Universidade de Aveiro Departamento de Biologia
2007

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Ecologia e selecção de *habitat* em crustáceos zooplanctónicos de lagos pouco profundos

tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Fernando José Mendes Gonçalves, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro

Apoio financeiro da Fundação para a Ciência e a Tecnologia e do Fundo Social Europeu no âmbito do III Quadro Comunitário de Apoio – bolsa de doutoramento SFRH/BD/6417/2001

Dedico esta tese à memória do avô Castro, por sempre nos ter incentivado na procura do conhecimento, e da avó Graça, por todos os mimos…

o júri

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agradecimentos

No decurso do presente trabalho, foram várias as pessoas e instituições que contribuíram para que este decorresse dentro da normalidade, atingindo os objectivos propostos. Em primeiro lugar, agradeço à Universidade de Aveiro e, em particular, ao Departamento de Biologia e ao Centro de Estudos do Ambiente e do Mar (CESAM) as condições e meios proporcionados durante a execução do plano de trabalhos. Agradeço também o financiamento dos meus estudos à Fundação para a Ciência e a Tecnologia, sob a forma de uma bolsa de doutoramento (SFRH/BD/6417/2001) e apoios associados.

Ao Prof. Doutor Fernando Gonçalves, orientador científico desta dissertação, estou grato pelo apoio e conselhos, bem como a sua participação activa no trabalho de campo. Agradeço sobretudo a confiança que sempre demonstrou ter em mim, bem patente no grau de independência que me concedeu ao longo do doutoramento, e no respeito pelas minhas convicções. Espero ter correspondido à altura das responsabilidades decorrentes de tal grau de confiança. Valorizo a estima e consideração recíproca que nos une.

A todos os colegas de laboratório, deixo um agradecimento pelos momentos de boa disposição e pela forma como conseguimos partilhar espaços e recursos muito concorridos. Aos “residentes” Ana Marta, Catarina, Joana, Nelson, Ruth, Sérgio e Sara deixo uma palavra de apreço por terem aturado o meu inconformismo. À Ruth, Joana e Sara agradeço também as valiosas trocas de impressões e desafios. Ao Sérgio, o meu muito obrigado pela ajuda fundamental no trabalho de campo, de dia ou de noite, à chuva ou ao sol. À Joana, agradeço ainda a confiança e o companheirismo demonstrados durante o nosso estágio em Frankfurt. Obrigado Sara, por todo o teu apoio!

Não posso deixar de expressar a minha gratidão ao Prof. Doutor José Vingada pelo auxílio imprescindível prestado durante as capturas dos peixes na Lagoa da Vela, bem como a forma sempre amigável como partilhou o seu vasto conhecimento da zona. À Laura Cerqueira e à Francisca Gouveia agradeço a colaboração prestada na captura e processamento dos exemplares piscícolas.

Estou grato a todos os que contribuíram para a minha formação pessoal e profissional enquanto investigador, especialmente ao Professor Rui Ribeiro, à Olímpia Sobral e à Isabel Lopes, que apadrinharam os meus primeiros passos nesta vida e que continuam a ser referências incontornáveis para mim.

Numa perspectiva mais pessoal, gostaria de deixar a minha gratidão a todos os amigos que contribuíram para a minha estabilidade mental (dentro e fora do Dept. de Biologia). O Joca, a Nath e a Sónia merecem destaque pela amizade duradoura e incondicional. Ao pessoal do NBIUA, agradeço os momentos de boa disposição e os sacrifícios pela causa. Os meus parceiros da bola também merecem uma referência pelos momentos de descontracção proporcionados.

À família, e sobretudo aos meus pais, devo-lhes muito do que sou hoje. À minha pequena família (Sara e Maria João) agradeço a compreensão pelas minhas ausências e os momentos de ternura que atenuam as frustrações. Obrigado por serem os sólidos alicerces em que assento…

palavras-chave

lagos pouco profundos, zooplâncton dulçaquícola, peixes de água doce, espécies alienígenas, ecologia de populações, interacções tróficas, selecção de *habitat*, defesas anti-predador, *Daphnia*

resumo

Apesar das evidências de que os lagos Mediterrânicos pouco profundos possuem características ecológicas distintas, o conhecimento sobre estes sistemas é limitado. Este facto é agravado pela invasão globalizada de peixes alienígenas em lagos mais quentes, cujos efeitos sobre as interacções tróficas são praticamente desconhecidos. Com o intuito de diminuir o grau de incerteza associado à ecologia destes sistemas em Portugal, foi estudada a ecologia do zooplâncton na Lagoa da Vela (Quiaios, Figueira da Foz), um lago natural pouco profundo e eutrófico que apresenta dominância de peixes alienígenas. O objectivo da investigação foi compreender os mecanismos que moldam a comunidade zooplanctónica, com particular enfoque nas suas interacções com os peixes planctívoros, dada a importância deste elo trófico de charneira.

Numa primeira fase, foram caracterizadas as comunidades de zooplâncton e peixes durante um ano. A lagoa revelou ser altamente produtiva e turva, exibindo níveis elevados de sólidos em suspensão, nutrientes e clorofila *a*. Foi observado um contraste evidente entre o período de água transparente, dominado por *Daphnia*, e o resto do ano, dominado por zooplanctontes de pequenas dimensões, incapazes de controlar o crescimento algal. A pressão predatória revelou ser uma força determinante do funcionamento da lagoa, a par com o nível de partículas em suspensão. Os peixes alienígenas exibiram um grau substancial de omnivoria, com o zooplâncton a constituir um dos principais componentes da dieta. A perca-sol (*Lepomis gibbosus*) foi o principal peixe planctívoro, exibindo populações muito densas, sobretudo no Verão, como resultado de um recrutamento contínuo de Julho a Setembro. A predação exercida por perca-sol e outros planctívoros contribuiu para o colapso da população de *Daphnia* (e consequente redução da transparência), mas o desaparecimento dos dafníideos durante o Verão e Outono não pode ser explicado sem recurso a outras variáveis ambientais para além da predação.

A segunda fase do estudo pretendeu avaliar as estratégias utilizadas pelos zooplanctontes para minimizar a pressão predatória a que estão sujeitos. Na Lagoa da Vela, o zooplâncton exibiu uma distribuição espacial heterogénea (no plano horizontal e vertical) dependente da transparência da água. Os padrões observados são consistentes com o evitamento de predadores visuais (i.e. peixes). A amplitude reduzida do refúgio no plano vertical e a ausência de refúgio proporcionada pela vegetação aquática tornam os zooplanctontes (sobretudo *Daphnia*) particularmente susceptíveis à predação. Em ambiente laboratorial, *Daphnia* reagiu a sinais químicos de duas espécies de peixes alienígenas presentes na lagoa através de incremento reprodutivo e redução do tamanho corporal, evidenciando uma estratégia que maximiza o seu desempenho ante predadores selectivos, conforme documentado para outras espécies de peixes planctívoros.

keywords

shallow lakes, freshwater zooplankton, freshwater fish, alien species, population ecology, trophic interactions, habitat selection, anti-predator defence, *Daphnia*

abstract

Few studies have focused on Mediterranean shallow lakes, although there is evidence of their distinctive features. This is particularly aggravated by the widespread invasion of alien fish predators in warmer lakes, whose effects on the trophic interactions largely remain unknown. To address the uncertainty associated with the ecology of these systems in Portugal, we studied the zooplankton ecology in a shallow polymictic lake (Lake Vela, Figueira da Foz), which is dominated by alien fish species and suffers from high nutrient loading (eutrophication). The purpose of the research was to understand the mechanisms shaping the zooplanktonic community, with particular emphasis on its interactions with planktivorous fish, given the importance of this pivotal trophic link.

In a first stage, surveys on the zooplankton and fish assemblages were conducted for a one-year period. Lake Vela was highly productive and turbid, displaying high levels of suspended solids, nutrients and chlorophyll *a*. An evident contrast was observed between a clear water phase, dominated by *Daphnia*, and the rest of the year, dominated by small-sized zooplankters, unable to control algal biomass. Predatory pressure was found to be a key determinant of the lake's condition and function, along with the level of suspended particles. Alien fish displayed a substantial degree of omnivory, where zooplankton was a main component in the diet. Pumpkinseed sunfish (*Lepomis gibbosus*) was the main planktivore, attaining very dense populations, mostly in the summer, as a consequence of continuous recruitment from July to September. Predation exerted by pumpkinseed and other planktivores contributed to the population collapse of *Daphnia* (and consequent reduction in transparency), but the disappearance of the daphniids during summer and autumn cannot be explained unless environmental variables other than predation are considered.

The second stage of this work intended to study how zooplankters cope with high predation pressure from the alien benthi-planktivorous fish. Zooplankton displayed a heterogeneous spatial distribution (both horizontal and vertically) in Lake Vela, which was directly dependent on transparency. The observed patterns were consistent with avoidance of visual predators (i.e. fish). The reduced amplitude of refuge in the vertical plane and the absence of refuge in aquatic vegetation make zooplankters particularly susceptible to predation. Under laboratorial environment, *Daphnia* responded to chemical cues from two alien fish species present in the lake by maximizing reproduction and reducing body size, as an anti-predator defence mechanism against size-selective predation, as reported for most fish taxa.

«Do que você precisa, acima de tudo, é de se não lembrar do que eu lhe disse; nunca pense por mim, pense sempre por você; fique certo de que mais valem todos os erros se forem cometidos segundo o que pensou e decidiu do que todos os acertos, se eles forem meus, não seus.»

Agostinho da Silva

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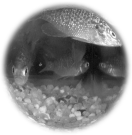

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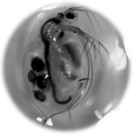

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Introdução geral

PREÂMBULO

O fascínio pelos lagos e rios, enquanto sistemas biológicos, e a preocupação acerca da interferência antropogénica sobre estes levaram ao surgimento de uma nova disciplina científica, no dealbar do século XX. A Limnologia (ou “Oceanografia dos lagos”) surgiu formalmente com o trabalho do suíço François-Alphonse Forel no Lago Genebra (Forel, 1882), publicado em três volumes entre 1882 e 1904. Do outro lado do Atlântico (EUA), o nascimento da Limnologia surgiu com os estudos de Edward Birge e Chancey Juday, que se dedicaram à pesquisa do Lago Mendota (e outros lagos do Wisconsin). Poucos anos antes, contudo, o trabalho de Stephen Alfred Forbes – prestigiado ecólogo americano – terá estabelecido as fundações da ciência dos ecossistemas aquáticos, bem presentes na sua influente publicação de 1887 (Forbes, 1887). O interesse crescente em torno da Limnologia originou o nascimento de prestigiadas sociedades científicas na primeira metade do século XX, como a *Netherlands Society of Aquatic Ecology* (fundada em 1921), a *Societas Internationalis Limnologiae* (SIL, fundada em 1922), a *Freshwater Biological Association* (fundada em 1929) ou a *Limnological Society of America* (fundada em 1936, dando origem em 1948 à actual *American Society of Limnology and Oceanography* - ASLO).

