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FOOD WEBS OF MEDITERRANEAN COASTAL WETLANDS

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2010

Universitat de Girona
Institut d'Ecologia Aquàtica

TESI DOCTORAL

FOOD WEBS OF MEDITERRANEAN COASTAL WETLANDS

Jordi Compte Ciurana

2010

PROGRAMA DE DOCTORAT D'ECOLOGIA FONAMENTAL I APLICADA

Dirigida per: Dra. Stéphanie Gascón García i Dr. Dani Boix Masafret

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

Universitat de Girona

Institut d'Ecologia Aquàtica

La Dra. Estefania Gascón García i el Dr. Dani Boix Masafret del Departament de Ciències Ambientals i de d'Institut d'Ecologia Aquàtica de la Universitat de Girona,

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Que aquest treball titulat Food webs of Mediterranean coastal wetlands, per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció i que compleix els requeriments per poder ser presentat com a compendi d'articles.

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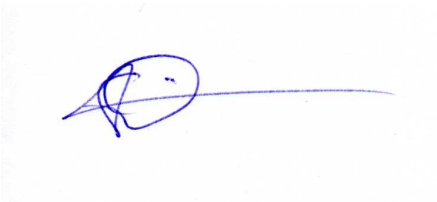

Girona, març del 2010

Agraïments

A diferència de moltes altres ocasions en què els agraïments es fan en l'últim moment de la tesi, aquesta pàgina va ser escrita ja fa uns mesos (encara que actualitzada recentment) ja que feia temps que tenia clar a les persones que volia agrair la seva ajuda. I és que aquesta tesi no hagués estat possible realitzar-la sinó fos que he estat envoltat dels millors companys que podia tenir que són uns grans professionals i, sobretot, unes grans persones i amics. Aquests companys són els que m'han ajudat i recolzat en tot moment (tant els bons com els dolents), i que m'han fet passar una molt bona etapa de la meva vida professional. Per això aquesta tesi no la considero només meva, sinó que és una mica de totes aquestes persones. En aquest sentit, m'agradaria agrair a tots el vostre suport.

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A tots i totes,

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La tesi presentada a continuació té un format de tesi com a compendi d'articles ja que compleix els requisits establerts per la Comissió d'Autorització de Defensa de Tesis Doctorals en la sessió de 16 de desembre de 2009. En base a les condicions establertes en l'esmentada sessió, tot seguit es mostra que es compleixen els mínims de qualitat i quantitat d'articles sol·licitats per presentar la tesi com a compendi d'articles.

L'àrea temàtica de la tesi és *Marine and Freshwater Biology* i en aquesta àrea la revista amb un factor d'impacte (FI) més alt és *Advances in Marine Biology* amb un 4.909 de FI. En el meu projecte de tesi presento tres articles publicats o acceptats dels quals dos en sóc el primer autor i un el segon autor. També presento un article en revisió com a primer autor. Les referències i FI de les revistes on han estat publicats o acceptats aquests articles segons l'ISI web de l'any 2008 són les següents:

- Brucet, S., Compte, J., Boix, D., López-Flores, R., Quintana, X. D., 2008. Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes. Marine Ecology Progress Series 355, 183-191. FI: 2.631.

- Compte, J., Brucet, S., Gascón, S., Boix, D., Sala, J., López-Flores, R., Quintana, X. D., 2009. Impact of different developmental stages of *Daphnia magna* (Straus) on the plankton community under different trophic conditions. Hydrobiologia 635, 45-56. FI: 1.449

- Compte, J., Gascón, S., Quintana X.D., Boix, D., In press. Top-predator effects of jellyfish *Odessia maeotica* in Mediterranean salt marshes. Marine Ecology Progress Series. FI: 2.631.

- Compte, J., Gascón, S., Quintana X.D., Boix, D., Submitted. Fish predation effects on benthos and plankton in a Mediterranean salt marsh. Marine Ecology Progress Series. FI: 2.631.

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ABSTRACT

Direct and indirect effects of key species were studied in the aquatic community of Empordà wetlands, a set of Mediterranean coastal wetlands and salt marshes with a food web characterized by a low number of species. To study the importance of key species in structuring these aquatic communities, two different types of organisms were analyzed: 1) grazer zooplanktonic species, such as calanoids or cladocerans, which may easily become dominant in zooplanktonic assemblages (dominances close to 100%); and 2) top predators feeding on aquatic invertebrates, which may achieve high, but monoespecific densities of predator. In this latter case, we compare the effects of an invertebrate (a jellyfish) and a vertebrate predator (a fish).

In the first part, the grazing effects of different developmental stages of zooplanktonic species are compared. The study attempts to check if exist a resource partitioning among different developmental stages of the same species which could be a strategy to reduce the intraspecific competition under resource limitations. Two experiments were performed to describe and compare the grazing effects of different developmental stages of two species (the calanoid *Calanipeda aquaedulcis* and the cladoceran *Daphnia magna*) which dominate the zooplankton in different conditions of resources limitation: in oligotrophic conditions (resource limiting) and eutrophic conditions (abundant resource).

The second part deals with the effects of top predators such as the jellyfish *Odessia maeotica* and the fish *Aphanius iberus* on the rest of the aquatic community. The different biology and ecology of these top predators suggests that their effects on aquatic community could be different. While *A. iberus* maintain long term stable populations in permanent waters, *O. maeotica* presents short term pulse appearances in temporary or semipermanent water bodies. Therefore, two more experiments one evaluating *O .maeotica* effects, and another analyzing *A. iberus* effects were performed.

All the experiments were carried out in the field and were located in different areas of the Emprodà wetlands (NE Iberian Peninsula). The experiments of *C. aquaedulcis* and *D. magna* were performed using microcosms whereas tanks and mesocosms were used in the experiments of *O. maeotica* and *A. iberus*. Different methodologies were used to describe the effects of the four studied species on aquatic community. To analyse the results, three approaches were used: taxonomic, functional and size-based approaches.

Results obtained from the experiments with *C. aquaedulcis* and *D. magna* confirmed the hypothesis that calanoids have recourse partitioning among their developmental stages, whereas different developmental stages of daphnids have not. Moreover, selective feeding behaviour in *C. aquaedulcis* and non selective feeding behaviour in *D. magna* were found. Hence, in Mediterranean coastal wetlands in situations of resource limitation, the selection of food and resources partitioning among developmental stages would allow *C. aquaedulcis* to decrease the intraspecific competition. In contrast, a strong intraespecific competence would exist among developmental stages of *D. magna*. Thus, *D. magna* would become dominant only in eutrophic waters with continuous nutrient pulses, the high availability of resource diminish the existence of intraspecific competence interactions.

Results obtained from the two experiments with the top predator species (*O. maeotica* and *A. iberus*) showed that, although both species trigger a trophic cascade in plankton, these have different top-down effects on community. The top-down effects observed in *O. maeotica* experiment are intense on zooplankton, but have a weak effect on phytoplankton by means of trophic cascade. However, their effects are expected to be short in time due to their characteristic populational dynamics. In contrast, *A. iberus* has stronger and more persistent effects on plankton (zooplankton and phytoplankton), as well as in benthos and in water characteristics. *A. iberus* effects imply a trophic cascade mainly related to body size, with a reduction of big sized invertebrates, an increase in smaller sized zooplankters and a decrease in picophytoplankton. Consequently, water transparency and macrophyte biomass increase.

According to our results, they suggested that trophic interactions between species of Mediterranean coastal wetlands may be a result of adaptation of these species in habitats with large salinity fluctuations and long periods without nutrient inputs. In this sense, similar resource partitioning than that found in *C. aquaedulcis* could be also attributed to *A. iberus*, because an ontogenetic shift of diet has been described previously in *A. iberus*. Therefore, the resource partitioning would allow these two species to have stable populations in situations of resource scarcity. Moreover, our results suggest a possible mutualism interaction between *A. iberus* and macrophytes. The presence of *A. iberus* in Mediterranean coastal wetlands would benefit the presence of macrophytes decreasing the phytoplankton competence for light and nutrients, and macrophytes, at their turn, would also beneficiate *A. iberus* since it provides him more refugee and availability of resource. On the other hand, species that inhabit in environments with

nutrient pulses (e.g. *D. magna*) or with bloom population dynamics (e.g. *O. maeotica*), would be characterized by high ingestion rates, speed growth, and the use of the same resource among their developmental stages that would suppose a strategy to exploit resource.

RESUMEN (en Castellano)

Se estudiaron los efectos directos e indirectos de especies claves de una comunidad acuática en los humedales de l'Empordà, que son un conjunto de marismas y humedales costeros Mediterráneos caracterizados por tener una red trófica con un número bajo de especies. Para estudiar la importancia de estas especies en la estructuración de esta comunidad acuática se analizaron dos tipos diferentes de organismos: 1) crustáceos zooplanctónicos filtradores, como calanoides y cladóceros, que pueden ser especies dominantes en el zooplancton (dominancias cerca del 100%); y 2) especies *top-predators* (depredadores del nivel trófico más alto de la red trófica) que pueden alcanzar densidades altas y llegar a convertirse en el único depredador de la comunidad acuática. En este último caso se compararon los efectos de un invertebrado (un cnidario) y un vertebrado (un pez ciprinodontido).

En la primera parte se comparan los efectos de la filtración realizada por los diferentes estadios de desarrollo de las especies de zooplancton. El estudio trata de comprobar si existe una división del recurso entre diferentes estadios de desarrollo de una misma especie, la cual podría ser una estrategia que permite reducir la competencia intraespecifica en condiciones de limitación de recurso. Se hicieron dos experimentos para describir y comparar los efectos de la filtración de los diferentes estadios de desarrollo de dos especies (el calanoide *Calanipeda aquaedulcis* y el cladócero *Daphnia magna*) que dominan el zooplancton en diferentes condiciones de limitación de recurso: en condiciones de oligotrofia (recurso limitado) y condiciones de eutrofia (recurso abundante).

La segunda parte se analizan los efectos de dos *top-predators*, el cnidario *Odessia maeotica* y el ciprinodontido *Aphanius iberus*, sobre el resto de la comunidad acuática. Su diferente biología y ecología sugería que estos *top-predators* tengan diferentes efectos sobre la comunidad acuática. Así, mientras que *A. iberus* mantiene poblaciones estables a lo largo del tiempo en aguas permanentes, *O. maeotica* presenta una dinámica poblacional caracterizada por la formación de *blooms* en aguas temporales y semipermanentes. Por esta razón se hicieron dos experimentos, uno para estudiar los efectos de *O. maeotica* y el otro para estudiar los efectos de *A. iberus*.

Todos los experimentos se realizaron en el campo en diferentes áreas de los humedales del Emprodà (NE de la Península Ibérica). Concretamente, para los experimentos de *C. aquaedulcis* y *D. magna* se utilizaron microcosmos, mientras que para los experimentos de *O. maeotica* y *A. iberus* se utilizaron tanques y mesocosmos.

Para estudiar los efectos de estas cuatro especies se utilizaron diferentes metodologías, y los resultados se analizaron mediante tres aproximaciones: la taxonómica, la funcional y la de tamaños.

Los resultados obtenidos en los experimentos de *C. aquaedulcis* y *D. magna* confirmaban la hipótesis de que los calanoides tienen una división del recurso entre sus estadios de desarrollo, mientras que los cladóceros no la tienen. Además, se observó un comportamiento selectivo de la alimentación de *C. aquaedulcis* y un comportamiento no selectivo en la alimentación de *D. magna*. Así, en los humedales costeros Mediterráneos en situaciones de limitación de recursos la selección diferencial del alimento entre los estadios de desarrollo permitiría a *C. aquaedulcis* disminuir la competencia intraspecífica. Por lo contrario, *D. magna* tendría una fuerte competencia intraespecifica entre sus estadios de desarrollo de tal forma que sólo sería dominante en aguas eutróficas con entradas continuas de nutrientes donde la disponibilidad de recurso es alta.

Los resultados de los experimentos de *O. maeotica* y *A. iberus* presentaban que, aunque las dos especies desencadenaban cascadas tróficas en el plancton, estas tienen diferentes efectos *top-down* sobre la comunidad. Los efectos *top-down* observados en el experimento de *O. maeotica* eran intensos sobre el zooplancton ya que reducía fuertemente su densidad, peró tenía un efecto debil de cascada trófica sobre el fitoplancton. Sin embargo, es de esperar que sus efectos sean cortos por su dinámica de poblacional. Por lo contrario, *A. iberus* tiene unos efectos más fuertes i persistentes sobre el plancton (zooplancton y fitoplancton) y también sobre el bentos y las características del agua. Los efectos de *A. iberus* implican una cascada trófica, principalmente relacionada con el tamaño corporal, con una reducción de los grandes invertebrados, un incremento del zooplancton de tamaño pequeño y una disminución del picofitoplancton. En consecuencia, la transparencia del agua y la biomassa de los macrófitos incrementan.

Según los resultados de los distintos experimentos, estos sugieren que las interacciones tróficas entre especies de los humedales costeros Mediterráneos podrían ser un resultado de la adaptación de estas especies en ambientes con grandes fluctuaciones de salinidad y largos periodos sin entrada de nutrientes. En este sentido una división del recurso similar a la encontrada en *C. aquaedulcis* podría ser atribuida a *A. iberus* ya que se le ha descrito diferentes dietas en las distintas fases de su crecimiento. De esta forma, la división del recurso permitiría a estas dos especies tener poblaciones estables en situaciones de falta de recurso. Además, nuestros resultados sugieren un posible mutualismo entre *A. iberus* y los macrófitos. La presencia de

A. iberus en los humedales costeros Mediterráneos beneficiaría la presencia de macrófitos, ya que disminuye la competencia del fitoplancton por la luz y los nutrientes y, a su vez, estos beneficiarían a *A. iberus* proporcionándole refugio y disponibilidad de recurso. Por otra parte, las especies que habitan en ambientes con entrada de nutrientes (por ejemplo *D. magna*) o con dinámicas poblacionales caracterizadas por la formación de *blooms* (por ejemplo *O. maeotica*), estarían especializadas en tasas de ingestión altas, un rápido crecimiento y la utilización del mismo recurso entre los distintos estadios de desarrollo que les permitiría explotar el recurso.

RESUM (en Català)

S'han estudiat els efectes directes i indirectes d'espècies claus de la comunitat aquàtica dels aiguamolls de l'Empordà, un conjunt de maresmes i aiguamolls costaners Mediterranis que es caracteritzen per tenir unes xarxes tròfiques amb un nombre baix d'espècies. Per estudiar la importància d'aquestes espècies en l'estructuració de la comunitat aquàtica, es van analitzar dos tipus d'organismes: 1) crustacis zooplanctònics filtradors, com els calanoides o els cladòcers, que dominen el zooplàncton (amb dominàncies properes al 100%); i 2) espècies *top-predators* (predadors del nivell tròfic més alt de la xarxa tròfica) que poden assolir densitats altes i esdevenir l'únic predador. En aquest darrer cas, es va comparar els efectes d'un invertebrat (un cnidari) i un vertebrat (un peix ciprinodont) predador.

En la primera part, es va comparar els efectes de la filtració realitzada pels diferents estadis de desenvolupament de les espècies zooplanctòniques. L'estudi tracta de comprovar si existia una segregació del recurs entre els diferents estadis de desenvolupament de la mateixa espècie, la qual cosa podría ser una estratègia per reduir la competència intraespecífica en condicions de limitació de recurs. Es van realitzar dos experiments per descriure i comparar els efectes de la filtració dels diferents estadis de desenvolupament de dos espècies (el calanoide *Calanipeda aquaedulcis* i el cladòcer *Daphnia magna*) que dominen el zooplàncton en condicions diferents de limitació de recurs: en condicions oligotròfiques (recurs limitat) i en condicions eutròfiques (recurs abundant).

En la segona part s'estudiava els efectes de dos *top-predators*, com el cnidari *Odessia maeotica* i el ciprinodont *Aphanius iberus*, sobre la resta de la comunitat aquàtica. La diferent biologia i ecologia d'aquests *top-predators* suggereix que els seus efectes sobre la comunitat podrien ser diferents. Mentre que *A. iberus* manté poblacions estables al llarg del temps en aigües permanents, l'*O. maeotica* presenta una dinàmica poblacional caracteritzada per la formació de *blooms* en aigües temporànies o semipermanents. Per aquesta raó es van realitzar dos experiments, un per a estudiar els efectes de l'*O. maeotica* i l'altre per a estudiar els efectes d'*A. iberus*.

Tots els experiments es van dur a terme en el camp en diferents àrees de dels aiguamolls de l'Empordà (NE de la Península Ibèrica). En els experiments de *C. aquaedulcis* i *D. magna* es van utilitzar microcosmos, mentre que en els experiments d'*O. maeotica* i d'*A. iberus* s'utilitzaren tancs i mesocosmos. Per descriure els efectes de

les espècies estudiades es van utilitzar diferents metodologies, i els resultats es van analitzar mitjançant tres aproximacions: la taxonòmica, la funcional i la de mides.

Els resultats obtinguts en els experiments de *C. aquaedulcis* i *D. magna* van confirmar la hipòtesis que els calanoides tenen una segregació del recurs entre els seus estadis de desenvolupament, mentre que aquesta segregació no és observada en els cladòcers. A més a més, es va observar que *C. aquaedulcis* té un comportament alimentari selectiu mentre que aquest no es va observar en *D. magna*. Per tant, en aiguamolls costaners Mediterranis en situacions de limitació de recurs, la selecció de l'aliment i la segregació del recurs entre els estadis de desenvolupament permetria a *C. aquaedulcis* disminuir la competència intraespecífica. En canvi, els diferents estadis de desenvolupament de la *D. magna* tindrien una forta competència intraespecifica la qual cosa restringiria la seva dominància en aigües eutròfiques amb entrada continua de nutrients on la disponibilitat del recurs fos alta.

Els resultats dels experiments amb els *top-predators* (*O. maeotica* i *A. iberus*) mostraven que, encara que les dos espècies desencadenin una cascada tròfica en el plàncton, aquestes tenen efectes *top-down* diferents sobre la comunitat. Els efectes *top-down* observats en l'experiment d'*O. maeotica* eren intensos sobre el zooplàncton ja reduïa fortament la seva densitat, però tenia un efecte feble per cascada trófica sobre el fitoplàncton. Tot i així, és d'esperar que els seus efectes siguin curts en el temps degut a la seva dinàmica poblacional. En canvi, *A. iberus* té efectes més forts i persistents sobre ambdues fraccions del plàncton (zooplàncton i fitoplàncton), com també sobre el bentos i sobre les característiques de l'aigua. Els efectes de l'*A. iberus* impliquen una cascada tròfica, relacionada principalment amb la grandària corporal, amb una reducció dels grans invertebrats, un increment del zooplàncton de mida petita i una disminució del picofitoplàncton. Conseqüentment, hi ha un increment de la transparència de l'aigua i de la biomassa de macròfits.

Els resultats dels diferents experiments suggereixen que les interaccions tròfiques entre les espècies dels aiguamolls costaners Mediterranis podrien ser un resultat de l'adaptació d'aquestes espècies a hàbitats amb grans fluctuacions de salinitat i llargs períodes sense entrada de nutrients. Així, una segregació del recurs semblant a la del *C. aquaedulcis* podria ser també atribuïda a *A. iberus* ja que prèviament s'han descrit canvis de dieta al llarg del seu creixement. Per tant, la segregació de l'aliment permetria a aquestes dos espècies tenir poblacions estables en situacions d'escassetat de recurs. A més a més, els nostres resultats suggereixen un possible mutualisme entre *A. iberus* i els

macròfits. La presència d' *A. iberus* en els aiguamolls costaners Mediterranis beneficiaria la presència de macròfits disminuint la competència del fitoplàncton per la llum i els nutrients, i a la vegada, aquests beneficiarien a *A. iberus* proporcionant-li refugi i disponibilitat de recurs. Per altra banda, les espècies que habiten en ambients amb entrada continua de nutrients (per exemple *D. magna*) o amb una dinàmica poblacional caracteritzada per la formació de *blooms* (com per exemple *O. maeotica*), serien especialitzades en taxes d'ingestió altes, un creixement ràpid i la utilització del mateix recurs entre els seus estadis de desenvolupament que els permetria explotar el recurs.

Chapter 1

General Introduction

GENERAL INTRODUCITON

Food web functioning

"Food web is a map that describes which kinds of organisms in a community eat which other kinds" (Pimm *et al*., 1991). Organisms of this map are related by mean of trophic interactions which can be controlled by resource (bottom-up) or by consumers (top-down) affecting the structure of community (McQueen *et al*., 1986). Bottom-up is the control of community by resource availability, which producers' (bottom levels of food web) regulate the consumers (top levels of food web) (White, 1978); whereas top-down is the control of community by predation, which the consumers regulate the producers (Hairston *et al.*, 1960). These controls usually occur simultaneously (McQueen *et al.*, 1986; Rosemond *et al*., 1993; Osenberg & Mittelbach, 1996), although only one control often dominates (Belovsky & Joern, 1995). For example, several authors have suggested that in simple food webs (food web with few species), top-down effects are very strong (Strong, 1992; Polis & Strong, 1996; Finke & Denno, 2004, Shurin *et al*., 2006). Another example is that top-down can be the key factor in the regulation the food webs of aquatic ecosystems (Strong, 1992; Polis, 1999; Halaj & Wise, 2001) by mean of direct (predation) and indirect (trophic cascade) effects.

In aquatic ecosystems, a predator of the highest trophic level (top predator) is often considered as keystone species (Carpenter & Kitchell, 1993; Lampert & Sommer, 1997). The keystone species is a species of high trophic status that had the capacity of change the species diversity predating and limiting the abundance of preys which would otherwise monopolize resources in its trophic level. Thus, it would affect competition process preventing the appearance of species with a well performance when competing with the rest (Paine, 1966, 1969; Kerfoort & DeMott, 1984).

Nevertheless, while this keystone effect is mechanistically simple and intuitive, its manifestation in natural communities is context-dependent and can be modulated by predation, food web complexity and resource availability (Brose *et al*., 2005). Regarding the predation effect, top predator can triggers a trophic cascade in ecosystem. Trophic cascade hypothesis (Carpenter *et al*., 1985, Carpenter & Kitchell, 1993) describes that, changes in each trophic level shows an opposite response in next trophic level because top level preys on bottom level. For example, the decrease of carnivores allows increase herbivores which reduce plants populations for increase of grazer. Additionally, the presence of predator can have indirect effects on environment. In this sense, predator

presence can change water characteristics as turbidity or dissolved nutrients in aquatic ecosystems (Scheffer, 1992; Vanni *et al*., 1997; Jeppesen *et al*., 1999) and structure of habitat (for example on macrophytes; Jeppesen *et al*., 1990; van Donk & Gulati, 1995; Moss *et al*., 1996). However, food web complexity has to be taken into account when studying keystone effects, since it has been described a buffering effect performed by distant species if long chains are considered (Strong, 1992; Schoener, 1993; Polis & Strong, 1996). This "buffering" effect make difficult to detect trophic cascades (Brose *et al*., 2005), due to either dampening effects in long chains (Schoener, 1993; Menge, 1995), or to multiple pathways of effects with opposite sign cancelling each other out (Berlow, 1999). As a consequence, keystone effects on complex food webs (both by high species richness and connectance among them) are usually difficult to predict, because of the many potentially strong influences of distant species (Yodzis, 2000).

Competitive interaction related to resource availability is another factor to consider when studying food web structure. Under resources limiting conditions, the competence for this resource has been suggested as one of main factors affecting the food web structure (Hairston *et al*., 1960; Menge & Sutherland, 1976; Abrams *et al*., 1995). Competition is a type of indirect trophic interaction defined as a negative effect of one species on the population growth rate or abundance of another species (Strauss, 1991; Wootton, 1994). It can occur that the two species compete directly for resources (Holt, 1977). Nevertheless, two types of competition exist: interspecific (among different species) and intraspecific (within the same species). To reduced the competence and maintain the coexistence of individuals of different or same species, different strategies have been described, mainly focused on some kind of resource partitioning: spatial, temporal or diet (Schoener, 1974; Armstrong & McGehee, 1980; Tilman & Pacala, 1993; Chesson, 2000). Although, resource partitioning has been often associated to a strategy to avoid interspecies competition, it has been also reported as a strategy to avoid intraspecific competition. For example, different body sizes of same species have different diet (Schoener, 1974; Peters, 1983; De Ross *et al*., 2008). This intraspecific competence may be strong in invertebrate communities dominated by one single species and the resource partitioning among different stages may allow the dominant species to reduce intraspecific competition (Werner & Gilliam, 1984).

Studying food webs: choosing appropriate methodological approach and suited habitat

The use of size-based approaches is important in study of food webs because provides complementary information to taxonomic and functional-based approach. The size-based approaches focus on the aggregation of the organisms according to their individual body weight regardless of their taxonomy. In fact, Quiñones (1994), Rodríguez (1994) and Gaedke (1995) suggested the use of biomass size distribution to predict the trophic position of organisms in a food web as simple method, relatively low cost efficient ratio, and reproducible. The biomass size spectrum is a method widely used to perform this approach and describes how biomass of organisms is distributed along size classes (Platt & Denman, 1977; Vidondo *et al.*, 1997). Nevertheless, this method is often merely used as a descriptive tool due to the complexity of working with non linear distributions characterising size spectra. To overcome this problem, a new metric named "size diversity" has been recently described (Quintana *et al*., 2008). Size diversity gives a unique value per size distribution, which integrates the amplitude of the size range and the evenness, that is, the relative distribution of sizes along the size range. Thus, it simplifies the comparison among samples. Size diversity has also the advantage of an intuitive interpretation of its ecological meaning as the concept of diversity is well established (Quiroga *et al*., 2005; Brucet *et al*., 2006; Quintana *et al*., 2008).

On the other hand, as it has been previously stated the manifestation of keystone species effects in natural environments is context-dependent (Brose *et al*., 2005). Thus, is important to choose a suitable habitat to perform food web studies. The suitability of the habitat may relay on the interests of the study. For example, when the interest focus in detect keystone species effect trough trophic cascade, species poor habitat systems seems especially interesting to study food web functioning. Short chains food webs may prevent some damping and buffering effects observed in complex food webs. In this sense, Mediterranean coastal wetlands are especially suitable habitats, since due to the large fluctuations of these systems (e.g. Britton & Crivelly, 1993; Álvarez-Cobelas *et al*., 2005; Beklioglu *et al*., 2007) only well-adapted species can successfully inhabit such environments (Bamber *et al*., 1992; Boix *et al*., 2007), and so are species poor habitats (Gray, 1974). Moreover, Mediterranean coastal wetlands are aquatic systems, free from tidal influence whose hydrological regime is determined by the occurrence of floods caused by meteorological disturbances in autumn and winter, and the process of desiccation during summer (Stora & Arnoux, 1983; Quintana, 2002). The hydrology of

these systems are characterized by prolonged periods of confinement, restricted water inputs, a low flushing rate and high marine influence (Guelorget & Perthuisot, 1983; Trobajo *et al*., 2002). Thus, during these prolonged confinement periods the external nutrient inputs are scarce (Guelorget & Perthuisot, 1983; Quintana *et al*., 1998a) and so bottom-up effects are not expected. Moreover, during these confinement periods resources availability decrease leading to situations of resource partitioning to avoid competition (Brucet *et al*., 2006) Therefore, these systems are especially interesting to study food web functioning because (1) are a poor species habitats, that may have short long chains preventing buffering effects that appears in complex food webs; (2) their oligotrophic state allows to focus the studies in top-down effects; and (3) the low resource availability also due to the oligotrophic state may facilitate to found resource partitioning situations.

General hypothesis

It has been shown that under stable conditions and in the absence of predators, succession in zooplankton communities leads mainly to the dominance of a single species, due to the elimination of the inferior competitors (Rothhaupt, 1990). In fact, zooplankton of salt marshes in confined periods, in which environmental conditions are stable, is dominated by single specie of calanoide (Quintana *et al*., 1998b; Frisch *et al*., 2006). Similarly, daphnids usually dominate the zooplankton community in eutrophic freshwaters (McNaught's, 1975) especially in periods of continuous nutrient pulses (Schulze *et al*., 1995). However, in both planktonic groups, daphnids and calanoids, all development stages coexist in situations when one of these organisms is highly dominant (Boersma, 1995; Brucet *et al*., 2005a; 2006). Consequently, intraspecific competition could play an important role in structuring zooplankton communities. To minimize this kind of competition different sizes (i.e. development stage) may partition their resources, for example, having different diet. The diet and the selective feeding in calanoids copepods have been widely described (e.g. Richman *et al*., 1980; Meyer *et al*., 2002). Also, it has found changes of diet during development in various calanoid species (Mullin & Brooks, 1967; Paffenhöfer & Lewis, 1989, Bonnet & Carlotti, 2001). Accordingly, in Mediterranean coastal wetlands during confinement and oligotrophic periods the dominance of a calanoid species but with different sizes (i.e. development stages) has been reported (Quintana *et al*., 1998b; Brucet *et al*., 2995; 2006). In these situations resource partitioning could be interpreted as a strategy to avoid or, at least,

minimized the intraspecific competition (Brucet *et al*., 2005a, 2006). In contrast, daphnids, which dominate in eutrophic environments (MacNaught's, 1975), were described as omnivorous organisms (e.g. Gophen *et al*., 1974; Lampert, 1987; Jürgens, 1994) and non-selective grazer (e.g. Reynolds, 1984; Freyer, 1991), or as less selective than copepods (DeMott, 1986). Moreover, differences of diet among their different development stages were not found Boersma (1995). Therefore, we hypothesis that zooplankonic grazer species whose stages feed on different food resources would be favoured under food limiting conditions, while those that do not have resource partitioning during ontogeny would be restricted to more productive or fluctuant environments, where resource competition between adults and juveniles will be less likely.

Situations of intraespecific competence usually happen in Mediterranean coastal wetlands with predator absence (Brucet *et al*., 2005a, 2006). However, when top predators are present, a weaker intraespecific competence would be expected and top-down control would be better expressed. Nevertheless, different types of predators may show a different predation role. In this sense, the differences in the main predator in temporary and permanent lagoons of Mediterranean coastal wetlands can imply a different predation role between these two lagoon types. In temporary lagoons, characterized by low fish densities, invertebrate predators can reach high densities (i.e. jellyfish *Odessia maeotica*; Quintana *et al*., 1998b), while in permanent lagoons benthivorous fish have stable populations (García-Berthou *et al*., 1991; Badosa *et al*., 2007). Jellyfish and planktivorous fish have been described as to top predators causing direct and indirect changes in lower trophic levels through cascading effects (Kerfoot & DeMott, 1984; Carpenter& Kitchell, 1993; Oguz *et al*., 2001; Pitt *et al*., 2007). However, jellyfish and fish have different biology and ecology. Therefore, our hypothesis is that two top predators with different biology and ecology may have different top-down effects on aquatic communities with similar complexity.

Study approach and objectives

To study these hypotheses, different field experiments were performed in a species poor habitat such are the Mediterranean coastal wetlands of Empordà wetlands (NE Iberian Peninsula).

Thus, regarding the resource partitioning hypothesis, two experiments using microscosms were carried out with objectives of (I) to describe the feeding behaviour of

a selective and a non selective zooplanktonic grazer species and (II) to compare the resource partitioning among developmental stage of both species. Calanoid *Calanipeda aquaedulcis* and cladoceran *Daphnia magna* were used as selective and non selective species respectively. Experiment with *C. aquaedulcis* was carried up in oligtorphic environments, whereas experiment with *D. magna* in eutrophic environments. In each experiment, different objectives were proposed:

a) The first experiment (*Calanipeda* experiment, chapter 3) was performed in a temporal lagoon in confined conditions during to autumn and spring with high abundance of *C. aquaedulcis*. With this experiment is proposed to (1) characterise the feeding behaviour of the different developmental stages of *C. aquaedulcis,* and (2) evaluate the possibility of food resource partitioning among developmental stages of this copepod.

This study has been published as a research article:

Brucet, S., Compte, J., Boix, D., López-Flores, R., Quintana, X. D., 2008. Feeding of nauplii, copepodites and adults of Calanipeda aquaedulcis *(Calanoida) in Mediterranean salt marshes. Marine Ecology Progress Series 355, 183-191.*

b) The second experiment (*Daphnia* experiment, chapter 4) was carried out in different trophic conditions in a permanent freshwater lagoon of wastewater treatment plant with continuous nutrient inputs. The objective of this experiment were to check: (3) if a non-selective feeding organism such as *D. magna* may significantly modify the structure of a microbial community through cascading trophic interactions, and (4) if the different sizes of *D. magna* have a similar feeding behaivour and consequently they will not have different effects on the microbial community.

This study has been published as a research article:

Compte, J., Brucet, S., Gascón, S., Boix, D., Sala, J., López-Flores, R., Quintana, X. D., 2009. Impact of different developmental stages of Daphnia magna *(Straus) on the plankton community under different trophic conditions. Hydrobiologia 635, 45-56.*

On the other hand, top predator hypothesis was tested performing two more field experiments using tanks and mesocosms. The objectives of these experiments were (I) to describe the effects of invertebrate and vertebrate top predator on plankton and/or benthos and (II) to compare their effects. In this case, jellyfish *O. maeotica* and fish

Aphanius iberus were used as invertebrate and vertebrate top predators. Objectives proposed in each experiment were:

a) The first experiment (*Odessia* experiment, chapter 5) was performed using tanks in temporary lagoon in period of confinement and oligotrophic conditions with high abundances of *O. maeotica*. Objectives were test that *O. maeotica*: (5) cause a strong direct effect on zooplankton by removing most of the plankton larger than 50 μm; and (6) causes indirect cascading effects on lower trophic levels, including small zooplankters, phytoplankton and bacteria, as a consequence of the depletion of the large zooplankters.

This study has been published as a research article:

Compte, J., Gascón, S., Quintana, X.D., Boix, D., In press. Top-predator effects of jellyfish Odessia maeotica *in Mediterranean salt marshes. Marine Ecology Progress Series.*

b) The fourth experiment (*Aphanius* experiment, chapter 6 and 7) was carried out in temporary lagoon where the endemic fish *A. iberus* was added in mesocosms. Objectives of this experiment were: (7) to compare the effects of *A. iberus* on zoobenthos and zooplankton (chapter 5), (8) to check direct and indirect effects of *A. iberus* on plankton (zoo- and phytoplankton) (chapter 6), and (9) to find effects on macrophyte community by indirect effect of trophic cascade (chapter 6).

The study of chapter 6 has been submitted as a research article:

Compte, J., Gascón, S., Quintana X.D., Boix, D., Submitted. Fish predation effects on benthos and plankton in a Mediterranean salt marsh.

The study of chapter 7 is in preparation as a research article:

Compte, J., Gascón, S., Quintana X.D., Boix, D., In preparation. Fish trophic cascade effects in Mediterranean salt marsh.

Chapter 2

Study site

STUDY SITE

The field experiments were conducted in different lagoons of Empordà wetlands in the northeastern Iberian Peninsula (Figure 1). The Empordà wetlands are a series of shallow coastal lagoons, with a Mediterranean hydrological regime. They are characterized by periods of flooding and nutrient inputs and prolonged periods of confinement, restricted water inputs, a low flushing rate and high marine influence (Quintana *et al*., 2002; Trobajo *et al*., 2002).

The hydrological regime had high intra-/-inter-annual variability (Britton & Crivelly, 1993; Álvarez-Cobelas *et al*., 2005; Beklioglu *et al*., 2007), but seasonal patterns are not frequently observed in aquatic invertebrate dynamics of the Mediterranean coastal marshes (Quintana *et al*., 2006). In contrast, aquatic invertebrate dynamics are primarily related to two hydrological conditions: flooding and confinement situations. In these sense, several studies has already noted that the degree of flooding and of confinement plays an important role in determining biological communities (Guelorget & Perthuisot, 1983; Pérez-Ruzafa & Marcos, 1992; Victor & Victor, 1997; Basset *et al*., 2006). Factors related to flooding and confinement such as salinity, water turnover, water permanence, and productivity; generally determine the composition and structure of zooplankton communities (e.g. Quintana *et al*., 1998b; Lam-Hoai & Rougier, 2001; Brucet *et al*., 2005a), primary producers (e.g. Trobajo *et al*., 2002; López-Flores *et al*., 2006; Reyes *et al*., 2007) and the spatial distribution of benthic communities (e.g. Santos *et al*., 1996; Gifre *et al*., 2002; Gascón *et al*., 2005).

The aquatic community of the Empordà wetlands

The aquatic community of the Empordà wetlands has been widely described in previous studies. The zooplankton is primarily composed of jellyfish (*Odessia maeotica*), calanoids (*Calanipeda aquaedulcis* and *Eurytemora velox*), cyclopoids (*Diacyclops bicuspidatus*), harpacticoids copepods (*Cleptocampus confluents*) and rotifers (*Brachionus plicatilis* and *Sinchaeta* sp.) (Quintana *et al*., 1998b; Brucet *et al*., 2005a). The phytoplankton is dominated by diatoms (*Amphora* spp., *Navicula* spp.), dinoflagellates (*Glenodinium foliaceum*) and haptophytes (López-Flores *et al*., in press). The zoobenthonic fraction is composed of quironomids (*Chironomus salinarius*), polychaetas (*Nereis diversicolor*), amphipods (*Gammarus aequicauda*), ostracodes (*Cyprideis torosa*), and nematodes (*Diplolaimella* sp., *Monhystrella* sp., *Thalassomonhystera* sp. and *Ptycholaimellus* sp.) (Gascón *et al*., 2006; 2008). *Ruppia*

cirrhosa and *R. maritima* are the main macrophytes in these lagoons (Gesti, 2000; Gesti *et al*., 2005). In permanent lagoons, *Aphanius iberus* and *Pomatoschistus microps* are the dominant fish species and *Atherina boyer* and *Anguilla anguilla* are occasionally present (García-Berthou *et al*., 1991).

Experiments

The *Calanipeda* experiment was conducted at the La Pletera salt marsh where long periods of confinement lead to scarcity of inorganic nutrients and dominance of heterotrophic nano- and microplankters (López-Flores *et al*., 2006) (Figure 1). The *Odessia* and *Aphanius* experiments were performed in a temporary and oligotrophic salt marsh lagoon (Quintana *et al*., 1998a), inside the reserve at the Empordà Wetlands Natural Park. The *Daphnia* experiment was performed in a wastewater treatment plant (hereafter WWTP) and a constructed wetland system of an urban area under heavy pressure from tourism during vacation periods (Empuriabrava) situated in the Empordà Wetlands Natural Park.

