U.PORTO
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

Ecology and trophic dynamics of the European eel, *Anguilla anguilla* (L.)

Sérgia Catarina de Amorim Costa Dias

Tese de doutoramento em Ciência Animal
Especialidade em Morfologia e Fisiologia

2010

Sérgia Catarina de Amorim Costa Dias

Ecology and trophic dynamics of the European eel, *Anguilla anguilla* (L.)

Tese de Candidatura ao grau de Doutor em Ciência Animal, Especialidade em Morfologia e Fisiologia submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto.

Orientador

Doutor José Carlos Fernandes Antunes

Categoria – Professor Auxiliar

Afiliação – Escola Superior *Gallaecia*

Co-orientador

Doutor Francisco Javier Lobón Cerviá

Categoria – Investigador

Afiliação – Museo Nacional de Ciencias Naturales – CSIC (Madrid)

Co-orientador

Doutor João José Oliveira Dias Coimbra

Categoria – Professor Catedrático

Afiliação – Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto

Cover: Playa de Cueva

Photo by S. Costa-Dias

To Alexandre,

the most special project in my life.

Agradecimentos

Ao longo deste trabalho são referidas as instituições e pessoas a quem são devidos agradecimentos, bem como os demais autores das publicações resultantes, todos eles essenciais para a tese agora apresentada. Por essa razão, e apesar de não ser excessivo estar também aqui a salientar o seu contributo, reservei-me o direito de tornar esta página a única realmente pessoal e livre do escrutínio científico!

Assim, nestes agradecimentos começo por mencionar os meus orientadores, pelos inúmeros momentos de discussão, por ter sentido que a minha opinião era considerada, pelo apoio no desenvolvimento e concretização de ideias… e muito, muito obrigada por todos aqueles instantes em que (quase) me esqueci de que estávamos no rio em trabalho!

Aos meus amigos (que têm sido como o vinho do Porto!), agradeço por me apoiarem nos dias bons, e principalmente nos menos bons. Obrigada pela vossa ajuda, fosse no rio com os músculos dormentes do frio ou o corpo gelado dentro de um barco encharcado, num exercício de imaginação para ultrapassar um problema qualquer ou a tentar não escorregar nas pedras dos ribeiros, no convívio delicioso de um pitéu ou no calor simples de um qualquer líquido à lareira! Ainda mais importante do que a ajuda em si, foi saber que estariam “lá” para mim!

Serei sempre grata à minha família. Aos meus pais e irmãos por me terem fadado há muitos anos, ainda não tinha saído desse nosso cantinho onde o verde é mais verde e nada se compara ao que quer que seja, que a curiosidade me levaria a fazer coisas diferentes e inesperadas! (e esquisitas…) Agradeço o apoio e o carinho da família enorme e fabulosa que tenho a sorte de ter, mas, porque não esperaria outra coisa de vós, acima de tudo agradeço a curiosidade, o interesse, e todas as perguntas sobre o meu trabalho.

Ao Alberto agradeço todas as pequenas (grandes) coisas que ultrapassamos, ter encarado algumas amostragens como uns dias bem passados e arregaçado as mangas para me ajudar, termos partilhado preocupações, mas principalmente ter partilhado o meu entusiasmo - afinal o que aqui está tem inevitavelmente um pouquinho de ti!

E porque me sinto leve no meu fardo, graças a todos vós, olhando para as dificuldades que surgiram posso dizer, (qual Nemo Knox)

“Pedras no caminho?
Guardo todas, um dia vou construir um castelo…”

Sérgia

Table of contents

	Page
Figures index	i
Tables index	ii
Abstract	iii
Resumo	vi
Introductory note	vii
BACKGROUND	
Chapter 1. The decline of European eel in the Iberian Peninsula	1
ESVA EUROPEAN EEL'S STOCK: ECOLOGY AND TROPHIC DYNAMICS	
Chapter 2. Eel recruitment to continental waters	19
Chapter 3. Eel's general condition: condition factor and infection by *Anguillicoloides crassus*	35
Chapter 4. Diel feeding activity and intensity patterns	47
Chapter 5. Feeding strategies along the environmental gradient of the river	59
WRAPPING UP	
Chapter 6. Food consumption and energy dynamics	77
REFERENCES	83

Figures index

	Simplified caption	Page
Figure 1.1.	European eel life cycle	4
Figure 1.2.	Glass eel recruitment in European rivers	4
Figure 1.3.	Natural distributional range of eel in mainland Spain, and Mediterranean and Atlantic Islands	5
Figure 1.4.	Eel distributional range in mainland Spain and Mediterranean Islands in 2001	6
Figure 1.5.	Portuguese mainland freshwater areas for eel colonization	7
Figure 1.6.	Glass eel and yellow + silver eel stages fishery catches in S. Juan de la Arena, Minho and Albufera de Valencia	8
Figure 2.1.	Geographical location of the study sites in Río Nalón and Río Minho	22
Figure 2.2.	Monthly occurrence of successive pigmentation stages (%) in the glass eels of the Río Nalón and Río Minho estuaries	24
Figure 2.3.	Mean monthly length (mm) and mass (g) of glass eels examined in Río Nalón and Río Minho	25
Figure 2.4.	Temporal decline in the physical condition of glass eels from Río Nalón and Río Minho	29
Figure 2.5.	Month-to-month variation in the pigmentation stages of Río Arno (Italy)' glass eels during 1978–1979	31
Figure 3.1.	Percentage of eels (%) infected by *A. crassus*	43
Figure 4.1.	Stream water temperatures (ºC) recorded at the time of sampling in Río LLorín over the sampling months	51
Figure 4.2.	Feeding activity index (A_I) in every sample (time of day) over the study months	52
Figure 4.3.	Feeding intensity index (I_I) for every sample (time of day) over the study months	54
Figure 4.4.	Mean monthly feeding activity and intensity as quantified by the indexes A_I and I_I, respectively, over the study months	56
Figure 5.1.	Feeding intensity index (I_I) for site and season	68
Figure 5.2.	Comparison between feeding patterns of eel and benthos composition for each site and season	70

Tables index

	Simplified caption	Page
Table 2.1.	Monthly length-to-mass relationships for the Río Nalón' glass eels	26
Table 2.2.	Results of ANCOVA for the length-to-mass relationships	27
Table 2.3.	Results from ANOVAs for the effects of river and month on the length and mass of glass eels	28
Table 2.4.	Comparison of pigmentation stages and size of glass eels across Atlantic and Mediterranean localities	30
Table 3.1.	Physico-chemical characteristics of the study sites	40
Table 3.2.	Number and mean (minimum – maximum) length and weight, and mean condition factor of eels examined. Levels of prevalence, mean intensity and mean abundance of pre-adults and adults of *A. crassus*. Levels of prevalence of larvae L3+L4; and presence of larvae L2 in relation to the number of individuals examined in each sampling site and season	41
Table 4.1.	Results of ANOVAs for the effects of the month and time of day on the feeding activity and feeding intensity	53
Table 4.2.	Results of ANOVA for the effects of the time of day on the feeding intensity quantified with the index I_I for each single month	55
Table 5.1.	Physico-chemical attributes across seasons for the four study sites	64
Table 5.2.	Benthos top three most abundant taxa per site and season	65
Table 5.3.	Number and mean (minimum – maximum in brackets) length and weight; mean condition factor; and feeding activity and intensity indices of eels examined	66
Table 5.4.	Numeric index (I_I) for all sites and seasons	69
Table 5.5.	Occurrence index (O_I) for all sites and seasons	69
Table 6.1.	Caloric values per area (cal. g wet $wgt^{-1}.m^{-2}$) according to taxonomic category across sites in May of 2007	80

Abstract

The European eel, *Anguilla anguilla* (L.), has been reported as in decline throughout its natural distribution range over the last decades. Along Chapter 1, general status and stocks temporal variation in the Iberian Peninsula are analyzed by reviewing pertinent literature and examining long term data sets. Major underlying causes for such decline appear related to human interventions on habitats, water quality deterioration, river regularization, introduction of invasive species, overexploitation and climate change. Furthermore, economical and ecological importance of eel on inland aquatic habitats is discussed and perspectives for the stocks changes commented.

Analyses of recruits to continental waters, the glass eel life stage, is conducted on Chapter 2 studying individual pigmentation stage and size during the arrival period to two northwestern Iberian estuaries (Río Nalón – a proxy for Río Esva, the main study area in this thesis; and Río Minho) and comparing the patterns elucidated in this study with those reported for other geographical locations of the Atlantic and Mediterranean ranges. Nine pigmentation stages ranging from practically transparent to fully pigmented were detected. Overall, stage VB was predominant but within a broad variance across rivers and seasons. Consistent with previous studies, pigmentation stages tended to increase when the season progressed whilst, for a given individual length, mass tended to be higher during the early months of arrival. Moreover, both mass and condition factor were lower in individuals of advanced stages. Whilst we found no significant differences on glass eels length among pigmentation stages, both mass and condition factor were lower in more pigmented individuals. Given that larger glass eels with better physical condition may reveal greater energy content implying improved upstream migration, earlier individuals may be better migrants than latter individuals.

Río Esva is a near pristine river, lacking most anthropogenic impacts, but with no previous study on the parasitic nematode *Anguillicoloides crassus*. Consequently, possible infection by the parasite was investigated on samples collected on a seasonal basis in distinct habitats along the river gradient (Chapter 3). This study highlighted the presence of the parasite and completion of the whole life cycle in eels from the Río Esva. Infection levels by *A. crassus* were high at three sites between the mid river to the estuary and also varied among seasons. Condition of eels was lower at upstream sites compared with downstream locations. Although high-quality environmental conditions in the Río Esva may buffer the effects of *A. crassus* on eels, potential impacts and limiting factors for the parasite are discussed.

Moreover, diel and seasonal shifts in the feeding activity and intensity of eels were assessed in the Río LLorín, a right-side tributary of the Río Esva drainage (Chapter 4). No consistent diel cycles were detected in either season (eels feed with similar intensity in the day and at night), but increased feeding activity was evidenced during the warmer months relative to weaker but still significant activity in winter. Heterogeneity in both feeding activity and intensity may stem from the high rates of secondary production in the form of benthic food available for eels, and mild temperatures all year round.

Eels' feeding strategy and food consumption was also investigated (Chapter 5) to track energy flow. Such information contributes to determine levels of production rates vis-à-vis the capacity of a system to support its eel stock. To this aim, we first determined eel feeding in relation to food availability in the form of benthos assemblage composition at four sites selected along the environmental gradient of the river. Yellow eels segregate spatially along the environmental gradient of Río Esva as do macro-invertebrates assemblage that constitutes the main food items fed upon by eel.

Finally, a brief merging of main outcomes is made on Chapter 6.

Resumo

A enguia europeia, *Anguilla anguilla* (L.), tem sido descrita como em declínio ao longo da sua área natural de distribuição durante as últimas décadas. O estado e a variação temporal dos stocks na Península Ibérica são analisados ao longo do Capítulo 1 através de uma revisão da literatura e examinando séries temporais de dados. Aparentemente, as principais causas do declínio estão relacionadas com a intervenção humana nos habitats, deterioração da qualidade da água, regularização dos rios, introdução de espécies invasoras, sobre-exploração e alterações climáticas. É ainda discutida a importância económica e ecológica da enguia em habitats de águas interiores e as perspectivas para alterações nos stocks comentadas.

A análise dos recrutas a águas continentais, a fase de enguia de vidro, foi levada a cabo no Capítulo 2 através do estudo do estado de pigmentação e tamanho individual durante o período de chegada a dois estuários do noroeste ibérico (Rio Nalón – uma aproximação para o Rio Esva, a área de estudo central desta tese; e o Rio Minho) e comparando os padrões elucidados neste estudo com os descritos para outras localizações geográficas no Atlântico e no Mediterrâneo. Foram detectados nove estados de pigmentação, desde praticamente transparente até completamente pigmentado, nos primeiros dois locais. O estado VB foi no geral predominante, mas com uma grande variação entre locais e estações do ano. Tal como em estudos anteriores, os estados de pigmentação tenderam a aumentar com o progredir da estação do ano, enquanto para um dado comprimento individual, a massa tendeu a ser maior durante os primeiros meses de chegada. Da mesma forma, tanto a massa como o factor de condição foram menores em indivíduos em estados mais avançados. Enquanto nenhuma diferença significativa no comprimento das enguias de vidro foi detectada entre estados de pigmentação, tanto a massa como o factor de condição foram menores em indivíduos mais pigmentados. Dado enguias de vidro maiores, com melhor condição física, poderem revelar maior conteúdo energético implicando uma migração para montante melhorada, os indíviduos que chegam mais cedo poderão ser melhores migradores que os indivíduos tardios.

O Rio Esva é um rio próximo do pristino, sem impactos antropogénicos relevantes, mas não existia um estudo anterior sobre o nemátode parasita *Anguillicoloides crassus*. Consequentemente, a possível infecção por este parasita foi investigada em amostras recolhidas com uma base sazonal em diferentes habitats ao longo do gradiente do rio (Capítulo 3). Este estudo evidenciou a presença do parasita e a ocorrência de todo o seu ciclo de vida em enguias do Rio Esva. Elevados níveis de infecção por *A. crassus* foram

detectados em três locais entre a zona média do rio e o estuário variando também entre estações do ano. A condição das enguias foi menor nos locais a montante. Embora a elevada qualidade ambiental do Rio Esva possa atenuar os efeitos de *A. crassus* nas enguias, os potenciais impactos e factores limitantes para o parasita são discutidos.

Adicionalmente, as alterações diárias e sazonais na actividade e intensidade alimentares da enguia foram avaliadas no Rio LLorín, um tributário da margem direita do Rio Esva (Capítulo 4). Não foram detectados ciclos diários consistentes em qualquer das estações do ano (as enguias alimentaram-se com intensidade semelhante durante o dia e a noite), mas foi evidenciada uma actividade alimentar aumentada durante os meses mais quentes relativamente a uma actividade mais fraca, mas ainda assim significativa, durante o Inverno. A heterogeneidade tanto na actividade como na intensidade alimentares podem advir das elevadas taxas de produção secundária na forma de alimento bentónico disponível para a enguia, e as temperaturas amenas durante todo o ano.

A estratégia alimentar e consumo alimentício da enguia foram também investigados (Capítulo 5) para detectar o fluxo energético. Essas informações contribuem para determinar níveis de taxas de produção relativamente à capacidade de um sistema para suportar o seu stock de enguia. Para alcançar este objectivo, foi primeiro determinada a alimentação da enguia em relação ao alimento disponível sob a forma de assembleias bentónicas em quatro locais seleccionados ao longo do gradiente ambiental do rio. No Rio Esva existe segregação espacial da enguia amarela ao longo do gradiente ambiental, acontecendo o mesmo com as assembleias de macro-invertebrados que constituem os principais itens alimentares de que a enguia se alimenta.

Finalmente, os principais resultados são integrados no Capítulo 6.

Introductory note

This introductory note has two main purposes: first, elucidate why this thesis exists as it is, and, secondly, introduce thesis structure and chapters arrangement.

The original PhD project divided focus between glass and yellow eel stages, aiming the period that the European eel, *Anguilla anguilla* (L.), spends in continental waters in different areas of the Iberian Peninsula. Abandoned approaches included otoliths analysis, with a strong chemical component, and isotopic analysis as a complementary examination of eel's diet. The expected sources of glass eels for geographical comparison failed mainly due to low catches in the selected rivers, thus preventing the intended meticulous comparison. Then again, after some well succeeded preliminary trials with glass eels and due to contingencies for which neither my supervisors nor I could be held responsible, isotopic analysis was no longer a possibility.

Furthermore, after one year of research as a provisional PhD student at ICBAS, students are asked to present their work so far to a committee whose positive evaluation allows the candidate to move on to the status of effective PhD student. By then students may propose changes on their project and be allowed to restructure the work plan according to that first year outcomes.

It was within this context that the work plan was altered. Part of previous and ongoing research by that time was incorporated in Chapter 2 and attention was then deviated towards the yellow eel stage during what is essentially an inland growing period. Due to the volume of field and laboratory work implied for this now much more detailed analysis on trophic matters, and with the shorten remaining working time, only Río Esva was considered to proceed with the PhD objective of contributing to the knowledge on the ecology and trophic dynamics of the European eel.

This thesis is divided into six chapters, some of them already published as independent studies but merging here as an all to allow a better understanding of an extraordinary species such as the European eel. Furthermore, the main research area, Río Esva, is virtually as pristine as you may get in Europe, and provided unique natural conditions for the work carried out.

Chapter 1 ("BACKGROUND: The decline of European eel in the Iberian Peninsula") introduces briefly the presence and importance of European eel, particularly in the Iberian Peninsula, potential causes of stocks decline and economical and ecological importance of the species on inland aquatic habitats. Nevertheless, eel recruits migrate from the Ocean into inland waters and if environmental conditions prior to recruitment are similar, inland conditions assume major importance. As mentioned before, Río Esva was

taken as the main study area and eels inhabiting it were looked into detail ("ESVA EUROPEAN EEL'S STOCK: ecology and trophic dynamics" – Chapters 2 through 5). In Chapter 2 ("Eel recruitment to continental waters") the main characteristics of recruits, the glass eel life stage, from the study region were investigated. Río Nalón was taken as a comparable reference for Río Esva recruits inflow due to geographical proximity. Once in freshwaters, the majority of the potential threats for eel pointed out in Chapter 1 are inexistent in Esva. Nevertheless, no study on the parasitic nematode *Anguillicoloides crassus* had ever been conducted. Consequently, key sites were selected within the basin and eels were analyzed to determine their physical condition and *A. crassus* burden, as described in Chapter 3 ("Eel's general condition: condition factor and infection by *Anguillicoloides crassus*"). After recruitment, the period eel remains in inland waters is basically a growing period that culminates with individuals' sexual maturation and migration back to ocean for reproduction. The role eel plays in the system, inter-specific relations, and carrying capacity of the system, largely determine stock maintenance and ability to increase. A critical factor to support a stock increase is the potential for energy acquirement. Most literature reports feeding behavior in captivity, in significantly altered systems, or regarding stocks attaining mean lengths much higher than Esva stock. Answer if eel is actively feeding all day long, a diel cycle, and all year round, an annual cycle, and variations on the intensity of feeding along both of those cycles was the purpose of Chapter 4 ("Diel feeding activity and intensity patterns"). On Chapter 5 ("Feeding strategies along the environmental gradient of the river") a further insight on trophic behavior of eel in Esva was taken. Benthos was considered as the source of potential prey and thus both benthos assemblages and eel feeding were investigated on the same four sites selected for eels' physical condition assessment. To wrap up this thesis, Chapter 6 ("WRAPPING UP: food consumption and energy balance") merges main outcomes from the previous chapters with complementary data to further enlighten the relevance of eel feeding strategies and the capacity of the system to support eel's stock.

Work not reported in this thesis:

The following articles are not included in this thesis due to approved changes on the working plan at the end of the first year of research, but the data used for their production were obtained during the period as a doctoral student of the University of Porto, developing research work at CIIMAR, and under the financial support of FCT:

Costa-Dias S., Freitas V., Sousa R. & Antunes C. 2010. Factors influencing epibenthic assemblages in the Minho Estuary (NW Iberian Peninsula). Marine Pollution Bulletin 61: 240-246. (doi:10.1016/j.marpolbul.2010.02.020)

Costa-Dias S., Sousa R. & Antunes C. 2010. Ecological quality assessment of the lower Lima Estuary. Marine Pollution Bulletin 61: 234–239.

(doi:10.1016/j.marpolbul.2010.02.019)

Sousa R., **Dias S.**, Freitas V. & Antunes C. 2008. Subtidal macrozoobenthic assemblages along the River Minho estuarine gradient (north-west Iberian Peninsula). Aquatic Conservation: Marine and Freshwater Ecosystems 18: 1063-1077. (doi: 10.1002/aqc.871)

Sousa R., **Costa-Dias S.**, Guilhermino L. & Antunes C. 2008. Minho River tidal freshwater wetlands: threats to faunal biodiversity. Aquatic Biology 3: 237-250.

BACKGROUND

Chapter 1. The decline of European eel in the Iberian Peninsula

Part of this chapter has been published as:

Costa Dias S., Sousa R., Lobón-Cerviá J., Laffaille P. 2009. The Decline of Diadromous Fish in Western European Inland Waters: Main Causes and Consequences, pp. 67-92. *In*: McManus N.F. and Bellinghouse D.S. (eds) Fisheries: Management, Economics and Perspective. NovaScience Publishers, Inc. (ISBN 978-1-60692-303-0).

Acknowledgments for this chapter

Acknowledgments are due to local Fishermen's Associations and Maritime Authorities of Nalón and Minho Rivers, and Albufera de Valencia for providing data sets. This study was supported by the Portuguese Foundation for Science and Technology – FCT (S.C.D. – grant SFRH/BD/16922/2004).

Scientific review of the above mentioned book chapter (Costa-Dias et al. 2009) was made by PhD Jonathan Wilson, researcher at CIIMAR, on behalf of NovaScience Publishers, Inc..

The decline of European eel in the Iberian Peninsula

Abstract

The European eel, *Anguilla anguilla* (L.), has been reported to be declining throughout its natural distribution range over the last decades. In this chapter, general status and stocks temporal variation in the Iberian Peninsula are analyzed by reviewing pertinent literature and examining long term data sets.

Major underlying causes for such decline appear related to human interventions on habitats, water quality deterioration, river regularization, introduction of invasive species, overexploitation and climate change.

The economical and ecological importance on inland aquatic habitats is discussed and perspectives for the stocks changes commented.

Introduction

The European eel, *Anguilla anguilla* (L.), is a catadromous teleost with reproduction grounds in the Sargasso Sea (Atlantic Ocean). After spawning, offspring in larval stage known as the “leptocephalii” migrate east towards Europe and North Africa where they colonize inland waters. A component metamorphose to glass eels prior to entrance in continental waters, where a new metamorphosis into a yellow stage takes place, and remain from around 3 (males) up to 20 (females) years (Vollestad 1992), growing until maturation to adult reproducing stage begins, developing into silver eels and migrating then downstream towards the Sargasso Sea (Tesch 2003) (Figure 1.1).

Highly appreciated and economically important, the unique eel population has been reported to be declining severely throughout its distributional range (Costa-Dias *et al.* 2009; Dekker 2003; ICES 2006). Its status was specified by the International Council for the Exploration of the Sea (ICES) as a species in regression, presenting some critical situations to the extent to be an endangered species.

Eel monitoring data in European rivers revealed general decreasing trends over the last two decades for all time-series analyzed, where the high levels of the late 1970s were followed by a rapid decline that continues to the present time (ICES 2008). This is shown on figure 1.2 for glass eels recruitment in estuarine areas.

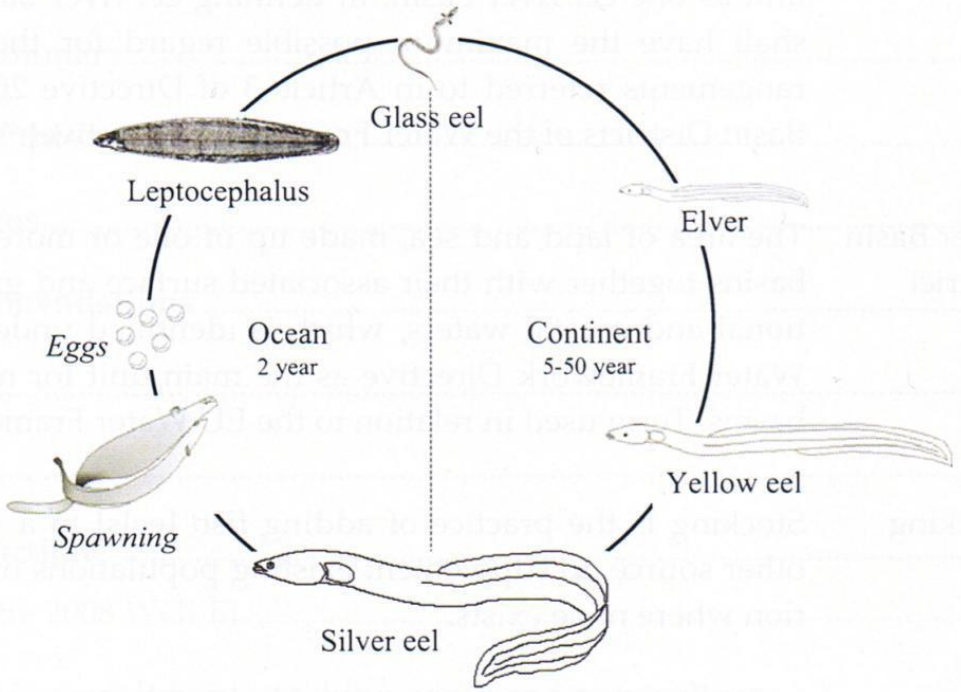


Figure 1.1. European eel life cycle. ICES (2008).

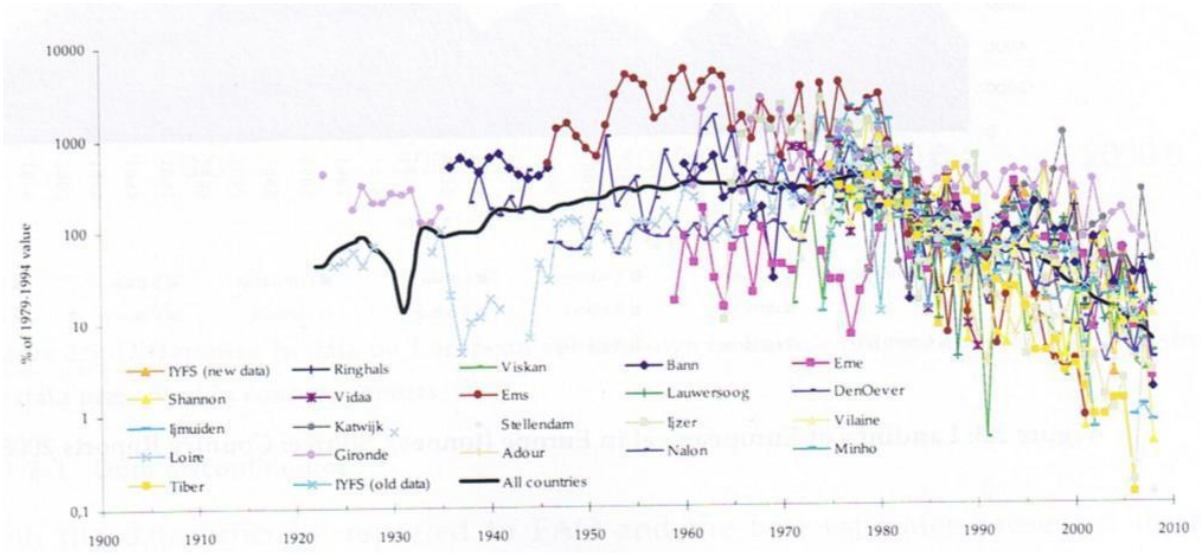


Figure 1.2. Glass eel recruitment in European rivers. Time series have been scaled to its 1979-1994 average (Log scale on the y-axis). Figure from ICES (2008).

In the Iberian Peninsula, where recruitment has been historically extremely high (Deelder 1984), eel stocks face concurrent declining trends observed across European waters and are considered “threatened” (Blanco & Gonzalez 1992; Doadrio 2001; Cabral *et al.* 2005). Declining trends are observed for both glass and yellow plus silver eel’s

fisheries. Several locations for which long term fisheries dataset exist were selected to explore temporal trends.

Presence *vs* absence data

A comparison between the natural distributional ranges in Spain with recent data, and in addition, with recent records for Portugal is instrumental to show the magnitude of habitat lost. Actually, the distribution of eel is reduced to a few coastal rivers where colonization upstream is unimpeded by human interventions, essentially by the construction of dams and weirs near the river mouths.

Spanish distributional ranges of eel recorded decades ago are presented in Figure 1.3, and the distribution in mainland Spain and Mediterranean Islands in 2001 (Doadrio 2001) in Figure 4. Eel was by then extinct in all large river drainages (e.g. Douro River - Lobón-Cerviá *et al.* 1989) and occurred only in a few unaltered coastal rivers (Lobón-Cerviá 1999). Eel is still present in Mediterranean and Atlantic Islands but only the Mediterranean group is included in Figure 1.4.