O pós-guerra trouxe a Limnologia moderna, cujo pai, responsável pelos primeiros tratados sobre Limnologia (Hutchinson, 1957; 1967), foi George Evelyn Hutchinson. A Limnologia era agora definida como o estudo das relações funcionais e da produtividade das comunidades de água doce (lagos, charcos, rios e ribeiras), reguladas pela dinâmica dos factores do seu ambiente físico, químico e biológico (Wetzel, 1983). Por definição, o estudo da Limnologia abrange todos os *habitats* de água doce, incluindo charcos, lagos e albufeiras (*habitats* lênticos), bem como rios e ribeiras (*habitats* lóticos), que diferem na ausência (lêntico) ou presença (lótico) de um fluxo unidireccional significativo da massa de água. Contudo, historicamente, as obras e as sociedades científicas de Limnologia (que deriva da palavra grega para lago, *limnos*) dedicaram a sua atenção quase exclusivamente aos sistemas lênticos. Esta divisão histórica ainda hoje tem repercussões nas reuniões e publicações científicas da especialidade, dado o distinto contexto abiótico e a diferente natureza das teias tróficas dos dois tipos de *habitats*.

Na Península Ibérica, a Limnologia e a Ecologia Aquática em muito progrediram a partir da década de 40 com o trabalho do notável ecólogo espanhol Ramón Margalef (Casado & Montes, 1992). Todavia, apenas em 1981 (pela mão de Margalef e seus seguidores) surgiu a primeira sociedade para a disseminação e debate do conhecimento científico sobre os recursos de água doce Ibéricos, a *Asociación Española de Limnología*. A reduzida expressão dos *habitats* lênticos Ibéricos, em comparação com os numerosos lagos das cadeias montanhosas do centro da Europa ou os vastos lagos da América do Norte, é uma das explicações plausíveis para a menor expressão da Limnologia na Península Ibérica. De facto, devido ao clima Mediterrânico, que se faz sentir sobretudo no centro e sul da Ibéria, os lagos naturais rareiam e os represamentos de rios (albufeiras) constituem os sistemas lênticos mais representativos (Colomer, 1996; Boavida & Marques, 1996; Boavida & Gliwicz, 1996; Velasco et al., 1999; Garcia-Berthou & Moreno-Amich, 2000; Boavida, 2000; Caramujo & Boavida, 2000b). A fraca implementação histórica da Limnologia na Península Ibérica (Casado & Montes, 1992) causou alguma descontinuidade nos registos limnológicos, sendo raros os casos de lagos na Península Ibérica com registos históricos mais ou menos contínuos das suas componentes abióticas e bióticas (e.g. Garcia-Berthou & Moreno-Amich, 2000; Romo et al., 2005), ao contrário do que se passa em muitos países onde a Limnologia adquiriu maior expressão (e.g. Perrow et al., 1994; Moss et al., 1996; Breukers et al., 1997; Benndorf et al., 2000; Carpenter et al., 2001; Hrbácek et al., 2003; Horn, 2003). Esta falta de dados históricos de base é particularmente notória em Portugal, o que dificulta a compreensão das consequências da interferência antropogénica sobre os sistemas aquáticos.

Foi no sentido de colmatar algumas das carências da investigação limnológica em Portugal, sobretudo na ecologia dos lagos pouco profundos, que surgiu a presente dissertação. Estas carências adquirem particular importância em virtude do agudizar da crise dos recursos de água doce, resultante do aumento do consumo e da degradação da qualidade de um bem já de si escasso (Wetzel, 1983; Boavida, 2000). A escassez dos recursos de água doce, mas também as ameaças que pairam sobre a sua integridade ecológica e biodiversidade, reforçaram a necessidade de um conhecimento mais profundo sobre estes sistemas. Só o incremento do conhecimento científico acerca dos *habitats* dulçaquícolas (com estudos de natureza fundamental ou aplicada) poderá proporcionar uma base sustentada e fundamentada para o desenvolvimento de ferramentas de monitorização mais adequadas, medidas de recuperação mais eficazes, e uma maior consciencialização das populações e dos gestores ambientais para os problemas que enfrentamos.

ENQUADRAMENTO DA DISSERTAÇÃO

As interacções tróficas como mecanismos reguladores

A primeira aproximação da Limnologia às comunidades dos lagos foi holística e multidisciplinar, dando grande ênfase aos processos físico-químicos enquanto modeladores das biocenoses. A categorização dos lagos em função da disponibilidade de nutrientes (estado trófico), por exemplo, rapidamente se tornou um paradigma (Vollenweider, 1976; Carlson, 1977; Williamson et al., 1999), especialmente porque adquiriu suporte nas evidências empíricas de uma forte relação entre a clorofila *a* e o fósforo (Vollenweider, 1976; Schindler, 1978). Progressivamente, contudo, a tendência para sobrevalorizar as condicionantes abióticas externas (clima, nutrientes, hidrologia) sobre os ecossistemas foi encontrando crescente oposição na Ecologia. Os trabalhos pioneiros de alguns ecólogos (Hrbácek et al., 1961; Brooks & Dodson, 1965; Hurlbert et al., 1972) demonstraram a importância das interacções ecológicas internas (no caso, a predação) no funcionamento dos sistemas aquáticos (ver mais adiante). Um novo paradigma, relativo ao papel determinante das interacções bióticas (competição, reciclagem de nutrientes e, sobretudo, predação) na dinâmica das comunidades biológicas, instalava-se assim na Ecologia (Paine, 1980; Brönmark & Hansson, 1998; Carpenter, 1998). Hoje em dia, é comummente aceite que a regulação dos sistemas aquáticos assenta numa combinação/interacção das condicionantes externas (factores abióticos) e dos processos internos (interacções bióticas).

A noção de que os predadores podem ter efeitos determinantes nos níveis tróficos inferiores (Hrbácek et al., 1961; Brooks & Dodson, 1965; Hurlbert et al., 1972; Hall et al., 1976; Mills & Forney, 1983) levou a que os mecanismos de regulação *top-down* (do topo da cadeia trófica para a sua base) fossem olhados com outra importância na Ecologia das águas doces (Carpenter et al., 1985). Estes mecanismos surgem por oposição aos mecanismos *bottom-up* (McQueen et al., 1986), que resultam da competição pelos recursos disponíveis (disponibilidade de nutrientes ou alimento). Do compromisso entre estes mecanismos reguladores resulta o equilíbrio ecológico da teia trófica de um lago (Wetzel, 1983; Brönmark & Hansson, 1998). Um exemplo ilustrativo da interacção dos mecanismos reguladores das teias tróficas do *habitat* lacustre é a dinâmica sazonal do plâncton, cujo modelo de sucessão para lagos temperados (Sommer et al., 1986) foi descrito por um grupo internacional de peritos (PEG – Plankton Ecology Group), assumindo que os eventos que regulam a sucessão do plâncton não ocorrem ao acaso e obedecem a uma sazonalidade mais ou menos previsível. Para melhor compreender o modelo sazonal do plâncton, há que enquadrar esta componente da teia trófica pelágica no seu lugar (Figura 1). Numa visão simplificada mas útil, é comum dividir o plâncton em dois grupos funcionais (produtores – fitoplâncton; herbívoros - zooplâncton), com elevado grau de interdependência, que sustentam os níveis tróficos superiores (sobretudo peixes).

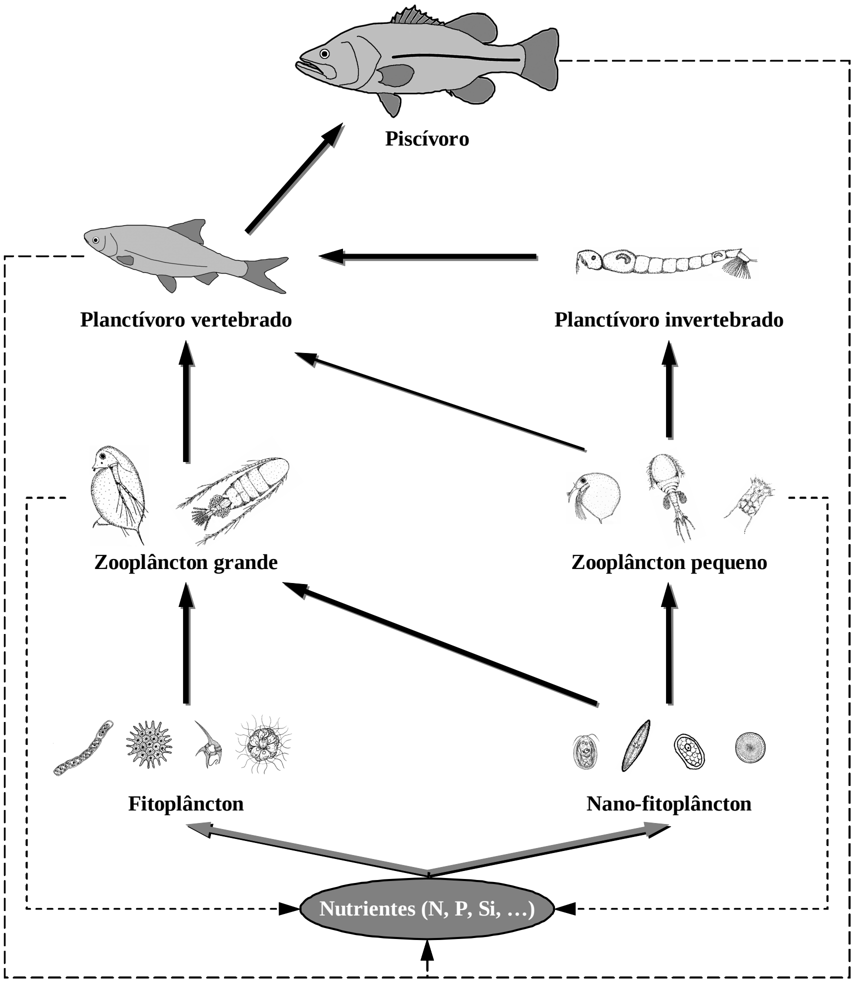


Figura 1 • Modelo conceptual simplificado de uma teia trófica pelágica de um lago típico (baseado em Carpenter et al., 1985; Brönmark & Hansson, 1998). A espessura das setas representa a intensidade da interacção e as setas a tracejado correspondem à reciclagem de nutrientes. As ilustrações a preto e branco são da autoria de Sara Antunes, excepto as relativas ao fitoplâncton, que foram obtidas no *site* educacional Biodidac (*Biodidac - A bank of digital resources for teaching biology*, http://biodidac.bio.uottawa.ca).