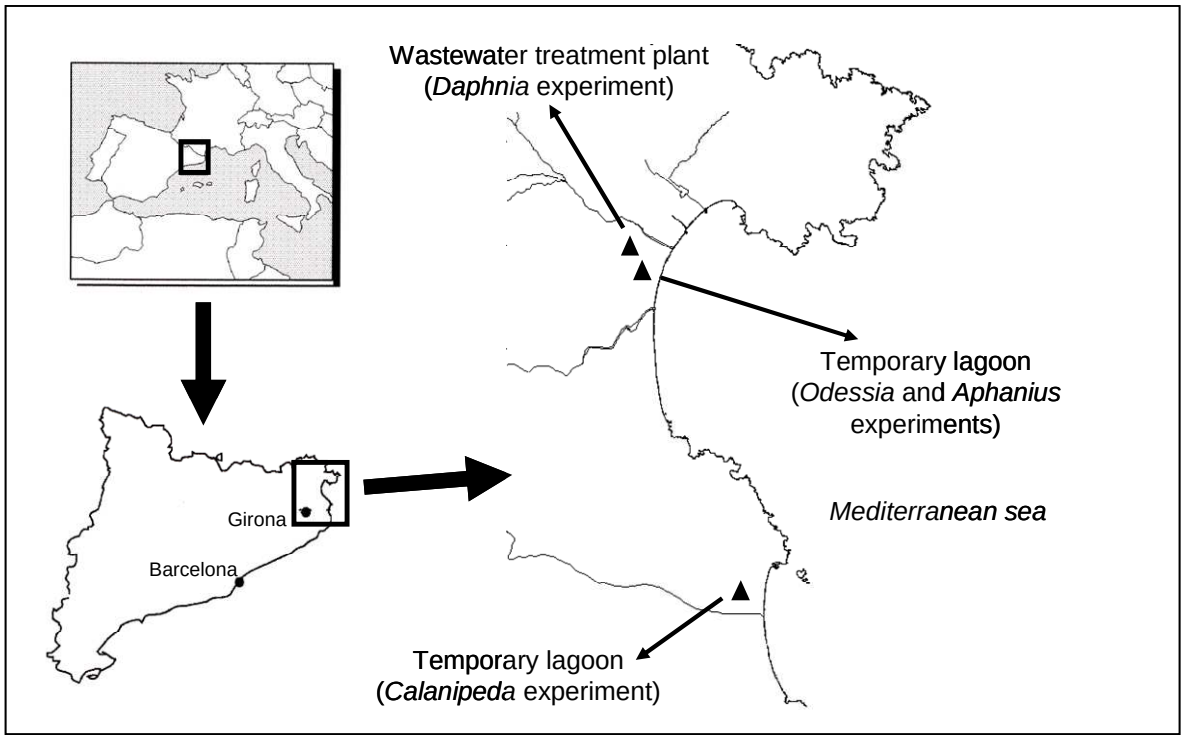
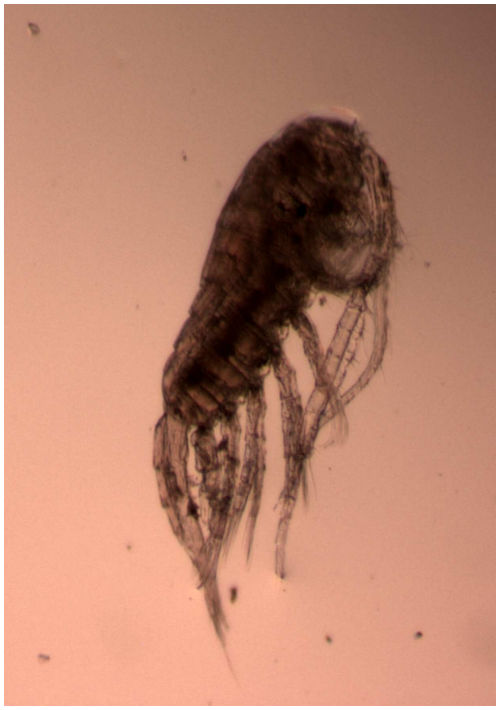


Fig. 1. Map of study site which the different experiments are showed.



Chapter 3

Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes

Brucet, S., Compte, J., Boix, D., López-Flores, R., Quintana, X. D. 2008. Feeding of nauplii, copepodites and adults of *Calanipeda aquaedulcis* (Calanoida) in Mediterranean salt marshes. Marine Ecology Progress Series 355, 183-191

ABSTRACT: Feeding of the different developmental stages of *Calanipeda aquaedulcis* on natural particles (bacterio-, phyto- and microzooplankton) was measured in a Mediterranean salt marsh (Empordà wetlands, NE Iberian Peninsula). Bottle incubations were performed in the field both in autumn and spring. The results showed differences in the diet of the different developmental stages due to both prey type and size. In general, the size of the ingested prey increased with increasing size of the *C. aquaedulcis* stage. While *C. aquaedulcis* adults had high ingestion rates and selection coefficients for large prey (micro- and nanoplankton), nauplii preferentially consumed smaller prey items (picoplankton). Copepodites showed the widest prey size range, including pico-, nano- and microplankton. Nevertheless, the lower size limit for particle capture was similar for all stages, i.e. between 1.7 and 2.1 μm. Omnivory was observed in all stages of *C. aquaedulcis*. Heterotrophic prey (picoplankton, dinoflagellates and ciliates) were the most ingested items. The ability to partition the available food among the different developmental stages could represent an advantage in times of food scarcity because it may reduce intraspecific competition. This may explain how *C. aquaedulcis* is able to predominate in the zooplankton community for several weeks during spring and summer even in situations of low food availability.

Key words: Feeding, Developmental stages, Ingestion, Omnivory, Selectivity, Zooplankton

INTRODUCTION

Due to their variations in size and bioenergetic needs during development, copepods must change their diet ontogenetically from nauplii to adult stages (Kleppel, 1993; Bonnet & Carlotti, 2001). Early stages invest their resources in growth while adults invest in reproduction. The morphology of the feeding appendages in nauplii differs from that of copepodites (Fernandez, 1979) and, therefore, their capture of particles is also likely to be different. However, there is little evidence regarding the ontogeny of the composition of copepod diet in nature (but see Poulet, 1977), most feeding studies concentrating on feeding and selectivity of adult stages. Research regarding feeding strategies in juvenile stages is still scarce, despite the fact that their abundances may equal or exceed those of adults in natural populations (Calbet *et al*., 2001).

Some laboratory studies have provided evidence regarding diet changes during development in various calanoid species. For instance, several authors have found differences in particle size consumed by calanoid copepodites and adults (Mullin & Brooks, 1967; Paffenhöfer & Lewis, 1989), while others found different optimal prey size and attributed it to differences in morphology of mouthparts at different ontogenic stages (Fernandez, 1979; Bonnet & Carlotti, 2001). Nevertheless, other authors have found similar selective behaviour and ingestion rates for all stages (Meyer *et al*., 2002) and concluded that copepod nauplii occupy the same feeding niche as adult stages (Conover, 1982).

Some authors have stated the need to undertake feeding experiments in the field, since results in the laboratory have been different from what has been found in field conditions (Donaghay & Small, 1979). The evidence from the few studies done using naturally occurring particles and freshly captured copepods show that selective feeding and diet in nature vary among naupliar, copepodite and adult stages (e.g. Poulet, 1977). Such trophic niche segregation among developmental stages favours copepod populations in terms of feeding efficiency increase, intraspecific competition decrease and an increase of immature survival (Poulet, 1977). Several copepods are known to be omnivorous. *Euryternora affinis,* for instance, can ingest ciliates or detritus as well as algae (Berk *et al.*, 1977; Heinle *et al*., 1977). There is increasing evidence that mixed-food diets are beneficial for copepod development (e.g. Stoecker & Egloff, 1987; Kleppel, 1993; Bonnet & Carlotti, 2001), however, information about the relative contribution of the different food sources to *in situ* ingestion is limited.

Calanipeda aquaedulcis is common in brackish and estuarine waters (e.g. Dussart & Defaye 1983). It regularly dominates the zooplankton community (e.g. Quintana *et al*., 1998) and its developmental stages may coexist for several weeks, especially during spring and summer (Brucet *et al*. 2006 and references therein). Since *C. aquaedulcis* is often found in conditions of low productivity (Brucet *et al*., 2006), resource partitioning among stages may be a way to reduce intraspecific competition and maintain stable populations over time. In this sense, a high intraspecific variability in amino acid composition has been found in *C. aquaedulcis*, which could indicate a gradual change in diet during the life cycle of this copepod (Brucet *et al*., 2005). However, to our knowledge, nothing is known about *C. aquaedulcis* feeding strategies.

The purpose of this study was to (1) characterize the diet of the different developmental stages of *C. aquaedulcis,* and (2) evaluate the possibility of food resource

partitioning among developmental stages of this copepod. The approach included using naturally occurring food particles (bacterio-, phyto- and microzooplankton), to cover the available diversity of food for the different developmental stages and to more closely approximate natural feeding conditions.

MATERIALS AND METHODS

Experimental setup, feeding experiments and sample processing

The study was carried out in the Empordà wetlands (NE Iberian Peninsula), a set of Mediterranean shallow coastal lagoons free from tidal influence and whose hydrological regime is determined by the occurrence of floods due to meteorological disturbances in autumn and winter and the process of desiccation (Brucet *et al*., 2006). Samples were taken in La Pletera salt marshes, where long confinement periods lead to scarcity of inorganic nutrients and dominance of heterotrophic nano- and microplankters (López-Flores *et al*., 2006). Expt 1 was carried out in the autumn (November 2003) and Expt 2 in the spring (May 2004) in order to include most potential pey types of *C. aquaedulcis* in these lagoons during two periods of different environmental conditions (flooding and confinement) (López-Flores *et al*., 2006).

Copepods were collected using a plankton net (50 µm mesh size). Twenty-two Winkler bottles were filled with 250 ml of ambient water filtered through 50 µm mesh, and then different stages of *C. aquaedulcis* were added. We checked that ciliates and chain-forming diatoms were not retained in the prefilters. Due to the difficulty in separating live individuals of each stage we performed the following grouping of stages: nauplii (from NII to NVI); copepodites (from CI to CV); and adults. Size and biomass ranges of each developmental stage are shown in (Table 1). Subsequent to identification under a microscope, nauplii, copepodites and adults were separated into groups of 30, 6 and 2 ind., respectively, and pipetted into distinct bottles. These proportions were equivalent to the natural densities of *C. aquaedulcis* in these lagoons. We incubated 5 bottles for each group and 5 controls. The bottles were incubated in the field for 24 h under natural conditions of temperature and light, corresponding to the sampling depth (10 to 15 cm). Two bottles without copepods were fixed immediately immediately using Lugol's iodine. After the 24 h incubation, copepod mortality was checked. The samples were then fixed with Lugol's iodine for microplankton taxonomic identification and cell-

counting with an inverted microscope, and stored in darkness prior to measurements. Bacterioplankton and autotrophic pico- and nanophytoplankton samples were filtered through 50 µm mesh, fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration) and immediately deep frozen in liquid nitrogen and stored frozen at -20°C. The abundance and biovolume were calculated with a FACSCalibur flow cytometer (BD Biosciences) with laser emitting at 488 nm (see López-Flores *et al*., 2006 for protocol details).

Table 1. *C. aquaedulcis*. Mean and range of size and dry weight of the different developmental stages of *C. aquaedulcis* in this study. N = 25 for each stage.

	Size (µm)		Dry weight (µg)	
Stage	Mean	Range	Mean	Range
Nauplii	215	110–440	0.12	0.02–0.49
Copepodites	729	290–1750	1.71	0.02–10.2
Adults	1212	900–1900	4.71	2.38–12.3

For heterotrophic pico- and nanoplankton taxonomic identification and cell-counting, 1 ml of sample fixed with glutaraldehyde was mixed by inversion and left to stain for 10 min with fluorochrome 4'-6-diamidino-2-phenylindole (DAPI; final concentration of 0.5 µg ml^{-1}). Then it was carefully filtered through a 0.2 µm polycarbonate filter (Millipore, Isopore membrane filters). Subsequently, filters were mounted on a glass slide and examined by epifluorescence microscopy with a UV excitation filter block and 1000× oil immersion, and more than 300 ind. were enumerated. By using this procedure, it was possible to locate and differentiate the heterotrophic from the autotrophic pico- and nanoplankton by visualizing the DAPI-stained nuclei (blue) and the chlorophyll*a* autofluorescence (red), respectively (Porter & Feig, 1980).

Biovolumes of microplankton, nano- and picoplankton were calculated from measurements of linear dimensions of cells under the inverted microscope or by means of cytometry using appropriate geometric formulae (Hillebrand *et al*., 1999). Carbon biomass was estimated using the equations of Lee & Fuhrman (1987) for bacterioplankton; Verity *et al*. (1992) for picoplankton and nanoplankton; Menden-Duer & Lessard (2000) for diatoms, chlorophytes and dinoflagellates; and Putt & Stoecker (1989) for ciliates.

Grazing coefficient, selectivity coefficient and ingestion rate

The grazing coefficient, selectivity and ingestion rate were calculated for each planktonic food type and for each food size group. In the first approach, the potential planktonic food was classified into food types according to taxonomy. We only used food types that were abundant enough to calculate the grazing coefficient, selectivity and ingestion rates and discarded those food types that were only occasionally present in some replicas. In the second approach, the potential planktonic food was subdivided into 3 prey size groups following the accepted decadal size classification: picoplankton (0 to 2 μm); nanoplankton (2 to 20 μm); and microplankton (20 to 50 μm). All prey size groups were based on the longest linear dimensions of planktonic organisms.

Grazing coefficient g (d^{-1}) was calculated according to Frost (1972):

$$g = \mu - \frac{\ln C_1^* - \ln C_0^*}{t_1 - t_0} \quad \text{with} \quad \mu = \frac{\ln C_1 - \ln C_0}{t_1 - t_0}$$

where μ is the gross growth rate of food organisms, C_1 and C_0 are the food concentrations at the end (t_1) and at the beginning (t_0) of the experiment in the controls, and C_1^* and C_0^* are the food concentrations in treatments with copepods.

Selectivity (*W'*) was calculated using the normalized selectivity coefficient *W'* defined by Vanderploeg & Scaria (1979) and modified after Vanderploeg *et al*. (1984):

$$W' = \frac{g_i}{g_{\max}}$$

where g_i is the grazing coefficient reached by a certain prey class and $g_{\max}$ is the grazing coefficient for the most preferred prey class ($0 < W' < 1$).

Similarly to the Katechakis *et al*. (2004) procedure, a *t*-test was used to test whether grazing coefficients (g) were significantly different from 0, if so, *W'* values were calculated. To test the possible effect of trophic cascade (Broglio *et al*., 2004), we compared growth rates in control and experimental bottles (*t*-test), so as to detect cases in which prey growth in control bottles was significantly lower than in experimental bottles, suggesting trophic cascade effects were masking grazing.

Clearance rate F (ml $ind.^{-1}$ d^{-1}) and ingestion rate I (pgC $ind.^{-1}$ d^{-1}) were calculated using the equations of Frost (1972). Following Nejstgaard *et al*. (1997), negative clearance rates were interpreted as zero ingestion.

RESULTS

Prey characteristics

Available food in the 2 experiments was relatively different (Table 2). In both experiments picoplankton was composed of bacterioplankton, auto- (APF) and heterotrophic (HPF) picoflagellates. The nanoplankton of Expt 1 included autotrophic nanoflagellates (ANF; chrysophytes and cryptophytes) and chlorophytes and diatoms of less than 20 μm in size. In Expt 2, nanoplankton included ANF and haptophytes. The microplankton of Expt 1 was composed of diatoms (*Navicula* sp., *Nitzschia* spp.) and chlorophytes between 20 and 49 μm in size. In Expt 2, microplankton was composed of autotrophic dinoflagellates (AD; *Glenodinium foliaceum*), heterotrophic dinoflagellates (HD; *Oxyrrhis marina*) and ciliates (*Strombidium* sp.).

In Expt 1, small size preys were the most dominant. Bacterioplankton, APF and HPF were the most abundant prey in terms of number cells and biomass (Table 2). In Expt 2, the most abundant prey were bacterioplankton and APF in terms of numbers of cells, and APF followed by HPF, ciliates, haptophytes and ANF in terms of biomass.

Ingestion rates

C. aquaedulcis showed an omnivorous feeding strategy, with bacterioplankton, autotrophic and heterotrophic phytoplankton and ciliates occurring in their diet (Figures 1 and 2). Individual clearance rates ranged from 1.1 ml d^{-1} for nauplii to 119 ml d^{-1} for adults (Table 3). In some cases, the high variability between replicates resulted in clearance and ingestion rates higher than 0 but grazing coefficients not significantly different from 0 (Tables 3 and 4). Consequently, although represented in Figures 1 and 2, we opted not to consider these ingestion rates in the analyses.

In Expt 1 (Figure 1A), all developmental stages had their highest ingestion rates on HPF (up to 0.14 μgC $ind.^{-1}$ d^{-1}). Nauplii were the only stage that consumed APF while copepodites showed some ingestion of ANF and chlorophytes. The width of the prey size spectra tended to increase with developmental stage, with adults having the widest prey size spectrum: apart from HPF, they showed also high ingestion of ANF, diatoms and chlorophytes.

In Expt 2 (Figure 1B), nauplii again presented maximum ingestion rates of HPF, but copepodites and adults showed their maximum ingestion rates for ciliates. Nauplii

Table 2. Mean (range) of size, volume and carbon content of all food types found in ambient water and offered as food in the experiments. The density and percentage of biomass of each food type in the initial conditions are also shown. APF: autotrophic picoflagellates; HPF: heterotrophic picoflagellates; ANF: autotrophic nanoflagellates; AD: autotrophic dinoflagellates; HD: heterotrophic dinoflagellates. C: cytometer; M: inverted microscopy; D: DAPI.

			Size (µm)		Volume (µm^3)		Carbon content (pgC cell^{-1})		Initial densities (cell ml^{-1})		Biomass percentage
Experiment	**Food type**	**Methodology**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**SE**	
Expt 1	Bacterioplankton	C	0.73	0.58–0.88	0.22	0.10–0.35	0.08	0.04–0.11	$2.81\cdot10^6$	$7.20\cdot10^5$	25.1
	APF	C	1.9	1.5–2.2	3.6	1.92–5.89	1.7	0.9–2.7	$3.47\cdot10^5$	$2.55\cdot10^4$	36.5
	HPF	D	2.0	1.7–2.1	4.1	2.6–4.99	2.0	1.2–2.3	$2.32\cdot10^5$	9147	38.1
	ANF	C	5.7	5.4–6.2	97.4	80.8–122	22.5	19.2–27.4	92.3	16.30	0.16
	Chlorophytes	M	30	12–49	892	153-$4.13\cdot10^3$	125	24–538	1.45	0.30	0.02
	Diatoms	M	30	6–49	$1.78\cdot10^3$	18–$3.69\cdot10^4$	104	3–$1.46\cdot10^3$	52.1	7.20	0.17
Expt 2	Bacterioplankton	C	0.34	0.28–0.51	0.02	0.01–0.07	0.01	0.01–0.02	$4.47\cdot10^5$	$1.08\cdot10^6$	0.73
	APF	C	2.6	2.4–2.7	8.8	6.9–9.9	4.2	3.2–4.7	$9.39\cdot10^5$	$2.19\cdot10^5$	77.7
	HPF	D	2.0	1.7–2.1	4.2	2.6–4.9	2.0	1.2–2.3	$1.71\cdot10^5$	$1.31\cdot10^5$	8.42
	ANF	C	5.9	5.5–6.4	109.9	89.3–134	25.0	20.3–29.7	$6.12\cdot10^3$	$1.32\cdot10^3$	2.37
	Haptophytes	C	11.7	10.6–15.7	871.4	614–$2.01\cdot10^3$	148.4	110–307	347	15.1	4.36
	AD	M	23	11–35	$6.12\cdot10^3$	611–$2.18\cdot10^4$	934	145-$2.72\cdot10^3$	60.2	0.00	0.55
	HD	M	24	17–33	$2.96\cdot10^3$	$1.68\cdot10^3$-$6.22\cdot10^3$	522	333–973	60.2	0.00	0.40
	Ciliate	M	22	12–42	$3.30\cdot10^3$	479-$1.44\cdot10^4$	1074	182–$4.57\cdot10^3$	360	46.5	5.44

did not consume ciliates. Copepodites had the widest prey size spectrum indicated by high ingestion rates for HPF, whereas adults did not consume HPF in this experiment. All stages consumed AD and HD and none of the stages consumed APF, ANF or haptophytes. Indeed in most of these cases (Table 3) the growth rate of APF, ANF and haptophytes in the treatment bottles was significantly higher than in the control bottles.

Results of ingestion rates based on food size groups (Figure 2A, B) showed that adults consumed mainly large prey: the highest ingestion rates in both experiments were for microplankton, and they also consumed nanoplankton in Expt 1. Copepodites showed the highest ingestion rates for picoplankton in both experiments but, while in Expt 1 they fed also on nanoplankton, in Expt 2 they ate microplankton. Nauplii preyed on the smallest sizes (picoplankton) in Expt 2, while in Expt 1 *g* was not significantly different from 0.

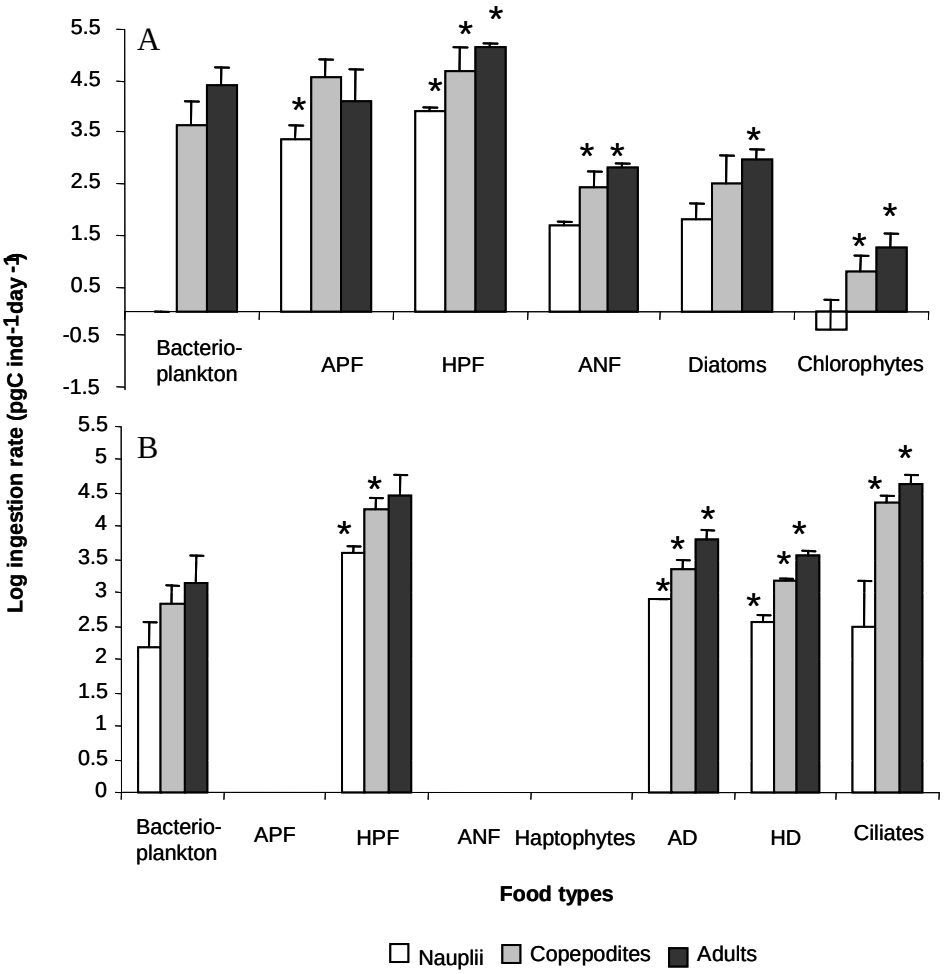


Fig. 1. *C. aquaedulcis.* Mean ingestion rates of the different developmental stages of *C. aquaedulcis* as a function of food type. (A) Expt 1, (B) Expt 2. *cases in which *g* was significantly different from 0; APF: autotrophic picoflagellates; HPF: heterotrophic picoflagellates; ANF: autotrophic nanoflagellates; AD: autotrophic dinoflagellates; HD: heterotrophic dinoflagellates; Error bars: +SE.

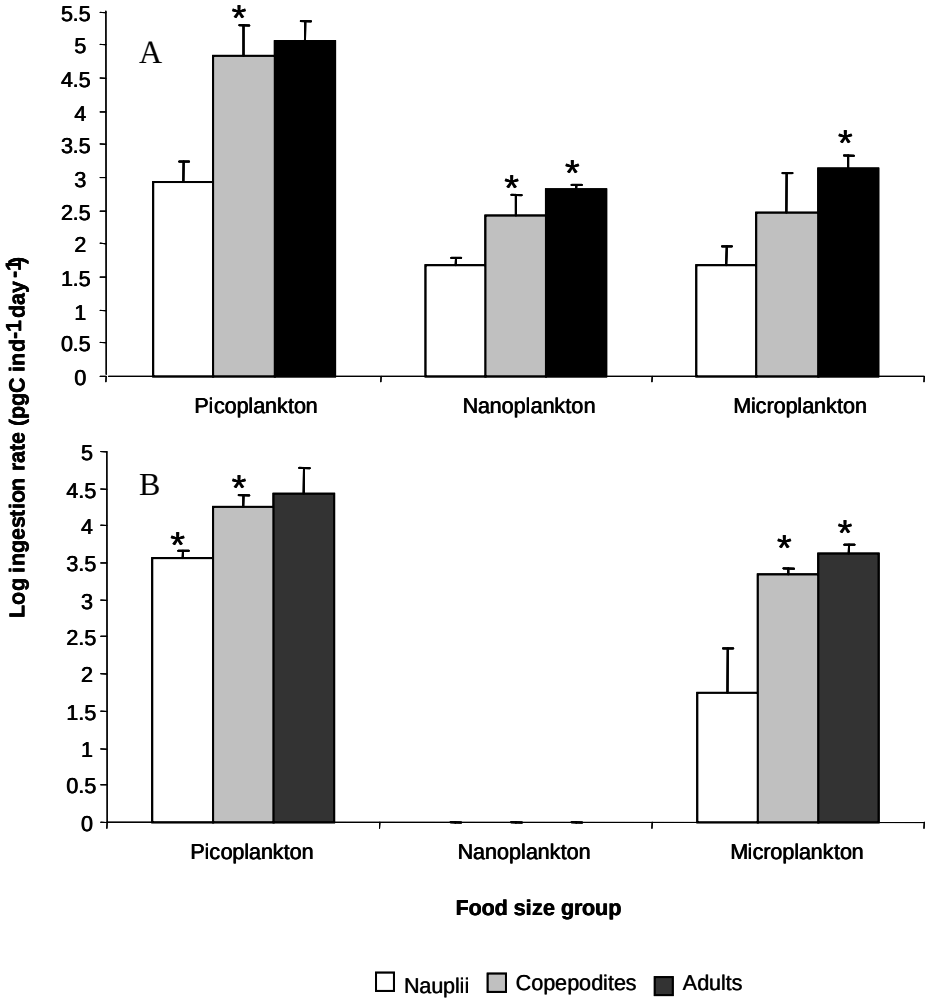


Fig. 2. *C. aquaedulcis.* Mean ingestion rates in the different treatments as a function of food size groups. (A) Expt 1, (B) Expt 2. *cases in which *g* was significantly different from 0.

Selectivity

The *W'* selective coefficients were different among nauplii, copepodites and adults and also between the 2 experiments (Table 3). During Expt 1, all stages showed maximum selectivity coefficients for HPF. Adults and copepodites had also high selectivity coefficients for chlorophytes (between 20 and 49 μm) and ANF. Adults also selected diatoms. Nauplii selected HPF and to a lesser extent, APF. In Expt 2, all stages had higher selectivity coefficients for HD than for HPF and only nauplii and copepodites showed some selection for HPF. Indeed, the most preferred prey for copepodites and adults were HD while nauplii selected mainly AD. Ciliates were selected by copepodites and adults but not by the nauplii.

When analyzing *W'* coefficients by means of the food size groups (Table 4), differences among copepod stages increased. In both experiments, adults showed the highest preference for microplankton and in Expt 1 they also selected nanoplankton. Copepodites selected mainly nanoplankton but also picoplankton in Expt 1 and picoplankton and microplankton in Expt 2. Nauplii did not select any prey size in Expt 1 even though they showed a high selection for picoplankton in Expt 2.

Table 3. *C. aquaedulcis*. Mean (SE) grazing coefficient *g* (d^{-1}), selectivity coefficient *W'* and clearance rate *F* (ml $ind.^{-1}$ d^{-1}) for each food type and for each *C. aquaedulcis* developmental stage in both experiments. Only values significantly different from 0 are indicated. *p < 0.05; **p < 0.01. [a]Prey number in the control bottles is significantly lower than in the experimental bottles (*t*-test, p < 0.05), suggesting possible trophic cascade effects. Acronyms as in Table 2.

		Nauplii			**Copepodites**			**Adults**		
Experiment	**Food types**	***g*** **(d^{-1})**	***W'***	***F*** **(ml $ind.^{-1}$ d^{-1})**	***g*** **(d^{-1})**	***W'***	***F*** **(ml $ind.^{-1}$ d^{-1})**	***g*** **(d^{-1})**	***W'***	***F*** **(ml $ind.^{-1}$ d^{-1})**
Expt 1	Bacterioplankton	0	0	0	0	0	0	0	0	0
	APF	0.13* (0.04)	0.21	1.06 (0.36)	0	0	0	0	0	0
	HPF	0.60* (0.18)	1.00	5.02 (1.57)	0.98** (0.27)	1.00	50.2 (26.5)	0.95** (0.04)	1.00	119 (11.9)
	ANF	0	0	0	0.67** (0.15)	0.69	31.8 (10.8)	0.42* (0.11)	0.50	51.5 (18.3)
	Diatoms	0	0	0	0	0	0	0.93* (0.31)	0.97	86.2 (22.8)
	Chlorophytes	0	0	0	0.70** (0.21)	0.92	24.4 (7.38)	0.87* (0.30)	0.96	70.0 (22.2)
Expt 2	Bacterioplankton	0	0	0	0	0	0	0	0	0
	APF	0	0	0	0	0	0	0[†]	0	0
	HPF	0.27* (0.04)	0.31	3.93 (0.54)	0.43* (0.11)	0.70	21.5 (6.93)	0	0	0
	ANF	0[a]	0	0	0[a]	0	0	0[a]	0	0
	Haptophytes	0[a]	0	0	0[a]	0	0	0[a]	0	0
	AD	0.85* (0.23)	1.00	10.7 (2.90)	0.48* (0.13)	0.78	22.2 (8.81)	0.52* (0.16)	0.89	63.5 (20.9)
	HD	0.64* (0.26)	0.66	7.07 (1.48)	0.84* (0.00)	1.00	28.0 (1.47)	0.84* (0.00)	1.00	70.0 (6.36)
	Ciliates	0	0	0	0.35* (0.02)	0.58	15.7 (1.37)	0.23* (0.04)	0.38	25.2 (4.48)

Table 4. *C. aquaedulcis.* Mean (SE) grazing coefficient *g* (d^{-1}), selectivity coefficient *W'* and clearance rate *F* (ml $ind.^{-1}$ d^{-1}) for each food size group and for each *C. aquaedulcis* developmental stage in both experiments. Only values significantly different from 0 are indicated. *p < 0.05; **p < 0.01. [a]Prey number in the control bottles is significantly lower than in the experimental bottles (*t*-test, p < 0.05), suggesting possible trophic cascade effects

		Nauplii			**Copepodites**			**Adults**		
Experiment	**Food size groups**	***g*** **(d^{-1})**	***W'***	***F*** **(ml $ind.^{-1}$ d^{-1})**	***g*** **(d^{-1})**	***W'***	***F*** **(ml $ind.^{-1}$ d^{-1})**	***g*** **(d^{-1})**	***W'***	***F*** **(ml $ind.^{-1}$ d^{-1})**
Expt 1	Picoplankton	0	0	0	0.24* (0.06)	0.03	13.86 (6.27)	0	0	0
	Nanoplankton	0	0	0	0.61** (0.14)	1.00	31.79 (10.8)	0.42* (0.11)	0.45	51.5 (18.3)
	Microplankton	0	0	0	0	0	0	0.92** (0.30)	1.00	312 (64.1)
Expt 2	Picoplankton	0.25* (0.05)	1.00	3.15 (0.60)	0.38* (0.08)	1.00	18.68 (5.07)	0	0	0
	Nanoplankton	0[a]	0	0	0[a]	0	0	0[a]	0	0
	Microplankton	0	0	0	0.36* (0.03)	0.95	16.14 (1.87)	0.25* (0.04)	1.00	27.7 (5.28)

DISCUSSION

Results of this study show that the diet of *C. aquedulcis* is diverse, which is in accordance with previous findings for other calanoid species (Kleppel, 1993). Furthermore, differences in the ingestion rates of certain prey between the 2 experiments support the hypothesis of a flexible feeding behaviour that may be modified with variability in the food environment. For example, in the presence of ciliates and HD and AD, adults did not ingest HPF.

Omnivory was observed in all stages of *C. aquaedulcis*. HPF were the most ingested and one of the most preferred prey items. Ciliates were also ingested in large quantities by copepodites and adults, and HD were selected by all stages. Previous findings also reported that copepods can ingest ciliates and HD at higher rates than phytoplankton, and may preferentially select the former (Stoecker & Egloff, 1987; Sanders & Wickham, 1993; Nejstgaard *et al.*, 1997). This fact has been attributed to the high nutritional value of ciliates and heterotrophic flagellates since they can produce essential copepod growth compounds (unsaturated fatty acids and/or sterols) that are not always found in phytoplankton (Oman & Runge, 1994) and they are relatively rich in

nitrogen and phosphorous (Sanders & Wickham, 1993). The mixture of phytoplankton and ciliates has been found to be the most favourable for development, growth and egg production for some copepods (Bonnet & Carlotti, 2001); however, this is not always the case, since the presence of ciliates and heterotrophic dinoflagelates in the diet may sometimes not provide copepods with an adequate nutrition for long-term survival (Koski *et al*., 1998). Our results showed that in the absence of ciliates and HD, *C. aquaedulcis* ingested high amounts of HPF. However, when different types of heterotrophic prey were present, adults of *C. aquaedulcis* preferred ciliates and HD to HPF, while copepodites ingested all 3 at similar rates.

According to our results, none of the developmental stages of *C. aquaedulcis* significantly prey on bacteria. Usually, bacterioplankton was considered too small to be efficiently ingested by most adult copepods, but nauplii of some species have been shown to feed upon bacterioplankton (Turner & Tester, 1992) and some feeding on free-living bacteria has been recorded in adults (Boak & Goulder, 1983).

In Expt 1, significant ingestion of APF and ANF was recorded, while in Expt 2 an increase of these prey items in experimental bottles with respect to control ones was observed. This could be due to trophic cascade effects, which have been observed in other feeding behaviour studies dealing with the whole size spectrum (Broglio *et al*., 2004; López-Flores *et al*., 2006). Grazing of *C. aquaedulcis* on ciliates and dinoflagellates, both of which consume small particles (Broglio *et al*., 2004), could result in a decrease in the grazing mortality of APF, ANF and haptophytes with a consequent increase in their growth rate. The fact that the increase in APF, ANF and haptophytes was only observed in Expt 2 when ciliates and dinoflagelates were present supports this hypothesis.

This study shows differences in the diet of the developmental stages of *C. aquaedulcis* due both to the size and type of prey. In general, the size of the ingested prey increases with an increasing size of the developmental stage, which is in accordance with other studies (Poulet, 1977; Berggreen *et al*., 1988). While *C. aquadulcis* adults had high ingestion rates and selection coefficients for large prey (micro- and nanoplankton), nauplii preferentially removed small prey (picoplankton). Copepodites showed the widest prey size range, including pico-, nano- and microplankton. Nevertheless, the lowest size limit of captured particles was similar for all stages (between 1.7 and 2.1 μm), and is similar to what has been found for other species: e.g. between 2 and 4 μm for all developmental stages of *Acartia tonsa*

(Bergreen *et al*., 1988), 1 μm for *Eurytemora affinis* copepodites and adults (Burkill & Kendall, 1982), 3 μm in *Pseudodiaptomus marinus* nauplii and copepodites (Uye & Kasahara, 1983) and 1.5 μm in *Pseudocalanus minutus* copepodites and adults (Poulet, 1977).

Studies dealing with the size spectra that each stage is capable of capturing have obtained contradictory results. Some authors have stated that nauplii are unable to capture prey effectively at the extreme ends of the size spectrum (Fernandez, 1979; Paffenhöfer & Lewis, 1989) since they do not develop mature feeding appendages until copepodid stage Cl (Björnberg, 1986). For example, nauplii of *Eucalanus* sp. were not able to capture small prey as efficiently as later stages (Paffenhöfer & Lewis, 1989) and nauplii of *Calanus helgolandicus* could not consume large diatoms that were fed upon by adults (Mullin & Brooks, 1967). In contrast, nauplii of *A. tonsa* were more efficient than adults in capturing small prey (Bergreen *et al*., 1988). Additionally, some studies have documented ingestion of protozoan microplankton by copepod nauplii (Fessenden & Cowles, 1994). For example, nauplii of *A. tonsa* and *Eurytemora* may ingest ciliates up to 40 μm (Stoecker & Egloff, 1987) and 35 μm (Merrell & Stoecker, 1998) in size, respectively. According to our results, *C. aqueadulcis* nauplii were not able to capture larger prey (chlorophytes, diatoms and ciliates) which were readily consumed by adults, but they did consume dinoflagellates between 11 and 35μm in size. Both copepodites and adults ingested prey within the same range, i.e.: 1.7 to 49 μm. Although it is generally reported that copepods tend to be inefficient filtering particles smaller than 5 to 10 μm (e.g. Berggreen *et al*., 1988), several authors have documented a significant ingestion of particles <5 μm by adults and copepodites (Boak & Goulder, 1983; Nejstgaard *et al*., 1997, Broglio *et al*., 2004). Nevertheless, the high preference for small cells found in copepodites and adults in this study appears not to be previously documented. It might be explained by the fact that, as already reported in other costal waters (Gasparini & Castel, 1997), in the Empordà wetlands, the largest particles were scarce compared to the large amount of smaller particles. In such conditions, Richman *et al*. (1977) demonstrated that copepods graze predominantly on small size prey, probably because they shift their grazing pressure to the size where the peak concentration of particles occurs (Poulet, 1977). This could explain the high ingestion of picoplankton in Expt 1 where larger particles were almost non-existent.

The different *C. aquaedulcis* life stages showed a different selective behaviour since the size of the selected prey increased with increasing size of the stage. According

to our selectivity metrics, prey type also determined selective behaviour since, in general, all stages showed a preference for heterotrophic forms among preys of similar size. These results would confirm the previous studies that show that adult copepods are able to select between particles of the same size but different nutritive value: plastic beads versus phytoplankton (Fernandez, 1979), fast-growing versus senescent cells or different growing states of the same species (Koski *et al.*, 1998) or toxic versus non-toxic strains of the same or similarly sized algae (Turriff *et al*., 1995). In contrast, Meyer *et al*. (2002) showed similar selection behaviour among stages of *Calanus* spp. and only depending on size.

These differences in feeding among developmental stages of *C. aquaedulcis* might be related to the changes in the amino acid composition during the life cycle of this copepod found in a previous study in the same lagoons (Brucet *et al*., 2005). Indeed, several studies have shown a high variation in the elemental composition during the life cycle of calanoids (e.g. Carrillo *et al.*, 2001), which would agree with ontogenic changes in their diet. On the other hand, the ability to partition the available food among the different developmental stages would represent an advantage when food is scarce since it reduces intraspecific competition. This could be the reason why *C. aquaedulcis* is able to dominate the zooplankton community for several weeks during spring and summer even in situations of low nutrient content (Quintana *et al*., 1998; Brucet *et al*., 2006 and references therein). The dietary differences among developmental stages have already been described to be important for reducing competition in environments where there is a scarcity or high temporal variability of food resources (Poulet, 1977).