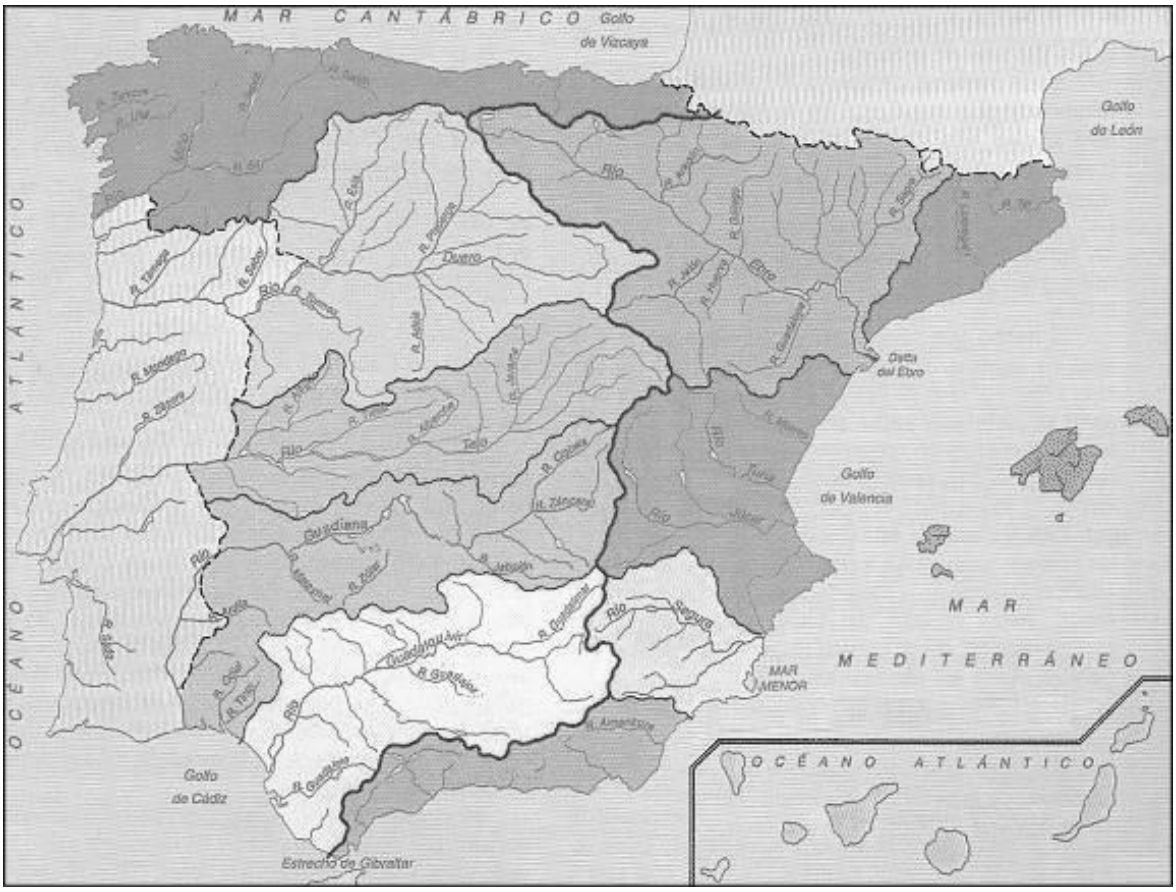


Figure 1.3. Natural distributional range of eel in mainland Spain, and Mediterranean and Atlantic Islands. Interrupted lines represent Spanish borders.

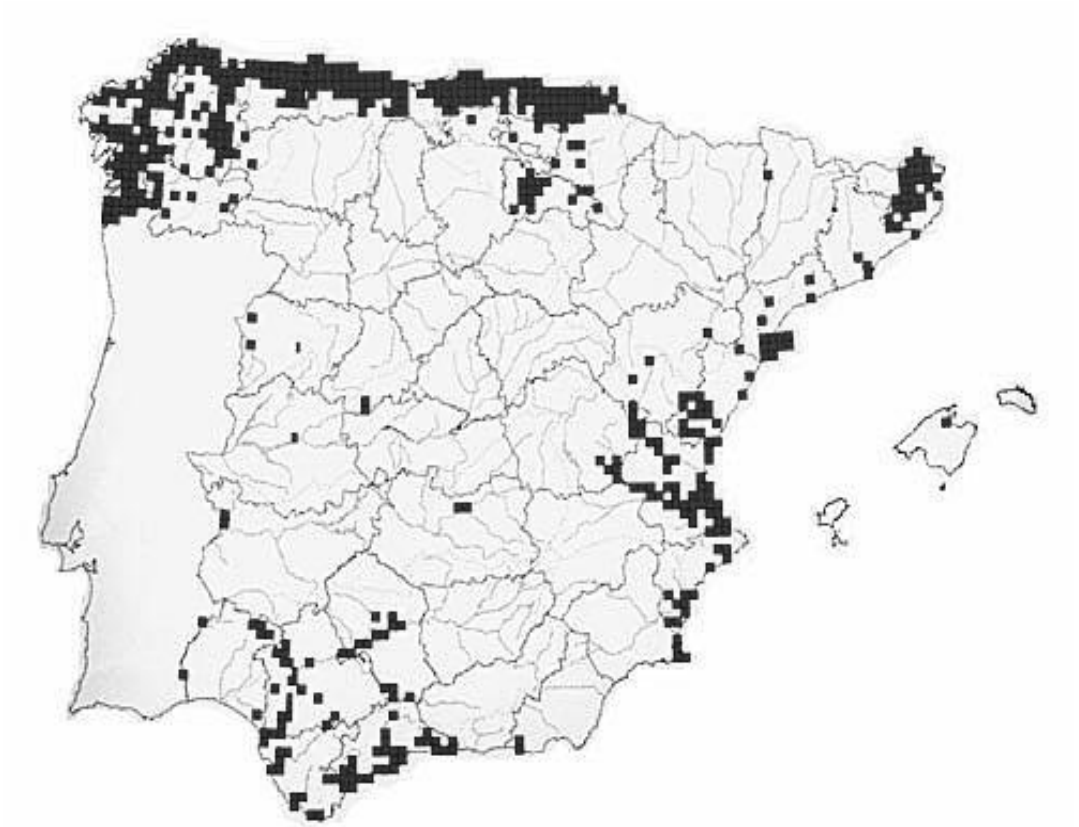


Figure 1.4. Eel distributional range in mainland Spain and Mediterranean Islands in 2001 (Doadrio 2001). Presence in Atlantic Islands not included.

Available areas for eel in Portuguese mainland have mainly been draw by physical barriers such as dams and weirs which constrain eel distribution to obstacles downstream areas (Figure 1.5; ICES *in press*). Information on the Portuguese islands of the Atlantic Ocean is scarce but eel was present in at least two islands (Terceira and S. Jorge) of the Azorean archipelago in 2002 (Antunes & Costa-Dias, personal observation). However, no further study has been conducted.

Fishery data sets

Three long term fisheries data sets, made available by local Fishermen's Associations and Maritime Authorities, were analyzed. The corresponding locations can be found in the map of Figure 1.3 and are as follows: Río Nalón, referred to as San Juan de la Arena where fisherman are settled (Northern margin of the Iberian Peninsula) and Río Minho (Western margin of the Iberian Peninsula; data comprises Portuguese and Spanish official records), both of them mouthing to the Atlantic Ocean, and the Albufera

de Valencia (Eastern margin of the Iberian Peninsula; Albufera lake fed by Río Júcar), that mouths into the Mediterranean Ocean.

The European eel recruits, the glass eel stage, arrive to European coasts all year round but enter estuaries essentially during late fall and winter (Tesch 2003). The legal fishing periods for that life stage are generally comprised within that period of entrance into continental waters (Korta & Díaz 2008) and their temporal trends are thus comparable between rivers.

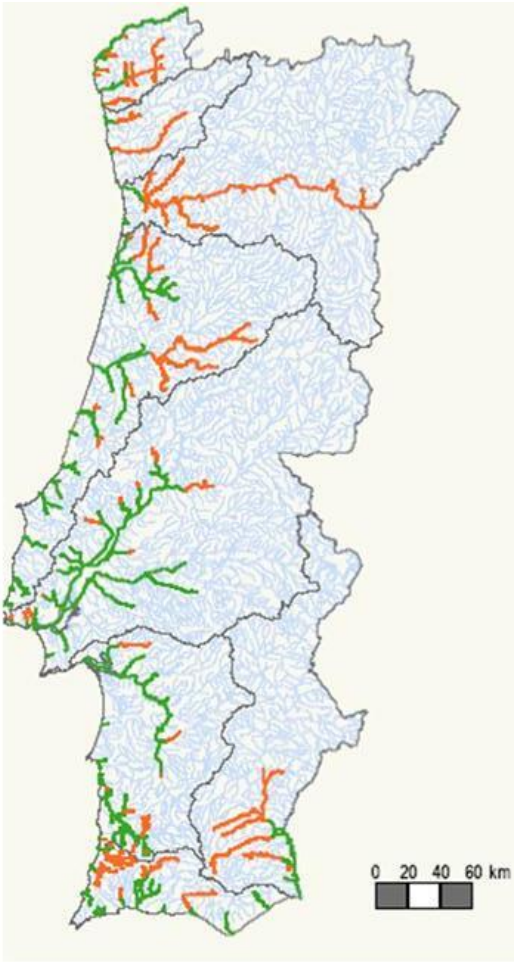


Figure 1.5. Portuguese mainland freshwaters (administrative limits with black line): available (in green), with limited access (in red) and inaccessible (in blue) areas for eel colonization (ICES *in press*).

The graphic representation of the three selected data sets for all life stages (glass eels and yellow plus silver eels) is presented in Figure 1.6, and shows decreasing trends over the last decades. Nonetheless, for S. Juan de la Arena (Figure 1.6a) only glass eel data exists with higher values recorded during the 1970s followed by a steep decline and extremely low values during the last two decades. Glass eel initial records for Minho River (Figure 1.6b) reefer to the 1970's, after which the fishery suffered a huge advance due to

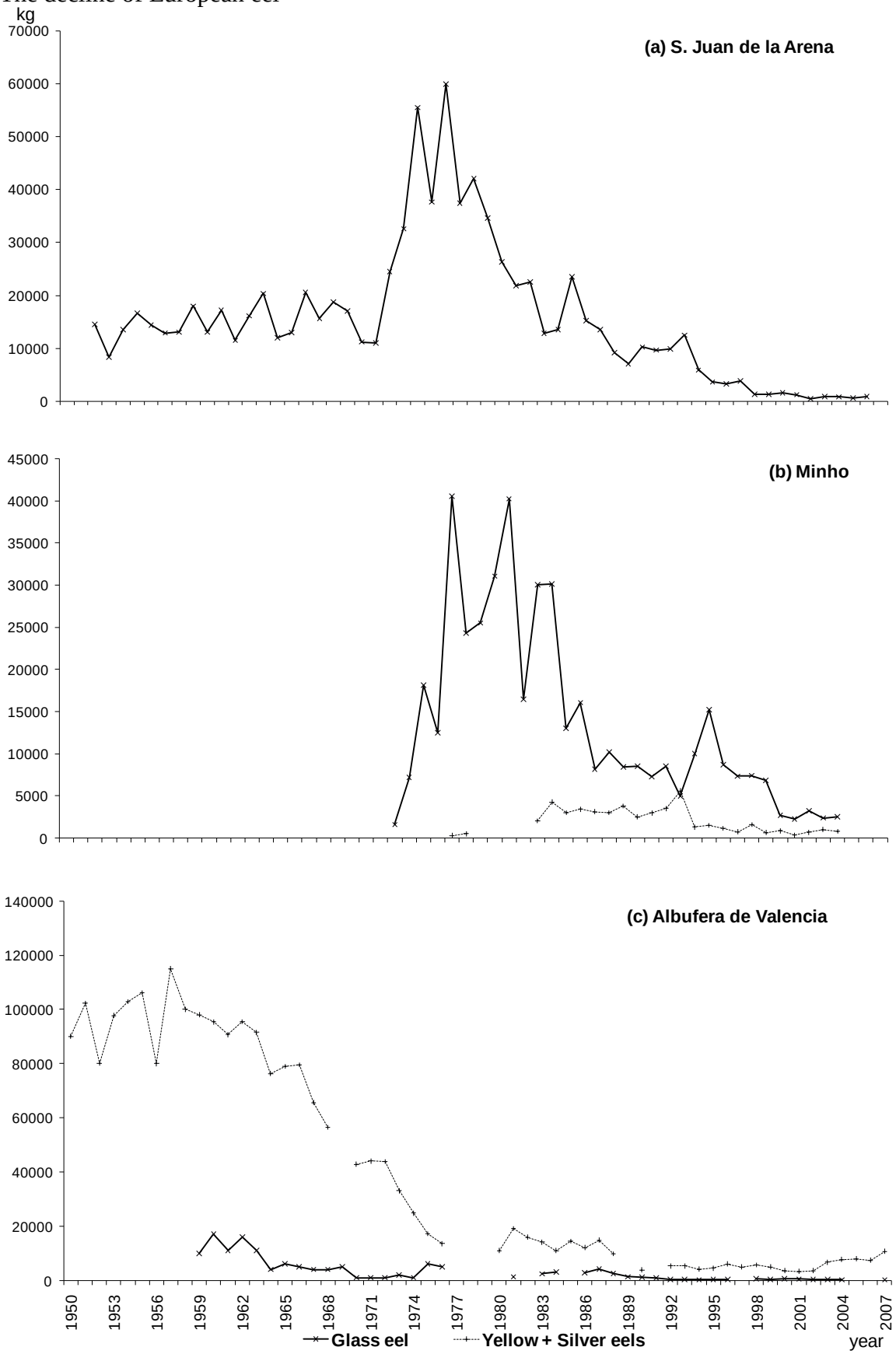


Figure 1.6. Glass eel and yellow + silver eel stages fishery catches in (a) S. Juan de la Arena, (b) Minho and (c) Albufera de Valencia. Data provided by local Fishermen's Associations and Maritime Authorities.

the implementation of a new and more efficient fishing gear, a stow net called “tela” (Weber 1986). Nevertheless, the glass eel data set from that period onwards seems to follow the same pattern as for S. Juan de la Arena. Yellow and silver eels’ fishery has been of lesser importance in Minho, which is reflected in low catches. In the Albufera (Figure 1.6c) the data set for yellow plus silver eel fishery shows much higher catches than for the glass eel stage but also exhibit declining values from the 60’s onwards.

In general, declines in eel catches have been assumed to reflect overall species decline but potential causes appear generally linked to anthropogenic activities and may have different local weights, as explored below.

Potential causes of stocks decline

The causes of eel decline and/or local extinctions track the same causes as pointed out for other diadromous species and are rarely due to just one factor in their origin, resulting probably from cumulative and/or interplay of several anthropogenic effects (Rochard *et al.* 1990; Jonsson *et al.* 1999; Feunteun 2002).

Habitat loss has been pointed out as the most common threat to biodiversity (Rodrigues *et al.* 2006) and in the case of aquatic ecosystems it may be a consequence of an alteration of land usage such as conversion to agricultural areas or construction on the margins, interventions on the riverbed such as channelization, or inaccessibility caused by physical barriers like ditches and dams. Probably the most significant anthropogenic influence on the decrease of diadromous fishes has been alteration, degradation and destruction of habitat (Jonsson *et al.* 1999). Effects are very difficult to estimate because they range from small (modification of micro-habitat characteristics) to large spatial scales (alteration of ecosystem processes) (Gregory & Bisson 1997).

In the case of ditches, weirs and dams, eels migration to suitable areas for feeding may be prevented. In addition, water impoundments interfere with both longitudinal and lateral connectivity, that is, with upstream-downstream and lateral movements, respectively. A decrease in longitudinal and lateral connectivity may result in fragmentation of the systems and alter the natural flooding patterns (Jones & Stuart 2008). In the case of large physical obstacles such as dams, further than preventing access to upstream areas, they may also prevent downstream migrations or lead to high mortality rates caused by individuals fall or turbines’ injuries in the case of hydroelectric facilities (Taverny *et al.* 2000), as would happen with eels maturing in dams’ upstream areas.

Loss of habitat, especially areas above barrages and dams (through the disconnection of available habitat from the stream), seems to be responsible for the

European eel decline, or even local extinction (Moriarty & Dekker 1997). Nowadays, migration of eels in many European rivers is obstructed by dams, and it has been estimated that 33% of the habitat within its natural range was not accessible for natural or artificial reasons more than one decade ago (Moriarty & Dekker 1997). Although the situation varies between countries, around 96% of habitat was lost in Portugal (ICES *in press*) and 90% in Spain with eel disappearing in more than 80% of the river catchments across the Iberian Peninsula (Lobón-Cerviá 1999).

Another factor reported as nocive to eel stocks was the outstanding spread of the parasitic nematode *Anguillicoloides crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda, Dracunculoidea), a swimbladder parasite of the Japanese eel *Anguilla japonica* Temminck & Schlegel, introduced at the beginning of the 1980s (Blanc 1989; Kirk 2003) that seriously affected European eel populations (Lefèbvre *et al.* 2004; Eel Rep. 2005). This parasite may cause inflammation, necrosis and scarring by both larvae living in the swimbladder tissue and pre-adults and adults feeding upon the host blood (Didžiulis, 2006), potentially causing the loss of functional role of swimbladder and affecting the physical condition of individual eels.

Furthermore, the European eel has been widely investigated as biomonitor of system's health and demonstrated to accumulate contaminants in accordance with concentrations in water or sediments (e.g. Batty *et al.* 1996; Bordajandi *et al.* 2003; Linde *et al.* 1999; Usero *et al.* 2003; Belpaire & Goemans 2007). Specific characteristics such as species size, life span, fat content, benthic feeding, habitat use, distribution, euryhyalinity and semelparity (one reproductive cycle) may be considered as unfavorable according to chemical contamination. Their important effects concern the decrease of the reproductive and/or migratory and/or resistance capacities (Bruslé 1994; Couillard *et al.* 1997; Eel Rep. 2005; Hodson *et al.* 1994). For instance, pollution might severely lower individual fitness by reducing the fecundity or the probability of reaching the Sargasso Sea for reproduction (Acou *et al.* 2008). As an indirect factor, in the Yser basin eutrophication has led to the impoverishment of the fish population and the habitat quality, with consequent decrease on the available food for eels (Denayer & Belpaire 1996). Additionally, even low but long-sustained levels of contamination may affect individuals and lead to physiological alterations or even increase their susceptibility to disease (e.g. see Feunteun 2002 and references therein).

Migratory fish species are especially vulnerable to some of the abnormalities caused by flow regularizations such as a decrease in freshwater outflow that otherwise would function as a strong river signature, as happens with the riverine recruitment of the early stage of the European eel juveniles (Briand *et al.* 2002). Glass eels enter estuaries mainly in autumn and winter, when high flow and floods naturally occur, and the flow may

quantitatively predict the fluvial recruitment (Acou 2006). Although there is some evidence of the influence of river flow on fish communities within estuaries (Costa *et al.* 2007) or freshwater catchments (Lasne *et al.* 2007a), upstream effects demand far more attention since lower estuarine areas function mainly as migratory corridors, with upstream areas being potentially more relevant for the vital life cycle requirements.

The European eel occupies a great variety of habitats in inland waters and Knights (2003) described the continental phase of this species as tolerant to a wide range of environmental conditions and with a broad choice of prey, which along with the fact that they do not spawn in fresh water, makes them less vulnerable to change. Being so, from all the factors reported as leading to a decline in the European eel population, ocean-climate changes interfering with larval (leptocephali) transport may be key influences leading to recruitment failure (Castonguay *et al.* 1994; Friedland *et al.* 2007; Knights 2003). Larval survival is possibly affected by starvation and/or unfavorable currents that prevent or prolong the duration of the oceanic migration (Friedland *et al.* 2007; Knights 2003).

Bonhommeau *et al.* (2008) also focused on the oceanic phase and particularly on the potential effect of oceanographic and trophic conditions encountered by eel larvae on the patterns of variability and the decline in glass eel recruitment over several decades. They investigated the relation between temperature and primary production, thus exploring that relation by using temperature as an inverse proxy for primary production over time. A negative correlation was found between glass eel recruitment and sea temperature, thus sustaining the hypothesis that variability in glass eel recruitment may be linked to food availability and/or composition for the early larval stage. Additionally, changes in the winds and temperature structure of the Sargasso Sea may affect the spawning location and consequently the efficiency of the leptocephali transport to continental habitats (Friedland *et al.* 2007).

Overfishing is not generally the sole cause of species extinction, but harvest is a contributing factor to extinction risk for many populations of diadromous fishes (Jonsson *et al.* 1999). Fishing the larger individuals frequently leads to an increased opportunity for reproduction of the smaller/younger individuals, potentially less fitted. For eel, fishing the larger (immature) yellow eels and (maturing) silver eels may put an increased pressure on one of the genus, females in this case, since they generally grow into larger sizes than males. Regarding the fact that all continental stages are targeted by fisheries must be considered. Nevertheless, the impact of fisheries on eel stocks seems to be site dependent, since overfishing tends to affect more seriously easily exploitable areas with limited recruitment (Knights 2003), that is, where recruitment does not exceed the carrying capacity of the ecosystem.

Consequences of stocks decline

The decline on diadromous fish is deemed responsible for economic and social losses and for considerable alteration in the ecosystem structure, processes and functions (Baisez & Laffaille 2005). Furthermore, inexistent or failed management plans allowed for intensive fisheries and economic structures depending on those resources were ruined when they collapsed. Besides the ecological and/or conservational importance of diadromous fish as an original component of biodiversity (Béguer *et al.* 2007), eel has been recognized for its role as an umbrella species (Feunteun 2002; Baisez & Laffaille 2005) or for its participation in organic matter exchanges (Laffaille *et al.* 2000). However, the ecological research focused on potential effects on ecosystem functioning derived from declines in migratory fish species still is in infancy. The number of studies addressing these and other related issues are considerably less than those on coastal areas and as a consequence, there is an urgent need to increase our knowledge and apply it in future management and conservation action plans.

Economic Consequences

Commercial and recreational fisheries of diadromous fish have been of economic and social importance for centuries (Dekker 2003; Elie *et al.* 2000; Marta *et al.* 2001). Despite the fact that the economic importance of inland fisheries is generally declining in Europe, at least in Western countries (Moriarty & Dekker 1997), some fisheries still maintain commercial and social importance (Baisez & Laffaille 2005). Furthermore, fish provides 15% of the total animal protein in human diets (Casal 2006). Although a single diadromous fish species may not be critical for most European local economies, they may still represent an important source of additional income.

In southern Europe, the exploitation of eel is one of the major components of the small-scale coastal and inshore fisheries. Juvenile eel are the third species in economic rank value, from the Loire (France) to the south of Portugal. Its economic importance is thus substantial and has a strong social impact within the framework of small-scale coastal, estuarine and inland fisheries. These fishing activities have a structuring effect on regional economies in the Atlantic Space, with around 25 000 people in Europe drawing an income from fishing eel (IFREMER–www.ifremer.fr; Moriarty & Dekker 1997). Furthermore, the total production amounts to 180 million Euro, plus 380 million Euro in added value (Feunteun 2002).

Despite all the actual scientific knowledge and advances on eel reproduction and development, it is not yet viable to complete the eel life cycle for aquaculture purposes.

Thus, eel aquaculture may only be used for growing individuals originating from wild stocks and is not an effective alternative to eel fisheries.

Nonetheless, direct income from fisheries is not the only benefit from migratory species since they may perform important functions in any given system and so contribute to the overall sustainability.

Ecological Consequences

The debate about loss of biodiversity and its consequences for the ecosystem functioning has been focused on the possibility that declines in assemblage diversity will be critical for the performance of important functions, and consequences will be greatest when species with disproportionately strong influences on nutrient, habitat, or assemblage dynamics are lost (Allan *et al.* 2005). In relation to migratory fish species, declines in abundances and diversity will be critical not only for the aquatic ecosystems colonized but also for adjacent terrestrial areas with possible consequences for a diverse assemblage of terrestrial and semiaquatic animals, and terrestrial plant assemblages (Allan *et al.* 2005). A reduction in the density of these fishes entering European rivers can have ecological consequences at least in food webs; nutrient cycling; abiotic properties of the ecosystem; and relationships with other organisms (predation, facilitation processes, parasitism).

In relation to the food webs the implications of losing a species depends on the complexity and diversity of each assemblage. Even so, a decrease or disappearance of a certain species may divert the flux of energy to their competitors and from their predators. Omnivorous fishes such as eel, consume variable amounts of organic matter not digestible for other species, which may be absorbed by the eel or expelled in a (partially) digested form becoming then available for other organisms. Furthermore, diadromous species are considered as biotic vector of nutrients traveling in different directions, from rivers to ocean and vice-versa (Laffaille *et al.* 1998; Lefeuvre *et al.* 1999).

Nutrient and organic matter fluxes have generally been assessed by measurements of quantities transferred by water. Nevertheless, the involvement of fish in these fluxes has rarely been studied. Though, Krokhin (1975), Durbin *et al.* (1979), Northcote (1988) and Elliott *et al.* (1997) have shown that diadromous species, e.g. salmonids, are responsible for major organic inputs in oligotrophic riverine systems. Fish migrations within fluvial basins are responsible for active transport of nutrients and energy at the landscape scale and declines in these migrations may result in the loss of an important flux of resources. In this way, the European eel export organic matter as body weight from continental waters (brackish and fresh) where they grow, to the Sargasso Sea where they spawn and die (Laffaille *et al.* 2000). Therefore, because spawning, nursery

and growth areas are located in different systems, important amounts of nutrients and organic matter are transferred during migrations.

In recent years, the functional importance of ecosystem engineers (i.e. organisms that directly or indirectly control the availability of resources to other organisms through the physical modification, maintenance, or creation of habitats; Jones *et al.* 1994, 1997) has gained importance in the ecological theory. In relation to freshwater migratory species a few studies have shown that some species can play important roles as ecosystem engineers (e.g. digging, foraging, and movement). Depending on the species, size, density and characteristics of the habitat (potentially, the largest impacts will be in habitats with intermediate to low hydrologic energy), the resulting disturbance may be high and potentially produce significant impacts on benthic habitats and communities. Changes such as coarsening of sediments by displacing fine sediments (Moore *et al.* 2004), increasing concentration of suspended particulate matter (Moore 2006), decreasing periphyton biomass (Moore *et al.* 2004) and increasing mortality of benthic invertebrates (Minakawa & Gara 2003) have been described. In addition, according to Montgomery *et al.* (1996) bioturbation may also decrease the susceptibility of streams to erosion from floods due to sorting of sediments into size classes since digging may increase critical shear stress of stream bottoms. Foraging activities also bioturbate the sediments which may result in increases in the movement of fine sediments, reductions in local sediment accumulations and release of nutrients (Power 1990; Flecker 1996; Statzner *et al.* 2003).

Perspectives

Some of the sources of impact on eel stocks are unlikely to reduce to a point of full species recovery in the short term, even if they are human induced and actions are taken. Reasons are numerous in nature and varied and include intrinsic characteristics of the species and the severity of the measures taken (e.g. reductions in the fisheries may not be sufficient and a complete stop may be needed).

Eel status was specified by the International Council for the Exploration of the Sea (ICES): species in the process of regression and presenting critical situations, mainly in the North of its distribution area. The Precautionary Approach (as defined by FAO Technical Guidelines in 1996) requires that any fishing action be justified within the framework of a global management of the fisheries and be periodically called into question according to observed population trends. In this context, the Advisory Committee for Fisheries Management of ICES proposed a recovery plan which requires a reduction in the exploitation of eel and must include a habitat-restoration plan (INDICANG 2004).

Studying a small Cantabrian stream, Río Esva, where eel is not commercially exploited, Lobón-Cerviá & Iglesias (2008) found a surprising trend for this species. Their data set refers to the period of 1986-2006 and they report that in-stream density of yellow eels declined from high values over 2000 ind.ha-1 during the mid 80's to a minimum value of about 400 ind.ha-1 by the end of the 90's, which then increased to reach values around the 2000 ind.ha-1 during the last sampled years. Nevertheless, recruitment (of glass eels) to the water basin and estuarine density of the youngest juveniles in the Río Esva kept declining along the entire period of study. These results suggest the occurrence of in-stream density-dependence influence towards the stabilization of the population. Yet, Lobón-Cerviá & Iglesias' (2008) results regard a stock of eel that has never been exploited. Furthermore, the water basin of the Río Esva, for which data for both eel and salmonids were analyzed, remains in a natural state, with no physical barriers or any other highly negative impact on the river or it streams. To the same extent, no interventions were taken that could explain the increased values for catch or density of those fish species.

Recent works on basin water quality improvement and with the perspective of many more interventions to fulfill the European Union Water Framework Directive objectives, diadromous fish now absent in some basins, may recover if gene pool is still available (Béguer *et al* 2007, and references therein) and the recent increases in some species density both in un- and intervened areas may be considered as recovery signs encouraging further studies and management actions.

ESVA EUROPEAN EEL'S STOCK: ECOLOGY AND TROPHIC DYNAMICS

Chapter 2. Eel recruitment to continental waters

Chapert 3. Eel's general condition:
condition factor and infection by *Anguillicoloides crassus*

Chapert 4. Diel feeding activity and intensity patterns

Chapter 5. Feeding strategies along the environmental gradient of the river

Chapter 2. Eel recruitment to continental waters

This chapter has been published as:

Iglesias T., Lobón-Cerviá J., Costa-Dias S. & Antunes C. 2010. Variation of life traits of glass eels of *Anguilla anguilla* (L.) during the colonization of Ríos Nalón and Minho estuaries (northwestern Iberian Peninsula). Hydrobiologia 651: 213 - 223.
(DOI: 10.1007/s10750-010-0300-1)

Acknowledgments for this chapter

Thanks are due to the fishermen of the two rivers and very especially to Pepín ‘‘El Ruleru’’ and Ester Diaz (staff of the Río Nalón Fishermen Association) and to Alfredo Oliveira and Eduardo Martins (members of the Río Minho fishery crew) for their continuous help during glass eels collection. Brian Knights gave valuable comments on an earlier draft and Maite Lavandeira made her best to improve the English. During this study T. Iglesias was funded by the INDICANG Project of the INTERREG IIB—EU Program whereas S. Costa Dias was funded by the Portuguese FCT through the grant Ref. No. SFRH/BD/16922/2004.

