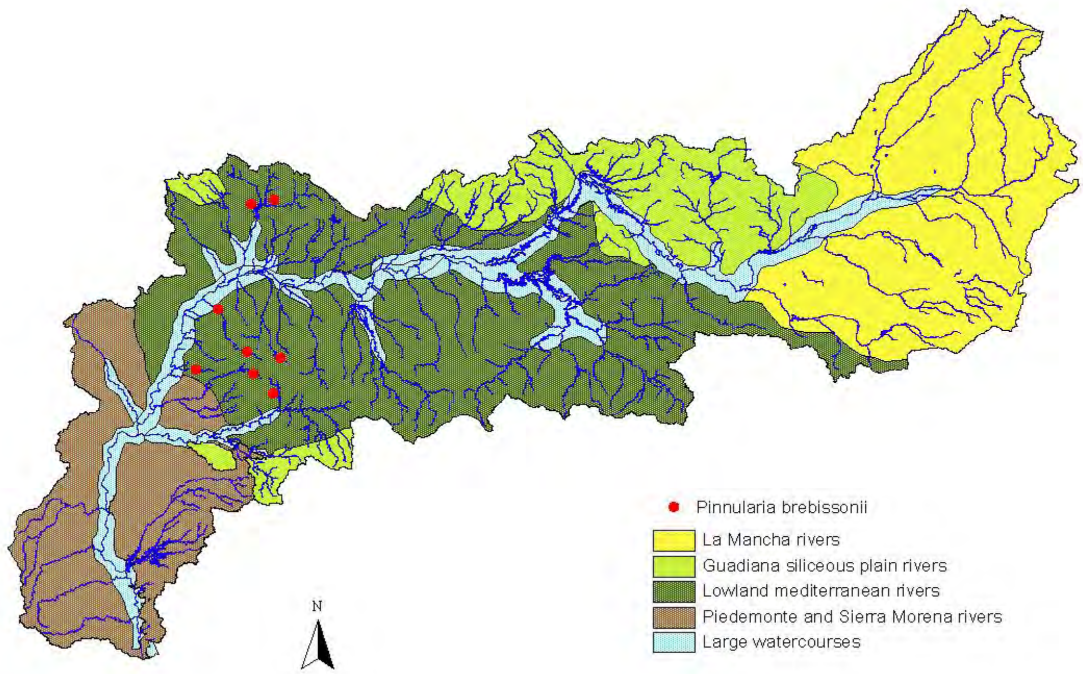
P. brebisonii is a cosmopolitan taxon inhabiting oligotrophic and cold waters (Patrick and Reimer, 1966) with moderate to high electrolyte content (Krammer and Lange–Bertalot, 1985).

It has been found in many waters form all over the world (Zong and Horton, 1999, Antoniades*, et al.*, 2004, Potapova*, et al.*, 2004, Salomoni*, et al.*, 2006). It has been reported in Pyrenean rivers (Margalef, 1948, Sabater and Roca, 1992, Gomà*, et al.*, 2005). It has also been found in numerous localities scattered around the Iberian Peninsula (Varela*, et al.*, 1991, Cambra, 1992a, Leira, 2005, Linares-Cuesta, *et al.*, 2007, Blanco, *et al.*, 2008, Luís, *et al.*, 2008, Tornés, 2009)

<u>Distribution and ecology in the Guadiana watershed:</u>

n=8	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	958.0	24.4	13.1	9.4	0.9	77.7	73.6	0.2	103.0	529.0	1.9	2.4	77.4	303.0
MIN	57.0	17.8	6.0	7.5	0.0	26.4	22.3	0.1	11.7	30.0	0.1	0.4	8.0	38.7
MEAN	462.8	21.0	9.7	8.2	0.2	58.5	41.3	0.1	44.7	92.4	0.8	0.8	44.9	193.5
SD	288.0	2.1	3.6	0.7	0.4	17.8	17.9	0.1	27.7	176.4	0.8	0.8	23.4	94.7

This taxon is not well represented in Guadiana watershed. It was found in siliceous sites with abundance not higher than 0.5%.



Pinnularia divergentissima (Grunow) Cleve [pdvg]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=7)	27.9 − 34.0 (30.2 ± 2.0)	5.1 − 5.3 (5.7 ± 0.2)	14 − 22 (17 ± 3)

Pinnularia divergentissima* cf. var. *subrostrata

General distribution and ecology:

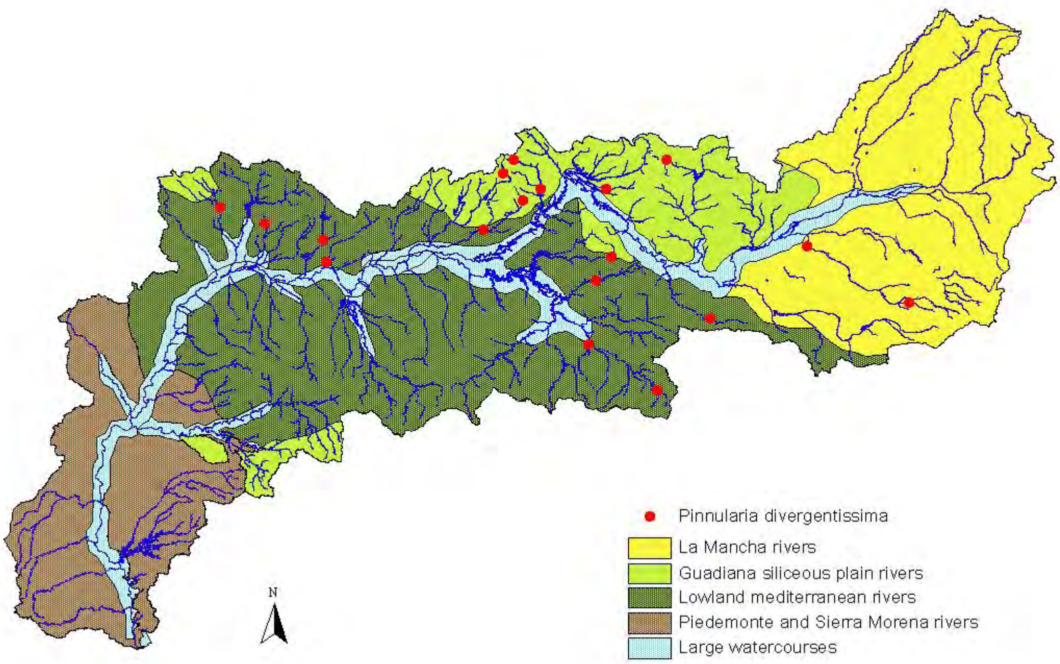
P. divergentissima is a cosmopolitan and widely distributed taxon frequent in freshwaters from all over the world (Weckström*, et al.*, 1997).

It has been cited as an epiphytic of macrophytes in some ponds in the north east of Iberian Peninsula (Cambra, 1991a, 1992b, Gómez, 1998) and epipelic in some biofilms in the Pyrenees (Massanell, 1966, Sabater and Roca, 1992) in the Mediterranean coast (Tomàs, 1988) as well as in some rivers in the West of the Peninsula (Varela, 1992, Almeida and Gil, 2001).

Distribution and ecology in the Guadiana watershed:

n_{loc} =39	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2610.0	29.6	11.6	9.7	2.6	92.9	100.0	3.3	834.0	866.0	5.3	26.4	177.4	257.5
MIN	38.0	2.2	1.4	6.8	0.0	0.0	6.8	0.0	2.3	4.6	0.0	0.4	2.1	15.0
MEAN	504.1	14.6	8.00	8.2	0.3	39.6	60.1	0.3	66.5	109.5	0.3	2.5	32.7	74.5
SD	634.9	8.3	1.9	0.6	0.5	24.2	24.3	0.8	168.7	215.9	1.1	6.1	47.3	56.8

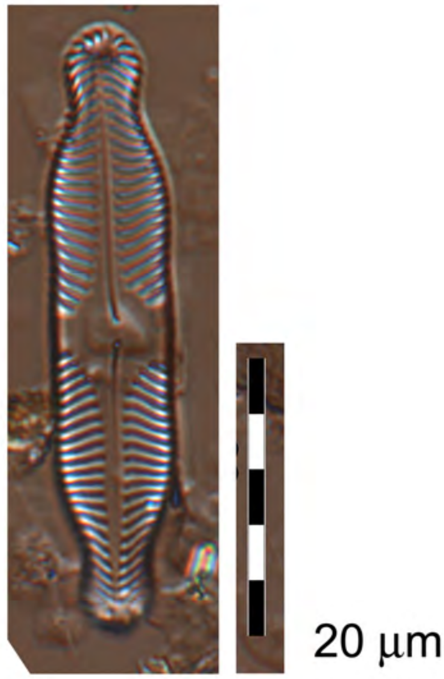
This taxon is not well represented in Guadiana watershed. Despite it was found in numerous localities, its relative abundance was never higher than 0.5%. Some specimens of our study should correspond to *P. divergentissima* var. *subrostrata*, but the low abundance of individuals has not allowed us to determine varieties.



Pinnularia lundii Hustedt [plun]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=4)	42.7 − 55.1 (49.0 ± 7.0)	8.7 − 10.1 (9.3 ± 0.6)	10 − 14 (12 ± 2)



Pinnularia lundii* cf. var. *linearis

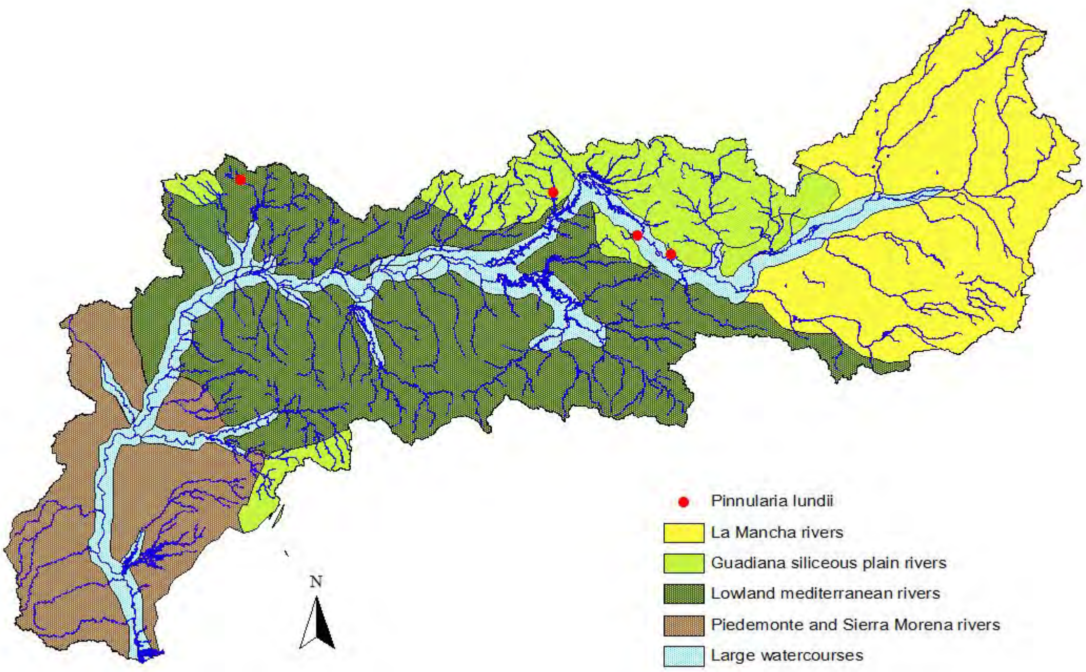
General distribution and ecology:

It has been reported in some alkaline waters from USA (Hill*, et al.*, 2003). It has also been found in some Mediterranean and semiarid streams from the Iberian Peninsula (Tomàs, 1988, Ros*, et al.*, 2009, Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n=4	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	397.0	26.8	10.7	8.4	0.2	36.7	91.4	0.2	35.9	30.0	0.1	2.1	8.0	53.3
MIN	89.0	17.0	8.7	7.2	0.0	8.6	63.2	0.0	9.0	9.0	0.0	0.4	2.7	15.0
MEAN	217.0	21.5	9.6	7.7	0.0	19.3	80.7	0.1	18.8	17.0	0.0	0.8	5.3	27.7
SD	134.4	4.2	0.9	0.6	0.1	13.2	13.3	0.1	11.9	10.1	0.0	0.9	2.2	18.1

This taxon is not well represented in Guadiana watershed. It was found in low proportion in small tributaries with high natural drainage area and oligotrophic waters. Some specimens of our study should correspond to *P. lundii* var. *linearis*, but the low abundance of individuals has not allowed us to determine varieties.



Pinnularia neomajor Kramer [pneo]

Taxonomical features:

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=3)	99.6 − 100.6 (100.2 ± 0.5)	18.0 − 18.3 (18.1 ± 0.2)	8 − 9 (9 ± 1)

General distribution and ecology:

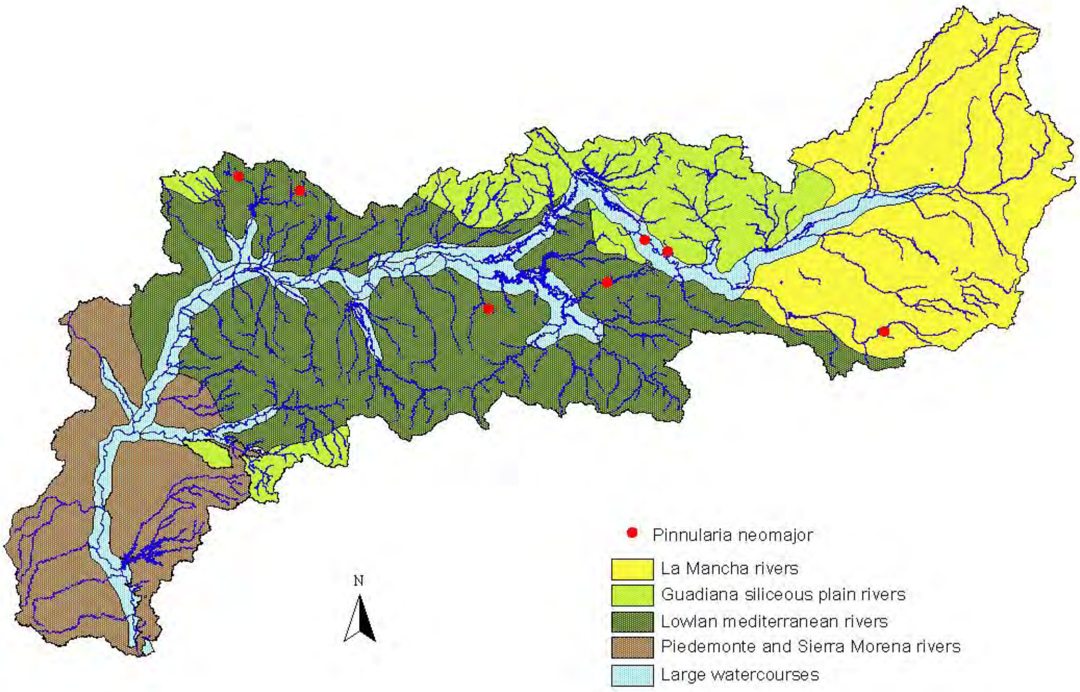
P. neomajor is recent taxa previously included in *P. maior* (Kützing) Rabenhorst. *P. maior* was described as a cosmopolitan taxon (Patrick and Reimer, 1966) frequent in upper section of rivers with low nutrient disturbances (Margalef, 1954, Sabater and Roca, 1992, Varela, 1992, Ndiritu*, et al.*, 2006). *P. neomajor* showed similar properties and ecological preferences.

It has been described in some lagoons of northern Peninsula (Leira, 1997).

Distribution and ecology in the Guadiana watershed:

n=8	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	3100.0	25.0	13.8	8.7	1.3	78.6	90.5	0.3	95.9	328.0	0.3	1.7	144.0	210.0
MIN	64.2	16.1	7.1	7.0	0.0	9.5	20.1	0.0	4.8	9.0	0.0	0.2	2.7	15.0
MEAN	706.4	20.6	10.0	7.8	0.2	43.8	56.0	0.1	41.5	65.8	0.1	0.7	34.5	86.0
SD	1037.2	3.3	2.1	0.5	0.5	25.0	25.3	0.1	33.8	107.7	0.1	0.6	47.9	75.6

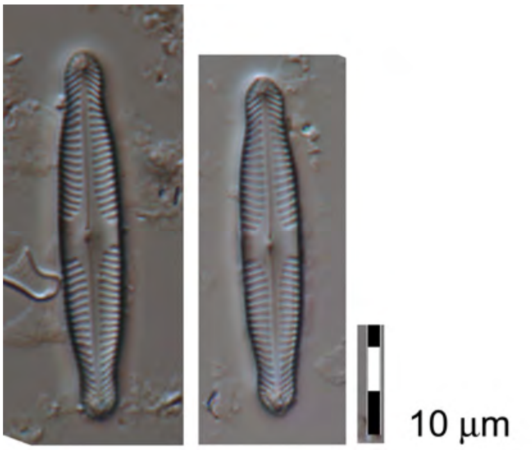
It has been found widespread along the watershed in samples with low nutrient content. Its frequency is very low, not higher than 0.5%.

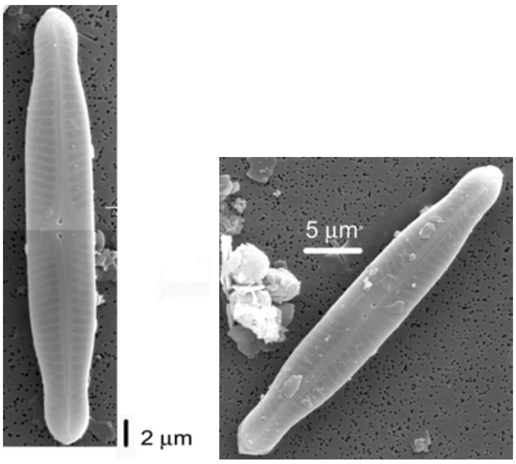


Pinnularia subcapitata Gregory [psca]

<u>Taxonomical features:</u>

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=10)	26.0 − 36.5 (31.3 ± 3.1)	4.3 − 6.1 (5.4 ± 0.4)	13 − 16 (14 ± 1)





General distribution and ecology:

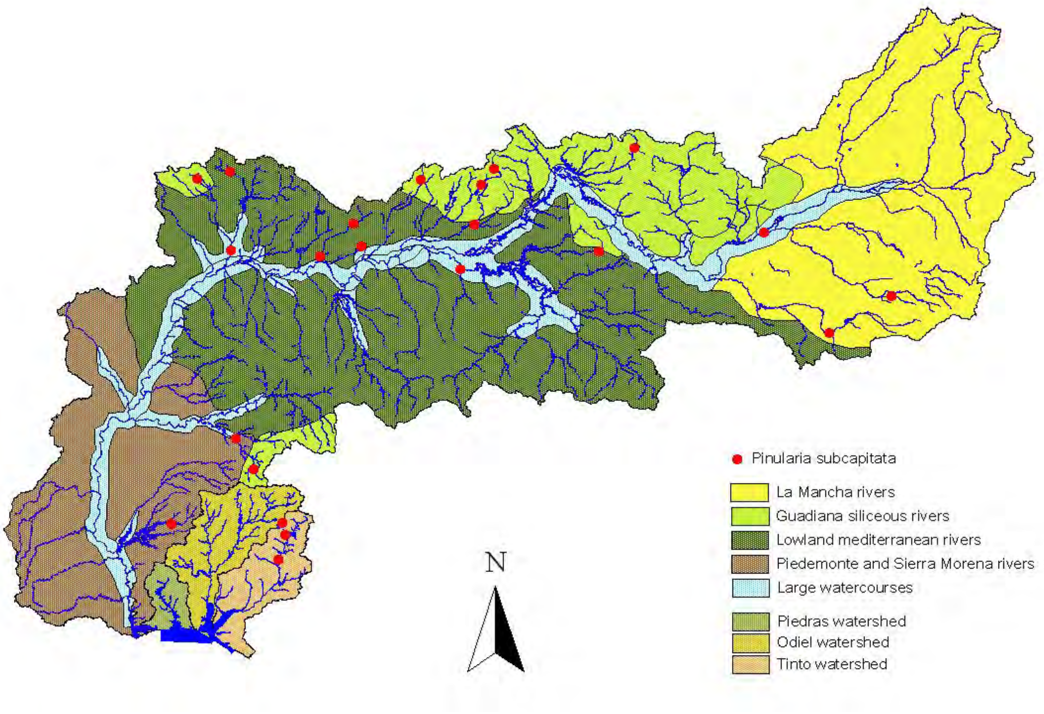
This taxon appears to be fairly widespread at pH values around 3.0, in sites receiving acid mine drainage, although it is not usually reported as abundant (Whitton and Diaz, 1981, Verb and Vis, 2000, Urrea and Sabater, 2009b).

It has been quoted in some Eastern (Margalef, 1955, Sabater and Roca, 1992, Gómez, 1998) and Western waters (Varela*, et al.*, 1991, Almeida and Gil, 2001) from the Iberian Peninsula.

Distribution and ecology in the Guadiana watershed:

n=23	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	3040.0	26.9	16.5	9.3	2.6	94.5	89.2	1.0	435.0	2380.0	1.8	9.4	773.0	452.0
MIN	40.4	6.3	4.3	6.4	0.0	10.8	4.7	0.0	2.0	9.0	0.0	0.2	2.5	15.0
MEAN	607.7	16.4	10.2	7.9	0.6	50.4	49.1	0.1	78.9	198.6	0.3	2.1	80.7	129.5
SD	761.0	6.5	3.6	0.7	0.8	23.9	23.9	0.2	111.4	567.9	0.4	3.1	180.3	129.9

This taxon appeared widespread along the watershed, in both siliceous and calcareous waters, and it seems to be indifferent to pH. In Tinto AMD affected localities we have found some isolated specimens of this taxon.



Pinnularia viridiformis Krammer [pvif]

Taxonomical features:

The dimensions of our specimens were larger than those described by Ciniglia*, et al.* (2007) in the revision of *Pinnularia* from acidic environments. However, they coincide with the size range described by Krammer (2000).

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=10)	70.2 − 107.1 (88.5 ± 17.9)	13.8 − 16.8 (15.0 ± 1.3)	8 − 10 (9 ± 1)

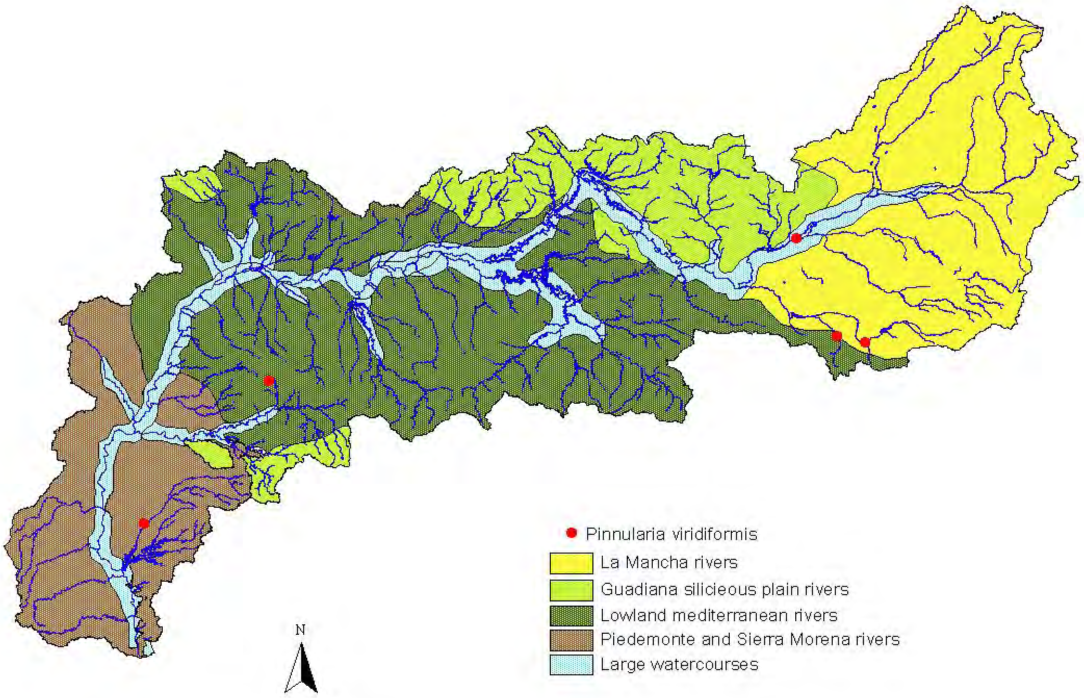
General distribution and ecology:

It has been described in the periphyton of shallow lakes of the northern part of Iberian Peninsula (Leira, 1997).

Distribution and ecology in the Guadiana watershed:

n=5	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	4140.0	24.7	16.5	10.6	1.4	85.1	73.9	0.8	270.2	1889.1	0.1	1.5	516.8	215.0
MIN	376.0	4.6	7.8	7.6	0.0	26.0	13.5	0.0	39.1	30.0	0.0	0.4	19.3	84.7
MEAN	1733.8	12.5	12.2	8.5	0.6	69.2	30.2	0.4	154.7	959.6	0.1	0.9	268.1	149.8
SD	1529.4	7.8	3.3	1.2	0.6	25.4	25.8	0.5	163.4	1314.6	0.0	0.7	351.8	92.1

This taxon is not well represented in Guadiana watershed. It was found only in several sites and its abundance was not higher than 0.5%.



Reimeria sinuata (Gregory) Kociolek & Stoermer and ***Reimeria uniseriata*** Guerrero & Ferrario complex

Taxonomical remarks:

The taxonomic study under the light microscope of *Reimeria* genus revealed some specimens with the following features:

a) uniseriate striae

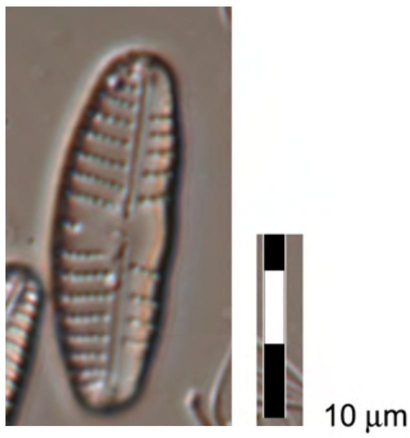
b)16–30 areolae in 10 µm

All this forms were placed under *R. uniseriata* following (Sala*, et al.*, 1993). The rarity and low abundance of this taxon has not allowed its study under SEM in order to determine the seriation of striae.

rsin	**L** (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=10)	11.4 − 15.5 (13.1 ± 1.1)	3.8– 5.5 (4.3 ± 0.5)	12 – 19 (14 ± 1)

runi	**L** (µm)	**W** (µm)	**Dorsal striae in 10 µm**	**Puncte in 10 µm**
LM (n_{in}=5)	16.4 − 21.5 (19.4 ± 2.0)	3.9– 5.3 (4.3 ± 0.5)	9 – 11 (10 ± 1)	17 – 25 (22 ± 3)

Reimeria sinuata



Reimeria uniseriata

General distribution and ecology:

Both taxa were widely distributed in the Duero (Álvarez–Blanco, 2009) and Ebro watersheds (Cambra*, et al.*, 2003). *R. uniseriata* has been also described in the North of Peninsula (Ortiz*, et al.*, 2005).

Distribution and ecology in the Guadiana watershed:

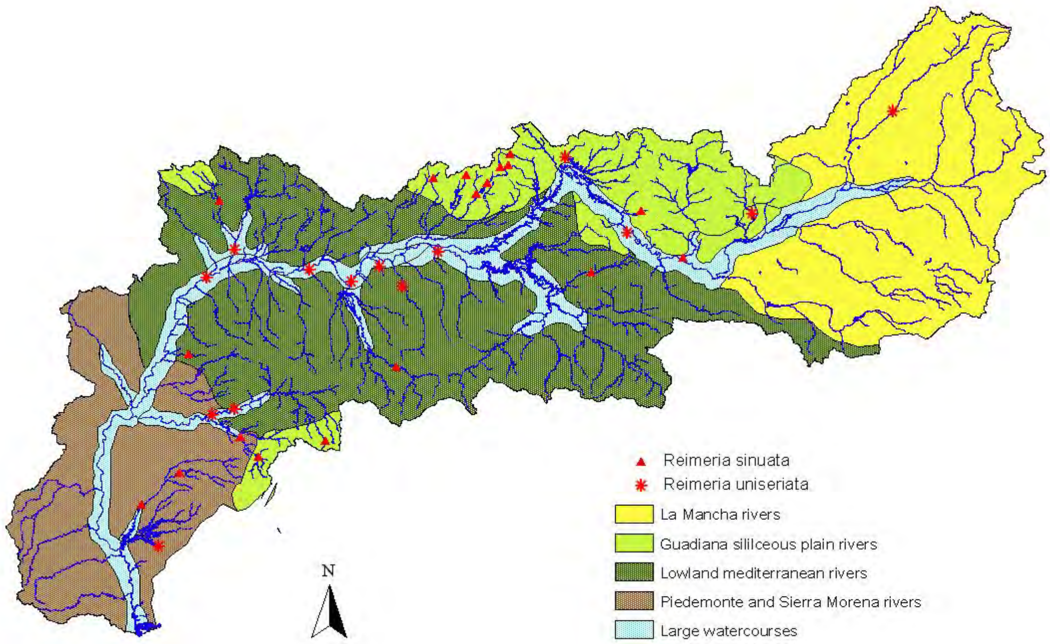
rsin

n=14	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	3100.0	25.4	11.9	9.1	1.4	79.8	98.7	2.7	126.0	1150.0	1.8	34.8	437.0	250.0
MIN	55.3	8.6	1.10	7.4	0.0	1.3	20.1	0.0	20.3	30.0	0.0	0.2	5.4	27.3
MEAN	892.7	19.0	8.8	8.4	0.6	58.5	40.9	0.4	72.3	193.7	0.4	5.5	80.2	147.5
SD	848.2	5.5	2.8	0.5	0.6	23.4	23.7	0.8	33.4	343.7	0.6	10.2	114.9	58.0

runi

n=21	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2500.0	29.6	11.8	9.1	2.6	87.9	86.4	0.9	124.0	184.0	0.4	2.7	51.6	236.0
MIN	40.4	8.9	4.4	6.8	0.0	13.6	11.8	0.0	2.0	20.0	0.0	0.2	2.5	15.0
MEAN	401.6	19.4	8.8	7.8	0.5	43.7	55.9	0.1	38.3	37.2	0.1	0.8	23.1	87.0
SD	533.2	5.0	1.6	0.7	0.7	21.6	21.6	0.2	32.5	34.8	0.1	0.7	16.0	56.8

R. sinuata is present in sites less eutrophized than *R. uniseriata* does in the Guadiana watershed. *R. sinuata* is associated to upper part of streams while *R. uniseriata* appeared near main courses in middle and lower part of streams.