In summary, *C. aquaedulcis* is feeding omnivorously on a wide spectrum of natural food particles and its diet can change throughout ontogeny and also with food availability. As previously reported for other copepod species (e.g. Poulet, 1977), early stages of *C. aquaedulcis* can have feeding niches partially separated from the adults. Hence, further studies should take into account the intraspecific variability in the feeding behaviour of copepod species in order to fully understand the mechanisms that structure pelagic food webs.

Chapter 4

Impact of different developmental stages of *Daphnia magna* Straus on the plankton community under different trophic conditions

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ABSTRACT: In situ 24-hour incubation experiments were performed to analyse the grazing effects of *Daphnia magna* on a planktonic microbial community. Three field grazing experiments under different nutrient concentrations were carried out on treated effluents of a wastewater treatment plant. The grazing effects of three different *D. magna* size classes (small (0.6 – 1.6 mm), medium (1.7 – 2.5 mm) and large individuals (2.6 – 3.7 mm)) were compared. The different sizes classes had similar effects on the plankton community. However, our results showed big differences in effects among experiments. Our findings suggest that, in spite of *D. magna*'s non-selective feeding behaviour and the fact that different developmental stages (i.e. its size) had similar effects on the microbial planktonic community, these effects can differ according to the initial structure and composition of the community and the resulting cascading trophic interactions. Moreover, *D. magna* effects can be direct, through grazing (as is the case with ciliates), or indirect through trophic cascade interactions (as is the case with bacteria).

Key words: *Daphnia*, Feeding, Ingestion, Trophic cascade, Wastewater, Ontogenetic diet shift.

INTRODUCTION

Many studies describe *Daphnia* spp. as a non-selective grazer (e.g. DeMott, 1988; Freyer, 1991) with an omnivorous diet that feeds on a wide range of particle types (e.g. Lampert, 1987; Jürgens, 1994). *Daphnia* consume a set of microorganisms integrated in a complex microbial community, composed of several species and their trophic interactions. Thus, *Daphnia* as consumers have the potential to modify the relative abundances of species and, thereby, affect the trophic interactions between them, as well as the structure of the food web (Zöllner *et al*., 2003). These effects may be caused by direct grazing, but also by indirect cascading interactions if the *Daphnia* remove smaller grazers such as ciliates or heterotrophic flagellates (Muylaert *et al.*, 2006).

Although the feeding ecology of *Daphnia* has been widely studied, there is some controversy surrounding its possible effect on lower trophic levels, such as bacteria. Some studies have determined that the filter mesh size of *Daphnia magna* is smaller than 1 μm and that they can therefore potentially feed on bacteria (Geller & Müller, 1981). Conversely, other studies have concluded that other species of *Daphnia* do not consume organisms smaller than 1 μm (Brooks & Dodson, 1965) or are inefficient at capturing

bacteria-sized particles (Pace *et al.*, 1983; Sanders *et al*., 1989). Besides this possible direct effect of *Daphnia* on bacteria through feeding, indirect trophic cascade effects have also been described (Zöllner *et al.*, 2003). Knowledge of these direct or indirect effects may be significant in wastewater management, since the use of *Daphnia* spp. has been considered as a promising tool for particle reduction in wastewater treatment plants (Groot, 1998; Rosenkranz, 2001).

Evidence of the different trophic effects of *Daphnia* spp. on some zooplankton communities with different body sizes is well known (e.g. Tessier *et al*., 2001). However, these comparisons have usually been made using different *Daphnia* spp. (Tessier *et al*., 2001; DeMott *et al*., 2001), and few of them have compared a single species at different developmental stages (e.g. Balayla & Moss, 2004). The information available on diet variation in *Daphnia* spp. at different developmental stages is confusing. Vanni and Lampert (1992) found differences between adults and juveniles of *Daphnia galeata* in terms of their ability to efficiently assimilate *Oocystis* sp., while Boersma (1995) found overlap in the use of resources between adults and juveniles of the same species. A microbial community's final structure and composition might change depending on whether the effects of grazing by the different development stages of *Daphnia* are similar or not.

We performed an *in situ* experimental approach to investigate the possible effects of *D. magna* on a plankton microbial community. The study was performed under different trophic conditions and the effects of the different developmental stages of *D. magna* were compared. We hypothesise that even a non-selective feeding organism such as *Daphnia* may significantly modify the structure of a microbial community through cascading trophic interactions, which, depending on the community's initial structure, will lead to different outcomes. Moreover, if the different sizes of *D. magna* have a similar diet, they will not have different effects on the microbial community.

MATERIALS AND METHODS

Study site

The study was performed at a wastewater treatment plant (hereafter WWTP) and a constructed wetland system in Empuriabrava (Empordà, NE Spain, 42º14'36"N, 3º6'29"E). Empuriabrava is an urban area under heavy pressure from tourism during

vacation periods. This WWTP (Fig. 1) was designed to treat of 8750 m^3 day^{-1} of urban wastewater (35000 equivalent inhabitants). After organic matter and nutrient reduction in the treatment plant, which has extended aeration and polishing lagoons, the wastewater is circulated through a constructed wetland system in order to reduce the concentration of inorganic nutrients.

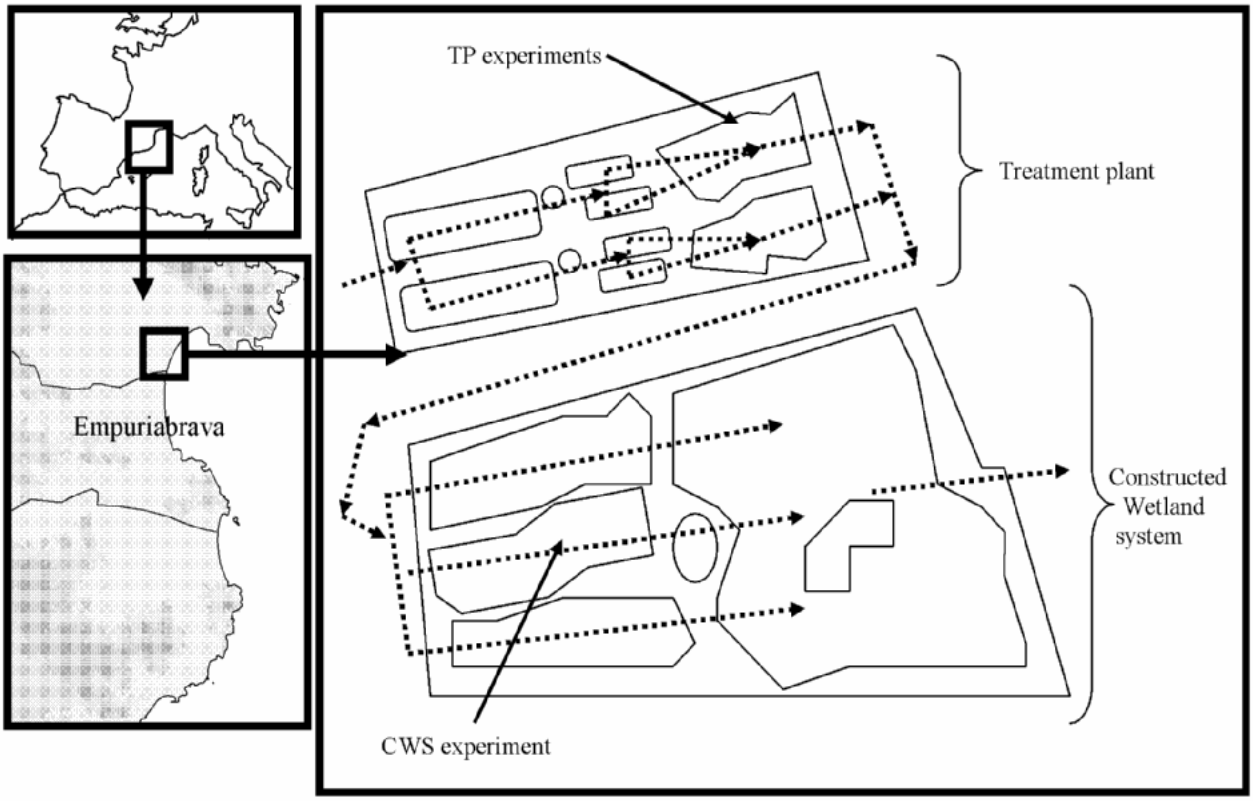


Fig. 1. Sketch map of the Empuriabrava wastewater treatment plant and constructed wetland system where the different experiments were performed. Discontinuous arrows indicate the water flow.

Three grazing field experiments were performed under different conditions representative of the wide range of trophic conditions in a wastewater treatment system, and in which *D. magna* is the dominant zooplankton organism. The first and second experiments were carried out in the WWTP polishing lagoons after secondary treatment. The first (hereafter referred to as the TP1 experiment) was performed before the Easter holidays (April 2006) when anthropic pressure on the area was lower (approximately 13000 inhabitants) and the second (hereafter referred to as the TP2 experiment) was performed after the Easter holidays when the population was close to 20000 inhabitants. The third experiment (hereafter referred to as the CWS experiment) was carried out in the constructed wetland system in summer (August 2005) during the peak vacation

period when there were approximately 39000 inhabitants in the area (the number of inhabitants was calculated using measured flows in the WWTP during the experiments, and per capita mean daily water consumption in Catalonia). In this way we were able to ensure that the three experiments corresponded to three conditions typical of wastewater treatment ecosystems where *D. magna* is especially abundant: conditions immediately after secondary treatment under low or high nutrient load (the TP1 and TP2 experiments respectively), and conditions characteristic of a constructed wetland, where low organic particle and high inorganic nutrient concentrations are expected (the CWS experiment).

Experimental set-up, feeding experiments and sample processing

Twenty-two Winkler bottles were filled with 250 ml of wastewater previously filtered through a 50 µm mesh, thereby assuring the presence of planktonic assemblages found in the field. *D. magna* were collected *in situ* (the constructed wetland system in the CWS experiment and the treatment plant in the TP experiments) using a plankton net (50 µm mesh size). They were identified and separated under a stereomicroscope into three groups according to size: small individuals (0.6-1.6 mm; mean biomass per bottle: 84 µgC), medium individuals (1.7-2.5 mm; mean biomass per bottle: 201 µgC), and large individuals (2.6-3.7 mm; mean biomass per bottle: 249 µgC). We considered these three size classes to be different developmental stages. The cladoceran were pipetted into experimental bottles in groups of eight small individuals (small-*Daphnia* treatment), four medium individuals (medium-*Daphnia* treatment), and two large individuals (large-*Daphnia* treatment). These proportions are similar to those in other grazing *Daphnia* studies such as Gilbert (1989) and DeMott *et al*. (2001).

The experiment consisted of five bottles with no *D. magna* (controls) and five bottles for each treatment. Additionally, two bottles with no *D. magna* were fixed immediately at the beginning of the incubations to provide the initial conditions. The bottles were incubated in the field for 24 h under conditions of temperature and ligh, naturally corresponding to the sampling depth (10-15 cm). Dissolved inorganic nutrients (ammonia, nitrite, nitrate and SRP) were analyzed in the WWTP laboratory. Water temperature, electrical conductivity (EC_{25}), pH and dissolved oxygen (O_2 in % of saturation) were measured *in situ* for each experiment before incubation.

After 24 hours, *D. magna* mortality was checked. A high mortality rate was observed (approximately 70%) in all the replicas of the small-*Daphnia* treatment in the

TP2 experiment. As a consequence, the results obtained with this treatment were disregarded and will not be discussed further.

Microplankton and large nanoplankton (>10 µm) were fixed with Lugol's iodine and stored under dark conditions until taxonomic identification and cell counting with an inverted microscope (Utermöhl, 1958). Bacterioplankton, pico- and nanophytoplankton samples were obtained after filtering through a 50 µm mesh, fixing with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration) and immediately deep frozen in liquid nitrogen and stored at -20ºC. Their abundance and biovolume were obtained using a flow cytometer (FACScalibur of Becton and Dickinson) with a laser emitting at 488 nm (for details and method, see López-Flores *et al*., 2006).

The taxonomic identification and cell counting of heterotrophic pico- and nanoplankton were carried out with a sample fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), mixed by inversion and left to stain for 10 minutes with fluorochrome 4'-6-diamidino-2-phenylindole (DAPI; final concentration of 0.5 µg ml^{-1}). After this, the stained sample was carefully filtered through a 0.2 µm polycarbonate filter (Millipore, isopore membrane filters). The filters were then mounted on a glass slide and examined by epifluorescence microscopy with a UV excitation filter block at 1000x with oil immersion. More than 300 individuals were counted (Liu *et al.*, 2005). Using this procedure, it was possible to locate and differentiate between heterotrophic from autotrophic pico- and nanoplankton by visualizing the DAPI-stained nuclei (blue) and the Chl-*a* autofluorescence (red) respectively (Porter & Feig, 1980).

Microplankton and large nanoplankton biovolumes were calculated from measurements of the linear dimensions of cells under the inverted microscope, using appropriate geometric formulae (Ruttner-Kolisko, 1977; Hillebrand *et al*., 1999). Amoebae and ciliate biovolumes were estimated by approximation of the body shape to geometric figures. Autotrophic nanoplankton and picoplankton biovolumes were calculated from flow cytometry measurements of the linear dimensions of cells using a calibration curve as described elsewhere (Olson *et al.*, 1989; Chisholm, 1992; Rodríguez *et al.*, 2002; López-Flores *et al.*, 2006). Finally, the bacterioplankton biovolume was calculated using the equation described in Gasol and Del Giorgio (2000). Carbon biomass was estimated using the equations of Lee and Fuhrman (1987) for bacterioplankton, and Verity *et al*. (1992) for picoplankton and nanoplankton. For microplankton organisms, the equation of Menden-Deuer and Lessard (2000) was used

for diatoms and chlorophytes, while those of Strathmann (1967), Putt and Stoecker (1989) and Telesh *et al*. (1998) were used for protozoa, ciliates and rotifers respectively.

Selectivity coefficient

To obtain the selectivity coefficient, the planktonic organisms were classified two approaches: 1) by size groups and 2) by organism types. In the first approach, the organisms were subdivided into three size groups in accordance with the accepted decadal size classification: picoplankton (0-2 μm); nanoplankton (2-20 μm); and microplankton (20-50 μm). All three size groups were based on the organism's longest linear dimensions. In the second approach the organisms were divided according to a functional classification. Thus, pico- and nanoplankton were identified according to an autotrophic (presence of autofluorescent chloroplasts) or heterotrophic (absence of autofluorescent chloroplasts) strategy. Microplankton was classified according to taxonomy.

Selectivity (*W'*) was calculated to analyse the preferences of different size classes of *D. magna* for different organism types and different organism sizes. The normalised selectivity coefficient *W'* defined by Vanderploeg and Scavia (1979) and modified after by Vanderploeg *et al*. (1984) was used:

$$W' = \frac{g_i}{g_{\max}}$$

where g_i is the grazing coefficient obtained for the organism class (i) and g_{max} is the grazing coefficient for the most preferred organism class ($0<W'<1$). The grazing coefficient g (day^{-1}) was calculated, in accordance with Frost (1972), to ascertain if a *D. magna* treatment feeds a certain organism type or an organism size:

$$g = \mu - \frac{\ln C_1^* - \ln C_0^*}{t_1 - t_0} \quad \text{with} \quad \mu = \frac{\ln C_1 - \ln C_0}{t_1 - t_0}$$

Where μ is the instantaneous growth rate of the organism type or size (day^{-1}), C_1 and C_0 are the organism concentrations at the end (t_1) and at the beginning (t_0) of the experiment in the controls, and C_1* and C_0* are the organism concentrations in treatments with *D. magna*.

Data analysis

In order to establish the effects of *D. magna*, the values obtained in the different treatments were compared and tested (control, small-*Daphnia*, medium-*Daphnia* and

large-*Daphnia* treatments) in each of the experiments (CWS, TP1 and TP2 experiment). All the tests were performed using a multivariate approach based on a Principal Correspondence Analysis (PCA) coupled to between-groups analyses (Dolédec & Chessel, 1989) considering plankton community structure.

For each experiment, each treatment (small-, medium- and large-*Daphnia* treatment) was considered a group. The between-group analysis allowed us to obtain the centroid of each treatment and the differences among treatments were checked using an available Monte-Carlo permutation test (999 unrestricted permutations under reduced model). The null hypothesis of this test stated that the relative proportion of plankton species in biomass did not differ among groups. To check the significance of the *D. magna* effects, several additional analyses (PCA + between-group analysis) were performed in each experiment as post-hoc tests. To carry out these multivariate post-hoc tests, only samples and plankton assemblages included in the compared treatments were used. Thus, six post-hoc tests were performed for each experiment: control *vs.* small-*Daphnia* treatment; control *vs.* medium-*Daphnia* treatment; control *vs.* large-*Daphnia* treatment; small-*Daphnia* treatment *vs.* medium-*Daphnia* treatment; small-*Daphnia* treatment *vs.* large-*Daphnia* treatment; medium-*Daphnia* treatment *vs.* large-*Daphnia* treatment. The Dunn-Sidak procedure $(1-(1-\alpha)^{1/6})$, where α is the significance level for each test (0.05), was used to adjust the significance level for each test and decrease the Type I error (Quinn & Keough, 2002). All multivariate analyses (the dudi.pca function) and the calculation of inertia for each taxa (the inertia.dudi function) were performed with the ade4 library (Drayet *et al.*, 2007) written in R language.

RESULTS

Trophic conditions

The initial conditions of nutrient and planktonic composition were different in the three experiments. The TP2 experiment, carried out after the Easter vacation period, had the highest concentrations of ammonia and SRP and the lowest dissolved oxygen level. The TP1 and CWS experiments had similar nutrient concentrations, but the CWS had the highest dissolved oxygen level (Table 1). The plankton composition was also different, depending on the trophic conditions (Table 2).

Table 1. Physical and chemical water conditions during the three experiments

	Experiment		
	CWS	TP1	TP2
Temperature (ºC)	24.9	17.2	17.3
Conductivity (mS cm^{-1})	1.59	2.05	1.06
pH	7.20	6.91	8.83
% O_2	42.0	18.2	7.50
NH_4^+ (mg N l^{-1})	2.95	2.20	27.7
NO_2^- (mg N l^{-1})	0.41	0.03	0.03
NO_3^- (mg N l^{-1})	0.42	0.50	0.50
SRP (mg P l^{-1})	3.13	2.54	7.90

Selectivity

Our results showed no main selectivity patterns when the different experiments and *D. magna* size were taken into account. The *D. magna* selectivity coefficients (*W'*) changed according to the different potential preys present in the plankton communities (Table 3). In the CWS experiment, high selection coefficients were obtained for autotrophic organisms (*Scenedesmus* sp. in all treatments, and euglenophytes in the small-*Daphnia* treatment) and bacterioplankton in the medium-*Daphnia* treatment. *Scenedesmus* sp. was the main prey item selected by small- and large-*Daphnia* while bacterioplankton was the main organism type selected by medium-*Daphnia*. In the TP1 experiment bacterioplankton was the main organism type selected by all *Daphnia* sizes. In the TP2 experiment, there was a high selection of heterotrophic organisms (large nanoflagellates (LNF), amoebae, ciliates and *Lecane* sp.) in medium- and large-*Daphnia* treatments, with Amoebae being the most selected.

Similarly to the results obtained with the organism type approach, selectivity coefficients (*W'*) were different between *Daphnia* treatments and experiments using the organism size groups approach (Table 4). In the CWS experiment no *Daphnia* sizes had a selection by any size group (i.e. they did not show a positive grazing rate in any size group). However, in the TP1 experiment, picoplankton was the size group most selected

Table 2. Mean values of size, initial densities and biomass percentage of all organism types found in natural water. Minimum and maximum size values for size and standard error for initial densities are shown in brackets. These values were calculated from initial samples. Legend: APF: autotrophic picoflagellates; HPF: heterotrophic picoflagellates; ANF: autotrophic nanoflagellates, HNF: Heterotrophic nanoflagellates; LNF: large nanoflagellates. The method used for particle counting is also shown. C: cytometer; M: inverted microscopy; D: DAPI. See the Material and Methods sections for details.

			CWS Experiment		**TP1 Experiment**		**TP2 Experiment**	
	Methodology	**Size (µm)**	**Initial densities (ind ml^{-1})**	**Biomass percentage**	**Initial densities (ind ml^{-1})**	**Biomass percentage**	**Initial densities (ind ml^{-1})**	**Biomass percentage**
Bacterioplankton	C	0.50 (0.26-1.33)	$1.18 \cdot 10^7$ ($2.08 \cdot 10^6$)	11.28	$8.35 \cdot 10^5$ ($4.56 \cdot 10^5$)	2.37	$6.36 \cdot 10^5$ ($2.94 \cdot 10^4$)	0.18
APF	C	1.76 (1.47-1.97)	$1.55 \cdot 10^5$ ($2.84 \cdot 10^4$)	38.21	-	-	-	-
HPF	D	2.00 (1.40-2.10)	$3.07 \cdot 10^4$	16.70	$7.13. \cdot 10^3$ ($2.14 \cdot 10^3$)	9.56	$5.81 \cdot 10^4$ ($1.18 \cdot 10^4$)	1.10
ANF	C	4.06 (2.70-7.27)	$6.64 \cdot 10^3$ (221)	13.46	-	-	-	-
HNF	D	4.50 (3.84-4.54)	930	5.76	-	-	-	-
LNF	M	10.8 (4.89-22.0)	-	-	$2.03. \cdot 10^3$ (88.3)	44.4	865 (203)	1.19
Cryptophytes	M	11.8 (7.34-17.1)	162 (26.1)	2.21	-	-	-	-
Chlorophytes (*Oocistys* sp.)	M	15.0 (7.34-34.2)	14.5 (2.90)	1.11	75.3 (0.00)	3.24	388 (214)	0.37
Chlorophytes (*Scenedesmus* sp.)	M	16.0 (2.90-48.9)	2.90 (0.00)	0.91	-	-	8.69 (5.79)	0.01
Euglenophytes	M	20.0 (12.2-48.9)	99.7 (18.6)	7.75	-	-	-	-
Ciliates (*Strombidium* sp.)	M	26.0 (12.2-48.9)	2.90 (0.00)	1.17	20.3 (0.00)	17.6	425 (57.9)	5.48
Diatoms	M	29.9 (9.78-48.9)	195 (24.6)	1.45	52.1 (14.5)	1.46	220 (185)	0.09
Ciliates (*Suctoria* sp.)	M	30.5 (22.0-48.9)	-	-	5.79 (0.00)	3.56	-	-
Ciliates undet sp. 1	M	30.7 (14.7-48.9)	-	-	43.4 (11.6)	17.8	287 (43)	0.43
Amoebae undet. sp.	M	32.3 (22.0-48.9)	-	-	-	-	66.6 (5.8)	1.36
Ciliates undet. sp. 2	M	36.9 (26.9-48.9)	-	-	-	-	75.3 (72.4)	20.81
Rotifer (***Lecane*** sp.)	M	41.6 (31.8-48.9)	-	-	-	-	37.6 (26.1)	68.98
Total biomass (pgC ml^{-1})	-	-	-	$3.62 \cdot 10^5$	-	$1.47 \cdot 10^5$	-	$1.04 \cdot 10^7$

by medium- and large-*Daphnia* while there was no selection by small-*Daphnia*. In the TP2 experiment, nanoplankton was the size group most selected by medium- and large-*Daphnia*, and microplankton was also highly selected in the large-*Daphnia* treatment.

Table 3. Selectivity coefficient *W'* for organism type selected in each treatment in the three experiments. *W'*=1, highest selection for an organism type; *W'*=0, lowest selection for an organism type; –, no selection. LNF: large nanoflagellates. Results of the small-*Daphnia* treatment in TP2 experiment are not discussed (see material and methods section).

		Small-*Daphnia* treatment	**Medium-*Daphnia* treatment**	**Large- *Daphnia* treatment**
Experiments	**Organism types**	***W'***	***W'***	***W'***
CWS	Bacterioplankton	-	1.00	-
	Scendesmus sp.	1.00	0.85	1.00
	Euglenophytes	0.60	-	-
TP1	Bacterioplankton	1.00	1.00	1.00
	Suctoria sp.	-	-	0.11
TP2	LNF	-	0.62	0.40
	Oocistys sp.	-	0.23	0.44
	Amoebae	-	1.00	1.00
	Ciliate undet. sp.2	-	-	0.56
	Lecane sp.	-	0.44	0.47

Table 4. Selectivity coefficient, *W'* for each size group selected in each treatment in the three experiments. *W'*=1, highest selection for an organism type; *W'*=0, lowest selection for an organism type; –, no selection. In CWS experiment there were no positive grazing rate on any size group and, therefore there was not selectivity by any food size group. Results of the small-*Daphnia* treatment in TP2 experiment are not discussed (see material and methods section).

		Small-*Daphnia* treatment	**Medium-*Daphnia* treatment**	**Large- *Daphnia* treatment**
Experiment	**Food size groups**	***W'***	***W'***	***W'***
TP1	Picoplankton	-	1.00	1.00
TP2	Picoplankton	-	-	-
	Nanoplankton	-	1.00	1.00
	Microplankton	-	0.55	0.99

Community structure

The first two axes of the PCA in the CWS experiment explained 49.46% of the total variance. In this experiment, all *Daphnia* treatments had similar effects on plankton structure, because the plankton communities of all treatments were significantly different from the control, but not from each other (Table 5 and Figure 2A). However, these effects were weak because the differences were marginal. In *Daphnia* treatments, the community was dominated by cryptophytes, ciliates, diatoms and heterotrophic pico- and nanoflagellates, while in the control the community was dominated by bacterioplankton and autotrophic microplankton (*Scenedesmus* sp., euglenophytes and *Oocystis* sp.).

In the TP1 experiment, the first two axes of the PCA explained 56.48% of the total variance. In this experiment no *D. magna* effect was detected, because no treatment showed significant differences against the control treatment. However, the plankton structure in the small-*Daphnia* treatment was marginally significantly different from the large-*Daphnia* treatment (Table 5). The large-*Daphnia* treatment was characterised by a higher abundance of *Oocystis* sp. and diatoms, whereas the small-*Daphnia* treatment was characterised by a higher number of bacterioplankton (Figure 2B).

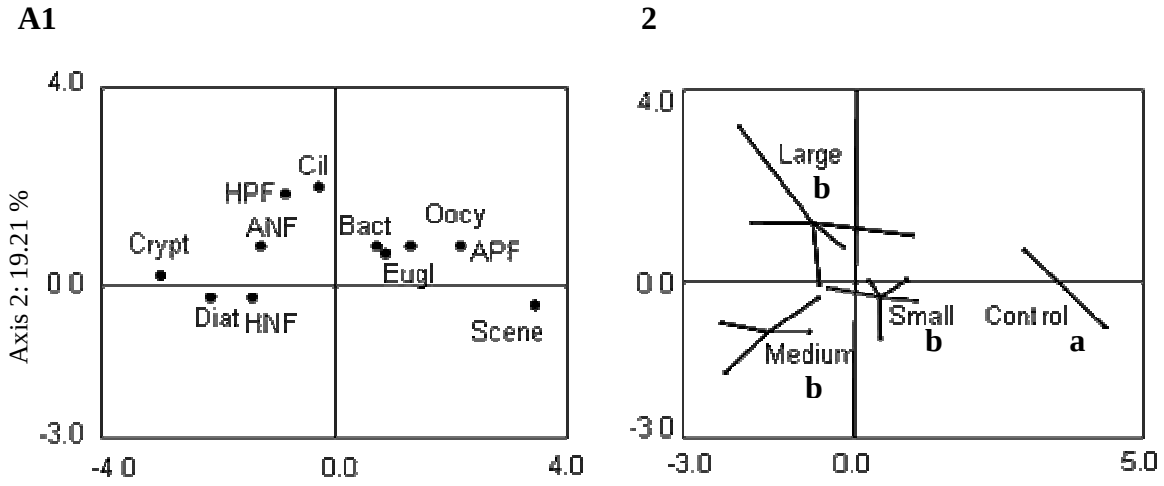
The first two axes of the PCA in the TP2 experiment explained 45.78% of the total variance. In the medium- and large-*Daphnia* treatments, the plankton structure showed significant differences compared with the ones found in the control but there were no significant differences between the two treatments (Table 5). Picoplankton (bacterioplankton and HPF), ciliates and *Scenedesmus* sp. dominated in medium- and large-*Daphnia* treatments, while LNF, diatoms, *Oocystis* sp., amoeba and *Lecane* sp. dominated in the control (Figure 2C).

Using the percentage of PCA inertia to identify the organisms that showed the highest variation during the experiments (those with higher inertia vaules), we observed that the main effects of *D. magna* were detected at low trophic levels. In all experiments, bacterioplankton was the taxa with the highest inertia percentage (30% in the CWS experiment, 34% in the TP1 experiment and 28% in the TP2 experiment). Other picoplankton (HPF and HNF) and nanoplankton (ANF and LNF) organisms also had a high inertia in all experiments (34% and 11% respectively in the CWS experiment, 22% and 14% respectively in the TP1 experiment, and 17% and 15% respectively in the TP2 experiment).

Table 5. Results of Monte-Carlo permutation test for different treatments. $^{+}$, $p < 0.1$ and **, $p < 0.02$ (significance level adjusted with the Dunn-Sidak procedure). C *vs.* S: control *vs.* small-*Daphnia* treatment; C *vs.* M: control *vs.* medium *Daphnia* treatment; C *vs.* L: control *vs.* large-*Daphnia* treatment; S *vs.* M: small-*Daphnia* treatment *vs.* medium-*Daphnia* treatment; S *vs.* L: small-*Daphnia* treatment *vs.* large-*Daphnia* treatment; M *vs.* L: medium-*Daphnia* treatment *vs.* large-*Daphnia* treatment. Results of the small-*Daphnia* treatment in TP2 experiment are not showed neither discussed (see material and methods section).

Experiment	**Treatment Test**	***p* value**
CWS experiment	C *vs.* S	0.086^{+}
	C *vs.* M	0.021^{+}
	C *vs.* L	0.073^{+}
	S *vs.* M	0.112
	S *vs.* L	0.484
	M *vs.* L	0.599
TP1 experiment	C *vs.* S	0.958
	C *vs.* M	0.109
	C *vs.* L	0.249
	S *vs.* M	0.131
	S *vs.* L	0.061^{+}
	M *vs.* L	0.817
TP2 experiment	C *vs.* M	0.002**
	C *vs.* L	0.008**
	M *vs.* L	0.598

Fig. 2. (next page) Principal Correspondence Analysis for three experiment: CWS experiment (A), TP1 experiment (B) and TP2 experiment (C). 1: ordination of taxa of each experiment; 2: ordination of samples analysed of each experiment. Different black subscripts (a or b) indicate significant differences for Monte-Carlo permutation test ($p<0.1$) among sample scores of the treatments on the first and second axis, respectively. Amo: amoebae undet. sp.; ANF: autotrophic nanoflagellates; APF: autotrophic picoflagellates; Bact: bacterioplankton; Cil1: ciliates 1; Cil2: ciliate 2; Crypt: cryptophytes; Diat: diatoms; Eugl: euglenophytes; HNF: heterotrophic nanoflagellates; HPF: heterotrophic picoflagellates; Lec: *Lecane* sp.; LNF: large nanoflagellates; Oocy: *Oocystis* sp.; Scene: *Scenedesmus* sp.; Stromb: *Strombidium* sp.; Suc: *Suctoria* sp. Control: Control; Large: Large-*Daphnia* treatment; Medium: Medium-*Daphnia* treatment; Small: Small-*Daphnia* treatment. In TP2 experiment, results of small-*Daphnia* treatment are showed in figure, but they are not discussed (see material and methods section).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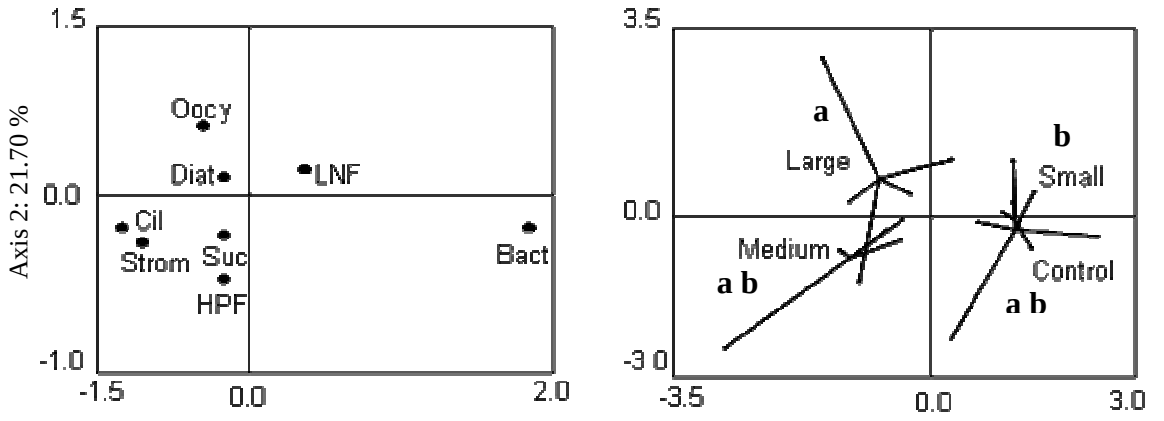
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3.0
Axis 2: 21.70 %
Axis 1: 34.78 %
Oocy
Diat
LNF
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Strom
Suc
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Bact
a
Large
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Medium
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Control
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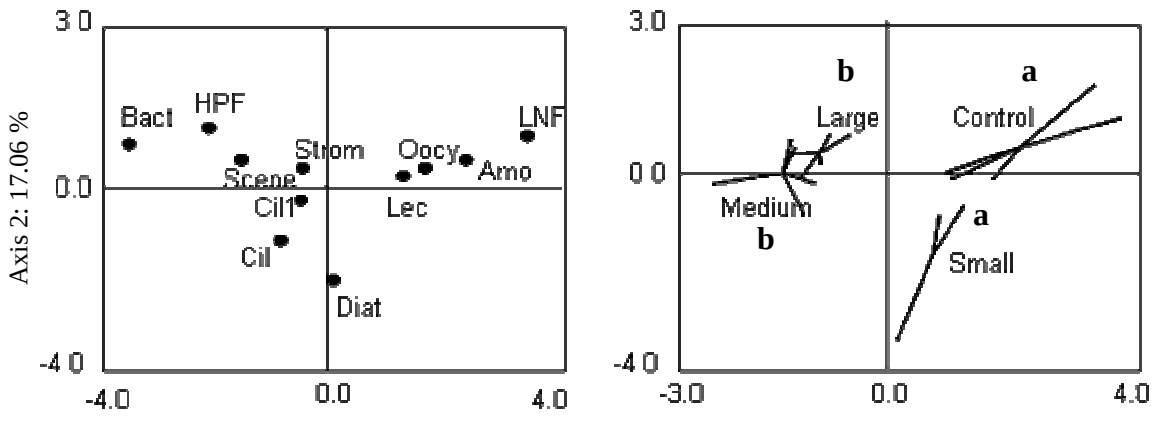
C1
2
3.0
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Axis 2: 17.06 %
Axis 1: 28.72%
Bact
HPF
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b
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Fig. 2.

DISCUSSION

Our results show that the effects of *D. magna* on aquatic communities depended heavily on the initial structure of the microbial community. Selectivity coefficients changed based on the different potential preys present in the plankton community, in accordance with an omnivorous non-selective feeding behaviour (e.g. DeMott, 1986; Carotenuto & Lampert, 2004). Furthermore, the microbial community structure that resulted after treatments differed among the experiments.

Similar selectivity coefficients were found for the different developmental stages (i.e. sizes) of *D. magna* in each experiment, which is in accordance with previous studies that found overlap in the use of resources between adult and juvenile cladocerans (Boersma, 1995). It has consistently been observed that filter mesh sizes are almost constant during daphnid body growth (Geller & Müller, 1981), meaning that all developmental stages can potentially feed on similar taxa. Therefore, it would be expected that if all sizes of *D. magna* showed a similar feeding behaviour, their effects on the microbial community would be similar. Our results are in accordance with this hypothesis. They show that particle composition after treatments differed mainly between control and *Daphnia* treatments while there were not differences between *Daphnia*-treatments. In summary, the lack of differences among *D. magna* developmental stages suggests that the body sizes of *D. magna* are not important when assessing the potential impacts of *D. magna* grazing on a microbial community.

Comparison of the PCA analyses showed differences among experiments of the grazing effects on some prey types. PCA coordinates of the three experiments show that bacterioplankton seemed to be greatly reduced by the presence of *D. magna* in the TP1 experiment (positive coordinates in axis 1), but favoured by *D. magna* presence in the TP2 experiment (negative coordinates). The CWS experiment showed intermediate effects on bacterioplankton (intermediate coordinates). Indirect trophic cascade interactions appear to be the most plausible explanation for these variations. The feeding of *Daphnia* spp. on ciliates and heterotrophic flagellates (e.g. McMahon & Rigler, 1965; Porter *et al*., 1979), both of which predate bacterioplankton (e.g. Hall *et al*., 1993; Christaki *et al*., 1999), could result in a decrease in the grazing mortality of bacterioplankton, with a consequent increase in their biomass. In addition, *Daphnia* spp. can mechanically interfere with rotifers (Gilbert & Stemberger, 1985; Gilbert, 1989), which are also bacterioplankton consumers (Agasild & Nõges, 2005; Fiałkowska &

Pajdak-Stós, 2008), and further contribute to an increase in bacterioplankton. In support of this hypothesis, we found high selection coefficients for ciliates and rotifers in the TP2 experiment, together with an increase in bacterioplankton. The importance of indirect effects via trophic cascade has already been observed in other feeding behavior studies dealing with the entire size spectrum of plankton food webs (e.g. Jürgens *et al*., 1994; Auer *et al*., 2004; Brucet *et al*., 2008).

The capacity of *Daphnia* spp. to reduce biomass particles and increase water transparency has been used to propose *Daphnia* as a biological treatment method for improving water quality in wastewater treatments (e.g. Sommer *et al*., 1986; Carpenter and Kitchell, 1993). Further, Schreijer *et al*. (2000) suggest that the consumption of pathogen bacteria by *Daphnia* spp. could contribute to the disinfection of treated wastewater. However, our results suggest that the net effects of *D. magna* on wastewater effluents could be strongly affected by the trophic cascade interactions of the planktonic community in the effluent. Being the smallest organism in the microbial community, bacterioplankton biomass would most probably be the most vulnerable to changes in the trophic cascade. Thus, to better assess their possible use for disinfection purposes, apart from their physical and chemical water properties, it is necessary to consider the planktonic food web in which *Daphnia* will be acting. In accordance with our findings, other authors have emphasised the importance of taking into account the food web structure when performing biological control assays, since unexpected results can arise (e.g. Matvevv *et al*., 2000; Radke & Kahl, 2002; Muylaert *et al*., 2006). In conclusion, our results show that a good knowledge of the planktonic structure and potential interactions in the microbial food web is necessary to understand the effects of *D. magna* on a microbial community.

Photo: Jordi Bas

Chapter 5

Top-predator effects of jellyfish *Odessia maeotica* in Mediterranean salt marshes

Compte, J., Gascón, S., Quintana, X. D., Boix, D., In press. Top-predator effects of jellyfish *Odessia maeotica* in Mediterranean salt marshes. Marine Ecology Progress Series.