Eel recruitment to continental waters

Abstract

Analyses of the pigmentation stages and the size of glass eels during the arrival period to two northwestern Iberian estuaries (Ríos Nalón and Minho) and comparison of the patterns elucidated in this study with those reported for other geographical locations of the Atlantic and Mediterranean ranges showed nine pigmentation stages ranging from practically transparent to fully pigmented in the two first locations. Overall, stage VB was predominant but within a broad variance across rivers and seasons. Consistent with previous studies, pigmentation stages tended to increase when the season progressed whilst, for a given individual length, mass tended to be higher during the early months of arrival. Moreover, both mass and condition factor were lower in individuals of advanced stages. Whilst we found no significant differences on glass eels length among pigmentation stages, both mass and condition factor were lower in more pigmented individuals. Given that larger glass eels with better physical condition may reveal greater energy content implying improved upstream migration earlier individuals may be better migrants than latter individuals.

Introduction

The abundance of riverine stocks of European eel *Anguilla anguilla* (L.) depends entirely on the annual recruitment of the youngest juveniles (i.e., glass eels) reaching continental waters to commence a new yearclass. Recent studies have emphasized the variability of the external factors during the oceanic migration of early life stages of the European eel (Kettle & Haines 2006; Friedland *et al.* 2007; Bonhommeau *et al.* 2008) so recruitment to rivers would, to a large extent, reflect larvae physiological conditions. Bureau du Colombier *et al.* (2007) have further suggested that estuarine colonization may depend on the energetic conditions of glass eels at their arrival that may also influence the dynamics of river stocks. Moreover, even if the ocean and estuary conditions may be indirectly influenced by human interventions, it is from recruitment onwards that both population assessment and management are best achieved. Therefore, understanding the arrival to the estuarine waters and the later recruitment to the stock is critical to evaluate the dynamics underlying riverine stocks. The relative abundance of recruiting glass eels (Dekker 2003) and their sizes and biometrics (Desaunay & Guerault 1997) have been

widely reported (Tesch 2003). In contrast, the pigmentation stages and size have been scarcely documented and only one previous study has assessed levels of variability in the pigmentation stages during the arrival period (Boëtius & Boëtius 1989) to several locations of northern Europe. Therefore, the objective of this study was to assess the pigmentation stages and the size of glass eels during the arrival period to two northwestern Iberian estuaries which are proximal to the eastward migratory route from the spawning grounds in the Sargasso Sea. Subsequently, we compared the patterns elucidated in this study with those reported for other geographical locations along the west-east colonization pathways of the Atlantic and Mediterranean ranges.

Study area and glass eels collections

Río Nalón is located in Asturias (Figure 2.1; drainage = 3692 km^2, length = 145 km; mean discharge = 100 $m^3.s^{-1}$) and flows north across the northern slope of the Cantabrian corridor of northwestern Spain to discharge into the Cantabrian Sea at San Juan de La Arena. The sources of Río Minho are also in Cantabrian corridor of northwestern Spain. However, Río Minho flows south-southwest to discharge into the Atlantic Ocean at the northern border between Rio Minho flows south-southwest from its sources in the Cantabrian corridor to discharge into the Atlantic Ocean at the northern border between Spain and Portugal (Figure 2.1; drainage = 17080 km^2, length = 340 km; mean discharge = 300 $m^3.s^{-1}$).

In the estuaries of the two rivers, monthly samples of glass eels were collected from November to April of the years 2004-2005 and 2005-2006 on new moon nights, when captures are more numerous. The fishing operations differed between the two estuaries. The professional fishing in Río Nalón has traditionally been based on fishing vessels operating along the estuary. Fishing vessels with two lateral 4–6 m long nets moving upstream, filter the 3 km long estuary waters. In contrast, Río Minho' glass eels were collected by experimental fishing procedures with stow nets. These nets had a maximum of 14–15 m width at the mouth, 10 m long (1–2 mm mesh size) and 8 m height at the mouth; maintained by fishing weights at the bottom and 10–20 l buoys at the surface along the sides. Net sides converge to a central posterior end fastened to a boat where fishermen scoop glass eels with a hand net. These stow nets were anchored facing the upstream current on rising tide for ~2 h and checked for glass eel captures every 15 min. Further details on these fishing techniques can be seen in Lara (1994) for Río Nalón and Weber (1986) and Antunes (2002) for Río Minho. Thus, Río Nalón' glass eels were sub-sampled from those captured by fishermen whereas those from Río Minho were collected directly from the experimental fishing procedures.

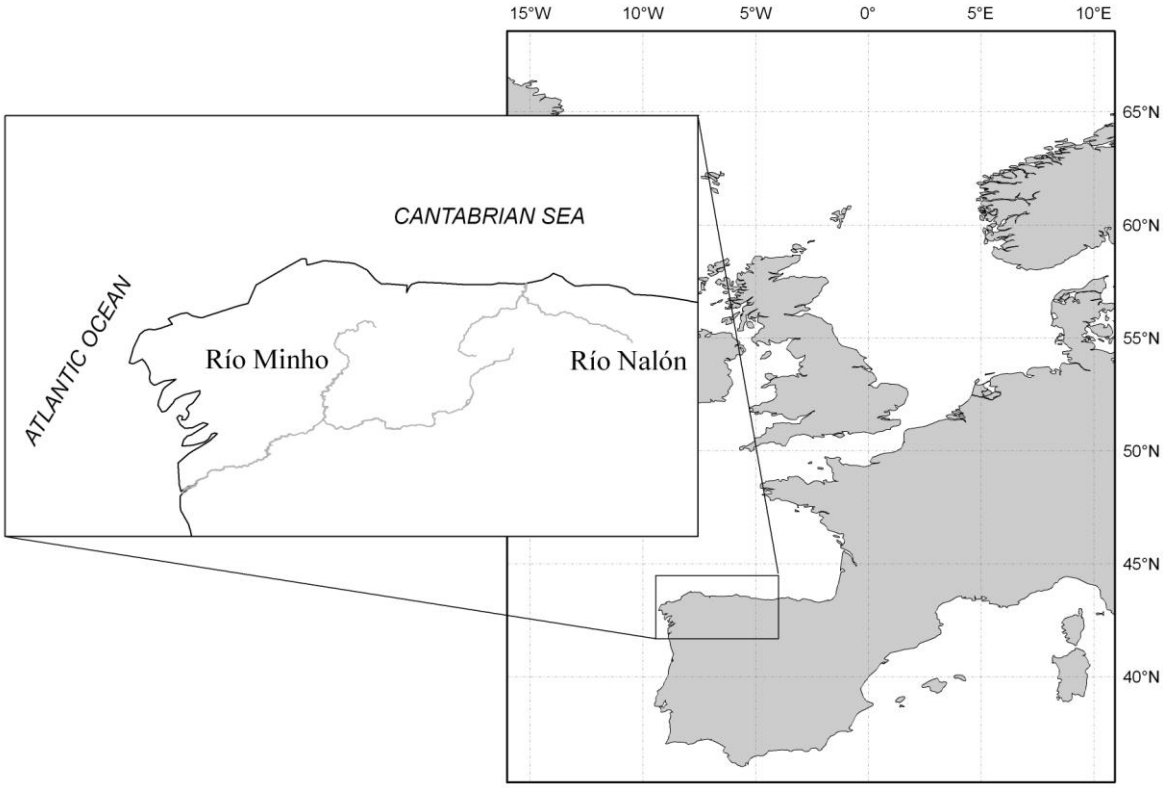


Figure 2.1. Geographical location of the study sites in Río Nalón (Asturias, Cantabrian Sea, northwestern Spain) and Río Minho (Atlantic Ocean, northern border between Spain and Portugal)

Since different workers collected and examined glass eels independently on each river, a methodological inter-calibration was carried out to prevent a potential mismatch in the assignment of pigmentation stages. Fresh glass eels just after capture, were measured (total length, mm), weighed (g) and their pigmentation stages were assigned following the classification established by Elie *et al.* (1982). Unfortunately, the individual assignment of pigmentation stages and size failed in Río Minho' glass eels in the year 2004–2005. In these samples the length and pigmentation stages were quantified separately for each individual glass eel.

Comparison among studies was hindered by the variety of methods used by different authors. For example, several authors described these stages based on preserved material (which causes a marked reduction in length and mass of glass eels, Iglesias, T. unpublished data) whereas other authors described pigmentation stages on different scales and/or used different classification systems as to those described by Strubberg (1913) and Boëtius (1976). To smooth the progress of our comparison we applied the equivalence of pigmentation stages defined by Briand *et al.* (2005) that made compatible our stages V to VI - as described by Elie *et al.* (1982) - with stages A to E as

proposed by Boëtius (1976). This equivalence among pigmentation stages is as follows: VB = A; VIA0 + VIA1 = B; VIA2 + VIA3 = C and VIA4 = D; stage E has no correspondence.

Data analysis

To infer the effects of river (R), year (Y), month (M) and pigmentation stage (PS) on glass eel length and mass we used Analysis of Variance (ANOVA, Zar 1999). In these ANOVAs, the explanatory variables were treated as categorical variables. Since the Río Minho data set did not include the individual assignment of size and pigmentation stage during the first sampling year (2004–2005) and also missed samples from December 2004 and March 2006, the interactions between size, pigmentation and river were disregarded and comparisons between rivers were focused on the 4 months common to the two rivers.

Length-to-mass relationships were explored in the log-linear form Log (mass, g) = $a + b$ Log (length, mm) where a, and b are constants. The physical conditions of glass eels were examined through the condition coefficient (K) calculated as $K = 10^6 * \text{mass/length}^b$, where the exponent b was obtained from the length-to-mass relationships. Both length-to-mass relationships and condition coefficient were explored for the effects of the month, river, year, and pigmentation stage. Among-month and between-river comparisons were made through Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA, Zar 1998). The null hypothesis that pigmentation stages are independent of the arrival month was tested through contingency tables as 9 pigmentation stages * 6 months for each single year in Río Nalón and as 9 pigmentation stages * 4 months for each single year when Río Minho was included in the analyses. For these comparisons, pigmentation stages with less than 5 individuals were omitted.

Results

Pigmentation stages

Monthly samples averaged 233 (range 55-365) and 68 (range 39-202) individuals within a total of 2798 and 683 total glass eels examined for Ríos Nalón and Rio Minho, respectively. Individuals in nine pigmentation stages and sub-stages IV, VA, VB, VIA0, VIA1, VIA2, VIA3, VIA4 and VIB were identified in the two rivers. These stages ranged from the least pigmented or completely transparent (i.e., stage IV) to the fully pigmented individuals (i.e, stage VIB). Overall, there was a predominance of individuals in stage VB that contributed a minimum monthly percentage of 55.8% and 33.3% for Ríos Nalón and Minho, respectively. Nonetheless, the relative contribution of each stage and sub-stage

varied widely among months (Figure 2.2). Contingency tables were conclusive to reject the null hypothesis that pigmentation stages were independent of the arrival month. Rejection of this hypothesis was consistent for each single year and for the two rivers. Contingency tables for each single year for Río Nalón, at least $\chi 2 > 100$, df = 30, $0.001 < P < 0.005$ and for Río Minho at least $\chi 2 > 62$, df = 18, $P < 0.001$.

In Rio Nalón, the monthly differences in the relative contribution of the pigmentation stages were expressed in the contribution of stage VB. The abundance of individuals in this stage declined over successive months to become replaced by individuals in more advanced sub-stages. By the end of the winter and early spring the number of individuals in advanced stages increased and very pigmented individuals in sub-stages VIA0 to VIA4 encompassed the bulk of the individuals whereas those in transparent or near transparent stages practically disappeared (Figure 2.2). Moreover, slight differences between years were reflected in a greater relative abundance of stages IV, VA, and VIA0 and a lower relative abundance of individuals in stage VIA2 in 2004–2005 relative to a total absence of stage IV; a decrease of VA and VIA0 and an increase of individuals in stage VIA2 in 2005– 2006. Thus, individuals of the second year tended to show a greater proportion of advanced stages. Overall the temporal patterns of pigmentation stages in Río Minho glass eels were similar (Figure 2.2) with the only exception in a less predominant stage VB in the winter of 2004–2005 and a markedly higher diversity of stages in December of 2005.

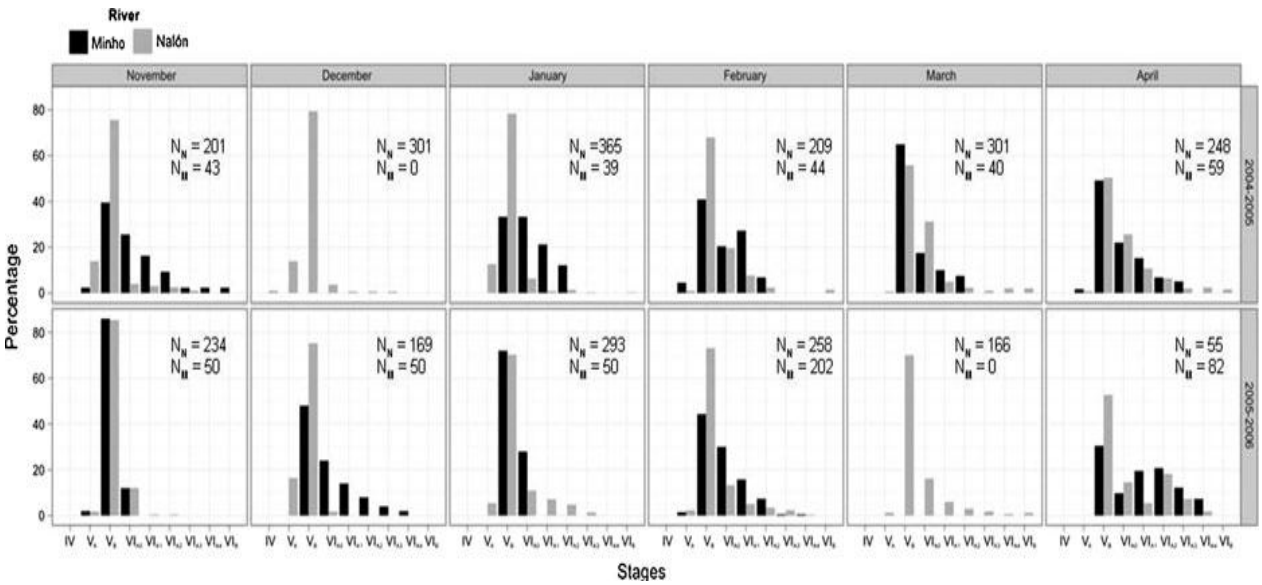


Figure 2.2. Monthly occurrence of successive pigmentation stages (%) in the glass eels of the Río Nalón (grey) and Río Minho (black)'estuaries over 2004–2005 and 2005–2006. NN and NM are the number of individuals examined in each estuary

Glass eels size

Visual inspections of the temporal variations in length and mass of glass eel from Río Nalón suggested concurrent declines over time in the two study years (Figure 2.3a). However, an ANOVA for the effects of year, month and pigmentation stage revealed a remarkable amount of unexplained variance (74.7 % for length and 69.9 % for mass, respectively). Nonetheless, the month had a significant effect on mass ($F_{5,2721}$ = 24.717; P<0.01, 26.1% of the variance explained) but not on length.

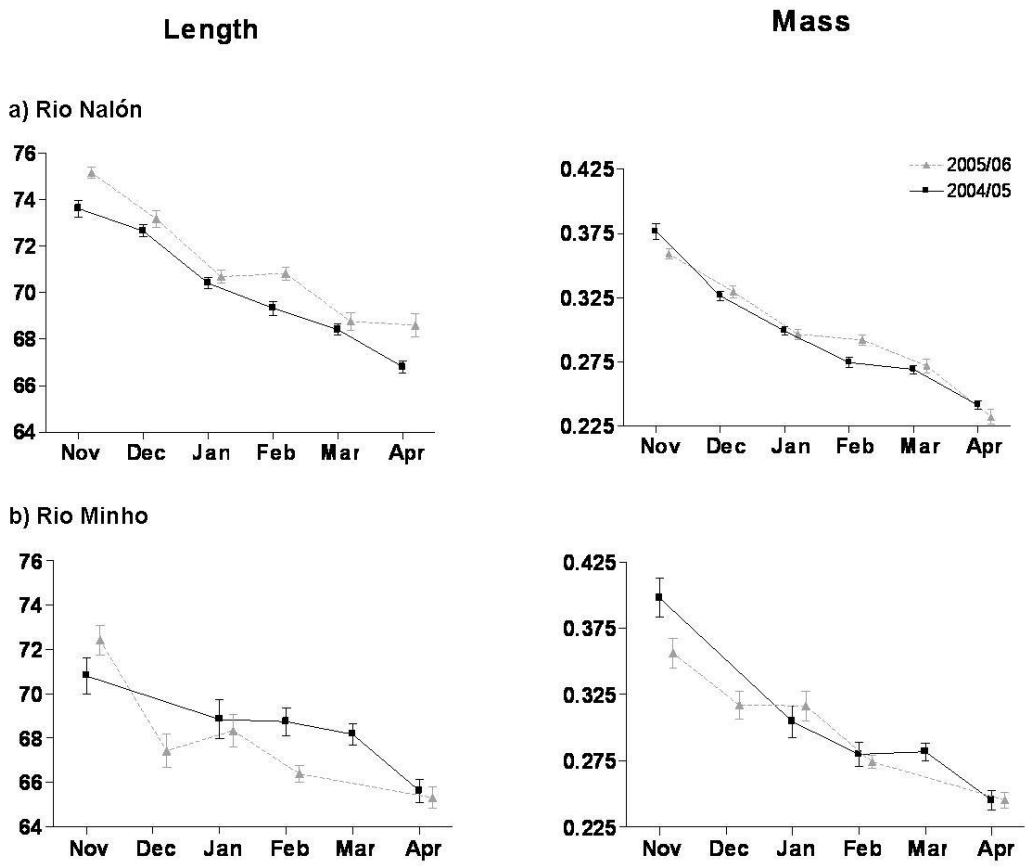


Figure 2.3. Mean monthly length (mm) and mass (g) (mean ± SE) of glass eels examined in **a** Río Nalón and **b** Río Minho over the years 2004–2005 and 2005–2006

Since length and mass are closely related, we explored whether length-induced effects were responsible for the temporal variations in mass. For any given month of the 2 years, log-transformed length and mass proved to be strongly related and the variations in log-transformed length explained between 52.5 and 82.1% of the variations in the log-transformed mass (Table 2.1). An ANCOVA for these 12 length-to-mass regressions with month and year as factors revealed no significant difference among slopes but highly significant differences among intercepts (Table 2.2a) and a Tukey-test revealed significant differences among practically all these 12 regressions. As a consequence, the monthly

length-to-mass regressions were assumed to be parallel to each other with a common slope equal to 2.795 (Table 2.1). Consistent with these results, an ANOVA for the condition factor (*K*) re-calculated for a common slope (*b* = 2.795) revealed significant effects of the year ($F_{1,2786}$ = 75.5; P<0.001), month ($F_{5,2786}$ = 73.8; P<0.001) and interaction year * month ($F_{5,2786}$ = 16.0; P<0.001). Accordingly, the mass of individuals of a given length tended to be higher during the early months of recruitment than in later months.

Table 2.1. Monthly (November to April of 2004-2005 and 2005-2006) length-to-mass relationships in the form log mass (g) = a + b log length (mm) for the Río Nalón' glass eels

Year	Month	N	a	b	r^2
2004/2005	Nov	201	-5.85	2.90	0.81
	Dec	301	-5.44	2.66	0.69
	Jan	365	-5.51	2.69	0.69
	Feb	209	-5.52	2.69	0.70
	Mar	301	-5.58	2.73	0.70
	Apr	248	-5.87	2.87	0.67
2005/2006	Nov	234	-5.37	2.62	0.52
	Dec	169	-5.62	2.75	0.82
	Jan	293	-6.42	3.18	0.80
	Feb	258	-5.79	2.83	0.72
	Mar	166	-6.13	3.02	0.76
	Apr	55	-5.38	2.58	0.59

All instances are significant in, at least, P<0.001. r^2 is the variance explained. An ANCOVA detected no significant differences with a common slope b = 2.795

In Río Nalón, an independent analysis of the effects of pigmentation stage and year (for stages VA to VIA4) on length indicated no significant effects of the pigmentation stage ($F_{6,2766}$ = 0.49, P = 0.82) but significant effects of the year ($F_{1,\ 2766}$ = 6.03, P = 0.01) and of the interaction year*pigmentation ($F_{6,\ 2766}$ = 2.67, P = 0.014). In contrast, significant effects of the pigmentation stage ($F_{6,2766}$ = 2.78, P =0.01) and of the interaction year*pigmentation stage ($F_{6,2766}$ = 4.02, P < 0.001) were detected on mass. Concurrently, an ANOVA for the condition coefficients revealed significant effects of the pigmentation stage ($F_{6,2766}$ = 7.94, P < 0.001) and a weak but yet significant effect of the interaction pigmentation stage*year ($F_{6,2766}$ = 2.21, P = 0.04).

Table 2.2. Results of ANCOVA for the length-to-mass relationships in the form log mass (g) = a + b log length (mm) for glass eels from **(a)** Rio Nalón, two successive years (2004-2005 and 2005-2006) and six months (November, December, January, February, March and April) and **(b)** Rio Nalón and Rio Minho combined, two successive years (2004-2005 and 2005-2006) and 4 months (November, January, February, and April)

		DF	P
(a)	**Year**	1	p > 0.05
	Month	5	p > 0.05
	Length	1	**p < 0.001**
	Y*M	5	**p < 0.05**
	Y*L	1	p > 0.05
	M*L	5	p > 0.05
	Y*M*L	5	**p < 0.05**
	Error	2774	
(b)	**River**	1	**p < 0.05**
	Year	1	p > 0.05
	Month	3	p > 0.05
	Length	1	**p < 0.001**
	R*Y	1	p > 0.05
	R*M	3	p > 0.05
	Y*M	3	p > 0.05
	R*L	1	**p < 0.01**
	Y*L	1	p > 0.05
	M*L	3	p > 0.05
	R*Y*M	3	p > 0.05
	R*Y*L	1	p > 0.05
	R*M*L	3	p > 0.05
	Y*M*L	3	p > 0.05
	R*Y*M*L	3	p > 0.05
	Error	2413	

Significant results in bold

Similar to Río Nalón, the length and mass of glass eels from Río Minho declined over time (Figure 2.3b). Moreover, an ANOVA for the 4 months common to the two rivers (November, January, February and April) also revealed a large proportion of unexplained

variance, 84.1% for length and 76.7% for mass, respectively (Table 2.3) and highlighted differences between the two rivers in length but not in mass and also emphasized the effects of the month on both, length and mass (Table 2.3).

Table 2.3. Results from ANOVAs for the effects of river (Nalón and Minho), year (2004-2005 and 2005-2006), and month (November, January, February and April) on the length and mass of glass eels

		Length		**Mass**	
	DF	**% V**	**P**	**% V**	**P**
River	1	3.2	**p < 0.001**	0.1	p > 0.05
Year	1	0.1	p > 0.05	0.1	p > 0.05
Month	3	11.4	**p < 0.001**	22.3	**p < 0.001**
R*Y	1	0.4	**p < 0.01**	0.0	p > 0.05
R*M	3	0.1	p > 0.05	0.2	p > 0.05
Y*M	3	0.4	**p < 0.05**	0.5	**p < 0.001**
R*Y*M	3	0.4	**p < 0.05**	0.2	p > 0.05
Residual	2429	84.1		76.7	

Significant results in bold

% V is the amount of variance explained in percent (%)

Interestingly, in Río Minho the month had a significant effect on both, length and mass ($F_{3,573}$ = 29.01, P<0.001 for length and $F_{3,57}$3 = 61.59, *P*<0.001 for mass) with smaller individuals predominating during the latest months of arrival but, like in Río Nalón, an ANCOVA for the length-to-mass relationships revealed no significant differences neither among months nor between years (Table 2.2 b). Hence, we compared the two sets of length-to-mass regressions for the two rivers and an ANCOVA highlighted no significant difference ($F_{1,2440}$ = 0.57, *P* = 0.45). As a consequence, we re-calculated the condition factors for all individuals across months, years and rivers based on a common slope equal to 2.915 and these coefficients differed significantly (at least *P*<0001) among months, rivers and years (Figure 2.4).

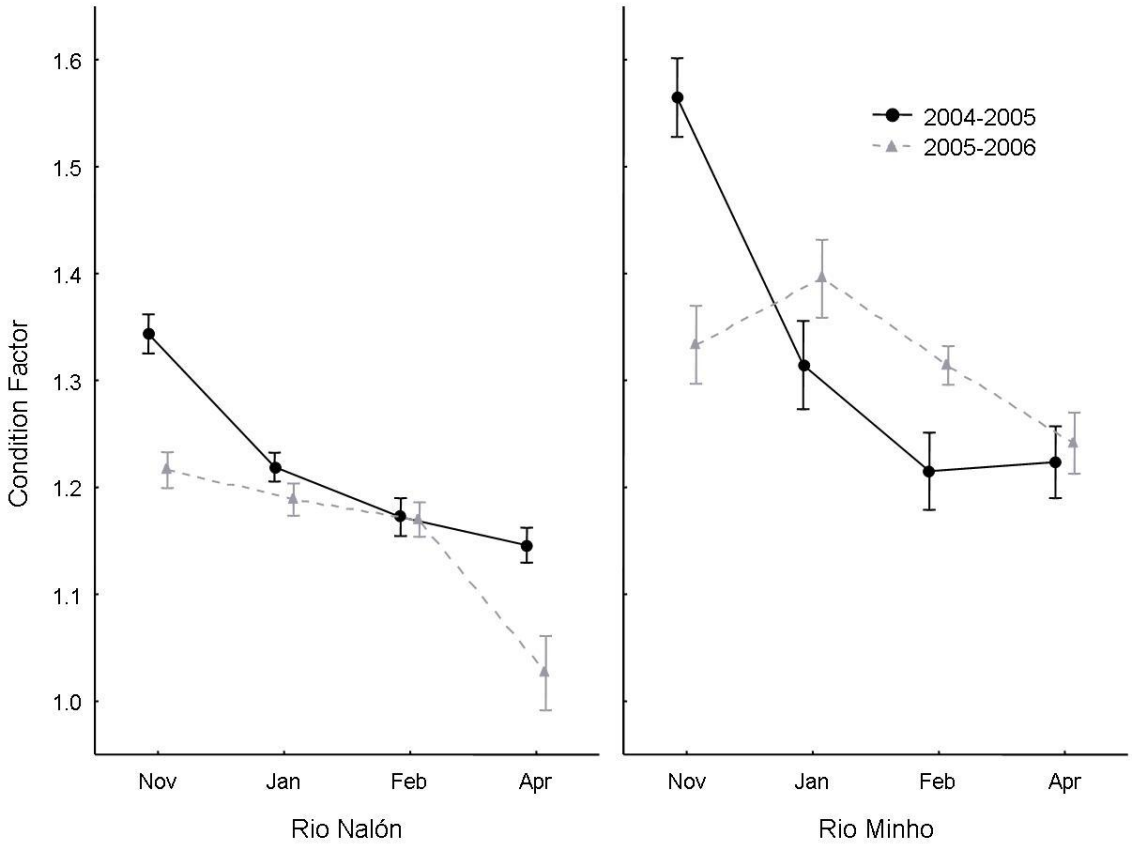


Figure 2.4. Temporal decline in the physical condition of glass eels as inferred from the condition factor calculated for a common slope b = 2.915 for Río Nalón and Río Minho combined

Correspondingly, an analysis of the effects of the pigmentation stage on length, mass and condition of glass eels for the only year available in Río Minho provided evidence of a similar pattern. An ANOVA highlighted no significant effect of the pigmentation stage on length ($F_{6,\ 376}$ = 1.19, P = 0.31) but a significant effect on mass ($F_{6,376}$ = 2.76, P = 0.01) and on the condition factor ($F_{6,\ 376}$ = 7.06, P < 0.001). Thus, whilst glass eel length did not vary significantly among pigmentation stages both, mass and condition factor were lower in individuals of increasingly advanced stages to an extent that individuals in the most advanced pigmentation stage VIA4 weighted, on the average, some 18% less than those in stage VB.

Comparison with other locations

Comparative data collected from the literature and the corresponding geographical locations (Table 2.4) include 15 studies on western Atlantic glass eels, two from the northern Atlantic area, one from the North Sea and six from the Mediterranean.