Devido à sua posição-chave na teia trófica pelágica (Figura 1), a dinâmica sazonal do plâncton resulta de um conjunto de interacções entre processos bióticos (competição pelos recursos e predação) e condicionantes abióticas (disponibilidade de nutrientes, temperatura), conforme ilustrado na Caixa A. O modelo PEG prevê que, devido às baixas temperaturas de Inverno e ao curto período diurno, o crescimento fitoplanctónico seja reduzido, permitindo a acumulação de nutrientes. Esta escassez de alimento compromete a fecundidade do zooplâncton e muitos recorrem a estratégias de diapausa, produzindo estruturas de dormência (Gilbert, 1974; Dahms, 1995; Hairston, 1996; Fryer, 1996). À medida que o Inverno chega ao fim, o incremento da intensidade luminosa, combinado com a disponibilidade de nutrientes, permite o crescimento ilimitado do fitoplâncton. Tem início a fase mais profícua do plâncton (Primavera), com o desenvolvimento de pequenas algas de crescimento rápido, que são alvo da herbivoria de espécies zooplanctónicas com tempos de geração curtos. Mais tarde, dá-se o aparecimento de espécies zooplanctónicas de crescimento mais lento e maior biomassa, como é o caso de *Daphnia* (*vide* Asaeda & Acharya, 2000). Estes zooplanctontes de grandes dimensões são capazes de uma herbivoria muito eficiente, podendo reduzir a biomassa algal a níveis muito baixos (Brooks & Dodson, 1965; Gliwicz, 1990; Scheffer, 1999), originando um período de água transparente (de duração variável) (Lampert et al., 1986). A vantagem competitiva de *Daphnia* (Gliwicz, 1990; Kreutzer & Lampert, 1999) sobre as espécies de cladóceros mais pequenas origina a exclusão competitiva destas últimas (Vanni, 1986). Os nutrientes são reciclados pela herbivoria e acumulam-se na água.

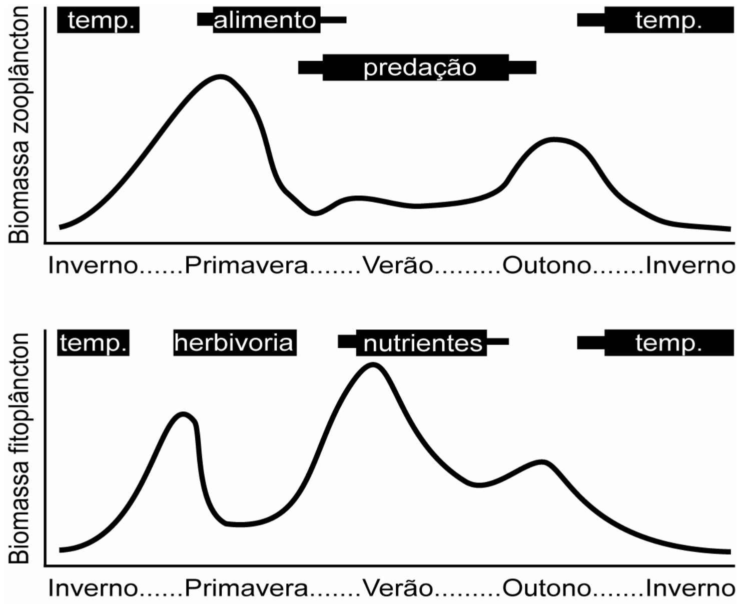
No final da Primavera, a supressão do crescimento do fitoplâncton pelos herbívoros origina um declínio das populações de *Daphnia* por escassez de alimento (Luecke et al., 1990; Hülsmann & Weiler, 2000). Nesta altura, o papel da predação no declínio das populações zooplanctónicas é residual (Luecke et al., 1990; Boersma et al., 1996; Hülsmann & Weiler, 2000), mas após o colapso, é a pressão predatória que mantém a abundância de herbívoros de grandes dimensões (sobretudo *Daphnia*) a níveis baixos, ao longo de todo o Verão (Mills & Forney, 1983; Luecke et al., 1990; Boersma et al., 1996; Saunders et al., 1999). Este controlo é particularmente forte na presença de estados larvares e juvenis de peixes (Boersma et al., 1996; Romare et al., 1999). É reconhecido que esta regulação dos peixes planctívoros sobre o zooplâncton depende muito da dinâmica populacional, quer de *Daphnia* (Hülsmann & Voigt, 2002; Hülsmann, 2003), quer dos peixes (Mills & Forney, 1983), podendo por isso produzir variações interanuais no mesmo sistema aquático (Mills & Forney, 1983; Saunders et al., 1999; Hülsmann, 2003) e variações entre diferentes sistemas (e.g. Sommer et al., 1986; Saunders et al., 1999).

O controlo exercido pelos peixes planctívoros sobre o zooplâncton origina uma mudança para uma comunidade zooplanctónica dominada por espécies de pequenas dimensões (*Bosmina*, *Chydorus*, copépodes ciclopóides e rotíferos), menos eficientes no controlo da biomassa algal (Brooks & Dodson, 1965; Declerck et al., 1997; Tessier et al., 2001). Por esse motivo, o Verão é caracterizado por uma diversificação e crescimento da comunidade fitoplanctónica e a uma forte redução da transparência. Entretanto, o crescimento descontrolado do fitoplâncton leva ao esgotamento progressivo dos nutrientes necessários ao seu crescimento (fósforo, sílica, azoto), promovendo uma intensa competição entre as espécies fitoplanctónicas. Nalguns lagos, este fenómeno é agravado pela estratificação térmica (*vide* Wetzel, 1983), que isola os nutrientes da parte mais funda do lago (abaixo da termoclina, *hypolimnion*), tornando-os indisponíveis para a comunidade fitoplanctónica (situada no *epilimnion*). Estas condições originam o aparecimento de uma comunidade fitoplanctónica de baixo valor nutritivo para o zooplâncton (Wetzel, 1983; Wilson et al., 2006), incluindo algumas cianobactérias causadoras de florescências (*blooms*). A dominância de cianobactérias (algumas delas tóxicas) que ocorre no fitoplâncton de muitos lagos eutróficos no Verão é um factor que também pode contribuir para a supressão de *Daphnia* durante este período (DeMott et al., 2001; Wilson et al., 2006), a par com a intensa pressão predatória.

Com a chegada do Outono, o lago sofre alterações físico-químicas resultantes do término da termoclina. Este acontecimento permite a remistura das águas (entre *epilimnion* e *hypolimnion*), o que origina um reabastecimento do *stock* de nutrientes e novo incremento de produção fitoplanctónica (sobretudo diatomáceas). O re-estabelecimento de recursos de boa qualidade e a redução da predação, como consequência do abaixamento da temperatura, levam ao ressurgimento de um novo máximo nas abundâncias zooplanctónicas (Sommer et al., 1986), que normalmente inclui formas de grandes dimensões como *Daphnia*.

CAIXA A – O modelo sucessional do plâncton

Devido à sua posição-chave na teia trófica pelágica, a dinâmica sazonal do plâncton resulta de um conjunto de interacções entre processos bióticos (competição pelos recursos e predação) e condicionantes abióticas (luz, temperatura), conforme ilustrado na figura abaixo (baseado em Sommer et al., 1986; Brönmark & Hansson, 1998; Saunders et al., 1999).



Mecanismos dominantes da sucessão sazonal do plâncton

Apesar de vários lagos temperados se adequarem ao modelo proposto pelo PEG, outros há que registam variações e excepções, mas isso não retira o valor a este modelo enquanto tentativa de estruturação e de generalização de um padrão sazonal (Sommer et al., 1986; Brönmark & Hansson, 1998). Aliás, a sua real utilidade é a de permitir avaliar a força relativa dos factores determinantes dos padrões de sucessão planctónica. Isso permite, por meio de análise comparativa, determinar quais os factores responsáveis pelos desvios ao modelo, ou levantar hipóteses que possam ser testadas em ambiente controlado ou semi-controlado.

Cascatas tróficas e biomanipulação

Os trabalhos pioneiros de Hrbácek et al. (1961), Brooks & Dodson (1965) e também de Hurlbert et al. (1972) ilustraram o contraste entre os sistemas com marcada dominância de peixes planctívoros e os sistemas com reduzido *stock* de peixes. Os primeiros exibiam comunidades zooplanctónicas desprovidas de *Daphnia* e copépodes calanóides, a par de elevada turbidez e biomassa fitoplanctónica; pelo contrário, os últimos apresentavam elevadas densidades de *Daphnia* e um grau de transparência acrescida. Estas evidências originaram uma hipótese científica (*size-efficiency hypothesis*), proposta por Brooks & Dodson (1965), que envolve o mecanismo de regulação dos níveis tróficos de base pelos peixes planctívoros e piscívoros, e que também está na base de alguns eventos do modelo sucessional do plâncton (ver acima; Sommer et al., 1986). Esta hipótese baseia-se na interacção entre duas estratégias diferentes: a dos peixes, como selectores de alimento (preferindo por isso presas de maiores dimensões), e a dos zooplanctontes como colectores generalistas de alimento (Brooks & Dodson, 1965). De forma a tornar mais clara esta relação, tomemos então como ponto de partida a teia trófica simplificada de um lago (Figura 1), que tem por base os nutrientes limitantes ao crescimento dos produtores pelágicos (fitoplâncton – representa o primeiro nível trófico). Os herbívoros (zooplâncton), planctívoros (macroinvertebrados planctónicos e peixes) e os piscívoros (peixes) constituem os níveis seguintes. O modelo contempla apenas os grupos funcionais do compartimento pelágico, dado ser aí que decorre a maior fracção de produtividade de um lago típico (Hutchinson, 1967; Wetzel, 1983). O diagrama apresentado é meramente indicativo das interacções tróficas mais fortes, não pretendendo abarcar a totalidade da reticulada teia trófica, que pode incluir componentes bênticas (Brönmark & Hansson, 1998).

Da interpretação do modelo conceptual (Figura 1), é possível inferir que um incremento na abundância dos piscívoros produzirá um decréscimo na abundância dos peixes planctívoros, resultando numa maior importância dos planctívoros invertebrados (como o díptero planctónico *Chaoborus*, na figura) e num incremento na densidade dos zooplanctontes de grandes dimensões (mormente *Daphnia*). Se a predação por parte dos peixes planctívoros for reduzida o suficiente, *Daphnia* predominará sobre as espécies de zooplanctontes mais pequenas, dada a sua superioridade enquanto competidor (Hall et al., 1976; Gliwicz, 1990; Kreutzer & Lampert, 1999) em regimes de reduzida predação (*size-efficiency hypothesis*; Brooks & Dodson, 1965). A presença abundante de *Daphnia* promove a redução da biomassa fitoplanctónica (ver mais adiante). O cenário oposto será esperado como consequência de uma redução na abundância de piscívoros, já que tal fará aumentar a abundância dos peixes planctívoros, que exercerão forte pressão predatória sobre o zooplâncton, particularmente *Daphnia* (*size-selective predation*; Brooks & Dodson, 1965; Hall et al., 1976; Gliwicz, 1994), o que promoverá o incremento da produção fitoplanctónica.