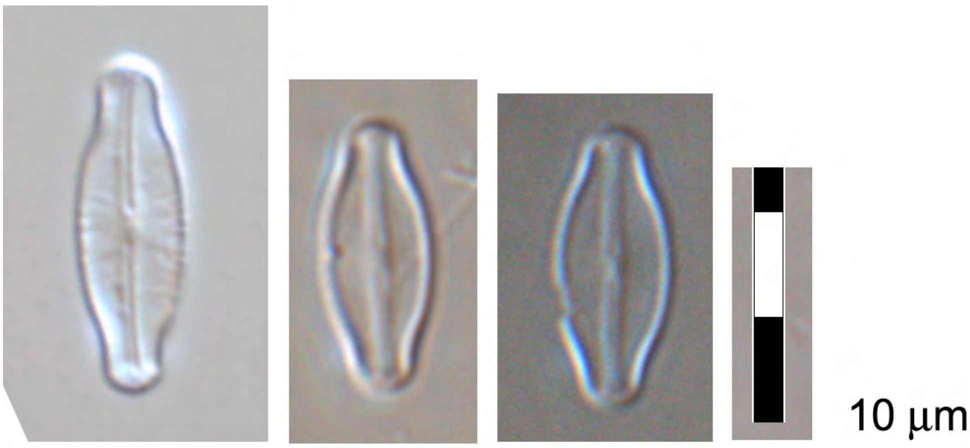
***Sellaphora* sp.1** [sesp1]

Taxonomical remarks:

Lanceolate valves with a conspicuous central area where striae are strongly radiate and considerably shortened and widely spaced. Included in this taxon are all forms of lanceolate shape related to *Navicula minuscula* Grunow in Van Heurck, 1880 group. However, they differ with respect to broadly capitated and rounded ends.

Striation density cannot be solved under LM. Ultrastructure of our individuals was not studied resulting from the low abundance of that taxon in the samples. A SEM revision of this taxon will allow determining the specific belongings of the taxon in question.

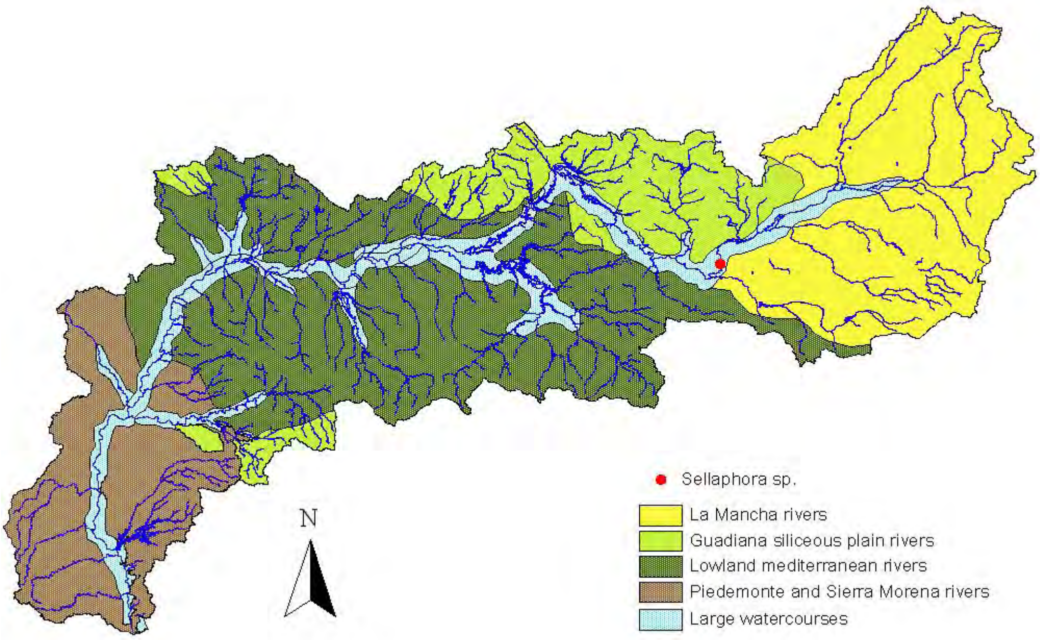
	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM (n_{in}=5)	12.0 − 15.2 (13.3 ± 1.3)	4.1 − 4.9 (4.4 ± 0.3)	–



Distribution and ecology in the Guadiana watershed:

n_{loc}=3	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2010.0	17.0	–	7.9	1.4	83.6	15.0	19.3	126.0	258.0	6.7	7.3	105.0	408.0
MIN	1384.0	7.6	–	7.7	1.4	83.6	15.0	1.6	120.0	161.0	5.5	0.4	101.0	388.0
MEAN	1678.3	11.0	2.7	7.8	1.4	83.6	15.0	13.4	124.0	225.7	5.9	5.0	103.7	394.7
SD	314.7	5.2	–	0.1	0.0	0.0	0.0	10.2	3.5	56.0	0.7	4.0	2.3	11.5

It has been found in only one site during the tree times it has been visited. Its maximum abundance was recorded in winter 2006. It is a site that receives waste water from sewage treatment plant and the waters are affected by high organic pollution.

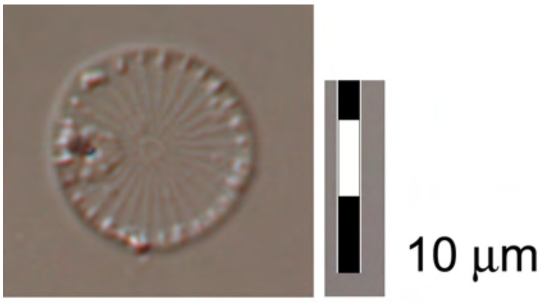


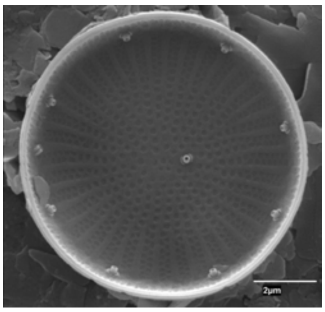
Stephanodiscus hantzschii Grunow in Cleve & Grunow [shan]

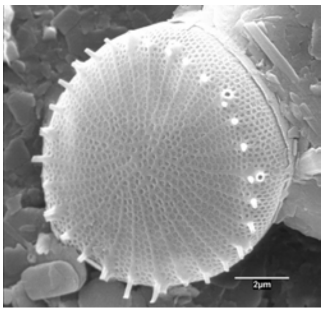
Taxonomical remarks:

There are several infraspecific taxa defined in the literature. These have been grouped under the nominate variety which coincide with the general description given by Krammer and Lange–Bertalot (1991a). The valves are circular, typically less than 15 µm in diameter with uniseriate puncta in the central region, becoming biseriate near the margin (SEM). It presents usually long spines, occurring at the end of every interfascicle. In most cases, there is a rosette of puncta, surrounded by a circular hyaline area in the centre of the valve.

	Diameter	**Striae in 10 µm**
LM (n_{in}=11)	6.1 − 11.1 (10.3 ± 1.4)	8 − 10 (9 ± 1)







General distribution and ecology:

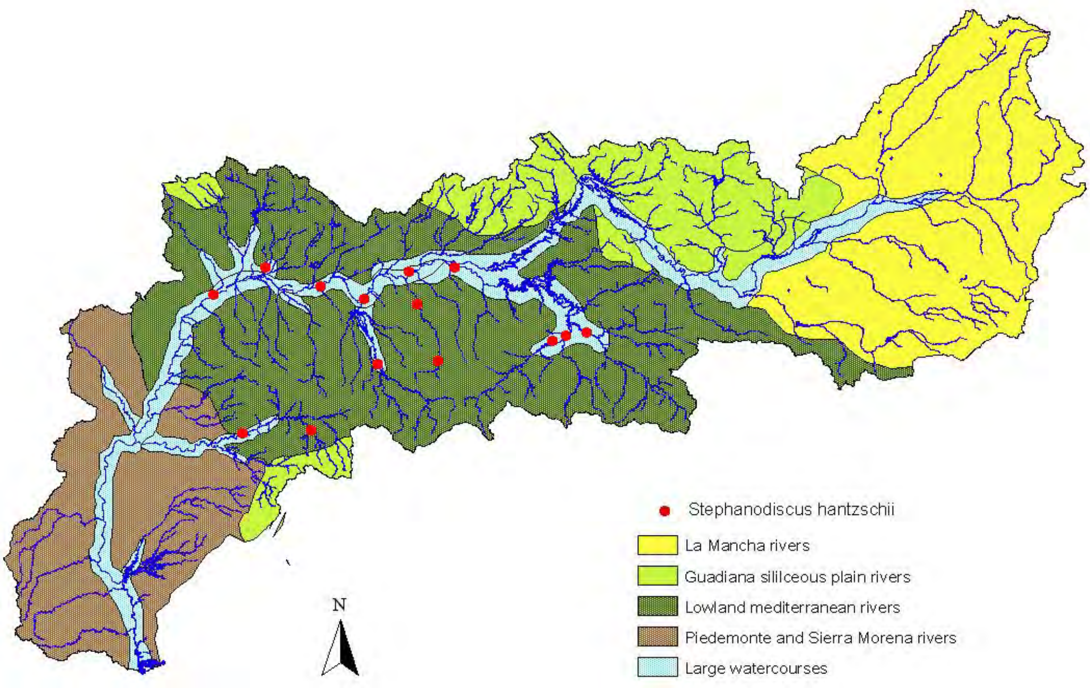
S. *hantzschii* is a planktonic and alcaliphilous taxon reported both in fresh and brackish waters (Hustedt, 1985). It is frequently inhabiting in the lower sites of the rivers supporting hypertrophic conditions associated to agricultural practices (Leland and Porter, 2000, Navarro*, et al.*, 2002).

It has been found in some ponds along Mediterranean coast of Iberian Peninsula (Vicente*, et al.*, 1984, Soria-García*, et al.*, 1987, Aboal*, et al.*, 1998, Saint-Martin*, et al.*, 2001) and in some reservoirs of the Mediterranean Iberian Peninsula (Margalef, 1956a, Sabater, 1987). It has also been reported in the lower part of some Iberian rivers (Sabater*, et al.*, 1987, Gomà*, et al.*, 2003, Álvarez–Blanco, 2009, Tornés, 2009).

Distribution and ecology in the Guadiana watershed:

n=19	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	1076.0	26.5	13.1	9.5	1.4	85.3	37.9	2.7	118.0	584.0	1.8	2.9	59.0	233.0
MIN	54.0	5.3	2.7	8.1	0.0	61.3	14.5	0.0	36.9	30.0	0.0	0.1	27.8	104.0
MEAN	682.1	12.5	8.5	8.7	0.8	71.1	28.1	0.6	59.9	103.5	0.6	0.9	42.7	162.4
SD	263.6	6.9	2.3	0.4	0.5	5.7	5.5	0.9	28.5	194.3	0.7	1.0	11.1	55.7

This taxon appeared widespread through the watershed in sites with low flow, both in winter and spring. In the present study, its maximum abundance was recorded in tail waters and in the lower parts of the main river sites where water flows quietly.

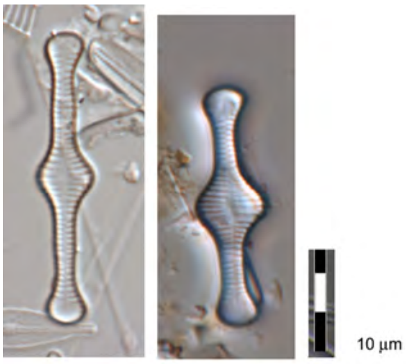


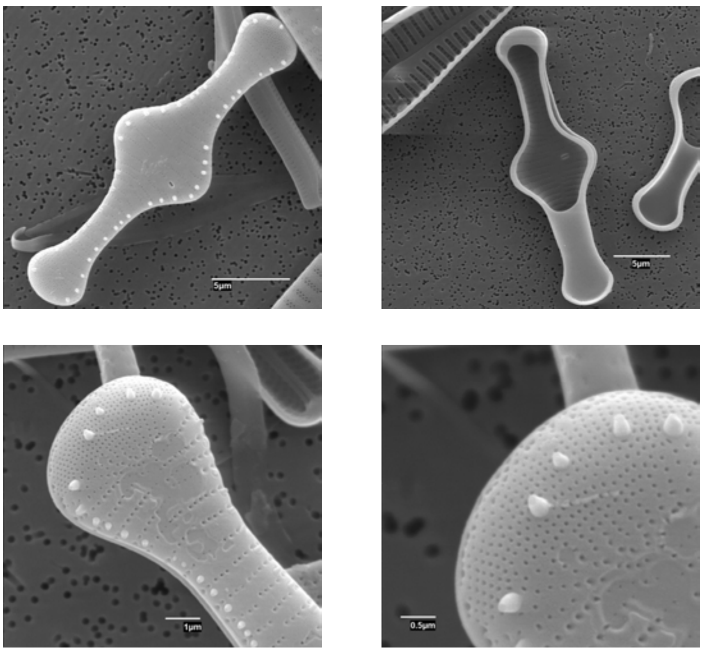
Tabellaria flocculosa (Roth) Kützing complex [tflo]

Taxonomical remarks:

In this work I have considered all forms of *Tabellaria* as *T. flocculosa.* Distinction between *T. fenestrata*, *T. flocculosa* and *T. flocculosa* varieties is difficult (Knudson, 1952, 1953a, 1953b), and some researchers have considered these species to be end–members of a single morphological series (Beyens, 1982, Kelly*, et al.*, 2005).

	L (µm)	**W** (µm)	**Dorsal striae in 10 µm**
LM n_{in}=8)	18.5 − 27.7 (23.9 ± 3.3)	6.5 − 9.0 (7.9 ± 0.9)	17− 20 (18 ± 1)





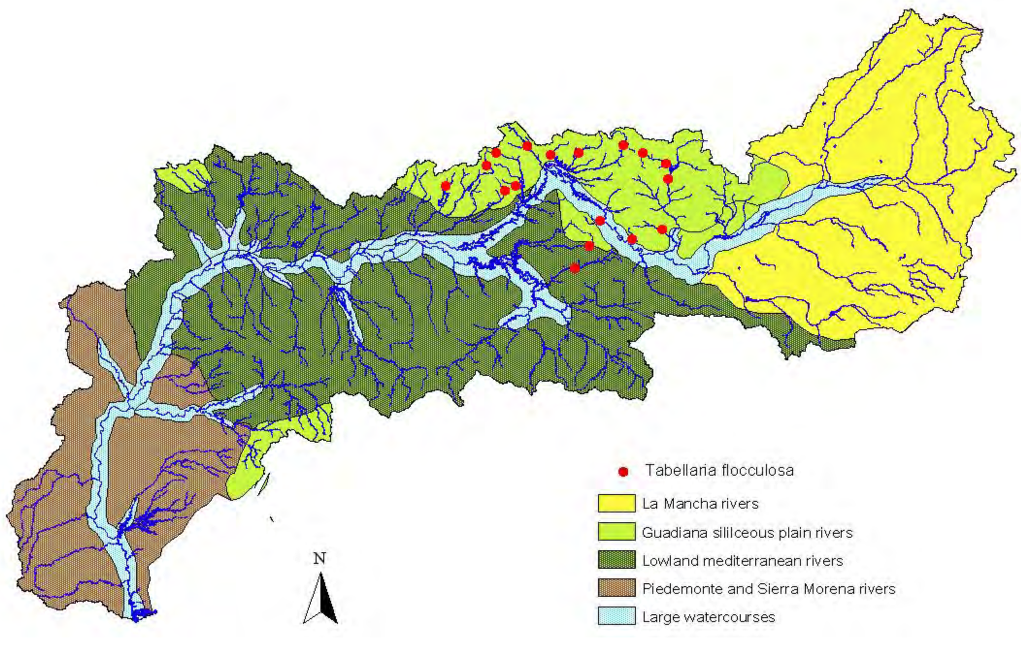
General distribution and ecology:

It is frequently reported in Iberian waters (Margalef, 1956a, Cambra, 1992b, Sabater and Roca, 1992, Ubierna and Sánchez–Castillo, 1992, Varela, 1992, Almeida and Gil, 2001, Álvarez–Blanco, 2009) and seems to be tolerant to high nutrient content.

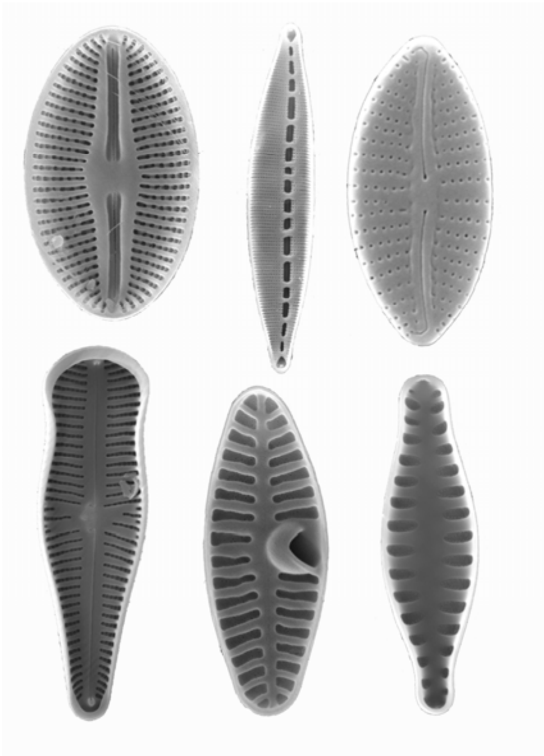
Distribution and ecology in the Guadiana watershed:

n=38	cond	temp	O2	pH	arti	cult	natu	NH4	Cl	SO4	PO4	NO3	Ca	alka
MAX	2300.0	28.1	13.0	9.3	2.9	78.8	100.0	26.4	87.7	1090.0	1.5	16.8	498.0	188.0
MIN	32.1	4.1	1.4	6.4	0.0	0.0	21.1	0.0	2.5	2.2	0.0	0.2	2.1	5.5
MEAN	223.4	15.6	9.5	7.8	0.3	30.8	68.9	1.1	19.9	52.0	0.1	2.7	27.5	47.6
SD	382.6	6.8	2.2	0.5	0.7	22.5	22.6	4.7	19.3	190.1	0.3	4.3	87.0	43.6

This taxon occurred in the upper siliceous tributaries. Its highest abundance was recorded in sites with natural land uses, low conductivities (<200µS) and low nutrient content.



CHAPTER V



EPILITHIC DIATOM ASSEMBLAGES AND THEIR RELATIONSHIP TO ENVIRONMENTAL CHARACTERISTICS IN THE GUADIANA WATERSHED

5.1. INTRODUCTION

Diatom assemblages have been widely related to specific environmental conditions in different geographical regions (Soininen, 2002, Martínez de Fabricius*, et al.*, 2003, Ndiritu*, et al.*, 2006). They differ both in their composition and relative abundance due to their ecological affinities and preferences (Potapova and Charles, 2003, Tornés*, et al.*, 2007). This variation is expressed spatially and temporally, based on the climate, geological settings, water chemistry and geomorphological conditions (Stevenson and Pan, 1999). The sensitivity of diatom communities has led them to be used as indicators of environmental conditions, such as water quality and habitat conditions in stream and river systems (Pan*, et al.*, 1999, Soininen*, et al.*, 2004). One of the major aims of the European Water Framework Directive (European Parliament, 2000, directive 2000/60/EC) is to evaluate deviations from the highest ecological quality expected in the absence of human induced stress, and subsequently maintain or restore the ecological status of aquatic ecosystems. This evaluation is based on applying specific metrics that relate biotic changes to alterations in the environment. However, these metrics need to be based on autoecological observations in different ecoregions in order to be reliable.

Although many studies have focused on the effects of human pressure on diatom communities (Descy and Ector, 1999, Potapova and Charles, 2003, Philibert*, et al.*, 2006), few have been developed in large geographic areas (Sabater and Roca, 1992, Potapova and Charles, 2002, Tison*, et al.*, 2005). Characterizing diatom assemblages of large watersheds requires considering a large number of sites; only with a large number of samples is possible to account for the multiplicity of situations that are included in a large area and that can shift with time. Multivariate statistical methods need to be applied in these situations to identify patterns in community ordination across the watershed (Soininen*, et al.*, 2004, Leira and Sabater, 2005, Pan*, et al.*, 2006). Multivariate techniques may help to separate the respective influences of the physicochemical and the morphometric factors on diatom distribution. The main purpose of this study is to determine how benthic diatom assemblages reflect the main environmental gradients that occur throughout the different tributaries and sections of a largely agricultural basin, the Guadiana.

The hydrology and the enduring human impacts determine large spatial variations in the flow regime, water chemistry, riparian vegetation and biological communities in the Guadiana watershed. The hydrology is related to the porosity of its geological substrata and the moderate rainfall in the upper part of its catchment, where groundwater plays an important role in the river flow (Sabater, *et al.*, 2009). The middle and lower parts of the watershed run on impermeable bedrock and depend on scarce and irregular rainfall. This spatial asymmetry provides the system with shifting environmental characteristics.

Identifying how diatoms can reflect the complexity of this system (which includes natural patterns and human influences) can shed light on the ecological state of geographical areas of similar complexity.

5.2. DATA ANALYSIS

Species richness (S) and diversity (H') (Shannon and Weaver, 1963) were calculated from the taxonomic composition of diatom samples. Relationships between diatom diversity and the environmental variables were analyzed by means of Pearson correlation using SPSS v.15 (SPSS-Inc., 2004).

An exploratory analysis of the available environmental data in the watershed was performed using principal component analysis (PCA). The environmental similarity between sampling sites was tested using the ANOSIM routine (Clarke, 1993), which tests *a priori* grouping designations against 1,000 random group designations in ordination space. Both analyses were performed using PRIMER–6 (Clarke and Gorley, 2005). Differences in chemical parameters between the two parts of the catchments area were analyzed thorough a one−way ANOVA analysis using SPSS v.15 (SPSS-Inc., 2004).

Patterns in diatom community composition were examined using canonical ordination techniques. Preliminary detrended correspondence analysis, DCA (Hill and Gauch, 1980) on the species data, revealed that the gradients lengths were greater than 2 standard deviation units (3.13 and 2.38 for the first two axis), indicating an unimodal response, which justified the use of unimodal ordination techniques (Leps and Smilauer, 2003). Consequently, a correspondence analysis (CA), which is an indirect–gradient ordination, was used to determine the major patterns of variation in the taxa without incorporating environmental variables. Since the sampling data were distributed between two different seasons and years, time was considered as a categorical co–variable in order to avoid the effect of seasonal differences between the two study periods (Tornés*, et al.*, 2007).

Constrained ordination was used to relate the structure of the diatom assemblage to predictor environmental variables, and to explore the relationships among and between species and the environment. A canonical correspondence analysis (CCA) was used for this purpose (Ter Braak and Verdonschot, 1995). Step–wise forward selection and Monte Carlo permutation test (999 permutations, $p<0.01$) were used to reduce further the environmental variables to those correlated significantly with the derived axes. Finally, partial CCAs were used to separate and examine the relative importance of the diatom community assemblages of several sets of explanatory variables (Borcard*, et al.*, 1992). We were interested in separating the effects of physical and chemical factors from physiographic factors and then testing whether these two different groups of variables were redundant to each other, or whether they each explained unique aspects of species composition.

Only those taxa with relative abundances greater than 1.5% at 5 sites or more were included in the analysis so as to minimize the influence of rare taxa. A total of 96 taxa were therefore selected. All data were scrutinized for normality, and some transformations were used in order to achieve the homogeneity of variances. A square root transformation was applied to diatom data rather than a log transformation, since it was desirable to retain zero values. Environmental variables, except for pH and percentage variables were also transformed. Hydraulic measurements, IHF and chemical concentrations were transformed by $\log_{10}(x+1)$ and calcium and ammonia concentrations were transformed by means of natural logarithms. The ordination analyses were performed using CANOCO version 4.5 (Ter Braak and Smilauer, 1998).

5.3. RESULTS

5.3.1. CHEMICAL CHARACTERISTICS

Water conductivity and alkalinity were higher in the upper part of the catchment due to the high content of calcium and sulphate ions. Conductivities at the Záncara and Gigüela could reach 3,000 μS/cm. Water conductivity decreased downstream due to inputs from poorly mineralized springs and water outflows from the reservoirs. Nitrate concentration was high, especially in winter as a result of increased runoff. River morphology in this part of the basin was highly modified, and values of river habitat condition were low, especially in the Záncara watershed. In this tributary, the river channel is reduced to a small watercourse flowing through cultivated fields.

The middle and lower part of the catchment have siliceous bedrock. In this section, calcium and sulphate concentrations, as well as alkalinity values, are lower. Conductivity had values near 500 μS/cm. Below wastewater treatment plants these values could rise up to 2,000 μS/cm, and ammonia and soluble reactive phosphorus (SRP) concentrations were also high (more than 2 mg/L or 15 mg/L respectively) (Table 5.1).

Table 5.1. Summary of selected variables with maximum, minimum and mean in the entire watershed, and mean values in calcareous and siliceous subcatchemnts. Results of one−way ANOVA between the two subcatchment areas are indicated

	Range	Entire w. Mean	Calcareous (n = 101)	Siliceous (n = 345)	F (n=446)	*p*
Conductivity (mS/cm)	39.00–3610	701.91	1561.79	537.47	149.41	<0.01
pH	2.75 – 9.75	7.98	8.01	8.12		n.s
Oxygen (mg/L)	1.10 – 12.35	9.83	7.56	8.67	10.31	<0.01
Temperature (°C)	4.00 – 26.90	17.95	12.34	19.03	60.95	<0.01
Ammonia (mg/L)	0.02 – 29.90	0.85	3.41	1.05		n.s
Chloride (mg/L)	2.29 834.00	61.72	104.08	55.53		n.s
Sulfate (mg/L)	5.75 – 2134.00	151.91	527.65	83.10	55.60	<0.01
Soluble Reactive Phosphorus (mg/L)	0.02 – 23.20	0.74	0.58	0.90		n.s
Nitrate (mg/L)	0.05 – 53.01	5.49	15.87	3.51	79.52	<0.01
Calcium (mg/L)	2.10 – 644.9	68.69	219.85	38.13	142.69	<0.01
Alkalinity (mg $CaCO_3$/L)	10.25 – 541.00	159.08	251.58	143.02	29.97	<0.01
Flow (m^3/s)	0.00 – 5.36	0.29	0.19	0.25		n.s
Wet width (m)	0.08 – 97.33	8.72	5.57	7.94	3.76	<0.05
Wet Perimeter (m)	0.79 – 368057	0.25	5.39	9.78		n.s
Hydraulic Radius (m)	1.28 – 21.9	0.27	19.18	23.76		n.s
Maximum Depth (cm)	4 – 188	0.36	3.2	3.3		n.s
River Index Habitat (IHF)	20 – 80	60.02	53.25	61.31	16.82	<0.01
% Urban land use	0.00 – 5.21	0.31	0.39	0.28		n.s
% Industrial land use	0.00 – 0.40	0.01	0.01	0.01		n.s
% Mining land use	0.00 – 0.73	0.02	0.00	0.01		n.s
% Cultivated land use	0.00 – 99.02	58.42	77.79	55.41	26.28	<0.01
% Forested land use	0.37 – 100.00	40.98	21.58	43.99	25.99	<0.01
% Water Bodies land use	0.00 – 7.60	0.22	0.22	0.16		n.s
Drainage Area (km^2)	0.06 – 49690.44	2596.30	2343.48	2027.89		n.s
Distance to the source (km)	5.34 – 685.39	69.12	76.93	56.90		n.s
Altitude (a.s.l)	80 – 984	429.35	745.40	368.92	273.10	<0.01

The PCA carried out with the physical and chemical variables separated localities of calcareous bedrock from those of siliceous bedrock (PC1, 25.9% of the variance). The analysis of similarities (ANOSIM) between these two groups of sites revealed a global R=0.327, $p<0.001$ (greater than 0.25) confirming significant differences between the two groups. The second axis of the PCA (PC2, 11.9% of the variance) was related to differences in nutrient concentrations. Localities with a high content of ammonia and SRP were pooled in opposition to upland localities (not shown).

5.3.2. DIATOM SPECIES RICHNESS AND DIVERSITY

A total of 278 diatom taxa were identified, from these, 12 were exclusive from the calcareous part, while 96 were present only in the siliceous subcatchment. Species richness varied from 6 to 69 in the analyzed set of samples. The Shannon–Wiener diversity index ranged from 0.39 to 5.16 (average 3.2). Correlation (Pearson's *r*) between biological (S and H') and physico−chemical parameters showed that species richness increased with conductivity and percentage of cultivated soil use and decreased in forested and cold headwaters. Species diversity increased in sites with permanent water flow and steady hydrological conditions (higher hydraulic radius and water depth), while it decreased in urbanized areas with high SRP content (Table 5.2).