Table 2.4. Comparison of pigmentation stages and size of glass eels across Atlantic and Mediterranean localities.

	Location	Date	Length	Mass	Pigm. Stage*	Preservation	Author
Atlantic locations							
1.	Minho (Portugal)	Jan-Jul 1982	72.0-65.0	0.36-027	Early	Formol	Weber (1986)
2.	Minho (Portugal)	Nov 2004-Apr 2006	73.5-65.3	0.40-0.25	V_B	Fresh	This study
3.	Nalón (Spain)	Nov 1989-Mar 1990	72.4-68.2	0.33-0.25	Early	Fresh	Lara (1994)
4.	Nalón (Spain)	Nov 2004-Apr 2006	75.2-66.8	0.38-0.23	V_B	Fresh	This study
5.	Santander (Spain)	Nov 1917-May 1918	76.5-67.5	0.53-0.36	VI_{AII}	Formol	Gandolfi-Hornyold (1918)
6.	Aguinaga (Spain)	Dec 1935-Jun 1936	78.4-68.4	0.58-0.30	---	Formol	Gandolfi-Hornyold (1936)
7.	Oria (Spain)	Oct 1928-Apr 1929	77.6-70.0	0.53-0.38	---	Formol	Gandolfi-Hornyold (1929)
8.	Oria (Spain)	Oct 1929-Apr 1930	76.1-69.7	0.51-0.30	---	Formol	Gandolfi -Hornyold (1930b)
9.	Adour (France)	Nov 1997-Mar 1998	73.5-67.4	0.35-0.26	---	Fresh	de Casamajor *et al.* (2000)
10.	Adour (France)	Dec 1999-Feb 2000	75.0-68.3	0.41-0.30	V_B	Fresh	de Casamajor *et al.* (2003)
11.	Adour (France)	Jan-Mar 2000	68.6-68.0	0.30-0.30	V_B	Fresh	Bardonnet *et al.* (2003)
12.	Adour (France)	Oct 1979-Aug 1980	76.0-70.3	0.45-0.29	Variable	Fresh	Charlon & Blanc (1982)
13.	Gironde (France)	Dec 1997-Apr 1998	70.0-66.0	0.32-0.23	Only V_B	Fresh	Lambert *et al.* (2003)
14.	Vilaine (France)	Feb + Mar 1992	69.7	---	V_B	Fresh	Lecomte-Finiger *et al.*(1996)
15.	Seudre (France)	Dec 1984-Jan 1987	70.0-68.1	0.31-0.29	A	Frozen	Boëtius & Boëtius (1989)
16.	Burrishoole (Ireland)	Feb-May 1988	72.8-70.6	0.36-0.29	V_B	Fresh	Poole (1994)
17.	Severn (England)	Apr 1985-Apr 1987	72.5-68.1	0.32-0.18	Variable	Frozen	Boëtius & Boëtius (1989)
18.	Vidå (Denmark)	Apr 1984-May 1987	72.2-69.9	0.22-0.12	C or D	Frozen	Boëtius & Boëtius (1989)
Mediterranean locations							
19.	Sebou (Morocco)	Nov1980-Jun1981	68.8-63.5	0.32-0.24	---	Formol	Yahyaoui *et al.* (1983)
20.	Mallorca (Spain)	Jun-Mar 1918	66.9-62.4	0.35-0.24	VI_{AIII}	Formol	Gandolfi-Hornyold (1920)
21.	Valencia (Spain)	Dec 1984	68.15	0.32	A	Frozen	Boëtius and Boëtius (1989)
22.	Camarge (France)	Jan 2004-Jan 2006	70.3-59.1	0.34-0.17	Variable	Fresh	Maes et al. (2009)
23.	Arno (Italy)	Dec 1978-Apr 1979	73.0-65.5	0.47-0.21	C	Formol	Gandolfi *et al.* (1984)
24.	Alexandrie (Egypt)	Mar 1928	60.7	0.14	VI_{AIII}	Formol	Gandolfi-Hornyold (1930a)

Length (mm), mass (g) and date represent the ranges and time periods of the collections.*Pigmentation Stage refers to the most abundant stage. In locations 1 and 3 "early" refers to combinations of stages V_A, V_B and VI_{A0} in the scale of Elie *et al.* (1982). Locations 5, 16 and 24 according to Strubberg (1913). Locations 15, 17, 18, 21 and 23 according to Boëtius (1976). All other locations according to Elie *et al.* (1982)

Apparently, glass eels from Atlantic locations appeared larger in size (range 68.6–78.4 mm) than those from the Mediterranean (60.7–73.0 mm, Table 2.4). However, western Atlantic glass eels show no marked differences in size or in pigmentation stages

among locations in Portugal, Spain, France and Ireland although Boëtius & Boëtius (1989) noted that pigmentation stages varied widely with no obvious temporal pattern among several northern Atlantic locations. Studies available for the Mediterranean [La Camargue, southern France (Maes *et al.* 2009) and Río Arno, northeastern Italy (Gandolfi *et al.* 1984)] also suggested temporal replacement of pigmentation stages with an increased predominance of more pigmented individuals as the season progressed and interestingly, an overall predominance of more pigmented stages than in the Atlantic glass eels (Table 2.4). Nonetheless, relative to the whole-season predominance of stage VB in the Iberian Ríos Nalón and Minho, in the Mediterranean Río Arno where pigmentation stages are well documented (Figure 2.5) such predominance only occurred in December–February and declined sharply from March to May to become replaced by the most pigmented individuals in stages VIA4 and VIB.

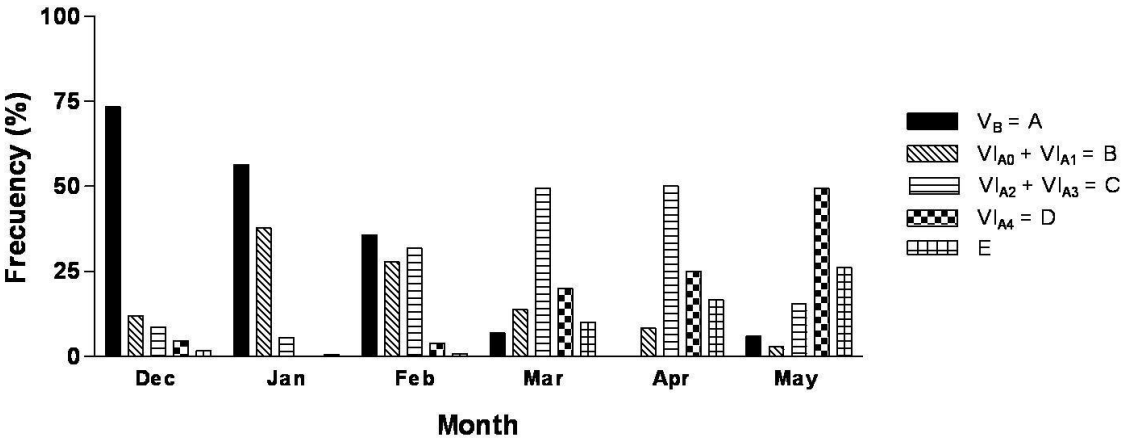


Figure 2.5. Month-to-month variation in the pigmentation stages of Río Arno (Italy)' glass eels during 1978–1979 as reported by Gandolfi *et al.*(1984) and re-calculated according to the pigmentation stages defined by Briand *et al.* (2005) that makes compatible the stages V to VI (Elie *et al.*1982) to the stages A to E (Boëtius 1976)

Discussion

This comparative study of the pigmentation stages and size of glass eels during the arrival period to two northwestern Iberian estuaries located some 400 km apart highlighted concurrent patterns of temporal variation and in turn, differences in these life history traits. In the two estuaries, the size and pigmentation stages of glass eels varied substantially from November to April. Although most of the temporal variation remained unexplained, several patterns were detected. Consistent with previous studies

(summarized by Tesch 2003), in the two estuaries, glass eels decreased in size and condition but augmented their pigmentation as the season progressed from November to April. Larger individuals in pigmentation stage VB predominated at the onset of the arrival period to contribute 50-75% of all individuals in November. The relative contribution of this pigmentation stage declined as the season progressed to become replaced by smaller, more pigmented individuals to the extent that in April, markedly-to-fully pigmented individuals (stage VI) contributed similarly to the bulk of the arriving glass eels. Although both rivers followed the same tendency, Río Minho shows a markedly higher diversity of stages in some early months, such as December of 2005. The reason for this phenomenon remains unclear. The existence of any structure that may block down glass eel migration leading to a mix of different migration waves and pigmented stages can be discarded. However, when analyzing the pigmentation stages of samples with very different number of individuals (for example, January 2005 – Figure 2.2) accuracy of results may be affected. Moreover, even if the analytical method was inter-calibrated, such phenomenon may be caused by different potential perceptions for visual assignments by the two different workers who assigned pigmentation stages on Ríos Nalón and Minho.

The pigmentation stage had a strong effect on mass and condition coefficient but not on length and because of the tendency of the advanced pigmentation stages to occur later in the season, the two factors, month and pigmentation stage, obscure one another.

Whilst glass eel length did not vary significantly among pigmentation stages, both mass and condition factor were lower in individuals of increasingly advanced stages to an extent that individuals in the most advanced pigmentation stage VIA4 weighted, on the average, some 18% less than those in stage VB. In turn, glass eels in the western Río Minho were smaller in size and showed higher condition than those in Río Nalón. Although we found no obvious explanation for such differences, larger glass eels with better physical condition may reflect greater energy content implying an enhanced upstream migration (Bureau du Colombier *et al.* 2007) but such differences may also be caused by the occurrence of different migration waves or by selective behaviour of tidal river transports. A possible explanation may be related to feeding. It is admitted that most glass eels do not feed during estuarine migration. However, Bardonnet & Riera (2005), using stable isotope analysis, reported that a small amount of individuals may feed. It could be hypothesized that fish in Río Minho were older and had a better condition because some individuals may stop migration to feed. The analyses of pigmentation results for Río Minho should be treated cautiously because only 4 months from one single year were considered. Current results, however, were confirmed by the conclusions drawn for the Río Nalón data set.

A comparison among studies highlighted consistent patterns in size and pigmentation stages among Atlantic locations from Portugal to Denmark with slightly larger sizes at the western localities and more advanced pigmentation stages in the northern locations. Moreover, Atlantic glass eels tend to be larger in size (range 68.6–78.4 mm) than those from the Mediterranean (59.1–73.0 mm). This pattern is consistent with those elucidated by Gandolfi-Hornyold (1920, 1930a, 1936)'s pioneering studies on Spanish Atlantic and Mediterranean locations. This author reported smaller glass eels in the Balearic Islands and remarkably smaller individuals in Alexandria (Egypt) located in the Far East Mediterranean (Gandolfi-Hornyold 1930b). Such counter-intuitive difference in the Mediterranean with smaller sizes at the eastern locations appears unrealistic and may have plausibly been caused by sampling bias involving at least sampling dates and different manipulation, measures and preservation techniques. Nevertheless, more advanced pigmentation stages in the Mediterranean Camargue and Río Arno may result from temperature dependent effects (Briand et al. 2005). Higher temperatures in the Mediterranean may accelerate the pigmentation process resulting in a predominance of more pigmented individuals relative to a predominance of the early stage VB in the Atlantic Ríos Nalón and Minho. For several Atlantic locations, Boëtius & Boëtius (1989) reported a replacement of weak pigmented glass eels by more pigmented stages than in the Mediterranean Río Arno (Gandolfi *et al.* 1984; Figure 2.5.) but here the pigmentation stages appeared in more advance stages since the beginning of the season.

Several causes have been suggested to explain the temporal reduction of length and mass. First, larger leptocephalus may swim faster and reduce the time needed to reach the European continent relative to the smaller, slower individuals. Second, fasting during the metamorphosis when leptocephalus larvae lose their teeth (Elie 1979; Desaunay & Guerault 1997) and reduce the length of the gastrointestinal tract, so glass eels may spend prolonged time periods without feeding and living at the expense of their lipid reserves (Tesch 2003). On the other hand, the larger size of Atlantic glass eels compared with the smaller Mediterranean individuals may be caused by a greater osmotic exchange cost in the markedly higher-salinity Mediterranean Sea. Nevertheless, a remarkable heterogeneity and a large proportion of the variance unexplained (~75%) may result from genetic heterogeneity (Maes *et al.* 2009) but still suggests that environmental factors such as water temperature and salinity may affect the pigmentations stages (Briand *et al.* 2005) and should be taken into account in future investigations on glass eel recruitment.

Chapter 3. Eel's general condition: condition factor and infection by *Anguillicoloides crassus*

This chapter has been published as:

Costa-Dias S., Aguilar E., Lobón-Cerviá J., Antunes C. & Coimbra J. 2010. Infection by *Anguillicoloides crassus* in a riverine stock of European eel, *Anguilla anguilla*. Fisheries Management and Ecology 17: 485 - 492.
(DOI: 10.1111/j.1365-2400.2010.00746.x)

Acknowledgments for this chapter
Administrative permissions for data collection were obtained by agreement between the CSIC and Principado de Asturias. The authors acknowledge all folks that help both in the field and lab. This study was supported by the Portuguese Foundation for Science and Technology - FCT (S.C.D., grant SFRH/BD/16922/2004).

Eel's general condition: condition factor and infection by *Anguillicoloides crassus*

Abstract

Infection by the parasitic nematode *Anguillicoloides crassus* Kuwahara, Niimi & Itagaki in a wild riverine stock of European eel, *Anguilla anguilla* (L.), in a near pristine river was investigated. Samples were collected on a seasonal basis in distinct habitats along the river gradient. This study highlighted the presence of the parasite and completion of the whole life cycle in eels from the Río Esva. Infection levels by *A. crassus* were high at three sites between the mid river to the estuary and also varied among seasons. Condition of eels was lower at upstream sites compared with downstream locations. Although high-quality, environmental conditions in the Río Esva may buffer the effects of *A. crassus* on eels, potential impacts and limiting factors for the parasite are discussed.

Introduction

The parasitic nematode *Anguillicoloides crassus* (formerly known as *Anguillicola crassus*) Kuwahara, Niimi & Itagaki, 1974 (Nematoda, Dracunculoidea) (Moravec 2006), a natural parasite of the Japanese eel, *Anguilla japonica* Temminck & Schlegel, was introduced into Europe during the early 1980's and spread rapidly through European freshwaters eel stocks (Peters & Hartmann 1986). Major causes of the invasion appear to be the introduction of infective parasite stages or infected hosts used to repopulate depleted stocks of European eel *Anguilla anguilla* (L.), escapement from eel farms or through lorry water exchanges during eels transportation (see Kirk 2003 for a review). Wide host parasite (intermediate or paratenic) specificity has accelerated the spread in non-infested waters (Würtz *et al.* 1998). Infections occur through the ingestion of intermediate or paratenic hosts (De Charleroy *et al.* 1990; Kirk 2003) and cause loss of functional role of the swimbladder where the parasites establish (Würtz & Taraschewski 2000; Kennedy 2007). Pathological reactions of tissues to the parasite include inflammation, necrosis and scarring caused by larvae living in the swimbladder tissue, and pre-adults and adults feeding on the host blood (Didžiulis 2006), leading to poor condition of affected eels (see Knopf 2006 for a review on the susceptibility and immune responses of both European and Japanese eels to *A. crassus*).

The impact of *A. crassus* on the survival of the European eel has long been recognized (see Kennedy 2007 for a review). Importantly, the freshwater stocks of

European eel have declined throughout its distributional range to an extent that numerous local or regional stocks are considered "vulnerable", "in danger" or "under safe biological limits" by the corresponding conservation agencies (ICES 2006).

The Iberian Peninsula is a major front to be faced by eel in its eastern migration towards the European and Mediterranean colonization regions and historically, the Iberian Peninsula has contained the largest stocks within its distributional range (Deelder 1984). Despite the severe decline of eel throughout the Iberian Peninsula during the 1980s and 1990s, through loss of >80% of eel habitat because of dam construction, river channelization and pollution (Deelder 1984; Granado-Lorencio 1991), eel still remain common-to-abundant in un-impounded coastal rivers (Lobón-Cerviá 1999). This is the case of the numerous rivers along the Cantabrian corridor of north western Spain that still hold some of the remaining natural stocks of eel. Given the threatened status of eel not only in the Iberian Peninsula (Blanco & Gonzalez 1992; Cabral *et al.* 2005) but also throughout its natural distributional range (ICES 2006), understanding the effects of *A. crassus* on the few wild stocks still occurring is imperative.

Despite the potential importance of *A. crassus* for the survival of eel (Kennedy 2007), only a few studies have addressed this issue on Iberian stocks. These studies have reported the occurrence of *A. crassus* in exploited stocks inhabiting tributaries or stream sections of Portuguese rivers as the Rios Liz (Silva *et al.* 1994) and Tejo (Silva *et al.* 1994; Neto 2008) and Ria de Aveiro (Silva *et al.* 1992, 1994); the international basin of Rio Minho (Antunes 1999; Aguilar *et al.* 2005); and in Spain, in several streams in País Vasco (Korta & Díaz 2008) and in the Mediterranean Río Júcar (Esteve & Alcaide 2009). These studies all failed to identify the origin of the parasite introduction and/or the ranges of distribution of *A. crassus* within and among rivers. The only documented instance (C. Antunes, unpublished data) concerns Rio Minho (north-western border between Portugal and Spain) where escape of infected, farmed individuals was the cause of the parasite spread. As a consequence, infection of eel by *A. crassus* in the Río Esva, a near pristine river drainage of the Cantabrian corridor of north-western Spain inhabited by a wild, unexploited stock of European eel was studied.

Material and methods

Study area

The Río Esva flows north (43º 33' N, 6º 32' W) across the mountainous and rugged landscape of the Cantabrian corridor of north-western Spain, towards the Cantabrian sea. The catchment area is about 500 km^2 and is fed mainly by rain (1000–1500 $mm.year^{-1}$) and runoff. Stream discharge fluctuates across seasons but shows rather

mild temperatures the year round (Lobón-Cerviá & Rincón 1998). The Río Esva is near pristine and for the most, safe of anthropogenic alterations such as fish stocking, farming, engineering activities, obstructions or pollution.

Four sites were selected along the environmental gradient of the Río Esva and tributaries representative of the environmental variability that typifies mountainous, Cantabrian river drainages. (1) The estuary, the 2-km inter-tidal river where marine waters fully occupy fresh waters during high tide (every 12 hours with a delay of 45 min a day). The main stem of Río Esva is wide (30-40 m) and surrounded by trees and meadows. (2) The Río Choudral, a low altitude, small rapidly flowing tributary of Río Esva, some 3 km upstream from the estuary. This stream is fully covered by dense vegetation (canopy). (3) The Chanona, located in the main stem of Río Esva some 21 km from the river mouth. This site is also wide and covered by abundant vegetation. Finally, (4) La Viella, one of the numerous tributaries of the Río Esva, typical of the mid reaches, located at some 27 km from the river mouth. This site is characterized by shallow and rapid waters, surrounded by meadows.

Sampling and laboratory procedures

Eels were collected by electric fishing in October 2006 (October 1), December, May, August and October 2007 (October 2). On every sampling occasion, attempts were made to collect ≥25 eels at each sampling site. After capture, eels were preserved in ice and maintained frozen until analysis. In addition, water temperature (ºC) was measured and water samples collected for determining major chemical attributes as pH, nitrites (NO_2), nitrates (NO_3), ammonia (NO_4), phosphates (PO_4) and calcium ($CaCO_3$) in the laboratory.

The swimbladder was extracted from each eel and examined under a stereomicroscope for infection by *A. crassus*. The number of parasites at life stages L3 and L4, pre-adult and adult stages, as defined by De Charleroy *et al.* (1990) and the presence of larvae L2 were checked to detect the level of infection by *A. crassus*.

Data analysis

Log-transformed, linear length-to-mass regressions were compared among sites and seasons with Analysis of Covariance (ANCOVA). In addition, the condition factor (K) calculated as $K = 10^3 \times mass/length^b$, where the exponent b was obtained from the length-to-mass relationships, were used to explore potential effects of *A. crassus* infection on eels across seasons and sites.

Parasitological indices were calculated following Bush *et al.* (1997), including the estimation of prevalence as the percentage of infected individuals; mean intensity, as the

mean number of parasites per infected fish; and the mean abundance, as the mean number of parasites per fish for each study site and sampling occasion. For all these estimates, the total number of *A. crassus* in pre-adult and adult stages were grouped and all other reference to *A. crassus* intensity refers to this combination unless otherwise stated. The prevalence (*P'*) of L3 and L4 (L3 + L4) larvae were also estimated.

The null hypothesis that the condition factor is independent of the *A. crassus* burden was tested for five levels of infection intensity (number of parasites per infected eel ranked as 0, 1, 2, 3, and ≥4) and four levels of infection by larvae L3 + L4 (number of parasites per infected eel ranked as 0, 1, 2, and ≥3), across study sites and seasons. Analysis of the Variance (ANOVA; Zar 1998) was used to detect effects for among seasons and among sites comparisons.

Results

Major characteristics of the study sites include low hardness, near neutral pH, high winter temperatures (minimum recorded 7.0 ºC in La Viella) and mild summer temperatures (maximum 20.5 ºC at Chanona, Table 3.1). Salinity in the Estuary was practically 0 at the sampling time (low tide) across seasons. There was no obvious relationship between the chemical attributes of the stream water and the physical features of the sites (tributaries vs main stem) nor was there any evidence of consistent longitudinal variations in relation to the distance of the study site to the river mouth into the Cantabrian Sea.

Table 3.1. Physico-chemical characteristics (mean ± SD) of the study sites in relation to distance from the river mouth: temperature (T), pH, nitrites (NO_2), nitrates (NO_3), ammonia (NO_4), phosphates (PO_4) and calcium ($CaCO_3$).

	Estuary	**Choudral**	**Chanona**	**Viella**
Distance up. (km)	1.0	3.0	21.0	27.0
T (ºC)	13.6 (4.7)	12.9 (2.5)	13.9 (5.3)	13.0 (5.0)
pH	7.8 (0.8)	6.5 (0.3)	7.9 (0.6)	7.0 (0.3)
NO_2 (mg/L)	0.006 (0.009)	0.004 (0.005)	0.002 (0.004)	0.004 (0.009)
NO_3 (mg/L)	2.0 (1.1)	2.0 (1.2)	2.1 (1.3)	1.4 (0.4)
NO_4 (mg/L)	0.09 (0.12)	0.09 (0.06)	0.09 (0.13)	0.03 (0.01)
PO_4 (mg/L)	1.1 (1.0)	0.7 (1.3)	0.8 (1.2)	1.7 (2.0)
$CaCO_3$ (mg/L)	25.7 (14.9)	10.0 (11.7)	30.0 (19.0)	13.0 (11.5)

The number, length, weight and condition for the eels sampled, together with the prevalence, intensity and abundance of *A. crassus* for all sites and seasons are summarized in Table 3.2. A severe flood prevented sampling Chanona site in winter. The size range of eel examined (from 6.5 to 35.7 cm), including two large-sized females (52.0 and 57.0cm long), typified the length structure of this riverine stock elucidated in previous studies: eel size differed significantly (F_3 = 43.9, P < 0.001) among sites and the smaller eels in the estuary (mean size across samples = 16.4 cm) increased in size with increased distance from the river mouth to attain 19.7 cm at Choudral (3.0 km), 21.0 cm at Chanona (21.0 km) and 23.4 cm at La Viella (27.0 km).

Table 3.2. Number (N) and mean (minimum – maximum in brackets) length (L) and weight (W), and mean condition factor (K) of eels examined. Levels of prevalence (P), mean intensity (MI) and mean abundance (MA) of pre-adults and adults of *A. crassus*. Levels of prevalence (P') of larvae L3+L4; and presence (L2) of larvae L2 (x = positive) in relation to the number of individuals examined in each sampling site and season. Mean (SD in brackets) values per sampling site were also calculated.

		October1	December	May	August	October2	Mean (SD)
Estuary	**N**	36	37	30	32	27	32.4 (4.2)
	L (cm) (min-max)	13.3 (6.6-19.8)	12.0 (7.2-19.9)	19.6 (12.3-28.7)	22.2 (13.4-52.0)	16.1 (12.2-25.6)	16.9 (6.2)
	W (g) (min-max)	5.1 (0.4-16.2)	3.3 (0.3-13.1)	16.1 (3.2-41.9)	29.5 (1.9-256.8)	8.7 (3.0-31.4)	12.3 (23.9)
	K	0.93	0.88	0.84	0.89	0.96	0.90 (0.12)
	P (%)	14	14	30	59	41	31.6 (19.1)
	MI	2.2	1.2	2.0	2.7	1.5	1.9 (0.6)
	MA	0.3	0.2	0.6	1.6	0.6	0.7 (0.6)
	P' (%)	3	0	7	44	11	13.0 (17.8)
	L2	x			x	x	
Choudral	**N**	36	30	29	35	19	29.8 (6.8)
	L (cm) (min-max)	19.7 (9.8-31.7)	21.0 (11.9-28.4)	20.4 (10.5-33)	17.9 (11.4-26.8)	20.2 (15.8-26.7)	19.7 (5.0)
	W (g) (min-max)	14.6 (1.0-61.1)	16.5 (16.3-38.3)	18.7 (1.2-52.1)	11.5 (1.3-36.4)	14.2 (6.5-29.8)	15.0 (11.9)
	K	0.79	0.76	0.80	0.89	0.79	0.80 (0.11)
	P (%)	56	40	34	51	42	44.6 (8.8)
	MI	2.1	2.8	1.5	1.8	1.6	2.0 (0.5)
	MA	1.1	1.1	0.5	0.9	0.7	0.9 (0.3)
	P' (%)	25	13	14	9	32	18.6 (9.6)
	L2	x	x		x	x	

(Table 3.2. - Continuation)

		October1	December	May	August	October2	Mean (SD)
Chanona	**N**	31	(no sample)	30	37	32	32.5 (3.1)
	L (cm)	23.2		23.2	19.4	18.9	21.0 (4.8)
	(min-max)	(15.5-30.6)		(13.4-35.8)	(11.8-29.1)	(13.3-25.3)	
	W (g)	23.6		24.7	15.2	11.1	18.4 (14.1)
	(min-max)	(5.7-49.6)		(4.2-86.8)	(2.7-46.2)	(3.4-29.9)	
	K	0.82		0.81	0.88	0.76	0.82 (0.11)
	P (%)	26		50	38	19	33.3 (13.6)
	MI	2.6		1.7	1.3	1.3	1.7 (0.6)
	MA	0.7		0.9	0.5	0.3	0.6 (0.3)
	P' (%)	10		3	16	3	8.0 (6.3)
	L2	x		x	x		
Viella	**N**	30	26	23	31	29	27.8 (3.3)
	L (cm)	22.9	27.4	21.4	23.5	22.9	23.4 (5.5)
	(min-max)	(15.2-32.1)	(16.3-57.0)	(12.3-28.7)	(14-32.5)	(11.5-31.0)	
	W (g)	23.8	44.1	17.6	24.1	22.3	26.1 (37.8)
	(min-max)	(6-64.4)	(5.9-431.2)	(2.7-42.7)	(3.7-68.7)	(2.3-63.7)	
	K	0.78	0.78	0.81	0.78	0.75	0.78 (0.10)
	P (%)	0	0	0	0	3	0.8 (1.5)
	MI	-	-	-	-	1.0	1.0 (-)
	MA	0	0	0	0	0.0	-
	P' (%)	0	0	0	0	0	0
	L2						

Infection was detected across a broad range of eel sizes being a 12-cm long eel the smallest infested. However, levels of infection by adult and pre-adult *A. crassus* varied widely along the river gradient. Overall, no relationships were found between the infestation burden and the physico-chemical features of the sites but a pattern of infestation was found related to river gradient. In the uppermost site (27 km from the river mouth), only one individual out of 139 eels examined was infected. By contrast, persistently high prevalence levels were recorded at all other sites that, in turn, varied widely among seasons in Chanona (19-50%); Choudral (34-56%) and the Estuary (14-59%) (Table 3.2). Nevertheless, the mean intensity and mean abundance of *A. crassus* adults and pre-adults were low with most individuals infected by only one or two parasites in all seasons, and slightly higher at the two sites downstream (Figure 3.1).