Existem actualmente numerosas evidências que corroboram o raciocínio e as inferências do modelo conceptual da Figura 1. Algumas destas observações são provenientes de estudos onde se acompanhou a dinâmica do sistema a longo prazo, a par com a evolução da ictiocenose (Breukers et al., 1997; Hrbácek et al., 2003; Horn, 2003). Estes trabalhos demonstraram que o aumento progressivo do *stock* piscícola reduziu a biomassa do zooplâncton e a transparência, ao mesmo tempo que a produção primária aumentava (eutrofização). O efeito mais pronunciado foi a redução da biomassa das espécies zooplanctónicas de maiores dimensões (sobretudo *Daphnia*) e/ou a sua menor expressão temporal. Num destes casos (Breukers et al., 1997), a espécie dominante *Daphnia pulex* foi mesmo suprimida e o seu lugar foi tomado por dafnídeos de menor dimensão (*D. cucullata*, *D. galeata* e os seus híbridos inter-específicos). Em dois destes casos (Breukers et al., 1997; Hrbácek et al., 2003), o incremento a longo prazo da clorofila *a* não teve correspondência no fósforo total, demonstrando claramente a cascata do topo (peixes planctívoros) para a base da teia trófica. Os efeitos contrários foram relatados em sistemas onde ocorreram eventos ocasionais de mortandade de peixes planctívoros, o que originou um incremento da biomassa zooplanctónica e consequente redução da turbidez e da produção primária, quando comparados com o estado prévio do sistema (Perrow et al., 1994; Jeppesen et al., 2000; Margaritora et al., 2001; Nagdali & Gupta, 2002; Abrantes et al., 2006a) ou com sistemas adjacentes onde não ocorreu a mortandade (Jeppesen et al., 2000; Korponai et al., 2003). Refira-se que, em dois dos casos aqui apresentados (Nagdali & Gupta, 2002; Abrantes et al., 2006a), o efeito foi transitório.

Ao conjunto de efeitos recíprocos do predador sobre a presa que alteram a abundância, biomassa ou produtividade de uma população, comunidade ou nível trófico, repercutindo-se através de mais do que um elo da teia trófica (Pace et al., 1999), denominou-se cascata trófica (Paine, 1980). Carpenter et al. (1985) foram pioneiros em dar destaque às cascatas tróficas em Ecologia Aquática, sugerindo que as interacções tróficas em cascata poderiam explicar a fracção da variância não explicada pela regressão entre a produção primária e o fósforo (ver acima), sobretudo quando se trata de justificar as diferenças de produtividade entre sistemas com níveis de nutrientes semelhantes mas com estruturas de teia trófica diferentes.

Para além dos exemplos empíricos acima mencionados, a grande maioria de exemplos de cascatas tróficas deriva de experiências de manipulação do stock piscícola sob condições controladas ou semi-controladas (e.g. Christofferson et al., 1993; Kurmayer & Wanzenböck, 1996; Romare et al., 1999; Annadotter et al., 1999; Karjalainen et al., 1999;

Carpenter et al., 2001; Lathrop et al., 2002; Williams & Moss, 2003; Tátrai et al., 2003a; 2003b; Schrage & Downing, 2004). Estas experiências vieram claramente corroborar a *size-efficiency hypothesis* de Brooks & Dodson (1965), confirmando que os peixes planctívoros têm um papel determinante na transparência e produção primária, via zooplâncton. Contudo, há que ressalvar que estes efeitos têm magnitudes diferentes consoante a densidade e os hábitos das espécies planctívoras manipuladas (Karjalainen et al., 1999; Williams & Moss, 2003; Tátrai et al., 2003a).

Com as evidências acumuladas das interacções tróficas em cascata, cedo foi reconhecido o potencial da biomanipulação das teias tróficas no controlo da biomassa e produtividade fitoplanctónica, em programas de gestão e recuperação de lagos (Shapiro & Wright, 1984; Carpenter et al., 1985). De facto, tem sido demonstrada a utilidade da biomanipulação como ferramenta útil para o controlo da eutrofização (e.g. Moss et al., 1996; Annadotter et al., 1999; Carpenter et al., 2001; Lathrop et al., 2002), muitas vezes em complementaridade com outras ferramentas, como a diminuição da carga de nutrientes do meio (Clasen et al., 1989). No entanto, o sucesso da biomanipulação depende de vários factores, entre eles a carga de nutrientes (sobretudo fósforo) do sistema, a profundidade e a ictiocenose (Moss, 1992; Moss et al., 1996; Jeppesen et al., 2005). Alguns casos de sucesso de biomanipulação podem ser encontrados na literatura, incluindo a remoção total ou parcial de peixes planctívoros (Moss et al., 1996; Annadotter et al., 1999; Tátrai et al., 2003b; Schrage & Downing, 2004) ou o aumento da densidade de piscívoros (Carpenter et al., 2001; Lathrop et al., 2002; Skov et al., 2002). Por vezes, as interacções tróficas em cascata permitem a manutenção de um estado de água transparente, com reduzida produção primária e presença de *Daphnia*, mesmo sob elevada carga de fósforo interna (Perrow et al., 1994). Contudo, o sucesso da biomanipulação é ainda incerto, dada a complexidade das interacções existentes no ecossistema lacustre (Moss, 1992). Em alguns trabalhos, por exemplo, foram relatados sucessos apenas temporários, com o rápido regresso às condições iniciais após a biomanipulação (Romo et al., 1996; Schrage & Downing, 2004) ou com resultados parcialmente insatisfatórios (Moss et al., 1996; Benndorf et al., 2000; Tátrai et al., 2003b). É necessário, por isso, um conhecimento mais profundo das interacções tróficas em cada caso particular e dos mecanismos de resistência (histerese, ver mais adiante) do sistema (Jeppesen et al., 2005), já que estes determinam o sucesso ou insucesso da biomanipulação.

O papel central de *Daphnia*

As evidências até aqui demonstradas e a argumentação que tem vindo a ser seguida culminam com a ideia de que o zooplâncton é o grupo chave no controlo da produção fitoplanctónica, na maioria dos lagos. São vários os autores (e.g. Lampert et al., 1986; Scheffer, 1998; Jeppesen et al., 1999; Scheffer, 1999) que concordam com esta ideia e os próprios princípios subjacentes à biomanipulação e recuperação de lagos assentam no fomento indirecto da herbivoria (ver acima). A importância do zooplâncton reside igualmente no seu papel central na transferência de massa e energia ao longo da teia trófica pelágica e, numa perspectiva mais aplicada, no seu potencial enquanto indicador do estado trófico (Caramujo & Boavida, 2000b) e da qualidade da água (Colomer, 1996) de reservatórios para uso humano. Contudo, o controlo efectivo do fitoplâncton (e consequente incremento da transparência) só é possível com a presença de cladóceros de grandes dimensões (Brooks & Dodson, 1965; Pace, 1984), nomeadamente *Daphnia* (Figura 2). Inclusivamente, o maior ou menor impacto sobre a biomassa algal está mais relacionado com a presença de *Daphnia*, do que com a abundância total do zooplâncton (Pace, 1984; McQueen et al., 1986; Scheffer, 1999). Isto deve-se, em parte, à capacidade destes microinvertebrados em explorar um leque mais amplo de células algais (em termos de dimensões), comparativamente às espécies zooplanctónicas mais pequenas (Brooks & Dodson, 1965; Tessier et al., 2001). Não obstante essa característica, o maior impacto de *Daphnia* sobre o fitoplâncton resulta da sua eficiência enquanto filtrador, sendo capaz de reduzir a biomassa algal a níveis extremamente reduzidos, dada a sua tolerância para baixos níveis de alimento (Gliwicz, 1990; Kreutzer & Lampert, 1999). Esta tolerância (*ergo* a eficiência de filtração) é tanto maior quanto maior o tamanho corporal (Gliwicz, 1990; Declerck et al., 1997; Kreutzer & Lampert, 1999), pelo que as espécies de maiores dimensões têm um potencial de filtração e um impacto mais forte na clorofila *a* e na transparência da água (Kasprzak et al., 1999; Tessier et al., 2001).

Apesar da sua competitividade, os períodos de dominância de *Daphnia* em lagos são limitados na dinâmica sazonal do zooplâncton de lagos (*vide* modelo PEG). Dentre os factores que contribuem para o menor desempenho de *Daphnia* durante alguns períodos do ano, destaca-se a fraca qualidade do alimento (reduzida digestibilidade, deficiência nutritiva, produção de toxinas, interferência mecânica), que tem sido alvo de bastante atenção na literatura (e.g. Vanni & Lampert, 1992; Brett, 1993; Kilham et al., 1997; Repka, 1997; DeMott et al., 2001; Becker & Boersma, 2003; Lürling, 2003; Wilson et al., 2006). Contudo, o factor preponderante no controlo das populações de *Daphnia* é a predação, na medida em que estas representam itens alimentares de grande importância para os peixes planctívoros (vide Roseman et al., 1996; Gliwicz et al., 2000; 2004; Scheuerell et al., 2005). Tessier & Woodruff (2002a) reconheceram o papel determinante da planctivoria na composição do fito- e zooplâncton, propondo uma categorização dos lagos em função do nível de planctivoria, intimamente relacionada com a comunidade de herbívoros (mormente *Daphnia*). De facto, os vários exemplos de cascatas tróficas acima ilustrados documentam o sucesso de *Daphnia* em regimes de reduzida predação, por oposição aos sistemas onde a pressão predatória é elevada. Não obstante, a coexistência entre *Daphnia* e elevadas densidades de peixes planctívoros ocorre na natureza, em parte devido a mecanismos de defesa que permitem às populações de *Daphnia* contrabalançar, até um certo nível, as perdas causadas pelos predadores.

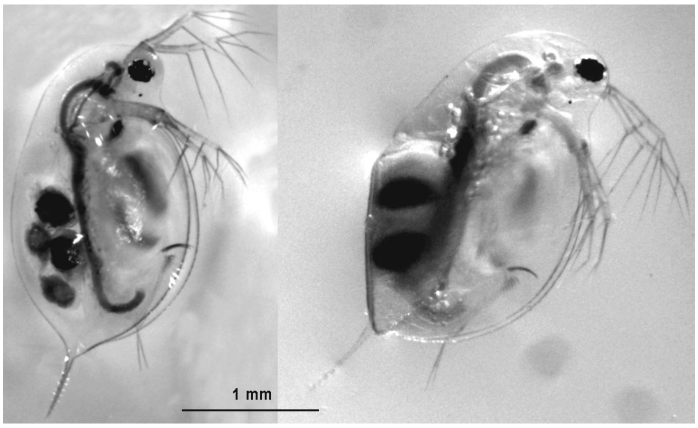


Figura 2 • Fotos de *Daphnia longispina* (O.F. Müller, 1776). Estes crustáceos pertencem a um grupo de organismos (cladóceros[1]) que, tipicamente, possui uma carapaça cuticular bivalve que envolve o corpo mas não a cabeça e que utiliza o seu 2º par de antenas como principal órgão de locomoção. À esquerda, fêmea carregando embriões partenogenéticos (assexuados) na câmara de incubação; à direita, fêmea carregando ovos de resistência, resultantes de reprodução sexuada, envoltos em cápsula protectora (*ephippium*).

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[1] O termo Cladocera tem sido historicamente utilizado para designar um grupo de crustáceos dentro da classe Branchiopoda (os organismos desta classe caracterizam-se por possuírem apêndices torácicos em forma de folha, que constituem a sua principal superfície respiratória). A revisão feita à classificação dos Branchiopoda (Fryer, 1987) rompeu com o termo e substituiu a ordem Cladocera por 4 novas ordens (Haplopoda, Ctenopoda, Anomopoda, Onychopoda), por considerar os cladóceros um grupo polifilético. O género *Daphnia* foi incluído nos Anomopoda. Aqui, mantemos a designação Cladocera pela forte implementação do uso do termo (Korovchinsky, 2006) e, sobretudo, porque têm sido trazidas à luz evidências de que os cladóceros, afinal, constituem um grupo monofilético, apesar de ser dúbia a posição dos Haplopoda (Schwenk et al., 1998; Negrea et al., 1999; Korovchinsky, 2006). Actualmente, algumas fontes colocam o género *Daphnia* na ordem Anomopoda, integrada na superordem Cladocera.