Table 5.2. Pearson correlation between H', species richness and the environmental variables measured. Only significant correlation at level $p < 0.01$ are shown

	S (species richness)		H' (diversity)	
		r		r
positive correlation	Nitrates	0.29	Hydraulic Radius	0.16
	Calcium	0.26	Maximum depth	0.15
	Conductivity	0.24		
	Alkalinity	0.18		
	Sulfates	0.18		
	Cultivated soil use	0.17		
	pH	0.15		
negative correlation	Temperature	–0.57	Altitude	–0.20
	Forested soil use	–0.17	Urban soil use	–0.16
			SRP	–0.16

5.3.3. DIATOM DISTRIBUTION IN THE GUADIANA WATERSHED

CCA analyses included 252 stations, 88 taxa and 26 environmental variables. The step–wise forward selection and Monte Carlo Permutation test ($p<0.01$) identified 10 environmental variables that explained a significant proportion of the variance in the diatom species assemblages (conductivity, SRP, nitrates, alkalinity, temperature, IHF, drainage area, percent of cultivated soil, altitude and geology; Table 5.3, Figure 5.1B). The first two CCA axes had eigenvalues of 0.16 and 0.07 respectively. Species–environmental correlations were higher than 0.79 for both axes, and explained collectively 56% of the species–environment variation. The first CCA axis summarized the distribution of the diatom communities throughout an eutrophication gradient. Localities in forested and shaded sites in mountain areas that were scarcely influenced by human activities were on one extreme of the gradient. *Brachysira vitrea*, *Eunotia bilunaris* and *Tabellaria flocculosa* were associated with this situation. All these taxa are sensitive to organic pollution and they occurred in waters with low conductivity rates. On the other extreme of the gradient were localities in agricultural, industrialized or urbanized sites, situated in lowlands with large drainage areas. Taxa associated with this situation were *Nitzschia amphibioides*, *Nitzschia capitellata* and *Nitzschia umbonata*. All of them are α–saprobic to polysaprobic taxa, and therefore are tolerant to organic pollution (Van Dam*, et al.*, 1994). The second CCA axis was related to the altitude and geology of the sites. This axis separated localities with calcareous bedrocks and high conductivity and pH, from those with siliceous bedrock and low conductivity and pH. In the calcareous part, there appeared alkaliphilous taxa like *Cymbella affinis*, *Cymbella cymbiformis* and *Diatoma moniliformis*. While acidophilous taxa like *Cyclotella atomus* and *Eunotia bilunaris* were characteristic of the siliceous part (Figure 5.2.a). These results are consistent with those performed in the previous CA analysis (unexplained).

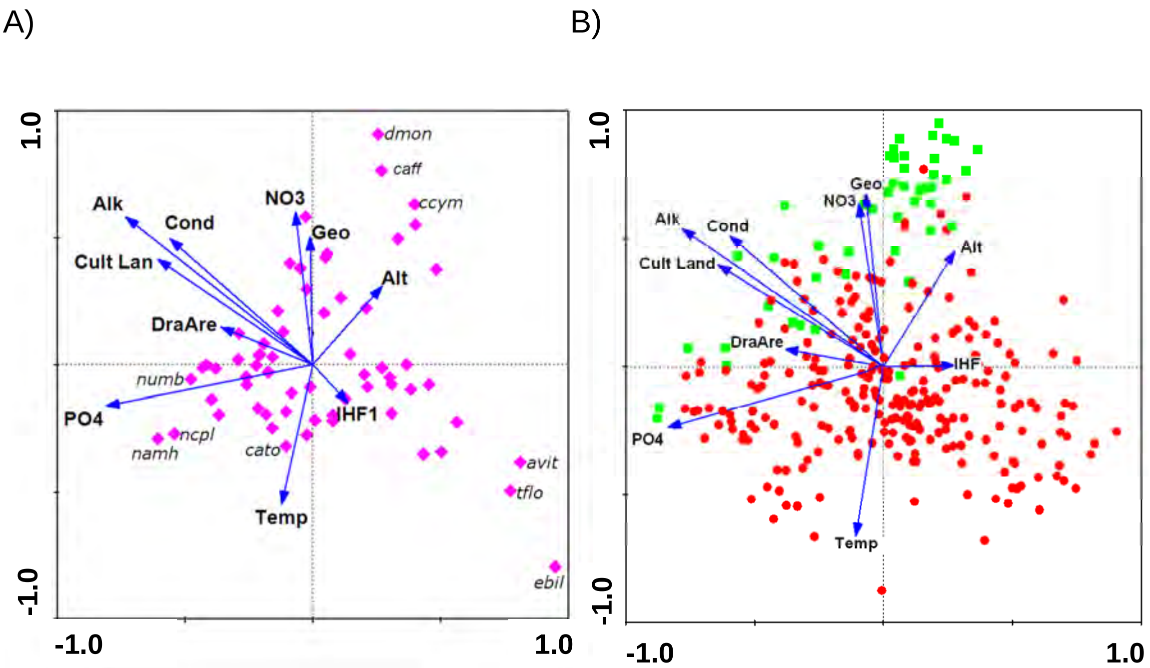


Figure 5.1. Distribution of the diatom species (a) and sampling sites (b) in an ordination diagram with the two first axes of the Canonical Correspondence Analysis (CCA). Independent variables detected as being significant by the analysis are indicated with arrows. Abbreviations of variables were listed in chapter IV. For clarity the plot represents only the diatom taxa with relative abundances higher than 5%. Species codes were listed in chapter IV.

The squares in (b) correspond to sites with hard water sites (calcareous bedrocks) while circles correspond to soft water sites (siliceous bedrocks)

The relative influence on the structure of diatom communities of water physical–chemistry *versus* physiography was analyzed by means of variance partitioning (partial CCA). Partial CCA analyses showed that physical–chemical variables explained a higher proportion (11.3%) of the variance, while physiographic factors explained 6.1% and the shared variance was only 1.4%.

Table 5.3. Summary of CCA analyses in the whole catchment, in the calcareous and siliceous subcatchment areas. Only environmental factors significantly involved in the distribution of diatom species are indicated. (sp–env = %variance of species–environment relationship; PQ = Physical−chemical factors; PG = Physiografical factors; alt = altitude (m asl); lat = latitude; geo = geology; the rest of the abbreviations were listed in chapter IV)

Whole watershed		Calcareous subcatchment		Siliceous subcatchemnt	
56.1 % sp–env		59.3 sp–env		57.9 sp–env	
PQ	PG	PQ	PG	PQ	PG
cond	DrAre	SRP	DrAre	cond	DrAre
SRP	cult	Cl^-	cult	SRP	cult
NO3	alt	pH	lat	NO3	alt
alk	geo	Flow		Cl^-	lat
temp				alk	
IHF				temp	
				IHF	

5.3.4. DIATOM DISTRIBUTION IN THE TWO SECTIONS OF THE CATCHMENT AREA

The multivariate analyses were carried out separately for the two main groups of sites identified by the previous PCA and confirmed by ANOSIM.

A first multivariate analysis (CCA) was carried out with the calcareous part of the catchment and included a subset of 50 localities, 55 species and 19 environmental variables. Seven environmental factors were associated with the distribution of diatom communities (Table 5.3). Partial CCA revealed that the two sets of predictor variables (physical–chemical and physiographical) captured a substantial portion of diatom variance (47.74%) that was distributed as 24% physical–chemical, 16% physiographic and 7.7% shared. The most relevant physical–chemical factors related to the diatom distribution in this section of the watershed were SRP and chlorides. *Craticula accomoda* and *Nitzschia umbonata* were positively influenced by these ions, while *Fragilaria capucina gracilis* avoided phosphate and chloride rich areas. In contrast, *Navicula gregaria* and *Planothidium frequentissimum* were mainly influenced by physiographic factors such as drainage area or percentage of cultivated soil (Table 5.4).

Table 5.4. Summary of the Partial CCA analyses carried out for the whole catchment, for the calcareous subcatchment and for the siliceous subcatchment. Diatom taxa significantly related to the physico−chemical and physiographical factors are presented

Physical−chemical factors	Physiografical factors
Whole watershed	
Physical−chemical factors (11.3% explained variance)	Physiografical factors (6.1 explained variance)
Diatoma vulgaris	*Stephanodiscus hantzschii*
Nitzschia dissipata	*Aulacoseira granulata*
Amphora inariensis	*Navicula reichardtiana*
Nitzschia palea	*Cyclotella ocellata*
Nitzschia capitellata	
Navicula tripunctata	
Calcareous subcatchment	
Physical−chemical factors (24.0% explained variance)	Physiografical factors (16.0% explained variance)
Craticula accomoda	*Navicula gregaria*
Nitzschia umbonata	*Planothidium frequentissimum*
Fragilaria capucina gracilis	
Cymbella cymbiformis	
Siliceous subcatchemnt	
Physical−chemical factors (11.5% explained variance)	Physiografical factors (5.8% explained variance)
Eolimna subminuscula	*Cyclotella distinguenda*
Cyclotella pseudostelligera	*Fragilaria parasitica subconstricta*
Encyonema minutum	*Aulacoseira granulata*
Achnanthidium biasolettianum	

The second set of analyses was performed with the 202 localities of the siliceous part of the catchment, 85 taxa and 23 environmental variables. Eleven environmental factors were involved in the distribution of diatom communities of this section (Table 5.3). The decomposition of the variance explained 21.02% of the total, from which 11.5% corresponded to physical–chemical, 5.8% to physiographic and 3.7% was shared. In addition to SRP and chlorides, the main physical–chemical variables were nitrates and conductivity. *Encyonema minutum* and *Eolimna subminuscula* occurred in nutrient rich sites, while *Achnanthidium biasolettianum* was characteristic of nutrient–poor sites in the opposite part. Drainage area and proportion of cultivated soil use (physiographic factors) were associated with the presence of *Aulacoseira granulata* and *Cyclotella distinguenda* that appeared in the lower parts of the river. In contrast, *Fragilaria parasitica subconstricta* was characteristic of upper mountains and fast flowing waters (Table 5.4).

5.4. DISCUSSION

The multivariate analyses performed with the data indicate that the distribution of diatom assemblages in the Guadiana River was sensitive not only to the water quality (mostly conductivity and nutrient concentration), but also to environmental descriptors related to the watershed scale (climate, geology and land soil use). On a watershed scale, the geological substrata may determine large changes in the water chemistry, while differences in land use may account for large differences in nutrient concentrations (Stevenson, 1997), which are expressed at the local scale.

Two large and different groups of sites were identified in the Guadiana basin according to their geology and chemistry. All the ordination analyses performed with diatom assemblages indicated an overall response to two main gradients. The first one was related to nutrient concentration, and defined a trend from pristine to polluted waters which was related to the percentage of cultivated soil. The second gradient was related to physiography, and geological substrata and altitude were the main factors involved. These two gradients provide information on how the river watershed is structured and how disturbances occurring at the large scale may affect diatom communities at the local scale.

Though both physical–chemical variables and physiographic factors provide information on how river basins are structured and how this can affect diatom communities (Pan*, et al.*, 1996, Winter and Duthie, 2000, Michels*, et al.*, 2006), their relative influence needs to be separated. Partial CCA analyses using the whole data set determined that the diatom community structure in the Guadiana was mostly affected by physical–chemical aspects (11.3%), and physiographic factors played a minor though relevant role (6.1%). Hence, *Nitzschia capitellata*, *N. dissipata* and *N. palea* were mostly influenced by water chemical characteristics (Table 5.4). These taxa occupied highly polluted sections, and their occurrence was irrespective of the physiography of the area (Lange–

Bertalot, 1979). In contrast, other taxa like *Aulacoseira granulata*, *Cyclotella ocellata* or *Stephanodiscus hantzschii* were mostly influenced by physiographic factors like distance from the source (drainage area) or location. These are planktonic taxa, typically found in the lower sections of the rivers (Fore and Grafe, 2002, Scuria*, et al.*, 2006). The value of intersection (1.4%), though smaller than the fraction of the variation independently explained by both sets of variables, indicated complex responses to species distribution. The intersection expresses the contribution of different variables that vary conjointly. It might be argued that this might be the case specially in cases where disturbance is not extreme, and ecoregional characteristics and responses to environmental stressors may combine to produce a confusing picture (Leira and Sabater, 2005). Some taxa showed a distribution that was an expression of the overlapping influence between the chemical and the physiographical factors. An example of this mixed influence was the occurrence of *Gomphonema truncatum*, which was found mainly in downstream sections occupying nutrient enriched, mineralized waters.

Nutrient–enrichment and human disturbances in the Guadiana watershed overshadow regional differences and large scale factors of diatom community distribution. The resulting diatom communities in these situations are homogeneous regardless of their regional context (Potapova and Charles, 2003, Leira and Sabater, 2005). An obvious consequence is that differences are more evident among undisturbed sites than among severely enriched sites. In our dataset, diatom communities in streams and river sections with intensive agriculture or in urban areas (scattered along the main reach of the Guadiana river) were characterized by nutrient–tolerant taxa like *Nitzschia palea* or *Mayamaea atomus permitis*, which indicate phosphate–enriched or organically polluted waters (Fore and Grafe, 2002). These taxa occurred in these situations irrespectively of the geological context of the river system.

The separate analysis of the whole data set in two geologically differentiated parts allowed us to define specific key factors affecting diatom species composition in each part of the basin, and to determine which species showed a certain preference for particular environmental conditions. *Cymbella affinis*, *Diatoma moniliformis*, *Diploneis oblongella*, *Encyonopsis microcephala* and *Gomphonema olivaceum* had a distinctly calcareous distribution, whereas *Melosira varians*, *Navicula capitatoradiata* and *Staurosirella pinnata*, occurred mainly or exclusively in the siliceous part.

Conductivity became a key factor to discriminate the distribution of the diatom assemblages in the siliceous part. Conductivity variations reflected the increase in ionic content that accompanied nutrient enrichment in that section (Leland and Porter, 2000, Leira and Sabater, 2005). Taxa associated with high conductivity were *Eolimna subminuscula*, *Encyonema minutum*, while *Achnanthidium biasolettianum* occurred in locations of low conductivity. The first two were tolerant to high nutrient content, while *A. biasolettianum* appeared in the oligotrophic siliceous headwaters (Tornés*, et al.*, 2007). Alkaliphilous (*Cymbella* sp.) and acidophilous (*Cyclotella atomus* or *Eunotia bilunaris*) diatoms respectively dominated at calcareous and siliceous sites. Moreover, indifferent taxa (*Achnanthidium minutissimum* or *Nitszchia palea*) dominated in the agricultural sites, similarly to other areas elsewhere (Zampella*, et al.*, 2007).

Physiographical factors showed relevant differences between the two parts of the watershed. The considered drained area and the percentage of cultivated soil were relevant to diatom distribution. Though the altitude is quite uniform in the upper part (Meseta Plateau), in the siliceous subcatchment it shows relevant differences between subcatchments. The most striking example is the presence of planktonic taxa like *Cyclotella distinguenda* or *Aulacoseira granulata* in the lowermost sites. However, *Fragilaria parasitica subconstricta*, which is known to prefer cold and oxygenated waters (Fore and Grafe, 2002, Potapova and Charles, 2002), was distributed in the headwaters.

In the upper calcareous part, river morphology was highly altered. Most river sections in this part do not have a riparian vegetation strip, and have oversimplified habitat conditions that lead to low and constant IHF values. This situation was associated with intensive agricultural practices and high nitrate concentrations. In contrast, in the siliceous part, both the IHF values and nitrate concentrations showed relevant variations that corresponded to shifts in diatom assemblage composition. *Luticola goeppertiana* was the taxon associated with higher nitrates and low IHF, a common situation in which this taxon develops (Potapova*, et al.*, 2004, Salomoni*, et al.*, 2006, Ponader*, et al.*, 2007).

5.5. CONCLUSIONS

Our study showed that multivariate analyses, especially variance partitioning, is a good tool for interpreting particular factors affecting diatom community composition in large river areas in spite of the homogenization due to disturbances. This might suggest that anthropogenic impacts can influence but not override the regional and large scale patterns in community structure. The Water Framework Directive recognizes the differences between ecoregions, but also needs to address those occurring within large and complex ecoregions, in particular where complex hydrology mix up with rampant human disturbances. The results presented here should clarify which are the factors determining diatom distribution within the Ibero–Macaronesian ecoregion.

CHAPTER VI

IDENTIFYING REFERENCE BENTHIC DIATOM COMMUNITIES IN THE GUADIANA WATERSHED

6.1. INTRODUCTION

Freshwater ecosystems are submitted to increasing disturbances due to intensive human use (Smith, *et al.*, 1999, Angeler, 2007, Weijters, *et al.*, 2009). Managing these ecosystems urgently implies assessing their ecological status and determining how much they are being affected (Hermoso, *et al.*, 2009). Legislative frameworks such as the European Water Framework Directive (WFD) (European_Commision, 2000) demand assessing the ecological status of freshwaters by comparing the actual to the potential (reference) condition.

Undisturbed or reference conditions with relatively low levels of human activity are difficult to establish in highly managed European freshwater ecosystems (Nijboer, *et al.*, 2004). This situation includes agricultural watersheds that have been extensively and intensively managed since historical times. The Guadiana River in SW Spain can be considered paradigmatic of long–lasting influences. Water extraction and heavily regulated reservoirs are spread disturbances in the entire river network (CHG, 2005). Drainage from agricultural land in river systems results in large inputs of nitrates and phosphates that eutrophy river waters (Johnson, *et al.*, 1997, Poor and McDonnell, 2007, Duff, *et al.*, 2008, Kvítek, *et al.*, 2009). Inputs and water extraction are especially remarkable in the North–East Guadiana watershed since nitrogen, phosphorus, and potassium–based fertilizers have been extensively applied to irrigated crops since decades (Moreno, *et al.*, 2006). These inputs are therefore being received by a river characterized by scarcity, where water is a shrinking resource (Sabater, *et al.*, 2009). Lacking reliable information in determining reference conditions and producing reliable assessment of impacts in biological communities is especially relevant in these conditions.

The taxonomic composition of benthic diatom communities is one of the biological quality elements in the definition of ecological status according to the WFD. Diatoms offer suitable indicator species for a variety of situations (Foerster, *et al.*, 2004). Benthic diatom assemblages in rivers are influenced by environmental descriptors not affected by human activities, such as the dominant geology of the river basin (Cantonati, 1998, Tison, *et al.*, 2004, Urrea and Sabater, 2009a), the altitude of the sampling site (Ndiritu*, et al.*, 2006, Rimet*, et al.*, 2007), the distance from the source (Potapova and Charles, 2002), but also by others related to human activities, such as the organic load or nutrient concentration of the water (Leira and Sabater, 2005, Kovács*, et al.*, 2006, Tornés*, et al.*, 2007). Therefore, the accurate characterization of diatom communities may allow the assessment of biological conditions and diagnosing stressors for the aquatic ecosystems (Stevenson*, et al.*, 2008b).

Diatom communities inhabiting the reference sites may be considered as target communities that could be used to asses the biological condition of each ecotype. Since diatom species composition varies among streams due to natural as well as anthropogenic factors, we can increase the accuracy of anthropogenic impact assessment by first accounting for natural variability among non–impacted sites.

In this chapter we aimed to identify the ecoregional reference conditions and determine which diatom species were the most relevant to illustrate the ecoregional variability characteristic of the Guadiana watershed. Because of the widespread disturbance characteristic of an agricultural watershed close–to–undisturbed conditions were formulated, having in mind that they should include the condition of a group of minimally disturbed sites in physical, chemical and biological terms (Reynoldson*, et al.*, 1997, Wallin*, et al.*, 2003). The diatom communities in the reference stations were determined once the reference conditions were established.

6.2. RIVER ECOTYPE CLASSIFICATION

Up to six river ecotypes were defined by the water authority: Confederación Hidrográfica del Guadiana by applying system B of the WFD (Annex II) (CHG, 2005) using hydromorphological, climatological and geographical factors (Table 6.1). The temporary and ephemeral watercourses were not included in the analysis, and therefore our analysis concerns the permanent ecotypes only (Figure 6.1).

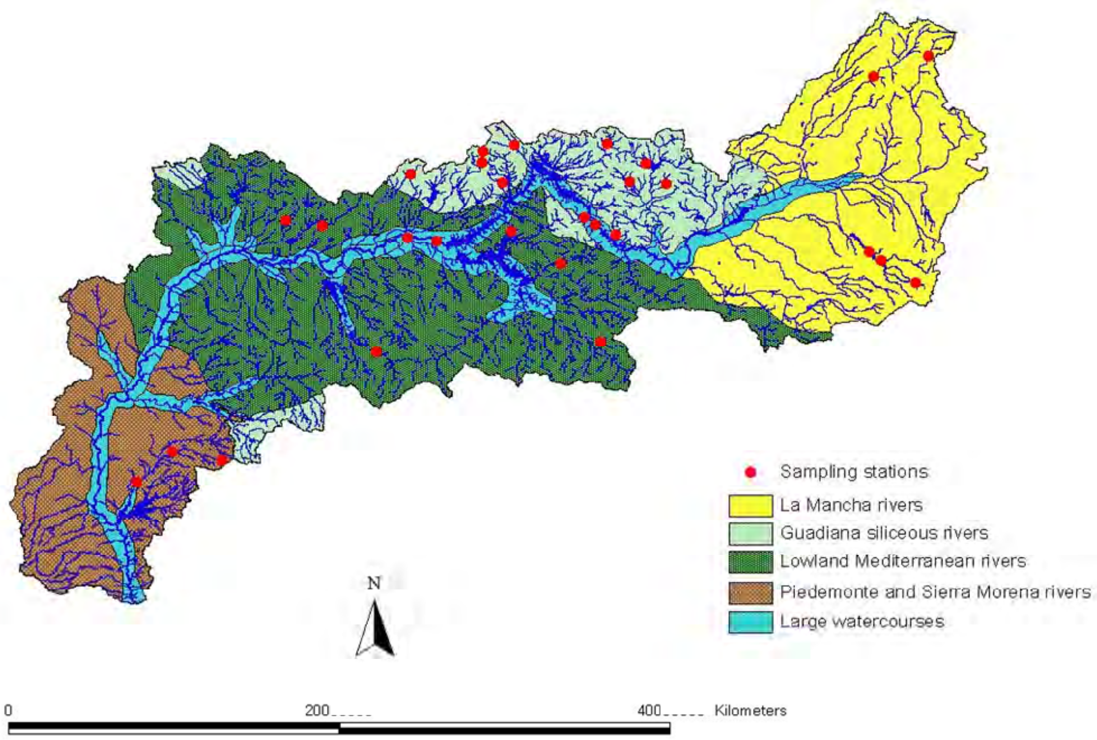


Figure 6.1. Geographical position of the reference sampling sites over the five ecotypes considered

Ecotype 1 La Mancha rivers. These are located in the north–eastern part of the catchment (*ca* 19000 km^2). This region is flat to undulated, ranging from 550 to 1000 m a.s.l. This area is made of Jurassic, Cretaceous and Tertiary limestones. Due to the porosity of its substrata and the moderate rainfall, surface water infiltrates in subterranean aquifers. The area is drained by slowly moving rivers connected to these aquifers, with an irregular flow. Groundwater pumping for irrigation has resulted in groundwater depletion which has caused reduced stream flow (Almagro–Costa, 2006).

Ecotype 2: Guadiana siliceous plain rivers. These are located in the north–western part of the catchment up to the border between Portugal and Spain (*ca* 24700 km^2). Geologically they are constituted by impermeable granitic batholites. The northern part of this area is flanked by Montes de Toledo with markedly stationary rainfall (600–800 mm/y) that cause river floods. The southern part is flatter and allows the development of the extensive Zujar's tributary where water flows quietly.

Ecotype 3: Lowland mediterranean rivers. These occupy the middle part of the catchment (*ca* 18800 km^2). The geological setting is constituted by slates and impermeable conglomerates. Precipitation range between 400–800 mm/year concentrated in spring and autumn, and summer is extremely dry and warm, when tributaries are commonly dry.

Ecotype 4: Piedemonte and Sierra Morena rivers. These are situated in the south part of the catchment (*ca* 2600 km^2). Geological setting is of metavolcanic rocks with acidic areas and Cenozoic deposits that origin the Iberian Pyrite Belt, one of the most extensive sulfide mining regions in the world. Precipitations are close to 1000 mm/year in the headwaters, and ca. 500 mm/year in the lower part . Tributaries in this part commonly get dry in summer.

Ecotype 5: Large watercourses. These develop on impermeable quaternary sands and silt deposits. The impermeability of the substrata and the low slope have made possible setting a large number of reservoirs scattered throughout its entire course. Several large and connected reservoirs (Cíjara, García de Sola and Orellana) in the middle part of the main Guadiana course are joined by La Serena reservoir of the Zújar tributary. In the lower part of the basin the Guadiana hosts the largest artificial lake in Europe, the Alqueva reservoir (4150 Mm3 capacity)

Table 6.1. Characteristics within each of the 5 ecotypes

	Area (Ha)	Precipitation (mm/year)	N. of sites considered
(1) La Mancha rivers	153924	510	34
(2) Guadiana siliceous rivers	74828	589	105
(3) Lowland mediterranean rivers	237075	589	78
(4) Piedemonte & Sierra Morena rivers	71985	567	11
(5) Large watercourses	85419	–	14

6.3. SPECIFIC METHODOLOGY

6.3.1. SELECTION OF REFERENCE STATIONS

Thirteen stressors were used to decide on the most appropriate reference stations in each ecotype. These stressors were divided into 4 categories following the IMPRESS (European_Commision, 2002) and REFCOND guidances (Owen*, et al.*, 2001, Wallin*, et al.*, 2003), modified and adapted to the Iberian rivers by Bonada *et al.* (Bonada*, et al.*, 2002) and Pardo *et al.* (Pardo*, et al.*, 2002) (Table 6.2).

1.- Channel alteration: Anthropogenic activities do not impede the hydrological continuum within a waterbody. Migration of aquatic organisms and sediment transport remain undisturbed.

2.- Hydrological pressures: The quantity and dynamics of waterflow and the resultant connection to ground waters reflect totally, or nearly totally, undisturbed conditions.

3.- Pollution pressures: The degree of urbanization, agriculture and silviculture and associated practices should be as low as possible for the reference sites, and nutrient concentrations should remain within the range of undisturbed conditions (annex II of WFD).

4.- River habitat alteration: The structure and substratum of the river bed following Pardo *et al.* (2002) and adjacent natural vegetation following Munné *et al.* (Munné*, et al.*, 2003) should be the one potentially characteristic in the type and geographical location of the river. The level of biodiversity and ecological functioning should be equivalent to unmodified and natural water bodies.

Table 6.2. 13 key stressors divided in 4 categories considered in identifying reference stations

Category	Key stressors
Channel alteration	1.- Dredging: sediment disposal, removal of substrate or change in water level (data from CHG) 2.- Flow regulation by physical barriers (dams, weirs…) (data from CHG) 3.- Channel modification by straightening (data from CHG)
Hydrological pressures	4.- Water abstraction (presence of wells, irrigation…) (data from CHG) 5.- Artificial recharge and increasing flow for water transfer (data from CHG)
Pollution pressures	6.- Discharge of wastewater from an urban area > 10 km^2 (data from CHG) 7.- Waste water from sewage treatment plant (data from CHG) 8.- Diffuse source of pollution: Natural land < 75% (Corine Land Cover, 2003) 9.- Ammonium > 0.5 mg/L NH_3 (ion chromatographic method, A.P.H.A.); log(x) 10.- Nitrates > 10 mg/L NO_3 (ion chromatographic method, A.P.H.A.); log(x) 11.- Phosphate > 0.05 mg/L P_2O_4 (ascorbic acid method, A.P.H.A.); log(x)
River habitat alteration	12.- Structure and condition of the riparian zones: QBR index < 75 (calculated after field observation) 13.- Measures of stream habitat: River habitat index: IHF < 70 (calculated after field observation)

For each of the 13 descriptors a 0–1 matrix was constructed where 1 indicated disturbance, and 0 indicated absence of disturbance. A site should be considered in reference condition when none of the 13 key stressors was labeled as 1. However, since undisturbed conditions meaning “completely lacking in any form of human impact” are virtually impossible to find in most rivers of Europe (Owen*, et al.*, 2001) reference thresholds were redefined for every ecotype.

6.3.2. DATA ANALYSIS

All data were scrutinized for normality and some transformations were used in order to achieve the homogeneity of variances. Diatom data set was square root transformed, while environmental variables, except for the pH and percentage variables were transformed by $\log_{10}$ (x+1).

Major gradients and patterns of variation among reference sites were detected by Principal Component Analysis PCA. ANOSIM analysis was used to validate differences between the groups.

Patterns in diatom community composition were examined using canonical ordination techniques. Preliminary detrended correspondence analysis (DCA) (Hill and Gauch, 1980) on the species data revealed that the gradient lengths were greater than 2 standard deviation units (3.07 and 2.59 for the first two axes), indicating an unimodal response which justified the use of unimodal ordination techniques (Leps and Smilauer, 2003). A canonical correspondence analysis (CCA) was therefore used to relate the structure of the diatom assemblages to predictor environmental variables and to explore relationships among and between species and the environment (Ter Braak and Verdonschot, 1995). Step–wise forward selection and Monte–Carlo permutation tests (499 permutations, $p<0.02$) were used to reduce the environmental variables to those correlated significantly with the derived axes. Since the sampling data were distributed between different seasons and years, time was considered as a categorical co–variable. Version 4.5 of CANOCO was employed (Ter Braak and Smilauer, 1998).

Finally, indicator species analysis (IndVal) was calculated to describe type–specific reference diatom community in each ecotype. Indicator species analysis provides a method of combining the relative abundance and relative frequency of each species into an indicator value (Dufrène and Legendre, 1997). Indicator values (IndVals) were tested for statistical significance using Monte−Carlo Permutations Test. Analyses were performed using PRIMER–6 (Clarke and Gorley, 2005) and PcOrd–5 (McCune and Mefford, 1999) statistical packages.

6.4. RESULTS

6.4.1. SELECTION OF REFERENCE STATIONS

A site was considered in reference condition when none of the 13 key stressors was labeled as 1. Since this exercise revealed that virtually no sites could be used as reference in the Guadiana watershed, stations were selected to fulfill as many criteria as possible (Table 6.3).

Table 6.3 Criteria to select reference or maximum ecological potential for each river typology (Σ = score results of stressors)

	Mancha	Siliceous	Lowland	Piedemonte	Large watercourses
Channel alteration	Σ ≤1	Σ = 0	Σ = 0	Σ = 0	Σ ≤2
Hydrological pressures	Σ = 0	Σ = 0	Σ = 0	Σ = 0	Σ ≤ 1
Pollution pressures	Σ ≤ 2	Σ ≤ 1	Σ≤ 1	Σ ≤ 1	Σ ≤ 2
River habitat alteration	Not considered	Σ ≤ 1	Σ = 0	Σ ≤ 1	Not considered
Number of stations satisfying all criteria	5 (14.71%)	6 (5.71%)	12 (15.38%)	3 (27.27%)	3 (21.43%)

Reference conditions could not be attributed in La Mancha ecotype and "maximum ecological potential" was defined following REFCOND recommendations (Wallin*, et al.*, 2003). Natural riparian conditions in this ecotype are highly altered because of long–term agricultural practices, and most of the streams in this region are open–channel systems. Therefore, river habitat alteration stressors were not considered in the decision process. Further, intensively irrigated agriculture contributes high nutrient concentrations so pollution pressures can score up to 2 (as the sum of scores of different stressor categories; Table 6.3).