ABSTRACT: Jellyfish can act as planktonic top predators, and their effects may cause drastic changes in the plankton structure of marine and freshwater systems. However, the top predator effects may not necessarily be the same in species-poor habitats as they are in species-rich habitats. The present study analyses the effects of the small lacunae jellyfish *Odessia maeotica* in a species-poor habitat, Mediterranean salt marshes in the wetlands of Empordà (NE Iberian Peninsula). A field experiment was carried out in March 2008 to assess the direct and indirect effects of *O. maeotica* on plankton composition. Our results show that the presence of *O. maeotica* changed the plankton composition through top-down effects. Changes were strong in zooplankton, because *O. maeotica* can suppress almost the entire trophic level of large zooplankton (>50 µm). Weak indirect effects on phytoplankton composition were observed as well. When *O. maeotica* was present, changes in the relative abundance of the phytoplankton species were found, but there was no net increase in phytoplankton biomass. Our results suggest that these weak indirect effects may be the result of trophic cascade effects coupled with the oligotrophic conditions of these salt marshes. Thus, trophic cascade effects lead to an increase in ciliate biomass, and these ciliates would feed on small algae (jellyfish–copepods–ciliates–small algae), while oligotrophic conditions would prevent increases in algal biomass.

Keywords: Jellyfish, *Odessia maeotica*, Top-down, Bottom-up, Brackish waters, Trophic cascade, Food web

INTRODUCTION

Jellyfish are widespread in both marine and limnic systems (e.g. Dumont, 1994), and can act as planktonic top predators, causing direct and indirect changes in lower trophic levels, through cascading effects (Oguz *et al.*, 2001, Pitt *et al*., 2007). Predation is the main direct effect on zooplankton (e.g. Larson, 1987; Stibor & Tokle, 2003; Smith & Alexander, 2008), since jellyfish can feed on copepods (e.g. Dodson & Cooper, 1983; Purcell *et al.*, 1999; Costello & Colin, 2002), cladocerans (Davis, 1955; Dodson & Cooper, 1983; Purcell, 2003) and fish eggs (e.g. Purcell, 1985; Dumont, 1994). Indirect effects may also appear, such as changes in the plankton structure of lower trophic levels due to cascade effects (Olsson *et al*., 1992; Granéli & Turner, 2002; Stibor *et al*., 2004). In this regard, some studies have pointed out an increase of phytoplankton in the presence of jellyfish (Lindahl & Hernroth, 1983; Jankowski & Ratte, 2001).

The effects of a top predator are variable and depend on the complexity of the aquatic community (Polis & Strong, 1996; Stibor *et al*., 2004). Most studies on the trophic role of jellyfish are carried out in marine or freshwater systems where medusae act as top predators affecting the zooplankton (e.g. Larson, 1987; Baird & Ulanowicz, 1989; Stibor & Tokle, 2003) and phytoplankton populations (e.g. Huntley & Hobson, 1978; Riisgård, 1998). However, the jellyfish's role as a top predator in simpler communities may be different, since, as pointed out by Strong (1992), the effects of a top predator in species-poor habitats would be expected to be different than in species-rich habitats. The large fluctuations in the salinity of brackish water systems (e.g. Barnes, 1989) mean that only well-adapted species can successfully inhabit such environments (Bamber *et al.*, 1992; Boix *et al.*, 2008). As a consequence, brackish waters are especially interesting systems because top predators can be studied in a poor-species habitat.

Daan (1986) and Purcell & Nemazie (1992) suggest negligible effects of jellyfish controlling the plankton population in brackish systems. However, the small jellyfish, *Odessia maeotica* (Ostroumoff, 1896) can dominate the plankton community in Mediterranean salt marshes in situations of confinement (low water turnover), reducing the plankton species diversity and the copepod populations to only a few isolated harpacticoids (Quintana *et al*., 1998b). Therefore, the top-predator behaviour of small jellyfish in brackish systems still needs to be clarified.

Despite the potential importance of jellyfish in the food web structure, there are relatively few experimental studies dealing with their effects on plankton communities. This could be due to some intrinsic difficulties that exist when studying these organisms (e.g. unpredictable occurrences, tank size effects; Toonen & Chia, 1993; Boero *et al.*, 2008). Nevertheless, many of these experimental difficulties are reduced when working with small-sized medusae, which make small jellyfish especially suitable for experimental approaches. In this sense, recent experimental studies carried out with *Craspedacusta sowerbii*, a small freshwater jellyfish species (mean bell diameter: <20 mm), have successfully demonstrated its effect (both direct and indirect) on a plankton community (Jankowski, 2004; Jankowski *et al*., 2005).

In the present study, we carried out a short-term field experiment using tanks in a Mediterranean brackish lagoon with the aim to finding out if *O. maeotica* acts as a top predator, exhibiting direct and indirect control on plankton communities. We compared

plankton structures in the presence of jellyfish with those in their absence. Because brackish habitats have simple communities, we would expect the top predator to exert a strong effect. In order to establish the strength of the effect, we simulated the strongest possible effects of *O. maeotica* by removing plankton organisms >50 µm (mainly zooplankton taxa), since small jellyfish, such as *O. maeotica* (e.g. *Craspedacusta sowerbyi*), feed on organisms >50 µm (e.g. Dodson & Cooper, 1983; Dumont, 1994; Spadinger & Maier, 1999). The hypotheses to be tested were that *O. maeotica* (1) cause a strong direct effect on zooplankton by removing most of the plankton >50 µm and (2) cause indirect cascading effects on lower trophic levels, including small zooplankters, phytoplankton and bacteria, as a consequence of the depletion of the large zooplankters.

MATERIALS AND METHODS

Study site and *O. maeotica*

The study was carried out in the wetlands of Empordà (NE Iberian Peninsula), a series of Mediterranean shallow coastal lagoons, free from tidal influence, whose hydrological regime is determined by the occurrence of floods caused by meteorological disturbances in autumn and winter, and the process of desiccation during summer (Quintana, 2002). In these coastal lagoons, the small jellyfish *O. maeotica* (mean bell diameter: 8 mm) has been captured in high densities in periods of confinement and under oligotrophic conditions (March to June) (Quintana *et al*., 1998b). This jellyfish is a Hydrozoa of the Moerisiidae family which exhibits alternation of generations; sessile polyps (asexual generation) and medusae (reproductive generation).

Stomach content

Prior to the experiment, the potential prey of *O. maeotica* were identified in the stomach contents of individuals captured in March 2007 in the same salt marsh in which the experiment was performed. Twenty-five *O. maeotica* individuals were captured using a net with a mesh size of 1.2 mm. Immediately, they were fixed with 4% formaldehyde solution and stored. *O. maeotica* stomachs were processed, identifying and counting the preys items found in each stomach using a stereomicroscope.

Experimental design

Our experiment to study the effects of *O. maeotica* on plankton was carried out in the field in March 2008, when the presence of potential prey for *O. maeotica* was detected. The lagoon was 60 cm deep and oligotrophic (2.08 µM dissolved inorganic nitrogen and 1.35 µM soluble reactive phosphorous) and was characterised by a conductivity of 53.30 mS cm^{-1} of conductivity. The dissolved oxygen at the start of the experiment was 70%.

Five samples of 8 l of water were collected from the lagoon and processed to provide information on the "initial conditions" of the plankton structure. Fifteen hermetically closed transparent PVC tanks (8 l capacity) were used in the experiment. Five of them were filled with 8 l of lagoon water without *O. maeotica* (hereafter "control"). Another 5 tanks were filled with lagoon water, and 15 *O. maeotica* individuals were added to each tank (hereafter "*Odessia* treatment"). This density of *O. maeotica* was similar to the maximum densities of medusae observed in these lagoons (Quintana *et al*., 1998b). The last 5 tanks were filled with lagoon water previously filtered through 50 µm mesh (hereafter "filtered treatment") in order to simulate the strongest possible jellyfish effect, i.e. total suppression of the large plankton trophic level.

The tanks were placed in the lagoon and fixed to the sediment by strings and tent pegs, and incubated for 72 h under the natural conditions of temperature (14 ºC at initial time of the experiment) and light at a depth of 10 to 15 cm. Finally 5 additional samples of 8 l from the same lagoon (hereafter "lagoon") were taken directly after incubation. After the 72 h incubation, all individuals of *O. maeotica* were found to be alive. They were then sorted and fixed with 4% formaldehyde. Their biomass was estimated measuring biovolume and converting it to dry weight (Malley *et al*., 1989) to check that predation pressure in all replica experiments was similar (ranging from 7.48 to 7.92 mg C l^{-1}).

Zooplankton was obtained from the retained material by filtering the 8 l of water through a 50 µm mesh and was immediately fixed with 4% formaldehyde solution. Taxonomic identification and counting of individuals were carried out using a stereomicroscope and an inverted microscope. Biomass was estimated using the equations of Malley *et al*. (1989) for Polychaeta larva and calanoids and cyclopoids

(nauplii, copepodites and adults); of Dumont *et al*. (1975) for harpacticoid nauplii, copepodites and adults; and of Telesh *et al*. (1998) for rotifers.

Microplankton and large nanoplankton between 5 and 50 µm in size were obtained by filtering 125 ml of water through a 50 µm mesh and were then fixed in the field with Lugol's iodine and stored under dark conditions until analysis. Taxonomic identification and cell-counting were performed using an inverted microscope (Utermöhl, 1958). Biovolumes were calculated from measurements of the linear dimensions of cells under the inverted microscope, using appropriate geometric formulae (Ruttner-Kolisko, 1977; Hillebrand *et al*., 1999). Ciliate biovolume was estimated by approximation of the body shape to geometric shapes. Biomass was estimated using the equations of Menden-Deuer & Lessard (2000) for diatoms and chlorophytes and of Putt & Stoecker (1989) for ciliates.

Bacterioplankton and autotrophic pico- and nanoplankton samples were obtained by filtration through a 50 µm mesh and were then fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), immediately deep frozen in liquid nitrogen and stored at -20ºC. Their abundance and biovolume were obtained using a flow cytometer (FACScalibur from Becton and Dickinson) with laser emission at 488 nm (for method details see López-Flores *et al*., 2006). The bacterioplankton biovolume was calculated using the equation described in Gasol & Del Giorgio (2000). Autotrophic pico- and nanoplankton biovolumes were calculated from measurements of linear dimensions of cells by means of cytometry through a calibration curve as described elsewhere (Olson *et al*., 1989; Rodríguez *et al*., 2002; López-Flores *et al*., 2006). Biomass was estimated using the equations of Lee & Fuhrman (1987) for bacterioplankton, and of Verity *et al*. (1992) for picoplankton and nanoplankton.

To differentiate heterotrophic from autotrophic pico- and nanoplankton, DAPI (4'-6-diamidino-2-phenylindole) fixation was used (Porter & Feig, 1980). The samples were mixed by inversion and left to stain for 10 min with the fluorochrome DAPI (final concentration of 0.5 µg ml^{-1}). Then, they were carefully filtered through a 0.2 µm polycarbonate filter (Millipore, Isopore membrane filters), and the filters were mounted on a glass slide and examined by epifluorescence microscopy with a UV excitation filter block and 1000x oil immersion. At least 300 individuals were enumerated per sample (Liu *et al*., 2005). By visualizing the DAPI-stained nuclei (blue) and the chlorophyll-*a* autofluorescence (red) it was possible to locate and differentiate heterotrophic from

autotrophic pico- and nanoplankton. Heterotrophic pico- and nanoplankton biovolumes were calculated from the measurements of the linear dimensions of cells taken under epifluorescence microscopy, using appropriate geometric formulae. Biomass was estimated using the equations of Verity *et al*. (1992).

Some experiments have provided evidence that indirect effects are better explained if size discrimination of lower trophic levels is performed (Stibor *et al*., 2004). Therefore, to study the direct and indirect effects of *O. maeotica*, 3 planktonic organism data sets were used: (1) data of mesozooplankton and microzooplankton >50 µm (hereafter "large plankton"), (2) data of pico-, nano- and microplankton <50 µm (hereafter "small plankton") and (3) data of both groups (hereafter "small + large plankton").

Comparisons between treatments and fractions allowed us to test the hypotheses listed below, as well as those regarding the direct effects (on large zooplankton) and indirect effects (on lower trophic levels; small plankton) of jellyfish presence in brackish environments:

Test 1 (tank effects): Hypothesis—The enclosure of water in a hermetically closed tank does not cause any effect on plankton composition. Lagoon samples versus control samples in final conditions were compared using the small + large plankton data set.

Test 2 (time effects): Hypothesis—No changes in plankton assemblages are expected between initial and final conditions, because our experiment covers a short time period (72 h). Initial conditions versus control samples were compared using the small + large plankton data set.

Test 3 (Odessia direct effects): Hypothesis—The presence of *O. maeotica* causes strong significant changes in large zooplankton organisms. Control samples versus *Odessia* treatment samples were compared using the large plankton data set.

Test 4 (strong Odessia effects): Hypothesis—*O. maeotica* acts as a top predator in a species-poor habitat causing an effect similar to the total suppression of the large zooplankton. *Odessia* treatment versus filtered treatment samples were compared using small plankton data set.

Test 5 (Odessia indirect effects): Hypothesis—The presence of *O. maeotica* causes changes in lower trophic levels by indirect cascading effects. Control samples versus *Odessia* treatment samples were compared using small plankton data sets.

Data analysis

All tests were performed using a multivariate approach (considering plankton structure) to check the effects of *O. maeotica* on the plankton community. Then, a univariate approach (taking into account 5 community parameters) was used to check if jellyfish affects these parameters. In both approaches, the biomass of *O. maeotica* added in the course of the experiment was not considered.

The multivariate approach was based on a correspondence analysis (CA), coupled with between-group analyses (Dolédec & Chessel, 1989). The between-group analyses allowed us to obtain the centroid of each group (i.e. each treatment) and to test the differences among these groups. Differences among groups were checked using the Monte-Carlo permutation test (999 unrestricted permutations under the reduced model). The null hypothesis of this test stated that the relative proportion in biomass of plankton taxa did not differ among groups (initial conditions, lagoon, control, *Odessia* and filtered treatments). This procedure was used to check the significance of the: (1) general effects (using the small + large plankton data set) (2) direct effects (using the large plankton data set) and (3) indirect effects (using the small plankton data set).

Additionally, 5 post hoc tests were carried out according to the 5 hypotheses previously described. Two of these post hoc tests were performed using the small + large plankton data set and tested the differences between lagoon and control treatment samples (tank effects) and between initial conditions and control treatment samples (time effects). Another post hoc test was carried out with the large plankton data set and tested the differences between control and *Odessia* treatment samples (*Odessia* direct effects). The last ones were carried out with the small plankton data set and tested the differences between *Odessia* and filtered treatment samples (strong *Odessia* effects), and between control and *Odessia* treatment samples (*Odessia* indirect effects). The significance between the groups compared in each post hoc test was assessed using Monte-Carlo permutation tests (999 unrestricted permutations under the reduced model). All multivariate analyses were performed with ade4 package (Dray & Dufour, 2007) written in R language.

The community parameters used in the univariate approach included species diversity, size diversity, species richness, total biomass and average body size. Species diversity was measured using the Shannon-Wiener index (Pielou, 1969) and calculated using biomass abundance. Size diversity and average body size were calculated using

the Kernel estimation (Quintana *et al*., 2008). Finally, species richness and total biomass were also calculated. These 5 community parameters were calculated considering the 3 organism data sets (large plankton, small plankton and small + large plankton) for control, *Odessia* and filtered treatments. Analyses of variance (ANOVA) and Welch statistics (when the assumption of variance homogeneity was violated) were used to test for significant differences among treatments for the 5 community parameters calculated. When a significant result was obtained in the ANOVA, the Tukey post hoc multiple comparison test was applied to identify which treatments were significantly different. All ANOVA were performed using SPSS 15.

Relationship between *Odessia maeotica* and plankton structure in natural conditions

Available data on *O. maeotica* and plankton abundances from a previous study (Quintana *et al*., 1998b) carried out from February to June (1989 to 1991) in the same salt marshes allowed us to find out whether, under natural conditions, increases in *O. maeotica* densities could be related to decreases of plankton abundances. Correlations between *O. maeotica* biomass and zooplankton biomass or chl-*a* as a surrogate of the biomass of primary producers biomass, were calculated by means of the Pearson correlation coefficient.

RESULTS

Stomach content

The stomach content analysis showed that the diet of *O. maeotica* was mainly composed of large zooplankton organisms (Table 1). Calanoid copepods at all stages of their developmental were the most abundant prey found in the stomach content of *O. maeotica*. Although less represented, harpacticoids and rotifers were also part of the *O. maeotica* diet.

Table 1. *Odessia maeoticas* Stomach analyses of 25 individuals jellyfish caught in open waters in March 2007 in the same area where the experiments were carried out during March 2008. Prey type is given and the number of *O. maeotica* stomachs in which they were found. Mean (SD) values of the number and size of individuals found in the stomachs are also shown.

Prey type	Nº of stomachs	Number	Size (µm)
Adult *Calanipeda aquaedulcis*	11	2.45 (1.62)	1140 (280)
Copepodite *Calanipeda aquaedulcis*	11	3.19 (2.29)	560 (140)
Nauplius *Calanipeda aquaedulcis*	10	3.90 (2.87)	210 (70)
Copepodite Harpacticoid	1	1	280
Brachionus sp.	2	1.5 (0.5)	110 (20)
Testudinella sp.	1	1	200
No prey	8	-	-

Effects on plankton structure

Twenty-three taxa present in the small + large plankton data set of all treatments were included in the CA (Figure 1A). The first 2 axes of the CA explained 78.57% of the total variance: the first axis explained 61.73% and the second axis explained 16.84%. The first axis separated samples with real and simulated effects of our *O. maeotica* experiment (including *Odessia* and filtered treatments) from those without *O. maeotica* experimental effects (initial conditions, lagoon and control) (Figure 1B). In these latter treatments, the community was characterised by a higher biomass of calanoids and euglenophytes, while in treatments with *O. maeotica* effects the community was characterised by higher biomass of smaller plankton taxa (i.e. ciliates, picoflagellates and bacterioplankton) (see Appendix 1 of the final of chapter 5). The second axis separates samples where *O. maeotica* was actually present (lagoon and *Odessia* treatment) from samples without *O. maeotica* (initial conditions, control and filtered treatments) (Figure 1B). When *O. maeotica* was present, a higher biomass of ciliates, rotifers and chlorophytes characterised the plankton community, while when *O. maeotica* was absent, a higher biomass of harpacticoid copepods, autotrophic picoplankton, diatoms and cryptophytes characterised the plankton community.

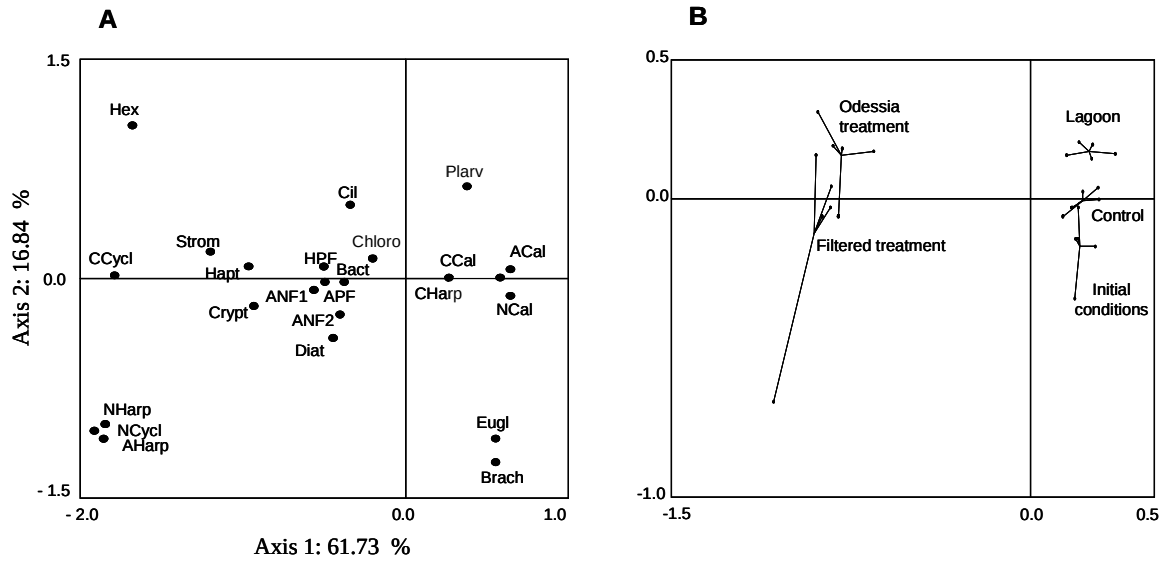


Fig. 1. Correspondence analysis for plankton taxa: ordination of (A) the 23 taxa and (B) the 25 samples analysed. APF: Autotrophic picoflagellates; ANF1: Autotrophic nanoflagellates 1; ANF2: Autotrophic nanoflagellates 2; ACal: adult calanoid (*Eurytemora velox*); AHarp: adult harpacticoid (*Mesochra* sp.); Bact: bacterioplankton; Brach: *Brachionus* sp.; Cil: ciliate; Chloro: chlorophytes; CCal: copepodite calanoid; CCycl: copepodite cyclopoid; CHarp: copepodite Harpacticoid; Crypt: cryptophytes; Diat: diatoms (*Amphora* sp., *Navicula* sp., *Nitzschia* sp.); Eugl: euglenophytes; Hapt: haptophytes; Hex: *Hexartra* sp.; HPF: heterotrophic picoflagellates; NCal: nauplii calanoid; NCycl: nauplii cyclopoid; NHarp: nauplii harpacitocid; Plarv: Polychaeta larvae; Strom: *Strombidium* sp.

When the large plankton data sets were analysed, the first 2 axes of the CA explained 79.54 % of the total variability observed (Figure 2A). The first axis explained 47.02% and separated samples of simulated *O. maeotica* effects (filtered treatment) from the rest of the treatments (initial conditions, lagoon, control and *Odessia* treatment) (Figure 2 B). The second axis explained 32.52% and separated samples with *O. maeotica* effects (*Odessia* and filtered treatment) from samples without *O. maeotica* experimental effects (initial conditions, lagoon and control). The gradients observed on both axes could be related to differences in zooplankton body sizes. The *Odessia* treatment was characterised as having only the smallest zooplankton taxa (rotifers and nauplii of harpacticoids). Similarly, filtered treatment samples were characterised by the presence of small zooplankton, although some larger organisms were also present (nauplii and copepodites of cyclopoids and adult harpacticoids). On the other hand, initial conditions, lagoon and control were characterised by a high biomass of large zooplankton (copepodites and adult of calanoids) (Appendix 1 of the final of chapter 5).

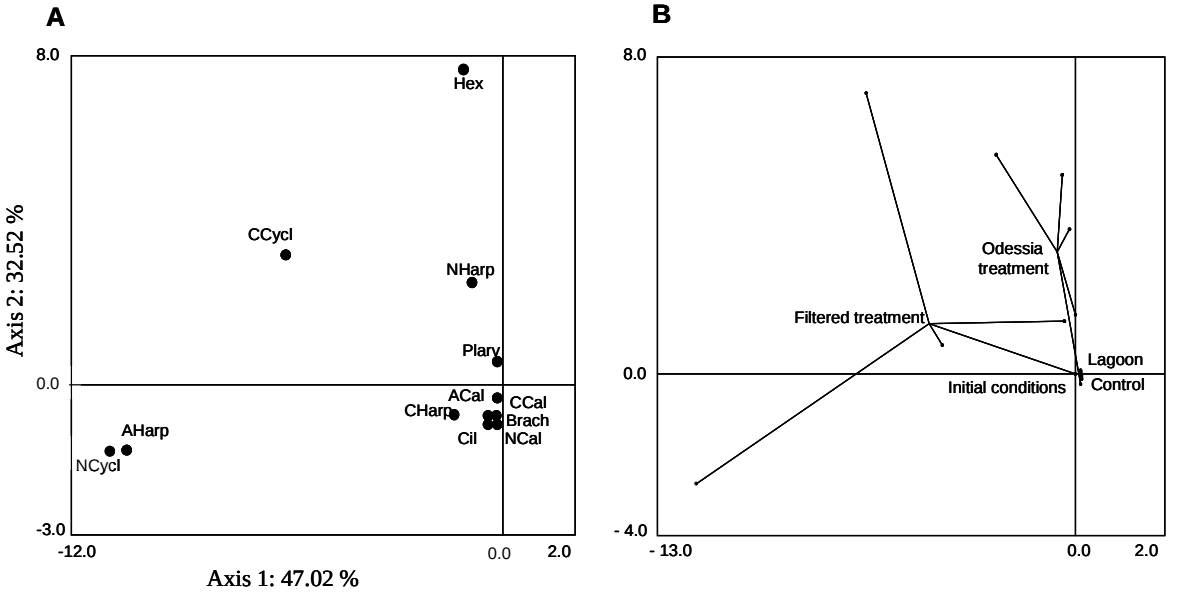


Fig. 2. Correspondence analysis for large plankton (>50 µm): ordination of (A) the 12 taxa and (B) the 25 samples analysed. ACal: adult calanoid (*Eurytemora velox*); AHarp: adult harpacticoid (*Mesochra* sp.); Brach: *Brachionus* sp.; Cil: ciliate; CCal: copepodite calanoid; CCycl: copepodite cyclopoid; CHarp: copepodite harpacticoid; Hex: *Hexartrha* sp.; NCal: nauplii calanoide; NCycl: nauplii cyclopoide; NHarp: nauplii harpacitocide; Plarv: Polychaeta larvae.

Taking only the small plankton data set into account, the first 2 axes of the CA explained 82.44% of the total variability observed (Figure 3A). The first axis explained 59.63% and separated initial conditions samples from those of final conditions (i.e. taken after 72 h: lagoon, control, *Odessia* and filtered treatments) (Figure 3B). The small plankton from initial condition samples was characterised by a higher biomass of only autotrophic organisms (euglenophytes, diatoms and autotrophic picoflagellates), while the one from the final conditions had a higher biomass of other organisms such as bacterioplankton, auto- and heterotrophic picoflagellates and ciliates (Appendix 1 of the final of chapter 5). The second axis explained 22.81% of total variance and separated samples with *O. maeotica* experimental effects (*Odessia* and filtered treatments) from samples without *O. maeotica* experimental effects (lagoon and control) (Figure 3B). The small fraction of the plankton community without *O. maeotica* effects was characterised by higher biomass of bacterioplankton, auto- and heterotrophic picoplankton and autotrophic nano- and microplankton, while in the small fraction of the plankton community with *O. maeotica* experimental effects was characterised by higher biomass of ciliates and haptophytes.

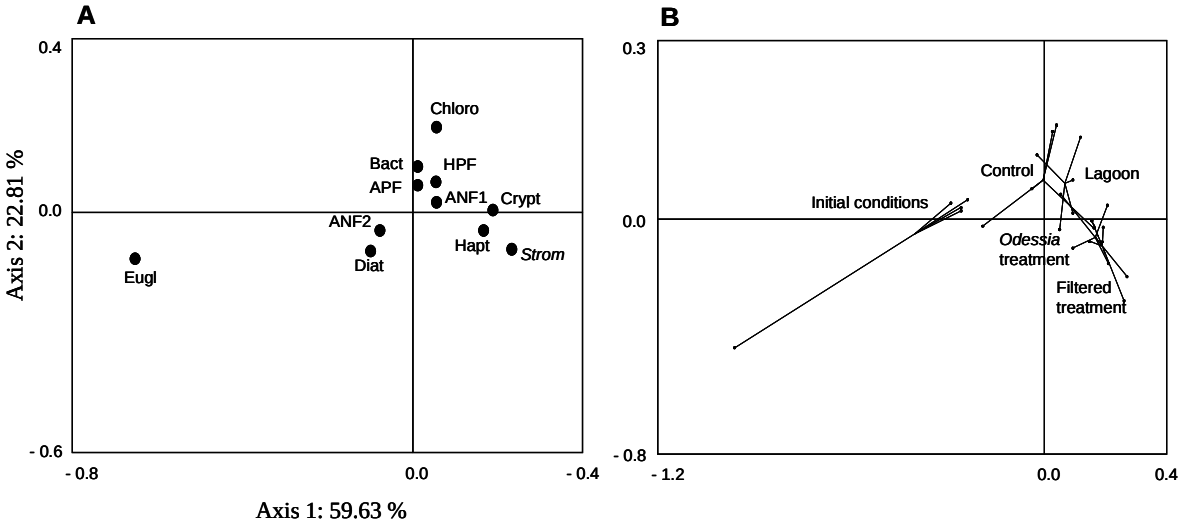


Fig. 3. Correspondence analysis for plankton small plankton (< 50 µm): ordination of (A) the 11 taxa and (B) the 25 samples analysed. APF: autotrophic picoflagellates; ANF1: autotrophic nanoflagellates 1; ANF2: autotrophic nanoflagellates 2; Bact: bacterioplankton; Strom: *Strombidium* sp.; Chloro: chlorophytes; Crypt: cryptophytes; Diat: diatoms (*Amphora* sp., *Navicula* sp., *Nitzschia* sp.); Eugl: euglenophytes; Hapt: haptophytes; HPF: heterotrophic picoflagellates.

Hypothesis testing

Test1: "tank effects"

A tank effect was detected on the plankton structure, because we obtained significant differences between the lagoon treatment and the control samples (Monte-Carlo permutation test; $p < 0.01$). This could be explained by the presence of small *Odessia maeotica* detected in the lagoon samples. In fact, lagoon samples were located in a similar position to *Odessia* treatment samples (both were positive values on Axis 2 when considering the small + large plankton data set (Figure 1B). In contrast, no tank effect was detected for any community parameters (Table 2).

Test 2: "time effects"

As in Test 1, time effects on plankton structure were detected, since a significant difference was found between the initial conditions and control samples (Monte-Carlo permutation test; $p = 0.02$). On the other hand, no time effects were detected for any community parameters (Table 2).

Test 3: "Odessia direct effects"

The between-group analyses performed with the large plankton data set revealed significant differences between the control and the *Odessia* treatment (Monte-Carlo permutation test; $p = 0.01$). In the control samples, there was a higher biomass of large organisms (mainly calanoids) than in the *Odessia* treatment samples (Figure 2A). Direct

effects of *O. maeotica* were also detected in community parameters (Table 2). Total planktonic biomass ($F_{1,7}$ = 282.65, p < 0.01) and average body size ($F_{1,7}$ = 14.64, p = 0.01) were significantly lower in the samples from the *Odessia* treatment than in the controls (Table 3).

Test 4: "Strong Odessia effects"

No significant differences were found in the small plankton data set (Monte-Carlo permutation test; p = 0.78) between the *Odessia* and filtered treatments. Similarly, no significant differences were found between the *Odessia* and the filtered treatments for any community parameter (Table 2). These results showed that *O. maeotica* exerts the strongest possible effect on the plankton community, since we did not find any significant differences between the *Odessia* treatment samples and the samples in which all organisms >50 µm had been artificially removed.

Test 5: "Odessia indirect effects"

The between-group analyses performed only with the small plankton data set showed significant differences between the control and the *Odessia* treatment samples (Monte-Carlo permutation test; p = 0.03). In the control samples there was a higher biomass of bacterioplankton, pico- and nanoplankton, while in the *Odessia* treatment samples there was a higher biomass of ciliates and haptophytes (Figure 3A). However, significant differences were not detected for any community parameter (Table 2). Therefore, our results showed significant but weak indirect effect of *O. maeotica* presence in lower trophic levels, since it was only detected at the community structure level.

Table 2. Results of ANOVA for the different tests—Test 1: tank effects; Test 2: time effects; test 3: *Odessia* direct effects; Test 4: Strong *Odessia* effects), Test 5: *Odessia* indirect effects. Samples and data sets used for each test are also indicated. µ: size diversity; *H*: species diversity; *S*: species richness; TB: total biomass (µg ml^{-1}); BS: average body size (µg ml^{-1}). (*W*): Welch test; ** $p \leq 0.01$ (after Bonferroni correction).

Test	**Parameters**	**df_1**	**df_2**	***F***	***p* value**
Test 1: Lagoon vs. Control	µ	1	4.61	0.68	0.45 (W)
(data set: small+large plankton)	H	1	8	3.97	0.08
	S	1	8	18.75	0.03
	TB	1	8	1.85	0.21
	Log BS	1	8	3.69	0.91
Test 2: Initial conditions vs. Control	µ	1	8	0.46	0.52
(data set: small+large plankton)	H	1	8	4.51	0.06
	S	1	8	0.93	0.37
	TB	1	8	2.93	0.13
	Log BS	1	8	0.19	0.68
Test 3: *Odessia* treatment vs. Control	µ	1	3.01	1.11	0.37 (*W*)
(data set: large plankton)	*H*	1	3.05	0.02	0.89 (*W*)
	S	1	7	2.78	0.14
	TB	1	7	282.65	< 0.01**
	Log BS	1	7	14.64	0.01**
Test 4: *Odessia* treatment vs. filtered treatment	µ	1	8	0.01	0.96
(data set small+large plankton)	*H*	1	8	0.13	0.73
	S	1	8	2.31	0.17
	TB	1	8	4.51	0.07
	Log BS	1	8	0.62	0.45
Test 5: *Odessia* treatment vs. Control	µ	1	8	0.27	0.62
(data set: small plankton)	*H*	1	8	1.25	0.29
	S	1	8	0.06	0.82
	TB	1	8	2.25	0.17
	Log BS	1	8	0.03	0.86

Table 3. Mean (SD) values of community parameters of plankton for initial conditions, lagoon, control, *Odessia* treatment and filtered treatment, taking into account plankton taxa. µ: size diversity; *H*: species diversity; *S*: species richness; TB: total biomass (µg ml^{-1}); BS: average body size (µg ml^{-1}). Parameters with the same superscript letter (a, b and c) do not differ significantly among treatments ($p < 0.05$, Tukey post hoc tests).

Data set	Parameters	Initial conditions	Lagoon	Control	*Odessia* treatment	Filtered treatment
Small+large plankton	µ	0.91 (1.34) [a]	1.42 (0.37) [a]	1.44 (1.10) [a]	1.80 (0.54) [a]	1.78 (0.35) [a]
	H	1.23 (0.15) [a]	1.27 (0.22) [a]	1.05 (0.11) [a]	1.36 (0.12) [a]	1.34 (0.11) [a]
	S	12.20 (1.30) [a]	15.20 (0.84) [a]	13.20 (1.92) [a]	11.80 (1.64) [a]	13.60 (2.07) [a]
	TB	147.27 (38.86) [a]	204.00 (84.85) [a]	190.33 (40.72) [a]	24.27 (4.57) [a]	19.04 (3.06) [a]
	BS	$1.39 \cdot 10^{-6}$ ($2.45 \cdot 10^{-6}$) [a]	$2.20 \cdot 10^{-7}$ ($4.63 \cdot 10^{-8}$) [a]	$3.82 \cdot 10^{-7}$ ($1.69 \cdot 10^{-7}$) [a]	$8.88 \cdot 10^{-7}$ ($1.22 \cdot 10^{-6}$) [a]	$4.41 \cdot 10^{-7}$ ($3.25 \cdot 10^{-7}$) [a]
Large plankton	µ	2.46 (0.18) [a]	2.58 (0.17) [a]	2.29 (0.09) [a]	1.59 (0.31) [a]	1.27 (0.01) [a]
	H	0.79 (0.09) [a]	1.02 (0.12) [a]	0.75 (0.04) [a]	0.62 (0.48) [a]	0.79 (0.52) [a]
	S	4.6 (0.89) [a]	6.8 (0.45) [a]	5.00 (1.22) [a]	3.40 (1.14) [a]	4.80 (1.30) [a]
	TB	125.46 (35.40) [a]	192.16 (84.14) [a]	175.20 (40.54) [a]	3.95 (6.05) [b]	0.38 (0.54) [b]
	BS	0.60 (0.12) [a]	0.47 (0.17) [a]	0.88 (0.09) [a]	0.32 (0.31) [b]	0.01 (0.01) [c]
Small plankton	µ	1.46 (2.02) [a]	1.18 (1.32) [a]	1.03 (2.14) [a]	0.28 (2.38) [a]	1.30 (1.46) [a]
	H	0.93 (0.03) [a]	1.10 (0.03) [a]	1.15 (0.06) [a]	1.20 (0.08) [a]	1.27 (0.03) [a]
	S	7.60 (0.55) [a]	8.40 (0.54) [a]	8.00 (1.87) [a]	8.40 (0.89) [a]	8.80 (0.84) [a]
	TB	21.81 (6.24) [a]	11.84 (3.39) [a]	15.14 (6.91) [a]	20.32 (3.46) [a]	18.67 (3.44) [a]
	BS	$3.00 \cdot 10^{-6}$ ($6.65 \cdot 10^{-6}$) [a]	$3.13 \cdot 10^{-6}$ ($1.77 \cdot 10^{-7}$) [a]	$1.72 \cdot 10^{-6}$ ($3.47 \cdot 10^{-6}$) [a]	$1.42 \cdot 10^{-6}$ ($1.19 \cdot 10^{-6}$) [a]	$4.41 \cdot 10^{-6}$ ($6.18 \cdot 10^{-6}$) [a]

Relationship between *O. maeotica* and plankton structure in natural conditions

A negative correlation was found in natural samples (data from 1989 to 1991, Quintana *et al*., 1998b) between *O. maeotica* biomass and total zooplankton biomass (r = -0.61, $p = 0.03$) and between *O. maeotica* biomass and chl-*a* (r = -0.80, $p < 0.05$) (Figure 4A, 4C). Because our experimental results indicate that the biomass of ciliates was higher when *O. maeotica* was present, we also tested the correlation between *O. maeotica* biomass and ciliate biomass. According to our results, in natural conditions, this relationship also exists, and a positive correlation (r = 0.61, $p = 0.05$) between ciliate biomass and *O. maeotica* biomass was obtained (Figure 4B). However, some caution has to be taken when interpreting this significant result since it is influenced by the presence of one extreme point. No correlation was found between *O. maeotica* biomass and

soluble reactive phosphorous (r = -0.19 p = 0.56) or between *O. maeotica* biomass and dissolved inorganic nitrogen (r = 0.06, p = 0.86) (Figure 4D, E).