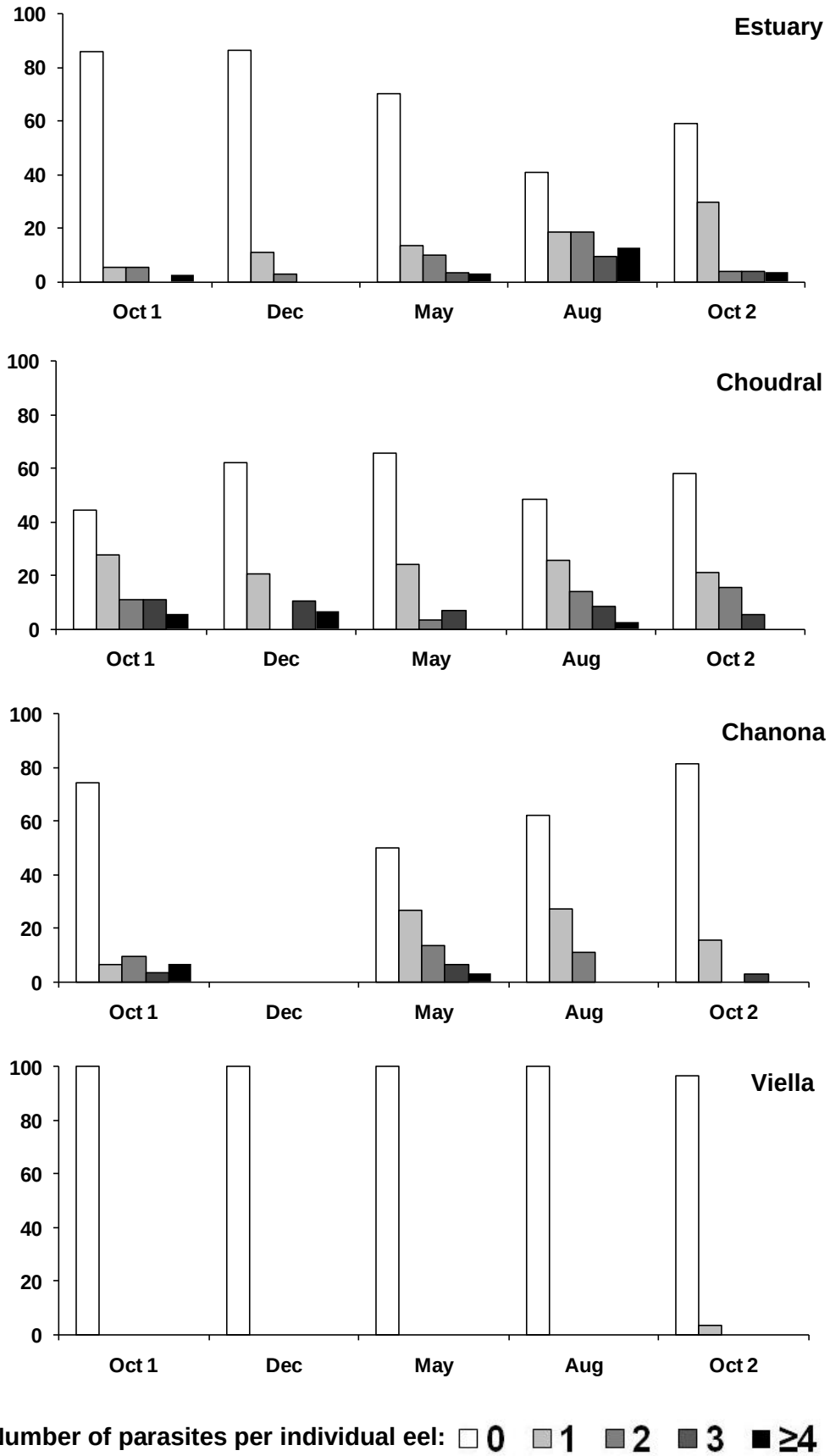


Figure 3.1. Percentage of eels (%) infected by *A. crassus* quantified as the total number of parasites in classes of abundance as 0, 1, 2, 3 and ≥4 parasites per individual eel. Eel data in Table 3.2.

Prevalence for larvae stages L3 + L4 was also detected at the same three sites where infection by adults and pre-adults predominated. Prevalence, however, varied somewhat among seasons with persistently high levels at the two lower-most sites: Choudral (9-32%) and Estuary (44 % in the summer, Table 3. 2). Larvae stage 2 was also detected at these three sites, but with only one exception in the Estuary (October1, Table 2), L2 was only detected in < 4 eels.

Log-transformed length and mass were highly correlated at all sites and seasons and an ANCOVA for the effects of site, season and site × season interaction showed no significant difference (site, $P = 0.509$; season, $P = 0.25$, and site × season, $P = 0.61$). As a consequence, a combined relationship was calculated for all individuals pooled in the form: log W (g) = -6.904 + 3.161 log L (cm); (n = 580 individuals; $r^2 = 0.98$; $P < 0.001$) and the value of b = 3.16 was used for calculating the condition factor.

The condition of eels decreased significantly with eel size, but this relationship only accounted for 2.1% of the variation ($P < 0.005$). In turn, eel condition did not differ among seasons ($P = 0.58$) but showed a highly significant difference among sites ($F_3 = 9.68$, $P = 0.002$) that underscore a consistent longitudinal pattern where poorer condition was at the upper-most La Viella site ($K = 0.78$) increased slightly at intermediate sites (Chanona, $K = 0.82$; Choudral $K = 0.80$) to maximize in the estuary ($K = 0.90$). The results of ANOVAs highlighted no effect of larvae L3 + L4 and L2 burdens on eel condition, but a counter-intuitive, positive effect ($F_4 = 3.21$, $P = 0.01$) of adult and sub-adult burden where eels with 2, 3 and ≥4 parasites exhibited higher condition ($K = 0.86$-0.88) than eels with only one or no parasite ($K = 0.82$).

Discussion

This study showed that a wild, unexploited stock of riverine eels, in a pristine environment was infected by *A. crassus*. The level of infection varied widely among sites over the longitudinal gradient of the river. Although the overall incidence of *A. crassus* was low in three out of four of the study sites, the prevalence of *A. crassus* attained values equal to, or higher than, 50%, in certain seasons of the year.

These results were unexpected but not surprising. Other instances of wild stocks infected by *A. crassus* have been reported in the literature. Morrissey and McCarthy (2007) detected *A. crassus* in a wild, unexploited stock of eels in a complex hydrological system in Ireland dominated by large-sized eels (mean = 44.6 cm). In this system, the eels were in poor condition (mean condition factor $K = 0.19$) that precludes comparisons with our small-sized, male-dominated stock (Lobón-Cerviá *et al.* 1995) where just a few >30 cm eels were recorded and mean condition factor attained an average $K = 0.83$.

When and how the colonization of Río Esva eels by *A. crassus* occurred is unclear. There was no apparent introduction or translocation of eels or exchange of waters from other systems. The propagation of *A. crassus* may occur through a number of transmission events including migration of intermediate or definitive hosts, or displacement by predators (herons, otters) of infected individuals (e.g. that may consume prey in a different place from capture) (Kirk 2003; Morrissey & McCarthy 2007). Although these hypotheses have not been previously explored along the rivers flowing north along the Cantabrian corridor of north-western Spain, it seems that even remote and well-preserved riverine eel stocks, unimpacted by anthropogenic activities, may be colonized and infected by *A. crassus*. Wild stocks of European eel, where stocking has not taken place and where no obvious mechanism may apparently increase the probability of infection, are infected by *A. crassus* is evident from not only this study for a north-western Spanish river but also by the Irish study (Morrissey & McCarthy 2007).

The life cycle of *A. crassus* is not seasonal-dependent (Didžiulis 2006) but may be affected by low temperatures. Knopf *et al.* (1998) performed a four-month experiment on the impact of low water temperature on the development of *A. crassus* in *A. anguilla*, and reported that long term exposure to a temperature of 4º C may not only retard the parasite life cycle but also injure several developmental stages. Moreover, although typical of fresh waters, *A. crassus* is able to cope with eel movements across different salinity gradients by osmo-conforming, and may overcome salinity stress due to acute, short-term and long-term host transfer to sea water without damage to body tissues (Kirk *et al.* 2002). Thus, the only limiting factors for *A. crassus* appear to be related to low temperatures and host absence (Kennedy 2007).

In the present study, the occurrence of *A. crassus* was assessed in eels inhabiting different sites along the environmental gradients of Río Esva, including different habitats (i.e., estuary vs river vs stream tributaries, with concurrent environmental and biological differences), differing in salinity levels but subject to similar temperature regimes. The highest infection occurred in the estuary where marine saline waters mixed with fresh water. In turn, with the only exception of the uppermost site (La Viella), the presence of larval stage L2 in the swimbladder of eels was detected at all sites including the estuary, main stem and streams, offering evidence of active reproduction (De Charleroy *et al.* 1990) at sites in the mid and lower reaches of the river. Conversely, the mild temperatures that typify winters in this Cantabrian region where stream waters only rarely attain temperatures below 5 ºC (Lobón-Cerviá & Rincón 1998) may plausibly cause delays in the life cycle but not operate as a limiting factor for the occurrence of *A. crassus*.

In the Río Esva, the absence of hosts is not likely to be a limiting factor. The literature reports a vast number of potential hosts (see Kennedy 2007; and references

therein) large enough to obscure the detection of *A. crassus*. Potential intermediate hosts were not assessed in this study, but the high diversity of invertebrates recorded in this and other nearby river drainages (Rincón & Lobón-Cerviá 1997) suggests that hosts are presumably present. Numerous species of aquatic insects have been described as paratenic hosts for *A. crassus* (Moravec & Skoríková 1998) and many of these species occur in Río Esva (Rincón and Lobón-Cerviá 1997; S. Costa-Dias & J. Lobón-Cerviá, unpublished data). Moreover, the omnivorous feeding strategy of eels (e.g. Tesch 2003) facilitates the consumption of potential vectors or paratenic hosts. Previous studies on these Río Esva eel stock have shown temporally persistent feeding activity with more than half of the individuals feeding year round (Costa-Dias & Lobón-Cerviá 2008). Thus, the presence of intermediate hosts and the consumption of infected organisms may also occur continuously.

Longitudinal differences in the size of eels examined in this study were consistent with the patterns of size segregation previously elucidated for this riverine stock (Lobón-Cerviá *et al.* 1995; Lobón-Cerviá & Iglesias 2008). With regard to individual fitness, current levels of infection by *A. crassus* did not apparently affect the condition of the Río Esva eels. The counter-intuitive pattern found with greater condition in highly infected individuals may result from co-variation factors not necessary related to the infection. A negative correlation between eel size and condition suggest that larger individuals at the upstream sites where infection is low are inherently associated with poorer condition whereas smaller, but markedly more infected individuals downstream are associated to higher condition. This further supports the results of studies that have reported no relationship between the condition of eels and the intensity of infection (Thomas & Ollevier 1992; Würtz *et al.*1998).

The infection by *A. crassus* predominated in the lower reaches of Río Esva and was practically absent in the uppermost reaches. García (2006, cited in Korta & Díaz 2008) examined a sample of 20 eels (mean size of 25.5cm) and reported that these eels were free of *A. crassus*. Sampling location in that study was not indicated but it is likely that those eels had been collected prior to the infection spreading and/or were collected from upstream reaches where the parasite may not have been present. It is hypothesised that colonization of the Río Esva by *A. crassus* may have occurred in recent years when the eel stock was at low density (Lobón-Cerviá & Iglesias 2008). In recent years, however, the Río Esva stock has shown high rates of recovery with marked increases in density (Lobón-Cerviá & Iglesias 2008). In this case, infected individuals from the lowermost reaches may be forced to migrate upstream as the stock increases and so colonization of the upstream reaches by *A. crassus* may be a question of time.

Chapter 4. Diel feeding activity and intensity patterns

This chapter has been published as:

Costa Dias S. and Lobón-Cerviá J. 2008. Diel feeding activity and intensity during an annual cycle of the European eel *Anguilla anguilla* (L.) in a Cantabrian stream. Knowledge and Management of Aquatic Ecosystems 390 - 391: 01 (9pp).
(DOI: 10.1051/kmae:2008010)

Acknowledgements for this chapter
These data were collected as part of long-term studies of the Río Esva fish populations. Administrative permissions for fish were obtained by agreement between the CSIC and Principado de Asturias. The senior author, Sérgia Costa Dias acknowledges the Portuguese Foundation for Science and Technology (FCT) for financial support trough a PhD grant (SFRH/BD/16922/2004).

Diel feeding activity and intensity patterns

Abstract

Diel and seasonal shifts in the feeding activity and intensity of a riverine stock of European eels, *Anguilla anguilla* (L.), were assessed in the Río LLorín, a right-side tributary of the Río Esva drainage (northwestern Spain). No consistent diel cycles were detected in either season (eels feed with similar intensity in the day and at night), but increased feeding activity was evidenced during the warmer months relative to weaker but still significant activity in winter. Heterogeneity in both feeding activity and intensity may stem from the high rates of secondary production in the form of benthic food available for eels, and mild temperatures all year round.

Introduction

In the course of its life cycle, the European eel, *Anguilla anguilla* (L.) spends a variable but significant period in freshwaters that may range from 3 (males) to 20 (females) years (Vøllestad 1992). Although not without exception (eels may remain in brackish waters, Vøllestad 1986), a development period is accomplished in rivers before maturation, energy acquisition being the main purpose of this period.

The European eel has been described as omnivorous and its feeding habits have been assessed in a diversity of habitats including estuaries (Moriarty 1987; Costa et al. 1992), streams and rivers (Sinha 1969; Rasmussen & Therkildsen 1979; Callaghan & McCarthy 1994; Lara 1994; Cullen & McCarthy 2007), and lakes (De Nie 1982; Moriarty 1987; Bergersen & Klemetsen 1988; Clarke *et al.* 1993). The few studies dealing with diel and seasonal patterns of feeding activity and intensity have reported increased activity with increased temperature (Tesch 2003) and nocturnal feeding (Lecomte-Finiger 1983; Clarke *et al.* 1993; Tesch 2003) with increased intensity at dawn and at dusk.

To further investigate this issue we analysed feeding activity and intensity in a stock of eels inhabiting a tributary of the Río Esva where eel is accompanied by brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.). Previous studies on the feeding ecology of these two salmonids in the Río Esva reported continuous day and night feeding patterns the year round (Rincón 1993; Utrilla 1997). The present study examines whether patterns of diel feeding activity and intensity of European eels track similar patterns to those exhibited by salmonids in Río Esva tributaries.

Material and methods

Study Area

The Río LLorín is a right-side tributary of the Río Esva drainage (Asturias, northwestern Spain), located about 11km upstream from the Esva mouth into the Cantabrian Sea. The Río LLorín flows over siliceous (quarzite) bedrock and its stony substratum is composed of sand (10%), gravel (50%), pebble (30%) and stones (10%). The Río LLorín is essentially fed by rain and run-off, and discharge fluctuates seasonally depending on rainy conditions. Overall, rainfall increases in spring and autumn but episodic or persistent rainfall may occur during prolonged time periods. Consistent with the mild climatic conditions that typify the coastal Cantabrian region, the stream temperatures are mild all year round, with winter stream temperatures that seldom reach the freezing level and summer temperatures with maxima at 20 °C (Lobón-Cerviá & Rincón 1998).

Sampling strategy

Eels were collected monthly from August 1990 to July 1991 (except February and September) with electrofishing in a 1-km long stream section (average width =10 m and depth = 30 cm). Sampling started at the lowermost stream reach and collections of ~ 10 individuals were made every three hours until the completion of a 24-hour daily cycle. During the following month, sampling was conducted by commencing the eel sampling just above the upper limit of the site previously sampled. Overall, we attempted to maintain a constant 30-minute fishing effort and attempted to collect 10 individuals every time period during the diel cycle. As a whole, we collected and analysed an average of 74 eels in each monthly sample, which amounted to a total of 735 eels, of which 413 were actively feeding (with stomach content) and they were used for subsequent analysis of feeding intensity. Stream water temperatures (ºC) were recorded at every sampling occasion (i.e., 8 temperature records per day).

To prevent digestion, eels were ice-preserved during transport to the laboratory where they were frozen and stored until analysis. Individual total weight (0.01 g) was obtained prior to dissection and stomach contents removed and weighed to 0.0001 g.

Data analysis

All individuals collected were in the range 10-34 cm. As a consequence, we focused our analysis on all individuals with no separation of size or age class. Feeding activity and intensity were quantified as follows:

(1) Feeding activity (A_I) was considered as the number of individuals with food in their guts (Ni) relative to the total number of individuals collected in each sample (N_T) and was quantified as:

$$A_I = 100 * (Ni / N_T)$$

(2) Feeding intensity (I_I) was considered to be reflected by the amount of food in the gut and was quantified for each single individual as the weight of food in the gut (Wi) relative to the total weight of the individual (W_T):

$$I_I = 100 * (Wi / W_T)$$

Analyses of variance (Zar 1999) was used to explore the effects of the month and time of day (hour) on both, feeding activity (A_I) and intensity (I_I).

Results

Stream water temperatures at the time of sampling (Figure 4.1) revealed a seasonal thermal regime typical of coastal Cantabrian streams: mild summers with temperatures around 15-16 ºC in July and August were followed by mild winters with lowest temperatures in December and January attaining, on average, ~ 6 ºC.

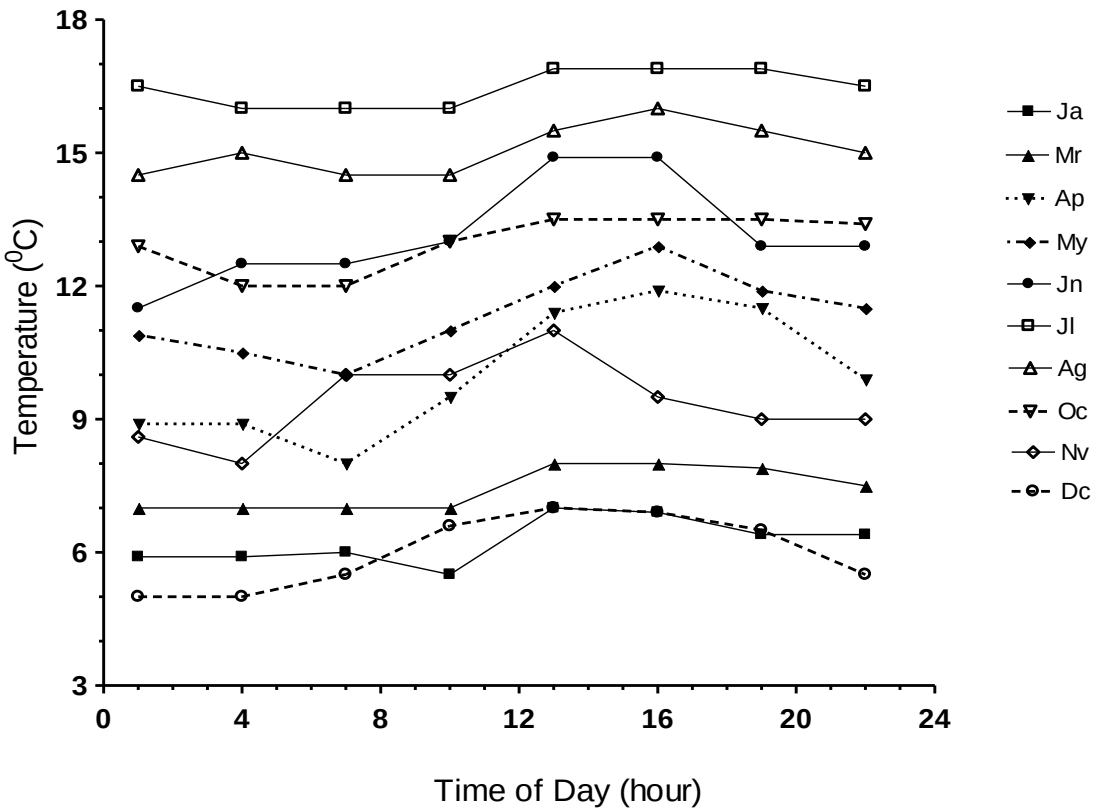


Figure 4.1. Stream water temperatures (ºC) recorded at the time of sampling in Río LLorín over the sampling months.

Feeding activity and intensity

Active eels were recorded across the whole range of temperatures and months with no obvious period of quiescence in evidence (Figure 4.2). However, both feeding activity and intensity varied somewhat across months. An ANOVA for the feeding activity quantified with the index A_I (Table 4.1A) revealed significantly lower numbers of feeding eels in October (33.7% of the individuals) and during the winter months (December, January and March; ~ 44.5% of the individuals). Markedly higher numbers were recorded in spring and summer including April, May, June and July (66.0 to 77.3 %). However, as revealed by non-significant effect of the time of day (Table 4.1A), no consistent pattern in diel activity was evidenced and a new ANOVA for the combined hours of day light *versus* night also revealed non-significant effect ($F_{1,9}$ = 0.07, P = 0.79).

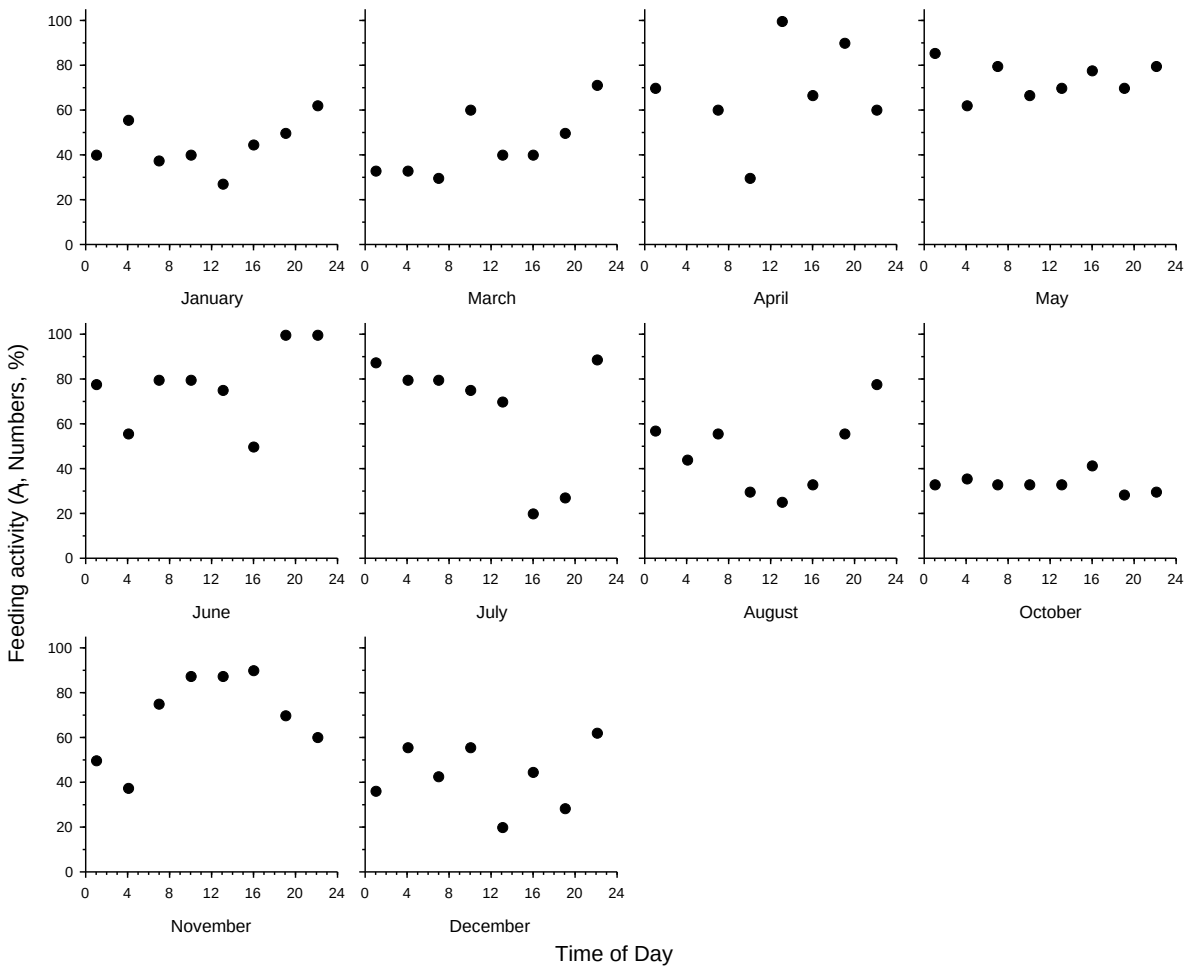


Figure 4.2. Feeding activity as quantified with the index (A_I, the percent of individuals with gut content) in every sample (time of day) over the study months.

Table 4.1. Results of ANOVAs (*F*-values and *P*, significance level) for the effects of the month (10) and time of day (8) on (**A**) the feeding activity quantified with the index A_I and (**B**) the feeding intensity quantified with the index I_I, for the eels *Anguilla anguilla* (L.) of Río Llorin (Asturias, northwestern Spain). Note that the first ANOVA is based on the total number of individuals pooled (Figure 2) whereas the second ANOVA is based on the analysis of single individuals (Figure 3). df are the degrees of freedom and V% is the amount of variance explained (in percent) by each effect.

(A)	df	F	p	V%
Month (M)	9	6.90	<0.001	47.1
Time of day (TD)	7	1.09	0.377	5.8
M*TD	62			47.1

(B)	df	F	p	V%
Month (M)	9	3.11	p = 0.001	6.5
Time of day (TD)	7	0.76	p = 0.621	1.2
M*TD	62	0.96	p = 0.569	13.9
Error				78.3

Likewise, an ANOVA for the feeding intensity, quantified with the index I_I (Figure 4.3), showed a significant effect of the month but a non-significant effect of the time of day (Table 4.1B) and a new ANOVA for the effects of the time of day for each single month separately also revealed a non-significant effect (Table 4.2). Thus, feeding intensity varied around a mean value I_I = 1.1 %, with the highest means in May (1.49%), June (1.22%) and November (1.38%) and lowest in March (0.65%) and October (0.76%).

As a consequence, the number of eels feeding actively increased in the warmer months but the relative numbers of eels feeding during the day and night remained similar across months. Both feeding activity and intensity described a consistent seasonal pattern

with higher numbers of individuals feeding more intensively in the warmer months and decreased numbers in winter (Figure 4.4). The only exception to this seasonal pattern was November when slightly decreased temperatures (i.e., ~ 9.4 ºC) were associated with a high percentage of actively feeding individuals (69.7%).

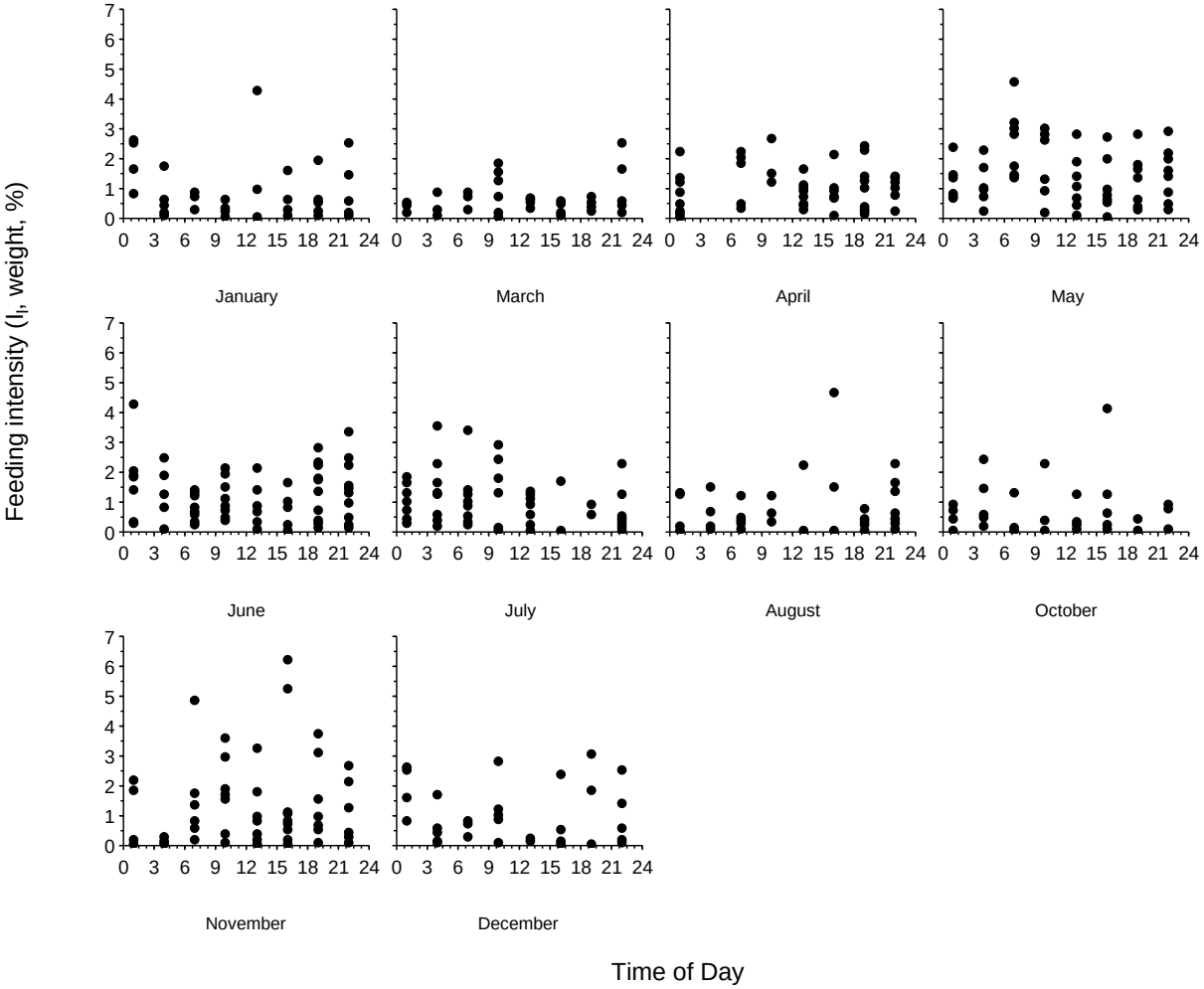


Figure 4.3. Feeding intensity index (I_I) for every sample (time of day) over the study months. Each point represents one single individual.