Sites in ecotypes 2, 3 and 4 were considered in reference condition when channel alteration and hydrological pressures scored 0 and pollution pressures and river habitat alteration punctuated maximum 1 (Table 6.3).

Reference and maximum ecological potential conditions could not be attributed to the large Watercourses ecotype, due to the intensive agricultural influences as well as the presence of large number of reservoirs. Less impacted sites were described in this ecotype, according to the criteria outlined in table 6.3.

Using the above described criteria of reference and less impacted sites up to 29 stations from the 244 sampling sites available in the sampling network were considered in the analysis (Table 6.3, Figure 6.1). These are named hereafter as the reference sites in the Guadiana watershed.

6.4.2. DATA ANALYSIS

A PCA was performed with the environmental data of the 29 reference sites. The first two principal components respectively accounted for the 30.24% and 16.10% of the environmental variation within the reference stations. Headwaters forested localities with low conductivities (ecotypes 2, 3 and 4) were arranged against localities with higher conductivities. The second axis separated mainland watercourses with high drainage area (ecotype 5) from calcareous bedrock localities with high calcium and alkalinity concentrations (ecotype 1) (Figure 6.2). ANOSIM analysis between the 5 ecotypes revealed no significant differences between groups of sites ($R=0.036$, $p=0.05$), and therefore a pair wise comparison between ecotypes was carried out. This showed no significant differences between ecotypes 2, 3 and 4 ($R<0.3$, $p=0.05$). After that these three ecotypes were grouped in a new ecotype encompassing rivers with siliceous bedrocks and low conductivities, which was redefined as the siliceous ecotype. A new ANOSIM analysis revealed a global $R=0.76$ ($p=0.05$), indicating a consistent separation between the new 3 groups. Physiographical and chemical characteristics of the ecotypes are detailed in tables 6.4 and 6.5.

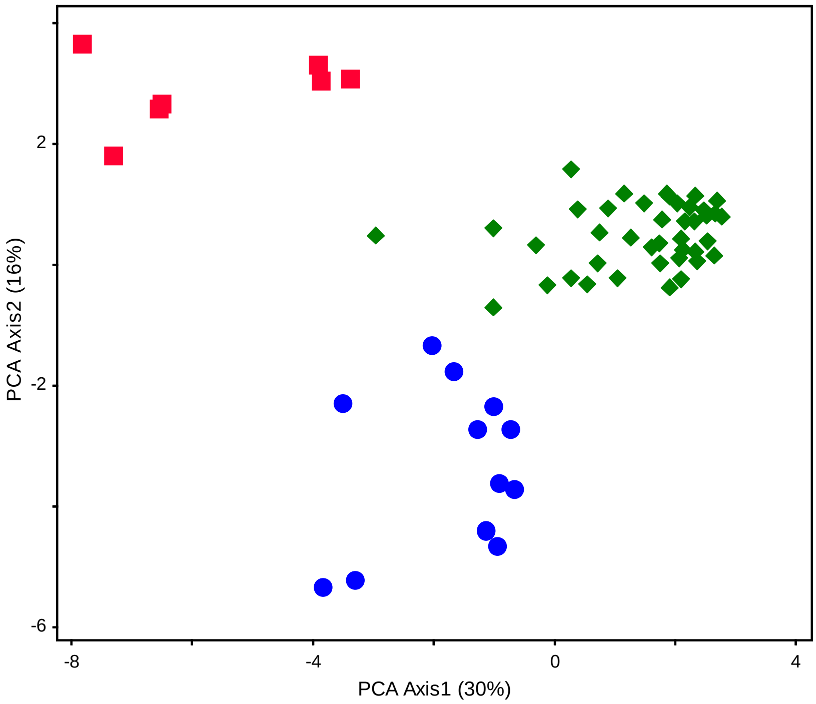


Figure 6.2. Plot of sites scores for the first 2 axes of PCA analysis carried out on the 24 environmental variables of 29 selected reference sites. Green diamonds correspond to siliceous localities, blue circles correspond to calcareous ones while red squares correspond to large watercourses

Table 6.4. Mean values and standard deviation of environmental variables for the 29 reference stations (visited 1 to 3 times) for the 3 ecotypes proposed after ANOSIM analysis

	Siliceous		Calcareous		Large watercourses	
	Range	Mean	Range	Mean	Range	Mean
Conductivity (µS/cm)	46.00 - 826.00	210.35	730 - 2210	1106.42	261 - 4140	1369.33
pH	4.65 - 9.42	7.87	7.45 - 8.52	8.09	7.62 - 9.31	8.50
Oxygen (mg/L)	1.10 – 12.35	8.70	1.10 – 10.90	7.56	2.29 – 12.59	8.67
Temperature (ºC)	3.40 - 27.20	13.97	2.70 - 20.00	11.56	6.80 - 31.10	13.98
Ammonia (mg/L)	0.01 - 26.38	0.73	0.01 - 6.50	0.82	0.02 - 0.96	0.22
Chloride (mg/L)	2 - 124	24.60	7.20 - 87.80	27.38	18.30 - 435.00	119.69
Sulfate (mg/L)	2.16 - 866.00	38.37	13.90 - 984.00	277.79	20 - 2380	509.34
Soluble Reactive Phosphorus (mg/L)	0.01 - 0.15	0.06	0.03 - 0.12	0.04	0.03 - 0.09	0.04
Nitrate (mg/L)	0.20 - 15.17	1.17	7.22 - 55.90	25.89	0.40 - 1.45	0.54
Calcium (mg/L)	2.70 - 51.40	14.95	72.30 - 400	166.95	18.30 - 773.00	172.24
Alkalinity (mg CaCO3/L)	5.50 - 207.00	60.64	158 - 313	222.69	65.70 - 335.00	154.71
Flow (m3/s)	0 - 0.69	0.08	0 - 1.92	0.42	0 - 3.59	1.12
Wet width (m)	90 - 1940	448.14	20 - 930	429.17	540 - 3170	1872.22
Wet Perimeter (m)	101.61 - 1940.68	433.23	25.16 - 940.35	439.04	546.32 - 3174.49	1879.32
Hydraulic Radius (m)	3.94 - 58.62	13.75	2.78 - 42.90	19.57	16.75 - 33.49	22.93
Maximum Depth (cm)	23924	22.42	22098	32.33	21 - 78	40.67
% Artificial land use	0 - 0.50	0.04	0 - 1.14	0.53	1.26 - 1.69	1.38
% Cultivated land use	8.57 - 87.91	32.49	62.30 - 79.07	70.08	68.46 - 85.13	77.30
% Forested land use	11.80 - 91.44	67.47	20.88 - 26.74	29.38	13.52 - 30.07	21.33
Drainage Area (km2)	9.76 - 989.45	163.10	86.08 - 873.63	434.84	13880.01 - 34939.41	22673.38
Distance from the source (km)	5.34 - 93.72	24.47	8.59 - 63.91	44.72	224.03 - 520.85	386.14
Altitude (a.s.l)	87.00 - 706.00	451.39	600 - 984	827.67	255 - 575	426.67

A CCA analysis included the 29 sampling stations visited 1 to 3 times (a total of 63 samples), 97 taxa (restricted to those taxa with relative abundances greater than 1%) and 24 environmental variables. The forward selection and Monte Carlo permutation test (p<0.02) identified 7 environmental variables to be significant. These were conductivity, percent of natural land use, drainage area, altitude, the geospatial UTMX and concentration of nitrates and calcium (Figure 6.3A). The first two axes of the CCA had eigenvalues of 0.236 and 0.184 respectively. Species environment variation was higher than 0.87 for both axes, and collectively explained 56.7% of the species–environment variation. The ordination identified the same 3 groups of sites described in the previous PCA analysis. From the seven environmental variables selected, conductivity and land use were the dominant variables in CCA axis 1, whereas the second CCA axis was related to the geomorphological setting. Large rivers with high drainage areas, low elevation and reduced canopy were opposed to higher altitude tributaries with high calcium content. *Tabellaria flocculosa*, *Gomphonema acuminatum, Brachysira vitrea* and *Encyonema silesiacum* formed assemblages in habitats with low conductivity and natural land uses (negative extreme of CCA axis 1). *Diatoma moniliformis* and some species of *Cymbella* (*C. helvetica*, *C. microcephala* or *C. affinis)* characterized sites with high conductivities due to high calcium content (negative part of CCA axis 2). , Sites with high drainage and agricultural areas and high conductivities were arranged in the positive part of CCA axis 2. These sites were inhabited by taxa tolerant to organic pollution like *Aulacoseira granulata, Staurosirella pinnata* or *Nitzschia amphibia* (Figure 6.3B).

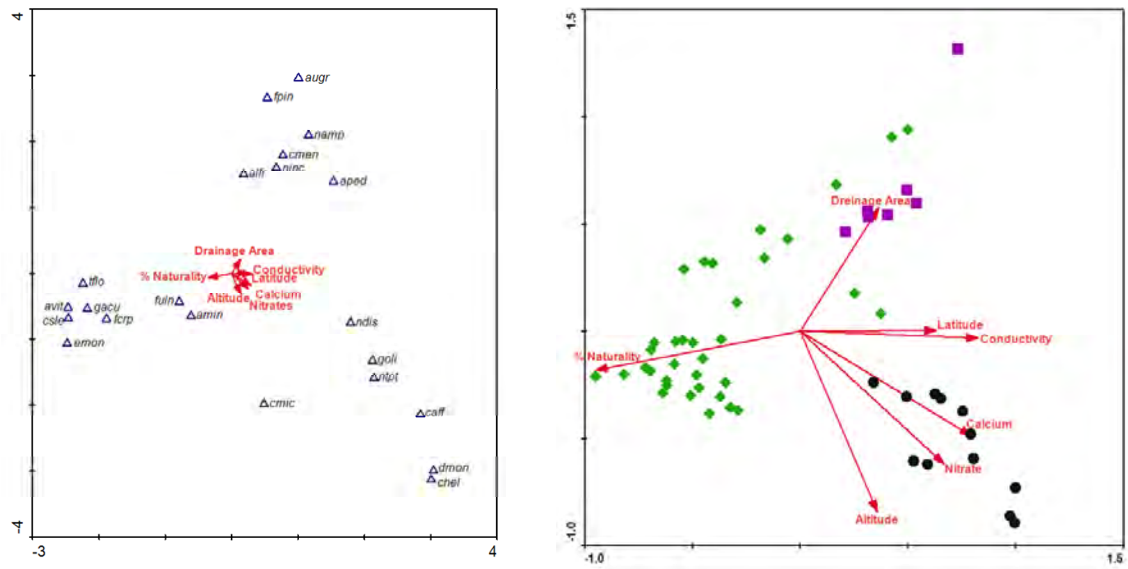


Figure 6.3. Distribution of the diatom species (A) and sampling sites (B) in an ordination diagram with the two first axes of the Canonical Correspondence Analysis (CCA). Independent variables detected as being significant by the analysis are indicated with arrows. The plot includes for clarity the diatom taxa with relative abundances higher than 20%. Codes in (A) are explained in table 6.5. Diamonds in (B) correspond to siliceous localities, circles correspond to calcareous ones and squares correspond to large watercourses

An Indicator Species Analysis (IndVal) was performed using all diatom taxa, including rare ones since they contribute to differences between groups (Nijboer*, et al.*, 2004). The most important indicator taxa for each 3 groups of reference sites, with associated indicator values are given in table 6.5. Results corroborated the previous CCA analysis. Indicator taxa for the siliceous ecotype included *Encyonema silesiacum, Tabellaria flocculosa* and *Navicula minuscula.* In the calcareous ecotype the indicator taxa were alkaliphilous and alkalibiontic species tolerant to high conductivities, like *Cymbella affinis*, *Navicula tripunctata*, *Diatoma moniliformis* and *Diploneis oblongella*. Finally, indicator taxa in large watercourses were *Nitzschia inconspicua*, *Gomphonema parvulum*, *Navicula veneta* and *Staurosirella pinnata* (taxa were listed in decreasing order of importance).

Table 6.5 Indicator value (IndVal) for the most important taxa in each of the three ecotypes. $^{**}p<0.01$; $^{*}p<0.05$

		Observed IndVal (%)			
		Siliceous	**Calcareous**	**Large w.**	
csle	*Encyonema silesiacum* (Bleisch in Rabenhorst) D.G. Mann	**38**	0	0	**
tflo	*Tabellaria flocculosa* (Roth) Kützing	**33**	0	1	*
nmis	*Navicula minuscula* Grunow in Van Heurck	**32**	0	0	*
gacu	*Gomphonema acuminatum* Ehrenberg	**28**	0	0	*
avit	*Brachysira vitrea* (Grunow) Ross	**26**	0	0	*
caff	*Cymbella affinis* Kützing	0	**63**	3	**
ntpt	*Navicula tripunctata* (O.F. Muller) Bory	1	**62**	13	**
dmon	*Diatoma moniliformis* Kützing	0	**55**	1	**
dobl	*Diploneis oblongella* (Naegeli) Cleve–Euler	2	**52**	0	**
ndis	*Nitzschia dissipata* (Kützing) Grunow	6	**46**	26	**
cmic	*Encyonopsis microcephala* (Grunow) Krammer	12	**42**	2	**
nsbh	*Fallacia subhamulata* (Grunow) D.G. Mann	1	**42**	0	**
aina	*Amphora inariensis* Krammer	3	**40**	17	*
cbac	*Caloneis bacillum* (Grunow) Cleve	0	**38**	1	**
goli	*Gomphonema olivaceum* (Hornemann) Brébisson	3	**36**	6	*
apel	*Amphipleura pellucida* Kützing	1	**32**	0	**
chel	*Cymbella helvetica* Kützing	0	**31**	0	**
nant	*Navicula antonii* Lange–Bertalot	2	**30**	10	*
nheu	*Nitzschia heufleriana* Grunow	0	**25**	7	**
ninc	*Nitzschia inconspicua* Grunow	13	8	**59**	**
gpar	*Gomphonema parvulum* (Kützing) Kützing	25	1	**56**	**
nven	*Navicula veneta* Kützing	7	6	**55**	**
fpin	*Staurosirella pinnata* Ehrenberg	4	1	**50**	**
alfr	*Planothidium frequentissimum* (Lange–Bertalot) Round & Bukhtiyarova	24	16	**43**	*
augr	*Aulacoseira granulata* (Ehrenberg) Simonsen	3	0	**40**	**
npal	*Nitzschia palea* (Kützing) W. Smith	28	15	**40**	*
ncpr	*Navicula capitatoradiata* Germain	9	7	**38**	*
ffas	*Fragilaria fasciculata* (Agard) Lange–Bertalot	6	4	**37**	*
nshr	*Navicula schroeteri* Meister	5	0	**37**	**
namp	*Nitzschia amphibia* Grunow	6	6	**32**	*
naco	*Craticula accomoda* (Hustedt) Mann	1	1	**31**	**
coce	*Cyclotella ocellata* Pantocsek	0	4	**29**	**
dvul	*Diatoma vulgaris* Bory	0	8	**29**	*
csol	*Cymatopleura solea* (Brébisson) W.Smith	0	1	**24**	*
nigr	*Nitzschia gracilis* Hantzsch	0	0	**20**	*
shan	*Stephanodiscus hantzschii* Grunow in Cleve & Grunow	1	0	**18**	*
bpar	*Bacillaria paradoxa* Gmelin	1	0	**17**	*

Non–diatom algae dominant in the siliceous ecotype were *Lemanea* cf. *fluviatilis*, *Draparnaldia* and some desmidiaceae. Mats of *Vaucheria* sp. were dominant in the calcareous waters accompanied by some mats of the filamentous cyanobacteria *Lyngbia* sp. *Cladophora* cf. *glomerata* and *Stigeoclonium* sp. were the dominant algae accompanying diatoms in large watercourses (Table 6.6).

Table 6.6: Non diatom algae and Cyanobacteria found in some reference stations. * refereed to abundance of the taxa

	Siliceous	Clacareous	Large w.
Chlorophyta			
Cladophora glomerata (L.) Kützing	*	*	**
Closterium sp.	**	*	
Cosmarium botrytis Meneghini ex Ralfs	**		
Draparnaldia sp.	**		
Oedogonium sp.	*	*	
Scenedesmus sp.	*		
Spirogyra sp.	*	*	
Stigeoclonium sp.			**
Vaucheria sp.		***	
Euglenophyta			
Euglena sp.	*		
Rodophyta			
Lemanea cf. *fluviatilis* (L.) C. Agardh	**		*
Cyanobacteria			
Chroococcus sp.			*
Lyngbia sp.		***	
Oscillatoria sp.	**	*	
Phormidium sp.	***	*	*
Pleurococcus sp.		*	
Tolypothrix sp.		*	

6.5. DISCUSSION

The combined use of ecological and pressure criteria should be sufficient for screening for sites representing potential reference conditions (Wallin*, et al.*, 2003). Once these sites are identified, biological elements should be used to corroborate the ecological high status. However, the Guadiana watershed drains a mostly agricultural zone which entails a great water demand that results in an increased competition among water uses and natural resources. Further, in the Upper–Guadiana catchment groundwater resources have been severely stressed by intensive exploitation since the 1980s to fed crop irrigation. The intensive use of groundwater, especially during long periods of low rainfall, has led to a dramatic decline in groundwater levels in places by up to 50m causing degradation of wetlands, reduction of river flows and destruction of riverbank habitats (Bromley*, et al.*, 2001, Conan*, et al.*, 2003). In addition to the hydric stress, the receiving nutrient content is high, and effects on primary producers might increase under low dilution conditions (Sabater*, et al.*, 2009). The conjoint occurrence of these situations make difficult to meet the reference criteria in both large watercourses and calcareous ecotypes. In those cases, more permissive criteria were applied to include partially impacted sites. Even applying the more permissive criteria, still a low number of reference sites could be included in the analysis. This was particularly relevant in the large watercourses, highly affected by long–lasting disturbances.

The PCA performed with the geomorphological and hydrological descriptors showed three main groups of reference localities in the Guadiana watershed. Two of them were located in the headwaters. One was in forested localities with low conductivities (defined as siliceous ecotype), and the second was made up of streams with high calcium content (defined as calcareous ecotype). The third group of reference sites included some locations in large watercourses. Multidimensional solutions showed similar relationships between diatom species and environmental variables, where conductivity, land use and calcium content were the explanatory variables accounting for the highest proportion of variation. Water conductivity is commonly reported to be the most important explanatory variable in large scale algal studies (Munn*, et al.*, 2002, Potapova and Charles, 2003, Urrea and Sabater, 2009a) because it summarizes the underlying geology and the agricultural practices (Biggs and Gerbeaux, 1993, Leland, 1995).

Achnanthidium minutissimum and *Cocconeis placentula* were found in most sites. They are ubiquitous taxa that appear to occupy a wide ecological range (Van Dam*, et al.*, 1994) and both have been found to tolerate different pollution levels from different sources (Ndiritu*, et al.*, 2006). The IndVal analysis identified the diatom taxa characteristic of each ecotype. In oligotrophic siliceous forested headwaters the type specific diatom indicator taxa were *Encyonema silesiacum* and *Tabellaria flocculosa*. Both taxa were associated to upstream stretches of siliceous rivers with low electrolyte content (≈200 μS/cm) (Gomà*, et al.*, 2005, de Jonge*, et al.*, 2008). Accompanying these taxa appeared desmidiaceae like *Cosmarium* cf. *botrytis* and *Closterium* sp., some *Euglena* species and *Lemanea* cf. *fluviatilis*. While most desmidiaceae are confined to oligo–mestrophic habitats (Coesel, 1993). *Lemanea* cf. *fluviatilis* has a cosmopolitan distribution in oligotrophic waters and a wide altitudinal gradient (Eloranta, 2004, Kucera*, et al.*, 2008).

The type specific diatom indicator taxa in calcareous localities were *Cymbella affinis*, *Navicula tripunctata* and *Diatoma moniliformis* accompanied by some other *Cymbella* species (e.g. *C. helvetica*). *Cymbella* showed the highest affinity towards calcium in a dataset collected from sites throughout USA (Potapova and Charles, 2003) and were dominant in the diatom communities of Pyrenean calcareous springs (Sabater and Roca, 1992, Tornés*, et al.*, 2007) and limestone regions of France (Rimet, 2009). Mat–forming *Vaucheria* was the dominant alga in the calcareous ecotype, and is commonly found in these environments (Foerster*, et al.*, 2004). They were accompanied by cyanobacteria filaments of *Lyngbia,* described as potential indicators of conductivity (Munn*, et al.*, 2002).

The geochemical characteristics of the large watercourse ecotype was masked by the pollution impacts (Tornés*, et al.*, 2007) occurring in this area. Though near–to–reference sites were selected only in case this pollution was not apparent, still nutrient enrichment and mineralized waters were general. The diatom community was characterized by *Nitzschia inconspicua* and *Gomphonema parvulum*, both characteristic in low elevation stretches supporting high irradiances and slow moving waters with high nutrient concentrations (Sabater and Sabater, 1988, Martínez de Fabricius*, et al.*, 2003). In these sites highly–tolerant species (e.g. *Nitzschia palea, Craticula accomoda*) were never dominant. The presence of some planktonic taxa like *Aulacoseira* *granulata* is also characteristic of lower rivers sections (where phytoplankton can develop) with moderate to high nitrate concentrations (Fore and Grafe, 2002, Tison*, et al.*, 2007, Urrea and Sabater, 2009a). Algae accompanying the above diatom community were the green algae *Cladophora glomerata* and *Stigeoclonium* sp. The first has been often reported under nutrient–rich, mineralized waters (Van der Hoek, 1976, Margalef, 1983, Gutowski*, et al.*, 2004) and well–lighted conditions (Ensiminger*, et al.*, 2000). Although it was not possible to identify *Stigeoclonium* to the species level their occurrence is also on nutrient–rich waters (Zanini Branco and Pereira, 2002, Gutowski*, et al.*, 2004). The presence of this algal community is close to potential reference communities observed in large rivers under natural conditions (Margalef, 1983).

6.6. CONCLUSIONS

While establishing reference conditions is possible in the headwaters of the Guadiana (though only after application of permissive criteria), determining reference diatom communities in the large watercourses was more difficult. Defining reference sites demanded very permissive criteria, and the algal community developing in those sites is (at least) present in eutrophic and polluted conditions. This anomaly is not unique of the Guadiana watershed, but widespread in agricultural landscapes that have been managed since long periods of time. In these cases an accurate distinction between natural and anthropogenic sources of stressors is extremely difficult. Since this remains an important criterion to assess river condition (Stevenson*, et al.*, 2008a) determining reference conditions in large river watercourses remains unreliable. When available, historical data may offer the most valuable information for this purpose. In these cases, reference diatom communities should be based on diatom collections or fossil records. While the use of fossil diatoms to infer the baseline state of environmental variables has been possible in lakes (Bennion*, et al.*, 2004, DeNicola*, et al.*, 2004, Taylor*, et al.*, 2006) such approaches are more difficult to apply in rivers, where erosion and sedimentation are usually too dynamic to permit accumulation of stable and lengthy sediment sequences.

Attempts have been made in fluvial lakes (Reavie*, et al.*, 1998, Edlund*, et al.*, 2009) or in low parts of main European rivers where paleomeanders could be identified (Hilt*, et al.*, 2008). All these attempts have limited success if suitable sites for such studies do not exist, which is commonplace in areas where long history of human settlement has influenced the river ecosystem. In these cases, applying autoecological criteria to selected sites remains as the only possibility to decide if an algal community can be considered as representative of near–reference conditions. This remains as the only applicable alternative to determine reference conditions in largely disturbed agricultural systems such as the Guadiana River.

CHAPTER VII

PATTERNS OF BENTHIC CHLOROPHYLL–*a* IN THE GUADIANA WATERSHED

7.1. INTRODUCTION

Benthic primary producers supports higher trophic level organisms through food web in shallow rivers (Carr*, et al.*, 2005). Algal biomass in river systems depends on a number of variables including availability of nutrients (Welch and Patmont, 1989, Veraart*, et al.*, 2008), quality and quantity of light (Mosisch*, et al.*, 2001, Sabater*, et al.*, 2005), temperature (Morin*, et al.*, 1999), geochemical properties of the water (Munn*, et al.*, 1989), and grazing pressure (Biggs*, et al.*, 1998, Anderson*, et al.*, 1999).

Algal–nutrient interactions in streams are complex since multiple ecological processes operate at different spatial and temporal scales (Stevenson, 1997). New approaches that recognize scale–dependent constraints are required to reveal patterns and processes at broad scales. Physical habitat variables including canopy shading (Mosisch*, et al.*, 2001), turbidity (Munn*, et al.*, 1989), water temperature (Munn*, et al.*, 1989), hydrologic disturbances (Powers, 1992, Biggs, 1995, Riseng*, et al.*, 2004) could affect algal production, as well as biological factors such as grazing (Lamberti and Resch, 1983, Powers, 1992). Further large regional factors, such as climate, geology, and land use, should be integrated with local processes to study algal–nutrient interaction (Biggs, 1995).

Several statistical approaches aimed to predict the effects of eutrophication on algal biomass (usually estimated as the chlorophyll−*a* content) have been extensively applied to lentic systems (Nogueira*, et al.*, 1998, Tufford and McKeller, 1999, Hakanson*, et al.*, 2003, Çamdevýren*, et al.*, 2005, Cho*, et al.*, 2009), and lesser to lotic systems (Biggs, 2000). Major difficulties affecting the predicting power of statistical expressions derived in lotic systems relies in the variable hydrological patterns as well as in the patchy nature of benthic algal communities in river ecosystems (Sellers and Buckvareckas, 2003, Carr*, et al.*, 2005, Sabater*, et al.*, 2008). Nutrients often explain only a moderate amount of the variation of algal biomass in many of these models (Lohman*, et al.*, 1992, Biggs, 2000, Dodds*, et al.*, 2002), that usually is even lower in large–scale studies (Leland, 1995).

The Guadiana has a complex watershed, with extensive agricultural influences, large human pressure, and relevant hydrological interruptions by natural and human–driven causes (Sabater*, et al.*, 2009). This makes the benthic chlorophyll biomass extremely complex to predict, and requires considering factors operating at different scales, from those relevant for the algae at the habitat scale to those operating at the watershed scale that define the climatic and physical framework.

In this chapter we aim to determine the relative influence of physico–chemical parameters (including nutrients) and physiographic parameters (including habitat characteristics and large scale factors) on algal biomass in the Guadiana watershed by using Partial Least Square Regressions (PLSR) models in the different river ecotypes of the watershed.

7.2. DATA ANALYSIS

Prior to all analyses, Kolmogorov–Smirnov normality test was applied to all variables. The ones transformed in order to improve normality are listed in table 7.1.

Partial Least Square Regressions (PLSR) models were used to interpret the parameters describing benthic chlorophyll. Partial Least Square Regression (PLSR) models were created by using chlorophyll as the dependent variable and environmental parameters as the predictor variables. PLSR is an extension of multiple regression analysis, in which the effects of linear combinations of several predictors on a response variable are analyzed. PLSR is especially useful when predictors are highly correlated, allowing to test for the combination of effects of continuous and categorical predictor variables together. PLSR aims to obtain a small number of relevant predictive factors. The method achieves a canonical decomposition of *X* (the predictor variables) in a set of orthogonal factors (called latent vectors) which are used for fitting *Y* (the predicted variable) (Spanos*, et al.*, 2008). PLSR has been shown to provide more reliable results than multiple regression or principal component regression (Garthwaite, 1994, Carrascal*, et al.*, 2009).

PLSR analyses were performed separately in the 3 ecotypes previously defined in the watershed (chapter VII). From each PLSR simulation, only components explaining more than 10% of original variance in the response variable (Y^2) were retained. In practice only two components were retained for the model. The sum of X^2 from each component provided the total explanatory capacity of PLSR model. PLRS analyses were run using STATISTICA 6 software (Stat-Soft, 2001).

Consistency of relationships between chlorophyll concentration (biomass) and community metrics (biological–response parameters) over two sampling periods was assessed by using ancova models, with the chlorophyll concentration as covariate, the sampling period as factor and each biological parameter as a dependent variable. Ancova analysis were ran using a two−step procedure: (i) after testing the homogeneity of slopes assumption through the significance of the interaction term (chlorophyll concentration * sampling period), the complete model was retained in case of significance, and (ii) when the interaction was not statistically significant it was deleted from the models, and standard ANCOVA analyses were run. All these analyses were performed using SPSS v.15 (SPSS−Inc., 2004).