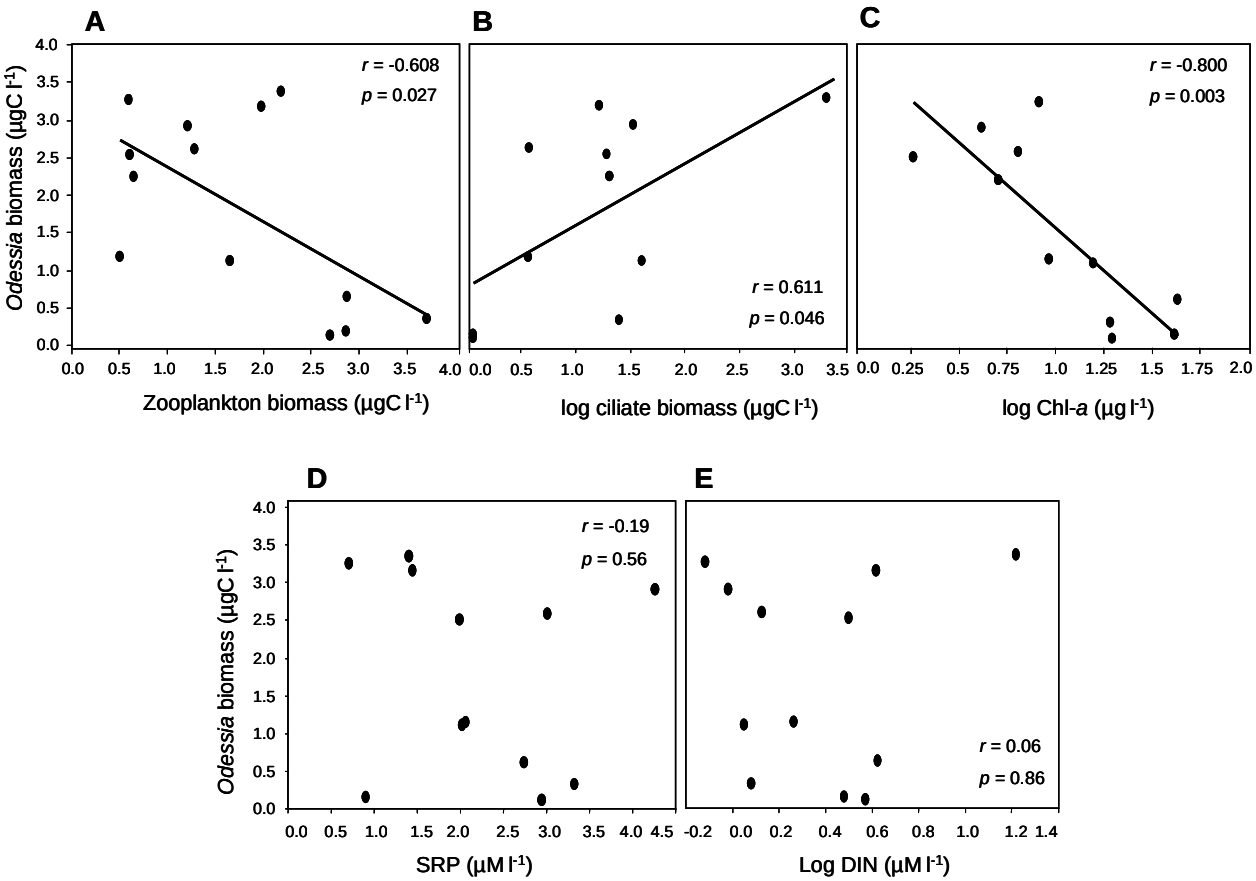


Fig. 4. Relationship between *O. maeotica* biomass and (A) zooplankton biomass, (B) the logarithm of ciliate biomass, (C) the logarithm chlorophyll-*a*, (D) soluble reactive phosphorous (SRP) and (E) the logarithm of dissolved inorganic nitrogen (DIN) calculated from field sample by Quintana *et al*., (1998b).

DISCUSSION

The direct effects of *O. maeotica* are mainly focused on large zooplankton through predation. The results of our experiments showed a decrease in zooplankton biomass and average body size in the presence of *O. maeotica*. These changes are related to a decrease in calanoid biomass. Calanoids are the dominant organisms in the experiment in the absence of the medusae, but were almost absent after 72 h of the *Odessia* treatment. The field data support this finding, since increases in *O. maeotica* were significantly related to decreases in zooplankton biomass. Moreover, stomach content analysis also supported the existence of a direct effect mediated by predation. Our results coincide with existing studies that also describe the predatory behaviour on zooplankton

by other jellyfish species (e.g. Hansson *et al*., 2005; Pitt *et al*., 2008; Smith & Alexander 2008), confirming the top-predator role of *O. maeotica* in brackish ecosystems.

Although large jellyfish can control the plankton population (Behrends & Schneider, 1995), smaller jellyfish species may fail to control the plankton population if copepod growth rates are higher than jellyfish grazing rates on them (Daan, 1986; Purcell, 1992). Thus, Purcell *et al*. (1994) concluded that the control of the copepod population was the result of a combination of different factors such as predation, bottom-up effects and physical effects. Similarly, an experimental study performed with a freshwater jellyfish species (*Craspedacusta sowerbii*), also described a possible bottom-up effect due to nutrient supplies (Jankowski *et al*., 2005). Although, our experiments showed copepod reduction values (98% depletion after 72 h of incubation) similar to those observed by Jankowski *et al*. (2005) (approx. 70 to 80% depletion after 48 h of incubation), in our case the experiment was performed without the addition of nutrients. Moreover, in these brackish systems, *O. maeotica* populations appear in very specific conditions of high values of salinity (30.74 $\pm$ 11.29 mS cm^{-1}), low dissolved inorganic nitrogen (2.25 $\pm$ 1.05 µM) and a low nitrogen:phosphorus ratio (2.15 $\pm$ 3.16); and when these physical and chemical conditions change *O. maeotica* populations disappear (Quintana *et al*., 1998a,b). Thus, the absence of nutrient inputs and the low nutrient concentration, especially of dissolved inorganic nitrogen, would make it difficult to have a bottom-up effect.

Weak indirect effects on small plankton and the microbial community were detected at the structure and composition levels. Nevertheless, our result did not show a significant increase of small autotrophic plankton biomass. Previous studies reported that the increase in total biomass of lower trophic levels (i.e. phytoplankton biomass) was due to evidence of an indirect effect of jellyfish (e.g. Jankowski & Ratte, 2001). It could happen that the duration of our experiment was too short to show significant differences in total phytoplankton biomass. However, similar experiments, also performed with small jellyfish species, found differences in phytoplankton biomass after only 48 h (Jankowski *et al*., 2005), so 72 h should be sufficient time to obtain significant results. Therefore, the lack of an increase in the total phytoplankton biomass in our study may be related to (1) a trophic cascade effect and/or (2) the oligotrophic conditions in which *O. maeotica* occurs, which would not allow significant phytoplankton increases

(López-Flores *et al*., 2006), even if phytoplankton grazers are suppressed. In our opinion, the most plausible explanation would be a combination of these 2 processes.

Regarding the trophic cascade effect, several studies have described changes in microplankton as being cascading trophic effects in the presence of jellyfish, and their top-down effect through several trophic levels (e.g. Pitt *et al*., 2007). For example, Lindahl & Hernroth (1983), Jankowski & Ratte (2001) and Jankowski (2004) showed that phytoplankton blooms can appear when grazing pressure by herbivorous zooplankton is reduced as a result of heavy predatory pressure by jellyfish. In our case, when *O. maeotica* was present, we found an increase of the ciliate *Strombidium* sp. and mixotrophic organisms (haptophytes and cryptophytes) and a decrease of autotrophic organisms (autotrophics picoflagellates, diatoms, chlorophytes and euglenophytes) and bacterioplankton. The increase of ciliates in the presence of jellyfish could be explained by an indirect effect, since jellyfish would prey on calanoids, which, in turn, prey on heterotrophic plankton (Brucet *et al*., 2008). Consequently, if calanoids are removed, small heterotrophic organisms such as ciliates may increase in density. Moreover, ciliates can feed on bacterioplankton (e.g. Kisand & Zingel, 2000) and autotrophic organisms of pico- and nanoplankton (e.g. Christaki *et al*., 1999). Therefore, an increase of ciliates due to cascading trophic effects could also indicate high grazing pressure on the phytoplankton and bacterioplankton community, and, therefore, no increases in the biomass of these planktonic organisms would be detected.

On the other hand, previous studies reported how nutrient concentration via bottom-up effects could alter the effect of trophic cascades (e.g. Danielsdottir *et al*., 2007). For example, in marine systems with low nutrient inputs, Sommer *et al*. (2002) and Stibor *et al*. (2004) described a trophic cascade effect similar to the one reported in our study (predator-copepods-ciliates-small algae), but this sequence changed when there was higher nutrient availability, with the final part ending with an increase in large algae. Stibor *et al*. (2004) related these differences to (1) low nutrient concentrations, which frequently exclude larger algae; and (2) the size-mediated predatory effect of ciliates, which is higher on small algae than it is on larger algae. As a consequence, they conclude that a positive effect of top predators over algal biomass is observed only in mesocosms with enhanced nutrient loading, whereas there are decreases in mesocosms receiving zero nutrient loadings. In fact, our experimental and field results agree with those by Stibor *et al*. (2004), in which a negative effect on phytoplankton was observed.

Summarizing, *O. maeotica* acts as top predator exerting top-down control on zooplankton and over the rest of the plankton community through a trophic cascade effect. When *O. maeotica* is present, the entire plankton community changes: through direct effects large zooplankton decrease and through indirect effects ciliates increase and autotrophic organisms decrease. Moreover, in contrast with previous studies in which the changes observed in planktonic communities with the presence of jellyfish species are explained by a combination of top-down and bottom-up controls (e.g. Jankowski *et al*., 2005), in our case, the observed direct and indirect effects may be due mainly to a top-down effect, since *O. maeotica* appears only under oligotrophic conditions, without any external nutrient input. Bottom-up effects could also appear due to excretion, mucus production and decomposition of jellyfish in oligotrophic environments (Pitt *et al*., 2009). Nevertheless our results suggest a strong top down effect of the jellyfish without any interaction with nutrient supplies. In fact, Mediterranean brackish marshes are characterised by pulses of nutrient inputs coinciding with sudden flooding due to sea storms or intense rainfall. After these pulses, the water remains confined, with no other water inputs, leading to a decrease in water level and an increase in salinity due to evaporation (Quintana *et al*., 1998a, Quintana 2002). In such environments, physical factors such as flooding intensity determine pulse events and, in turn, nutrient loadings during pulses. Our results suggest that these 2 environmental situations (pulse and confinement) correspond to a change in the successional process associated with a change in the food web control mechanism. Thus, the pulse situation implies an allogenic succession when the food web is bottom-up controlled, whereas the confinement situation implies an autogenic succession when the food web is top-down controlled (situations of *O. maeotica* dominance). Abrupt shifts in the food web control mechanisms according to different environmental situations have been reported previously in brackish ecosystems (e.g. Petersen *et al*., 2008). Moreover, the existence of allogenic succession after a resource pulse and the posterior substitution by an autogenic process has been considered a general pattern in other aquatic ecosystems, such as freshwater temporary ponds (e.g. Lake *et al*., 1989; Boix *et al*., 2004).

Annex 1. Mean (SE) values of size, initial densities and biomass of all organism types found in different treatments. APF: autotrophic picoflagellates; ANF1: autotrophic nanoflagellates 1; ANF2: autotrophic nanoflagellates 2; HPF: heterotrophic picoflagellates. SP: small plankton; LP: large plankton.

			Initial conditions		**Lagoon**		**Control**		***Odessia* treatment**		**Filtered treatment**	
	Data set	**Size (µm)**	**Density (ind ml^{-1})**	**Biomass**	**Density (ind ml^{-1})**	**Biomass**	**Density (ind ml^{-1})**	**Biomass**	**Density (ind ml^{-1})**	**Biomass**	**Density (ind ml^{-1})**	**Biomass**
Bacterioplankton	SP	0.38	$8.78 \cdot 10^{6}$	0.07	$1.24 \cdot 10^{7}$	0.08	$1.43 \cdot 10^{7}$	0.10	$1.28 \cdot 10^{7}$	0.09	$1.29 \cdot 10^{7}$	0.08
		(0.10)	($5.80 \cdot 10^{6}$)	(0.04)	($6.09 \cdot 10^{6}$)	(0.04)	($1.17 \cdot 10^{7}$)	(0.08)	($8.68 \cdot 10^{6}$)	(0.06)	($6.92 \cdot 10^{6}$)	(0.05)
APF	SP	1.95	$3.09 \cdot 10^{6}$	6.62	$3.67 \cdot 10^{6}$	6.83	$3.72 \cdot 10^{6}$	7.05	$6.03 \cdot 10^{6}$	10.05	$4.77 \cdot 10^{6}$	7.96
		(0.12)	($8.92 \cdot 10^{5}$)	(1.98)	($5.98 \cdot 10^{5}$)	(2.14)	($7.66 \cdot 10^{5}$)	(2.43)	($1.88 \cdot 10^{6}$)	(2.54)	($1.25 \cdot 10^{6}$)	(1.57)
HPF	SP	2.01	$7.14 \cdot 10^{3}$	0.01	$9.92 \cdot 10^{4}$	0.01	$1.03 \cdot 10^{4}$	0.01	$1.34 \cdot 10^{4}$	0.01	$9.60 \cdot 10^{3}$	0.01
		(0.32)	($2.45 \cdot 10^{3}$)	($2.75 \cdot 10^{-3}$)	($5.80 \cdot 10^{3}$)	(0.01)	($6.39 \cdot 10^{3}$)	(0.01)	($7.72 \cdot 10^{3}$)	(0.01)	($4.11 \cdot 10^{3}$)	($4.57 \cdot 10^{-3}$)
ANF1	SP	3.76	$1.37 \cdot 10^{5}$	1.72	$1.19 \cdot 10^{5}$	1.81	$2.30 \cdot 10^{5}$	2.93	$2.95 \cdot 10^{5}$	3.75	$3.58 \cdot 10^{5}$	4.42
		(0.22)	($3.62 \cdot 10^{4}$)	(0.48)	($2.94 \cdot 10^{4}$)	(0.78)	($8.30 \cdot 10^{4}$)	(1.16)	($9.64 \cdot 10^{4}$)	(1.04)	($1.47 \cdot 10^{5}$)	(1.34)
ANF2	SP	4.15	$5.94 \cdot 10^{5}$	13.12	$1.64 \cdot 10^{5}$	2.72	$2.76 \cdot 10^{5}$	4.71	$3.35 \cdot 10^{5}$	5.47	$5.94 \cdot 10^{5}$	5.23
		(0.31)	($2.16 \cdot 10^{5}$)	(3.91)	($6.22 \cdot 10^{4}$)	(0.89)	($1.82 \cdot 10^{5}$)	(3.66)	($1.25 \cdot 10^{5}$)	(1.18)	($2.16 \cdot 10^{5}$)	(1.53)
Chlorophytes	SP	8.70	4.02	$1.30 \cdot 10^{-4}$	20.76	$4.12 \cdot 10^{-3}$	4.02	$2.10 \cdot 10^{-4}$	8.03	$1.40 \cdot 10^{-4}$	12.05	$2.18 \cdot 10^{-4}$
		(1.78)	(8.98)	($2.91 \cdot 10^{-4}$)	(34.77)	($7.65 \cdot 10^{-3}$)	(8.98)	($4.70 \cdot 10^{-4}$)	(17.96)	($3.13 \cdot 10^{-4}$)	(17.96)	($3.45 \cdot 10^{-4}$)
Haptophytes	SP	8.78	642.42	0.02	$4.42 \cdot 10^{3}$	0.16	$5.79 \cdot 10^{3}$	0.18	$1.11 \cdot 10^{4}$	0.35	$1.18 \cdot 10^{4}$	0.37
		(2.59)	(107.17)	(0.01)	($1.95 \cdot 10^{3}$)	(0.09)	($1.98 \cdot 10^{3}$)	(0.05)	($7.67 \cdot 10^{3}$)	(0.22)	($1.67 \cdot 10^{3}$)	(0.06)
Cryptomonas	SP	23.85	0.00	0.00	0.00	0.00	4.02	$2.16 \cdot 10^{-3}$	4.02	$1.48 \cdot 10^{-3}$	8.03	$2.29 \cdot 10^{-3}$
		(2.34)					(8.98)	($4.82 \cdot 10^{-3}$)	(8.98)	($3.30 \cdot 10^{-3}$)	(11.00)	($3.20 \cdot 10^{-3}$)
Ciliates (*Strombidium* sp.)	SP	29.56	4.02	0.01	168.64	0.22	32.12	0.11	473.00	0.58	521.96	0.57
		(13.65)	(8.98)	(0.02)	(126.64)	(0.16)	(30.44)	(0.16)	(301.00)	(0.28)	(261.37)	(0.35)
Euglenophytes	SP	55.32	48.18	0.21	0.00	0.00	4.02	0.03	0.00	0.00	4.02	0.00
		(19.12)	(107.74)	(0.48)			(8.98)	(0.06)			(8.98)	
Diatoms (A*mphora* sp.,	SP	76.18	248.94	0.02	176.67	0.02	28.11	$2.36 \cdot 10^{-3}$	76.29	0.01	256	0.02
Navicula sp., *Nitzchia* sp.)		(45.35)	(130.57)	(0.01)	(82.04)	(0.01)	(33.59)	($2.48 \cdot 10^{-3}$)	(16.80)	(0.01)	(122.44)	(0.01)
Nauplis Harpacticoid	LP	88.33	0.00	0.00	0.00	$7.9 \cdot 10^{-5}$	0.08	$2.8 \cdot 10^{-5}$	0.13	$2.32 \cdot 10^{-4}$	17.20	$3.89 \cdot 10^{-3}$
		(16.22)				($1.77 \cdot 10^{-4}$)	(0.11)	($6.2 \cdot 10^{-5}$)	(0.22)	($4.03 \cdot 10^{-4}$)	(21.51)	($4.20 \cdot 10^{-3}$)

Hexarthra sp.	LP	114.28 (29.92)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18 (0.28)	$4.17 \cdot 10^{-3}$ ($6.44 \cdot 10^{-3}$)
Naupli Cyclopoid	LP	186.90(44.41)	0.00	0.00	0.00	0.00	0.00	0.00	0.03 (0.06)	$2.72 \cdot 10^{-3}$ ($6.09 \cdot 10^{-3}$)	0.00	0.00
Brachionus sp.	LP	189.40 (45.34)	0.05 (0.11)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Naupli Calanoid	LP	0.02 (83.43)	37.65 (12.93)	6.96 (3.29)	39.95 (10.06)	4.19 (1.21)	23.50 (1.24)	3.63 (0.45)	0.35 (0.65)	0.01 (0.02)	0.78 (0.27)	229.87 (0.01)
Polychaeta larvae	LP	270.78 (142.80)	0.45 (0.21)	0.11 (0.16)	1.90 (0.68)	0.54 (0.15)	0.45 (0.27)	0.06 (0.05)	0.10 (0.16)	0.06 (0.09)	0.00	0.00
Ciliates	LP	295.00 (85.21)	0.20 (0.21)	0.10 (0.13)	0.45 (0.21)	0.18 (0.10)	0.15 (0.22)	0.06 (0.10)	0.48 (0.42)	0.20 (0.16)	0.08 (0.11)	0.02 (0.02)
Copepodit Harpacticoid	LP	323.00 (112.24)	0.00	0.00	0.15 (0.14)	0.01 (0.01)	0.00	0.05 (0.10)	0.00	0.00	0.03 (0.06)	0.01 (0.02)
Copepodite Cyclopoid	LP	446.50 (99.76)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03 (0.06)	0.37 (0.35)
Adult Harpacticoid (*Mesochra* sp.)	LP	537.80 (116.81)	0.00	0.00	0.00	0.00	0.00	0.00	0.03 (0.06)	0.04 (0.09)	0.03 (0.06)	0.04 (0.09)
Copepodite Calanoid	LP	717.48 (215.18)	41.60 (9.89)	80.34 (31.78)	62.90 (28.54)	99.40 (46.99)	68.90 (15.81)	109.42 (33.51)	0.13 (0.22)	0.39 (0.81)	0.05 (0.07)	0.03 (0.04)
Adult Calanoid (*Eurytemora velox*)	LP	1448.01 (99.67)	5.65 (3.38)	37.96 (14.87)	10.90 (5.23)	73.76 (34.97)	7.13 (4.31)	61.97 (12.73)	0.00	0.00	0.00	0.00

Photo: Carlos González Revelles

Chapter 6

Fish predation effects on benthos and plankton in a Mediterranean salt marsh

Compte, J., Gascón, S., Quintana, X. D., Boix, D., Submitted. Fish predation effects on benthos and plankton in a Mediterranean salt marsh.

ABSTRACT: Here we study how predation by *Aphanius iberus*, an endangered cyprinodontic species, affects plankton and benthos community. A field experiment using mesocosms in a Mediterranean salt marsh was carried out to assess if *A. iberus* effects are stronger on benthos or plankton. We observed weak effects of *A. iberus* on benthos, possibly because they use macrophytes as a refuge from predators. However, the presence of *A. iberus* decreased the abundance of large plankton, such as gammarids and jellyfish, and increased the abundance of medium-sized plankton, such as harpacticoids and rotifers, suggesting that *A. iberus* has a visual predatory behaviour. *A. iberus* changed the species richness and diversity in benthos and species composition and size distribution in plankton. These results suggest that *A. iberus* is a keystone species.

Keywords: *Aphanius iberus*, Keystone species, Field experiment, Coastal wetlands, Mesocosm

INTRODUCTION

Predation is a key factor shaping natural communities (Sih *et al*., 1998) with an important role structuring aquatic communities (Lynch, 1979; Carpenter *et al*., 1985; Jeppesen *et al*., 2000). Fish predation reduces the biomass of plankton assemblages and decreases the abundance of large-bodied species (Lynch, 1979; Vanni, 1987). Due to indirect effects, the abundance of small-bodied zooplankton increases (Brooks & Dodson, 1965; Vanni, 1988; Carpenter & Kitchell, 1993). Additionally, fish predation can results in changes in the demography, morphology and behaviour of the planktonic species (Gliwicz, 1990; Hansen & Jeppesen, 1992; Jacobsen *et al*., 1997; Moss, 1998). Fish also prey on benthic organisms but in this case their effects are usually more complex to asses because the presence of physical refuges can alter the interaction fish-prey (Diehl, 1992). In these sense, the use of macrophytes as refugee for invertebrates to avoid fish predation has been widely described (Diehl & Eklöv, 1995; Schriver *et al*., 1995; Paukert & Willis, 2003). Consequently, the effects of fish predation may be less evident in benthic than in planktonic assemblages, because refugee decreases the fish capture ability (Strayer, 1991). However, some fish do prey on benthos, reducing both biomass and density of benthos (Mittelbach, 1988; Diehl, 1992; Aarnio, 2000). Moreover, recent works has suggested that when the predators are small-bodied fish,

macrophytes do not offer a refuge for the benthos, because these fish can also move and live within the vegetation (Meerhoff *et al.*, 2007). Thus, it remains unknown whether a small predatory fish will have a greater impact on plankton or on benthos.

To answer this question we performed a field experiment, using of small fish *Aphanius iberus* (total length usually <5 cm, García-Berthou & Moreno-Amich, 1992). Several characteristics make *A. iberus* an ideal target species for this study. Firstly, *A. iberus* is a cyprinodontic species endemic from the Mediterranean coast of the Iberian Peninsula, and it is considered in danger of extinction (Doadrio, 2001). Consequently, it would be of general interest to know which kind of changes it would expect in aquatic communities due to their extinction. Secondly, its actual distribution is reduced to brackish and hyperhaline shallow waters in salt marshes and coastal lagoons, but there it use to be the main fish species, with stable populations achieving high densities around 30 individuals m^{-2} (García-Berthou *et al.*, 1991; Badosa *et al.*, 2007). It means that its effects might be not negligible. Thirdly, *A. iberus* is a benthic species strongly related to macrophyte mats (Moreno-Amich *et al.*, 1999, Rincón *et al.*, 2002). This is especially interesting when studying if macrophytes support refugee to invertebrates in front small fish predators. Finally, fish diet are very broad and dynamic as the result of the variability and availability of larger prey (Eggers, 1982). *A. iberus* have an omnivorous behaviour with benthic (Vargas & de Sostoa, 1999; Doadrio, 2001; Rincón *et al.*, 2002), and planktonic prey (Alcaraz & Garcia-Berthou, 2007). Hence, it has the potential ability to exploit both fractions of the aquatic community, and so to impact both fractions.

Using mesocosms, we investigated the effects of the presence of *A. iberus* on both benthic and planktonic invertebrate fractions. Our main objective is focussed on possible changes in the benthic and planktonic fraction associated to the fish presence. *Aphanius iberus* has traditionally been described as a benthic species, and consequently a stronger effect on benthos is expected if macrophytes really not reduce the capture ability of this small fish.

MATERIALS AND METHODS

Study site

The study site was a temporary oligotrophic salt marsh lagoon in the Empordà wetlands (NE Iberian Peninsula). The Empordà wetlands are a set of Mediterranean shallow coastal lagoons free from tidal influence. Their hydrological regime is determined by the occurrence of floods due to meteorological disturbances in autumn and winter and desiccation in summer (Quintana *et al*., 1998a, Quintana, 2002). In the wetlands, the zooplankton assemblage is composed mainly by calanoids (*Calanipeda aquaedulcis* and *Eurytemora velox*), cyclopoids (*Diacyclops bicuspidatus*), harpacticoids (*Cleptocampus confluents*) and rotifers (*Brachionus plicaitlis* and *Synchaeta* spp.) (Quintana *et al*., 1998b; Brucet *et al*., 2005). The zoobenthos is dominated by chironomids (*Chironomus salinarius*), polychaetes (*Nereis diversicolor*), amphipods (*Gammarus aequicauda*), ostracods (*Cyprideis torosa*), harpacticoids (*Cleptocampus confluents*) and nematodes (*Diplolaimella* sp., *Monhystrella* sp., *Thalassomonhystera* sp. and *Ptycholaimellus* sp.) (Gascón *et al*., 2005; 2006; 2008). *Aphanius iberus* and *Pomatoschistus microps* are the dominant fish in the permanent lagoons of Empordà wetlands; and *Atherina boyeri* and *Anguilla anguilla* are occasionally present (García-Berthou, *et al*., 1991).

Experimental design

The experiment was done in the field from March to May 2006. We installed six mesocosms (fibreglass cylinders; diameter: 1.2 m; high: 1.0 m) in the lagoon. To prevent any exchange with the outside of mesocosms, each mesocosm was 5 cm deep in the sediment. To prevent predation by birds, the open top was covered by a net (2 cm of mesh. We randomly assigned the mesocosms to one of two treatments (fish presence and fish absence) with three mesocosms for each treatment. One week after installation (March), we added 20 adult females *A. iberus* from a nearby (up to 800 m) permanent lagoon to each mesocosm for the "fish treatment". The *A. iberus* density within the mesocosms was similar to densities in nearby permanent lagoons (Badosa *et al*., 2007). We limited our study to females because males are territorial and spend a lot of energy on defending their territory and on mating (Oliva-Paterna *et al*., 2007), which could coincide with a non-feeding period (Wootton, 1998). To obtain groups of 20 individuals with similar size and biomass in each mesocosm, measured the total length of the fish.

We estimated the biomass of each individual using the equation of Verdiell-Cubedo *et al*. (2006). Fish biomass ranged from 2.63 to 3.31 g ind^{-1} was not significantly different among the fish treatment mesocosms (ANOVA; $F_{2,57} = 0.61$; $p = 0.54$) assuring a similar predation pressure per fish treatment mesocosm. In the remaining three mesocosms, we did not add any fish ("control treatment"). We measured the macrophyte biomass within each mesocosm to estimate the availability of refugee. The average macrophyte biomass in the mesocosms was 5.92 mg cm^{-2}. *Ruppia cirrhosa* was the main macrophyte species (96.45% of macrophyte biomass), and *Lauprothamnium populosum* was also present (3.55% of total macrophyte biomass). Significant differences among treatments were not found in macrophyte biomass (ANOVA; $F_{1,4} = 7.36$; $p = 0.06$) assuring a similar refugee per mesocosm. All *A. iberus* survived the duration of the experiment and were released at its conclusion.

Sampling procedure

Before fish addition, we determined the "initial conditions" in each mesocosm. We measured water characteristics as well as benthonic and planktonic fractions. To control the intra-mesocosms variability we took three samples from each mesocosm. Additionally, we collected three samples from the lagoon ("lagoon") to determine if there is a possible mesocosm effect. Two months after fish addition we repeated the sampling to obtain the "final conditions". Samples were stored, identified and counted by means of the different methods described bellow.

Several water characteristics were measured (Table 1). Water temperature (ºC), electrical conductivity (EC_{25}), pH, and dissolved oxygen (%) were measured *in situ*. Dissolved inorganic nutrients (ammonia, nitrite, nitrate, and soluble reactive phosphorous) and total nutrients (total nitrogen and phosphorous) were analyzed following Grasshoff *et al*. (1983). Water chlorophyll-*a* was measured according to Talling and Driver (1963).

We randomly collected three sediment samples per mesocosm. To obtain population estimates from benthic organisms of a range of sizes (meio- to macrobenthos), each sediment sample consisted of two captures: one with a core (internal diameter = 5.2 cm) and the other with a grab (Ekman grab= 225cm^2). All organisms captured in the sediment samples (core or grab) were considered as the benthonic fraction. Each core sample was taken 5 cm deep in the sediment and sieved through 500 µm mesh. To sort meiobenthos organisms, the sieved material was

suspended in a sugar-water solution (1:1) for 3 h. The sugar suspension was filtered through a sieve (50 µm mesh-size), stained with rose Bengal, and preserved in 4% formalin (modified from de Jonge & Bouwman, 1977) until taxonomic identification. Three sugar extractions were successively carried out per sample. We estimated the population density (individuals per cm^2) using the removal method for closed populations (Seber, 1982). Macrobenthos organisms were obtained using an Ekman grab (225 cm^2). To identify the macrobenthic organisms, we sorted the sediment obtained from the Ekman grab (225 cm^2) using a 500 µm mesh-size sieve, counted the number of animals, and preserved each animal in 4% formalin until it was identified.

Table 1. Physical and chemical water characteristics of the lagoon in the beginning and end of the experiment. Chla-*a*: chlorophyll *a*; NH_4^+: Amonia; NO_2^-: Nitrite; NO_2^{3-}: Nitrate; OM: Organic matter; SRP: soluble reactive phosphorous. [a] : under detection level.

	March	May
Water level (cm)	55.55	22.11
Conductivity (mS cm^{-1})	37.02	49.87
Temperature (ºC)	10.83	17.77
pH	7.68	9.08
Dissolved Oxygen (%)	120.69	97.53
OM (ppm)	11.15	24.00
Total nitrogen (mg l^{-1})	0.95	3.01
NH_4^+ (mg l^{-1})	0.03	<0.01[a]
NO_2 (mg l^{-1})	<0.01[a]	<0.01[a]
NO_3^-(mg l^{-1})	0.01	0.01
SRP (mg l^{-1})	<0.01[a]	0.01
Total phosphorous (mg l^{-1})	0.03	0.23
Chla-*a* (µg l^{-1})	2.97	20.08

We obtained the planktonic fraction by filtering 5 l of water trough a 50 µm mesh-size and the organisms retained was immediately fixed with 4% formaldehyde solution. All individuals captured in these samples were considered as planktonic fraction. We counted and identified the plankton using a stereomicroscope and an inverted microscope.

We classified each benthic and planktonic specimen to the species level when possible, except for nematodes, microturbellaris, rotifers, and ciliates that were identified

to genera level. For data analysis, nematodes and copepods were grouped into functional groups. Nematodes genera were grouped according to feeding behaviours following the Weiser (1953) classification: selective deposit feeders (*Thalassomonhystera* sp. and *Monhystrella* sp.), unselective deposit feeders (*Theristus* sp.), epigrowth feeders (*Ptycholaimellus* sp., *Dichromadora* sp. and *Calomicrolaimus* sp.) and omnivorous and/or predatory nematodes (*Sphaerolaimus* sp. and *Fictor* sp.). Copepods were grouped by development stages (nauplii, copepodites, and adults) and by order: calanoids (*C. aquaedulcis* and *E. velox*), cyclopoids (*D. bicuspidatus* and *Halicyclops rotundipes*), and harpacticoids (*Canuella perplexa*, *Cletocampus confluens*, *Mesochra heldti*, *Mesochra lilljeborgi*, and *Nitocra spinipes*).

We measured 30 random individuals of each taxa of benthos and plankton fraction and estimated their biomass using the following equations: Quintana (1995) for gammarids; Smock (1980) for chironomids; Linton and Taghon (2000) for capitellids; Lingegaard (1992) for gastropods; Johnston (1995) for ostracods; Wieser (1960) and Jensen (1983) for nematodes; Gradinger *et al*. (1999) and Nozais *et al*. (2001) for turbellarians; Malley *et al*. (1989) for copepods, rotifers (*Notholca* sp.), and jellyfish; Ruttner-Kolisko (1997) for rotifers; and Putt and Stoecker (1989) for ciliates.

Measured community parameters

We measured five community parameters for the benthic and the planktonic fractions: species diversity (*H*), size diversity (μ), species richness (*S*), total biomass (TB), and average body size (BS). Species diversity was measured using the Shannon-Wiener index (Pielou, 1969) and was calculated using biomass as abundance. Size diversity and average body size were calculated using Kernel estimation (Quintana *et al*., 2008). Total biomass was the sum of each organism's biomass in a sample.

Data analysis

We tested the possible effects of *Aphanius iberus* and mesocosm on benthonic and planktonic fractions separately. We used two approaches to test these effects: multivariate one when considering taxa matrices, and univariate one when using community parameters. Finally, we used a variation partitioning to compare the strength of fish effects on benthonic and planktonic fractions.

a) Fish effect: multivariate approach

Using Canonical Correspondence Analysis (CCA), we tested how the presence of *Aphanius iberus* affected assemblage matrices using biomass as abundance data. We determined the significance of fish effects using restricted Monte-Carlo permutation tests (499 restricted permutations for split-plot design). To allow the correct comparison between treatments (repeated measures design), we restricted the permutations using a split-plot design to restrict comparisons to the same mesocosm in initial and final conditions. The null hypothesis of this test stated that the relative proportion in biomass of the analysed fraction (benthos or plankton) do not differ between treatments (i.e. no fish effect detected). To do so, the interaction between fish and time was used as explanatory variable (Lepš & Šmilauer, 2003). The response variables were the log-transformed taxa abundances expressed in biomass. Additionally, using CANOCO 4.5 software (ter Braak & Smilauer, 2002), Correspondence Analyses (CA) were used for illustrative purposes, to show the samples position.

b) Fish effect: univariate approach

To analyze data with both fixed and random effects, we used linear mixed-effects (lme) models with the "lme" function found in nlme package (Pinheiro *et al*., 2007) wrote in R language (R 2.5.0; Development Core Team, 2007). We estimated the parameters for two fixed variables (treatment and time) and an interaction term (treatment per time). Similarly to the approach used in the multivariate analyses, we used interaction term to asses the fish effects. We considered the interaction term rather than the treatment variable, because the interaction term also takes into account the time changes due to "natural" succession processes (i.e. differences in the control treatment between initial and final conditions). Thus, if the trend observed in fish treatment is not the same to the one observed in the control treatment would mean that the changes observed in fish treatment are not the same as the observed on a "natural" succession process (i.e. without fish presence). We took three samples per mesocosm and we considered mesocoms as random effects in the models to avoid problems of spatial pseudo-replication (Crawle, 2002). With lme, we also used heteroscedastic models. To fit these models, we used the 'VarIdent' variance function to allow for different variance in each mesocosm. We determined if heteroscedastic model fit the data with a likelihood ratio test (Pinheiro & Bates, 2000). Finally, to check if parameters were not significantly different between treatments in initial conditions, we also used lme.

c) Comparison between fish effect on plankton and benthos

To determine whether the presence of *A. iberus* affected planktonic or benthonic fraction more, we employed a variation partitioning technique using CANOCO 4.5. This method divides the variation of species biomass data into independent components (Borcard *et al*., 1992) decomposing the variation of dependent variables in unique (or pure) and shared (or joint) effects of a set of predictors. To partition the variation, we used partial regression and redundancy analysis for multiple dependent variables (taxa matrices). We used two sets of explanatory variables: time (including two dummy variables indicating initial and final condition samples) and fish (including two dummy variables indicating control and fish treatments samples). This allowed decomposing the variation in the following components: (1) pure fish effect, (2) pure time effect, and (3) shared effects (time+fish).

d) Mesocosm effect: multi and univariate approaches

We also analyzed mesocosm effect with CCA (multivariate) and lme models (univariate) as described above. To determine the net mesocosm effect on assemblage matrices, we compared the initial and final conditions in lagoon and control samples using CCA and Monte-Carlo permutation test (499 restricted permutations). To determine if the changes in community parameters over time (interaction mesocosm-time) were significantly different between lagoon and control samples, we used lme models.

RESULTS

Mesocosm effects on community structure

We detected a mesocosm effect on the assemblage matrices of benthic organisms (Monte-Carlo permutation test, $F = 2.22$; $p = 0.03$). Only two benthic community parameters, total biomass ($F_{2,17} = 5.78$; $p = 0.01$) and average body size ($F_{2,17} = 9.28$; $p < 0.01$), had a significant mesocosm effect. We did not observe mesocosm effects on plankton assemblage matrices (Monte-Carlo permutation test; $F= 1.16$; $p= 0.27$) or planktonic community parameters.

Fish effects on community structure

We did not find significant fish effects in the assemblage matrices of the benthonic fraction (Monte-Carlo permutation test; $F = 1.35$; $p = 0.16$), and the sample position in CA plot of fish and control treatments are not clearly separated in final conditions (Figure 1).

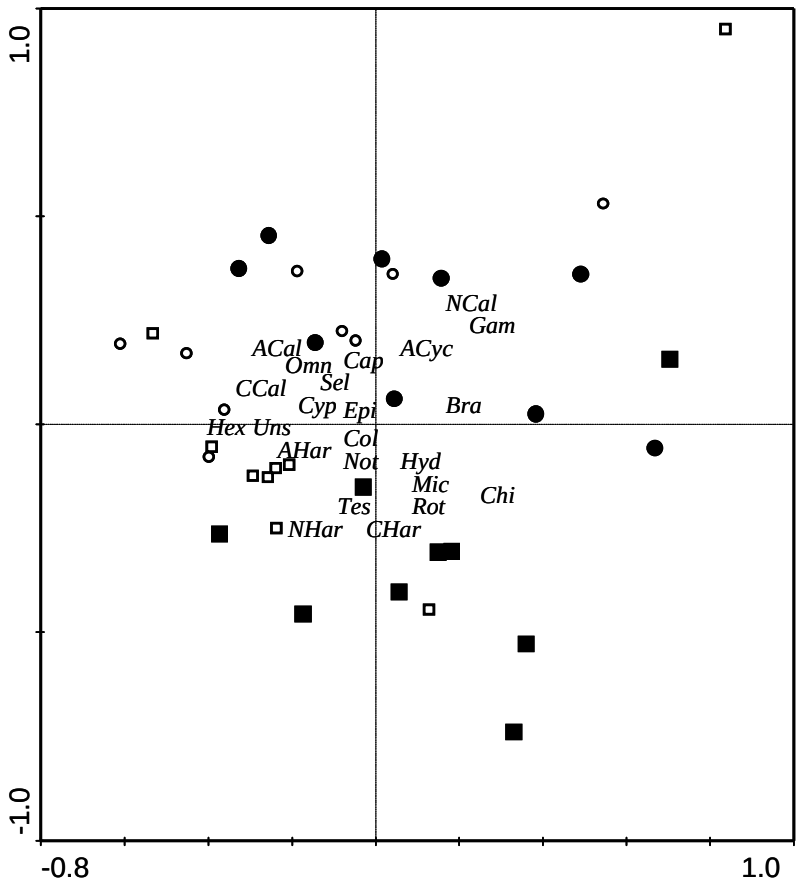


Fig. 1. Correspondence Analysis for benthos taxa. Open circles: control treatment, initial conditions; open squares: fish treatment, initial conditions; black circles: control treatment, final conditions; black squares: fish treatment, final conditions. ACal: calanoid adults; ACyc: cyclopoid adults; AHar: harpacticoid adults; Bra: *Brachionus* sp.; Cap: Capitellids; CCal: calanoid copepodites; CHar: harpacticoid copepodites; Col: *Colurella* sp.; Cyp: *Cyprideis torosa*; Epi: Epigrowth feeder nematodes; Hex: *Hexarthra* sp.; Hyd: *Hydrobia acuta*; Gam: *Gammarus aequicauda*; Mic: Microturbellaria; NCal: calanoid nauplii; NHar: harpacticoid nauplii; Not: *Notholca* sp.; Omn: Omnivorous / predatoyr nematodes; Chi: *Chironomus salinarius*; Rot: unidentified Rotifer; Sel: Selective deposit feeder nematodes; Tes: *Testudinella* sp.; Uns: Unselective deposit feeder nematodes.