Os mecanismos de defesa anti-predatórios de *Daphnia* são numerosos e incluem alterações comportamentais, morfológicas e populacionais (Lass & Spaak, 2003). As defesas comportamentais são induzidas numa escala temporal muito curta e envolvem a alteração de comportamentos de evitamento, fototaxia ou agregação (Lass & Spaak, 2003), que constituem a base de comportamentos mais complexos como, por exemplo, a migração vertical diária (Lampert, 1989; Loose & Dawidowicz, 1994; Ringelberg, 1995; Han & Straskraba, 2001; Ringelberg & Van Gool, 2003). O padrão mais habitual deste fenómeno consiste na permanência de uma população de zooplanctontes em águas profundas (refúgio hipolimnético) durante o dia, na ascensão para águas superficiais durante o crepúsculo, na permanência nessas águas ricas em alimento durante a noite, e na descida para águas frias e escuras ao amanhecer. Este comportamento tem como objectivo último evitar os predadores visuais durante o dia e resulta da percepção de alterações diárias no gradiente de luz em profundidade (Ringelberg & Van Gool, 2003). Este e outros comportamentos têm reflexos na distribuição dos zooplanctontes num lago (Pinel-Alloul, 1995; Folt & Burns, 1999; Hembre & Megard, 2003), podendo originar estruturação espacial e fenómenos de selecção de *habitat*. Em *Daphnia*, as alterações morfológicas a predadores invertebrados (selectivos para indivíduos menores) envolvem um aumento do volume corporal (cristas, elmos, espinhos), ao passo que o contrário é norma para os peixes planctívoros (selectivos para indivíduos maiores), o que constata o valor destas defesas enquanto adaptações a diferentes regimes predatórios (Stabell et al., 2003; Lass & Spaak, 2003). Apesar do prolongamento do espinho caudal de *Daphnia* também ser frequentemente registado na presença de peixes (Spaak & Boersma, 1997; Caramujo et al., 1997; Stabell et al., 2003), a defesa mais comum é a redução do tamanho corporal (Engelmayer, 1995; De Meester & Weider, 1999; Sakwinska, 2000; Weber, 2003; Sakwinska & Dawidowicz, 2005). A este mecanismo de defesa morfológica, é comum estarem associadas alterações populacionais que visam o incremento da fecundidade (maturação precoce, produção de mais descendência), para fazer face às perdas causadas por predadores.

Todas estas respostas a predadores são mediadas por sinais químicos exsudados pelos peixes planctívoros (Burks & Lodge, 2002; Lass & Spaak, 2003) e todas têm custos para o indivíduo, à escala metabólica, reprodutiva ou outra (Dawidowicz & Loose, 1992; Loose & Dawidowicz, 1994; Spaak & Boersma, 1997; Caramujo & Boavida, 2000a). Não

sendo mutuamente exclusivas, qualquer combinação de respostas é possível mediante um compromisso entre a estratégia defensiva menos onerosa e a mais eficaz, consoante a situação (Boersma et al., 1998; De Meester & Weider, 1999; Sakwinska & Dawidowicz, 2005). A origem, natureza e percepção do estímulo químico que estabelece a comunicação entre predador e presa (Burks & Lodge, 2002; Lass & Spaak, 2003) são campos de investigação com respostas ainda pouco concretas.

Lagos pouco profundos vs. lagos profundos

Tradicionalmente, o estudo da Limnologia nasceu e floresceu nos lagos profundos. Segundo Margalef (1983), um «verdadeiro lago» é aquele cujo fundo não recebe luz suficiente para ficar, todo ele, coberto de macrófitas e no qual acaba por se formar, durante alguma fase do seu ciclo anual, uma termoclina e consecutivo isolamento relativo das camadas mais profundas da coluna de água. Todavia, os lagos que podem albergar vegetação aquática em quase toda a sua extensão e cuja coluna de água sofre mistura constante (lagos polimícticos) constituem a maioria (em número) dos lagos na Biosfera (Wetzel, 1983). Só recentemente (últimas décadas) estes lagos pouco profundos foram alvo de mais atenção na literatura especializada. Segundo Scheffer (1998), os lagos pouco profundos distinguem-se do objecto tradicional da Limnologia pela maior interacção entre o sedimento e a coluna de água (originando uma contínua libertação de fósforo) e pela potencialmente maior influência da vegetação aquática no funcionamento do sistema (ver Tabela 1). As suas características distintas têm implicações nos mecanismos de regulação trófica abordados até aqui.

Em condições de elevada transparência, a reduzida profundidade dos lagos pouco profundos permite a penetração de luz até ao fundo do lago, permitindo o desenvolvimento de macrófitas submersas. As macrófitas e as epífitas associadas competem com o fitoplâncton na obtenção de nutrientes e produzindo substâncias alelopáticas (Scheffer et al., 1993; Beklioglu & Moss, 1996; Scheffer & Jeppesen, 1998; Burks & Lodge, 2002). As macrófitas estabilizam o sedimento, reduzindo a sua ressuspensão, sustentam uma miríade de invertebrados filtradores (Scheffer et al., 1993; Beklioglu & Moss, 1996; Scheffer & Jeppesen, 1998; Jeppesen et al., 2002) e proporcionam refúgio ao zooplâncton herbívoro (Jeppesen et al., 1998; Perrow et al., 1999; Burks et al., 2002). Num lago dominado por macrófitas submersas, estes mecanismos reforçam ainda mais o controlo da biomassa fitoplanctónica e estado de elevada transparência do lago, mesmo em regimes de elevada entrada de nutrientes. Em oposição, níveis muito elevados de nutrientes fomentam o crescimento algal, promovendo o incremento da turbidez e reduzindo a penetração da energia luminosa na coluna de água. Nestas condições, as macrófitas não se conseguem desenvolver e o lago exibe uma condição turva.

Tabela 1 • Quadro resumo das principais características distintivas entre os lagos profundos e os lagos pouco profundos (baseado em Jeppesen et al., 1997; 1998; Scheffer, 1998).

Lagos profundos	Lagos pouco profundos
• as flutuações do nível da água têm um impacto desprezível nas comunidades biológicas e no funcionamento do sistema	• as flutuações no nível da água podem ter um impacto drástico nas comunidades biológicas e no funcionamento do sistema
• a interacção da água com o sedimento é pouco importante devido à profundidade e à estratificação	• a intensa mistura da coluna de água resulta numa contínua ressuspensão de partículas finas e de fósforo do sedimento
• o impacto da vegetação aquática é pouco significativo, na medida em que se restringe a zonas marginais	• o impacto da vegetação é potencialmente muito grande, mediando a alternância de estados de transparência e produtividade
• a elevada profundidade e a estratificação térmica providenciam um refúgio anti-predador para o zooplâncton herbívoro	• o zooplâncton tem de fazer face à predação intensa na ausência do refúgio hipolimnético, dada a reduzida profundidade
• regra geral, os nutrientes são limitativos ao crescimento fitoplanctónico, originando a predominância do controlo *bottom-up*	• os mecanismos de controlo *bottom-up* estão enfraquecidos por causa dos níveis contínuos e elevados de nutrientes
• os peixes desempenham um papel secundário (controlo *top-down*) sobre a teia trófica pelágica e estrutura das comunidades	• o controlo *top-down* é substancial devido à elevada biomassa (por unidade de volume) de peixes benti-planctívoros

Estes dois estados alternativos (Scheffer et al., 1993; Scheffer & Jeppesen, 1998; Dokulil & Teubner, 2003; Schroder et al., 2005), um dominado por macrófitas submersas (água transparente) e outro dominado pelo fitoplâncton (água turva), são auto-reforçados pelos mecanismos de regulação mediados pela presença ou ausência das macrófitas aquáticas. Estes mecanismos fazem com que a resistência à mudança de estado seja elevada, um fenómeno denominado de histerese (Scheffer et al., 1993; Scheffer & Jeppesen, 1998; Schroder et al., 2005). Como consequência, não é linear que um incremento ou diminuição na carga de nutrientes tenha como consequência a passagem de um estado para o outro (Scheffer et al., 1993), devido aos vários factores de resistência (não só relacionados com as macrófitas – ver abaixo e Tabela 1). Em particular, o retorno de um lago no estado turvo (dominância do fitoplâncton) para um estado de maior transparência (dominância de macrófitas submersas) é particularmente difícil (Scheffer et al., 1993; Scheffer, 1998), apesar de uma fase transitória de água transparente poder ocorrer em lagos turvos, na Primavera (conforme previsto no modelo PEG, ver acima). O regresso a um estado de dominância de macrófitas submersas (água transparente) é o objectivo último da biomanipulação (ver acima) dos lagos pouco profundos (Moss, 1992; Scheffer et al., 1993; Perrow et al., 1997; Dokulil & Teubner, 2003).

Para além das macrófitas, também os peixes podem desempenhar um papel central na alternância entre estados e nos mecanismos de histerese. Directamente, os peixes contribuem para o aumento de nutrientes na coluna de água através da sua reciclagem (excreção de metabolitos) e promovem a turbidez ao facilitarem a ressuspensão do sedimento (Scheffer et al., 1993; 2003). Adicionalmente, os peixes planctívoros promovem o crescimento descontrolado do fitoplâncton ao exercerem forte pressão predatória sobre o zooplâncton herbívoro de grandes dimensões (sobretudo *Daphnia*, conforme explicado acima). Este facto é agravado pela importância do controlo *top-down* pelos peixes (especialmente as formas juvenis) ser consideravelmente mais elevada em lagos pouco profundos, por vários motivos (ausência de refúgio hipolimnético do zooplâncton, maior biomassa de peixes por unidade de volume, entre outros), conforme postulado por Jeppesen et al. (1997). Por outro lado, o efeito estimulante indirecto dos peixes planctívoros sobre a produção fitoplanctónica é mais significativo a altos níveis de nutrientes, quando comparado com os efeitos observados a reduzidos níveis de fósforo (Jeppesen et al., 2003). Deste modo, é possível identificar uma cascata trófica nos lagos pouco profundos e eutróficos que fomenta a ocorrência e permanência (histerese) de um estado de dominância algal (elevada turbidez). Este fenómeno é comprovado pelas experiências de Tessier & Woodruff (2002a; 2002b) que mostraram que os recursos algais são mais ricos nos lagos pouco profundos (com pressão predatória mais elevada sobre o zooplâncton) do que nos lagos profundos, demonstrando que o zooplâncton é incapaz de controlar o crescimento do fitoplâncton nos lagos pouco profundos.