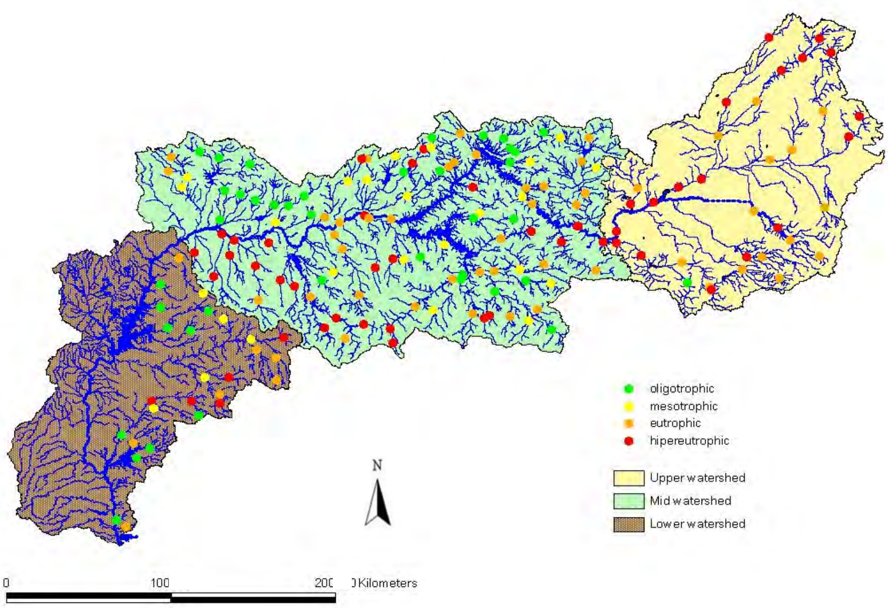


Figure 7.1: Trophic condition of sampling sites was also described estimated from mean chlorophyll concentration all over the year

Table 7.1. Summary of 24 measured variables with range and mean in the 297 stations used for the PLRS analyses. Values of chlorophyll–*a* were also shown

		Calcareous ecotype (n = 42)		Sileceous ecotype (n = 231)		Main watercourses (n = 24)	
	transf.	range	mean	range	mean	range	mean
Chlorophyll–*a* (mg/m^2)		12.03 – 1297.70	221.21	1.97 – 1425.00	78.39	6.07 – 1060.58	163.53
Conductivity (µS/cm)	$\log_{10}$(X)	550 – 3230	1666.60	32 – 2500	494.85	261 – 5500	1076.13
pH		7.35 – 8.59	8.02	0.15 – 10.63	7.93	7.62 – 8.93	8.20
Dissolved oxygen (mg/L)		5.40 – 12.59	10.55	1.10 – 11.87	9.00	1.10 – 9.89	7.96
Temperature (ºC)		2.20 – 22.6	11.64	2.90 – 29.60	19.04	6.70 – 24.50	19.14
Ammonia (mg/L)	$\log_{10}$(X+1)	0.01 – 6.50	0.54	0.01 – 29.90	0.74	0.01 – 19.3	1.84
Nitrate (mg/L)	$\log_{10}$(X+1)	0.40 – 55.90	17.11	0.05 – 43.85	3.30	0.05 – 22.15	3.55
Soluble reactive phosphorus (mg/L)	$\log_{10}$(X+1)	0.03 – 4.20	0.29	0.01 – 23.20	0.80	0.03 – 6.70	0.89
Sulfate (mg/L)	$\log_{10}$(X+1)	13.90 – 1499.05	501.63	2.16 – 1500	76.97	20 – 2380	275.91
Chloride v	$\log_{10}$(X+1)	7.20 – 834.00	77.52	2.00 – 250.00	55.33	18.30 – 435.00	89.55
Calcium (mg/L)	$\log_{10}$(X+1)	42.40 – 504.00	218.11	2.10 – 318.00	38.12	18.3 – 773.00	102.19
Alkalinity	$\log_{10}$(X+1)	81.40 – 81.40	241.35	5.50 – 514.00	141.37	65.70 – 408.00	173.77
Flow m^3/s	√X	0 – 1.92	0.24	0 – 4.89	0.13	0 – 14.32	2.30
Maximum depth (cm)		4 – 62	29.74	5 – 200	34.53	20 – 400	64.36
% Natural land use		2.56 – 49.32	24.04	0.37 – 100	45.88	13.52 – 37.97	25.56
% Human land use		0 – 0.82	0.34	0 – 5.21	0.31	0.22 – 0.68	0.52
% Water bodies		0 – 0.98	0.23	0 – 7.60	0.15	0.59 – 1.13	0.80
% Dry agriculture		44.69 – 96.71	69.77	0 – 99.02	51.83	61.08 – 79.61	68.88
% Irrigated agriculture		0 – 26.64	5.63	0 – 82.40	1.56	0.14 – 5.58	4.23
Drainage area (km^2)	$\log_{10}$(X)	26 – 10422	1076	2 – 2709	338	8355 – 49690	27222
Distance from the source (km)	$\log_{10}$(X)	8.59 – 185.87	62.55	5.34 – 225.73	34.31	212.50 – 685.50	417.46
Stream order		1 – 4		1 – 4	1.80	4 – 6	4.65
Altitude (m a.s.l.)	√X	600 – 984	762.33	80 – 750	374.21	162 – 584	359.26
Slope (‰)	$\log_{10}$(X+1)	0 – 33.82	5.89	0 – 37.57	6.67	0 – 2.64	1.06
Population density (ind/km^2)	$\log_{10}$(X+1)	0 – 46.36	13.62	0 – 459.28	22.65	21.89 – 31.30	26.79

7.3. RESULTS

7.3.1. BENTHIC ALGAL BIOMASS AND OTHER COMMUNITY METRICS

Benthic algal biomass (in terms of chlorophyll–*a*) was lower during spring (ranging from 0.16 mg m^{-2} to 1300 mg m^{-2}) than during winter (ranging from 0.62 mg m^{-2} to 3171 mg m^{-2}). About 30% of the samples presented low chlorophyll concentration (<20 mg/m^2, within the range of oligotrophic sites). All these samples correspond to headwater streams. About 22% of the samples could be considered mesotrophic since the chlorophyll concentrations ranged between 20 and 70 mg/m^2. These occurred in localities situated in the middle part of streams, in rather undisturbed conditions. The highest concentrations of benthic chlorophyll (eutrophic sites) occurred in areas of high population density and relevant agricultural practices. While most of these sites were located in the mid river reaches of the siliceous ecotype, in the calcareous ecotype all the sampling localities presented eutrophic values of chlorophyll, including the first order headwaters (Figure 7.1). Finally, hypereutrophic conditions were described as those presenting chlorophyll–*a* values higher than 600 mg/m^2, and these principally occurred in main watercourses, independently of the substrate type. Algal biomass was significantly higher in winter; more than 75% of the sites had higher chlorophyll–*a* concentrations in winter than in spring period.

Diatom density showed significant differences between sampling periods, being higher during spring season. The covariance analyses showed that the increase in chlorophyll–*a* concentration was related to the increase in diatom density in the two periods. That the regression slope was significantly higher during spring indicates that diatom community was growing faster during this period. The increase in Chlorophyll–*a* was correlated to a decrease in Margalef ratio in spring whereas in winter Margalef ratio increased (Tables 7.2 and 7.3, Figures 7.2 A, and B). Diatom richness and IPS index showed no differences between sampling periods, however both parameters exhibited a significant linear decrease with increasing chlorophyll concentration (Tables 7.2 and 7.3, Figures 7.2 C and D). Finally, macroinvertebrate richness and ASPT index were significantly higher in winter, showing a linear decrease with increasing chlorophyll concentration (Tables 7.2 and 7.3, Figures 7.2 E and F).

Table 7.2. Descriptive statistics for the biological variables measured. Results of one–way ANOVA testing for differences in the 174 sampling points visited twice (*$p < 0.05$; **$p < 0.01$)

	SPRING (n=174)		WINTER (n=174)		ANOVA	
	range	mean	range	mean	F	p
Algal biomass (Chl−*a* mg/m^2)	0.2 – 1299	117	0.6 – 1425	291	35.2	**
Margalef Ratio D_{430}/D_{665}	1.3 – 8.5	2.7	1.6 – 6.3	2.5	3.8	0.06
Diatom density (cells/cm^2)	0.8 – 1230	114.3	0.8 – 680	48.8	17.1	**
Diatom richness	7 – 60	32.5	6 – 56	32.3	0.1	0.84
Diatom Shannon–Wiener diversity	0.4 – 5.2	3.2	0.4 – 4.7	3.2	0.2	0.62
IPS	1.2 – 19.8	11.1	1.8 – 20	11.1	0.0	0.99
Macroinvertebrate richness	3 – 35	14	2 – 34	15.2	4.1	*
ASPT	1.7 – 6.2	4.1	1.5 – 6.65	4.4	18.6	**

Table7.3. Analysis of covariance testing for a relationship between chlorophyll–*a* and community metrics (biological parameters) over two sampling periods: winter and spring across the 425 samples obtained (** p<0.001)

	chlorophyll (mg/m^2)		time		chl * time	
	F	p	F	p	F	p
D_{430}/D_{665}	14.80	**	7.29	**	8.18	**
Diatom density	16.44	**	3.48	0.06	18.18	**
Diatom richness	25.32	**	1.85	0.17	2.18	0.14
Diatom Shannon–Wiener diversity	1.12	0.29	0.02	0.88	0.15	0.70
IPS	8.84	**	0.84	0.36	1.53	0.22
Macroinvertebrate richness	28.60	**	13.39	**	2.20	0.14
ASPT	38.83	**	38.17	**	0.12	0.73

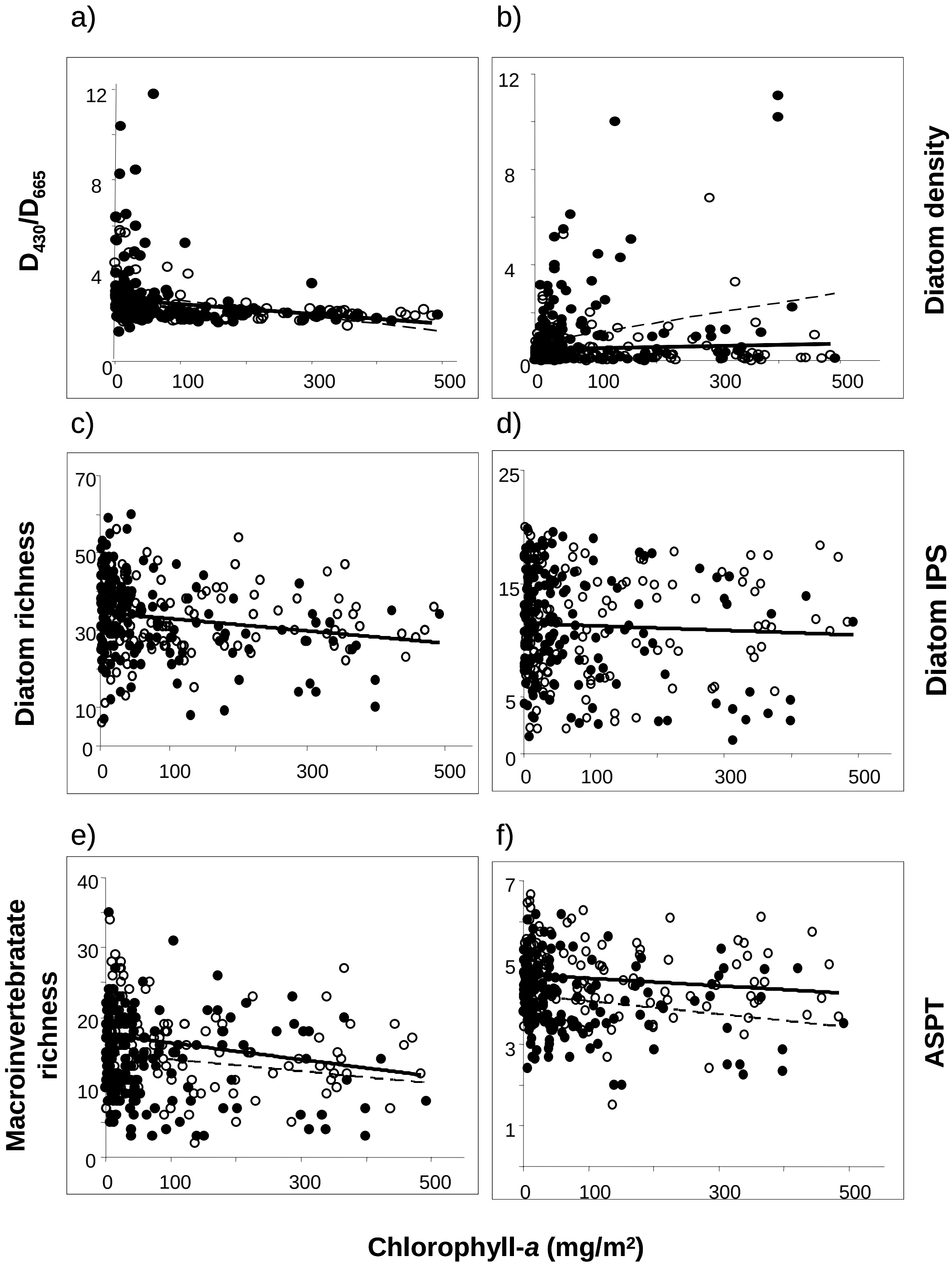


Figure 7.2: Relationship of chlorophyll–*a* concentration (mg/m^2) with community metrics during (●) spring and (○) winter periods. Dotted lines are the regression for the spring period, whereas solid lines are the regression for winter period. The slopes of A and B are significantly different (ancova $p<0.05$), while the slopes of C, D, E and F lines are not significantly different (ANCOVA, $p>0.05$). Every data point in the figure represents the average of three replicates

7.3.2. ALGAL BIOMASS MODELS

A matrix of 297 chlorophyll–*a* samples could be retained and related to the physical, chemical and physiographical variables. The descriptive statistics from this matrix is detailed in table 7.1.

Results of the PLSR analysis performed on the calcareous ecotype (n=42 samples) provided two significant latent vectors explaining 11 and 14% of the variance respectively. The first vector is positively related to pH and flow and negatively to temperature. The most important contributor to the second latent vector was the ammonia (related positively), while the presence of reservoirs upstream was negatively related to chlorophyll concentration (Table 7.4). The first two vectors resulting from the PLRS analysis performed on the siliceous ecotype (n=231 samples) explained 23 and 12 % of the variance respectively. Nitrate was the main positive contributor to both vectors, whereas temperature was negatively related to both vectors (Table 7.4).

The two latent vectors retained in the PLRS analysis performed on the main watercourses (n=24 samples) explained 42 and 16 % of the variance respectively the first one is positively related to conductivity, chloride, alkalinity and altitude and negatively related to naturalness of the catchment area. The second vector was positively related to nitrate concentration. Temperature was negatively influencing the two vectors (Table 7.4).

Relationships between mean chlorophyll values (observed) and the values predicted by the model, were represented in Figure 7.3. The R^2 of the regression slope represented to the total explanatory capacity of each latent vector (Y^2).

Table 7.4. Results of three PLRS performed on the calcareous, siliceous and main watercourses ecotypes. The table shows the weight of the original variables for the two first latent vectors extracted by each one of the PLSR analyses, as well as the regression coefficient (β). Higher β values (in absolute values) are highlighted in bold. Bottom rows show R^2 of X and and R^2 of Y

	calcareous ecotype (n = 42)			siliceous ecotype (n = 231)			main watercourses (n = 24)		
	V1	V2	β	V1	V2	β	V1	V2	β
Conductivity (µS/cm)	0.14	0.19	0.79	0.21	-0.07	0.12	**0.25**	-0.02	0.54
pH	**0.39**	0.23	0.29	0.16	0.15	0.03	-0.13	0.09	0.85
Dissolved oxygen (mg/L)	-0.07	-0.15	-0.03	0.12	0.19	0.00	-0.04	0.24	0.11
Temperature (ºC)	**-0.51**	-0.22	0.00	-0.47	**-0.72**	-0.05	**-0.42**	**-0.76**	-0.08
Ammonia (mg/L)	0.18	**0.36**	1.25	0.14	0.07	0.08	0.19	-0.03	0.09
Nitrate (mg/L)	0.27	0.19	0.20	**0.38**	0.34	0.27	0.24	**0.30**	0.29
Soluble reactive phosphorus (mg/L)	-0.11	0.12	-1.48	0.14	-0.07	0.04	0.16	-0.06	1.77
Sulfate (mg/L)	0.11	-0.04	0.18	0.02	-0.25	-0.08	0.27	0.09	-0.71
Chloride v	-0.14	0.07	-0.37	0.19	-0.11	0.06	**0.29**	0.05	-0.20
Calcium (mg/L)	0.08	-0.10	-0.85	0.19	-0.15	-0.03	0.27	0.10	5.24
Alkalinity	-0.05	0.18	-0.40	0.17	-0.16	0.02	**0.28**	0.03	-5.03
Flow m^3/s	**0.44**	0.14	0.78	0.22	0.19	0.06	-0.18	-0.10	0.05
Maximum depth (cm)	0.28	-0.13	0.00	-0.12	-0.16	0.00	-0.02	0.06	0.00
% Natural land use	-0.03	-0.18	0.85	-0.20	0.10	0.02	**-0.26**	0.03	0.10
% Human land use	-0.09	0.25	1.42	0.23	0.08	0.14	0.10	-0.21	0.84
% Water bodies	-0.14	**-0.51**	0.01	0.12	0.19	0.10	-0.11	0.12	-0.67
% Dry agriculture	-0.07	0.23	0.85	0.18	-0.09	0.02	0.25	-0.05	-0.16
% Irrigated agriculture	0.22	-0.14	0.85	0.11	0.00	0.01	0.13	0.10	0.78
Drainage area (km^2)	0.06	-0.07	0.38	0.24	0.02	0.02	-0.08	0.19	2.10
Distance from the source (km)	0.03	-0.07	0.27	0.22	0.01	0.11	-0.13	0.13	-0.89
Stream order	0.13	0.07	-0.09	0.12	0.01	0.01	-0.11	0.19	0.77
Altitude (m a.s.l.)	0.15	0.26	0.10	0.03	0.22	0.01	**0.28**	0.10	0.22
Slope (‰)	-0.08	0.03	0.13	-0.14	0.04	-0.12	0.01	-0.07	4.33
Population density (ind/km^2)	0.01	0.26	0.03	0.22	-0.01	-0.01	0.05	-0.21	15.09
R^2 of X	0.52	0.10		0.24	0.13		0.47	0.24	
R^2 of Y	0.11	0.14		0.23	0.12		0.42	0.16	

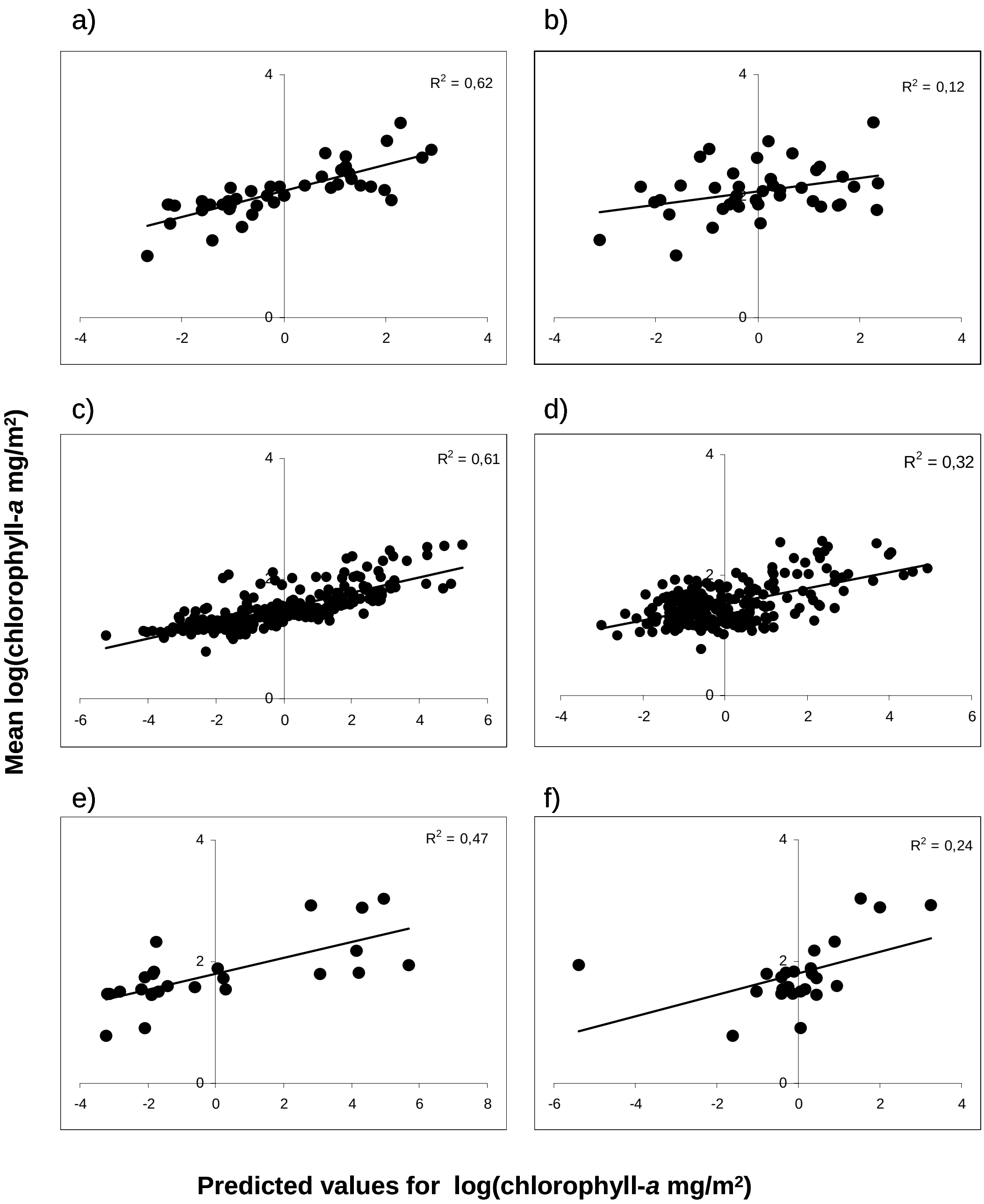


Figure 7.3: Linear relationships between predicted values of chlorophyll−*a* for each retained latent vector and observational chlorophyll−*a* values (R^2 of the slope correspond to the Y^2 of the model). A and B correspond to calcareous ecotype, C and D to siliceous ecotype, and E and F to main watercourses

7.4. DISCUSSION

The values of benthic algal biomass in the Guadiana are within the range of agricultural watersheds (Munn*, et al.*, 2010), which are usually characterized by large nutrient concentrations (Welch*, et al.*, 1988, Flinders*, et al.*, 2009). Many parts of the Guadiana watershed, presented chlorophyll values characteristic of eutrophic water masses, including the first order headwaters of the calcareous ecotype. These observations may be a consequence of the extensive pollution that these waters have been receiving through nitrate–rich fertilizers (Moreno*, et al.*, 2006).

The positive relationship between algal biomass and diatom density reinforces the relevance of diatoms as the major photosynthetic component of the periphyton. The significantly higher regression slope during spring season indicated the higher growth during this period, and was related to the lowest values of Margalef ratio index, indicating the active growth of benthic algal community during that period (Estrada, 1978, Sabater, 1988).

Nutrients are the most common key variables explaining benthic chlorophyll–*a* concentration (Sabater and Admiraal, 2005). However, results from the literature range from models showing no relationships between nutrients and benthic algal biomass (Munn*, et al.*, 1989, Kjeldsen, 1994) to others linking nutrient inputs with increasing periphyton biomass. Amongst those r^2 values range from 0.05 to 0.6 (Biggs and Close, 1989, Lohman*, et al.*, 1992, Dodds*, et al.*, 1998b, Dodds*, et al.*, 2002, Flinders*, et al.*, 2009), showing the relative lower or higher relevance of these empirical expression. Although these models could be useful, the strength of the model is commonly low because many other factors including hydrodynamics, grazing, turbidity, riparian shading, human impacts and large–scale factors, such as land uses or geology are not considered (Stevenson, 1997). In order to avoid this constraint we have used a model including 24 factors operating at different scales.

Nitrates were the key factor explaining chlorophyll–*a* variation in the three ecotypes of the Guadiana watershed when the whole set of physical, chemical and physiographical factors were considered. The three studied areas contained elevated SRP concentration, greater than 30 µg/L, and arguably this nutrient was not limiting the algal biomass (Dodds*, et al.*, 1997, Sabater and Admiraal, 2005). This could justify that the SPR did not play an important role in any developed model, as succeeded in some other agricultural streams (Munn*, et al.*, 2010). Streams receiving agricultural and urban sewage waters have a surplus of nutrients that can produce even higher growths of benthic algae (Leland, 1995). Chlorophyll–*a* values higher than 100 mg/m^2, and peaking up to 1000 mg/m^2 have been reported in rivers subjected to high pollution (Odum, 1957, McConnell and Sigler, 1959, Bombowna, 1972, Margalef, 1983). The very high chlorophyll–*a* detected in some areas or sites of the Guadiana may be the result of combined nutrient (phosphates and nitrates) concentrations, high light irradiances in shallow waters, and steady hydrological conditions. The period when the sampling occurred was characterized by the absence of remarkable rain episodes and extremely low water flows (MMA, 2006b, 2006c, 2006a, 2006d). This situation probably reinforced the high nutrient concentrations and allowed the mass algal growth in open areas.

Water temperature was an important factor in the three models developed, being negatively related to chlorophyll–*a*. Temperature is behind the chlorophyll–*a* values being distinctly lower in spring (mean = 117.02) than in winter (mean = 368.35), when the temperature was lower. Other observations of higher chlorophyll–*a* during winter months (Dubé and Scrimgeour, 1997) could be related with the dynamics of the riparian vegetation foliation. This might be mostly applicable to the headwaters of the Guadiana watershed. While in spring there is a dense canopy that shades out the stream, resulting in a light limitation of the periphyton community (Acuña*, et al.*, 2004), in winter there is not such a limitation. It has been described in Mediterranean forested streams, that pulses of light availability are highly significant for primary producers, mostly because water temperature does not limit algal growth (Guasch and Sabater, 1995) and natural variation in light, temperature, and water flow in the stream conflicts with the potential effect of nutrients on algal communities (Veraart*, et al.*, 2008). Further, winter water temperatures are unlikely limiting algal activity in the Guadiana.

Watershed land uses were revealed as another key factor in the three developed models. In the calcareous ecotype the presence of reservoirs enhancing fine sediments, and retaining nutrients resulted in lower values of chlorophyll–*a* downstream. The percent of natural land was inversely related to chlorophyll–*a* content in the main watercourses type as well as in the siliceous ecotype, where human activities also play an important role in determining chlorophyll–*a* content. These findings are in agreement with earlier studies (Allan*, et al.*, 1997, Stendera and Johnson, 2006) that showed that urban land use, or naturalness of the catchment were important factors in explaining variability in stream water chemistry. Effects of nutrients, light, and stream–size on benthic algae were evident in some studies only after accounting for large–scale ecological constraints, such as land use, geology and climate (Johnson*, et al.*, 1997, Stevenson, 1997).

Even though some variables were not considered in this study (e.g. grazing or substrata type), our approach confirmed the relevance of using both local and large–scale factors in models used to predict algal biomass. While conductivity and nutrient content were the main local or proximate factors related to algal biomass distribution, large−scale factors such as flow and land uses were necessary to understand the biomass patterns in the agricultural, Mediterranean watersheds.

7.5. CONCLUSIONS

This study demonstrated the importance of including local and large–scale factors in models that predict algal biomass. In Guadiana watershed, as was the case in Mid−Atlantic and eastern streams of United States (Pan*, et al.*, 1999, Hill*, et al.*, 2003), ion content (summarized in conductivity and nutrient content) was the main local −proximate− factor related to algal biomass distribution. Regarding to large−scale −ultimate− factors algal distribution depends on flow, and land uses as it was also shown for large agricultural basins of USA (Munn*, et al.*, 2010).

CHAPTER VIII

COMPARATIVE STUDY OF ALGAL COMMUNITIES IN ACID AND NON–ACID WATERS FROM TINTO–ODIEL SYSTEM AND PIEDRAS RIVER BASINS

8.1. INTRODUCTION

The Iberian Pyrite Belt (IPB) is one of the most extensive sulfide mining regions in the world, and it ranges from SW Spain to the Portuguese Atlantic coast. Associated with the complex sedimentary materials in this area, many massive sulphide deposits occur, and they have been explored and mined for more that 5000 years (Nocete*, et al.*, 2005). Minas de Río Tinto has been intensively exploited during the Phoenician and Roman periods and again during the 19th century, but about 80 mines have been operative during the last hundred years (Sáez*, et al.*, 1999).

The Tinto and Odiel river systems have their source in the Sierra de Aracena, at an altitude of 900 m a.s.l. Part of their courses run over the IPB, and they are seriously affected by acid mine drainage. Even though there is no active mining nowadays, pollution continues to arrive to the watercourses due to the oxidation of mining wastes (Nieto*, et al.*, 2007). These waters can be defined as an extreme habitat in terms of their very low mean pH (near 2.5) and high concentration of heavy metals, especially ferric iron, copper and zinc, as well as some anions such as sulfate (López–Archilla and Amils, 1999). Only extremophilous taxa can survive on these situations of very low pH (López–Archilla*, et al.*, 2001) and high heavy metals precipitation (Niyogi*, et al.*, 2002, Gerhardt*, et al.*, 2008). The Tinto and Odiel rivers converge into a common coastal wetland, with marked tidal influence. Around this salt marsh zone there is an intensive agricultural, industrial and urban development (MMA, 2005).

The Piedras river basin is a short stream with very restricted fluvial catchment located between the Guadiana and the Tinto–Odiel system. The final part of this river ends into an extensive and well delimited estuary. Regarding the human occupation, the river is divided in two main zones: the upper part which is of low density and with scarce crops, and the middle–lower part which is more densely populated and includes extensive irrigations (MMA, 2005). In this area, two reservoirs enormously alter the natural fluvial regime.

This study will analyze the relationship between the physico–chemical characteristics of the three fluvial systems and their respective algal floras (diatom, other microalgae and cyanobacteria). This study includes both zones influenced by AMD (López–Archilla*, et al.*, 2001, Sabater*, et al.*, 2003a, Aguilera*, et al.*, 2006, Aguilera*, et al.*, 2007a), and areas unaffected by AMD of which algae communities have not been described to present.

8.2. DATA ANALYSIS

All data were scrutinized for normality, and some transformations were used in order to achieve the homogeneity of variances. A square root transformation was applied to diatom data rather than a log transformation, since it was desirable to retain zero values. Environmental variables, except for pH and percentage variables were also transformed by $\log_{10}$ (x+1).

Species richness (R), Shannon–Wiener diversity (H') (Shannon and Weaver, 1963) and density (D=number of cells/cm^2) were calculated from the taxonomic composition of diatom samples.

Relationships from these biological parameters (R, H', E and D) between them and among the environmental variables were analyzed by using Spearman rank correlations for the non–parametric variables (biological), and Pearson rank correlations for parametric variables (physico–chemical and land uses). All these analyses were performed using SPSS v.15 (SPSS-Inc., 2004).

Sampling stations were classified into groups based on environmental data using a hierarchical cluster analysis applying the farthest neighborhood method and Bray–Curtis similarity distance. One–way ANOVA was used to test for significant differences in environmental data among the main cluster groups.

The association between diatom community assemblages and environmental data was analyzed at station level, and the attributes were plotted in an ordination by non–metric multidimensional scaling analysis (NMDS).

Finally, the floristic data for the stations were classified using a two way indicator species analysis (TWINSPAN), and indicator taxa for each previous group was determined by the IndVal analysis (Dufrène and Legendre, 1997).

A presence/absence matrix was constructed with the non–diatom algal community. A new cluster analysis was performed with this data using UPGMA method and Jaccard similarity distance.

All previous analyses were performed using Pc–Ord v.4 statistical package (McCune and Mefford, 1999).

8.3. RESULTS

8.3.1. PHYSICOCHEMICAL VARIABLES *VERSUS* BIOLOGICAL PARAMETERS

AMD streams in this study were well characterized by environmental parameters, pH and conductivity being the most influential which exhibited extreme variations between the systems. Stations affected by AMD had significantly lower pH (average value of 3.44, ANOVA $p<0.05$ $F_{1,16}=102.75$) and high conductivity (average value of 2122 μS/cm, ANOVA $p<0.01$ $F_{1,16}=63.12$), while in stations not affected by AMD pH ranged from 7.95 to 8.95, and conductivity was near 400 μS/cm. Both parameters have been commonly used as indicators of AMD (e.g. Verb and Vis, 2000).