In contrast, we observed significant fish effects in the plankton fraction (Monte-Carlo permutation test; $F = 15.19$; $p < 0.01$). The CA plot discriminated three distinct sample groups (Figure 2). The first cluster included initial samples from both treatments. These samples were characterized by higher biomass of calanoids and *Hexarthra* sp. The

second cluster included all control samples in final conditions. These samples were characterised by higher biomass of large (*Odessia maeotica* and *G. aequicauda*) and small organisms (ciliates). The third cluster included all samples from the fish treatment in the final conditions. These samples had a higher biomass of medium-sized organisms (harpacticoids and rotifers).

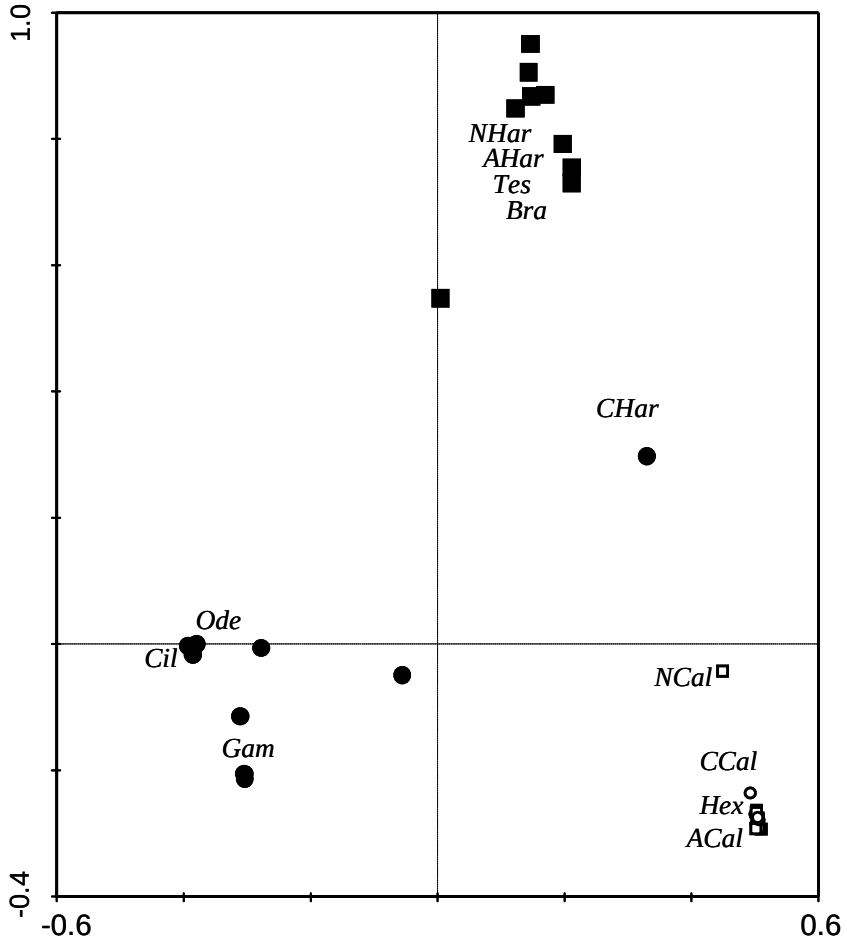


Fig. 2. Correspondence Analysis for plankton taxa. Open circles: control treatment, initial conditions; open squares: fish treatment, initial conditions; black circles: control treatment, final conditions; black squares: fish treatment, final conditions. ACal: calanoid adults; AHar: harpacticoid adults; Bra: *Brachionus* sp.; Cil: Ciliate; CCal: calanoid copepodites; CHar: harpacticoid copepodites; Gam: *Gammarus aequicauda*; Hex: *Hexarthra* sp.; NCal: calanoid nauplii; NHar: harpacticoid nauplii; Ode: *Odessia maeotica*; Tes: *Testudinella* sp.

At initial conditions, neither benthic nor planktonic community parameters were significantly different between treatments. At the final conditions, the presence of *A. iberus* had a significant effect (significant interaction term) in for both benthonic and planktonic fractions. In the benthonic fraction, species diversity, species richness and total biomass increased in fish treatment, whereas in control treatment, species diversity and specie richness decreased and the increase of total biomass was significantly lower

in the control treatment (lower slope; Figure 3). In the planktonic fraction, size diversity increased in control treatment and decreased in fish treatment, while species diversity had a significantly stronger decrease in control than fish treatment (Figure 4).

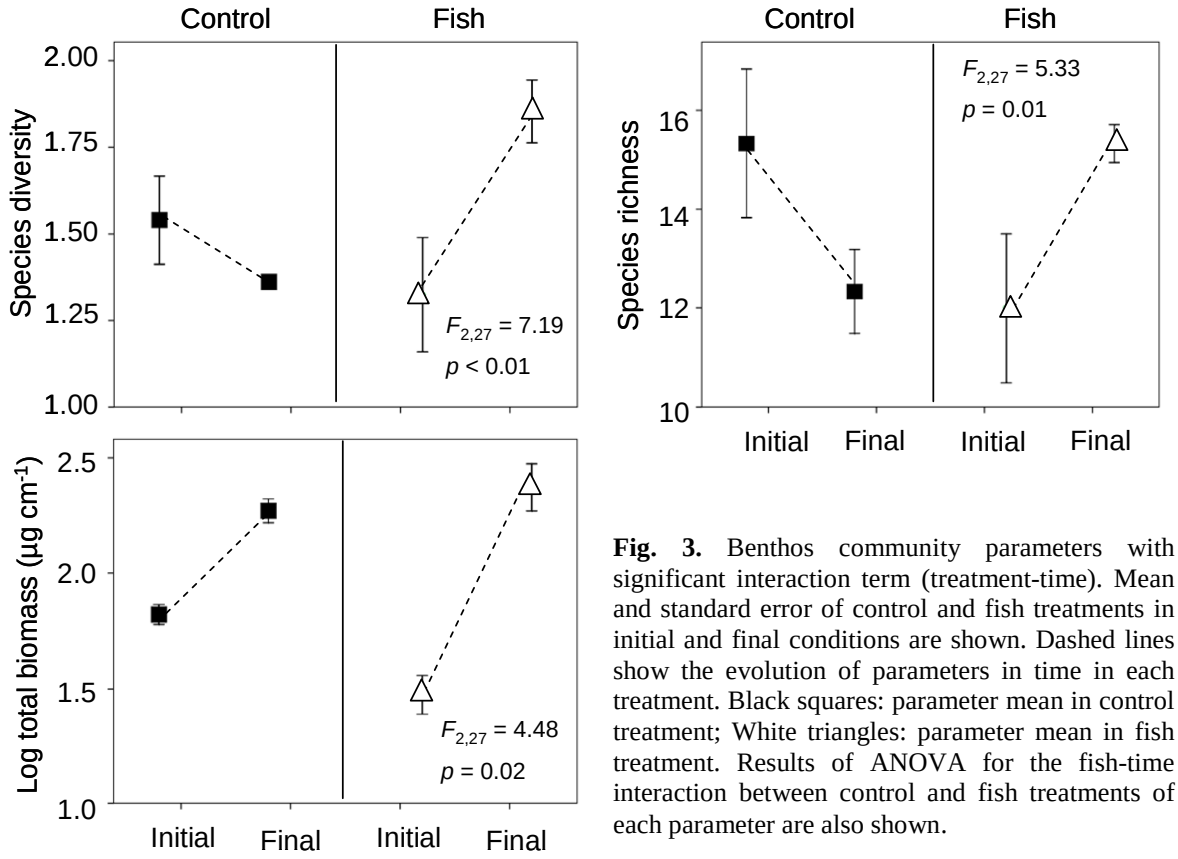


Fig. 3. Benthos community parameters with significant interaction term (treatment-time). Mean and standard error of control and fish treatments in initial and final conditions are shown. Dashed lines show the evolution of parameters in time in each treatment. Black squares: parameter mean in control treatment; White triangles: parameter mean in fish treatment. Results of ANOVA for the fish-time interaction between control and fish treatments of each parameter are also shown.

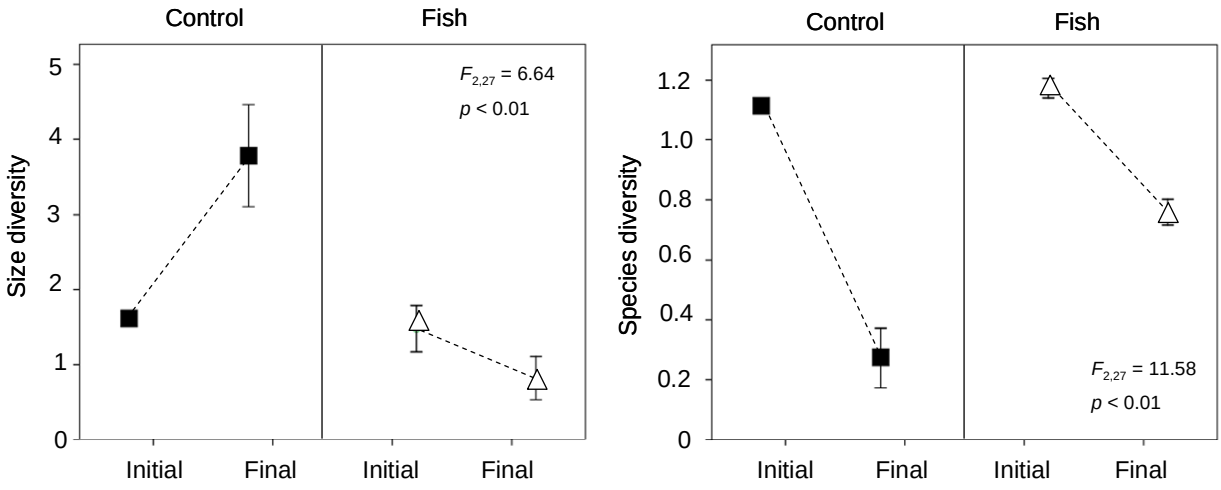


Fig. 4. Plankton community parameters with significant fish-time interaction. Mean and standard error of control and fish treatments in initial and final conditions are shown. Dished lines are the evolution of parameters in time in each treatment. Black squares: parameter mean in control treatment; White triangles: parameter mean in fish treatment. Results of ANOVA for the fish-time interaction between control and fish treatments of each parameter are also shown.

In the planktonic fraction, size diversity was significantly different between treatments, whereas the average body size was similar between treatments. Thus is explained because the most abundant size class is the same in both treatments, but the number of size classes was different among treatments (Figure 5). In the control treatment, the planktonic fraction had a wider range of sizes (from size class 0.01 to 1.1) with a higher abundance of smallest organisms (size class 0.1; mainly corresponding to ciliates). In fish treatment the plankton had a narrower range of sizes (from size class 0.01 to 0.2) but with higher relative abundance, corresponding of harpacticoids copepodits and rotifers (also size class 0.1).

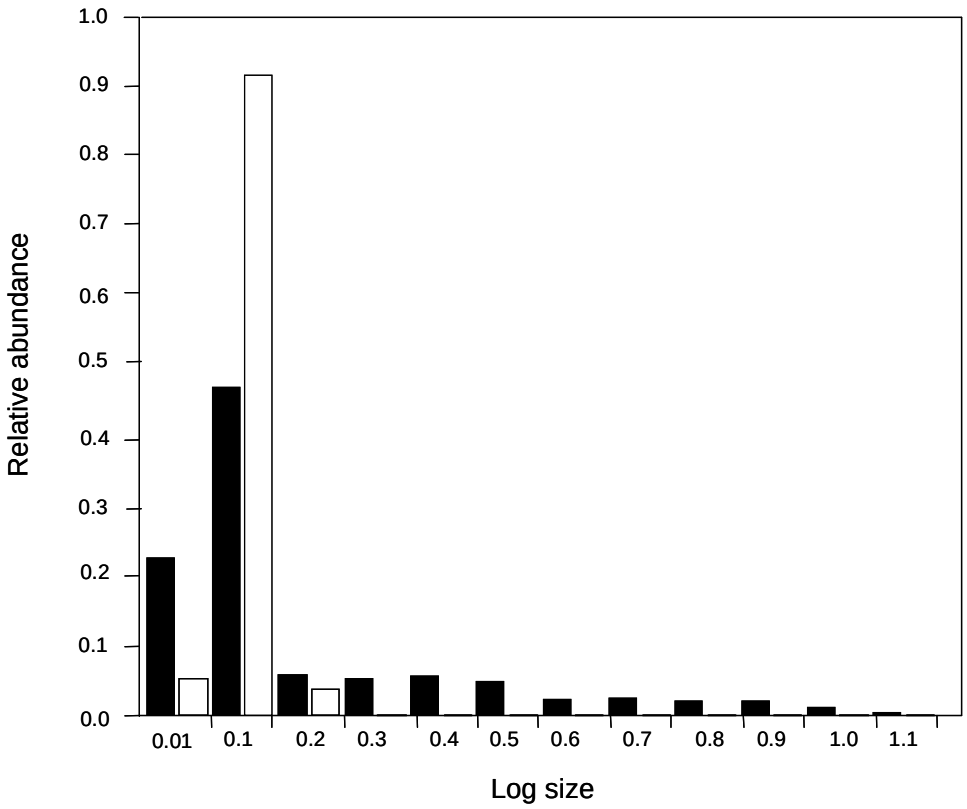


Fig. 5. Relationship between log-transformed body size and their relative abundance per treatment in planktonic fraction. Black bars: control treatment; white bars: fish treatment.

Comparison of relative importance of fish effects on benthos and plankton

Using variation partitioning, we determined that the importance of fish and time effects differ for the two fractions (Figure 6). Together, fish and time variables explained less than 20% of the variability in the benthonic fraction. In contrast, they explained more than 50% of the variability in the planktonic fraction. In benthonic fraction, fish

effect had higher importance (10.04%) than time (7.04%). However, in the planktonic fraction, time had higher importance (32.37%) than fish (19.70%). Nevertheless, when looking to the fish effect in both fractions, its importance is higher in the planktonic fraction (19.70%) than in the benthonic fraction (10.04%), which supports results from permutation test on the fraction matrices.

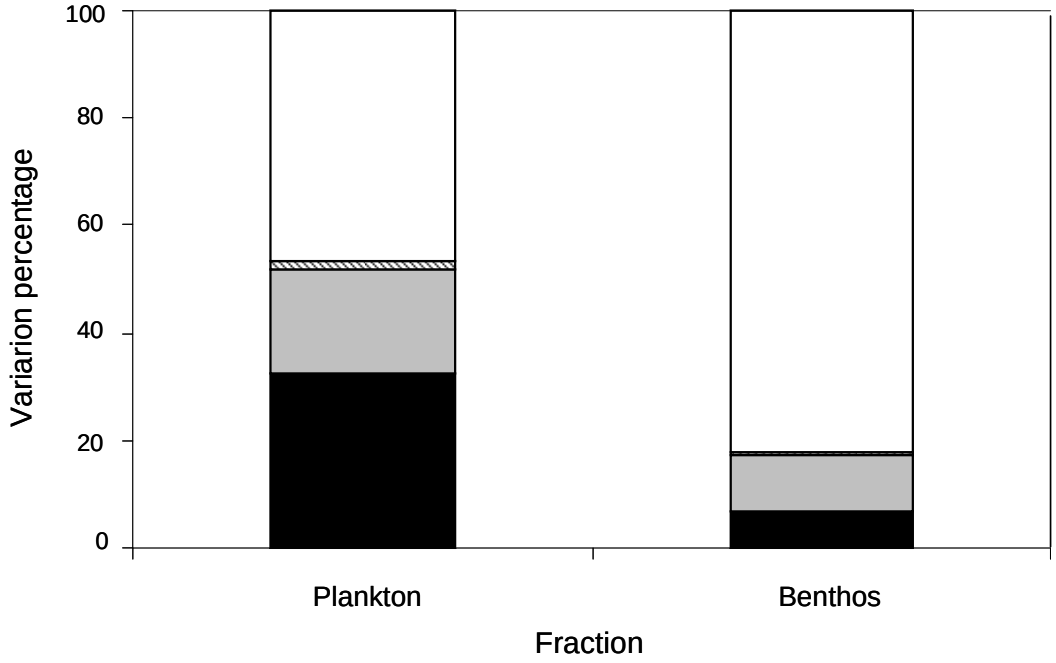


Fig. 6. Variation partitioning used to determine the percentage of variability explained by fish and time variables. Each column shows the different contribution of the variable in the variation of planktonic or benthonic fractions in the fish treatment samples. The different colours show the factors that explain the variation. Black: variation explained by time effect; Grey: variation explained by fish effect; grated pattern: variation explained by shared effects (time and fish); White: unexplained variation.

DISCUSSION

To generalize our results, we must account for two factors. First, we observed a mesocosm effect in the benthonic fraction, where spatial heterogeneity is noticeable in the initial conditions. In contrast, no such difference was observed in the planktonic fraction. Additionally, the variation partitioning analysis revealed a higher percentage of unexplained variance in the benthonic fraction than planktonic fraction. This unexplained variance could be attributed to a higher spatial heterogeneity in the benthonic fraction, which is expected when comparing benthonic organisms with more motile plankton (Beisner *et al.*, 2006). Second, we only used adult females in the

experiments and changes on the diet among different developmental stages of *A. iberus* have been described (Alcaraz & García-Berthou, 2007). We consider that the results of this study could be different in natural conditions when all developmental stages *A. iberus* population are presents. Still, it is important to note that the effect found can be exportable to natural conditions in periods with dominance of adult fish, it means out of the breeding season.

The strength of fish effects was different between the benthonic and planktonic fractions. These effects were more evident in the planktonic fraction, since it showed differences on assemblage composition and community parameters, as well as a higher percentage of variation explained by pure fish effects. In the absence of fish, we observed high size diversity and low species diversity because the planktonic fraction was composed of a few species with very different body sizes, such as large *O. maeotica* and *G. aequicauda* and small ciliates. In contrast, in the presence of fish, we observed that the size range of planktonic organisms was characterized by the dominance of a higher number of species with relatively similar body sizes (harpacticoids and rotifers). Fish presence had a stronger effect on free swimming invertebrates than on those more related to substrate.

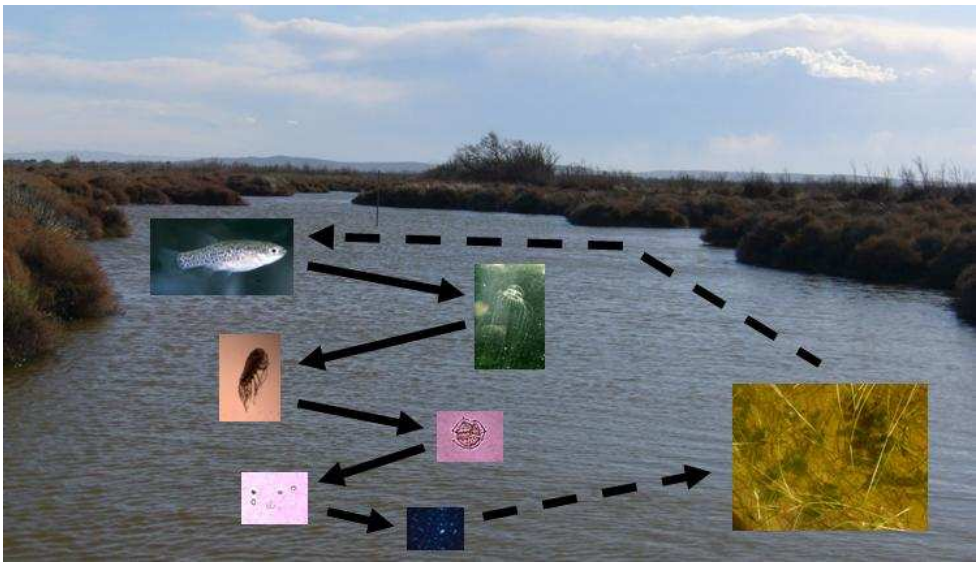
Fish effects may by due to direct but also to indirect effects. Both direct and indirect effects are not rare fish predation studies (e.g. Mancinelli *et al*., 2002). In indirect effects, competitive interactions may favour smaller sized zooplankton over larger ones if the latter are subjected to stronger predation pressures. In that sense, several evidences have been reported (Brooks & Dodson, 1965; Vanni, 1988; Carpenter & Kitchell, 1993). The absence of *O. maeotica* in the fish treatment could have presented another indirect effect. *O. maeotica* is a top-predator in this system and can exert a top-down control on the planktonic food web eventually arriving to suppress copepods from the food web (Compte *et al*., in press).

Direct effects may also occur since *Gammarus* is a prey of *A. iberus* (Alcaraz & García-Berthou, 2007). We observed that *G. aequicauda* changed its spatial distribution avoiding open waters, possibly as an adaptive defence against fish predation. These results are in line with previous studies that found that an increase in habitat complexity leads to a decrease of predation on gammarids (Dahl & Greenberg, 1996). Furthermore, in the presence of a predator, gammarids reduce their time in the water column and remain within the benthos (Wudkevich *et al*., 1997, Kaldonski *et al*., 2009). Additionally, the increase in total benthic biomass in the presence of *A. iberus* may also

be a consequence of an escape behaviour induced by the presence of fish, as has been widely described in other aquatic environments (e.g. Tjossem, 1990; Pijanowska, 1997; Åbjörnsson *et al*., 2004). Taken together, these behaviours suggest that visual detection of prey is important for *A. iberus*.

Macrophytes may provide refuges from fish predation for invertebrates to avoid fish predation (Dielh, 1992; Jacobsen *et al*, 1997), decreasing the predation ability of fish (Diehl, 1988; Bean & Winfield, 1995; Tatrai & Herzig, 1995). However, small fish can manoeuvre within the vegetation reducing its effectiveness as a refuge for invertebrates (Meerhoff *et al*., 2007). However, we observed few significant effects on the benthic fraction, and a lack of changes on its size distribution, together with invertebrate movements, as described before. These results, could be interpreted as even in the case of small fish predators associated with macrophytes, the habitat complexity provided by macrophytes represent a refuge for invertebrates against a visual predatory fish and may help to decrease the fish effects on preys.

The classical definition describes that the keystone species is a species of high trophic level that regulate species diversity predating and limiting the abundance of preys that would otherwise monopolize resources in its trophic level. Thus, it would affect competition process preventing the appearance of species with a well performance when competing with the rest (Paine 1966, 1969; Kerfoort & DeMott, 1984). In agreement with keystone species definition, *A. iberus* is a species of a higher trophic level that has the capacity to change the species diversity. This designation is supported by the higher species diversity and richness observed, even in the benthonic fraction. Furthermore, the presence of *A. iberus* decreased the abundance of large organisms, such as *G. aequicauda* and *O. maeotica*, and increased of smaller organisms (harpacticoids and rotifers), suggesting a trophic cascade effect (Carpenter *et al*., 1985; Carpenter & Kichell, 1993; Pace *et al*., 1999), that could affect the overall aquatic community structure, including both invertebrates and phytoplankton.



Chapter 7

Fish trophic cascade effects in Mediterranean salt marsh

Compte, J., Gascón, S., Quintana, X. D., Boix, D., In preparation. Fish trophic cascade effects in Mediterranean salt marsh.

ABSTRACT: Trophic cascade effects trigged by an endangered cyprinodontic species, *Aphanius iberus*, are studied. A field experiment using mesocosms in a Mediterranean salt marsh was carried out to assess if *A. iberus* have indirect effects trough trophic cascade on plankton and macrophytes. The *A. iberus* presence has effects on plankton structure increasing the species diversity and decreasing the size diversity. Stable isotops allowed us to differentiated two trophic levels in the studied food web. Nevertheless, our results showed that, *A. iberus* presence produces a larger trophic cascade as it would be expected by the few trophic levels differentiated. The observed trophic cascade could exist because the interactions among organisms are mainly size-dependent and not trophic level dependent. Additionally, and in contrast with our expectations, the *A. iberus* presence supposes a smaller increase of phytoplankton trough trophic cascade effects. Moreover, macrophytes biomass shows a smaller decrease than the observed one in absence of *A. iberus*. This fact suggests a possible co-evolution of *A. iberus* and the macrophyte *R. cirrhosa* which would benefice both organisms.

Keywords: *Aphanius iberus*, Stable isotops, Log ratios, Coastal wetlands, Mesocosm, Phytoplankton

INTRODUCTION

Trophic interactions among organisms determine the community structure (Kerfoot & Lynch, 1987). According to their effects on the community two kind of trophic interactions exist: direct and indirect (Schmitz *et al*., 1997). Predation is an example of direct trophic interaction (e.g. Sih *et al*., 1985; Paine, 1992; Snyder *et al*., 2005); whereas trophic cascades are examples of indirect ones (e.g. Rosenzweig, 1973; Carpenter *et al*., 1985; Schmitz & Sutlle, 2000). Nevertheless, direct and indirect trophic interactions often occur simultaneously. For instance, fish predation can trigger to trophic cascade (Carpenter *et al*., 1985, Carpenter & Kitchell, 1993). Trophic cascade hypothesis describes that, changes in each trophic level shows an opposite response in next trophic level because top level prey on bottom level. Therefore, the introduction or disappearance of fish in lakes can have important consequences in these environments.

On the other hand, macrophytes play a key role in species trophic interactions because can modulate the predator-prey relationship providing refugee to invertebrates in order to avoid fish predation (Dielh, 1992; Jacobsen *et al*, 1997; Meerhoff *et al*., 2007; Farina *et al*., 2009). Consequently, the presence of macrophytes is related to a decrease of the predator forage ability (Diehl, 1988; Bean and Winfield, 1995; Tatrai and Herzig, 1995). Moreover, macrophytes can also be related with water turbidity. Macrophytes and phytoplankton compete for nutrients and light (Ozimek *et al*., 1990; Sand-Jensen & Borum, 1991; Van Donk *et al*., 1993), hence a negative relationship between macrophytes and phytoplankton has been found (Scheffer *et al*., 1993; Scheffer & Jeppesen, 1997). Additionally, macrophytes have the capacity to reduce sediment resuspension and thus also allow increases of water transparency (Scheffer *et al*., 1993; Barko & James, 1998; James & Barko, 1990). In this sense, different studies have described that macrophyte biomass increase due to a phytoplankton reduction as result of trophic cascade originated by piscivorous fish (Jeppesen *et al*., 1990, van Donk & Gulati, 1995; Moss *et al*., 1996). Similarly, increase of water transparency resulting of a trophic cascade effect for fish has been reported (Carpenter, 1988; Scheffer, 1992; Søndergaard *et al*., 1997; Jeppesen *et al.*, 1999). Therefore, the presence of macrophytes may imply a positive relationship with aquatic invertebrates such as zooplankton (refugee) and negative ones with phytoplankton (competition). However, if trophic cascade is triggered by planktivorous fish, a negative relationship between planktivorous fish and macrophytes would be expected since, at first sight, an increase of phytoplankton populations is expected due to the reduction of zooplankton organisms.

We performed a field experimental approach to investigate the possible effects of small fish on plankton and macrophytes. The study was performed with mesocosms where the effects of fish on water characteristics, plankton and macrophytes were analysed. *Aphanius iberus* was the target fish species. *A. iberus* is a cyprinodontic fish endemic of the Iberian Peninsula Mediterranean coast and it is considered in danger of extinction (Doadrio, 2001). In now-a-days, the *A. iberus* distribution is reduced to brackish and hyperhaline shallow waters in salt marshes and coastal lagoons where is the main fish species with stable populations achieving high densities (around 30 individuals m^{-2}; García-Berthou *et al*. 1991, Badosa *et al*. 2007). It is a benthic species strongly related to macrophyte mats (Moreno-Amich *et al*., 1999, Rincón *et al*., 2002). Although adults of *A. iberus* have an omnivorous diet mainly based of benthonic organisms (Vargas & de Sostoa, 1999; Doadrio, 2001; Rincón *et al*., 2002) several studies suggest

that diet of *A. iberus* is more related with zooplanktonic organisms (Alcaraz & Garcia-Berthou, 2007). In this sense, a negative relationship between their densities and size diversity and planktonic organisms has been observed (Badosa *et al*., 2007). We hypothesized that the presence of *A. iberus* would changes the structure of plankton community through both direct (predation) and indirect (trophic cascade) effects causing at the end an increase of phytoplankton and consequently a decrease of macrophyte biomass.

MATERIALS AND METHODS

Study site

The study was carried out in a temporary and oligotrophic salt marsh lagoon located in the Empordà wetlands (NE Iberian Peninsula). Empordà wetlands are a set of Mediterranean shallow coastal lagoons free from tidal influence and whose hydrological regime is determined by the occurrence of floods due to meteorological disturbances in autumn and winter, and the process of desiccation during summer (Quintana *et al*., 1998a, Quintana, 2002). The zooplankton is composed mainly by jellyfish *Odessia maeotica*, calanoids (*Calanipeda aquaedulcis* and *Eurytemora velox*), cyclopoids (*Diacyclops bicuspidatus*), harpacticoids copepods (*Cleptocampus confluents*) and rotifers (*Brachionus plicaitlis* and *Sinchaeta* spp.) (Quintana *et al*., 1998b; Brucet *et al*., 2005). Phytoplankton is dominated by diatoms (*Amphora* spp., *Navicula* spp.), dinoflagellates (*Glenodinium foliaceum*) and haptophytes (López-Flores, in press). *A. iberus* and *Pomatoschistus microps* are the dominant fish in the permanent lagoons of Empordà wetlands; although, *Atherina boyeri* and *Anguilla anguilla* can be occasionally present (García-Berthou *et al*., 1991). *R. cirrhosa* and *Ruppia maritima* are the main macrophyte in these lagoons (Gesti, 2000; Gesti *et al*., 2005).

Experimental design

The experiment was done in the field along March-May 2006. Six fibreglass cylinders (diameter: 1.2 m; high: 1.0 m) were installed in the lagoon. To prevent any exchange with the outside of mesocosms, each mesocosm was 5 cm deep in the sediment. To prevent predation by birds, the open top was covered by a net (2 cm of mesh. Two treatments (fish presence and fish absence) were assigned randomly to the

mesocosms, with three mesocosms for each treatment. One week after installation (March), we added 20 adult females *A. iberus* from a nearby permanent lagoon were added per mesocoms (hereafter "fish treatment"). On the other three mesocosms, no fishes were added (hereafter "control treatment").

Sampling procedure

Before fish addition, water characteristics as well as plankton and macrophytes were sampled to provide "initial conditions". All organisms captured in the water column samples, were considered plankton organisms. Three samples were taken in each mesocosm in order to control the intra-mesocosms' variability. Three additional samples of the lagoon out of the mesocosms were collected to provide possible mesocosm effect (hereafter "lagoon"). Two months after fish addition the same sampling procedure was repeated providing "final conditions". At the end of the experiment, all individuals of *A. iberus* were alive and were captured and released. For more details, see Compte *et al*. (submitted).

In order to establish the water characteristics, water level, water temperature, electrical conductivity (EC_{25}), pH and dissolved oxygen (O_2 in % of saturation) were measured *in situ*. Dissolved inorganic nutrients (ammonia, nitrite, nitrate and soluble reactive phosphorous) total nutrients (total nitrogen and phosphorous), organic matter and water chlorophyll-*a* were analyzed according to the methods of described in Compte *et al*. (submitted).

Planktonic samples were collected, identified and counted using different methods: meso- and large microzooplankton (larger than 50 μm) following the methods of described in Compte *et al*. (submitted); and micro-, nano- and picoplankton (smaller 50 μm) according to the methods used in Compte *et al*. (2009). Meso- and large microzooplankton was identified to species level when possible or else to genera level or functional group. However, to data analysis copepods were grouped to different stages (nauplii, copepodits and adults) and to order level (calanoid, cyclopoid and harpacticoid). Micro-, nano- and picoplankton were identified a level of functional group. Thirty random organisms of each taxa were measured and their biomass was estimated using equations referenced in Brucet *et al*. (2008); Compte *et al*. (in press; submitted).

Three macrophytes samples were randomly taken using an Ekman grab (225 cm^2). Plants were identified, separated from the sediment, dried to 60 ºC during 48 hours and weighted.

Stable isotopes

In order to analyze the trophic position of different organisms of the pelagic food web and macrophytes according to presence of *A. iberus*, samples of plankton, fish and macrophytes were taken in each mesocosm at the end of the experiment.

Plankton was divided in different fractions according to size: plankton larger than 500 µm (large mesozooplankon), plankton between 50 and 500 µm (meso- and large microzooplankton), plankton between 20 and 50 µm (small microplankton), and smaller than 20 µm (nano- and picoplankton). Moreover, plankton larger than 500 µm was separate taxonomically. Samples were obtained filtering 10 l of water samples for different filters: 500-, 50- and 20-µm-mesh, and finally a pre-combusted (450ºC; 4 h) glass fibber filter (GF/F). Previously to filtered, samples were stored in fridge at -8ºC during 12 hours to allow the organisms to empty their guts. Fibber filters (GF/F) were acidified with HCl 1M. All samples were rinsed with distilled water and stored to at -20°C until analysis.

Fish samples were obtained using a 20 cm diameter dip-net (mesh size: 1.7 mm) and stored in fridge at -8ºC during 12 hours to allow the fish to empty their guts. After, a sample of muscle tissue was extracted of each fish and sorted at -20ºC. To collect macrophytes samples, a grab was used (Ekman grab= 225cm^2). Macrophytes were separated by taxonomy and rinsed with distilled water and stored to at –20°C until analysis.

To analyze carbon and nitrogen isotopic composition, all samples were dried at 60ºC between 24 and 72 hours. After, samples were homogenized through a mortar and placed into preweighed tin boats for be analyzed. In samples of plankton of <20 µm, fibber filter (GF/F) was separated of sample and Vanadium Oxide was added. Three replicates per sample were analysed.

Isotopic analysis were carried out using a Delta C Finnigan MAT mass spectrometer with a elemental analyzer Carlo Erba Flash 1112, polarizer TC-EA, breath bench and interface Conflo III Finnigan MAT (Barcelona, Spain). All estimates of isotopic composition were based on at least two measurements and results are expressed in δ notation:

$$\delta I = [(R_{sample}/R_{standard})-1] \times 1000$$

$$R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N$$

where I is the isotope of interest (either ^{13}C or ^{15}N) and R is the ratio of this isotope to the lighter isotope (either ^{12}C or ^{14}N). δI is expressed as the permille (‰) deviation of that sample from the recognised isotope standard (Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta\ ^{15}N$). Typical precision for a single analysis was $\pm$ 0.1‰ for $\delta^{13}C$ and $\pm$ 0.3‰ for $\delta^{15}N$.

Data analysis

The possible effects of *A. iberus* and mesocosm on water characteristics, plankton and macrophytes were tested separately in each assemblage. The effects on water characteristics, community parameters of plankton and macrophytes were analyzed by mean of univariate approach (linear mixed-effects models; lme), whereas effects on assemblage matrices were analyzed using multivariate approach (Canonical Correspondence Analysis; CCA) (see Compte *et al*., submitted; for more details).

To estimate the effects of *A. iberus* on each planktonic group we used the log ratio {ln(NP+/NP-)} of preys biomass in the presence (NP+) and absence (NP-) of predator (*A. iberus*) (hereafter, prey log ratio). The prey log ratio is a meta-analysis metric often used in studies of trophic cascade (e.g. Osenberg *et al*., 1997; Hedges *et al*., 1999). Species with positive values are favoured by the presence of *A. iberus*, whereas negative log ratio values indicate the opposite. Values of near to 0 indicate that the species was equal in fish treatments and control treatments. To avoid a time confounding effect due to succesional community composition changes, only samples of control and fish treatments in final conditions were used to calculate log ratios.

The effects of *A. iberus* on $\delta\ ^{15}N$ and $\delta^{13}C$ were assessed also using linear mixed effect models (lme) where the mean of each sample of nitrogen and carbon isotope signatures were compared between control and fish treatments. Moreover, the number of trophic levels and position of each analysed fraction (TL) was estimated based on its mean $\delta^{15}N$ using the model proposed by Caban and Rasmussen (1996) in which:

$$TL_{consumer} = 1 + (\delta\ ^{15}N_{consumer} - \delta\ ^{15}N_{baseline}) / \Delta$$

where $\delta\ ^{15}N_{baseline}$ is the average $\delta\ ^{15}N$ of all collected primary consumers, as primary consumers constitute a more suitable baseline to estimate the trophic levels than potential food sources (Cabana and Ramussen, 1996). Δ is the enrichment factor per trophic level and following the general assumption (Peterson & Fry, 1987; Post, 2002),

we accepted that $\delta^{15}N$ increase 3-4‰ for each additional trophic level due to isotopic fractionation.

RESULTS

Mesocosm effects

No significant differences in water characteristics, except for conductivity ($F_{1,2}$ = 26.96; p = 0.04) that was higher into mesocosms, were detected in initial conditions between lagoon and control treatment. Similarly, no significant differences in community structure, composition and community parameters were detected in initial conditions. Moreover, macrophyte biomass was not significantly different between lagoon and mesocosm in initial conditions ($F_{1,2}$ = 0.24; p = 0.67).

Mesocosm effects were detected in final conditions at different levels from water characteristics to planktonic community structure and composition, but not for macrophytes ($F_{2,17}$ = 0.68; p = 0.522). Mesocosm effects were more evident in water characteristics (Figure 1). Conductivity, pH, soluble reactive phosphorous and chlorophyll-*a* had a significantly higher increase in mesocosms than in the lagoon; while temperature, organic matter and total nitrogen and phosphorous had a lower increase. Dissolved oxygen decreased in controls and increased in lagoon. Nitrite and ammonia were under detection level. Regarding community structure and composition, mesocosm effect were detected by means of the CCA (Monte-Carlo permutation test, F= 11.29; p < 0.01). In contrast, community parameters did not show effects, with the exception of total biomass that had a significantly higher increase in control treatment than lagoon (ANOVA, $F_{2,16}$ = 5.83; p = 0.01).