A compreensão da interacção peixes-zooplâncton é vital para a biomanipulação e nos lagos pouco profundos este elo ganha uma nova dimensão com a presença de macrófitas (mediadoras da alternância de estados). Esta área tem sido motivo de alguma investigação (Schriver et al., 1995; Beklioglu & Moss, 1996; Jeppesen et al., 1998; Perrow et al., 1999; Burks et al., 2002), já que essa informação é necessária para que os planos de recuperação dos lagos pouco profundos sejam bem sucedidos. Contudo, é ainda contraditória a informação disponível sobre a forma como as macrófitas influenciam a interacção entre peixes e zooplâncton. Um bom exemplo é o refúgio contra predadores (peixes) supostamente proporcionado pelas macrófitas ao zooplâncton. Vários trabalhos relataram evidências de que o zooplâncton pode formar agregações em zonas de macrófitas durante o dia (Kvam & Kleiven, 1995; Lauridsen & Buenk, 1996; Masson et al., 2001; Wojtal et al., 2003), como alternativa face à ausência do refúgio hipolimnético dos lagos profundos. Durante a noite, o zooplâncton tende a dispersar-se pela zona pelágica adjacente – migração horizontal diária (ver revisão por Burks et al., 2002). Este fenómeno tem sido registado em lagos pouco profundos do Norte da Europa de forma pouco consistente por vários autores, que defendem que o refúgio proporcionado pelas macrófitas parece depender do estado trófico (Lauridsen et al., 1999), mas também de um equilíbrio entre a pressão predatória e a densidade das macrófitas (Schriver et al., 1995; Lauridsen et al., 1996; Jeppesen et al., 1998; Perrow et al., 1999; Burks et al., 2002). Todavia, muitas espécies de peixes juvenis também recorrem às macrófitas como refúgio contra predadores de maiores dimensões (Burks et al., 2002; Romare et al., 2003; Romare & Hansson, 2003), podendo provocar perdas severas nas populações zooplanctónicas nessas áreas (Perrow et al., 1999; Hülsmann et al., 1999; Masson et al., 2001), reduzindo a eficiência do refúgio proporcionado ao zooplâncton.

Os lagos pouco profundos no contexto Mediterrânico

A vasta maioria dos estudos em lagos pouco profundos foi realizada em países temperados do norte da Europa e nos Estados Unidos. Em particular na Europa, a Dinamarca e a Bélgica/Holanda lideraram durante muito tempo a investigação no campo da ecologia dos lagos pouco profundos, acompanhados de perto pela Grã-Bretanha e pelos países escandinavos (Suécia/Finlândia). Tal facto deve-se à abundância e importância destas zonas húmidas nesses países, por oposição aos países mais montanhosos do centro da Europa, onde os lagos profundos (normalmente de origem glaciar) adquiriram maior destaque. No sul da Europa, devido à influência do quente clima Mediterrânico, os lagos naturais rareiam e os poucos lagos pouco profundos que existem estão sob a frequente ameaça da seca. Talvez por isso, e não obstante a importância das zonas húmidas enquanto motores de biodiversidade, a pesquisa científica nos lagos pouco profundos Mediterrânicos tenha adquirido pouco destaque. Contudo, existem evidências de desvios à norma aos mecanismos de regulação trófica abordados anteriormente para lagos temperados profundos, ainda mais evidentes do que é possível observar em outros lagos pouco profundos. Por exemplo, tem sido registado em lagos pouco profundos mais quentes (sobretudo tropicais e subtropicais) que a biomassa fitoplanctónica não é controlada pelo zooplâncton herbívoro (Havens et al., 1996) e que a recuperação deste tipo de lagos é mais difícil (Jeppesen et al., 2005). Consequentemente, há necessidade de informação mais específica sobre os mecanismos de regulação trófica destes sistemas.

O mecanismo de resistência biológica mais apontado à recuperação dos lagos eutróficos de zonas mais quentes é a sua distinta ictiocenose, dominada por espécies com reduzido tamanho corporal e de hábitos omnívoros (Blanco et al., 2003; Jeppesen et al., 2005). Daqui resulta uma ictiocenose caracterizada por uma elevada densidade de pequenas espécies altamente fecundas (como resultado do aproveitamento de vários recursos alimentares), exercendo uma pressão predatória sobre o zooplâncton mais elevada e mais prolongada no tempo do que sucede em lagos temperados (Blanco et al., 2003; Jeppesen et al., 2005; Gyllstrom et al., 2005). Como agravante, Burks et al. (2002) consideram que esta predominância de espécies benti-planctívoras de pequenas dimensões deverá reduzir drasticamente a eficiência do refúgio proporcionado pelas macrófitas (ver também Meerhoff et al., 2006), dado os hábitos litorais destes pequenos peixes. Outra característica distintiva das ictiocenoses tropicais é a reduzida expressão de grandes piscívoros, o que diminui substancialmente o controlo exercido por estes sobre os peixes benti-planctívoros quando comparado com os lagos temperados (Jeppesen et al., 2005). Esta situação é também partilhada pela ictiofauna Ibérica (Almaça, 1995; Blanco et al., 2003), o que posiciona as teias tróficas dos lagos Ibéricos e Mediterrânicos num contexto ecológico próprio dentro da Europa. Mais, o estudo de Blanco et al. (2003) sugere que a introdução de espécies alienígenas (ou exóticas) como a gambúzia (*Gambusia holbrooki*) e a perca-sol (*Lepomis gibbosus*) tem agravado o grau de omnivoria da ictiocenose dos lagos Ibéricos e a aproximado das características das ictiocenoses tropicais.

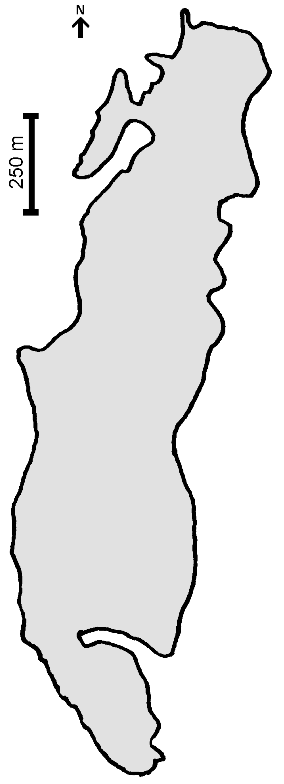
A posição especial dos países Mediterrânicos no contexto dos lagos pouco profundos, e em particular da Península Ibérica, tem sido reconhecida em vários trabalhos recentes. Estudos comparativos realizados em larga escala (Nöges et al., 2003; Gyllstrom et al., 2005; Declerck et al., 2005) incluíram diversos locais de amostragem em Espanha, ao passo que uma recente experiência pan-europeia (e.g. Stephen et al., 2004; Moss et al., 2004) teve em consideração a latitude ao avaliar os efeitos do incremento da carga de nutrientes e de peixes em lagos pouco profundos (simulações experimentais em mesocosmos). Os resultados desta experiência em larga escala vieram confirmar que os lagos pouco profundos da zona Mediterrânica são mais sensíveis ao incremento da carga de nutrientes do que os lagos temperados (ver também Romo et al., 2004). Paralelamente, num interessante caso de estudo, Romo et al. (2005) demonstraram também que, mesmo após a diminuição da carga de nutrientes, não foi possível fazer regressar o Lago Albufera (Valência) ao estado anterior, nem tampouco a um estado de dominância de macrófitas capaz de estabilizar a transparência da água.

Em suma, os lagos pouco profundos do sul da Europa (sob a influência do clima Mediterrânico) parecem estar sob efeitos *top-down* mais acentuados do que os lagos pouco profundos mais a norte, aumentando a sua vulnerabilidade à eutrofização e a sua resistência à mudança para um estado de água transparente. Tal como os outros lagos pouco profundos, também os lagos Mediterrânicos desempenham um papel ecológico e socio-económico (em alguns casos) importante, particularmente se olhados como componente de um sistema terrestre mais vasto enquanto zona húmida (Scheffer, 1998). Estes *habitats* suportam uma considerável biodiversidade, mas o seu valor encontra-se habitualmente comprometido pelo enriquecimento em nutrientes (agravado por actividades humanas). Do ponto de vista científico, levanta-se o desafio de melhor compreender os mecanismos de interacção trófica subjacentes a esta maior vulnerabilidade à eutrofização dos lagos pouco profundos Mediterrânicos. Numa perspectiva mais aplicada, o uso desta informação permitirá decisões de gestão melhor enquadradas e com maiores probabilidades de sucesso a longo prazo.

Um bom exemplo da problemática dos lagos pouco profundos da região Mediterrânica é a Lagoa da Vela, local escolhido como objecto de estudo do presente trabalho. Esta pequena massa de água permanente (ver Caixa B), outrora transparente e com grande variedade de peixes (segundo os habitantes locais), encontra-se actualmente sob forte carga de nutrientes, elevada turbidez e reduzida cobertura de macrófitas (Fernandes, 1999; Antunes et al., 2003; Abrantes et al., 2006a; 2006b). Este estado de água turva avançado é ainda agravado pela presença recorrente de cianobactérias, muitas delas altamente tóxicas (Vasconcelos, 1990; Barros, 1994; Figueiredo et al., 2006; Abrantes et al., 2006a). Apesar da falta de dados históricos que pudessem funcionar como base de partida para um plano de recuperação adequado, foram já produzidas duas teses (Pereira, 1997; Fernandes, 1999) com recomendações e ferramentas para a gestão das Lagoas de Quiaios, mormente a Lagoa da Vela. O trabalho de Fernandes (1999) é particularmente pormenorizado na caracterização do sistema (ver Caixa B) e propõe um modelo para a análise e gestão do problema da eutrofização. Apesar deste estudo referir a biomanipulação como medida adicional no combate à eutrofização, nenhum estudo até à data analisou de forma integrada as potenciais cascatas tróficas resultantes da predação pelos peixes na Lagoa. Mais, os dados sobre a ictiofauna da Lagoa da Vela são muito limitados (o único estudo é o de Ferreira, 1997), apesar das evidências de um forte controlo *top-down* sobre o zooplâncton (elemento chave no controlo da produção fitoplanctónica, ver acima). De facto, o papel estruturante da ictiocenose sobre a comunidade planctónica da Lagoa ficou bem patente em 2001, quando se verificou uma fase de dominância de *Daphnia* no Verão após uma mortandade massiva de peixes no final de Maio, originando um controlo efectivo do crescimento fitoplanctónico (Antunes et al., 2003; Abrantes et al., 2006a).

A ictiocenose da Lagoa da Vela possui há largas décadas uma componente forte de espécies alienígenas, cuja introdução foi promovida por motivos piscatórios (sobretudo a carpa, *Cyprinus carpio*, e o achigã, *Micropterus salmoides*) ou como ferramentas de controlo do mosquito da malária (gambúzia ou gambuzino, *Gambusia holbrooki*). Estas espécies invasoras rapidamente formaram populações estáveis, promovendo a eliminação de algumas espécies indígenas. O estudo de Ferreira (1997) revelara já uma ictiocenose pouco diversificada, mas desde então a comunidade íctica tornou-se mais homogénea com a rápida proliferação da perca-sol, *Lepomis gibbosus*, ausente à data deste estudo pioneiro. Daqui resultou uma comunidade quase totalmente alienígena, dominada por espécies omnívoras de pequenas dimensões (sobretudo gambúzia e perca-sol), cujos efeitos nos níveis tróficos inferiores não são ainda totalmente compreendidos. Apesar da Lagoa da Vela constituir um exemplo extremo ao nível da ictiocenose, a alteração das comunidades piscícolas (por homogeneização biótica) e consequente modificação das interacções tróficas é um problema comum a outros sistemas lênticos (e.g. Rodríguez, 1989; Garcia-Berthou & Moreno-Amich, 2000; Blanco et al., 2003) e lóticos (e.g. Godinho & Ferreira, 1998; 2000; Vila-Gispert et al., 2005) Ibéricos.