From the biological parameters, diatom diversity (ANOVA $p<0.01$ $F_{1,16}=19.73$) and taxa richness (ANOVA $p<0.01$ $F_{1,16}=61.59$) were significantly lower in waters affected by AMD (Table 8.1). Applied to non–diatom taxa, the two parameters were also lower in AMD sites.ç

Correlation analyses between physico–chemical and biological parameters were summarized in table 8.1. Percentage of mining land use, and conductivity affected negatively richness ($p<0.01$) and diatom diversity ($p<0.05$), while pH affected positively both parameters ($p<0.01$ for richness, and $p<0.05$ for diversity).

By contrast, diatom density was positively correlated with flow ($p<0.01$), distance from the source and drainage area ($p<0.05$).

Finally, algal biomass became independent from the physical parameters studied.

Table 8.1 Maximum, minimum and mean values of physico–chemical and biological variables for the entire watershed, for the acid affected stations, and alkaline ones. Parameters with significant differences between acid and non acid group were also shown. ANOVA (*p<0.05; **p<0.01)

	Entire watershed		Acid waters		Alkaline waters		
	Range	Mean	Range	Mean	Range	Mean	***p***
Conductivity (µS/cm)	145 - 3000	960	1520 - 3000	2122	145 - 926	379	** $F_{1,16}$=63.12
pH	2.71 - 8.95	6.72	2.71 - 4.67	3.44	7.67 - 8.95	8.19	$F_{1,16}$=102.75
Oxygen (mg/L)	2.50 – 13.70	11.59	2.50 - 13.70	10.87	10.20 - 13.20	11.59	
Temperature (ºC)	5.10 - 15.00	8.98	5.10 - 15.00	8.25	6.40 - 13.10	9.35	
Flow (m^3/s)	0.00 - 0.17	0.03	0.00 - 0.03	0.01	0.00 - 0.17	0.03	
Wet width (cm)	30 - 1825	487.19	250 - 1825	966.25	30 - 830	327.50	** $F_{1,16}$=6.44
Wet Perimeter (cm)	1833.45	430.08	1833.45	783.26	41.62 - 833.78	312.35	* $F_{1,16}$=5.92
Hydraulic Radius (cm)	2.99 - 59.81	12.45	2.99 - 59.81	18.24	3.60 - 39.50	10.52	
Maximum Depth (cm)	8 - 60	19.88	8.00 - 60.00	24.50	10.00 - 40.00	18.33	
IHF	34 - 81	62.06	34 - 53	47.75	49 - 81	66.83	** $F_{1,16}$=16.08
% Urban land use	0 - 2.59	0.33	0 - 0.32	0.14	0.00 - 2.59	0.42	
% Mining land use	0 - 52.17	6.04	0.73 - 52.17	17.97	0.00 - 0.73	0.08	* $F_{1,16}$=5.83
% Cultivated land use	0 - 36.86	14.81	0 - 36.17	12.59	2.57 - 36.86	15.92	
% Forested land use	47.52 - 97.43	78.57	47.52 - 90.12	69.02	62.67 - 97.43	83.34	
% Water Bodies land use	0 - 1.39	0.15	0 - 0.09	0.01	0.00 - 1.39	0.22	
Drainage Area (km^2)	11.03 - 572.73	114.00	572.73	195.48	25.13 - 227.70	73.26	
Distance from the source (km)	4.83 - 52.39	18.18	4.83 - 52.39	22.8	10.34 - 23.46	15.86	
Altitude (m a.s.l)	35 - 500	171.72	35 - 500	197	35 - 300	159.08	
Diatom richness (R)	4 - 43	23.44	4 - 12	6.67	11 - 43	31.83	** $F_{1,16}$=61.59
Diatom diversity (H')	0.26 - 3.98	2.20	0.26	0.99	1.10 - 3.98	2.80	** $F_{1,16}$=19.73
Diatom density ($cells/cm^2$)	518 - 1339.10	27224	518	18833	1096 - 1E+05	31420	
Algal biomass (mg/m^2)	4.04 - 168.85	38.60	4.04	24.51	8.20 - 168.85	51.80	

8.3.2. BENTHIC DIATOM COMMUNITY STRUCTURE

A total of 108 diatom taxa were identified from the 18 stations, but only those taxa with relative abundances higher than 1.5% were included in the analyses in order to minimize the influence of rare taxa. A total of 62 taxa were therefore included in the analysis.

Final stress for the NMDS analysis was 12.33 (<17) indicating a reliable ordination (McCune and Mefford, 1999). Axis 1 and 2 explained 61.4% and 16.6% of the total variance respectively, indicating a strong ordination in the first axis, where the acidic stations were delineated from the non–acidic stations as it was expected because of the importance of pH.

AMD stations consistently grouped together on the left side of the NMDS axis–1 where the % of mining soil use and the conductivity were higher while diversity and diatom richness were lower. Non–affected AMD localities were situated on the right part of the axis–1 where pH, diversity and richness were higher. The second axis was positively correlated ($p<0.05$) with the spatial components latitude and altitude indicating that even in rather small spatial scales, regional factors had some influence on diatom distribution (Table 8.2, Figure 8.1).

Table 8.2 Summary of physico–chemical parameters that significantly affect biological variables. Significant correlation of these physico–chemical variables with the axes of NMDS graph are also shown (*$p<0.05$; **$p<0.01$)

	Spearman's *r*			**Kendall's τ**	
	R	**H'**	**D**	**Axis 1**	**Axis 2**
Conductivity (µS/cm)	-0.61**	-0.57*		-0.29**	
pH	0.61**	0.56*		0.43**	
Flow (m^3/s)			0.59**		
Altitude (m asl)					0.30*
% Mining	-0.77**	-0.72**		-0.60**	
Drainage area (km^2)			0.50*		
Distance to source (km)			0.56*		
UTM Y					0.53*
Diatom diversity (H')				0.66**	

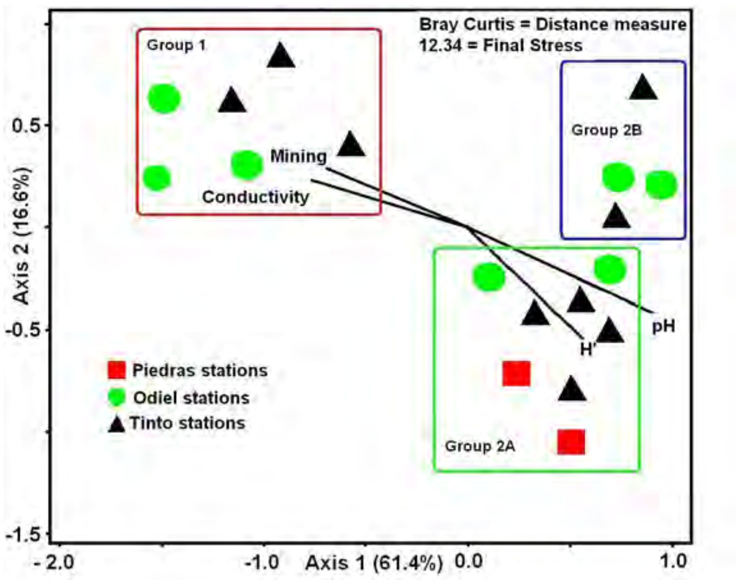


Figure 8.1. NMDS ordination graph based on diatom community. Three groups of localities were detected. Group 1 corresponded to the localities with pH< 4. The localities with pH> 6 were located to the right of the first axis. Among them we can discriminate two groups, 2B corresponded with upland localities with small and forested drainage areas, whereas 2A integrated stations situated in the lower part of the catchment with agricultural soil uses

The first TWINSPAN division primarily separated sampling stations according to pH (Figure 8.2, Table 8.3), i.e. the acidic–water stations (group 1) from the alkaline stations (group 2). IndVal analysis indicated that *Eunotia exigua*, *Pinnularia acoricola* and *P. subcapitata* were the most representative taxa for the acidic group of sites. A major division in the group 2 separated two further groups according to land use: group 2A which was integrated by stations placed in lowlands with high proportion of cropland and high human occupation. Indicator taxa for this group were *Fragilaria capucina*, *F. ulna*, *Navicula radiosa*, *N. schroeteri*, *N. veneta*, *N. viridula* and *Nitzschia palea*. The group 2B was integrated by upland and lowly human occupation sites, with clean waters. Representative taxa for this group were *Cocconeis placentula* var. *lineata*, *Gomphonema angustum*, *Planothidium frequentissimum and Rhoicosphenia abbreviata*.

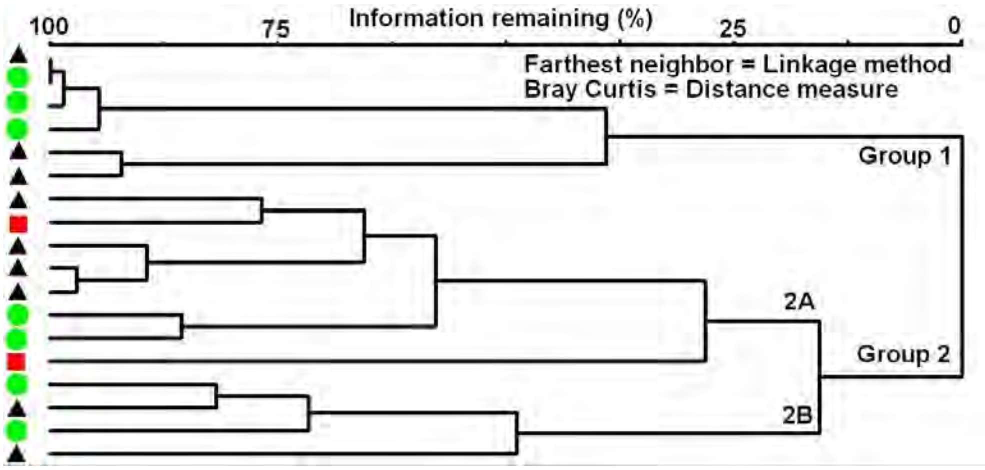


Figure 8.2. Dendrogram resulting of the hierarchic cluster analysis based on physico–chemical parameters

Table 8.3. Indicator diatom taxa from the 3 clusters. IndVal = Indicator Value in %. Only taxa with statistically significant Indicator Values were shown (*p<0.05 **p<0.01), based on Monte–Carlo test

Group 1 (n=6) Acid waters	**IndVal**
Eunotia exigua	97.2**
Pinnularia acoricola	83.3*
Pinnularia subcapitata	66.7*

Group 2A (n=8)	**IndVal**	**Group 2B (n=4)**	**IndVal**
Fragilaria ulna	78.3**	*Planothidium frequentissimum*	70.2**
Fragilaria capucina	75.0**	*Rhoicosphenia abbreviata*	68.1*
Navicula viridula	75.0**	*Cocconeis placentula*	68.1*
Nitzschia palea	71.2**	*Gomphonema angustum*	67.9**
Navicula veneta	63.2*		
Navicula radiosa	62.5**		
Navicula schroeteri	62.5*		

8.3.3. ALGAL (NON–DIATOM) COMMUNITY STRUCTURE

A total of 31 non–diatom taxa were identified from the 18 sampled sites (Table 8.4). A presence/absence matrix was constructed, and the resulting dendrogram broadly corroborated the previous diatom community analysis: the algal flora from acidic sampling stations was significantly poor and different from the algal flora present in alkaline waters.

Table 8.4 List of non–diatom algae found in Tinto–Odiel system and Piedras river watershed

Division	Order	Taxa
Heterokontophyta	Vaucheriales	*Vaucheria* sp.
Euglenophyta	Euglenales	*Euglena* sp01
		Euglena cf. *mutabilis* Schmitz
Chlorophyta	Volvocales	*Chlamydomonas* cf.
	Klebsormidiales	*Klebsormidium flaccidum* (Kützing) P.C. Silva, K.R. Mattox & W.H. Blackwell
	Chlorococcales	*Scenedesmus* cf. *magnus* Meyen
	Ulothricales	*Ulothrix* sp.
	Chaetophorales	*Draparnaldia glomerata* (Vaucher) C. Agardh
		Chaetophora sp.
		Gongrosira sp.
		Coelochaete sp.
	Oedogoniales	*Oedogonium* sp.
	Cladophorales	*Cladophora glomerata* (Linnaeus) Kützing
	Zygnematales	*Mougeotia* sp01
		Mougeotia sp02
		Spirogyra sp.
		Zygnema sp.
	Desmidiales	*Cosmarium botrytis* Meneghini ex Ralfs
		Closterium cf. *ehrenbergii* Meneghini ex Ralfs
		Micrasterias sp.
		Staurastrum cf. *dilatatum* Ehrenberg ex Ralfs
Rhodophyta		*Lemanea* sp.
Cyanophyta		*Aphanocapsa* sp.
		Calothrix sp.
		Coelosphaerium sp.
		Gloeocapsa sp.
		Gomphosphaeria sp.
		Lyngbia sp.
		Nostoc sp.
		Oscillatoria sp.
		Phormidium sp.

The algal community present in acidic waters was composed almost exclusively by *Klebsormidium flaccidum*, *Euglena mutabilis* and some unicellular chlorophyta like *Chlamydomonas* sp. All this taxa did not occur in alkaline waters. The algal community in alkaline waters was composed by a higher number of taxa. The assemblage included cyanobacteria (*Oscillatoria* and *Anabaena*), and chlorophyta: several genera of zygnemataceae (*Spirogyra*, *Zygnema* and *Mougeotia*) and desmidiaceae (*Cosmarium botrytis*, *Closterium ehrenbergii*, *Staurastrum dilatatum* and *Micrasterias* sp.).

8.3.4. BENTHIC CHLOROPHYLL CONCENTRATION

Chlorophyll–*a* in AMD stations ranged between 4 to 12 mg/m^2 whereas it ranged between 8 to 160 mg/m^2 in non–affected AMD sites.

In spite of that, there were no significant differences in chlorophyll–*a* (expressed as algal biomass in Table 8.1) between the two type of sites stations. In all the stations the values of chlorophyll–*a* did not exceed 200 mg/m^2, so the whole set of sites can be considered as mesotrophic (Dodds*, et al.*, 1998a)..

8.4. DISCUSSION

The Tinto–Odiel system constitutes an exteme environment for the aquatic life where water pH plays a very important role organizing the development of biological communities. The sites affected by AMD produce both chemical stress (low pH, dissolved heavy metals) as well as physical stress (deposition of metal oxides) on stream biota (Gerhardt*, et al.*, 2008).

As described in similar situations (Verb and Vis, 2000, Niyogi*, et al.*, 2002, Ross*, et al.*, 2008) diversity (H') and taxa richness (R) are significantly low in AMD affected systems, since few taxa are able to adapt to such situations. However, algal biomass is stable along all the sampling stations, probably because tolerant species compensate for the loss of sensitive species by increasing their biomass (Margalef, 1983). The existence of indirect effects, such as the suppression of grazing because of stress on the herbivores, may allow greater autotrophic biomass to develop than would be found in the absence of stress (Elwood and Mulholland, 1989, Niyogi*, et al.*, 2002). Algal biomass values in AMD stations are close to that observed in other nutrient rich systems elsewhere (Dodds*, et al.*, 1998a, Romaní and sabater, 2000) indicating that algae would significantly contribute to primary production of those acidic systems.

Heavily impacted sites consistently grouped together and were well characterized by environmental parameters being pH and conductivity the most influential. *Pinnularia acoricola* was the dominant taxon in AMD affected stations, accompanied by *Eunotia exigua* and *Pinnularia subcapitata*. All of these taxa have a cosmopolitan distribution in this low pH environments (DeNicola, 2000, Ivorra*, et al.*, 2000, Verb and Vis, 2000, Lörh*, et al.*, 2006). As water pH increases some other accompanying taxa like *Achnanthidium minutissimum* or *Nitzschia capitellata* appeared, both frequently reported in low–pH waters (Verb and Vis, 2000, Gerhardt*, et al.*, 2008). Some *Eunotia* species have been described as facultative heterotrophs (Hill, 1996). Such capacity may be important in acidic environments in order to significantly enhance scarce carbon and phosphorus supplies (Lessmann*, et al.*, 2000).

In not AMD–affected sampling sites and in the Piedras river basin, the diatom community is mostly distributed throughout an eutrophication gradient. In upstream sections, not highly affected by human activities, the sites could be considered as reference stations (Tison*, et al.*, 2005). In these situations pollution–sensitive taxa whose distribution is associated to geochemical variables (Leira, 2005) dominated. Amongst these occurred *Planothidium frequentissimum*, *Rhoicosphenia abbreviata* and *Cocconeis placentula* var. *lineata*. In localities situated in the middle and lower sections of the river nutrient–tolerant taxa like *Nitzschia palea* and *Navicula veneta*, dominated. Their occurrence could be related with the existence of phosphate–enriched or organically polluted waters (Fore and Grafe, 2002, Tornés*, et al.*, 2007), that can be associated to agricultural land use in these sections.

In AMD affected localities, the non–diatom algal community had low diversity. These were mainly dominated by *Klebsormidium* that produced long greenish filaments, and by motile cells of *Euglena mutabilis*. Both taxa are frequent in these environments (Olaveson and Nalewajko, 2000, Lörh*, et al.*, 2006). *Euglena mutabilis* have been shown to growth heterotrophically in culture (Tuchman, 1996) and in absence of light (Johnson, 1998).

Macroinvertebrate samples were collected in parallel with our study (Red Control, unpublished data). The macroinvertebrate taxa richness in AMD stations was lower than in non affected stations (Gray and Delaney, 2008). Only the chironomid genus *Lymnophyes* was present in all AMD affected stations, together with AMD tolerant coleopteran or hemiptera. *Lymnophyes* was previously described in the Tinto river basin (Sabater*, et al.* 2003) and in other AMD affected environments (Gerhardt*, et al.*, 2004, Lörh*, et al.*, 2006, Ross*, et al.*, 2008).

8.5. CONCLUSIONS

Our study confirmed the importance of pH on algal distribution by means of different multivariate analysis both in diatom and non–diatom algal communities.

AMD systems host few taxa capable to survive, but the relevance of primary producers in terms of biomass is not greatly affected.

CONCLUSIONS; CONCLUSIONES; CONCLUSIONS

CONCLUSIONS (ENGLISH)

1. The high diatom richness found confirms the wide geological and hydrodynamic heterogeneity of the Guadiana and related watersheds

2. Multivariate analyses, especially variance partitioning, is a reliable tool to interpret particular factors affecting diatom community composition in large river areas, in spite of the homogenizing role of disturbances. The anthropogenic impacts can influence but not override the regional and large scale patterns in community structure

3. Although water chemistry consistently played the most important role in structuring diatom assemblages in the Guadiana watershed, spatial factors such as altitude or geographic location also explained a relevant part of variation in diatom distribution

4. Establishing reference conditions is possible in the headwaters of the Guadiana only after application of permissive criteria. However, determining reference conditions in large river watercourses remains unreliable

5. In oligotrophic siliceous forested headwaters the type specific diatom indicator taxa were *Encyonema silesiacum* and *Tabellaria flocculosa.* The type specific diatom indicator taxa in calcareous localities were *Cymbella affinis*, *Navicula tripunctata* and *Diatoma moniliformis* accompanied by some other *Cymbella* species (e.g *C. helvetica*). The geochemical characteristic of the large watercourse ecotype was masked by the pollution impacts. The diatom community was characterized by *Nitzschia inconspicua* and *Gomphonema parvulum*

6. Ion content (summarized in conductivity and nutrient content) was the main local factor related to algal biomass distribution in the Guadiana watershed. Water flow and land uses were the main large−scale factors affecting algal biomass distribution. Altogether, the study showed the importance of including local and large–scale factors in models to predicting algal biomass

7. pH was revealed as a key factor on algal distribution in systems affected by AMD. The AMD systems host few taxa capable to survive, but the relevance of primary producers in terms of biomass is not greatly affected

CONCLUSIONES (CASTELLANO)

1. El elevado número de taxones hallados confirma la gran heterogeneidad geológica e hidrodinámica de las cuencas del Guadiana y asociadas

2. Los análisis multivariantes, y en concreto la partición d la variaza, se manifiestan como una buena herramienta que permite interpretar los patrones de distribución de las comunidades de diatomeas en las grandes cuencas a pesar de la homogeneidad de sus distribuciones. Los impactos antrópicos influencian, aunque sin emmascarar los patrones de de distribución de las comunidades algales a escala regional

3. A pesar que la química del agua juega un papel muy importante en la estructuración de las comunidades de diatomeas en la cuenca del Guadiana, factores espaciales tales como la altitud o la propia localización geográfica también contribuyen a la distribución de dichas comunidades

4. Es posible establecer condiciones de referencia en las zonas de cabecera de la cuenca del Guadiana utilizando criterios suficientemente permisivos. En los cursos principales de agua se hace imposible determinar dichas condiciones de referencia

5. En las cabeceras forestadas de los ríos silícicos, la comunidad de diatomeas de referencia incluye *Encyonema silesiacum* y *Tabellaria flocculosa*. Para los ríos calizos, al comunidad de referencia está integrada por *Cymbella affinis*, *Navicula tripunctata* y *Diatoma moniliformis* a menudo acompañadas por otras especies del género *Cymbella* (*C. silesiaca* y *C. helvetica*). Las características geoquímicas de los cursos principales de agua quedan totalmente enmascaradas por la polución de origen antrópico. La comunidad de diatomeas de estas

masas de agua incluye taxones como *Nitzschia inconspicua* y *Gomphonema parvulum*

6. El contenido iónico (resumido en la conductividad y los nutrientes) es el principal factor relacionado con la distribución de la biomasa algal en la cuenca del Guadiana. El caudal y los usos del suelo se revelan como los principales factores a gran escala que afectan a la distribución de la biomasa algal. Este estudio demuestra la importancia de incluir en los modelos predictivos tanto los factores locales como los generales

7. El pH se manifiesta como un factor clave en la distribución de las comunidades algales afectadas por el AMD. Son pocas las especies de productores primarios capaces de sobrevivir en los ambientes afectados por el AMD, aunque su biomasa no se vea severamente afectada

CONCLUSIONS (CATALÀ)

1. El gran nombre de tàxons presents en la zona confirma la gran heterogeneïtat geològica i hidrodinàmicade la conca del Guadiana i conques associades

2. Els anàlisi multivariants, i concretament la tècnica de la partició de la variança, han esdevingut una eina fonamental per permetre la interpretació dels patrons de distribució de les comunitats de diatomees en les grans conques, malgrat l'aparent homogeneïtat que presenten. Els impactes antropogènics poden influenciar, però no emmascarar, els patrons de distribució de les comunitats algals a escala regional

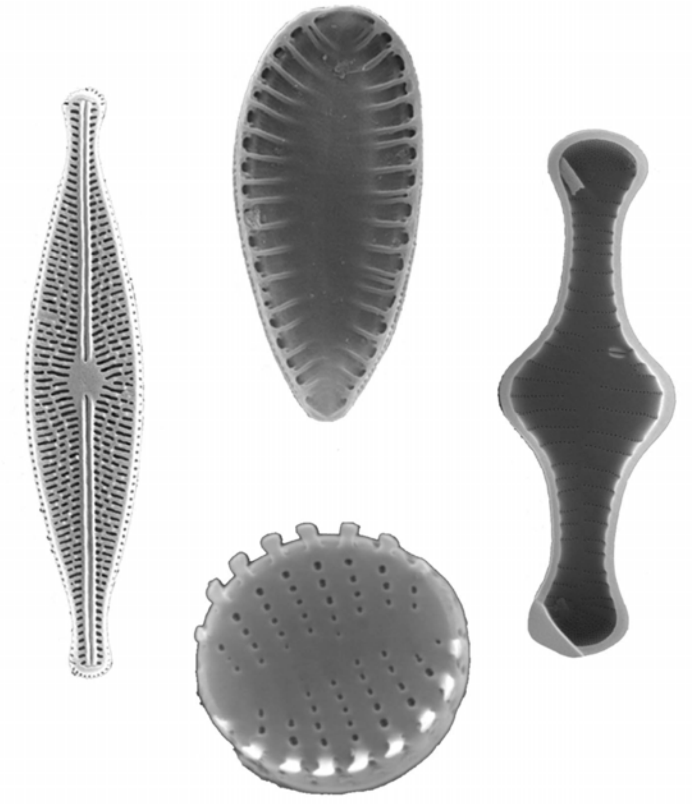
3. Malgrat que la química de l'aigua jugui un paper molt important en l'estructuració de les comunitats de diatomees de la conca del Guadiana, factors espacials com poden ser l'altitud o la pròpia localització geogràfica també estan influint en la distribució d'aquestes comunitats

4. És possible establir condicions de referència en las zones de capçalera de la conca del Guadiana si s'utilitzen criteris suficientment permissius. En els cursos principals es fa impossible determinar estacions de referència

5. En les capçaleres forestades dels rius silícics, la comunitat de diatomees de referència inclou *Encyonema silesiacum* i *Tabellaria flocculosa*. La comunitat de diatomees pròpia de les zones de referència dels rius calcaris està integrada per *Cymbella affinis*, *Navicula tripunctata* i *Diatoma moniliformis*, sovint acompanyades per altres espècies del gènere *Cymbella* (*C. silesiaca* y *C. helvetica*). Les característiques geoquímiques dels cursos principals d'aigua queden totalment emmascarades per la pol·lució d'origen antropogènic. La comunitat de diatomees que habita en aquestes masses d'aigua inclou tàxons com *Nitzschia inconspicua* i *Gomphonema parvulum*

6. El contingut iònic (resumit en la conductivitat i els nutrients) és el principal factor relacionat amb la distribució de la biomassa algal a la conca del Guadiana. El cabal i els usos del sòl es revelen com un dels principals factors a gran escala que afecten la distribució de la biomassa algal. Aquest estudi demostra la importància d'incloure en els models predictius tant els factors locals com els de caràcter més general

7. El pH es manifesta com un factor clau en la distribució de les comunitats algals afectades per l'AMD. Són molt poques les espècies de productors primaris que poden sobreviue en els ambients afectats per l'AMD, tot i que la seva biomassa no se'n vegi severament afectada



REFERENCES

Aboal, M. and Llimona, X., 1984. Aportación al estudio algológico del sistema de sierra de Ponce y Quípar (N.O. de Múrcia, S.E. de España). Anales de Biología 2: 1–17.

Aboal, M., 1987. Flora algal epicontinental de la cuenca del río Segura, SE de España. PhD. thesis, in Departamento de Botánica. Universidad de Múrcia, Murcia. pp.290

Aboal, M., 1989a. Epilithic algal communities from river Segura Basin, Southeastern Spain. Archiv für Hydrobiologie 116: 113–124.

Aboal, M., 1989b. Flora algal del río Benamor (Cuenca del Segura, SE España). Limnética 5: 1–11.

Aboal, M., Puig, M. A. and Prefasi, M., 1998. Diatom assemblages in springs in Castellón province, Eastern Spain. Algological Studies 90: 79–95.

Acuña, V., Giogi, A., Muñoz, I., Uehlinger, U. and Sabater, S., 2004. Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. Freshwater Biology 49: 960–971.

Aguilera, A., Manrubia, S. C., Gómez, F., Rodríguez, N. and Amils, R., 2006. Eukaryotic community distribution and its relationship to water physicochemical parameters in an extreme acidic environment, Río Tinto (southwestern Spain). Applied and Environmental Microbiology 72: 5325–5330.

Aguilera, A., Souza–Egipsy, V., Gómez, F. and Amils, R., 2007a. Development and structure of eukaryotic biofilms in an extreme acidic environment, Río Tinto (SW, Spain). Microbial Ecology 53: 294–305.

Aguilera, A., Zettler, E., Gomez, F., Amaral–Zettler, L., Rodriguez, N. and Amils, R., 2007b. Distribution and seasonal variability in the benthic eukaryotic community of Rio Tinto (SW, Spain), an acidic, high metal extreme environment. Systematic and Apllied Microbiology 30: 531–46.

Aizaki, M. and Sakamoto, K., 1988. Relationships between water quality and periphyton biomass in several streams in Japan. Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 23: 1511–1518.

Aldaya, M. M. and Llamas, R. M., 2008. Water footprint analysis for the Guadiana river basin. (eds.) Fundación Marcelino Botín Madrid, pp.114

Almagro–Costa, J., 2006. Las huellas del Guadiana. Madrid, pp.223

Almeida, S. and Gil, M. C., 2001. Ecology of freshwater diatoms from the central region of Portugal. Cryptogamie, Algologie 22: 109–126.

Álvarez–Blanco, I., 2009. Las diatomeas fluviales epilíticas de la parte española de la cuenca del Duero. Aspectos ecológicos y biogeográficos. PhD. Thesis, in Biodiversidad y Gestión Ambiental. Universidad de León, León.

Allan, J. D., Erickson, D. L. and Fay, J., 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshwater Biology 37: 149–161.

Alles, E., Nörpel–Schempp, M. and Lange–Bertalot, H., 1991. Zur Systematik und ökologie characteristicher *Eunotia*-Arten (*Bacillariophyceae*) in elektrolytarmen Bachoberlaufen. Nova Hedwigia 53: 171–213.

Amaral–Zettler, L., Gómez, F., Zettler, E., Keenan, B. G., Amils, R. and Sogin, M. L., 2002. Eukaryotic diversity in Spain's River of Fire. Nature 417: 137.

Amaral–Zettler, L., Messerli, M., Laatsch, A., Smith, P. and Sogin, M. L., 2003. From genes to genomes: beyond biodiversity in Spain's Río Tinto. Biological Bulletin 204: 205–209.

Anderson, E. L., Welch, E. B., Jacoby, J. M., Schimek, G. M. and Horner, R. E., 1999. Periphyton removal related to phosphorus and grazer biomass level. Freshwater Biology 41: 633–651.

Angeler, D. G., 2007. Resurrection ecology and global climate change research in freshwater ecosystems. Journal of North American Benthological Society 26: 12–22.

Antoniades, D., Douglas, M. S. V. and Smol, J. P., 2004. Diatom species–environment relationships and inference models from Isachsen, Ellef Ringnes Island, Canadian High Arctic. Hydrobiologia 529: 1–18.

Antonie, S. E. and Benson–Evans, K., 1983. Morphological variation in *Cocconeis placentula* Ehr. from the River Wye, Wales, U.K. Nova Hedwigia 38: 555–567.