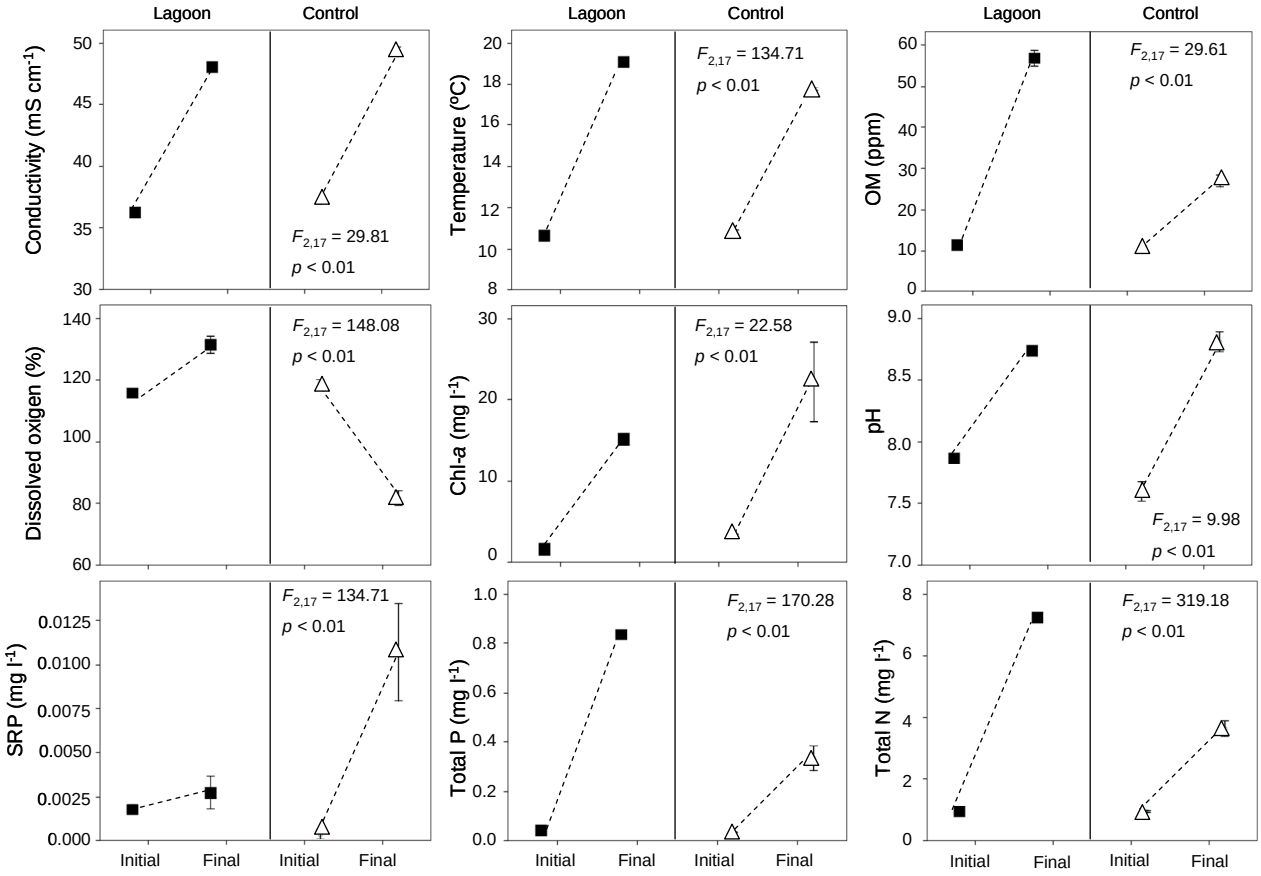


Fig 1. Water characteristics with significant interaction term (treatment-time). Mean and standard error of lagoon and control treatments in initial and final conditions are shown. Dashed lines show the evolution of parameters in time in each treatment. Black squares: parameter mean in lagoon; White triangles: parameter mean in control treatment. Results of ANOVA for the fish-time interaction between control and fish treatments of each parameter are also shown. Chl-*a*: Chlorophyll-*a*; OM: Organic matter; Total N: total nitrogen; Total P: total phosphorous; SRP: Soluble reactive phosphorous.

Fish effects on planktonic community structure

All mesocosm have similar planktonic community structure and composition at initial conditions (Figure 2). However, after two month fish presence effects were detected since the results of CCA analysis using all planktonic organisms showed significant differences in the plankton structure between control and fish treatments (Monte-Carlo permutation test; $F = 5.97$; $p < 0.01$). Therefore, three groups were discriminated from the samples position: initial samples (control and fish treatments), control treatment samples in final conditions and fish treatment samples in final conditions (Figure 2). Planktonic structure in the initial conditions of both control and fish treatments was characterised by higher biomass of calanoids, *Hexarthra* sp., chlorophytes and crysophytes. Control samples in final conditions were characterised by a higher biomass of largest mesozooplankton species (*O. maeotica* and *G. aequicauda*).

Microplankton and nanoplankton were characterised by hetero- and mixotrophic organisms (ciliates, heterotrophic dinoflagellates and haptophytes) and the picoplankton by autotrophic picoflagellates (hereafter APF) and bacterioplankton. In contrast, in fish treatment samples in final conditions meso- and microplankton were characterized by a higher biomass of harpacticoids, rotifers (*Testudinella* sp. and *Brachionus* sp.) and auto- and mixotrophic organisms (diatoms, euglenophytes and autotrophic dinoflagellates); nanoplankton by autotrophic nanoflagellates (hereafter ANF); and picoplankton by heterotrophic picoflagellates (hereafter HPF).

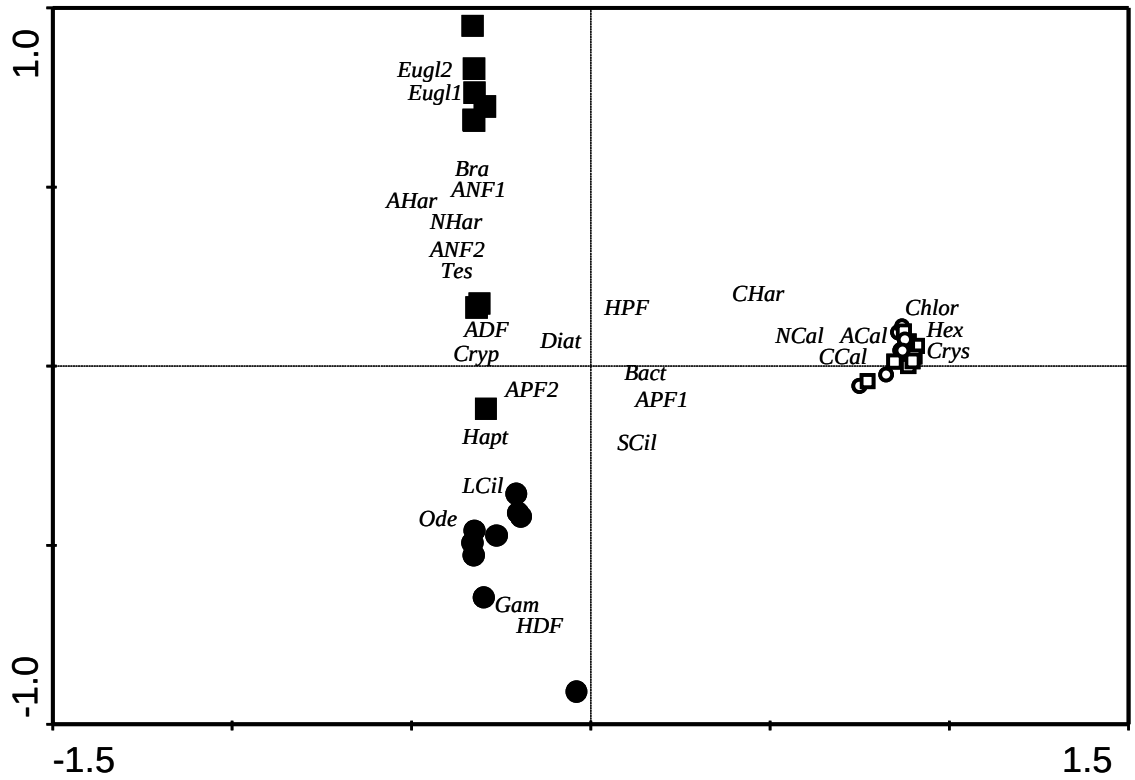


Fig. 2. Correspondence Analysis for plankton taxa. Open circles: control treatment, initial conditions; open squares: fish treatment, initial conditions; black circles: control treatment, final conditions; black squares: fish treatment, final conditions. ACal: calanoid adults; ADF: autotrophic dinoflagellates; AHar: harpacticoid adults; ANF1: autotrophic nanoflagellates undet. 1; ANF2: autotrophic nanoflagellates undet. 2; APF1: autotrophic picoflagellates undet. 1; APF2: autotrophic picoflagellates undet. 2; Bact: bacterioplankton; Bra: *Brachionus* sp.; CCal: calanoid copepodites; CHar: harpacticoid copepodites; Chlo: chlorophytes; Cryp: cryptophytes; Crys: crysophytes; Diat: diatoms; Eugl1: euglenophytes undet 1; Eugl2: euglenophytes undet. 2; Gam: *G. aequicauda*; Hapt: haptophytes; HDF: heterotrophic dinoflagellates; Hex: *Hexarthra* sp.; HPF: heterotrophic picoflagellates; LCil: large ciliates; NCal: calanoid nauplii; NHar: harpacitocid nauplii; Ode: *O. maeotica*; SCil: small ciliates; Tes: *Testudinella* sp.

Results of prey log ratio analyses showed similar results to CCA since negative or positive fish effects on each plankton organisms coincided with high or low biomass of these organisms in the CCA (Figure 3). Prey log ratios showed groups of alternant

positive and negative values when organisms were sorted by their size and trophic strategy. The largest organisms such as *G. aequicauda*, *O. maeotica* and adult calanoids (range size: from $1.14 \cdot 10^3$ to $3.59 \cdot 10^3$ µm) had negative log ratios, therefore were negatively affected by fish presence. The next size of organisms (range size: from $1.07 \cdot 10^2$ to $7.87 \cdot 10^2$ µm) had positive log ratios (calanoids copepodites and harpacitoids and some rotifers such as *Testudinella* sp. and *Brachionus* sp.), being positively affected by fish presence. Among the small micro- and nanoplankters (range size: from 4.62 to 75.10 µm) log ratios were positive or negative according to their trophic strategy. Heterotrophic organisms (ciliates and heterotrophic dinoflagellates) had negative values, while autotrophic organisms (autotrophic dinoflagellates, euglenophytes, ANFs and haptophytes) had positive values. The smallest organisms (range size: from 0.40 to 3.38 µm) had negative log ratios (APFs and bacterioplankton). Finally, log ratios of *Hexarthra* sp., diatoms, cryptophytes and HPF were close to 0.

All mesocosms had similar community parameters in initial conditions. In contrast, the analysis of the interaction term (between fish and time factors) showed significant results all community parameters measured. Thus, the presence of *A. iberus* was related to a higher increase of species diversity and species richness, a lower increase average body size and total biomass, and, finally, a decrease of size diversity than the observed in control treatment (Figure 4).

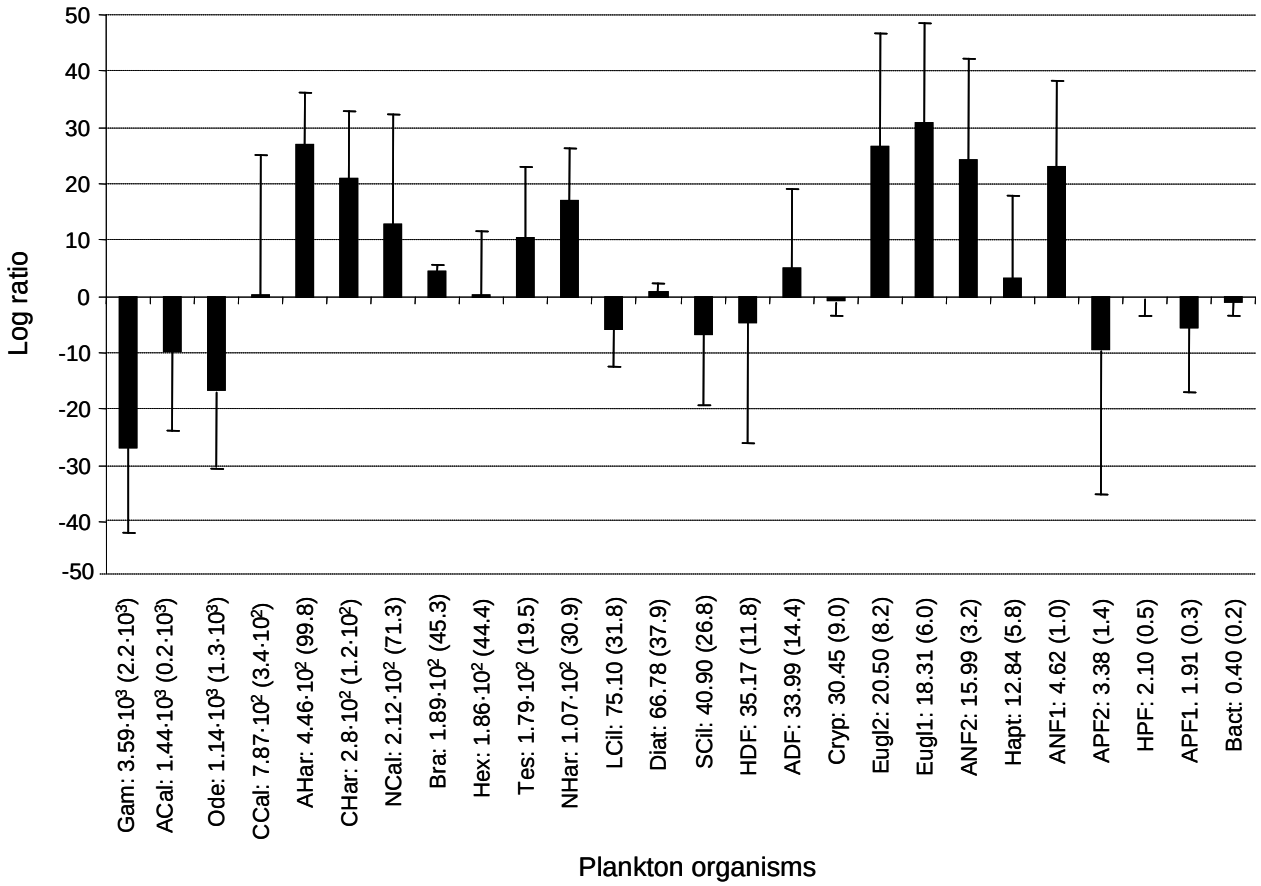


Fig. 3. Prey log ratio for each planktonic organism. Positive values suggest that the presence of *A. iberus* had a positive effect on population density, whereas negative values suggest that the presence of *A. iberus* had an adverse effect on plankton. Values near to 0 indicate that plankton density was equal in treatments and control treatments. The different planktonic organisms are ordered by size and their size means (µm) and standard deviation (in brackets) are also included in the organisms label. ACal: calanoid adults; ADF: autotrophic dinoflagellates; AHar: harpacticoid adults; ANF1: autotrophic nanoflagellates undet. 1; ANF2: autotrophic nanoflagellates undet. 2; APF1: autotrophic picoflagellates undet. 1; APF2: autotrophic picoflagellates undet. 2; Bact: bacterioplankton; Bra: *Brachionus* sp.; CCal: calanoid copepodites; CHar: harpacticoid copepodites; Cryp: cryptophytes; Diat: diatoms; Eugl1: euglenophytes undet 1; Eugl2: euglenophytes undet. 2; Gam: *G. aequicauda*; Hapt: haptophytes; HDF: heterotrophic dinoflagellates; Hex: *Hexarthra* sp.; HPF: heterotrophic picoflagellates; LCil: large ciliates; NCal: calanoid nauplii; NHar: harpacitocid nauplii; Ode: *O. maeotica*; SCil: small ciliates; Tes: *Testudinella* sp.

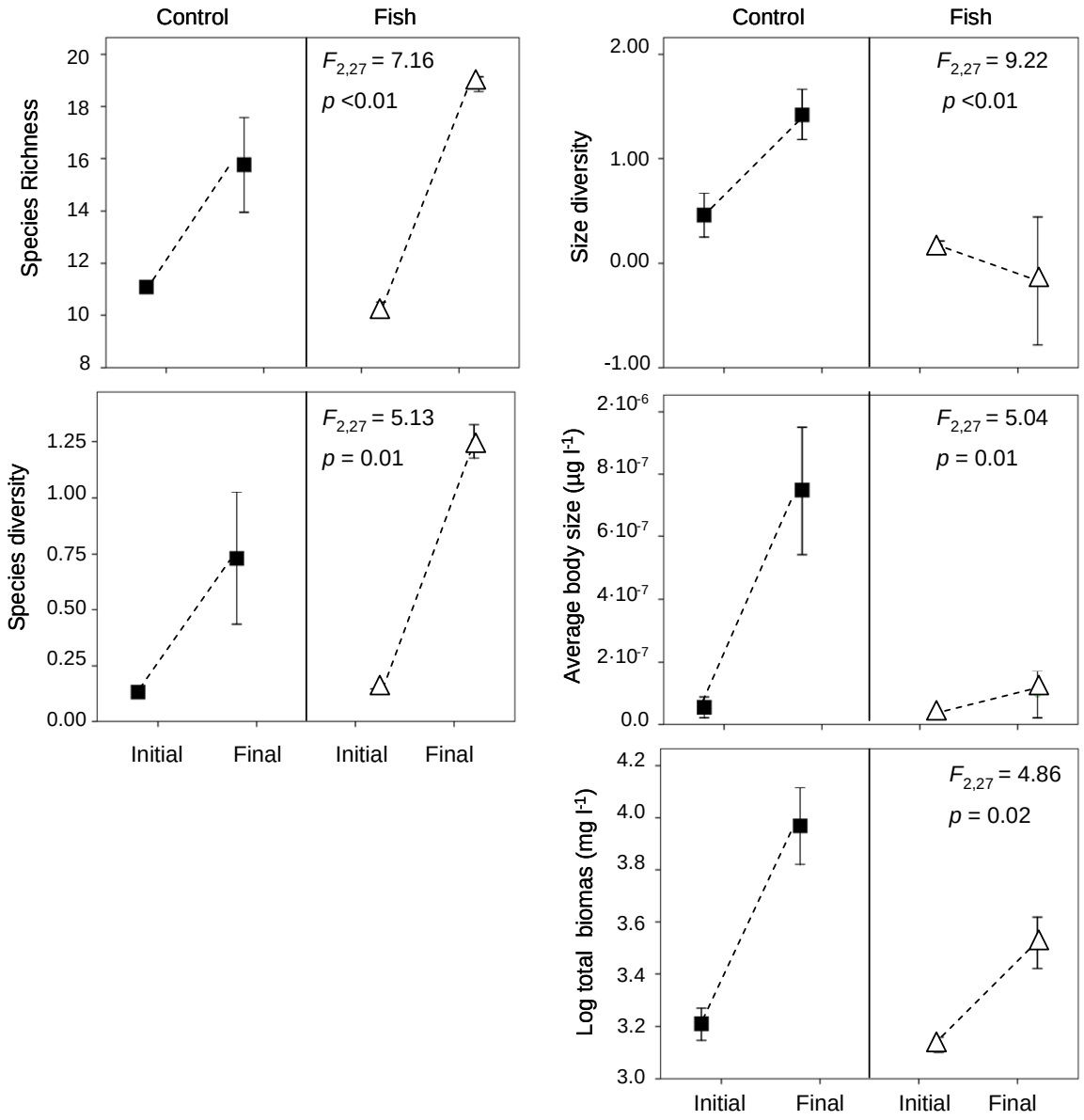


Fig. 4. Plankton community parameters with significant interaction term (treatment-time). Mean and standard error of control and fish treatments in initial and final conditions are shown. Dashed lines show the evolution of parameters in time in each treatment. Black squares: parameter mean in control treatment; White triangles: parameter mean in fish treatment. Results of ANOVA for the fish-time interaction between control and fish treatments of each parameter are also shown.

Effects on macrophytes and water characteristics

In initial conditions, all water charactersitics did not have significant differences between control and fish treatments with the exception of total phsophorous ($F_{1,4}$ = 13.05; p = 0.02) which in control treatments was higher than in fish treatments. Significant effects of *A. iberus* on the evolution of some water characteristics were detected (Figure 5). In fish treatments, chlorophyll-*a* and total nutrients (total nitrogen

and phosphorus) had a higher increase in control treatment than in fish ones. Moreover, pH had a lower increase in control treatment than fish ones whereas dissolved oxigen had higher decrease in control treamtment that in fish ones. No significant differences were found in the other water characteristics analysed and nitrite and amonia concentration remain under the detection level in both treatments.

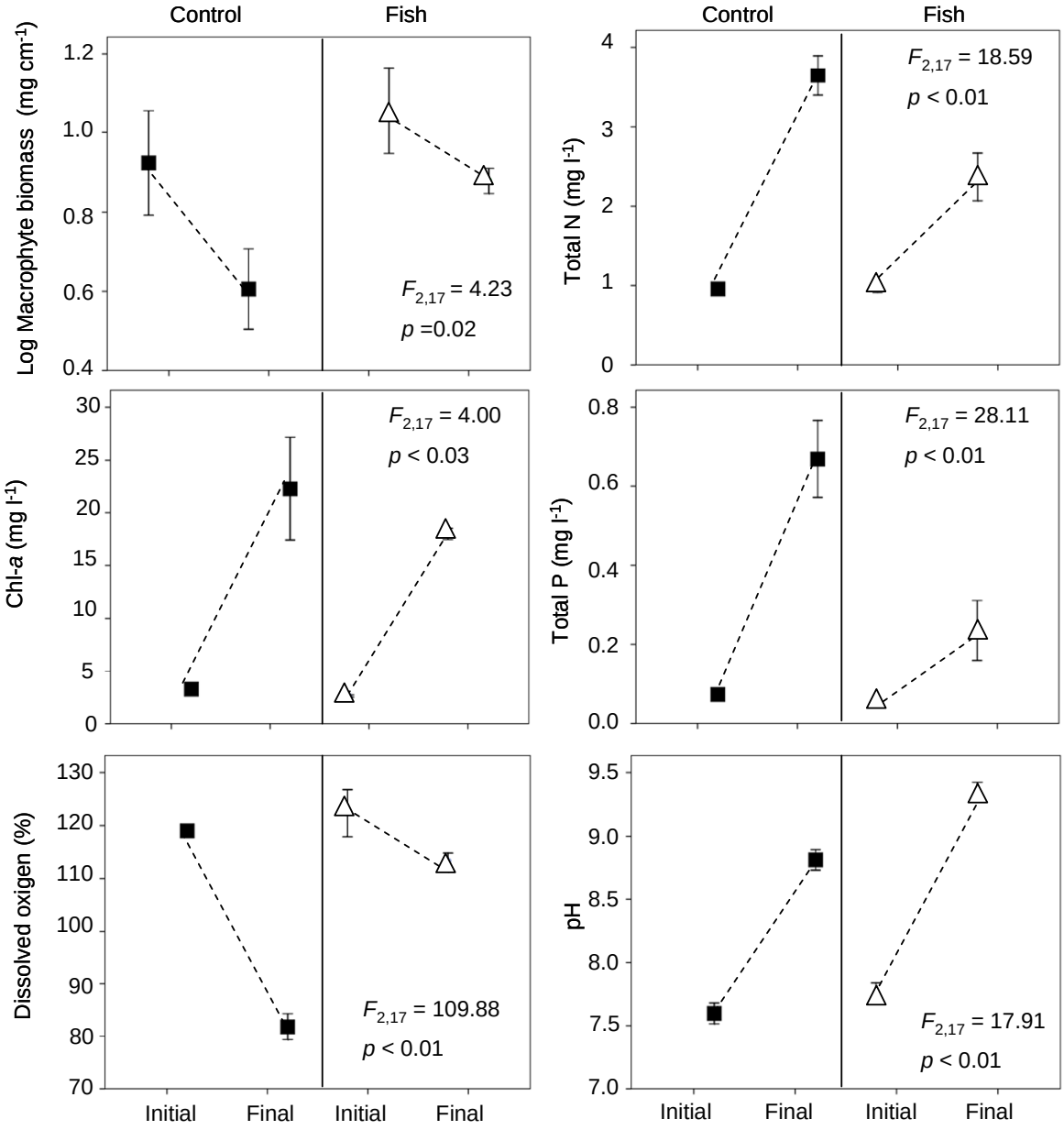


Fig. 5. Water characteristics and macrophytes biomass with significant interaction term (treatment-time). Mean and standard error of control and fish treatments in initial and final conditions are shown. Dashed lines show the evolution of parameters in time in each treatment. Black squares: parameter mean in control treatment; White triangles: parameter mean in fish treatment. Results of ANOVA for the fish-time interaction between control and fish treatments of each parameter are also shown. Total N: Total nitrogen; P: Total phosphorus; Chl-*a*: Chlorophyll-*a*.

Two species of macrophytes were identified: *R. cirrhosa* and *Laprothamnimum populosum* representing 96.45% and 3.55% of macrophyte total biomass, respectively. Significant differences between control and fish treatments in initial conditions were not detected ($F_{1,4}$ = 1.05; p =0.36). However, the interaction fish-time of macrophyte biomass was significantly different between control and fish treatments samples (Figure 5). The reduction of macrophytes biomass was higher in control treatment than in fish treatment.

Stable isotope composition of different aquatic organisms

In the isotope composition analyse, the $\delta^{13}C$ or $\delta^{15}N$ of plankton smaller 20 µm fraction was not measured since his concentration remain under the detection level in both treatments. Significant differences between control and fish treatments in the relationships between the $\delta^{13}C$ or $\delta^{15}N$ values of fractions were not found in any fraction (Figure 6). Nevertheless, two carbon sources were differentiated in isotope analyses: macrophytes (*R. cirrhosa* and *L. papulosum*) with high values of $\delta^{13}C$, and plankton smaller 500 µm with low values of $\delta^{13}C$. To note the high $\delta^{15}N$ of *R. cirrhosa* observed in both treatments. *O. maeotica* in control treatment and *A. iberus* in fish treatment has similar $\delta^{15}N$.

To estimate the number of trophic levels, the mean $\delta^{15}N$ of plankton between 20 and 50 µm was considered as $\delta^{15}N$ baseline since the most primary consumers of plankton are in sized between 1 and 50 µm (López-Flores *et al*., in press). Unfortunately, fraction smaller than 20 µm was not measured and so it was not be used as baseline. Two trophic levels were differentiates in control and fish treatments (Figure 6). In both treatments, plankton smaller than 500 µm and *L. papulosum* were in first trophic level and *R. cirrhosa* was in the second trophic level. In control treatment *O. maeotica* were also in the second trophic level, whereas, in fish treatment this position was occupied by *A. iberus*. *G. aequicauda* was the only organism that showed a change in its trophic position according to fish presence. Thus, in control treatment *G. aequicauda* was positioned at the first trophic level whereas in fish treatment was at the second trophic level.

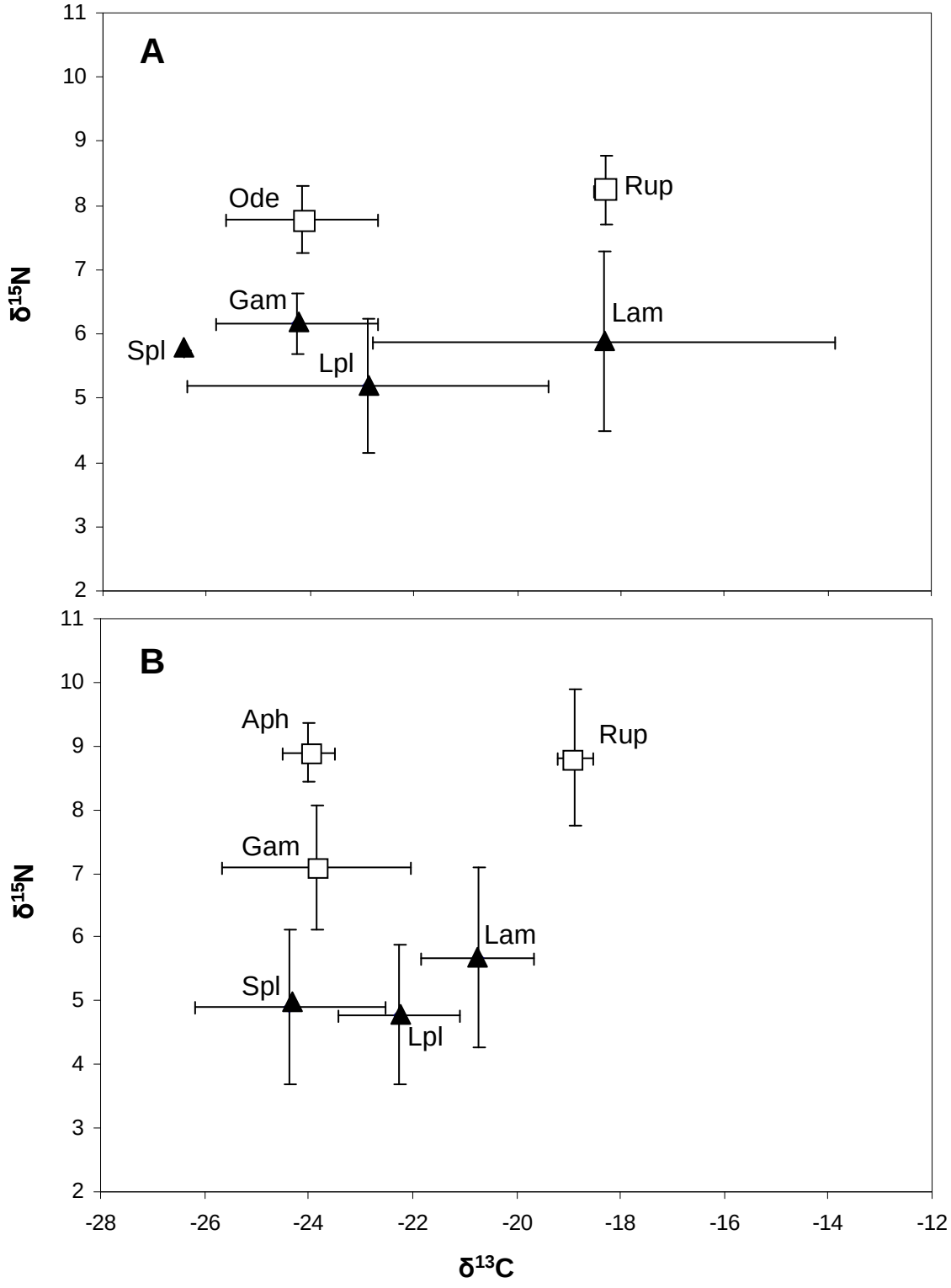


Fig. 6. Plot of $\delta^{13}C$ and $\delta^{13}N$ values of different fractions of Mediterranean coastal lagoon community. A: control treatment; B: Fish treatment. Aph: *A. iberus*; Gam: *G. aequicauda*; Lpl: >50 µm plankton; Lam: *L. populosum*; Ode: *O. maeotica*; Rup: *R. cirrhosa*; Spl: 20-50 µm plankton. Black triangle: Trophic level 1; White square: Trophic level 2.

DISCUSSION

Classical trophic cascade hypothesis (Carpenter *et al*., 1985, Carpenter & Kitchell, 1993) describes four trophic levels in lakes (piscivorous fish – planktivorous fish – zooplankton – phytoplankton). However, in our pelagic food web only two trophic levels were detected by mean of stable isotopes analyses (fish/jellyfish – zoo/phytoplankton). In contrast, using log ratios, a large trophic cascade was observed. This fact point to that the pelagic food web of a Mediterranean salt marsh has complex trophic interactions with many interactions between organisms. In this sense, many organisms of this food web have an omnivore diet (e.g. Kleppel, 1993; Kelly *et al*., 2002; Brucet *et al*., 2008) or are mixotrophs (e.g. Stoecker, 1998; Flöder *et al*., 2006¸ Unrein *et al*., 2007) which would not allow differentiate different trophic levels. Consequently, the trophic cascade effects observed may responds to size dependent predation behaviour of the organisms. Only the range of body size of microplankton was divided in different levels according to their trophic strategy (heterotrophic and autotrophic). This division may be explained by the feed preference of mesozooplankton (manly copepods) on heterotrophic organisms since previous studies have found that copepods can ingest ciliates and heterotrophic dinoflagellates at higher rates than phytoplankton (Stoecker & Egloff, 1987; Sanders & Wickham, 1993; Nejstgaard *et al*., 1997; Brucet *et al*., 2008).

Trophic cascade based on body size would also explain the differences found in community parameters. The decrease of size diversity, and the low increase of average body size and total biomass in presence of *A. iberus* can be attributed to a reduction of large-bodied species (*O. maeotica*, *G. aequicauda* and calanoid adult) and increase of small-bodied zooplankton species of zooplankton (harpacticoids and rotifers). Similar changes in size structure of plankton with the presence of size selective predators have been reported in other studies (Brooks & Dodson, 1965; Lynch, 1979, Carpenter & Kitchell, 1993). Moreover, the higher increase of specie diversity and richness in presence of *A. iberus* can be explained by the elimination of possible large invertebrate predators as *O. maeotica* and *G. aequicauda* which its potential predation could explain the reduction of small zooplankton organisms (MacNeil *et al*., 1997; Kelly *et al*., 2002; Compte *et al*., in press).

Our experiment was made using only the adult *A. iberus*. Nevertheless, differences of diet among adult and juvenile *A. iberus* individuals and among habitats have been reported (Alcaraz & Garcia-Berthou, 2007). Juvenile fish selects small organisms of water column while adult fish selects large organisms of benthos. Therefore, different

effects on plankton caused by different size of *A. iberus* are expected and the results of the study could be different in natural conditions with all developmental stages of *A. iberus*. However, it is important to note that the effect found in this study can be similar to natural conditions in periods with dominance of adult fish, and so out of the breeding season. On the other hand, we detected mesocoms effect in our experiment, because water characteristics and plankton structure within mesocosms were different from those of the lagoons. Nevertheless, water characteristics and plankton structure within mesocosms were similar to the observed ones in periods of maximum confinement described in this salt marsh (Quintana *et al*., 1998a; 1998b). Thus, our experiment would explain how *A. iberus* adults can structure the plankton especially in conditions of maximum confinement.

Phytoplankton and macrophytes had different source of carbon since latter contained a higher proportion ^{13}C than phytoplankton. This difference can be related to the thickness of the boundary water layer, which affects the diffusion of nutrients into the cells and causes fractionation of heavy isotopes (France, 1995; Raven *et al*. 2002; Bode *et al*., 2006). This difference of carbon isotope signature is used as a procedure for distinguishing between benthic and planktonic food sources for coastal animals (e.g. Hobson, 1993; Hobson *et al*., 1994; France, 1995). In this sense, in our pelagic food web, phytoplankton appeared to be the main source of carbon. On the other hand, the results of stable isotope analyses showed that *R. cirrhosa* has values of $\delta^{15}N$ similar to top predators (*A. iberus* and *O. maeotica*). Salt marshes are environments with high denitrification rates (Kaplan *et al*., 1979; Valiela, 1984; Thompson *et al*., 1995; Ericsson *et al*., 2003) and this process increase the ^{15}N of substrate (Delwiche & Steyn, 1970; Shearer *et al*., 1974; Kendall, 1998). Moreover, *Ruppia* sp. has epiphytes algae in their blades and bacteria in their roof which can fix nitrogen inorganic (Flores-Verdugo *et al*., 1988; Currin *et al*., 1990) and thus, can increase the concentration of ^{15}N in these organisms (Henn & Chapela, 2001; Spriggs *et al*., 2003). Therefore, these both process could be the causing of high values of $\delta^{15}N$ of *R. chirrosa*.

Although macrophytes were not involved in the pelagic food web, a significant smaller decrease of its biomass in presence of fish was found in our experiment. This smaller decrease could be explained for indirect effects of the observed trophic cascade. A smaller increase of chlorophyll-*a* was detected in fish treatment because there was a lower density of APF in final conditions respect to control treatment (mean in control treatment $2 \cdot 10^7$ ind l^{-1}; mean in fish treatment $3.69 \cdot 10^6$ ind l^{-1}). The small increase of

chlorophyll-*a* can be attributed to trophic cascade effects of *A. iberus* since its presence coincide with an increase of APF predators. The small increase of chlorophyll-*a* and total nutrients in water would also help to increase water transparency and can favour to maintain a higher macrophyte biomass, since the competence for light and nutrients would decrease (Ozimek *et al*., 1990; Sand-Jensen & Borum, 1991; Van Donk *et al*., 1993; Sfriso *et al*., 2003). Thus, in contrast with the observed effects of some planktivorous fish (Brett and Goldman, 1997; Jeppesen *et al*., 2003), *A. iberus* has slight positive effects on macrophytes slowing macrophytes biomass reduction.

The above results could also be seen a mutualism relationship since the presence of macrophytes would suppose also advantages for *A. iberus* providing him with refugee and food supply (Moreno-Amich *et al*., 1999; Alcaraz *et al*., 2007). Mediterranean coastal wetlands are habitats with large environmental fluctuations (e.g. Britton & Crivelly, 1993; Álvarez-Cobelas *et al*., 2005; Beklioglu *et al*., 2007) characterized by periods of flooding and nutrients inputs and prolonged periods of confinement, restricted water inputs, a low flushing rate and high salinity (Guelorget & Perthuisot, 1983; Trobajo *et al*., 2002). In these conditions, only well-adapted species can successfully inhabit such environments (Bamber *et al*., 1992; Boix *et al*., 2007) as *A. iberus* and *R. cirrhosa* (Verhoeven, 1979; Sanz-Brau, 1985; Moreno-Amich *et al*., 1999; Gesti *et al*., 2005; Oliva-Paterna, 2006; Oliva-Paterna *et al*., 2009). In this sense, our results suggest a possible strategy between *A. iberus* and *R. cirrhosa* to overcome these adverse conditions. This strategy could understand as an example of co-evolution but based on a mutualism relationship between *A. iberus* and *R. cirrhosa* both organisms are reciprocally beneficiated.

Chapter 10

General discussion

GENERAL DISCUSSION

Resource partitioning in Mediterranean coastal wetlands

The first hypothesis of this study proposed that resource partitioning among the developmental stages of the dominant zooplankton species was a strategy to avoid the intraspecific competition in environments with resource limitation. The results of the experiments (*Calanipeda* and *Daphnia* experiments) showed that dominant species of zooplankton in environments with resource limitation had a partial segregation of resource among their developmental stages. However, when resources were not limited, the resource partitioning was not observed among developmental stages of dominant zooplanktonic species.

In accordance with previous studies (e.g. Kleppel, 1993; Calbet *et al*., 2006; Stemberger & Miller, 1998), the results revealed that *Calanipeda aquaedulcis* and *Daphnia magna* were omnivorous and could change their feeding behaviour depending on resource availability. However, *C. aquaedulcis* mainly selected heterotrophic prey, whereas *D. magna* did not have a defined pattern of selectivity. The selective feeding behaviour in most copepods (e.g. Richman *et al*., 1980; Meyer *et al*., 2002), besides a non-selective one in daphnids (DeMott, 1988b; Feyer, 1991), had been widely reported.

Mediterranean coastal wetlands have periods of confinement with high salinity and low nutrient inputs (Quintana *et al*., 1998a). During this situation of confinement, the resource availability becomes limiting. In these conditions, some calanoid copepods, such as *Eurytemora velox* and *C. aquaedulcis*, maintain stable populations, leading to a monospecific zooplankton community (Brucet *et al*., 2006), although a strong intraespecific competence is expected under these limiting conditions. Brucet *et al*. (2005a) suggested the existence of food selection and resource partitioning among the different developmental stages of these calanoid populations as a strategy to avoid intraspecific competition. They argued that spatial and temporal segregation among stages would not affect intraspecific competition because all stages coexist in time and space. The *Calanipeda* experiment showed that nauplii, copepodites, and adults have partially different diet and selectivity according to food types and food sizes. These results agree with the described niche segregation between young and adult copepods (Mullin & Brooks, 1967; Poulet, 1977). Thus, the food segregation as a type of resource partitioning, allows to *C. aquaedulcis* maintain stable populations in confinement situations with low resources availability. Although not directly shown, *E. velox* likely

had similar dietary segregation over its ontogeny, based on indirect evidence, such as ontogenic changes in amino acid composition (Brucet *et al*., 2005b).