CAIXA B – Caracterização do local de estudo: Lagoa da Vela



Lagoa da Vela

A Lagoa da Vela é um pequeno lago natural e permanente, resultante da acumulação de água numa depressão superficial, localizada na interface entre o cordão dunar de Quiaios (a oeste) e a planície da Gândara (a leste). Esta zona de interface compreende ainda outros três pequenos lagos de água doce mais ou menos permanentes (Fernandes, 1999). As dunas de Quiaios são parte de um extenso cordão dunar que se estende desde o Cabo Modego (Figueira da Foz) até ao sul da Ria de Aveiro (Mira e Vagos) e que alberga vegetação essencialmente constituída por matos e por pinhal (*Pinus* spp.), este último plantado com o objectivo de impedir o avanço das areias para o interior (Pereira, 1997). Esta zona da costa Atlântica Portuguesa, recentemente incorporada na lista nacional de sítios da Rede Natura 2000* (PTCON0055 - sítio das Dunas de Mira, Gândara e Gafanhas), alberga *habitats* diversificados e vasta biodiversidade (ver caracterização ecológica em Pereira, 1997; Fernandes, 1999). Englobada nesta área, a Lagoa da Vela proporciona recursos (alimento) e condições (locais de nidificação e desova) para numerosas espécies aquáticas e terrestres (nomeadamente aves), contribuindo de forma significativa para a biodiversidade da área.

A Lagoa da Vela é a maior das lagoas de Quiaios, ocupando uma área máxima inundável de 70 ha (1 ha = 0,01 km^2). Enquanto lago natural, está em continuidade com o aquífero freático local, cuja recarga depende exclusivamente da precipitação atmosférica. Um sistema de valas e canais interliga esta massa de água com as outras lagoas da zona, sendo que, nos períodos mais húmidos, a drenagem da lagoa é feita para o Rio Mondego, via vala do Zurrão, no extremo sul da lagoa (ver caracterização hidrológica em Fernandes, 1999).

Toda a bacia drenante da Lagoa da Vela tem uso rural do solo, distribuída em ocupação florestal-natural na margem oeste e ocupação agrícola e pecuária (incluindo habitações) na margem este. As actividades humanas na área adjacente à lagoa geram fontes difusas de poluentes. Como consequência, a lagoa tem mantido uma carga de nutrientes muito grande nas últimas décadas, típica de lagos eutróficos e proveniente sobretudo do escoamento superficial dos terrenos agrícolas adjacentes (no caso do P e N) e por deposição atmosférica (no caso do N) (ver caracterização das fontes poluentes em Fernandes, 1999; Abrantes et al., 2006b).

Características morfométricas da Lagoa da Vela (adaptado de Fernandes, 1999):

Largura máxima	500 m
Comprimento máximo	2000 m
Linha de costa – perímetro	5000 m
Profundidade máxima	2,4 m
Profundidade média	0,9 m
Área máxima inundável	70 ha
Volume máximo armazenável	700 000 m^3

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* A Rede Natura 2000 é uma rede ecológica que resulta da política de Conservação da Natureza da União Europeia (UE) e da aplicação das Directivas nº 79/409/CEE (Directiva Aves) e nº 92/45/CEE (Directiva *Habitats*). O objectivo desta rede é o de contribuir para assegurar a biodiversidade através da conservação dos *habitats* naturais e da fauna e flora selvagens no território europeu dos Estados-membros da UE.

OBJECTIVOS E ESTRUTURA DA DISSERTAÇÃO

Dada a natureza da estrutura trófica dos lagos pouco profundos Mediterrânicos e a sua vulnerabilidade à eutrofização, este trabalho pretendeu estudar as interacções tróficas na Lagoa da Vela e os reflexos dessa interacção na sua ecologia. Mais concretamente, o presente estudo pretendeu explorar e melhor compreender a influência da comunidade piscícola da Lagoa, dominada por espécies alienígenas de hábitos omnívoros, sobre os níveis tróficos inferiores, com particular ênfase no zooplâncton, por representar um nível trófico de charneira. Os objectivos específicos foram:

- compreender a dinâmica sazonal do zooplâncton e os mecanismos reguladores dessa dinâmica (Capítulo 1);
- caracterizar o actual estado da ictiocenose e os seus padrões de recrutamento, bem como a ecologia alimentar das principais espécies de peixes (Capítulo 2);
- analisar em detalhe a planctivoria das principais espécies de peixes e compreender o seu papel nas interacções tróficas entre os peixes planctívoros e o zooplâncton (Capítulo 3);
- avaliar o papel da transparência da água e da pressão predatória na distribuição espacial (vertical e horizontal) do zooplâncton (Capítulo 4);
- identificar alguns mecanismos populacionais utilizados pelo zooplâncton para minimizar o impacto da ictiocenose alienígena (Capítulo 5).

Como está patente na descrição dos objectivos, a presente dissertação assenta numa estrutura dividida em capítulos, que representam unidades com objectivos próprios bem definidos, integrados no âmbito global do trabalho de investigação. Cada capítulo é autónomo, possuindo as suas próprias secções de Introdução, Material e Métodos, Resultados, Discussão e Referências. Esta é uma opção clara de apresentar a tese num formato mais próximo da publicação, facilitando a compreensão e interpretação do leitor. Aliás, cada um dos capítulos corresponde a um artigo submetido ou aceite para publicação, resultante do trabalho experimental conducente à elaboração desta tese. Por este motivo, o corpo da tese (Capítulos 1-5) está redigido na língua inglesa. Os **Capítulos 1** (*Ecologia e dinâmica sazonal do zooplâncton*) e **4** (*Selecção de habitat em crustáceos zooplanctónicos*) dão a conhecer a dinâmica do zooplâncton em termos espaciais e temporais, avaliando a influência da predação como mecanismo regulador dessa dinâmica.

A informação relativa à ecologia da comunidade de peixes da Lagoa da Vela é apresentada nos **CAPÍTULOS 2** (*Recrutamento e ecologia alimentar da ictiocenose*) e **3** (*Dinâmica sazonal da planctivoria na ictiocenose*). Por fim, o **CAPÍTULO 5** (*Respostas populacionais de Daphnia a predadores*) tenta compreender (em ambiente laboratorial) como as duas principais espécies de peixes planctívoros moldam as respostas populacionais de *Daphnia*. O corpo da tese é precedido pela presente **INTRODUÇÃO GERAL**, onde são definidos os objectivos (gerais e específicos) e o âmbito do presente trabalho, situando-o à luz dos conhecimentos actuais na área científica em que se enquadra. A última secção da tese (**CONSIDERAÇÕES FINAIS**) tenta resumir, de uma forma global e integrada, a relevância do novo conhecimento gerado.

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Capítulo 1

Ecologia e dinâmica sazonal do zooplâncton

Dinâmica sazonal dos crustáceos zooplanctónicos de um lago pouco profundo e eutrófico da zona Mediterrânica

In: Castro B.B. & Gonçalves F. (submetido). Seasonal dynamics of the crustacean zooplankton of a shallow eutrophic lake from the Mediterranean region.

RESUMO

O conhecimento acerca das interacções tróficas em lagos pouco profundos da região Mediterrânica é muito limitado, apesar destes apresentarem algumas características distintivas quando comparados com lagos temperados do Norte da Europa (sobretudo ao nível da ictiocenose). Por forma a abordar esta carência de informação, realizámos um estudo sobre a dinâmica sazonal do zooplâncton na Lagoa da Vela, um lago que exibe dominância marcada de peixes exóticos. Este lago pouco profundo e eutrófico revelou ser altamente produtivo e turvo, excepto durante um período de maior transparência de Janeiro a Abril. O aumento de transparência deveu-se ao incremento do nível da água, o que acarretou uma diminuição da ressuspensão de partículas. A transparência foi adicionalmente melhorada por um pico na densidade de *Daphnia longispina* (em Fevereiro e Março), que originou uma redução temporária da clorofila *a* (fase de água transparente). No resto do ano, a comunidade zooplanctónica foi dominada por zooplanctontes de pequenas dimensões (sobretudo *Bosmina* e copépodes ciclopóides). De Agosto a Dezembro, praticamente não foi registada a ocorrência de *Daphnia*. Com o recurso a análises de regressão e multivariável foi possível identificar o *seston* e a predação como os principais gradientes ambientais que modelam a comunidade zooplanctónica. Durante a fase de água transparente, a competição pelos recursos também pareceu contribuir para a dinâmica de *Daphnia*. O padrão sazonal observado é concordante com o modelo PEG, apesar de serem perceptíveis pequenas diferenças no momento de ocorrência do pico de *Daphnia* e na influência mais prolongada dos peixes sobre o zooplâncton, comparativamente com os lagos mais a norte. Os nossos resultados sugerem que a implementação futura de programas de recuperação da Lagoa da Vela terá que contabilizar a influência quer da planctivoria quer das partículas em suspensão (seston) sobre o zooplâncton herbívoro.

PALAVRAS-CHAVE

zooplankton dulçaquícola, dinâmica sazonal, modelo PEG, lagos Mediterrânicos, *Daphnia*

O presente capítulo constitui um manuscrito submetido para publicação em revista internacional com arbitragem científica e é, por esse motivo, apresentado na língua inglesa.

Seasonal dynamics of the crustacean zooplankton of a shallow eutrophic lake from the Mediterranean region

ABSTRACT

Little is known on the trophic interactions of shallow lakes from the Mediterranean region, although there is evidence of their distinctive features compared to northern temperate shallow lakes (particularly when considering their ichthyocenosis). To address this gap, we conducted a study on the seasonal dynamics of the zooplankton community in Lake Vela (Portugal), a turbid and eutrophic shallow lake, which exhibits marked dominance of alien fish species. Lake Vela was shown to be highly productive and turbid, except during a period of higher transparency, from January to April. The increase in transparency was attributable to a rise in water level, which reduced the amount of resuspended particles. Transparency was additionally improved by a peak in *Daphnia longispina* densities (February and March), which transiently reduced chl *a* to low levels (clear water phase). In the remainder of the year, the zooplanktonic assemblage was dominated by small-sized zooplankters (mainly *Bosmina* and cyclopoid copepods). *Daphnia* were virtually absent from August to December. Regression and multivariate analyses identified seston and fish predation as the main environmental gradients shaping the zooplanktonic assemblage. During the clear water phase, competition for resources also seemed to contribute to *Daphnia* dynamics. The observed pattern of seasonal dynamics of the zooplankton of Lake Vela was overall concordant with the PEG model, although different timings were perceptible, namely concerning the *Daphnia* peak and the more prolonged influence of fish, relatively to northern temperate lakes. Our results suggest that both planktivory (by alien fish) and turbidity (seston) are issues that need to be addressed if adequate restoration programs are to be implemented.