APHA, 1989. Standard Methods for the examination of water and wastewater. (eds.) Washington, pp.1134

Armitage, P. D., Moss, D., Wrigh, J. F. and Furse, M. T., 1983. The performance of a new biological water quality score system based on macroinvertebrate over a wide range of unpolluted running–water sites. Water Research 17: 333–347.

Barbosa, A. B., Domingues, R. B. and Galvao, H. M., 2010. Environmental Forcing of Phytoplankton in a Mediterranean Estuary (Guadiana Estuary, South–western Iberia): A Decadal Study of Anthropogenic and Climatic Influences. Estuaries and Coasts 33: 324–341.

Bennion, H., Fluin, J. and Simpson, G. L., 2004. Assessing eutrophication and reference conditions for Scottish freshwater lochs using subfossil diatoms. Journal of Applied Ecology 41: 124–138.

Beyens, L., 1982. On the Difference in Ecology between *Tabellaria fenestrata* and *Tabellaria focculosa* (Diatomophyceae). Bulletin De La Societe Royale De Botanique De Belgique 115: 6–13.

Biggs, B. J. F. and Close, M. E., 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshwater Biology 22: 209–231.

Biggs, B. J. F. and Gerbeaux, P., 1993. Periphyton development in relation to macro–scale (geology) and micro–scale (velocity) limiters in two gravel bed rivers, New Zealand. New Zealand Journal of Marine and Freshwater Research 27: 39–53.

Biggs, B. J. F., 1995. The contribution of flood disturbance, catchment geology and land use to the habitat template of perphyton in stream ecosystems. Freshwater Biology 33: 419–438.

Biggs, B. J. F., 1996. Patterns in benthic algae of streams. In: Algal ecology. Stevenson, R. J. (eds.): 31–56. Academic Press, Inc. San Diego, California

Biggs, B. J. F., Stevenson, R. J. and Lower, R. L., 1998. A habitat matrix conceptual model for stream periphyton. Archiv für Hydrobiologie 143: 21–56.

Biggs, B. J. F., 2000. Eutrophication of streams and rivers: dissolved nutrient–chlorophyll relationships for benthic algae. Journal of North American Benthological Society 19: 17–31.

Blanco, S., Ector, L., Huck, V., Monnier, O., Cauchie, H., Hoffman, L. and Bécares, E., 2008. Diatom assemblages and water quality assessment in the Duero basin (NW Spain). Belgium Journal of Botany 141: 39–50.

Bombowna, M., 1972. Primary production in a montane river. Productivity problems of freshwater. In: Proceedings of the IBP-UNESCO Symposium on productivity problems of freshwater. Kajak, Z. and Hillbricht–Ilkowska, A. (eds.):

Bonada, N., Prat, N., Munné, A., Rieradevall, M., Alba–Tercedor, J., Álvarez, M., Avilés, J., Casas, J., Jáimez–Cuéllar, P., Mellado, A., Moyà, G., Pardo, I., Robles, S., Ramón, G., Suárez, M. L., Toro, M., Vidal–Abarca, M. R., Vivas, S. and Zamora–Muñoz, C., 2002. Criterios para la selección de condiciones de referencia en los ríos mediterráneos. Resultados del proyecto GUADALMED. Limnética 21: 99–114.

Borcard, D., Legendre, P. and Drapeau, P., 1992. Partialling out the spatial components of ecological variation. Ecology 73: 1045–1055.

Bort, S., Rojo, C., Rodrigo, M. A. and Maidana, N., 2005. El fitoplancton de Lagunas de Ruidera (Parque Natural, Ciudad Real, España). Limnetica 24: 33–46.

Bourrelly, P., 1957. Les algues d'eau douce. initiation à la systematique. I Les Algues Vertes. (eds.) Paris, pp.182

Bromley, J., Cruces, J., Acreman, M., Martínez, L. and Llamas, M. R., 2001. Problems of sustainable groundwater management in an area of over–exploitation: The Upper Guadiana catchment, Central Spain. Water Resources Development 17: 379–396.

Buczkó, K., 2007. The occurrence of the epiphytic diatom *Lemnicola hungarica* on different European Lemnaceae species. Fottea 7: 77–84.

Cambra, J. and Perera, M., 1988. Nota sobre la flora acuática de los embalses deVall–Llosera (Barcelona, ESpaña). Anales de Biología 15: 131–134.

Cambra, J., 1991a. Contribució a l'estudi de les algues epifítiques dels estanys de Banyoles, Basturs i Estanya. Orsis 6: 27–44.

Cambra, J., 1991b. Contribució al coneixement de les algues epifítiques en estanys dels Pirineus. SCIENTIA gerundensis 17: 17–27.

Cambra, J., 1992a. Variaciones estacionales de las algas epifíticas de macrófitos en algunos sistemas lacustres de Cataluña. Acta Botanica Malacitana 17: 5–18.

Cambra, J., 1992b. Distribución de las algas epifíticas en sistemas lacustres de Cataluña (N.E. de España). Botanica Complutensis 17: 23–45.

Cambra, J., 1993. Contribució al coneixement de les algues epifítiques en llacunes litorals. Collectanea Botanica 22: 25–38.

Cambra, J. and Gomà, J., 1997. Flood effects on algal biodivesity in a mediterranean river. Lagascalia 19: 463–478.

Cambra, J., Gomà, J., Huck, V. and Ector, L., 2003. 2ª fase de diseño de la red de diatomeas en la cuenca del Ebro. CHE. Zaragoza. pp.52

Cambra, J., Ector, L. and Sabater, S., 2005. Protocolos de muestreo y análisis para Microalgas Bentónicas. Métodos Para el establecimiento del Estado Ecológico según directiva Marco del Agua. pp.33

Cambra, J. and Ortiz–Lerin, R., 2005 Red de intercalibración, red de referencia y red básica de diatomeas en la cuenca del Ebro. Resultados verano 2005. CHE, Zaragoza. pp.92

Çamdevýren, H., Demýr, N., Kanik, A. and Keskýn, S., 2005. Use of principal component scores in multiple linear regression models for prediction of Chlorophyll–*a* in reservoirs. Ecological Modeling 181: 581–589.

Cantonati, M., 1998. Diatom communities of springs in the Southern Alps. Diatom Research 13: 201–220.

Cantoral–Uriza, E. A. and Aboal, M., 2008. Diatomeas (Bacillariophyceae) del marjal Oliva–Pego (Comunidad Valenciana, España). Anales del Jardín Botánico de Madrid 65: 111–128.

Carr, G. M., Chambers, P. A. and Morin, A., 2005. Periphyton, water quality, and land use at multiple spatial scales in Alberta rivers. Canadian Journal of Fisheries and Aquatic Sciences 62: 1309–1319.

Carrascal, L. M., Galván, I. and Gordo, O., 2009. Partial least squares regression as an alternative to current regression methods used in ecology. Oikos 118: 681–690.

Cassie, V. and Cooper, R. C., 1989. Algae of New Zealand thermal areas. Cramer, J. (eds.) Berlin, Stuttgart. ,

CEN, E. C. F. S., 2002. Water quality–Guidance standard for the routine sampling and pretratment of benthic diatoms from rivers for water quality assessment.

CEN, E. C. F. S., 2004. Water Quality - Guidance Standard For The Identification, Enumeration And Interpretation Of Benthic Diatom Samples From Running Waters.

Ciniglia, C., Cennamo, P., De Stefano, M., Pinto, G., Caputo, P. and Pollio, A., 2007. *Pinnularia obscura* Krasske (Bacillariophyceae, Bacillariophyta) from acidic environments: characterization and comparison with other acid–tolerant *Pinnularia* species. Fundamental and Applied Limnology 170: 29–47.

Clarke, K. R., 1993. Non–parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143.

Clarke, K. R. and Gorley, R. N., 2005. Plymouth Routines In Multivariate Ecological Research (PRIMER).

Cleve, 1894. Synopsis of the naviculoid diatoms. Svenska, K. (eds.) pp.194

Coesel, P. F. M., 1993. Poor physiological adaptation to alkaline culture conditions in *Closterium acutum* var. *variable*, a planktonic desmid from eutrophic waters. European Journal of Phycology 28: 53–57.

Comín, F., 1984. Características físiscas y químicas de las lagunas costeras, Enañizada, Tancada y Buda (Delta del Ebro). Oecologia aquatica 7: 79–162.

Conan, C., de Marsily, G., Bouraoui, F. and Bidoglio, G., 2003. A long–term hydrological modelling of the Upper Guadiana river basin (Spain). Physics and Chemistry of the Earth 28: 193–200.

Coste, M. and Richard, M., 1980. Observation in microscopipe photonique de quelques *Nitzschia* nouvelles ou interessantes. Cryptogamie Algologie 1: 187–212.

Coste, M., 1982. Etude des méthodes biologiques quantitatives d'appréciation de la qualité des eaux. Cemagref

Chen, G., Dalton, C., Leira, M. and Taylorchem, D., 2008. Diatom-based total phosphorus (TP) and pH transfer functions for the Irish Ecoregion. Journal of Paleolimnology 40: 143–163.

Chételat, J., Pick, F. R., Morin, A. and Hamilton, P. B., 1999. Periphyton biomass and community composition in rivers of different nutrient status. Canadian Journal of Fisheries and Aquatic Sciences 56: 560–569.

CHG, 2005. Caracterización de las masas de aga superficiales. Informe resumen de los artículos 5 y 6 de la Directiva Marco del Agua. Demarcación del río Guadiana (parte española) y ámbito complementario de los ríos Tinto, Odiel y Piedras. MMA pp.40

Cho, K. H., Kang, J. H., Ki, S. J., Park, Y., Cha, S. M. and Kim, J. H., 2009. Determination of the optimal parameters in regression models for the prediction of chlorophyll–*a*: A case study of the Yeongsan Reservoir, Korea. Science of the Total Environment 407: 2536–2545.

de Jonge, M., de Vijuer, B. V., Blust, R. and Bervoets, L., 2008. Responses of aquatic organisms to metal pollution in a lowland river in Flanders: A comparison of diatoms and macroinvertebrates. Science of the Total Environment 407: 615–629.

de la Pena, S. and Barreiro, R., 2008. Biomonitoring acidic drainage impact in a complex setting using periphyton. Environmental Monitoring and Assessment

Dell'Uomo, A., 2004. L'indice diatomico di eutrofizzazione/pollutione (EPI-D) nel monitoraggio delle acque correnti. Agenzia per la protezione dell'ambiente e per i servizi tecnici.

DeNicola, D. M., 2000. A review of diatoms found in highly acidic environments. Hydrobiologia 433: 111–122.

DeNicola, D. M., Eyto, E., Wemaere, A. and Irvine, K., 2004. Using epilithic algal communities to asses trophic status in Irish lakes. Journal of Phycology 40: 481–495.

Descy, J. P., 1979. A new approach to water quality estimation using diatoms. Nova Hedwigia 64: 305–323.

Descy, J. P. and Ector, L., 1999. Use of diatoms for monitoring rivers in Belgium and Luxemburg. In: Use of algae for monitoring rivers, III. Prygiel, J., Whitton, B. A. and Bukowska, J. (eds.): 128–137. Agence de l'eau Artois – Picardie.

Denys, L., 2009. Palaeolimnology without a core: 153 years of diatoms and cultural environmental change in a shallow lowland lake (Belgium). Fottea 9: 317-332.

Dodds, W., Smith, V. H. and Zander, B., 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. Water Research 31: 1738–1750.

Dodds, W., Jones, J. R. and Welch, E. B., 1998a. Suggested classification of stream trophic state: distributions of temperate stream types bu clorophyll, total nitrogen, and phosphorus. Water Research 32: 1455–1462.

Dodds, W., Smith, W. H. and Zander, B., 1998b. Developiong nutrient targets to control benthic chlorophyll levels in streams a case study of the Calrk fork river. Water Research 31: 1738–1750.

Dodds, W., Smith, V. H. and Lohman, K., 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences 59: 865–874.

Domingues, R. B., Barbosa, A. and Galvao, H., 2005. Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south–western Iberia). Estuarine Coastal and Shelf Science 64: 249–260.

Domingues, R. B. and Galvao, H., 2007. Phytoplankton and environmental variability in a dam regulated temperate estuary. Hydrobiologia 586: 117–134.

Domingues, R. B., Sobrino, C. and Galvao, H., 2007. Impact of reservoir filling on phytoplankton succession and cyanobacteria blooms in a temperate estuary. Estuarine Coastal and Shelf Science 74: 31–43.

Dubé, M. G. and Scrimgeour, G. J., 1997. Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent. Canadian Journal of Fisheries and Aquatic Sciences 54: 2584–2595.

Duff, J. H., Tesoriero, A. J., Richardson, W. B., Strauss, E. A. and Munn, M. D., 2008. Whole–stream response to nitrate loading in three streams draining agricultural landscapes. Journal of Environmental Quality 37: 1133–44.

Dufrène, M. and Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67: 345–366.

Edlund, M. B., Engstrom, D. R., Triplett, L. D., Lafrancois, B. M. and Leavitt, P. R., 2009. Twentieth century eutrophication of the St. Croix River (Minnesota–Wisconsin, USA) reconstructed from the sediments of its natural impoundment. Journal of Paleolimnology 41: 641–657.

Eloranta, P., 2004. Indicator value of freshwater red algae in running waters for water quality assessment. Oceanological and Hydrobiological Studies 33: 47–54.

Elosegi, A. and Sabater, S., 2009. Conceptos y técnicas en ecología fluvial. Elosegi, A. and Sabater, S. (eds.) Fundación BBVA Bilbao, pp.444

Elwood, J. W. and Mulholland, P. J., 1989. Effects of acidic precipitation on stream ecosystems. In: Acidic precipitation; vol 2. Biological and ecological effects. Adriano, D. C. and Johnson, A. H. (eds.): 88–135. Springer–Verlag. New York

Ensiminger, I., Hagen, C. and Breune, W., 2000. Strategies providing success in a variable habitat: I. Relationships of environmental factors and dominance of *Cladophora glomerata*. Plant, Cell and Environment 23: 1119–1128.

Estrada, M., 1978. Estudios sobre poblaciones de algas sometidas a gradientes continuos en la concentración de nutrientes. Oecologia aquatica 3: 133–151.

European_Commision, 2000. Directive 2000 ⁄ 60 ⁄EC of the European Council and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L327: 1–72.

European_Commision, 2002. Guidance for the analysis of pressures and impacts in accordance with the Water Framework Directive. Common Implementation Strategy of the Water Framework Directive. pp.153

Fishman, M. J., Schroder, L. J. and Shockey, M. W., 1986. Evaluation of methods for preservation of water samples for nutrient analysis. International Journal of Environmental Studies 26: 231–238.

Flinders, C. A., Minshall, G. W., Hall, T. J. and Rodgers, J. H., 2009. Spatial and temporal patterns of periphyton chlorophyll–*a* related to pulp and paper mill discharges in four US receiving streams. Integrated Environmental Assessment and Management 5: 259–269.

Fore, L. S. and Grafe, C., 2002. Using diatoms to assess the biological condition of large rivers in Idaho (U.S.A.). Freshwater Biology 47: 2015–2037.

Foerster, J., Gutowski, A. and Schaumburg, J., 2004. Defining types of running waters in Germany using benthic algae: A prerequisite for monitoring according to the Water Framework Directive. Journal of Applied Phycology 16: 407–418.

Garthwaite, P. H., 1994. An Interpretation of Partial Least Squares. Journal of the American Statistical Association 89: 122–127.

Gaul, U., Geissler, U., Henderson, M., Mahoney, R. and Reimer, C. W., 1993. Bibliography on the fine–structure of diatom frustules (Bacillariophyceae). Proceedings of the Academy of Natural Sciences of Philadelphia 144: 69–238.

Geitler, L., 1932. Der Formweschsel der Pennaten Diatomeen (Kieselalgen). Archiv für Protistenkunde 78: 1–226.

Geitler, L., 1982. Die infraspezifischen Sippen von *Cocconeis placentula* des Lunzer Seebachs. Archiv für Hydrobiologie Supplementen 63 (Algological Studies 30): 111.

Gerhardt, A., Janssens de Bisthoven, L. and Soares, A. M. V. M., 2004. Macroinvertebrate response to acid mine drainage: community metrics and on–line behavioural toxicity bioassay. Environmental Pollution 130: 263–274.

Gerhardt, A., de Bisthoven, L. J., Guhr, K., Soares, A. M. and Pereira, M. J., 2008. Phytoassessment of acid mine drainage: *Lemna gibba* bioassay and diatom community structure. Ecotoxicology 17: 47–58.

Germain, H., 1981. Flore des diatomées (Diatomophycées). Eaux douces et saumâtres du Massif Armoricain et des contrées vosines d'Europe Occidentale. Boubée, N. (eds.) Paris, pp.443

Gomà, J., Cambra, J., Tudesque, L. and Ector, L., 2003. Red de diatomeas en la Cuenca del Ebro. Campaña de muestreo verano 2002. Informe final. pp.37

Gomà, J., Rimet, F., Cambra, J., Hoffman, L. and Ector, L., 2005. Diatom communities and water quality assessment in mountain rivers of the upper Segre basin (La Cerdanya, Oriental Pyrenees). Hydrobiologia 551: 209–225.

Gómez, N., 1998. About the Bacillariophyceae from natural wells in the Delta of the River Ebro. Oecologia aquatica 44: 67–71.

Gray, N. F. and Delaney, E., 2008. Comparison of benthic macroinvertebrate indices for the assesment of the impact of acid mine drainage on an Irish river below an abandoned Cu–S mine. Environmental Pollution 155: 31–40.

Guasch, E. and Sabater, S., 1995. Seasonal variations in photosynthesis–irradiance responses by biofilms in Mediterranean streams. Journal of Phycology 31: 727–735.

Gutowski, A., Foerster, J. and Schaumburg, J., 2004. The use of benthic algae, excluding diatoms and charales, for the assessment of the ecological status of running fresh waters: a case history from Germany. Oceanological and Hydrobiological Studies 33: 3–15.

Hakanson, H., 1989. A light and electron microscopical investigation of the type species of *Cyclotella* (Bacillariophyceae) and related forms, using original material. Diatom research 4: 255–267.

Hakanson, L., Malmaeus, J. M., Bodemer, U. and Gerhardt, V., 2003. Coefficients of variation for chlorophyll, green algae, diatoms, cryptophytes and blue–greens in rivers as a basis for predictive modelling and aquatic management. ecological Modeling 169: 179–196.

Hermoso, V., Clavero, M., Blanco–Garrido, F. and Prenda, J., 2009. Assessing freshwater fish sensitivity to different sources of perturbation in a Mediterranean basin. Ecology of freshwater fish 18: 269–281.

Hilt, S., Schonfelder, I., Rudnickaa, A., Carls, R., Nikolaevich, N., Sukhodolov, A. and Engelhardt, C., 2008. Reconstruction of pristine morphology, flow, nutrient conditions and submerged vegetation of lowland river spree (Germany) from palaeomeanders. River Research and Applications 24: 310–329.

Hill, B. H., Herlihy, A. T., Kaufmann, P. R., DeCelles, S. and Vander Borgh, M. A., 2003. Assessment of streams of the eastern United States using a periphyton index of biotic integrity. Ecological Indicators 2: 325–338.

Hill, M. O. and Gauch, H. E. J., 1980. Detrended correspondence analysis: an improved ordination technique. Vegetatio 42: 47–58.

Hill, W. R., 1996. Effecs of light. In: Algal ecology. Stevenson, R. J., Bothwell, M. L. and Lowe, R. L. (eds.): 121–148. Academic Press. San Diego

Hoffmann, G., 1994. Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie in Seen. Biblioteca Diatomologica 30: 1–241.

Horner, R. E. and Welch, E. B., 1981. Stream periphyton development in relation to current velocity and nutrients. Canadian Journal of Fisheries and Aquatic Sciences 38: 449–457.

Hustedt, F., 1985. The pennate diatoms (A trasnlation of *Die Kieselalgen Flora von Deutschland, Osterreich und Schweiz, 2.* Teil, Leipzig 1959) Koeltz Scientific Books Koenigstein, Germany 918 pp.

Ivorra, N., Bremer, S., Guasch, E., Kraak, H. S. and Admiraal, W., 2000. Differences in the sensitivity of benthic microalgae to Zn and Cd regarding biofilm development and exposure history. Environmental Toxicology and Chemistry 19: 1332–1339.

Jeffrey, S. W. and Humphrey, U. G., 1975. New spectrophotometric equations for determining chlorophylls *a*, *b* and *c* in higher plants, algae and natural phytoplankton. Biochemie Physiologie Pflanzen 167: 191–194.

Johnson, D. B., 1998. Biodiversity and ecology of acidophilic microorganisms. FEMS Microbiology Ecology 27: 307–317.

Johnson, L. B., Richards, C., Host, G. E. and Arthur, J. W., 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshwater Biology 37: 193–208.

Jones, J.G. 1978. Spatial variation in epilithic algae in a stony stream (Wilfin Beck) with particular reference to *Cocconeis placentula*. Freshwater Biology 8: 539–546.

Jones, J. R., Smart, M. M. and Burroughs, J. N., 1984. Factors related to algal biomass in Missouri Ozark streams. Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 22: 1867–1875.

Kawecka, B., 1980. Sessile algae in European mountain streams. Acta Hydrobiologica 23: 17–46.

Kelly, M., Bennion, H., Cox, E. J., Goldsmith, B., Jamieson, J., Juggins, S., Mann, D. G. and Telford, R. J., 2005. Common freshwater diatoms of Britain and Ireland: an interactive key.

Kelly, M. G. and Whitton, B. A., 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. Journal of Applied Phycology 7: 433–444.

Kjeldsen, K., 1994. The Relationship between Phosphorus and Peak Biomass of Benthic Algae in Small Lowland Streams. In: International Association of Theoretical and Applied Limnology, Proceedings, Vol 25, Pt 3. Sladeckova, A. (eds.): 1530–1533. E Schweizerbart'sche Verlagsbuchhandlung. Stuttgart

Knudson, B. M., 1952. The diatom genus *Tabellaria* I. Taxonomy and morphology. Ann Bot NS 16: 421–440.

Knudson, B. M., 1953a. The diatom genus *Tabellaria* III. Problems of intra–specific taxonomy and evolution in *T. flocculosa*. Ann Bot NS 17: 598–609.

Knudson, B. M., 1953b. The diatom genus *Tabellaria*. II. Taxonomy and morphology of the plankton varieties. Annals of Botany N.S 17: 131–155.

Komárek, J. and Anagnostidis, K., 1998. Cyanoprokariota: Chroococcales. Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. (eds.) Fischer, G Stuttgart, pp.548

Komárek, J. and Anagnostidis, K., 2005. Cyanoprokariota: Oscillatoriales. Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. (eds.) Fischer, G Stuttgart,

Kotlash, A. R. and Chessman, B., 1998. Effects of water sample preservation and storage on nitrogen and phosphorus determination: implications for the use of automated sampling equipment. Water Research 32: 3731–3737.

Kovács, C., Kahlert, M. and Padisák, J., 2006. Benthic diatom communities along pH and TP gradients in Hungarian and Swedish streams. Journal of Applied Phycology 18: 105–117.

Krammer, K. and Lange–Bertalot, H., 1985. Bacillariophyceae I: Naviculaceae. Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. (eds.) Fischer, G. Stuttgart, Krammer, K. and Lange–Bertalot, H., 1988. Bacillariophyceae II: Bacillariaceae, Ephitemiaceae, Surirellaciaceae. Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. (eds.) Fischer, G. Stuttgart,

Krammer, K. and Lange–Bertalot, H., 1991a. Bacillariophyceae III: Centrales, Fragilariaceae, Eunotiaceae. Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. (eds.) Fischer, G. Stuttgart,

Krammer, K. and Lange–Bertalot, H., 1991b. Bacillariophyceae IV: Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis. Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. (eds.) Fischer, G. Stuttgart,

Krammer, K., 2002. Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 3. *Cymbella*. A.R.G. Gantner Verlag K.G, Ruggell. 584pp.

Krammer, K., 2000. Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 1. The genus *Pinnularia*. A.R.G. Gantner Verlag K.G, Ruggell. 703pp.

Kucera, P., Grulich, V., Frankova, M. and Bures, P., 2008. Distribution of freshwater red algal family Lemaneaceae (Rhodophyta) in the Czech Republic: an update. Fottea 8: 125–128.

Kvítek, T., Zlábek, P., Bystricky, V., Fucík, P., Lexa, m., Gergel, J., Novák, P. and Ondr, P., 2009. Changes of nitrate concentrations in surface waters influenced by land use in the crystalline complex of the Czech Republic. Physics and Chemistry of the Earth 34: 541–551.

Lackey, J. B., 1938. The fauna and flora of surface water polluted by acid mine drainage. U.S. Public Health Reports 53: 1499–1507.

Lamberti, G. A. and Resch, V. H., 1983. Stream periphyton and insect herbivores: an experimental study of grazing by caddisfly population. Ecology 64: 1124–1135.

Lange–Bertalot, H., 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64: 285–305.

Lange–Bertalot, H., 1993. 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süßwasserflora von Mitteleuropa Vol. 2/1–4 (eds.) pp.454

Lange–Bertalot, H. and Moser, G., 1994. *Brachysira*– Monographie der Gattung. Wichtige Indikator–Species fuer das Gewaesser–Monitoring und *Naviculadicata* nov.gen. Ein Loesungsvor– schlag zu dem Problem *Navicula* sensu lato ohne *Navicula* sensu stricto. (eds.) pp.212

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

Lavoie, I., Campeau, S., Grenier, M. and Dillon, P. J., 2006. A diatom–based index for the biological assessment of eastern Canadian rivers: an application of correspondence analysis (CA). Canadian Journal of Fisheries and Aquatic Sciences 63: 1793–1811.

Leira, M., Bao, R. and Vidal-Romaní, J. R., 1997. Evolución postglaciar de la laguna de Villaseca (NW de la Península Ibérica) a partir del análisis de diatomeas. Caderno Lab. Xeolóxico de Laxe Coruña 22: 81-97.

Leira, M., 2005. Diatom responses to Holocene environmental changes in a small lake in northwest Spain. Quaternary International 140–141: 90–102.

Leira, M. and Sabater, S., 2005. Diatom assemblages distribution in catalan rivers, NE Spain, in relation to chemical and physiographical factors. Water Research 39: 73–82.

Leland, H. V., 1995. Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use and other environmental factors. Canadian Journal of Fisheries and Aquatic Sciences 52: 1108–1129.

Leland, H. V. and Porter, S. D., 2000. Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. Freshwater Biology 44: 279–301.

Lenoir, A. and Coste, M., 1996. Developemnt of a practical diatom index of overall water qulity applicable to the French national water Board network. In: Use of Algae for Monitoring Rivers II. Whitton, B. A. and Rott, E. (eds.): Insbruck

Leps, J. and Smilauer, P., 2003. Multivariate analysis of ecological data using canoco. (eds.) United Kingdom, pp.110

Lessmann, D., Fyson, A. and Nixdorf, B., 2000. Phytoplankton of the extremely acidic mining lakes of Lusatia (Germany) with pH<3. Hydrobiologia 433: 123–128.

Linares–Cuesta, J. E., Olofsson, L. and Sánchez–Castillo, P., 2007. Comunidades de diatomeas epipélicas en las lagunas de alta montaña de Sierra Nevada (Granada, España) Limnética 26: 99–113.

Lohman, K., Jones, J. R. and Perkins, D., 1992. Effects of nutrient enrichment and flood frequency on periphyton biomass in northern Ozark streams. Canadian Journal of Fisheries and Aquatic Sciences 49: 1198–1205.

López–Archilla, A. I. and Amils, R., 1999. A comparative ecological study of two acidic rivers in southwestern Spain. Microbial Ecology 38: 146–156.

López–Archilla, A. I., Marin, I. and Amils, R., 2001. Microbial community composition and ecology of an acidic aquatic environment: the Tinto River, Spain. Microbial Ecology 41: 20–35.

López–Rodríguez, M. C. and Penalta, M., 2004. Aportación al conocimiento de la flora ficológica del Macizo Central Gallego (N.O. España). Anales de Biología 26: 79–91.

Lörh, A. J., Sluik, R., Olaveson, M. M., Ivorra, N., Van Gestel, C. A. M. and Van Straalen, N. M., 2006. Macroinvertebrate and algal communities in an extremely acidic river and the Kawah Ijen crater lake (pH < 0.3), Indonesia. Archives für Hydrobiologia 165: 1–21.

Luís, A. T., Teixeira, P., Almeida, S., Ector, L., Matos, J. X. and Ferreira da Silva, E., 2008. Impact of Acid Mine Drainage (AMD) on water quality, stream sediments and periphytic diatom communities in the surrounding streams of aljustrel mining area (Portugal). Water Air and Soil Pollution 200: 147–167.

Llamas, M. R., Varela–Ortega, C., de la Hera, A., Aldaya, M. M., Villarroya, F., Martínez–Santos, P., Blanco–Gutiérrez, I. M. M., Carmona–García, G., Esteve–Bengoechea, P., de Stefano, M., Hernández–Mora, N. and Zorrilla, P., 2010. The Guadiana basin. In: The adaptative water resource management handbook. Mysiak, J., Henrikson, H. J., Sullivan, C., Bromley, J. and Pahl–Wostl, C. (eds.): 216. earthscan. London

Margalef, R., 1944. Datos para la flora algológica de nuestras aguas dulces. Publicaciones del Instituto Botánico de Barcelona 4: 128.

Margalef, R., 1945. Primera nota sobre la biología de las aguas estancadas del bajo Urgel. Ilerda 5: 253–313.

Margalef, R., 1948. Flora, fauna y comunidades bióticas de las aguas del Pirineo de la Cerdaña. CSIC_Instituto_de_estudios_Pirenaicos (eds.) Zaragoza, pp.226

Margalef, R., 1950a. Datos para la biología del estanque de Montcortés (Lérida). Boletín de la Real Sociedad Española de Historia Natural 48: 209–218.

Margalef, R., 1950b. Datos para la hidrobiología de la cordillera cantábrica, especialmente del macizo de los Picos de Europa. Publicaciones del Instituto de Biología Aplicada 8: 37–76.

Margalef, R., 1951. Materiales para la hidrobiología de la Isla de Ibiza. Publicaciones del Instituto de Biología Aplicada 7: 5–70.

Margalef, R., 1954. Materiales para una flora de las algas del NE de España V: *Bacillariophyceae*. Collectanea Botanica 4: 53–79.