In contrast, in environments with high food abundance, daphnids are benefited because they have higher ingestion rates than calanoids (McNaught, 1975; Richman & Dodson, 1983; Schulze *et al*., 1995). These conditions with high resource availability would lead to a weak intraspecific competition, reducing the need for spatial-temporal segregation and resource partitioning (Brucet *et al*., 2006). The results did not indicate resource partitioning in among *D. magna* stages, supporting the hypothesis that species adapted to situations of high resource availability do not have resource partitioning among development stages.

Top-down and top predators in Mediterranean coastal wetlands

The second hypothesis of this study proposed that jellyfish and fish have strong but different top-down effects on aquatic communities of Mediterranean coastal wetlands. The results of the experiments (*Odessia* and *Aphanius* experiments) revealed that top-down and trophic cascade had an important role in the control of aquatic communities of these environments. However, their effects were different according to the top predator.

Odessia maeotica had strong top-down effects on the community. However, their effects may be temporary since high densities of *O. maeotica* are present in the medusa stage for a few weeks in oligotrophic confinement conditions (Quintana *et al*. 1998b). According to these experimental results and those from previous studies (Quintana *et al*. 1998b), *O. maeotica* greatly reduced medium-sized zooplankton (copepods and rotifers) directly through predation. Additionally, there were indirect effects from trophic cascades that explained both the increase of small zooplankton (ciliates) and weak changes found in phytoplankton with *O. maeotica* presence.

Aphanius iberus had a very different effect on the aquatic community, since their diet is based mainly on benthonic organisms (Vargas & De Sostoa, 1999; Alcaraz & García-Berthou *et al*., 2007), whereas *O. maeotica* has a planktonic diet. Additionally, *A. iberus* maintain dense and stable populations (García- Berthou *et al*., 1991; Moreno-Amich *et al*., 1999; Badosa *et al*., 2007). In contrast to *O. maeotica*, *A. iberus* reduced the abundance of large-sized invertebrates, such as *Gammarus aquicauda* and *O. maeotica*, and increased the abundance of medium-sized zooplankton (copepods and rotifers). As a result of trophic cascade effects, it also reduced the abundance of ciliates, dinoflagellates and autotrophic picoflagellates. These *A. iberus* effects differed from

those caused by other planktivorous species, whose predation on zooplankton increases phytoplankton density and, in turn, in water turbidity (e.g. Arcifa *et al*., 1986, Jeppesen *et al*., 1997; Jeppesen *et al*., 1998).

The presence of *A. iberus* was also associated with changes to water and benthos characteristics. Results obtained in the *Aphanius* experiment showed that the presence of fish increased water transparency and slowed macrophyte biomass decrease. This slow decrease in macrophyte biomass could suppose an increase of refugee for benthos when fish was present (Dielh, 1992; Jacobsen *et al*., 1997; Meerhoff *et al*., 2007b) explaining the weak effect of *A. iberus* on zoobenthos.

Although *O. maeotica* and *A. iberus* have different effects on aquatic community, both species can be considered a keystone species based on how their presence affected the structure of aquatic community (Paine, 1966, 1969; Kerfoort & DeMott, 1984). In absence of these predators (e.g. temporary lagoons) copepods and daphnids can substitute for *O. maeotica* and *A. iberus* as the role of the keystone species by affecting the dynamics of the microbial community.

Trophic adaptations of the organisms to fluctuating environments

Mediterranean coastal wetlands are characterized by large fluctuations in the salinity (e.g. Barnes, 1989) and long periods without nutrient inputs (e.g. Quintana *et al*., 1998a). This means that only well-adapted species can successfully inhabit such environments (Bamber *et al*., 1992; Boix *et al*., 2007). This, organisms have several strategies that allows them to successfully inhabit this kind of systems. Some of these strategies can be related to trophic interactions.

For example, *D. magna* maintains stable populations in environments with continuous pulses of nutrients (e.g. Schulze *et al*., 1995), whereas *O. maeotica* inhabits environments of specific conditions of salinity and oligotrophy with high abundance of its potential preys, but its populations are not stable during a long time period (Quintana *et al*., 1998b). The high ingestion rates known for *D. magna* (McNaught, 1975; Richman & Dodson, 1983) may exploit the abundant resources in the stable environments. We also observed high ingestion rates in *O. maeotica*, which may allow for fast growth when their resources are abundant but unstable. This strategy of fast growth is not rare, since is a usual strategy in cnidarians (Boero *et al*., 2008) and other organisms of fluctuating environments as snails of intertidal zone (Zeldis *et al*., 1979). In contrast this strategy do not allow the species to persist during a long period of time, since no

resource partitioning is observed and so all their sizes and/or developmental stages feeds on the same resource until its finish.

On the other hand, *C. aquaedulcis* and *A. iberus* have stable populations in temporary and permanent lagoons of the Mediterranean coastal wetlands (García-Berthou *et al*., 1991; Quintana *et al*., 1998b; Badosa *et al*., 2007). In these stable conditions during confinement, resource partitioning between developmental stages may have evolved as a strategy to overcome resource limitation. Such dietary segregation among developmental stages in copepods reduced competition in environments with limited resources or high temporal variability (Poulet, 1977). Furthermore, *A. iberus* reproduce in spring (Fernández-Delgado *et al*., 1988; Vargas & De Sostoa, 1997; Oliva-Paterna *et al*., 2009), coinciding with the confinement periods of the coastal lagoons (Quintana *et al*., 1998a; 1998b). As a result, the highest density of *A. iberus* occurs in periods of low resource availability. However, differences on the diet as well as on habitat selection between *A. iberus* adults and juveniles exist (Alcaraz & Garcia-Berthou, 2007). Juvenile fish selects small organisms of water column, while adult fish selects large organisms of benthos. Although the *Aphanius* experiment only included *A. iberus* adults, the results likely reflect the dietary segregation among development stages, because the presence of adults increased the abundance of small planktonic species that dominate the juvenile diet. Such ontogenetic shifts in diet are common in fish (Persson & Greenberg 1990; Eggleston *et al*., 1998).

In addition to dietary segregation, *A. iberus* may maintain populations in the resource-limited Mediterranean coastal wetlands by establishing a mutualistic relationship with macrophyte *R. cirrhosa*. Macrophytes compete with phytoplankton for nutrients and light (Ozimek *et al*., 1990; San-Jensen & Borum, 1991; Van Donk *et al*., 1993). Consequently, if *A. iberus* reduces small phytoplankton abundances by trophic cascade effects, macrophytes benefit from decreased competition for light and nutrients. *R. cirrhosa* increases prey availability for *A. iberus* (Alcaraz *et al*., 2008) and acts as a refuge from bird predation (Moreno-Amich *et al*., 1999). Such mutualisms between animals and aquatic plants are common. For example, the presence of bivalves, which reduce epiphytes and phytoplankton, benefitted macrophytes in salt marshes (Bertness 1984; Peterson & Heck (2001a,b). In return, macrophytes provide bivalves with increased food sources and a refuge from predators. Additionally, in marine environments, Hay *et al*. (2004) suggest that seagrasses provide refuge to fish and macroinvertebrates, whereas the latter consumed epiphytes on the seagrass blades.

Organisms' trophic adaptations to Mediterranean salt marsh also could be related to the food webs characteristics of this environment. Omnivore diet is common among the key species (e.g. Kleppel, 1993; Kelly *et al*., 2002) and mixotrophy is often reported (e.g. Stoecker, 1998; Flöder *et al*., 2006, Unrein *et al*., 2007). Therefore, many trophic interactions are established among organisms, which are difficult to assign to different trophic levels. In agreement our results pointed out that the food web of Empordà coastal wetlands is relatively short (with two trophic levels) but with complex trophic cascade effects because trophic interactions would be mainly size depended.

Food web control in Mediterranean coastal wetlands

The results of this study suggest that changes in the successional process of Mediterranean coastal wetlands associated with changes in the food web control mechanisms could be related to two different environmental situations: nutrient pulse and aquatic confinement. Physical factors, such as flooding intensity, determined pulse events and, in turn, nutrient loadings pulses (Guelorget & Perthuisot, 1983; Quintana *et al*., 1998a). The frequency and intensity of flooding hydrological periods differentiates the periods of sudden flooding and confinement periods. In sudden flooding, the nutrient inputs are due to sea storms or intense rainfall. In confinement, there are not water inputs, leading to a decrease in water level and an increase in salinity due to evaporation (Quintana *et al*., 1998a, Quintana, 2002). During the pulse situations, there is an allogenic succession and the food web is controlled by bottom-up mechanisms. In contrast, confinement periods are characterized by autogenic succession and top-down controls on the food web. Changes in environmental conditions can result in abrupt shifts in food web control mechanisms in brackish ecosystems (e.g. Petersen *et al*., 2008). Allogenic succession after a resource pulse and the posterior substitution by an autogenic process has been considered a general pattern in other aquatic ecosystems, such as freshwater temporary ponds (e.g. Lake *et al*., 1989, Boix *et al*., 2004).

Chapter 10

General conclusions

GENERAL CONCLUSIONS

1. Resource partitioning among different developmental stages were noticed for planktonic grazers that characteristically appears in oligotrophic situations on Empordà salt marshes under confinement conditions (e.g. *Calanipeda aquaedulcis*). In contrast, planktonic grazer that characteristically appears in more eutrophic lagoons with continuous nutrient pulses (e.g *Daphnia magna*) did not show resource partitioning among different developmental stages.

2. *C. aquaedulcis* had omnivore and selective diet. However, different developmental stages showed different diet, which were mainly related to size and type of prey. In general, prey size increased with an increasing size of the developmental stages. Thus, nauplii and copepodites showed high ingestion rates and selection coefficients for hetrotrophic picoflagellates, whereas adults had higher ingestion rate and selection coefficients for heterotrophic dinoflagellates.

3. The effects of *D. magna* on aquatic communities mainly depended on the initial structure of the microbial community. Therefore, selectivity coefficients changed based on the different potential preys in the plankton community, in accordance with omnivorous non selective feeding behaviour. Moreover, no significant differences among developmental stages were found either in ingestion rates or in selective coefficients.

4. Top predators effects were strong in Empordà coastal wetlands. Both direct and indirect effects were detected independently of top predator species studied. However, according to the top predator type (vertebrate and invertebrate) different responses of aquatic community were observed.

5. Effects of jellyfish *Odessia maeotica* as top predator were intense, because their direct effects were not significantly different from those observed after artificially removed zooplankton. However, their indirect effects on phytoplankton were less evident, because a trophic cascade increased ciliates populations that, at their turn, preyed on phytoplankton.

6. The presence of fish *A. iberus* triggers high trophic cascade effects on plankton which respond to size dependent predation behaviour of the organisms. In contrast with *O. maeotica*, this effect was detectable even in phytoplankton.

7. *A. iberus* predation effects were stronger on zooplankton than zoobenthos, since zoobenthos can use macrophytes as refuge in order to avoid fish predation.

8. *A. iberus* presence reduced the increase of phytoplankton density which competes with macrophytes for light and nutrients. Therefore, macrophytes were benefit by fish, and their turn, provide refugee and resource availability to fish, thus suggesting a mutualism relationship.

9. The studied food web of Empordà coastal wetland was relatively short (only two trophic levels were detected). Nevertheless, complex trophic cascade effects existed because trophic interactions were mainly size depended.

CONCLUSIONES GENERALES (en Castellano)

1. Una división del recuso entre diferentes estadios de desarrollo de una misma especie se observó en organismos filtradores planctónicos que aparecen en situaciones de oligotrofia en las marismas de l'Empordà durante el confinamiento (por ejemplo *Calanipeda aquaedulcis*). Por el contrario, organismos filtradores planctónicos que aparecen en lagunas más eutróficas con entradas continuas de nutrientes (por ejemplo *Daphnia magna*) no presentaban división del recurso entre sus estadios de desarrollo.

2. *C. aquaedulcis* tenía una dieta omnívora y selectiva. Sin embargo sus diferentes estadios de desarrollo presentaban diferente dieta que estaba relacionada con el tamaño y el tipo de presa. En general, el tamaño de las presas incrementaba con el tamaño del estadio de desarrollo. De esta forma, los nauplios y los copepoditos presentaban una tasa de ingestión y coeficiente de selección altos por los picoflagelados heterotróficos, mientras que los adultos tenían una tasa de ingestión y coeficiente de selección altos por los dinoflagelados heterótrofos.

3. Los efectos de *D. magna* sobre la comunidad acuática dependían principalmente de la estructura inicial de la comunidad microbiana. Así, los coeficientes de selección cambiaban según las diferentes presas potenciales en la comunidad planctónica de acuerdo con su comportamiento omnívoro y no selectivo. Además, no se encontraron diferencias significativas ni en las tasas de ingestión ni en los coeficientes de selección entre sus estadios de desarrollo.

4. Los efectos de los *top-predators* (depredadores del nivel trófico más alto de la red trófica) eran fuertes en los humedales costeros de l'Empordà. Los efectos directos e indirectos fueron detectados independientemente de la especie de *top-predator* estudiada. Sin embargo, según el tipo de *top-predator* (vertebrado e invertebrado) se observaron respuestas diferentes de la comunidad acuática.

5. Los efectos de la medusa *Odessia maeotica* como *top-predator* eran intensos ya que sus efectos no eran significativamente diferentes de los observados después de retirar el zooplancton artificialmente. Sin embargo, sus efectos indirectos sobre el fitoplancton

eran menos evidentes porque la cascada trófica que generaba *O. maeotica* incrementaba las poblaciones de ciliados que se alimentaban de fitoplancton.

6. La presencia del pez *Aphanius iberus* desencadenaba una cascada trófica con efectos intensos sobre el pláncton basada en el tamaño de los organismos. A diferencia de la *O. maeotica,* estos efectos eran detectados en el fitoplancton.

7. La depredación de *A. iberus* era más fuerte en el zooplankton que en el zoobentos ya que éste último podría usar los macrofitos como refugio para evitar la depredación de *A .iberus*.

8. Se observó un menor incremento de la densidad de fitoplancton, competidor con los macrófitos por la luz y los nutrientes, con la presencia de *A. iberus*. Como consecuencia, los macrófitos eran beneficiados por el pez y, a la vez, estos últimos proporcionaban al pez refugio y disponibilidad de recurso, sugiriendo una relación de mutualismo.

9. La red trófica estudiada en los humedales del Empordà era relativamente corta (con solo dos niveles tróficos detectados). Aun así, existía una compleja cascada trófica ya que las interacciones tróficas dependían del tamaño de los organismos.

CONCLUSIONS GENERALS (en Català)

1. Una segregació del recurs entre els estadis de desenvolupament es va observar en organismes planctònics filtradors que apareixen en situacions d'oligotròfia en les maresmes de l'Empordà en condicions de confinament (com per exemple el *Calanipeda aquaedulcis*). En canvi, organismes filtradors planctònics que apareixen en llacunes més eutròfiques amb entrades contínues de nutrients (com per exemple *Daphnia magna*) no presentaven segregació del recurs entre els seus estadis de desenvolupament.

2. *C. aquaedulcis* tenia una dieta omnívora i selectiva. Tot i així, els seus estadis de desenvolupament presentaven una dieta diferent relacionada amb la grandària i el tipus de presa. En general, la grandària de la presa incrementava amb la grandària de l'estadi de desenvolupament. D'aquesta forma, els nauplis i els copepodits presentaven unes taxes d'ingestió i coeficients de selecció alts pels picoflagel·lats heterotròfics, mentre que els adults tenien una taxa d'ingestió i coeficient de selecció més alts pels dinoflagel·lats heterotròfics.

3. Els efectes de la *D. magna* sobre la comunitat aquàtica depenien principalment de l'estructura inicial de la comunitat microbiana. Per tant, els coeficients de selecció canviaven segons les preses potencials en la comunitat aquàtica, d'acord amb el comportament omnívor i no selectiu de la *D. magna*. A més a més, no es van trobar diferencies en les taxes d'ingestió i els coeficients de selecció dels diferents estadis de desenvolupament.

4. Els efectes dels *top-predators* (predadors del nivell tròfic més alt de la xarxa tròfica) eren forts en els aiguamolls costaners de l'Empordà. Es van detectar efectes directes i indirectes independentment de l'espècie *top-predator* estudiada. Tot i així, es van observar diferents respostes de la comunitat aquàtica en funció del tipus de to predator.

5. Els efectes de l' *Odessia maeotica* com a *top-predator* eren intensos ja que els seus efectes directes no eren significativament diferents amb els observats després de retirar el zooplàncton artificialment. Tot i així, els efectes indirectes sobre el fitoplàncton eren poc evidents ja que per efectes de cascada tròfica incrementaven les poblacions de ciliats que s'alimentaven de fitoplàncton.

6. La presència d'*A. iberus* desencadenava cascada tròfica important sobre el plàncton basada en la predació segons la grandària corporal dels organismes. A diferència de l'*O. maeotica*, els efectes eren detectas en el fitoplàncton.

7. Els efectes de la predació de l'*A. iberus* eren més forts en el zooplàncton que en el zoobentos, ja que aquest últim podria utilitzar els macròfits per evitar la depredació de l'*A. iberus*.

8. Quan l'*A. iberus* era present, es va observar una reducció de l'increment de la densitat de fitoplàncton el qual competia per la llum i els nutrients amb els macròfits. Per tant, els macròfits eren beneficiats pel peix, i a la vegada, aquests proporcionaven refugi i disponibilitat de refugi al peix, la qual cosa suggeria un relació de mutualisme.

9. La xarxa tròfica estudiada ens els aiguamolls costaners de l'Empordà era relativament curta (amb només dos nivells tròfics detectats). Tot i així, existia una xarxa tròfica complexa ja que les interaccions tròfiques eren depenents de la grandària dels organismes.

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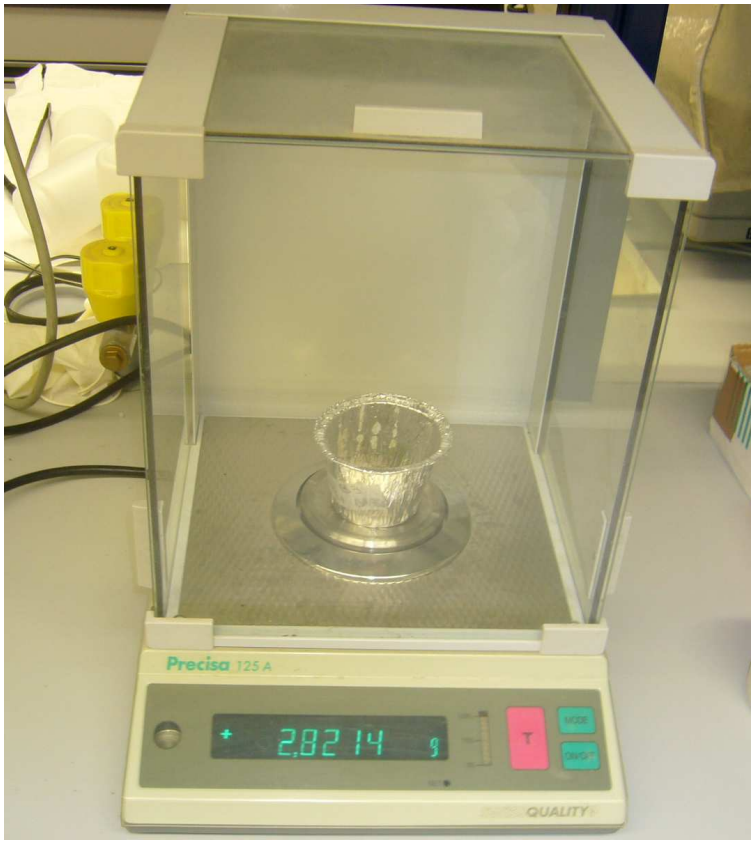

Appendices

APPENDIX 1

Organism types found in lagoons in different experiments. CA: *Calanipeda* experiment; DA: *Daphnia* experiment; OD: *Odessia* experiment; AP: *Aphanius* experiment. W: weight (µg); DWA: dry weight ash (µg); FW: fresh weight (µg); V: volume according to Hillebrand *et al*., 1999 (μm^3); Vc: volume calculated by cytometer (μm^3); Vn: Volume according to Warwick & Price, 1979 (mm^3); L = large longitude or diameter (mm); a: small longitude (mm); * volume estimated by approximation of the body shape to geometric figures.

Taxa	Experiments	Weight	Reference
Bacterioplankton	CA, DA, OD, AP	$W = (Vc \cdot 350)$	Lee & Fuhrman (1987)
Autotrophic picoflagellates (APF)	CA, DA, OD, AP	$W = (Vc \cdot 470)$	Verity *et al*. (1992)
Heterotrophic picoflagellates (HPF)	CA, DA, OD, AP	$W = (Vc \cdot 470)$	Verity *et al*. (1992)
Autotrophic nanoflagellates (ANF)	CA, DA, AP	$W = 0.433 \cdot (Vc^{0.863})$	Verity *et al*. (1992)
Phylum Tubulinea			
Order Tubulinea			
Family Amoebidae undet. ssp.	DA	$W = 0.437 \cdot \log V^{0.866}$ $V = ((((\pi/6) \cdot (a^2) \cdot L) \cdot 0.29)/0.42) + (\pi/6) \cdot (a^2) \cdot L)$*	Strathman (1967)
Phylum: **Cryptophyta**			
Class Cryptophyceae			
Order Cryptomonadales			
Family Scarabaeoidea			
Chryptomonas sp.	OD AP	$W = 0.216 \cdot V^{0.939}$; $V = (\pi/6) \cdot (a^2) \cdot L$	Menden-Duer & Lessard (2000)
Chryptophyte undet. ssp.	DA	$W = 0.216 \cdot V^{0.939}$; $V = (\pi/6) \cdot (a^2) \cdot L$	Menden-Duer & Lessard (2000)
Phylum: Heterokontophyta			
Class Crysophyceae undet. ssp.	CA, AP	$W = 0.216 \cdot V^{0.939}$; $V = (\pi\ 6) \cdot (a^2) \cdot L$	Menden-Duer & Lessard (2000)

Phylum: Hatpophyta	CA, OD, AP	$W = 0.216 \cdot V^{0.939}$; $V = (\pi /6) \cdot (a^2) \cdot L$	Menden-Duer & Lessard (2000)
Phylum Chlorophyta			
Class Chlorophyceae			
Order Chlorococcales			
Family Scenedesmaceae			
Scenedesmus sp.	DA	$W = 0.216 \cdot V^{0.939}$	Menden-Duer & Lessard (2000)
Class Trebouxiophyceae			
Order Chlorellales			
Family Oocystaceae			
Oocistys sp.	DA	$W = 0.216 \cdot V^{0.939}$	Menden-Duer & Lessard (2000)
Chlorophyte undet. ssp.	CA, OD, AP	$W = 0.216 \cdot V^{0.939}$	Menden-Duer & Lessard (2000)
Phylum: Euglenozoa			
Class Euglenophyceae			
Order Euglenales			
Family Euglenaceae			
Trachelomonas sp.	AP	$W = 0.216 \cdot V^{0.939}$; $V = (\pi /6) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Euglenophyte undet. ssp.	DA, OD, AP	$W = 0.216 \cdot V^{0.939}$; $V = \pi/12 \cdot L \cdot a \cdot (L+a)$	Menden-Duer & Lessard (2000)
Phylum Heterokontophyta			
Class Bacillariophyceae			
Order Naviculales			
Family Naviculaceae			
Navicula sp. 1	CA, DA, OD, AP	$W = 0.288 \cdot V^{0.811}$; $V = (\pi /4) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Navicula sp. 2	CA, DA	$W = 0.288 \cdot V^{0.811}$; $V = (\pi /4) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)

Order Bacillariales			
Family Bacillariaceae			
Nitzschia closterium	CA, OD, AP	$W = 0.288 \cdot V^{0.811}$; $V = (1/2) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Nitzschia pellucida	CA, DA	$W = 0.288 \cdot V^{0.811}$; $V = (1/2) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Nitzschia sp. 1	CA	$W = 0.288 \cdot V^{0.811}$; $V = (1/2) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Nitzschia sp. 2	DA, OD, AP	$W = 0.288 \cdot V^{0.811}$; $V = (1/2) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Nitzschia sp. 3	DA, AP	$W = 0.288 \cdot V^{0.811}$; $V = (1/2) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Nitzschia sp. 4	DA	$W = 0.288 \cdot V^{0.811}$; $V = (1/2) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Order Rhopalodiales			
Family: Rhopalodiaceae			
Rhopalodia constricta	DA	$W = 0.288 \cdot V^{0.811}$; $V = ((4/6) \cdot \pi \cdot a^2 \cdot L)/1080$	Menden-Duer & Lessard (2000)
Order Thalassiophysales			
Family: Catenulaceae			
Amphora sp. 1	CA	$W = 0.288 \cdot V^{0.811}$; $V = ((4/6) \cdot \pi \cdot a^2 \cdot L)/1080$	Menden-Duer & Lessard (2000)
Amphora sp. 2	OD, AP	$W = 0.288 \cdot V^{0.811}$; $V = ((4/6) \cdot \pi \cdot a^2 \cdot L)/1080$	Menden-Duer & Lessard (2000)
Order Centrales			
Family Chaetocerotaceae			
Chaetoceros sp.	CA	$W = 0.288 \cdot V^{0.811}$; $V = (\pi/4) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Phylum Myzozoa			
Class Dinophyceae			
Order Peridiniales			
Family Peridiniaceae			
Glenodinium sp.	CA, AP	$W = 0.760 \cdot V^{0.81}$; $V = (\pi/6) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Order Gymnodiniales			
Family Gymnodiniaceae			
Gymnodinium sp.	AP	$W = 0.760 \cdot V^{0.819}$; $V = (\pi/6) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Family Pronoctilucaceae			
Oxyrrhis marina	CA, AP	$W = 0.760 \cdot V^{0.819}$; $V = (\pi/6) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Autotrophic Dinoflagellate undet. ssp. 1	CA, AP	$W = 0.760 \cdot V^{0.819}$; $V = (\pi/6) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)
Autotrophic Dinoflagellate undet. ssp. 2	CA, AP	$W = 0.760 \cdot V^{0.819}$; $V = (\pi/6) \cdot L \cdot a^2$	Menden-Duer & Lessard (2000)

Division Charophyceae			
Class Charophyceae			
Order Charales			
Family Characeae			
Lamprothamnium papulosum	AP	W= Dried to 60 ºC during 24 hours and weighted	
Division Magnoliophyta			
Class Liliopsida			
Order Najadales			
Family Ruppiaceae			
Ruppia sp.	AP	W = Dried to 60 ºC during 24 hours and weighted	
Phylum Ciliophora			
Class Ciliatea			
Order Oligotrichida			
Family Strombidiidae			
Strombidium sp.	OD, AP	$W = 0.19 \cdot V$; $V = (\pi /6) \cdot (a^2) \cdot L$	Putt & Stoecker (1989)
Class Phyllopharyngea			
Suctoria undet. ssp.	DA	$W = 0.19 \cdot V$; $V = (1/3) \cdot a^2 \cdot L$	Putt & Stoecker (1989)
Ciliate undet. ssp. 1	DA	$W = 0.19 \cdot V$; $V = (\pi /4) \cdot L \cdot a^2$	Putt & Stoecker (1989)
Ciliate undet. ssp. 2	DA, OD, AP	$W = 0.19 \cdot V$; $V = (\pi /12) \cdot L^2 \cdot (a+L)$	Putt & Stoecker (1989)
Ciliate undet. ssp. 3	CA, DA, AP	$W = 0.19 \cdot V$; $V = (\pi /12) \cdot L^2 \cdot (a+L)$	Putt & Stoecker (1989)
Ciliate undet. ssp. 4	AP	$W = 0.19 \cdot V$; $V = (\pi /12) \cdot L^2 \cdot (a+L)$	Putt & Stoecker (1989)

Phylum Rotifera			
Class Rotatoria			
Order Ploimida			
Family Lecanidae			
Lecane sp.	DA, AP	$W = 0.19 \cdot V$; $V = (\pi/6) \cdot (a^2) \cdot L$	Putt & Stoecker (1989)
Family Brachionidae			
Brachionus sp.	OD, AP	$W = 36.4 \cdot L \cdot (a^2) + 3.64 \cdot L \cdot (a^2)$	Ruttner-Kolisko (1977)
Notholca sp.	AP	$W = 5.46 \cdot (L^2) \cdot a + 0.29 \cdot L^3$	Malley *et al*. (1989)
Family Colurellidae			
Colurella sp.	AP	$W = 12.38 \cdot (L^2) \cdot a + 1.38 \cdot L^3$	Ruttner-Kolisko (1997)
Order Gnesiotrocha			
Family Testudinellidae			
Testudinella sp.	AP	$W = 7.28 \cdot (L^2) \cdot a$	Ruttner-Kolisko (1977)
Family Hexarthridae			
Hexarthra sp.	OD AP	$W = 24.22 \cdot L \cdot (a^2)$	Ruttner-Kolisko (1977)
Rotifera undet. ssp. 1	AP	$W = 7.28 \cdot (L^2) \cdot a$	Ruttner-Kolisko (1977)
Phylum Cnidaria			
Class Hydrozoa			
Order Anthomedusae			
Family Moerisiidae			
Odessia maeotica	OD, AP	$W = 018.2 \cdot L^3$	Malley *et al*. (1989)

Phylum Nematoda			
Class Nematoda			
Order Enoplida			
Family Oncholaimidae			
Oncholaimus sp	AP	DW = 0.25·FW; FW = 1.13·Vn; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Order Monhysterida			
Family Monhysteridae			
Monhystrella sp.	AP	FW = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Thalassomonhystera sp.	AP	W = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Family Sphaerolaimidae			
Sphaerolaimus sp.	AP	FW = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Family Xyalidae			
Theristus sp.	AP	FW = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Order Rhabditida			
Family Neodiplogastridae			
Fictor sp.	AP	FW = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Order Chromadorida			
Family Chromadoridae			
Dicrhomadora sp.	AP	FW = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Ptycholaimellus sp.	AP	FW = 1.13·V; DW = 0.25·FW; Vn = 530·L·å	Wieser (1960); Jensen (1983)
Family Microlaimidae			
Calomicrolaimus sp.	AP	FW = 1.13·V; DW = 0.25·FW, Vn = 530·L·å	Wieser (1960); Jensen (1983)
Phylum Platyhelminthes			
Class Turbellaria			
Microtruberrari undet. ssp. 1	AP	DW = 5·FW; FW= 1070·V; V = 0.785·(å)·L*	Wieser (1960); Jensen (1983)
Microtruberrari undet. ssp. 2	AP	DW = 5·FW; FW= 1070·V; V = 0.785·(å)·L*	Wieser (1960); Jensen (1983)
Microtruberrari undet. ssp. 3	AP	DW = 5·FW; FW= 1070·V; V = 0.785·(å)·L*	Wieser (1960); Jensen (1983)

Phylum Annelida			
Class Polychaeta			
Order Capitellida			
Family Capitellidae			
Capitella sp.	AP	$DW = 0.558 \cdot L^{2.945}$	Linton & Taghon (2000)
Polychaeta larvae undet. ssp.	OD		
Phylum Mollusca			
Class Gastropoda			
Order Neotaenioglossa			
Family Hydrobiidae			
Hydrobia acuta	AP	$DWA = 27.3 \cdot L^{2.4992}$	Lingegaard (1992)
Phylum Arthropoda			
Class Copepoda			
Order Calanoida			
Family Temoridae			
Eurytemora velox	OD, AP	$W = e^{1.0968} + 2.195 \cdot \ln L$	Malley *et al*. (1989)
Family Pseudodiaptomidae			
Calanipeda aquaedulcis	CA, AP	$W = e^{1.0968} + 2.195 \cdot \ln L$	Malley *et al*. (1989)
Order Harpacticoida			
Family Canuellidae			
Canuella perplexa	AP	$W = e^{0.6154} + 2.034 \cdot \ln L$	Malley *et al*. (1989)
Family Cletodidae			
Cletocamptus confluens	AP	$W = e^{0.6154} + 2.034 \cdot \ln L$	Malley *et al*. (1989)
Family Canthocamptidae			
Mesochra lilljeborgii	AP	$W = e^{0.6154} + 2.034 \cdot \ln L$	Malley *et al*. (1989)
Mesochra heldti	AP	$W = e^{0.6154} + 2.034 \cdot \ln L$	Malley *et al*. (1989)
Family Ameiridae			
Nitocra spinipes	AP	$W = e^{0.6154} + 2.034 \cdot \ln L$	Malley *et al*. (1989)
Harpacticoida undet. ssp.	OD	$W = e^{0.6154} + 2.0343 \ln L$	Malley *et al*. (1989)

Order Cyclopoida			
Family Cyclopidae			
Halicyclops rotundipes	AP	$W = e^{0.6154}+2.034 \cdot lnL$	Malley *et al*. (1989)
Diacyclops bicuspidatus	AP	$W = e^{0.6154}+2.034 \cdot lnL$	Malley *et al*. (1989)
Cyclopoida undet. ssp.	OD	$W = e^{0.6154}+2.034 \cdot lnL$	Malley *et al*. (1989)
Class Malacostraca			
Order Amphipoda			
Family Gammaridae			
Gammarus aequicauda	AP	$W = (e^{-5.1605}+2.721 \cdot lnL) \cdot 10^3$	Quintana (1995)
Class Euentomata			
Order Diptera			
Family Chironomidae			
Chironomus salinarius	AP	$W= (e^{-5.2785}+2.32 \cdot lnL) \cdot 1000$	Smock (1980)
Class Ostracoda			
Order Podocopida			
Family Cytheroidea			
Cyprideis torosa	AP	$DW = 28.42 \cdot L^{2.8}$	Johnston (1995)
Class Branchiopoda			
Order Diplostraca			
Family Daphniidae			
Daphnia magna	DA	$W = e^{1.4660}+3.1932 \cdot lnL$	Botrell *et al*. (1976)
Phylum Chordata			
Class Actinopterygii			
Order Cyprinodontiformes			
Family Cyprinodontidae			
Aphanius iberus	AP	$W = \log(0.01673) + 2.981 \cdot logL$	Verdiell-Cubedo *et al*. (2006)

APPENDIX 2

Photos of the study.

1. *Calanipeda* and *Daphnia* experiments.

Photo 1. Pletera lagoon where *Calanipeda* experiment was carried up.

Photo 2. Bottles incubating in *Daphnia* experiment.

Photo 3. Processing samples in laboratory.

2. *Odessia* experiment.

Photo 4. Lagoon of Alt Empordà wetlands where *O. maeotica* was captured.

Photo 5. Tanks and material used in *Odessia* experiment.

3. *A. iberus* experiment.

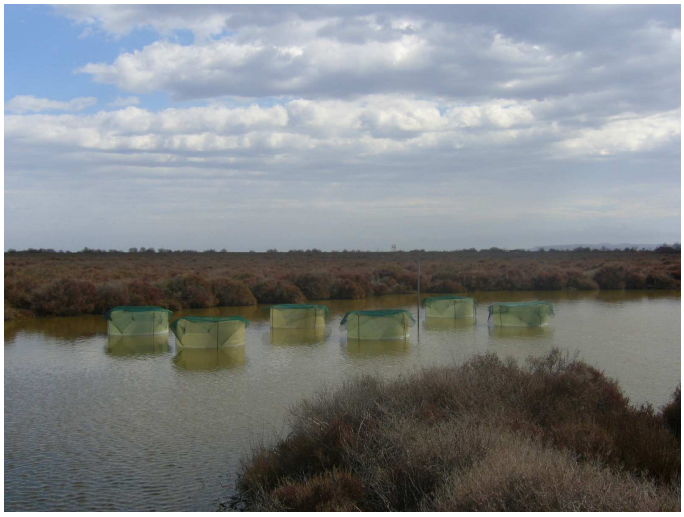

Photo 6. Connectada and the mesocosms of *Aphanius* experiment.

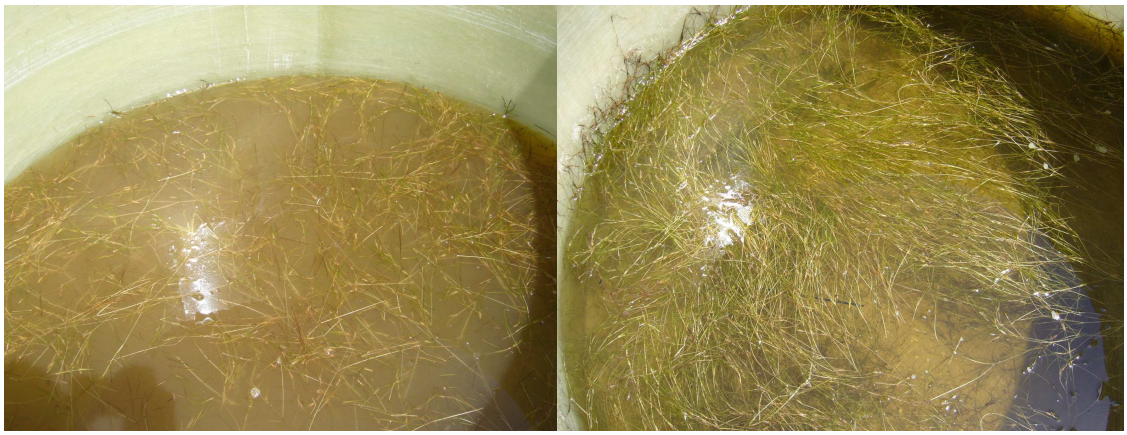

Photo 7. Water transparency. In left control mesocosm and in right fish mesocosm.

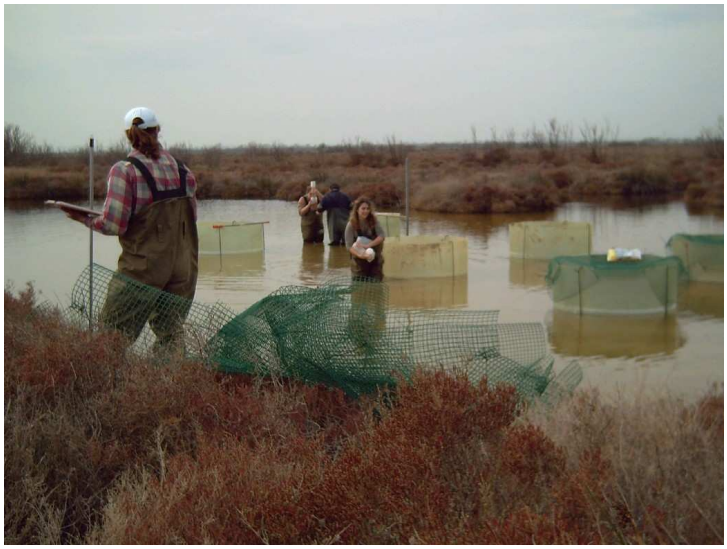

Photo 8. Working in the capture of samples.

Photo 9. Cleaning the sediment samples.

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