Table 4.2. Results of ANOVA for the effects of the time of day (8) on the feeding intensity quantified with the index I_I for each single month. N is the number of individuals and other symbols as in Table 1. Note that no instance was significant.

	N	df	F	p	V%
January	33	7	1.43	0.238	28.6
March	32	7	1.06	0.417	23.6
April	45	6	1.02	0.426	13.9
May	54	7	1.96	0.081	23.0
June	59	7	0.97	0.464	11.7
July	48	7	0.70	0.673	10.9
August	33	7	1.14	0.373	24.1
October	29	7	0.45	0.857	13.1
November	49	7	0.53	0.804	8.4
December	31	7	1.22	0.334	27.0

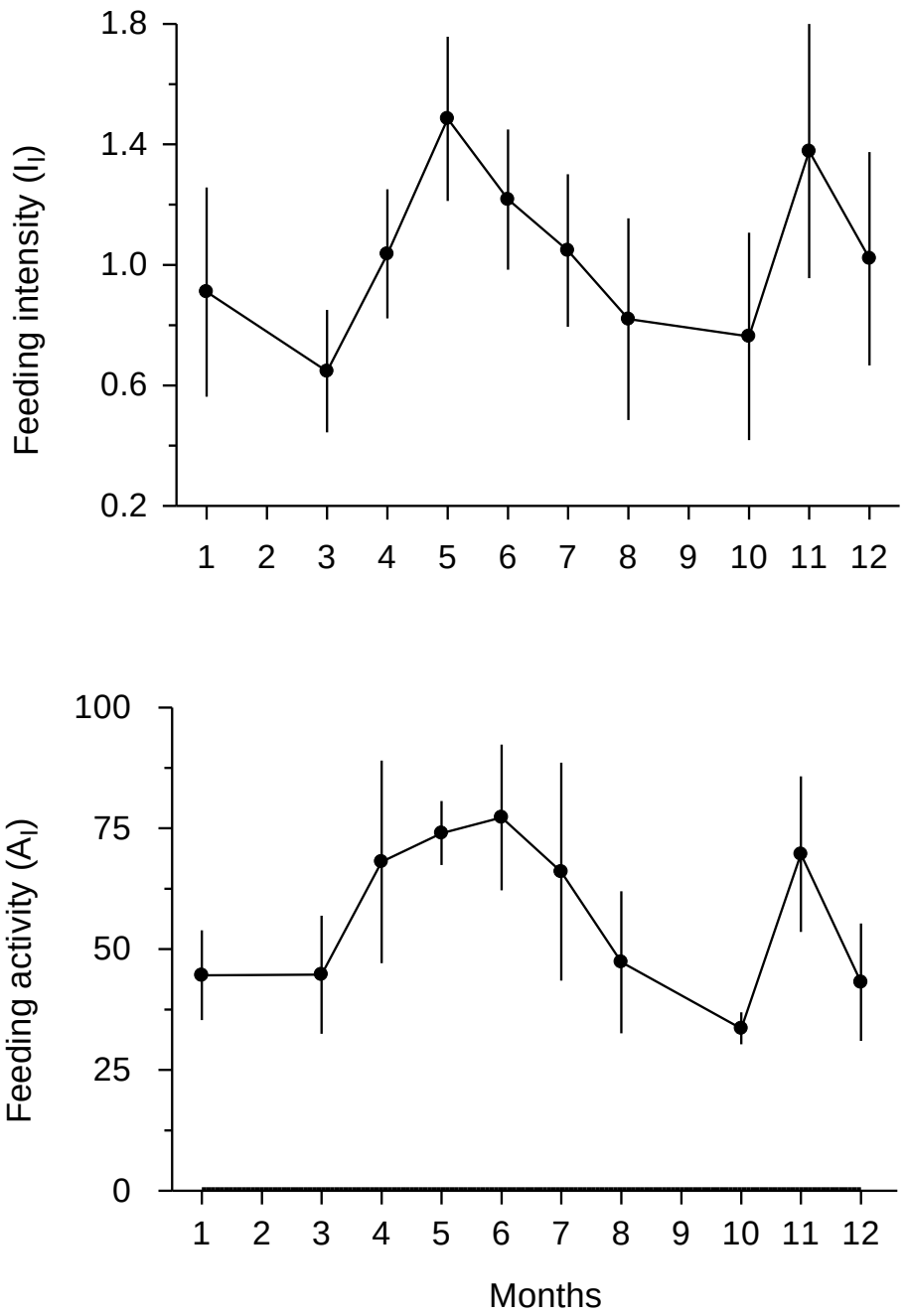


Figure 4.4. Mean (± 95 % CL) monthly feeding activity and intensity as quantified by the indexes A_I and I_I, respectively, over the study months.

Discussion

This study shows that, in the Río LLorín, a typical coastal stream of the Cantabrian corridor of northwestern Spain that is fully occupied by eels, and where the temperatures regime is rather mild with no high or low temperature extremes, the eels remain active the year round, showing only slight differences in their feeding activity and intensity across

months and no obvious diel pattern, with apparently similar amounts of individuals feeding during the day and night throughout the year. Although no consistent diel cycles were detected in either season, increased feeding activity and intensity were evidenced in the warmer months.

The notion that European eels are active and feed intensively during late spring and summer and become inactive and cease feeding in autumn and winter has been reported for a number of European regions including Wales (Thomas 1962; Sinha & Jones 1967), Ireland (Moriarty 1982; Cullen & McCarthy 2007), Scotland (Hussein 1981), France (Lecomte-Finiger 1983), Denmark (Rasmussen & Therkildsen 1979), the Netherlands (De Nie 1987), and temperate Europe (Tesch 2003). Moreover, Lecomte-Finiger (1983) described diel feeding patterns with a predominance of nocturnal feeding activity in spring and summer. Clearly, none of those patterns match the patterns elucidated for the studied Cantabrian stock. However, discrepancies may be simply caused by the different thermal regimes in northern and central Europe watercourses relative to Cantabrian coastal streams. Eel activity has been suggested to be temperature-dependent (Tesch 2003). This being the case, a temporally persistent feeding activity in the Cantabrian eels may be caused by the combined effects of mild temperatures and high rates of secondary production (Rincón & Lobón-Cerviá 1997). Warmer temperatures and abundant food available across seasons may permit eels not only to be active but also feed the year round. Heterogeneity in both feeding activity and intensity may stem from the combined effects of high rates of secondary production in the form of benthic food available for eels all year around, and mild winters.

Nevertheless, a study conducted in Río Nivelle (Neveu 1981), a small French river located at a similar latitude to the Río Esva, showed a similar pattern of feeding activity. In that study, the author also questions the latitudinal factor and the possible influence of mild temperatures. The conclusions, however, were based on a single daily cycle in June with samples collected every 6 h. Neveu (1981) reported that, in June, smaller eels (< 30cm) showed continuous feeding, while larger individuals (> 30cm) exhibited nocturnal feeding activity. The practically non-occurrence of eels > 30cm in Río Esva preclude further comparisons (Lobón-Cerviá et al. 1995).

Nevertheless, despite substantial differences in the temporal patterns of feeding among stocks across the European distributional range, the values of the feeding intensity index appear similar to those reported by Lecomte-Finiger (1983). Lower values of the feeding intensity index in Río LLorín can be expected because, according to studies on prey items of eel in streams of the Río Esva drainage (Rincón & Lobón-Cerviá 1997), these are mainly small-sized insects.

Feeding activity and intensity

Interestingly, the feeding patterns of the European eel in the Río Esva are quite similar to those described for the accompanying salmonids, Atlantic salmon (Utrilla 1997) and brown trout (Rincón 1993). Consistency among patterns allows us to hypothesize that the underlying environmental factors related to mild temperatures operate similarly on the three co-occurring species in this geographical region.

Chapter 5. Feeding strategies along the environmental gradient of the river

This chapter in under preparation for publication as:

Costa-Dias S. and Lobón-Cerviá J. (in prep.). Benthos assemblages along the environmental gradient of Río Esva.

Costa-Dias S. and Lobón-Cerviá J. (in prep.). Feeding strategies of the European eel along the environmental gradient of Río Esva.

Acknowledgments for this chapter

Acknowledgments are due to Ester Dias, Tania Iglesias, Micaela Mota, Joana Barreira and Carla Olim for assistance in either field sampling or laboratory procedures; and to the Principado de Asturias for administrative permissions for data collection. This study was supported by the Portuguese Foundation for Science and Technology - FCT (S.C.D., grant SFRH/BD/16922/2004).

Feeding strategies along the environmental gradient of the river

Abstract

The feeding tactics of European eel were assessed along the environmental gradient of a Cantabrian river. The feeding patterns in relation to food availability (i.e., benthos assemblages) were examined at four sites along the environmental gradient. Eels were actively feeding the year round at the four sites but more intensively during the warmer months. Overall eels feed on a wide range of organisms. However, at the scale of site, prey items determined in the eels stomachs reflect, to a large extent, the benthos composition across seasons suggesting that whilst they behave as an omnivorous fish they actually feed on the most common prey at a local scale.

Introduction

The feeding patterns of the European eel, *Anguilla anguilla* (L.), have been investigated in contrasting aquatic systems throughout its natural distributional range. Several studies have emphasized omnivorous behaviour, nocturnal feeding, increased feeding intensity with increased temperature and ontogenetic diet shifts (please see Chapter 1. Introduction).

Previous studies on the feeding patterns of Río Esva eels (Chapter 1) revealed no diel patterns but increased feeding activity and intensity during the warmer (summer) months. Indeed, a 45% active feeding individuals in winter also indicated a continuous feeding across seasons within a temperature range of 5-17 ºC. Subsequently, other related issues for this Esva stock arise as for example: is the eel feeding omnivorous? Does eel select specific food items or alternatively feed primarily on most abundant prey available? And is eel productivity limited by the amount of prey available?

Previous studies indicated that eel essentially feed on benthic organisms (e.g. Costa *et al.* 1992; Cullen & McCarthy 2007) and as a consequence, to answer the questions above, eels and benthos were collected simultaneously along the environmental gradient of Río Esva over a year period.

Material and Methods

Study area

Río Esva is located in the Cantabrian corridor of northwestern Spain, flowing north across a mountainous and rugged landscape towards the Cantabrian sea (43º 33' N, 6º 32' W). The catchment area (c. 500 km^2) is mainly fed by rain (1000–1500 $mm.year^{-1}$) and runoff, with discharge fluctuating widely among months and seasons, and shows mild temperatures the year round (Lobón-Cerviá & Rincón 1998). Río Esva is well preserved and for the most, safe of human-induced alterations as fish stocking, farming, weirs, obstructions or pollution.

For the purpose of this study, four stream sections (henceforth sites) were selected to represent the environmental variability visually detectable from the estuary to the uppermost river reaches. These four sites are as follows: (1) the lowest site at the estuary at km = 0 (henceforth Estuary site); (2) a low altitude tributary near the estuary located at some 3 km from the estuary (henceforth Choudral); (3) an upstream site within the main stem of Río Esva located at some 21 km (henceforth Chanona), and (4) the uppermost study site at La Viella stream located at some 27 km from the estuary (henceforth La Viella). These sites were selected on stream sections where eel density has been previously quantified and previous studies on invertebrate assemblages (Rincón & Lobón-Cerviá 1998) forecast different benthos composition.

Characterization of sites included determination of water temperature (ºC) "in situ" and major chemical attributes including pH, nitrites (NO_2), nitrates (NO_3), ammonia (NO_4), phosphates (PO_4) and calcium ($CaCO_3$) in the laboratory, for each sampling occasion and site.

Benthos assemblages

Benthos composition was assessed on a seasonal basis including five samples collected in October 2006, December 2006, May 2007, August 2007 and October 2007. A type surber net of approximately 0.1 m^2 was used to collect two benthos replicates at each study site and season. All macro-invertebrate and related species collected in the samples were assigned to the lowest taxonomic level and were counted for density estimations expressed as $individuals.m^{-2}$.

Eel sampling

Just after the completion of the benthos samples, eels were collected with electro fishing to analyse eel's diet. Samples were conducted to collect a minimum of 25 eels per site. All eels were immediately ice-preserved for transport and frozen during the following hour to prevent stomach content digestion.

Total length (nearest 0.1cm) and weight (nearest 0.1g) were determined at the laboratory. After extraction, eels' stomachs were opened and their content was weighted (nearest 0.1mg) and prey items were determined under the stereomicroscope.

Data analysis

The mean length of eels studied per site varied between 16.9 (± 6.2) cm at the Estuary and 23.4 (± 5.5) cm at La Viella. Although the size of eels collected varied among sites, most individuals were ~20 cm. Consequently, (see Chapter 4), no separation per eel size or age class was considered.

Eel's feeding activity and intensity were quantified as described by Costa-Dias & Lobón-Cerviá (2008):

(1) Feeding activity (A_I) was considered the number of individuals with food in their guts (Ni) relative to the total number of individuals collected in each sample (N_T) and was quantified as:

$$A_I = 100 * (Ni / N_T)$$

(2) Feeding intensity (I_I) was considered the amount of food in the gut across individuals and was quantified for each single individual as the weight of food in the gut (Wi) divided by the total weight of the individual (W_T):

$$I_I = 100 * (Wi / W_T)$$

To express the feeding tactic of eel the following indices were used:

(3) Numeric index (N_I) was considered the number of prey of a certain taxa (Ni) relative to the total number of prey taxa (N_T) and was quantified as:

$$N_I = 100 * (Ni / N_T)$$

(4) Occurrence index (O_I), the number of individual eels with a certain prey in their gut (Ni) relative to the total number of individuals in the sample (N_T):

$$O_I = 100 * (Ni / N_T)$$

Results

The chemical attributes including temperature determined on the study sites (Table 5.1) were rather similar and provide no evidence of consistent gradients related to the distance of the study sites to the river mouth.

Table 5.1. Physico-chemical attributes across seasons (mean + SD in brackets) for the four study sites in relation to distance from the river mouth: temperature (T), pH, nitrites (NO_2), nitrates (NO_3), ammonia (NO_4), phosphates (PO_4) and calcium ($CaCO_3$).

	Estuary	**Choudral**	**Chanona**	**Viella**
Distance up. (km)	1.0	3.0	21.0	27.0
T (ºC)	13.6 (4.7)	12.9 (2.5)	13.9 (5.3)	13.0 (5.0)
pH	7.8 (0.8)	6.5 (0.3)	7.9 (0.6)	7.0 (0.3)
NO_2 (mg/L)	0.006 (0.009)	0.004 (0.005)	0.002 (0.004)	0.004 (0.009)
NO_3 (mg/L)	2.0 (1.1)	2.0 (1.2)	2.1 (1.3)	1.4 (0.4)
NO_4 (mg/L)	0.09 (0.12)	0.09 (0.06)	0.09 (0.13)	0.03 (0.01)
PO_4 (mg/L)	1.1 (1.0)	0.7 (1.3)	0.8 (1.2)	1.7 (2.0)
$CaCO_3$ (mg/L)	25.7 (14.9)	10.0 (11.7)	30.0 (19.0)	13.0 (11.5)

Benthos assemblages

Benthos composition shared several common taxonomic groups with marked differences on their relative contributions among sites. To avoid overload of figures such data are presented in Figure 5.2. In several instances a single taxonomic group accounted for a high proportion of the total number of individuals.

For example, Amphipoda attained the highest relative density at the Estuary but appeared almost absent at all other sites. Similarly, Gastropoda was exceptionally abundant at Chanona but not at other sites.

Table 5.2 summarizes densities for the top three, most abundant prey. At the Estuary, Amphipoda and Diptera showed top densities along the year but varied widely for among seasons: for example, Amphipoda showed low density at 28 ± 39 individuals.m^{-2} in the second autumn (October 2) and up to 11042 ± 4578 individuals.m^{-2} in December. Similarly, Diptera showed lower density in October 1 and 2 (46 ± 13 and 64 ± 65 individuals.m^{-2}, respectively) and attained >2300 individuals.m^{-2} in May and August. At Choudral, the top three most abundant species were Diptera and Ephemeroptera for most seasons and Tricoptera during October 1 through May, with comparable values throughout the year. At Chanona, Gastropoda (density between ~ 2500 and 5600 individuals.m^{-2}) and Tricoptera (lower mean densities at 225 ± 149 individuals.m^{-2} in May, > 1300 individuals.m^{-2} during the rest of the year) showed the top densities while in La Viella, Ephemeroptera and Tricoptera predominated with lower densities in December and May.

Table 5.2. Density of the top three most abundant taxa across sites and seasons. Density (individuals.m^{-2}) in brackets (mean ± SD).

	October 1	**December**	**May**	**August**	**October 2**
Estuary	Amphipoda (202±13)	Amphipoda (11042±4578)	Diptera (2337±409)	Diptera (2332±974)	Coleoptera (110±91)
	Diptera (46±13)	Gastropoda (647±149)	Amphipoda (776±33)	Coleoptera (289±149)	Diptera (64±65)
	Gastropoda (23±6)	Coleoptera (257±117)	Oligochaeta (643±13)	Tricoptera (41±7)	Amphipoda (28±39)
Choudral	Coleoptera (1488±649)	Diptera (455±136)	Ephemeroptera (450±143)	Diptera (1226±1227)	Diptera (551±260)
	Diptera (776±539)	Tricoptera (303±169)	Diptera (395±442)	Oligochaeta (308±370)	Coleoptera (537±176)
	Tricoptera (487±78)	Ephemeroptera (243±175)	Tricoptera (202±169)	Ephemeroptera (220±39)	Ephemeroptera (349±117)
Chanona	Gastropoda (5051±472)	Gastropoda (2456±759)	Gastropoda (2755±235)	Diptera (4316±3117)	Gastropoda (5615±1967)
	Tricoptera (1313±727)	Tricoptera (1882±740)	Coleoptera (262±149)	Tricoptera (3182±552)	Tricoptera (1841±1201)
	Diptera (794±84)	Coleoptera (303±26)	Tricoptera (225±149)	Gastropoda (2759±396)	Diptera (1065±442)
Viella	Tricoptera (1635±1260)	Ephemeroptera (680±247)	Ephemeroptera (712±903)	Ephemeroptera(3118±357)	Tricoptera (1024±980)
	Ephemeroptera (900±195)	Plecoptera (115±20)	Diptera (234±279)	Tricoptera (634±0.0)	Ephemeroptera (794±279)
	Diptera (468±299)	Tricoptera (110±39)	Tricoptera (96±110)	Aracnidae (624±13)	Diptera (748±136)

Eel feeding patterns

A total of 580 eels were analyzed (Lengths (cm) and Weights (g) in Table 5.2). Eels length differed among sites (F_3 = 43.9, P < 0.001) with larger individuals at the upstream locations. The mean size of eels varied between 16.9 (± 6.2) cm at the lowermost site i.e., Estuary and 23.4 (± 5.5) cm at the uppermost site, i.e, La Viella. Whilst most individuals were around 20 cm, the total size range was 6.5-35.7 cm, plus two large-sized females with 52.0 and 57.0 cm.

Apparently, eels feed the year round with the feeding activity index (A_I) varying between 25% (Choudral, October 1) and 96% (Estuary, in October 2) (Table 5.3). Within each site, the highest feeding activity was recorded in October 2 at the Estuary, Choudral and Chanona; and in May at La Viella. On the contrary, a lower percentage of eels were actively feeding in October 1 at Choudral and in December at the Estuary and La Viella. In December no eels were collected at Chanona.

Table 5.3. Number (N); mean (minimum – maximum in brackets) length (L) and weight (W); and feeding activity (A_I) and intensity (I_I, SD in brackets) indices of eels examined. Mean (SD in brackets) values per sampling site were also calculated.

		October 1	December	May	August	October 2	Mean (SD)
Estuary	N	36	37	30	32	27	32.4 (4.2)
	L (cm)	13.3 (6.6-19.8)	12.0 (7.2-19.9)	19.6 (12.3-28.7)	22.2 (13.4-52.0)	16.1 (12.2-25.6)	16.9 (6.2)
	W (g)	5.1 (0.4-16.2)	3.3 (0.3-13.1)	16.1 (3.2-41.9)	29.5 (1.9-256.8)	8.7 (3.0-31.4)	12.3 (23.9)
	A_I (%)	58	26	81	53	96	62.9 (26.9)
	I_I (%)	1.4 (1.2)	0.6 (0.6)	1.4 (1.9)	1.3 (1.1)	1.2 (2.2)	1.2 (0.6)
Choudral	N	36	30	29	35	19	29.8 (6.8)
	L (cm)	19.7 (9.8-31.7)	21.0 (11.9-28.4)	20.4 (10.5-33)	17.9 (11.4-26.8)	20.2 (15.8-26.7)	19.7 (5.0)
	W (g)	14.6 (1.0-61.1)	16.5 (16.3-38.3)	18.7 (1.2-52.1)	11.5 (1.3-36.4)	14.2 (6.5-29.8)	15.0 (11.9)
	A_I (%)	25	60	69	71	84	61.9 (22.4)
	I_I (%)	1.1 (0.9)	0.8 (0.5)	1.5 (1.7)	1.6 (0.9)	1.1 (0.7)	1.2 (0.5)
Chanona	N	31	---	30	37	32	32.5 (3.1)
	L (cm)	23.2 (15.5-30.6)		23.2 (13.4-35.8)	19.4 (11.8-29.1)	18.9 (13.3-25.3)	21.0 (4.8)
	W (g)	23.6 (5.7-49.6)		24.7 (4.2-86.8)	15.2 (2.7-46.2)	11.1 (3.4-29.9)	18.4 (14.1)
	A_I (%)	65		57	57	72	62.5 (7.3)
	I_I (%)	1.3 (1.6)		1.7 (1.4)	0.7 (0.4)	0.4 (0.4)	1.0 (0.6)
Viella	N	30	26	23	31	29	27.8 (3.3)
	L (cm)	22.9 (15.2-32.1)	27.4 (16.3-57.0)	21.4 (12.3-28.7)	23.5 (14-32.5)	22.9 (11.5-31.0)	23.4 (5.5)
	W (g)	23.8 (6-64.4)	44.1 (5.9-431.2)	17.6 (2.7-42.7)	24.1 (3.7-68.7)	22.3 (2.3-63.7)	26.1 (37.8)
	A_I (%)	57	35	87	71	55	60.9 (19.5)
	I_I (%)	1.0 (0.9)	0.3 (0.1)	2.9 (1.6)	1.0 (1.1)	1.3 (0.8)	1.3 (0.5)

Moreover, the majority of the feeding intensity index (I_I) values were <2% to attain 7.9% at the Estuary in October 2, and with samples from May drawing a generally wider range (Table 5.3 and Figure 5.1). Thus, actively feeding individuals were consuming low mass prey relative to their body mass.

Overall eels feed on a broad range of invertebrates and almost exclusively on benthic organisms with occasional intake of adult insects and small fish. Varying amounts of detritus were found in around half of the feeding eels but with only vestigial traces on most of them, and were not included on diet analysis. With the only exception of the

lowermost site (i.e. Estuary), Diptera, Tricoptera and Ephemeroptera were the most common prey. However, at the Estuary, Amphipoda was particularly important throughout the year and in August when the abundance of Amphipoda was negligible eels feed upon Diptera, Tricoptera and Odonata.

Feeding patterns in terms of numerical (N_I) and occurrence (O_I) indices are detailed in Tables 5.4 and 5.5, respectively. The maximum values of N_I = 97.8% and O_I = 92.6% were found for Amphipoda at the Estuary in October 2 and points out Amphipoda as the most common prey in all seasons except in the summer whereas O_I also revealed the importance of other prey in specific seasons. As for example, in May, Amphipoda was the most abundant prey with N_I = 45.3% followed by 17.0% for Diptera, but both occurring with an equal O_I value of 30.0% and was still higher i.e., 43.3% for Oligochaeta. On the other hand, Diptera, Tricoptera and Ephemeroptera were important both in number and occurrence along the year at all other sites i.e., Choudral, Chanona and Viella.

Comparison between feeding patterns and benthos composition

On this section analysis concerns whether eel actually feed upon prey depending on their abundance across ites and seasons or alternatively, eel select or prefer specific prey depending on their abundance in the benthos.

Taken into account that several taxa present in the benthos may be unedible for eels as Coleoptera or Aracnidae, eel seems to feed upon the most common benthic taxa across sites and seasons (Figure 5.2). At the Estuary, Amphipoda was always important in the eels diet even when they appeared less abundant in the benthos (Figure 5.2) and in August, when amphipoda showed lower abundances in the benthos, eels shifts to feed upon Diptera, Tricoptera and Odonata.

In contrast, at Choudral, eels show no apparent preference in either season. At this site, where the abundance of Amphipoda was negligible, eel fed on the most common prey including Diptera, Tricoptera and Ephemeroptera whereas small-sized prey as Coleoptera were not much consumed even in seasons when they were most abundant. A quite similar pattern was evident in La Viella where the feeding pattern also matches the benthos composition, with the only exception of August when Diptera was more abundant in the eel's diet than in the benthos.

Also in Chanona, the eel diet was essentially composed of the most dominant Diptera, Tricoptera and Ephemeroptera. However, at this site eels apparently prefer Ephemeroptera which appeared in high percentage in eel' diet but seldom occurred in the benthos; a pattern shared with Plecoptera in October 1 but not at other seasons. In addition, at this site, Gastropoda was most abundant in benthos but only occurred scattered in the eel diet.

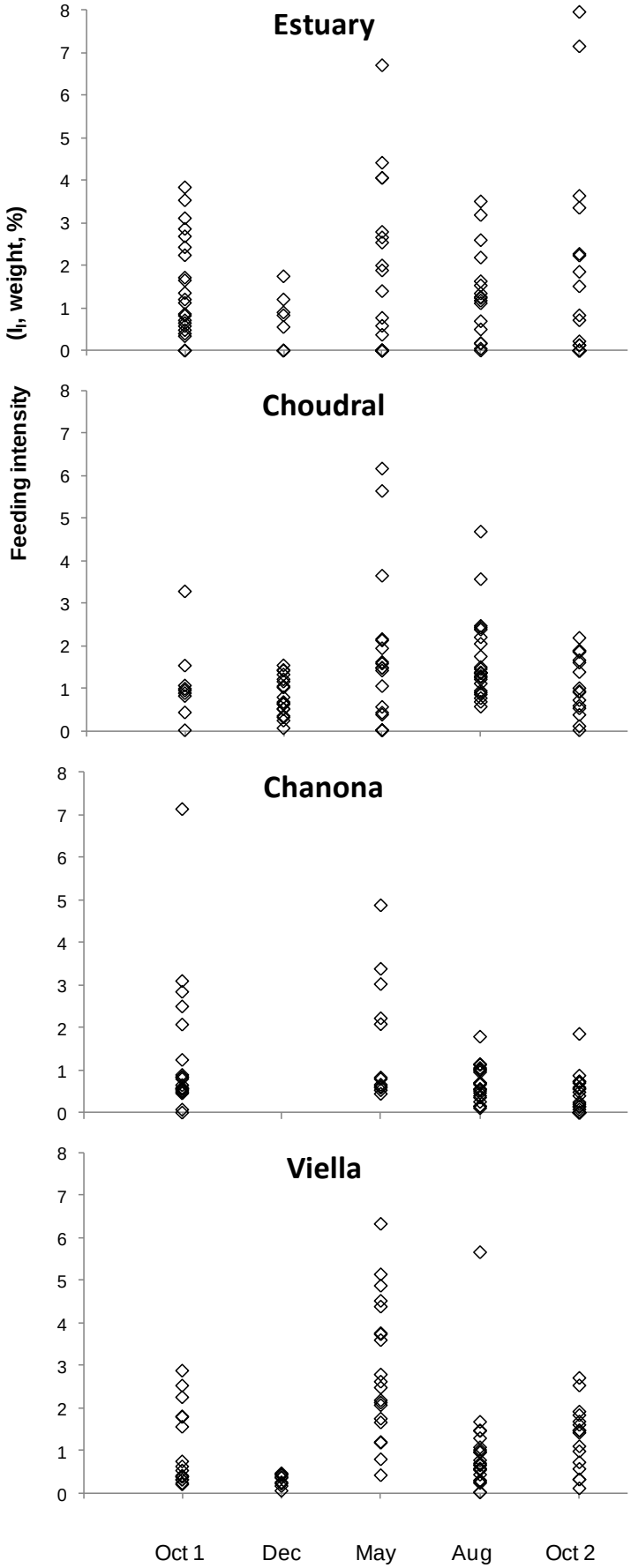


Figure 5.1. Feeding intensity index (I_i) for all sites and seasons. Each point represents one single individual.