Margalef, R., 1955. Materiales para una flora de las algas del NE de España VI: *Bacillariophyceae*. Collectanea Botanica 4: 53–80.

Margalef, R., 1956a. Materiales para una flora de las algas del NE de España, VII Suplemento. Collectanea Botanica 5: 87–107.

Margalef, R., 1956b. Algas de agua dulce del noroeste de España. Publicaciones del Instituto de Biología Aplicada 22: 43–152.

Margalef, R., 1958. Materiales para el estudio de las comunidades bióticas de las aguas dulces y salobres, principalmente del NE de España. Publicaciones del Instituto de Biología Aplicada 28: 5–47.

Margalef, R. 1964. Correspondence between the classic types of lakes and the structural and dynamic prop-erties of their populations. Verhandlungen der Internationale für Limnologie 15: 169-175.

Margalef, R., 1983. Limnologia. Ed. Omega, Barcelona 1024 pp.

Martínez de Fabricius, A. L., Maidana, N., Gómez, N. and Sabater, S., 2003. Distribution patterns of benthic diatoms in a Pampean river exposed to seasonal floods: the Cuarto River (Argentina). Biodiversity and Conservation 12: 2443–2454.

Massanell, M. A., 1966. Algues aquàtiques del Parc d'Aigües Tortes. Treballs de l'Institutu d'Estudis Catalans, arxius de la secció de ciències 31: 1–42.

Mayama, S. and Kobayasi, H., 1991. Observations of *Eunotia arcus* Ehr., type species of the genus *Eunotia* (Bacillariophyceae). The Japanese Society of Phycology 39: 131–141.

Mayama, S., 1992. Morphology of *Eunotia multiplastidica* sp. nov. (Bacillariophyceae) examined throughout the life cycle. The Korean Journal of Phycology 7: 45–54.

Mayama, S., 1997. *Eunotia valida* and *E. pseudovalida* spec. nov. (Bacillariophyceae): Ultrastructure of the valve and girdle. Nova Hedwigia 65: 165–176.

McConnell, W. J. and Sigler, W. F., 1959. Chlorophyll and productivity in mountain rivers. Limnology and Oceanography 4: 335–351.

McCune, B. and Mefford, M. J., 1999. PC–ORD. Multivariate analyses of ecological data.

Michels, A., Umaña, G. and Raeder, U., 2006. Epilithic diatom assemblages in rivers draining into Golfo Dulce (Costa Rica) and their relationship to water chemistry, habitat characteristics and land use. Archives für Hydrobiologia 165: 167–190.

MMA, 2005. Informe resumen de los artículos 5 y 6 de la Directiva Marco del Agua. Demarcación hidrográfica del río Guadiana (parte española) y ámbito complementario de los ríos Tinto, Odiel y Piedras. pp.92

MMA, 2006a. Informe sobre el estado hidrológico de las cuencas en España (período abril 2006 – junio 2006). MMA pp.112

MMA, 2006b. Informe sobre el estado hidrológico de las cuencas en España (periodo octubre 2005–diciembre 2005). MMA pp.76

MMA, 2006c. Informe sobre el estado hidrológico de las cuencas en España (período enero 2006–marzo 2006). MMA pp.90

MMA, 2006d. Informe sobre el estado hidrológico de las cuencas en España (período julio 2006 – septiembre 2006). MMA pp.136

Moreno, J. L., Navarro, C. and De las Heras, J., 2006. Abiotic ecotypes in south–central Spanish rivers: Reference conditions and pollution. Environmental Pollution 143: 388–396.

Morin, A., Lamoureux, W. and Busnarda, J., 1999. Empirical models predicting primary productivity from chlorophyll a and water temperature for stream periphyton and lake and ocean phytoplankton. Journal of the North American Benthological Society 18: 299–307.

Mosisch, T. D., Bunn, S. E. and Davis, P. M., 2001. The relative importance of shading and nutrients on algal production in subtropical streams. Freshwater Biology 46: 1269–1278.

Munn, M., Osborne, L. L. and Wiley, M. J., 1989. Factors influencing periphyton growth in agricultural streams of central Illinois. Hydrobiologia 174: 89–97.

Munn, M., Frey, J. and Tesoriero, A., 2010. The influence of nutrients and physical habitat in regulating algal biomass in agricultural streams. Environmental Management 45: 603–615.

Munn, M. D., Black, R. W. and Gruber, S. J., 2002. Response of benthic algae to environmental gradients in an agriculturally dominated landscape. Journal of North American Benthological Society 21: 221–237.

Munné, A., Prat, N., Solà, C., Bonada, N. and Rieradevall, M., 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquatic Conservation: Marine and Freshwater Ecosystems 13: 147–163.

Murphy, J. and Riley, J. P., 1956. The storage of sea–water samples for the determination of dissolved inorganic phosphate. Analytica Chimica Acta 14: 318–319.

Navarro, E., Guasch, E. and Sabater, S., 2002. Use of microbenthic algal communities in ecotoxicological tests for the assessment of water quality: the Ter river case study. Journal of Applied Phycology 14: 41–48.

Ndiritu, G., Gichuki, N. and Triest, L., 2006. Distribution of epilithic diatoms in response to environmental conditions in an urban tropical stream, Central Kenya. Biodiversity and Conservation 15: 3267–3293.

Negoro, K., 1985. Diatom flora of the mineralogenous acidotrophic inland waters of Japan. Diatom 1: 1–8.

Nieto, J. M., Sarmiento, A. M., Olías, M., Canovas, C. R., Riba, I., Kalman, J. and Delvalls, T. A., 2007. Acid mine drainage pollution in the Tinto and Odiel rivers (Iberian Pyrite Belt, SW Spain) and bioavailability of the transported metals to the Huelva Estuary. Environment International 33: 445–455.

Nijboer, R. C., Johnson, R. K., Verdonschot, P. F. M., Sommerhauser, M. and Buffagni, A., 2004. Establishing reference conditions for European streams. Hydrobiologia 516: 91–105.

Niyogi, D. K., Lewis, W. M., Jr and McKnight, D. M., 2002. Effects of stress from mine drainage on diversity, biomass, and function of primary producers in Mountain Streams. Ecosystems 5: 554–567.

Nocete, F., Álex, E., Nieto, J. M., Sáez, R. and Bayona, M. R., 2005. An archaeological approach to regional environmental pollution in the south–western Iberian Peninsula related to Third millennium BC mining and metallurgy. Journal of Archaeological Science 32: 1566–1576.

Nogueira, E., Perez, F. F. and Ríos, A. F., 1998. Modelling nutrients and Chlorophyll–*a* time series in an estuarine upwelling ecosystem (ria de Vigo, NW Spain) using the Boxjenkins approach. Estuarine Coastal and Shelf Science 46: 267–286.

Noguerol–Seoane, A., 1990. Estudio ficológico de la fuente termanl de Torneiros (Lovios, Orense, España). Anales Jardín Botánico de Madrid 47: 295–300.

Noguerol–Seoane, A., 1994. Sucesión algal en una charca de agua dulce del NO de España. Studia Botanica 13: 69–73.

Odum, H. T., 1957. Trophis structure and productivity of Silver springs, Florida. Ecological Monographs 27: 55–112.

Olaveson, m. m. and Nalewajko, c., 2000. Effects of acidity on the growth of two *Euglena* species. Hydrobiologia 433: 39–56.

Olay, A., Cosme, N., Álvarez, R., Sousa, S., Loredo, J. and Estrela, M., 2004. Environmental data on a case study from the Transboundary Catchment of Guadiana River.

Ortiz, R., Huck, V., Cambra, J. and Ector, L., 2005. Diseño de la red de diatomeas de la Cuenca Hidrográfica del Norte. Confederación_Hidrográfica_del_Norte pp.59

Ortiz, R. and Cambra, J., 2007. Distribution and taxonomic notes of *Eunotia* Ehrenberg 1837 (Bacillariophyceae) in rivers and streams of Northern Spain. Limnetica 26: 415–434.

Oscoz, J., Gomà, J., Ector, L., Cambra, J., Pardos, M. and Durán, C., 2007. Estudio comparativo del estado ecológico de los ríos de la cuenca del Ebro mediante macroinvertebrados y diatomeas. Limnetica 26: 143–158.

Owen, R., Duncan, W. and Pollard, P., 2001. Definition and establishment of reference conditions. pp.20

Pan, Y., Stevenson, R. J., Hill, B. H., Herlihy, A. T. and Collins, G. B., 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. Journal of North American Benthological Society 15: 481–495.

Pan, Y., Stevenson, R. J., Hill, B. H., Kaufmann, P. R. and Herlihy, A. T., 1999. Spatial patterns and ecological determinants of benthic algal assemblages in Mid–Atlantic streams. Journal of Phycology 35: 460–468.

Pan, Y., Hill, B. H., Husby, P., Hall, R. k. and Kaufmann, P. R., 2006. Relationships between environmental variables and benthic diatom assemblages in California Central Valley streams (USA). Hydrobiologia 561: 119–130.

Pardo, I., Álvarez, M., Casas, J., Moreno, J. L., Vivas, S., Bonada, N., Alba–Tercedor, J., Jiménez–Cuéllar, P., Moyà, G., Prat, N., Robles, S., Suárez, M. L., Toro, M. and Vidal–Abarca, M. R., 2002. El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. Limnetica 21: 115–133.

Patrick, R. and Reimer, C. W., 1966. The diatoms of United States. Vol 1: *Fragilariaceae, Eunotiaceae, Achnanthaceae, Naviculaceae*. In: The

Academy of Natural Sciences of Philadelphia. (eds.): 688. Philadelphia

Philibert, A., Gell, P., Newall, P., Chessman, B. and Bate, N., 2006. Development of diatom–based tools for assessing stream water quality in south–eastern Australia: assessment of environmental transfer functions. Bioassesment Methods 572: 103–114.

Ponader, K. C., Charles, D. F. and Belton, T. J., 2007. Diatom–based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. Ecological Indicators 7: 79–93.

Poor, C. J. and McDonnell, J. J., 2007. The effects of land use on stream nitrate dynamics. Journal of Hydrology 332: 54– 68.

Potapova, M. and Charles, D. F., 2002. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. Journal of Biogeography 29: 167–187.

Potapova, M. and Charles, D. F., 2003. Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. Freshwater Biology 48: 1311–1328.

Potapova, M., Charles, D. F., Ponader, K. C. and Winter, D. M., 2004. Quantifying species indicator values for trophic diatom indices: a comparison of approaches. Hydrobiologia 517: 25–41.

Powers, M. E., 1992. Hydrologic and trophic controls of seasonal algal blooms in northern california rivers. Archiv für Hydrobiologie 125: 285–410.

Prygiel, J. and Coste, M., 2000. Guide Méthodologique pour la mise en oeuvre de l'Indice Biologique Diatomées. 134

Prygiel, J., Carpentier, P., Almeida, S., Coste, M., Druart, J. C., Ector, L., Guillard, D., Honoré, M. A., Iserentant, R., Legeganck, P., Lalanne–Cassou, C., Lesniak, C., Mercier, I., Mmoncaut, P., Nazart, M., Nouchet, N., Peres, F., Peeters, V., Rimet, F., Rumeau, A., Sabater, S., Straub, F., Torrisi, M., Tudesque, L., Van de Vijver, B., Vidal, H., Vizinet, J. and Zydek, N., 2002. Determination of the biological diatom index (IBD NF T 90–354): results of an intercomparison exercise. Journal of Applied Phycology 14: 27–39.

Rakowska, B. and Sitkowska, M., 2010. Algae assemblages in the Rąbień peat-bog reserve. Oceanological and Hydrobiological Studies 39: 63-74.

Reavie, E. D., Smol, J. P., Carignan, R. and Lorrain, S., 1998. Diatom paleolimnology of two fluvial lakes in the St. Lawrence river: a reconstruction of environmental changes during the last century. Journal of Phycology 34: 446–456.

Reed, J. M., 1996. The potential of diatoms, ostracods and other indicators for holocene palaeoclimate research in southern spanish salt lakes. Limnetica 12: 25–39.

Reed, J. M., 1998a. A diatom–conductivity transfer function for Spanish salt lakes. Journal of Paleolimnology 19: 399–416.

Reed, J. M., 1998b. Diatom preservation in the recent sediment record of Spanish saline lakes: implications for palaeoclimate study. Journal of Paleolimnology 19: 129–137.

Revenga, C. and Kura, Y., 2003. Status and trends of biodiversity of inland waters ecosystems. Technical series number 11. Secretariat of the Convention on Biological Diversity, Montreal, Quebec, Canada. 122.

Reynoldson, T. B., Norris, R. H., Resh, V. H., Day, K. E. and Rosenberg, D. M., 1997. The reference condition: A comparison of multimetric and multivariate approaches to assess water–quality impairment using benthic macroinvertebrates. Journal of North American Benthological Society 16: 833–852.

Rieradevall, M. and Cambra, J., 1994. Urban freshwater ecosystems in Barcelona. Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 25: 1369–1372.

Rimet, F., Goma, J., Cambra, J., Bertuzzi, E., Cantonati, M., Cappelletti, C., Ciutti, F., Cordonier, A., Coste, M., Delmas, F., Tison, J., Tudesque, L., Vidal, H. and Ector, L., 2007. Benthic diatoms in western European streams with altitudes above 800 M: Characterisation of the main assemblages and correspondence with ecoregions. Diatom Research 22: 147–188.

Rimet, F., 2009. Benthic diatom assemblages and their correspondence with ecoregional classifications: case study of rivers in north–eastern France Hydrobiologia 636: 137–151.

Riseng, C. M., Wiley, M. J. and Stevenson, R. J., 2004. Hydrologic disturbance and nutrient effects on benthic community structure in mid–western US streams: a coveriance structure analysis. Journal of North American Benthological Society 23: 309–326.

Robinson, C. T., Kawecka, B., Füreden, L. and Peter, A., 2009. Biodiversity of flora and fauna in Alpine waters. In: The handbook of environmental chemistry, vol 6. Alpine waters. Bundi, U. (eds.). Springer. Dübendorf, Switzerland, 278 pp.

Rocha, C., Galvao, H. and Barbosa, A., 2002. Role of transient silicon limitation in the development of cyanobacteria blooms in the Guadiana estuary, south–western Iberia. Marine ecology progress series 228: 35–45.

Rodrigo, M. A., Vicente, E. and Aboal, M., 2000. The physical, chemical and biological characteristics of the holomictic sulphated Lake Arcas–2 (Cuenca, Spain). Hydrobiologia 418: 153–168.

Rojo, C., Rodrigo, M. A., Salazar, G. and Alvarez–Cobelas, M., 2008. Nitrate uptake rates in freshwater plankton: the effect of food web structure. Marine and Freshwater Research 59: 717–725.

Romaní, A. and sabater, S., 2000. Variability of heterotrophic activity in Mediterranean stream biofilms: A multivariate analysis of physical–chemical and biological factors. Aquatic Sciences 62: 205–215.

Ros, M. D., Marin–Murcia, J. P. and Aboal, M., 2009. Biodiversity of diatom assemblages in a Mediterranean semiarid stream: implications for conservation. Marine and Freshwater Research 60: 14–24.

Ross, R. M., Long, E. S. and Dropkin, D. S., 2008. Response of macroinvertebrate communities to remediation–simulating conditions in Pennsylvania streams influenced by acid mine drainage. Environmental Monitoring and Assessment

Rott, E., 1991. Methodological aspects and perspectives in the use of periphyton for monitoring and protecting rivers. In: Use of algae for monitoring rivers. Whitton, B. A., Rott, E. and Friedrich, G. (eds.): 9–16. Institut für Botanik, Universität Innsbruck. Innsbruck, Austria

Round, F. E., Crawford, R. M. and Mann, D. G., 1990. The Diatoms. Biology and morphology of the genera. (eds.) Cambridge University Press Cambridge, pp.747

Round, F. E. and Basson, P. W., 1997. A new monoraphid diatom genus (*Pogoneis*) from Bahrain and the transfer of previously described species *A. hungarica* and *A. taeniata* to new genera. Diatom Research 12: 71–81.

Sabater, S., 1987. Estudi de les poblacions d'algues del riu Ter. PhD Thesis, in Departament d'Ecologia. Universitat de Barcelona, Barcelona. pp.481

Sabater, S., Sabater, F. and Tomàs, X., 1987. Water quality and diatom communities in two catalan rivers (NE Spain). Water Resources 21: 901–911.

Sabater, S., 1988. Composición y ciclo de pigmentos clorofílicos en las poblaciones del fitobentos del río Ter durante un período de nueve meses. Oecologia aquatica 9: 61–75.

Sabater, S. and Sabater, F., 1988. Diatom assemblages in the river Ter. Archives für Hydrobiologia 111: 397–408.

Sabater, S. and Roca, J. R., 1990. Some factors affecting distribution of diatom assamblages in Pyrenean springs. Freshwater Biology 24: 493–507.

Sabater, S. and Roca, J. R., 1992. Ecological and biogeographical aspects of diatom distribution in Pyrenean springs. British Phycological Journal 27: 203–213.

Sabater, S., 2000. Diatom communities as indicators of environmental stress in the Guadiamar River, S–W. Sapin, following a major mine tailings spill. Journal of Applied Phycology 12: 113–124.

Sabater, S., Buchaca, T., Cambra, J., Catalan, J., Guasch, E., Ivorra, N., Muñoz, I., Navarro, E., Real, M. and Romaní, A., 2003a. Structure and function of benthic algal communities en a extremely acid river. Journal of Phycology 39: 481–489.

Sabater, S., Tornés, E., Leira, M., Trobajo, R., Cambra, J., Gomà, J. and Ortiz, R., 2003b. Anàlisi de la viabilitat i proposta d'indicadors fitobentonics de la qualitat de l'aigua per als cursos fluvials de Catalunya. Aplicació de la Directiva Marc en Política d'Aigües de la Unió Europea (2000/60/CE). pp.113

Sabater, S., Acuña, V., Giogi, A., Guerra, E., Muñoz, I. and Romaní, A., 2005. Effects of nutrient inputs un a forested Mediterranean stream under moderate light availability. Archives für Hydrobiologia 163: 479–496.

Sabater, S. and Admiraal, W., 2005. Periphyton as Biological Indicator in Managed Aquatic Ecosystems. In: Periphyton: Ecology, Exploitation and Management. Azim, M. E. (eds.)

Sabater, S., Artigas, J., Duran, C., Pardos, M., Romani, A. M., Tornes, E. and Ylla, I., 2008. Longitudinal development of chlorophyll and phytoplankton assemblages in a regulated large river (the Ebro River). The Science of the Total Environment 404: 196–206.

Sabater, S., Feio, M. J., Graça, M. A. S., Muñoz, I. and Romaní, A., 2009. The Iberian rivers. In: Rivers of Europe. Tockner, K., Robinson, C. T. and Uehlinger, U. (eds.): 113–149. Academic Press. London

Sáez, R., Pascual, E., Toscano, M. and Almodóvar, R., 1999. The Iberian type of volcano–sedimentary massive sulphide deposits. Mineralium Deposita 34: 549–570.

Saint–Martin, J. P., Pestrea, S. and Conesa, G., 2001. Les assemblages de diatomées des niveaux infra–gypseux du bassin messinien de Sorbas (Espagne). Cryptogamie Algologie 22: 127–149.

Sala, S. E., Guerrero, J. M. and Ferrario, M. E., 1993. Redefinition of *Reimeria sinuata* (Gregory) Kociolek & Stoermer and recognition of *Reimeria uniseriata nov*. spec. Diatom research 8:

Salomoni, S. E., Rocha, O., Callegaro, V. L. and Lobo, E. A., 2006. Epilithic diatoms as indicators of water quality in the Gravataí river, Rio Grande do Sul, Brazil. Hydrobiologia 559: 233–246.

Schaumburg, J., Schranz, C., Stelzer, D. and Hofmann, G., 2007. Action Instructions for the ecological Evaluation of Lakes for Implementation of the EU Water Framework Directive: Makrophytes and Phytobenthos. pp.69

Scuria, S., Torrisi, M., Cocchionia, M. and Dell'Uomo, A., 2006. The European Water Framework Directive 2000/60/EC in the evaluation of the ecological status of watercourses. Case study: the river Chienti (central Apennines, Italy). Acta Hydrobiologica 34: 498–505.

Sellers, T. and Buckvareckas, P. A., 2003. Phytoplankton production in a large regulated river: a modelling and mass balance assessment. Limnology and Oceanography 48: 1476–1487.

Shannon, C. E. and Weaver, W., 1963. The mathematical theory of communication. (eds.) University of Illinois Press Urbana, pp.125

Sládecek, V., 1973. System of water quality from the biological point of view. Archiv für Hydrobiologie 7: 1–218.

Smith, V. H., Tilman, G. D. and Nekola, J. C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine and terrestrial ecosystems Environmental Pollution 100: 179–196.

Soininen, J., 2002. Responses of epilithic diatom communities to environmental gradients in some Finnish rivers. International Review für Hydrobiologie 87: 11–24.

Soininen, J., Paavola, R. and Muotka, T., 2004. Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. Ecography 27: 330–342.

Soininen, J., 2007. Environmental and spatial control of freshwater diatoms – A review. Diatom Research 22: 473–490.

Soria–García, J. M., Miracle, M. R. and Vicente, E., 1987. La albufera de Valencia: comunidades fitoplanctónicas en la campaña 1982–83. Actas del VI Simposio Nacional de Botánica Criptogámica 165–172.

Spanos, T., Simenov, V., Simenova, P., Apostolidou, E. and Stratis, J., 2008. Environmetrics to evaluate marine environment quality. Environmental Monitoring Assessment 143: 215–225.

SPSS–Inc., 2004. SPSS for windows.

Stat–Soft, 2001. Statistica (data analysis software system).

Steinman, A. D. and Sheath, R. G., 1984. Morphological variability of *Eunotia pectinalis* (Bacillariophyceae) in a softwater Rhode and stream and in culture. Journal of Phycology 20: 266–276.

Stendera, S. and Johnson, R. K., 2006. Multiscale Drivers of Water Chemistry of Boreal Lakes and Streams. Environmental Management 38: 760–770.

Stevenson, R. J., 1997. Scale–dependent determinants and consequences of benthic algal heterogeneity. Journal of North American Benthological Society 16: 248–262.

Stevenson, R. J. and Pan, Y., 1999. Assessing environmental conditions in rivers and streams using diatoms. In: The diatoms: Application for the environmental and earth sciences. Stoermer, E. F. and Smol, J. P. (eds.): 11–40. Cambridge university Press. Cambridge

Stevenson, R. J., Hill, B. H., Herlihy, A. T., Yuan, L. L. and Norton, S. B., 2008a. Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. Journal of the North American Benthological Society 27: 783–799.

Stevenson, R. J., Pan, Y., Manoylov, K. M., Parker, C. A., Larsen, D. P. and Herlihy, A. T., 2008b. Development of diatom indicators of ecological conditions for streams of the western US. Journal of the North American Benthological Society 27: 1000–1016.

Taylor, D., Dalton, C., Leira, M., Jordan, P., Chen, G., León–Vintró, L., Irvine, K., Bennion, H. and Nolan, T., 2006. Recent histories of six productive lakes in the Irish Ecoregion based on multiproxy palaeolimnological evidence. Hydrobiologia 571: 237–259.

Ter Braak, C. J. F. and Verdonschot, P. F. M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquatic Sciences 57: 153–187.

Ter Braak, C. J. F. and Smilauer, P., 1998. CANOCO for windows.

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

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

Tison, J., Park, T. S., Coste, M., Wasson, J. G., Rimet, F., Ector, L. and Delmas, F., 2007. Predicting diatom reference communities at the French hydrosystem scale: A first step towards the definition of the good ecological status. Ecological Modeling 203: 99–108.

Tolotti, M., 2001. Phytoplankton and littoral epilithic diatoms in high mountain lakes of the Adamello-Brenta Regional Park (Trentino, Italy) and their relation to trophic status and acidification risk. Journal of Limnology 60: 171-188.

Tomàs, X. and Sabater, S., 1985. The diatom flora of the Llobregat river and its relation to water quality. Verh. Internat. Verein. Limnol. 22: 2348–2352.

Tomàs, X., 1988. Diatomeas de las aguas continentales saladas del litoral mediteráneo de la Península Ibérica. PhD Thesis, in Departament d'Ecologia. Universitat de Barcelona, Barcelona.

Tornés, E., Cambra, J., Gomà, J., Leira, M. and Sabater, S., 2007. Indicator taxa of benthic diatom communities: a case study in Mediterranean streams. Annales de Limnologie – International Journal of Limnology 43: 1–11.

Tornés, E., 2009. Distributional patterns of diatoms communities in Mediterranean rivers. PhD Thesis, in Departament de Ciències Ambientals. Universitat de Girona, Girona. pp.157

Trobajo, R., 2003. Ecological analysis of periphytic diatoms in mediterranean coastal wetlands (Empordà wetlands, NE Spain). PhD. Thesis, in Ecologia. Universitat de Girona, Girona. pp.200

Trobajo, R., Cox, E. J. and Quintana, X., 2004. The effects of some environmental variables on the morphology of *Nitzschia frustulum* (Bacillariophyta), in relation its use as a bioindicator. Nova Hedwigia 79: 433–445.

Troya, A. and Bernués, M., 1990. Humedales españoles en la lista del convenio de Ramsar. (eds.) Instituto para la conervación de la Naturaleza, Ministerio de Agricultura, Pesca y Alimentación Madrid,

Tuchman, N., 1996. The role of heterotrophy in algae. In: Algal ecology. Stevenson, R. J., Bothwell, M. L. and Lowe, R. L. (eds.): 299–319. Academic Press. San Diego

Tufford, D. L. and McKeller, H. N., 1999. Spatial and temporal hydrodynamic and water quality modelling analysis for a large reservoir on the south Carolina (USA) coastal palin. Ecological Modeling 114: 137–173.

Ubierna, M. A. and Sánchez–Castillo, P., 1992. Diatomoflora de varias lagunas de aguas mineralizadas de las provincias de Málaga y Granada. Anales del Jardín Botánico de Madrid 41: 171–185.

Urrea, G. and Sabater, S., 2009a. Epilithic diatom assemblages and their relationship to environmental characteristics in an agricultural watershed (Guadiana River, SW Spain). Ecological Indicators 9: 693–703.

Urrea, G. and Sabater, S., 2009b. Comparative study of algal communities in acid and non–acid waters from Tinto, Odiel and Piedras river basins (SW Spain). Limnetica 28: 261–272.

Van Dam, H., Mertens, A. and Sinkeldam, J., 1994. A coded checklist and ecological indicator value of freshwater diatoms from the Nederlands. Netherlands journal of aquatic ecology 28: 117–133.

Van der Hoek, C., 1976. Revision of the European species of Cladophora. (eds.) Koeningstein, pp.248

Varela, M., Rodríguez, B. and Costas, E., 1991. Inventario de diatomeas de auga doce de Galicia (Bacillariophyceae). Cadernos da Area de Ciencias Biolóxicas (Inventarios). Seminario de Estudos Galegos ix: 11–55.

Varela, M., 1992. Adiciones a la flora de diatomeas de agua dulce de Galicia. Collectanea Botanica 13: 977–985.

Veraart, A. J., Romaní, A., Tornés, E. and Sabater, S., 2008. Algal response to nutrient enrichment in forested oligotrophic stream Journal of Phycology 44: 564–572.

Verb, R. G. and Vis, M. L., 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and nonimpacted sites. Journal of north American Benthological Society 19: 274–288.

Vicente, E., M.P, G. and Miracle, M. R., 1984. Sucesión estacional del fitoplancton de la albufera de Valencia. Anales de biología. Sección especial 2: 91–100.

Von Edith, K., 1978. Systematik und Ökologie der Alguen österreichischer Bergbäche. archives für Hydrobiologia 53: 405–643.

Wallin, M., Wiederholm, T. and K. Johnson, R., 2003. Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters. Common Implementation Strategy of the Water Framework Directive. pp.93

Watanabe, T. and Asai, K., 1995. *Pinnularia acoricola* Hustedt var. *acoricola* as an environmental frontier species occurred in organic acid water with 1.1 – 2.0 in pH value. Diatom 10: 9–11.

Weckström, J., Korhola, A. and Blom, T., 1997. Diatoms as quantitative indicators of pH and water temperature in subarctic Fennoscandian lakes Hydrobiologia 347: 171–184.

Weijters, M. J., Janse, J. H., Alkemade, R. and Verhoeven, T. A., 2009. Quantifying the effect of catchment land use and water nutrient concentrations on freshwater river and stream biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems 19: 104–112.

Welch, E. B., Jacoby, J. M., Horner, R. R. and Seely, M. R., 1988. Nuisance biomass levels of periphytic algae in streams. Hydrobiologia 157: 161–168.

Welch, E. B. and Patmont, C. R., 1989. Prediction of nuisance periphytic biomass. A management approach. Water Research 23: 401–405.

Wendker, S., 1990. Morphologische Untersuchungen an Populationen aus dem Formenkreis um *Nitzschia frustulum* (Kützing) Grunow. Diatom Research 5: 179–187.

Whitton, B. A. and Diaz, B. M., 1981. Influence of environmental factors on photosynthetic species composition in highly acidic waters. verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 21: 1459–1465.

Winter, J. G. and Duthie, H. C., 2000. Epilithic diatoms as indicators of stream total N and total P concentration. Journal of North American Benthological Society 19: 32–49.

Woelfl, S. and Whitton, B. A., 2000. Sampling, preservation and quantification of biological samples from highly acidic environments (pH <3) Hydrobiologia 433: 173–180.

Wolfel, A. P. and Kling, H. J., 2001. A consideration of some North American soft–water *Brachysira* taxa and description of *B. arctoborealis sp. nov*. In: Lange–Bertalot–Festschrift. Jahn, R., Kociolek, J. P., Witkowski, A. and Compere, P. (eds.): 243–264. Ruggell. Gantner

Zampella, R. A., Laidig, K. J. and Lowe, R. L., 2007. Distribution of diatoms in relation to land use and pH in blackwater coastal plain streams. Environmental Management 39: 369–384.

Zanini Branco, L. E. and Pereira, J. L., 2002. Evaluation of seasonal dynamics and bioindication potential of macroalgal communities in a polluted tropical stream. Archives für Hydrobiologia 155: 147–161.

Zong, Y. and Horton, B. P., 1999. Diatom–based tidal–level transfer functions as an aid in reconstructing Quaternary history of sea–level movements in the UK. Journal of Quaternary Science 14: 153–167.