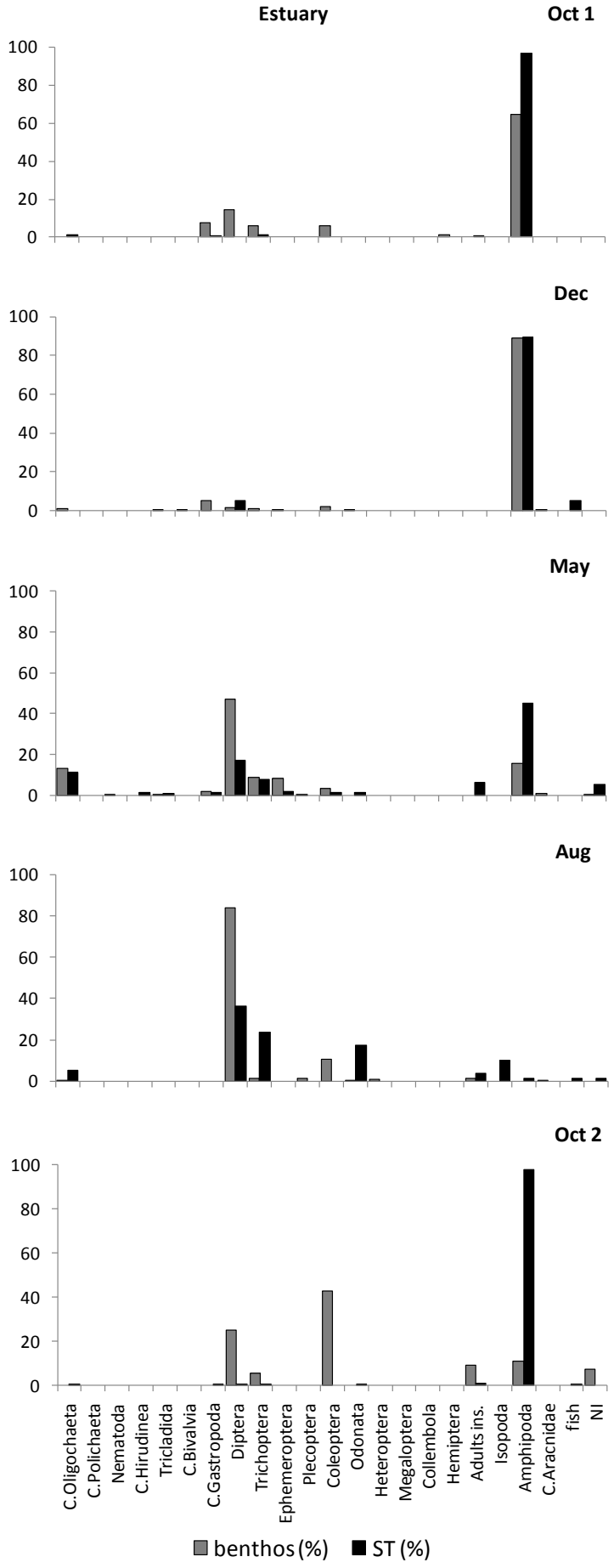
Table 5.4. The numerical index (N_I) of the eel feeding in Rio Esva across study sites and seasons.

	Estuary					Choudral					Chanona					Viella				
	Oct 1	Dec	May	Aug	Oct 2	Oct 1	Dec	May	Aug	Oct 2	Oct 1	Dec	May	Aug	Oct 2	Oct 1	Dec	May	Aug	Oct 2
C.Oligochaeta	1.3		11.3	5.0	0.3	2.9		7.5		2.0			2.9	0.2		0.6		0.9		1.8
C.Hirudinea			1.3																	
Diptera		5.3	17.0	36.3	0.3	34.3	13.6	19.6	62.8	54.7	2.0		18.3	4.3	4,4	37.5	36.4	4.4	76.6	48.5
Trichoptera	1.3		7.5	23.8	0.1		24.7	12.1	9.4	12.2	11.7		10.6	8.0	29.6	48.1	13.6	10.6	8.5	36.7
Ephemeroptera			1.9			37.1	48.1	27.1	26.8	18.1	12.9		50.0	77.8	50.7	10.6	50.0	73.9	14.0	9.5
Plecoptera										5.9	66.7		8.7		0.4	0.6		4.0	0.5	
Coleoptera			1.3			2.9	7.4				1.2		1.9		0.4			0.9	0.2	
Odonata			1.3	17.5	0.2	8.6		5.6	0.3				1.9	0.2				2.7		2.4
Heteroptera																				
Megaloptera																				
Collembola																				
C.Bivalvia																				
C.Gastropoda	0.4		1.3		0.1						5.0		4.8	8.8	14.1					
Amphipoda	96.6	89.5	45.3	1,3	97.8	11.4	3.7	11.2		2.0								0.4		
Isopoda				10,0			1.2			3.5				0.6	0.4	1.3				
C.Polichaeta																				
Nematoda																				
Hemiptera																				
C.Aracnidae																				
Tricladida			0.6																	
Adults ins.	0.4		6.3	3.8	0.9	2.9		0.0	0.8	1.6				0.2				1.8	0.2	0.6
Pisces		5.3		1.3	0.1			0.9			0.3		1.0							
NI			5.0	1.3			1.2	15.0			0.3					1.3		0.4		0.6

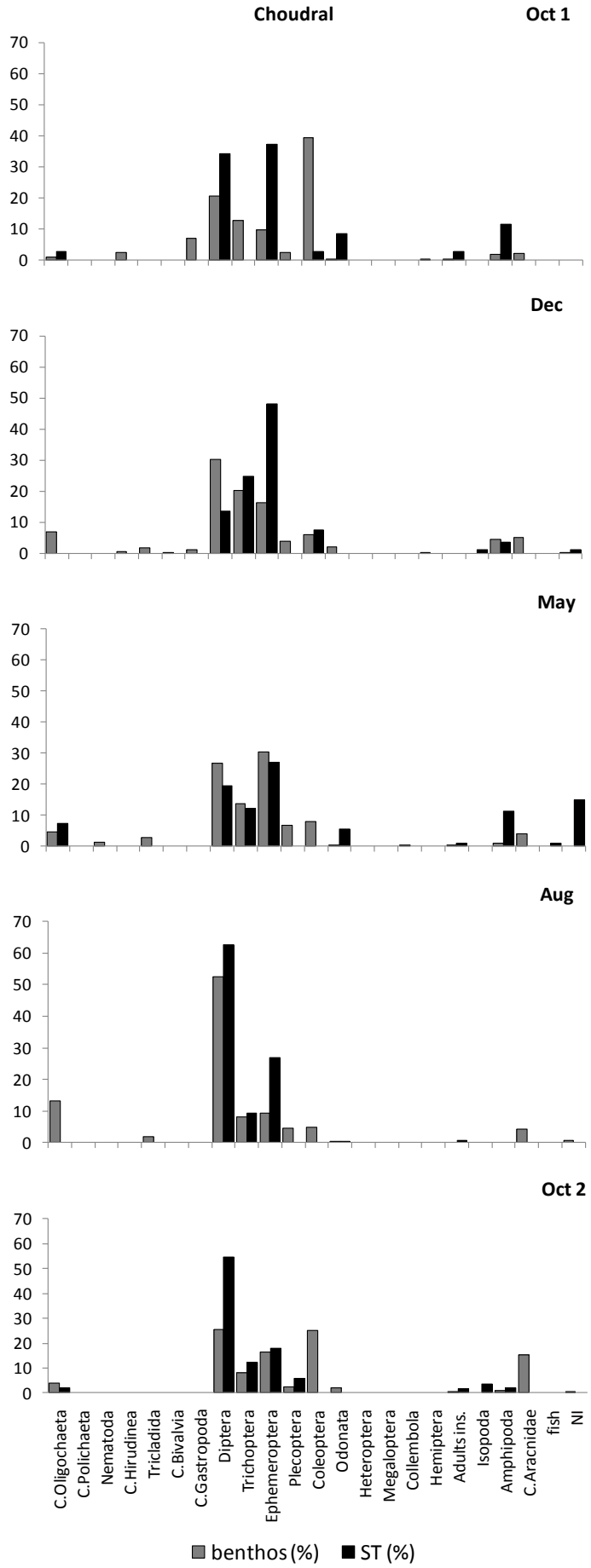
Table 5.5. The Occurrence index (O_I) of the eel feeding in Rio Esva across study sites and seasons.

	Estuary					Choudral					Chanona					Viella				
	Oct 1	Dec	May	Aug	Oct 2	Oct 1	Dec	May	Aug	Oct 2	Oct 1	Dec	May	Aug	Oct 2	Oct 1	Dec	May	Aug	Oct 2
C.Oligochaeta	5.6		43.3	12.5	11.1	2.8		13.8		15.8			10.0	2.7		3.3		4.3		10.3
C.Hirudinea			3.3																	
Diptera		2.7	30.0	15.6	11.1	11.1	33.3	34.5	54.3	73.7	12.9		20.0	21.6	31.3	33.3	11.5	17.4	41.9	20.7
Trichoptera	8.3		16.7	21.9	3.7		26.7	17.2	48.6	52.6	29.0		26.7	32.4	25.0	33.3	11.5	47.8	45.2	34.5
Ephemeroptera			6.7			8.3	23.3	27.6	54.3	47.4	22.5		23.3	51.4	62.5	16.7	15.4	43.5	48.4	27.6
Plecoptera										15.8	16.1		6.7		3.1	3.3		8.7	6.5	
Coleoptera			6.7			2.8	3.3				3.2		3.3		3.1			4.3	3.2	
Odonata			6.7	18.8	7.4	2.8		10.3	2.9				6.7	2.7				21.7		13.8
Heteroptera																				
Megaloptera																				
Collembola																				
C.Bivalvia																				
C.Gastropoda	2.8		3.3		3.7						16.1		3.3	37.8	43.8					
Amphipoda	58.3	21.6	30.0	3.1	92.6	2.8	3.3	13.8		10.5								4.3		
Isopoda				9.4			3.3			10.5				2.7	3.1	3.3				
C.Polichaeta																				
Nematoda																				
Hemiptera																				
C.Aracnidae																				
Tricladida			3.3																	
Adults ins.	2.8		13.3	9.4	7.4	2.8		3.4	5.7	15.8				2.7				8.7	3.2	3.4
Pisces		2.7		3.1	3.7			3.4			3.2		3.3							
NI			20.0	3.1			3.3	20.7			3.2					6.7		4.3		3.4

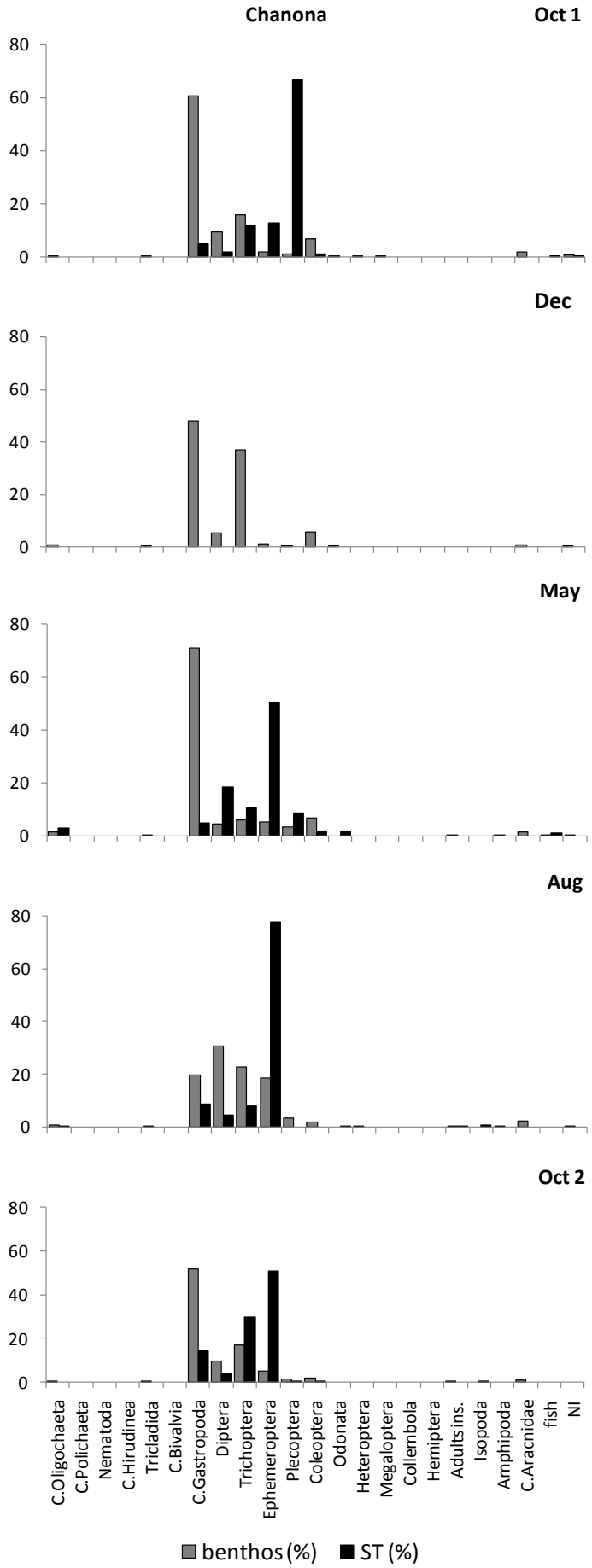
(A)


Estuary
Oct 1
Dec
May
Aug
Oct 2
100
80
60
40
20
0
C.Oligochaeta
C.Polichaeta
Nematoda
C.Hirudinea
Tricladida
C.Bivalvia
C.Gastropoda
Diptera
Trichoptera
Ephemeroptera
Plecoptera
Coleoptera
Odonata
Heteroptera
Megaloptera
Collembola
Hemiptera
Adults ins.
Isopoda
Amphipoda
C.Aracnidae
fish
NI
benthos (%)
ST (%)

(B)


Choudral
Oct 1
Dec
May
Aug
Oct 2
70
60
50
40
30
20
10
0
C.Oligochaeta
C.Polichaeta
Nematoda
C.Hirudinea
Tricladida
C.Bivalvia
C.Gastropoda
Diptera
Trichoptera
Ephemeroptera
Plecoptera
Coleoptera
Odonata
Heteroptera
Megaloptera
Collembola
Hemiptera
Adults ins.
Isopoda
Amphipoda
C.Aracnidae
fish
NI
benthos (%)
ST (%)

(C)


Chanona
Oct 1
Dec
May
Aug
Oct 2
80
60
40
20
0
C.Oligochaeta
C.Polichaeta
Nematoda
C.Hirudinea
Tricladida
C.Bivalvia
C.Gastropoda
Diptera
Trichoptera
Ephemeroptera
Plecoptera
Coleoptera
Odonata
Heteroptera
Megaloptera
Collembola
Hemiptera
Adults ins.
Isopoda
Amphipoda
C.Aracnidae
fish
NI
benthos (%)
ST (%)

(D)

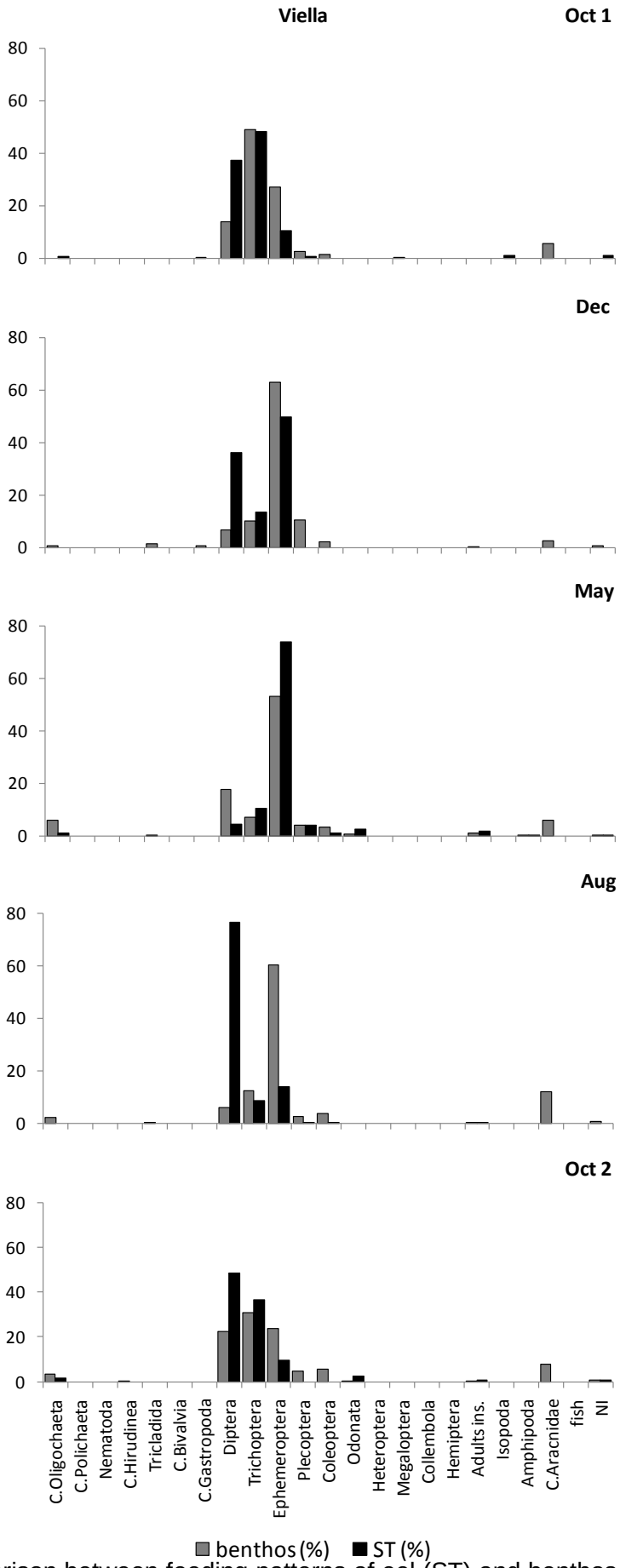


Figure 5.2. Comparison between feeding patterns of eel (ST) and benthos composition (in percentage) for each site and season. (A) Estuary, (B) Choudral, (C) Chanona and (D) La Viella. No sample for eels at Chanona in December. (NI = non-identified)

Discussion

The spatial segregation of eel size recorded in this study fully coincide with previous studies on this very same eel stock: smaller, younger individuals predominate on downstream sections; larger (older) individuals predominate upstream whereas the estuary is inhabited by all size and ages (Lobón-Cerviá *et al.* 1995, Lobón-Cerviá & Iglesias 2008). Consequently, our samples are actually representative of the Río Esva eel stock.

The feeding activity index (A_I) revealed that eels were feeding the year round and with the only exceptions of Choudral in the first autumn, and Estuary and La Viella in winter, the values of A_I were always >50%. These results are consistent with a previous study on the feeding activity patterns of the Rio Esva eels (Chapter 4) where a minimum value of A_I of 34% was observed in October, and values of A_I over the mean winter value of 45% during the rest of the year. The only obvious difference detected in this study concerns the first and the second autumn at Choudral where values of A_I attained 25 and 84%, respectively. This was probably caused by different environmental/climatic conditions between the two years that could not be captured in our analysis. Interestingly however, mean annual values were quite similar with A_I = 61 to 63% for the four study sites.

Considering feeding intensity (I_I), an I_I<2% was calculated for most cases, which is concurrent with the I_I values reported in Chapter 4 where monthly means varied between 0.65 and 1.49%. As evidenced by previous (Rincón & Lobón-Cerviá 1997) and the present study, eel in this river prey mainly upon small sized species or life stages. Thus, low I_I values are to be expected even for well feed individuals.

The composition of the benthos assemblages both in taxa diversity and their relative contribution in density differed among sites and varied along the year. Simultaneous diet analysis revealed that aquatic invertebrates were the main prey at all sites and seasons reflecting an omnivorous behaviour. Clearly, this pattern reinforces the commonly accepted description of the European eel as an omnivorous species which is furthermore capable to feed opportunistically depending on prey availability. However, several abundant taxa appeared unedible or at least do not form part of the eel diet. For example, although Gastropoda was always very abundant in Chanona, only appeared scattered in the eel's diet. It is suggested that most likely, given the high abundance of other accompanying abundant and edible species and the potentially difficult digestion of their shells, gastropods were not an intended prey but ingested while feeding upon other taxa.

Disregarding probably unedible taxa as Aracnidae or Gastropoda, and thus potentially less appealing to be eaten, numeric and occurrence indices revealed a considerable list of prey making up eels diet. Given a set of prey, eel seems to feed primarily on most common taxa revealing occasionally preference for particular taxa. This may be caused by the presence of bigger and thus more appealing prey, such as some large Tricoptera or Odonata, or to the benthos patchiness, such as occurs with some Diptera that may aggregate according to preferred positions relative to river flow.

Prior to this study, Esva eel stock was at low density. However in recent years eel has shown high recovery rates with marked increases in density (Lobón-Cerviá & Iglesias 2008). Given that there are no apparent diet restrictions and the local high secondary production (Rincón & Lobón-Cerviá 1997), Esva eel stock does appear to have potential for recovery. Detailed studies on benthos, particularly allowing for determination of aquatic invertebrates' production, and thus energy availability, could provide further insight into the potential to further increases in eel densities within Esva basin to attain the high densities recorded during the 80ies.

WRAPPING UP

Chapter 6. Food consumption and energy balance

Acknowledgments for this chapter

As for all other chapters, financial support was given by the Portuguese Foundation for Science and Technology - FCT (S.C.D., grant SFRH/BD/16922/2004).

Food consumption and energy balance

Along the previous chapters, some aspects of the European eel, *Anguilla anguilla* L., ecology were looked into and its trophic dynamics in Río Esva (Cantabrian corridor) deserved great attention. Eel is a generally declining species (Chapter 1) with expected recruitment inflow into the cantabric area within the overall pattern (Chapter 2). The study area was a near pristine river, but not free of the parasitic nematode *Anguillicoloides crassus* (Chapter 3). Nevertheless, Esva stock does not show severe local impacts (neither anthropogenic nor due to *A. crassus*) for the time being.

The yellow eel stage, a crucial growing period mainly occurring in inland waters, depends on the carrying capacity of the system which determines stock maintenance. A critical factor to support a stock increase is the potential for energy acquirement. Most literature reports feeding behavior in captivity, in significantly altered systems, or regarding stocks attaining mean lengths much higher than Esva stock. This subject was considered in Chapter 4, where eel is described as actively feeding all day long, a diel cycle, and all year round, an annual cycle. Although feeding activity was not different on diel cycle analyses, increased values were found in the warmer months. Furthermore, the intensity of feeding along both of those cycles was in general low, mainly attributed to small-sized prey items. On Chapter 5, a further insight on throphic behavior of eel in Esva revealed its opportunistic feeding behavior by mainly eating the most abundant benthic macro-invertebrates at each site and season.

Furthermore, eel's food consumption allows for energy acquirement assessment. In order to estimate the energy potentially available for eel in the form of benthic prey, the work of Cummins and Wuycheck (1971) was used as a reference for invertebrates' caloric value. Although concerning different species of the same taxonomic groups, this procedure is a proxy to Esva species allowing for an estimation of eel's daily energy acquirement. The benthic assemblages of the study sites were analyzed in Chapter 5 with relative densities (%) elucidated on figure 5.2 while table 5.2 shows the density (individuals.m^{-2}) of the top three most abundant taxa across sites and seasons. Data relating to May of 2007 were used to estimate the caloric value (calories per gram of wet weight) of the main prey taxa for the four sites (Estuary, Choudral, Chanona and La Viella). From the most abundant benthic taxonomic groups, as assessed in the previous chapter, Ephemeroptera has the highest energy content, 1124 cal. g wet wgt^{-1}, whereas Mollusca has the lowest, 480 cal. g wet wgt^{-1}. Furthermore, the caloric value available for eels per area (cal. g wet $wgt^{-1}.m^{-2}$) was estimated according to benthos biomass

(unpublished data) of the most important species (until a total of >90% was achieved) across sites (table 6.1).

Table 6.1. Caloric values per area (cal. g wet $wgt^{-1}.m^{-2}$) according to taxonomic category across sites in May of 2007.

	Estuary	Choudral	Chanona	Viella
C.Oligochaeta	322	35	30	39
C.Gastropoda	1134		30931	
Diptera	1187	201	84	119
Trichoptera	4367	2089	2326	997
Ephemeroptera	3251	3540	1626	5599
Plecoptera		150	198	82
Coleoptera	193	136	298	52
Odonata				28
Amphipoda	3298	59	59	20
Total	13753	6208	35550	6936

It is thus apparent that benthos assemblage at Chanona has a much higher caloric value than the other three sites, with close values at Choudral and Viella. Nonetheless, the total caloric value for benthos at Chanona was largely attained by the contribution of Gastropoda, calculated on the basis of the value reported by Cummins & Wuycheck (1971) for Mollusca. Taken into consideration that Mollusca were not a common prey, the total caloric value available as prey in benthos would then have comparable values at Choudral, Chanona and Viella, and a substantially higher value at the Estuary.

Furthermore, most relevant benthic species for eel diet were discussed on Chapter 5 providing a quantitative measure of taxa preyed upon by eel. Based on the items identified from eel's stomach content, and considering the stomach content as equivalent to one day feeding, the mean caloric value ingested per day (cal. g wet $wgt^{-1}.day^{-1}$) was estimated for each site during May of 2007. Considering only eels actively feeding, around 20 to 25 calories were ingested per eel at the Estuary and Choudral, 35 at Chanona and

80 at La Viella. This latter value seems to result from the high weight Ephemeroptera had on eels' diet.

Additionally, from the balanced energy equation from Wooten (1990), food consumption (C) relates to species use of energy as:

$$C = P + R + F + U$$

where P is production (growth and reproduction), R is metabolism, F is fecal wastes, and U is excretory wastes.

For the period eel remains in continental waters, and particularly for the Esva stock lengths, production mainly concerns growth rather than gonads development. Previous studies like Lobón-Cerviá *et al.* (1995) give an insight on eel productivity in Esva according to eel's density, but if C is considered in terms of prey caloric value, eel productivity might also be estimated from the above equation.

Regarding R, eel metabolism has been reported as low in comparison with other teleost fishes and Iversen et al. (2010) reviewed values for standard metabolic rate ranging between 16.8 and 63.6 µmol O_2. kg^{-1}. min^{-1} but corresponding to different temperatures and body mass. Preliminary results from experimental work carried out with different size classes of eels <20cm and at temperatures of 5 – 20ºC (unpublished data) also point out to low values of R but need further analysis.

Next step would then be to fill in the gaps identified and if stock biomass or local densities are determined the total energy consumed by the stock might me estimated. According to Lammens et al. (1985), the daily maintenance energy requirements of eel population is 0.8% of the eel biomass in terms of fresh weight, with an assimilation efficiency of 80%. Being so, maximum productivity could also be estimated.

Final comments

In the light of the subjects discussed, definition of a management plan for eel would theoretically be able to aim viable stock increases for a given area. Nevertheless, if eel stock increases, as aimed at the European level, how would that affect other species? How would that affect the ecosystem and the energy flow through the food web? Answering those questions with knowledge on the ecosystem functioning is by far a difficult task, but may lead us to the point of defining management strategies that take into consideration the all system instead of a species oriented management plan.

For Río Esva most of those considerations on stock management are somewhat pointless since no commercial fishery occurs for any of the life stages (glass, yellow or silver eel). On the contrary, understanding how environmental constrains in a natural population inhabiting a well preserved river shapes eel stock is priceless.

Main accompanying fish species in Esva are the salmonids *Salmo salar* and *Salmo trutta*, feeding close to the water surface and thus not representing feeding competitors. On the contrary, if an increase on eel density led to a decrease on benthic macro-invertebrates on which aquatic insects are included, salmonids feeding resources could be affected.

Accordingly, eels would be limited by benthos productivity. Rincón & Lobón-Cerviá (1997) reported high rates of secondary production but eel stock in Esva has been decreasing over last decades with a slight recovery in recent years (Lobón-Cerviá & Iglesias 2008). Given historical eel and benthos production values and nowadays benthos productivity it is expected that, if recruitment increases, eel stock in Esva has conditions for recovery.

Nevertheless, if feeding conditions proved not to be a limiting factor, other environmental constraints must be considered. As enlighten in previous chapters, Esva eels mature at small sizes and mostly into males. Due to the small dimensions of the river and its tributaries a hypothesis would then be that the habitat available for growing eels is somewhat limited for the bigger sizes (and thus for females). As so, the maximum number of silver eels per year leaving Esva for spawning would be mostly environmentally determined and stock increases would only increase the number of reproducers to a given plateau with a small percentage of females.

REFERENCES

Acou A. 2006. Bases biologiques d'un modéle pour estimer la biomasse féconde de l'anguille européenne en fonction des recrues fluviales et du contexte de croissance: approche comparative a` l'échelle de petits bassins versants. PhD. thesis. Rennes: University of Rennes 1. 316pp.
URL http:// www.diadfish.org/francais/documents_f.htm.

Acou A., Robinet T., Lance E., Gerard C., Mounaix B., Brient L., Le Rouzic B. & Feunteun E. 2008. Evidence of silver eels contamination by microcystin-LR at the onset of their seaward migration: what consequences for breeding potential? Journal of Fish Biology, 72: 753–762.

Aguilar A., Álvarez M.F., Leiro J.M. & Sanmartin M.L. 2005. Parasite populations of the European eel (*Anguilla anguilla* L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). Aquaculture 249: 85–94.

Allan J.D., Abell R., Hogan Z., Revenga C., Taylor B.W., Welcomme R.L. & Winemiller K. 2005. Overfishing of Inland Waters. BioScience 55: 1041-1051.

Antunes C. 1999. *Anguillicola* infestation of eel population from the Rio Minho (North of Portugal). ICES-EIFAC Working Group on Eel, 20-24 September, Silkeborg, Denmark.

Antunes J. C. 2002. Monitoring of glass eels recruitment in Portugal. In. Monitoring of glass eels recruitment. (Dekker, W. ed), pp. 219-226. Netherlands Institute of Fisheries Research, Ijmuiden, Report C007/02-WD.

Baisez A. & Laffaille P. 2005. Un outil d'aide à la gestion de l'anguille: le tableau de bords anguille du bassin Loire. Bulletin Français de Pêche Piscicole, 378-379: 115-130.

Batty J., Pain D. & Caurant F. 1996. Metal concentrations in eels *Anguilla anguilla* from the Camargue region of France. Biological Conservation 76: 17-23.

Bardonnet A., Dasse S., Parade M. & Heland M. 2003. Influence de l´alternance jour/nuit sur les desplacements de civelles en fluvarium. Bulletin Français de la Pêche et de la Pisciciculture 368: 9-20.

Bardonnet A. & P. Riera 2005. Feeding of glass eels (*Anguilla anguilla*) in the course of their estuarine migration: new insights from stable isotope analysis. Estuarine, Coastal and Shelf Science 63: 201–209.

Béguer M., Beaulaton L. & Rochard E. 2007. distribution and richness of diadromous fish assemblages in Western Europe: large-scale explanatory factors. Ecology of Freshwater Fish 16: 221-237.

Belpaire C. & Goemans G. 2007. Eels: contaminant cocktails pinpointing environmental contamination. ICES Journal of Marine Science 64: 1423-1436.

Bergersen R. & Klemetsen A. 1988. Freshwater Eel *Anguilla anguilla* (L.) from North Norway, with Emphasis on Occurrence, Food, Age and Downstream Migration. Nordic J. Fresw. Res. 64: 54-66.

Blanc G. 1989. L'anguillicolose. Répartition géographique. Mise au point d'une méthode de diagnostic par échotomographie. Moyens de lutte. Thèse vétérinaire ENV Nantes.

Blanco J.C. & Gonzalez J.L. 1992. Libro rojo de los vertebrados de España. Ministerio de Agricultura Pesca y Alimentacion Madrid & Instituto Nacional para la Conservacion de la Naturaleza, ICONA, Madrid.

Boëtius J. 1976. Elvers, *Anguilla anguilla* and *Anguilla rostrata* from two Danish localities. Size, body mass, developmental stage and number of vertebrae related to time of ascent. Meddelelser fra Danmarks Fiskeri-Og Havundersogelser 7: 199-220.

Boëtius I. & Boëtius J. 1989. Ascending elvers, *Anguilla anguilla*, from five European localities. Analyses of pigmentation stages, condition, chemical composition and energy reserves. Dana 7: 1-12.

Bonhommeau S., Chassot E. & Rivot E. 2008. Fluctuations in European eel (*Anguilla anguilla*) recruitment resulting from environmental changes in the Sargasso Sea. Fisheries Oceanography 17: 32-44.

Briand C., Fatin D. & Legault A. 2002. Role of Eel Odour on the Efficiency of an eel, *Anguilla anguilla*, Ladder and Trap. Environmental Biology of Fishes 65: 473-477.

Briand C., Fatin D., Cicotti E. & Lambert, P. 2005. A stage-structure model to predict the effect of temperature and salinity on glass eel *Anguilla anguilla* pigmentation development. Journal of Fish Biology 67: 993-1009.

Bruslé J. 1994. L'anguille européenne *Anguilla anguilla*, un poisson sensible aux stress environnementaux et vulnérable à diverses atteintes pathogènes. Bulletin Français de Pêche Piscicole 335: 237-262.

Bureau du Colombier S., Bolliet V., Lambert P. & Bardonnet, A. 2007. Energy and migratory behavior in glass eels (*Anguilla anguilla*). Physiology & Behavior 92: 684-690.

Bush A.O., Lafferty K.D., Lotz J.M. & Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al, revisited. Journal of Parasitology 83 (4): 575-583.

Cabral M.J., Almeida J., Almeida P.R., Dellinger T., Ferrand de Almeida N., Oliveira M.E., Palmeirim J.M., Queiroz A.I., Rogado L. & Santos-Reis M. (eds.) 2005. Livro Vermelho dos Vertebrados de Portugal. Instituto de Conservação da Natureza, Lisboa.

Callaghan R.M. & McCarthy T.K. 1994.The Food and Feeding of Eels (*Anguilla anguilla* L.) in an Irish River System. EIFAC Working Party on Eel, Oviedo, Spain.

Casal C.M.V. 2006. Global documentation of fish introductions: the growing crisis and recommendations for action. Biological Conservation 8: 3-11.

Castonguay M., Hodson P.V., Moriarty C., Drinkwater K.F. & Jessop B. M. 1994. Is there a role of ocean environment in American and European eel decline? Fisheries Oceanography 3: 197-203.

Clarke D.A., McCarthy T.K. & Cowx I. 1993. Foraging activity patterns and diets of yellow eels *Anguilla anguilla* (L.) in Lough Derg. EIFAC 8th Session, Olsztyn, Poland.

Costa M.J., Vasconcelos R., Costa J.L. & Cabral H.N. 2007. River flow influence on the fish community of the Tagus estuary (Portugal). Hydrobiologia 587: 113-123.

Costa-Dias S. & Lobón-Cerviá J. 2008. Diel feeding activity and intensity during an annual cycle of the European eel *Anguilla anguilla* (L.) in a Cantabrian stream. Knowledge and Management of Aquatic Ecosystems 390-391 (01): 1-9.

Costa-Dias S., Sousa R., Lobón-Cerviá J. & Laffaille P. 2009. The Decline of Diadromous Fish in Western European Inland Waters: Main Causes and Consequences. In Fisheries: Management, Economics and Perspectives (ed. McManus, N.F. and Bellinghouse, D.S.), 23 pp. Nova Science Publishers, Inc, NY.

Costa J.L., Assis C.A., Almeida P.R., Moreira F.M. & Costa M.J. 1992. On the food of the European eel, *Anguilla anguilla* (L.), in the upper zone of the Tagus estuary, Portugal. J. Fish Biol. 41: 841-850.

Couillard C.M., Hodson P.V. & Castonguay M. 1997. Correlations between pathological changes and chemical contamination in American Eels, *A. rostrata*, from the St Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences 54(8): 1916-1927.

Cullen P. & McCarthy T.K., 2007. Eels (*Anguilla anguilla* (L.)) of the lower River Shannon, with particular reference to seasonality in their activity and feeding ecology. Biology and Environment: Proceedings of the Royal Irish Academy 107B (2): 87-94.

Cummins K.W. & Wuycheck J.C. 1971. Caloric equivalents for investigations in ecological energetic. Int. Ver. Theor. Angew Limnol. Suppl. 18: 1-158.

de Casamajor M.N., Prouzet P. & Lazure P. 2000. Identification des flux de civelles (*Anguilla anguilla*) à partir des relations d´allométrie en fonction des conditions hydrodynamiques de l´estuarie de l´Adour. Aquatic Living Resources 13: 411-420.

de Casamajor M. N., Lecomte-Finiger R. & Prouzet P. 2003. Caracteristiques biologiques des civelles (*Anguilla anguilla*) lors de la transition en estuaire. Bulletin Français de la Pêche et de la Pisciciculture 368: 109-124.

De Charleroy D., Grisez L., Thomas K., Belpaire C. & Ollevier F. 1990 The life cycle of *Anguillicola crassus*. Diseases of Aquatic Organisms 8: 77–84.

De Nie H.W. 1982. A note on the significance of larger bivalve molluscs (*Anodonta* spp. and *Dreissena* sp.) in the food of the eel (*Anguilla anguilla*) in Tjeukemeer. Hydrobiol. 95: 307-310.

De Nie H.W. 1987. Food, feeding periodicity and consumption of the eel *Anguilla anguilla* (L.) in the shallow eutrophic Tjeukemeer (The Netherlands). Arch. Hydrobiol. 109(3): 421-443.

Deelder C.L. 1984. Synopsis of biological data on the eel *Anguilla anguilla* (Linnaeus, 1758). FAO Fisheries Synopsis, Nº 80 (Revision 1): i-vii: 1-73.

Dekker W. 2003. Status of the European eel stock and fisheries. In Eel Biology (Aida K., Tsukamoto K. & Yanauchi K., eds), pp 237-254. Tokyo, Springer-Verlag.

Denayer B. & Belpaire C. 1996. Bottle-necks for restoration of the eel population, *Anguilla anguilla* (L.) of the river Yser basin (Flanders). Archives of Polish Fisheries, 4: 175-186.

Desaunay Y. & Guerault D. 1997. Seasonal and long-term changes in biometrics of eel larvae: a possible relationship between recruitment variation and North Atlantic ecosystem productivity. Journal of Fish Biology 51 (Suppl. A): 317-339. doi:10.1111/j.1095-8649.1997.tb06106.x

De Charleroy D., Grisez L., Thomas K., Belpaire C. & Ollevier, F. 1990. The lyfe cycle of *Anguillicola crassus*. Diseases of Aquatic Organisms 8: 77-84.

Didžiulis V. 2006. NOBANIS – Invasive Alien Species Fact Sheet – *Anguillicola crassus* – From: Online Database of the North European and Baltic Network on Invasive Alien species – NOBANIS www.nobanis.org.

Doadrio I. (Ed.) 2001. Atlas y Libro Rojo de los Peces continentales de España. Dirección General de Conservación de la Naturaleza - Museo Nacional de Ciencias Naturales. Xx pp.

Durbin A.G., Nixon S.W. & Oviatt C.A. 1979. Effects of the spawning migration of the alewife, Alosa pseudoharengus, on freshwater ecosystems. Ecology 60: 8-17.

EELREP 2005. Estimation of the reproduction capacity of European eel. Final report. http://www.fishbiology.net/eelrepsum.html

Elie P. 1979. Contribution à l'étude des montées de civelles d'*Anguilla anguilla* L. dans l'estuaire de la Loire : Pêche, écophysiologie et èlevage. Thèse de 3eme Cycle Universitè Rennes I, 383 pp.

Elie P., Lecomte-Finiger R., Cantrelle I. & Charlon N. 1982. Définition des limites des différentes stades pigmentaires durant la phase civelle d'*Anguilla anguilla* L (Poisson, Téléostéen, Anguilliformes).Vie et Mileu 32 : 149-157.

Elie P., Taverny C., Mennesson-Boisneau C. & Sabatié M.R. 2000. L'exploitation halieutique. In: Baglinière J.L. & Elie P. (eds.), Les Aloses (*Alosa alosa* et *Alosa fallax* spp.). INRA, Paris, pp. 199-226.

Elliott J.M., Lyle A.A. & Campbell R.N.B. 1997. A preliminary evaluation of migratory salmonids as vectors of organic carbon between marine and freshwater environments. The Science of the Total Environment 194/195: 219-223.

Esteve C. & Alcaide E. 2009. Influence of diseases on the wild eel stock: The case of Albufera Lak. Aquaculture 289: 143-149.

FAO 1996. Technical guidelines for responsible fisheries / Food and Agricultural Organization of the United Nations, Rome.

Feunteun E. 2002. Management and restoration of European eel population (Anguilla anguilla): An impossible bargain. Ecological Engineering 18: 575-591.

Flecker A.S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology 77: 1845-1854.

Friedland K.D., Miller M.J. & Knights B. 2007. Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. ICES Journal of Marine Science 64: 519-530.

Gandolfi G., Pesaro M. & Tongiori P. 1984. Environmental factors affecting the ascent of elvers, *Anguilla anguilla* (L.), in to the Arno river. Oebalia 10: 17-35.

Gandolfi-Hornyold A. 1918. Algunas medidas de las angulas de Santander. Boletín de Pescas 21: 153-192.

Gandolfi-Hornyold A. 1920. Algunas observaciones sobre la angula en Palma de Mallorca. Boletín de Pescas 41-42-43: 1-128.

Gandolfi-Hornyold A. 1929. Mensurations de civelles de l'Oria pendant la saison de pêche 1928-29. Bulletin de la Société d'Océanographie de France 49: 897-908.

Gandolfi-Hornyold A. 1930a. Mensurations de civelles de Mex pêchées en mars 1928, près d'Alexandrie. Bulletin de la Société d'Océanographie de France 51: 936-940.

Gandolfi-Hornyold A. 1930b. Mensurations de civelles de l'Oria pendant les saisons de pêches 1928-1929 et 1930-1931. Bulletin de la Société d'Océanographie de France 56: 1012-1023.

Gandolfi-Hornyold A. 1936. La civelle d'Aguinaga de 1935 à 1936. Bulletin de la Société d'Océanographie de France 92: 1597-1599.

Granado-Lorencio C. 1991. The effect of man on the fish fauna of the River Guadalquivir, Spain. Fisheries Research 12: 91-100.

Gregory S.V. & Bisson P.A. 1997. Degradation and loss of anadromous salmonid habitat in the pacific Northwest. In: Stouder D.D.J., Bisson P.A. & Naiman R.J. (Eds.) Pacific salmon and their ecosystems: status and future options. Chapman & Hall, New York, pp 277-314.

Hodson P.V., Castonguay M., Couillard C.M., Desjardins C., Pelletier E. & McLeaod R. 1994. Spatial and temporal variations in chemical contamination of American eels,

Anguilla rostrata, captured in the estuary of the St Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences 51: 464-478.

Hussein S.A. 1981. The population density, growth and food of eels *Anguilla anguilla* L. in some tributaires of the river Tweed. Proceedings 2nd Britain Freshwater Fisheries Conference: 120-128.

ICES 2006. Report of the Joint EIFAC/ICES Working Group on Eels. ICES Document CM 2006/ACFM 16. 352 pp..

ICES 2008. Report of the Joint EIFAC / ICES Working Group on Eels. EIFAC Occasional Paper nº 43. ICES Document CM 2009 / ACOM 15. 192p.

ICES *in press*. Report of the Joint EIFAC / ICES Working Group on Eels. EIFAC Occasional Paper.

IFREMER – www.ifremer.fr.

INDICANG, the newsletter of - Nov/2004, IFREMER – www.ifremer.fr.

Jones M.J. & Stuart I.G. 2008. Regulated floodplains – a trap for unwary fish. Fisheries Management and Ecology 15: 71-79.

Jones C.G., Lawton J.H. & Shachak M. 1994. Organisms as ecosystem engineers. Oikos 69: 373-386.

Jones C.G., Lawton J.H. & Shachak, M. 1997. Positive and negative effects of organisms as physical ecosystem engineers. Ecology 78: 1946-1957.

Jonsson B., Waples R.S. & Friedland K.D. 1999. Extinction considerations for diadromous fishes. ICES Journal of Marine Science 56: 405-409.

Kennedy C.R. 2007. The pathogenic helminth parasites of eels. Journal of Fish Diseases 30: 319-334.

Kettle A.J. & Haines K. 2006. How does the European eel (*Anguilla anguilla*) retain its population structure during its larval migration across the Northern Atlantic Ocean? Canadian Journal of Fisheries and Aquatic Sciences 63: 90-106.

Kirk R.S. 2003. The impact of *Anguillicola crassus* on European eels. Fisheries Management and Ecology 10: 385-394.

Kirk R.S., Morritt D., Lewis J.W. & Kennedy C.R. 2002. The osmotic relationship of the swimbladder nematode *Anguillicola crassus* with seawater eels. Parasitology 124: 339-347.

Knights B. 2003. A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. The Science of the Total Environment 310: 237-244.

Knopf K. 2006. The swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla* and the Japanese eel *Anguilla japonica*: differences in susceptibility

and immunity between a recently colonized host and the original host. Journal of Helminthology 80: 129-136.

Knopf K., Würtz J., Sures B. & Taraschewski H. (1998). Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. Diseases of Aquatic Organisms 33: 143-149.

Korta M. & Díaz E. 2008. Report on the Eel Stock and Fishery in Spain. AZTI – Tecnalia, 29 pp. Available at:(http://www.azti.es).

Krokhin E.M. 1975. Transport of nutrients by salmon migration from the sea to lake. In: Hasler A.D. (ed.) Coupling of land and water systems. Springer-Verlag, New York, pp. 153-156.

Laffaille P., Feunteun E., Acou A. & Lefeuvre J.C. 2000. Role of European eel (*Anguilla anguilla* L.) in the transfer of organic matter between marine and freshwater systems. Verh. Internat. Verein. Limnol. 27: 616-619.

Laffaille P., Brosse S., Feunteun E., Baisez A. & Lefeuvre J.C. 1998. Role of fish communities in particulate organic matter fluxes between salt marshes and coastal marine waters in the Mont Saint-Michel bay. Hydrobiologia 373/374: 121-133.

Lambert P., Sabih M., Rochard E., Marchelidon J., Dufour S. & Elie P. 2003. Variabilite morphlogique et du taux d'hormone de croissance des civelles d'anguilles europeennes (*Anguilla anguilla*) dans l'estuaire de la Gironde au cours de la saison 1997-1998. Bulletin Français de la Pêche et de la Pisciciculture 368: 69-84.

Lammens E.H.R.R., Nie H.W., Vijverberg J. & van Densen W.L.T. 1985. Resource partitioning and niche shifts of bream (*Abramis brama*) and eel (*Anguilla anguilla*) mediated by predation of smelt (*Osmerus eperlanus*) on *Daphnia hyalina*. Canadian Journal of Fisheries and aquatic Sciences 42 (8): 1342 – 1351.

Lara M.J. 1994. Feeding of eels (*Anguilla anguilla* L.) in the Narcea basin (Asturias, NW Spain). EIFAC Working Party on Eel, Oviedo, Spain.

Lara M. J. 1994. Catch statistics, capture methods, size, and development stages of glass eels in Asturias (northwestern Spain). Bulletin of the Sea Fisheries Institute 1: 31-39.

Lasne E., Lek S. & Laffaille P. 2007b. Patterns in fish assemblages in the Loire floodplain: the role of hydrological connectivity and implications for conservation. Biological Conservation 139: 258-268.

Lecomte-Finiger R. 1983. Régime alimentaire des civelles et anguillettes (*Anguilla anguilla*) dans trois étangs saumâtres du Roussillon. Bull. Ecol. 14: 297-306.

Lecomte-Finiger R., Desaunay Y., Guerault D. & Grellier P. 1996. The inmigration of *Anguilla anguilla* (L.) glass eels in coastal waters: questions about the determinism of the otolith structures. Archives of Polish Fisheries 4: 235-243.

Lefèbvre F., Mounaix B., Poizat G. & Crivelli A.J. 2004. Impacts of the swimbladder nematode *Anguillicola crassus* on *Anguilla anguilla*: variations in liver and spleen masses. Journal of Fish Biology 64: 435-447.

Lefeuvre J.C., Laffaille P. & Feunteun, E. 1999. Do fish communities function as biotic vectors of organic matter between salt marshes and marine coastal waters? Aquatic Ecology 33: 293-299.

Linde A.R., Sánchez-Galán S., Klein D., Garcia-Vázquez, E. & Summer K.H. 1999. Metallothionein and Heavy Metals in Brown Trout (Salmo trutta) and European Eel (*Anguilla anguilla*): A Comparative Study. Ecotoxicology and Environmental Safety 44: 168-173.

Lobón-Cerviá J. 1999. The decline of eel *Anguilla anguilla* (L.) in a river catchment of northern Spain 1986-1997. Further evidence for a critical status of eel in Iberian waters. Archiv für Hydrobiologie 144: 245-253.

Lobón-Cerviá J. & Iglesias T. 2008. Long-term numerical changes and regulation in a river stock of European eel *Anguilla anguilla*. Freshwater Biology 53: 1832-1844.

Lobón-Cerviá J. & Rincón P.A. 1998. Field assessment of the influence of temperature on growth rate in a brown trout population. Transactions of the American Fisheries Society 127: 718-728.

Lobón-Cerviá J., Utrilla C.G. & Rincón P.A. 1995. Variations in the population dynamics of the European eel *Anguilla anguilla* (L.) along the course of a Cantabrian river. Ecology of Freshwater Fish 4: 17-27.

Maes J., Stevens M. & Breine J. 2007. Modelling the migration opportunities of diadromous fish species along a gradient of dissolved oxygen concentration in a European tidal watershed. Estuarine Coastal Shelf Science 75: 151-162.

Marta P., Bochechas J. & Collares-Pereira M.J. 2001. Importance of recreational fisheries in the Guadiana River Basin in Portugal. Fisheries Management and Ecology 8: 345-354.

Minakawa N. & Gara R.I. 2003. Effects of chum salmon redd excavation on benthic communities in a stream in the Pacific Northwest. Transactions of the American Fisheries Society 132: 598-604.

Montgomery D.R., Buffington J.M., Peterson N.P., Schuett-Hames D. & Quinn T.P. 1996. Stream-bed scour, egg burial depth, and the influence of salmonid spawning on bed surface mobility and embryo survival. Canadian Journal of Fisheries and Aquatic Sciences 53: 1061-1070.

Moore J.W. 2006. Animal ecosystem engineers in streams. BioScience 56: 237-246.

Moore J.W., Schindler D.E. & Scheuerell M.D. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia 139: 298-308.

Moravec F. & Skoríková B. 1998. Amphibians and larvae of aquatic insects as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea), a swimbladder parasite of eels. Diseases of Aquatic Organisms 34: 217-222.

Moriarty C. 1982. Experiences in Eel management in Europe. Proceeding 1980' North American Eel Conference. Ontario Technical Reporting Series, 4: 56-65.

Moriarty C. 1987. The eel in Ireland. Royal Dublin Society, Occasional Papers in Irish Science and Technology, nº 4, 9p.

Moriarty C. & Dekker W. (Eds.) 1997. Management of the European eel. Fisheries Bulletin (Dublin) 15: 1-110.

Morrissey M. & McCarthy T.K. 2007. The ocurrente of *Anguillicola crassus* (Kuwahara, Niimi & Itagaki, 1974), an introduced nematode, in an unexploited Western Irish eel population. Biology and Environment: Proceedings of the Royal Irish Academy 107B (1): 13-18.

Neto A.F.G. 2008. Susceptibilidade da enguia-europeia (*Anguilla anguilla*) à degradação ambiental no estuário do Tejo: contaminação biológica pelo parasita *Anguillicola crassus* e contaminação química por metais pesados. Master thesis, Faculdade de Ciências, Universidade de Lisboa, 81p.

Neveu A. 1981. Variations saisonnières et journalières de l'alimentation de l'anguille (*Anguilla anguilla* L.) dans des conditions naturelles. Acta Oecol. - Oecol. Applic. 2(2): 99-116.

Northcote T.G. 1988. Fish in the structure and function of freshwater ecosystems: a 'top-down' view. Canadian Journal of Fisheries and Aquatic Sciences 45: 361-379.

Peters G. & Hartmann F. 1986. *Anguillicola*, a parasitic nematode of the swimbladder spreading among eel populations in Europe. Diseases of Aquatic Organisms 1: 229-230.

Poole W.R. 1994. A population study of the European Eel (*Anguilla anguilla* (L.)) in the Burrishoole System, Ireland, with special reference to growth and movement. PhD Thesis, Dublin University, Ireland, 416pp.

Power M.E. 1990. Resource enhancement by indirect effects of grazers: Armored catfish, algae, and sediment. Ecology 71: 897-904.

Rasmussen G. & Therkildsen B. 1979. Food, growth, and production of *Anguilla anguilla* L. in a small Danish stream. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 174: 32-40.

Rincón P.A. 1993. Utilización integrada de diferentes recursos: patrones en la alimentación y el uso del microhabitat de una población de Trucha común (Salmo *trutta* L.) en el Río Negro (Asturias). Tesis Doctoral. Departamento de Biologia Animal I. Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, 179 p.

Rincón P.A. & Lobón-Cerviá J. 1997. Temporal patterns in macroinvertebrate drift in a northern Spanish stream. Marine and Freshwater Research 48: 455-64.

Rochard E., Castelnaud, G. & Lepage, M. 1990. Sturgeon (Pisces: Acipenseridae); threats and prospects. Journal of Fish Biology 37: 123–132.

Rodrigues A.S.L., Pilgrim J.D., Lamoreux J.F., Hoffmann, M. & Brooks, T.M. 2006. The value of the IUCN Red List for conservation. Trends in Ecology and Evolution 21: 71-76.

Silva C.M.P., Freitas M.S.G. & Varela M.C. 1992. First report of *Anguillicola crassus* in the European eel in Portugal. Bulletin of the European Association of Fish Pathologists 12(5): 155-156.

Silva C.M.P., Fagulha T.M., Freitas M.S.G., Albuquerque M.T. & Varela M.C. 1994. Some notes on the distribution and effects of *Anguillicola crassus* in European-eel in Portugal. 6th International Colloquium on Pathology in Marine Aquaculture, 28-30 April, Montpellier, France.

Sinha V.R.P. 1969. A note on the feeding of larger eels *Anguilla anguilla* (L.). J. Fish Biol. 1: 279-283.

Sinha V.R.P. & Jones J.W. 1967. On the food of freshwater eels and their relationship with the salmonids. Journal of Zoology 150: 371-385

Statzner B., Sagnes P., Champagne J.Y. & Viboud, S. 2003. Contribution of benthic fish to the patch dynamics of gravel and sand transport in streams. Water Resources Research 39: 1-17.

Strubberg A. 1913. The metamorphosis of elvers as influenced by outward conditions. Meddelelser fra Kommissionen for Havundersogelser, Serie Fiskeri 4: 1-11.

Taverny C., Belaud A., Elie P. & Sabatié M.R. 2000. Influence des activités humaines. In: Baglinière, J.L., Elie, P. (Eds.), Les Aloses (*Alosa alosa* et *Alosa fallax* spp.). INRA, Paris, pp. 227-248.

Tesch F.W. 2003. The Eel. Biology and management of anguillid eels. 5th Edition. Oxford, Blackwell Science. 434p.

Thomas J.D. 1962. The food and growth of brown trout (*Salmo trutta* L.) and its feeding relationship with the salmon parr (*Salmo salar* L.) and the eels (*Anguilla anguilla* L.) in the River Teify, West Wales. J. Anim. Ecol. 31: 175-205.

Thomas K. & Ollevier, F. 1992. Population biology of *Anguillicola crassus* in the final host Anguilla anguilla. Diseases of Aquatic Organisms 14: 163-170.

Usero J., Izquierdo I., Morillo J. & Gracia, I. 2003. Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. Environment International 29: 949-956.

Utrilla C.G. 1997. Desarrollo y uso de recursos en los juveniles de Salmón atlantico (*Salmo salar* L.): interacciones entre la estrategia de vida adoptada y el comportamiento exhibido. Tesis Doctoral. Departamento de Biología Animal I. Facultad de Biología, Universidad Complutense de Madrid, 190 pp.

Vøllestad L. 1986. Growth and production of female yellow eels (*Anguilla anguilla* L.) from brackish water in Norway. Vie et Millieu 34: 267-271.

Vøllestad L.A. 1992. Geographic variation in age and length at metamorphosis of maturing European eel: environmental effects and phenotypic plasticity. J. Anim. Ecol. 61: 41-48.

Weber M. 1986. Fishing method and seasonal occurrence of glass eels (*Anguilla anguilla* L.) in the river Minho, west coast of the Iberian Peninsula. Vie et Milieu 36: 243-250.

Wooten R.J. 1990. Ecology of teleost fishes. London, Chapman & Hall.

Würtz J., Knopf K. & Taraschewski H. 1998. Distribution and prevalence of *Anguillicola crassus* (Nematoda) in eels *Anguilla anguilla* of the rivers Rhine and Naab, Germany. Diseases of Aquatic Organisms 32: 137-143.

Würtz J. & Taraschewski H. 2000. Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. Diseases of Aquatic Organisms 39: 121-134.

Yahyaoui A., Aguesse P. & Beaubrun P. 1983. Etude du recrutement en civelles (*Anguilla anguilla* L., 1758) dans l'estuaire du Sebou au de la saison de peche 1980-1981. Boletin de l'Institute Scientifique, Rabat, 7: 149-164

Zar J.H. 1999. Biostatistical Analysis (4th Edition), Prentice-Hall Inc., Upper Saddle River, NJ, 620 p.