KEYWORDS

freshwater zooplankton, seasonal dynamics, PEG model, Mediterranean lakes, *Daphnia*

INTRODUCTION

According to Scheffer (1998), shallow lakes possess distinctive features from deep thermally-stratified lakes, as the result of a more intense sediment-water interaction (due to polymixis) and a larger impact of aquatic vegetation. Studies on shallow lakes are common in northern temperate regions, but few works have focused on warmer areas, such as Mediterranean countries. A recent pan-European project (Romo et al., 2004; Moss et al., 2004) has provided evidence that lakes from warmer regions of the temperate zone are more vulnerable to nutrient loading (i.e. eutrophication). Furthermore, some resemblances in the features of the fish community between tropical lakes and some Iberian lakes have been recognised (Blanco et al., 2003), due to the predominance of small omnivorous species and low abundance of piscivores. These latter features are particularly aggravated by the vulnerability of Iberian fish faunas to invaders, such as pumpkinseed sunfish (*Lepomis gibbosus*) or mosquitofish (*Gambusia holbrooki*). The implications of such distinct features on trophic cascades and alternating stable states in shallow lakes have still been largely unexplored.

Although rare in Iberia (because of the dry climate), shallow lakes constitute important pools of biodiversity and provide unique ecological niches. However, these habitats usually face the threat of eutrophication, fish fauna homogenisation and man-induced change. Lake Vela (Portugal) represents an interesting case study in Iberia, as its native cyprinid assemblage has been completely replaced by alien fish, over the course of the last century. However, data on the dynamics of its biota and food web interactions are very limited (see e.g. Fernandes, 1999; Antunes et al., 2003; Abrantes et al., 2006a), which is particularly serious because this eutrophic and turbid lake requires urgent restoration. Restoration measures may include food web manipulation, which depends on the key role of grazers (mainly *Daphnia*) as controllers of phytoplankton biomass and water transparency (Carpenter et al., 1985). However, this control is only effective once zooplankton is released from fish predation, as shown in several biomanipulation experiments (e.g. Christofferson et al., 1993; Carpenter et al., 2001) and as predicted by the size-efficiency hypothesis (Brooks & Dodson, 1965). In Lake Vela, the structuring role of fish on the zooplankton community was evident in 2001, when a massive fish kill preceded a large *Daphnia* peak and low phytoplanktonic production during that summer (Antunes et al., 2003; Abrantes et al., 2006a).

In this study, we intend to provide additional information on trophic interactions and regulation mechanisms of the zooplankton assemblage of Lake Vela. To do so, we conducted an annual survey on its limnology and zooplankton assemblage, and we collected seasonal data on its fish assemblage (see also Castro et al., submitted manuscript[1]). Our main goals were: i) to identify the seasonal patterns of the crustacean zooplankton and their underlying regulation mechanisms, with emphasis on *Daphnia* dynamics; ii) to understand the role of grazing (chiefly by *Daphnia*) in the formation of a clear water phase; iii) to assess the cascading effects of predation (i.e. top-down forces)

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[1] CAPÍTULO 2 da tese

from alien omnivorous fish on the zooplankton dynamics and on key water parameters (transparency and chlorophyll *a*); iv) to compare the zooplankton dynamics of Lake Vela with studies conducted at different latitudes.

MATERIALS AND METHODS

Study site

Lake Vela is located in the west coast of central Portugal (Figure 1.1), lying in a transition between Mediterranean (southwards) and Atlantic (northwards) bioclimates. Lake Vela is a small (maximum floodable area 70 ha), shallow (0.9 m average depth; 2.4 m maximum depth) and polymictic water body, being the largest lake of a system of small lakes located within a coastal pine wood forest.

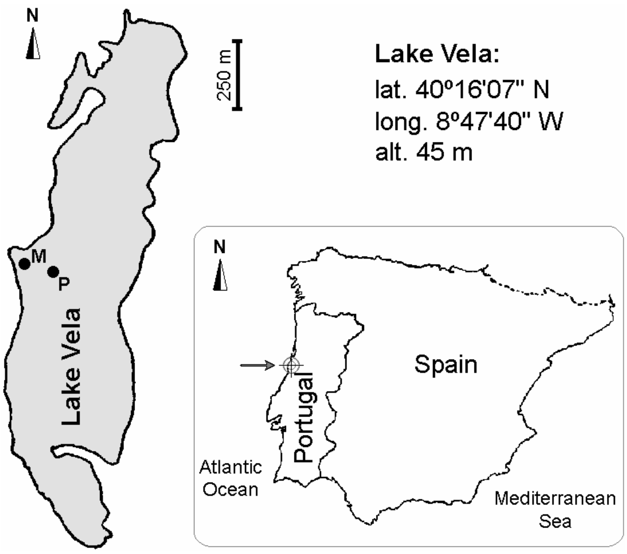

Figure 1.1 • Location and outline of Lake Vela (Figueira da Foz, Portugal). M (macrophyte) and P (pelagial) represent sampling stations (sites).

High nutrient levels are recorded all year-round, mostly derived from agriculture and livestock activities (diffuse pollution) (Fernandes, 1999; Abrantes et al., 2006b). The main planktivorous fish in Lake Vela are pumpkinseed, mosquitofish and age-0 largemouth bass (*Micropterus salmoides*). They exert substantial predatory pressure on zooplankton, particularly during the summer due to very high densities of juvenile pumpkinseed (see Results). More detailed data on the recruitment patterns and feeding ecology of fish taxa is presented elsewhere (Castro et al., submitted manuscript). Predatory pressure from pelagic invertebrates (such as *Chaoborus*) is negligible, as these predators are seldom found in Lake Vela. Cyanobacterial blooms are frequent and occasional episodes of oxygen shortage and fish kills have occurred in recent years (Antunes et al., 2003; Abrantes et al., 2006a). The water is usually very turbid due to high primary productivity, suspended particles and dissolved organic substances. Submerged aquatic vegetation is scarce in the lakebed and the main macrophyte species is the alien water-lily (*Nymphaea alba*), which is restricted to patches of variable size. More details on Lake Vela can be found in Fernandes (1999), Antunes et al. (2003) and Abrantes et al. (2006a; 2006b).

Sampling strategy

Sampling was carried out fortnightly using a small boat, at two adjacent points in the lake: a pelagial zone (macrophyte-free) and a macrophyte (*Nymphaea alba*) patch (P and M in Figure 1.1, respectively). Sampling in M was performed in the periphery of the macrophyte bed, where some zooplankters tend to aggregate (Lauridsen & Buenk, 1996). The macrophyte bed was dense (coverage area > 75%) and large (*circa* 20 x 10 m), although seasonal variation in size occurred. Water temperature, pH, conductivity and dissolved oxygen levels were measured *in situ* with appropriate portable meters, whilst transparency and depth were determined using a Secchi disk. Air temperature was measured with a thermometer, while precipitation data for the region were obtained from the Portuguese Water Institute (INAG - http://snirh.inag.pt). A 1.5-L water sample was collected and transported (in the dark, at 4ºC) to the laboratory for determination of colour at 320 nm (CDOC) – an indirect measure of dissolved organic carbon (Williamson et al., 1999), total nitrogen (TN), total phosphorous (TP), suspended solids (seston) and phaeophytin-corrected chlorophyll *a* (chl *a*). A preliminary oxidation step was necessary for the determination of TN and TP (Ebina et al., 1983). For zooplankton, composite water samples were collected with a vertical Van Dorn bottle and concentrated with a 55-μm-mesh plankton net. The entire water column was sampled, with the exception of the layer closest to sediments, as this would represent noise and bias from the re-suspension of organic particles and dead zooplankters, respectively. The sampled volume ranged between 16L and 25.6L, depending on the total zooplankton densities. Each sample was immediately preserved in sucrose-saturated 4% formalin, stained with Bengal rose, and stored until further examination. In the laboratory, cladocerans were identified to the genus/species level (following the nomenclature of Alonso, 1996) and copepods were separated into nauplii and cyclopoid or calanoid (copepodites + adults). Counting by subsampling was used for the most abundant taxa, with at least 100 individuals of the dominant zooplankton species being counted and the total number of counts always being higher than 400. When necessary, the whole sample was screened in order to enumerate large organisms (especially for *Daphnia*). Zooplankton abundance data were expressed as densities (ind L^{-1}).

Fish were caught using seine nets and electrofishing (with identical sampling effort) and their abundance, size structure and diet were assessed (additional details in Castro et al., submitted manuscript). Because fish sampling was not carried out in the same temporal scale as zooplankton, we defined fish predation as a function of the recruitment patterns and feeding ecology of the main planktivores jointly with the abiotic frame (temperature, water level and daylight duration). Thus, a categorical variable (FISH) representing the theoretical fish predation pressure on zooplankton was allowed to vary discretely from 1 (lowest predation pressure) to 5 (highest predation pressure) – see Results.

Environment and community data analysis

To assess which abiotic and biotic variables were the most important determinants of key limnological features of Lake Vela (transparency, seston, chl *a* and *Daphnia* density), simple linear regression and stepwise multiple regression were used to measure the proportion of variation explained by the predictor variables. Data were log-transformed whenever linearity was substantially improved. In the multiple regression analysis, a 0.10 cut-off value was used in the stepwise selection procedure. Simple regression analysis was also used to assess the strength of the relationship between FISH and the main zooplanktonic taxa densities.

Detrended correspondence analysis (DCA) was used to describe the main sources of variation in the zooplankton assemblage data (excluding rare taxa). DCA is an improved eigenvector ordination technique based on reciprocal (weighted) averaging, which is commonly used in community ecology (Gauch, 1982; ter Braak, 1995). Its purpose is to reduce a species x sample matrix to a few dimensions (the eigenvectors) that explain the highest proportion of total variation in the data. Similar taxa are grouped based on common occurrence and the ordination can be interpreted with external data (see ter Braak, 1995), identifying potential sources of variation or environmental gradients. We evaluated the contribution of seasonal and spatial variation in the ordination with a two-way ANOVA using month and site (macrophyte vs. pelagial) as factors and each of the sample scores (for axis 1 and axis 2) as the dependent variables. In addition, correlation analysis was used to measure the association between log-transformed environmental variables (including FISH) and the first two DCA dimensions.

Daphnia dynamics

The average body size (from the top of the carapace to the base of the tail spine) of *Daphnia longispina* was estimated by measuring a random sub-sample of 100 individuals in each sample or all individuals in the sample, if $N<100$. The proportion of ovigerous (subitaneous and ephippial eggs) females relatively to the total number of *Daphnia* was also estimated from this sub-sample or from the whole sample (if $N<100$). Based on an initial assessment of the *Daphnia* data (see Results), we pooled the reproductive and body size data into two periods (corresponding to different predation pressure and transparency levels) and we determined size at maturity (SAM), for each combination of period and site (pelagial/macrophyte), as the smaller size-class of egg-bearing females, using a 5% cut-off value to discard precocious primiparae (Caramujo et al., 1997). A two-way analysis of variance (ANOVA) was used to test for differences in body size and proportion of females between period (using dates as replicates within period) and site, as well as for period x site interaction. Data on the proportion of females were arcsine square-root transformed, arcsin ($\sqrt{x}$), prior to the ANOVAs. In order to determine which abiotic and biotic factors exerted a control on the *Daphnia* dynamics, we individually plotted the data on body size, proportion of ovigerous females and density against *a priori* selected variables (temperature, chl *a* and *Daphnia* densities) for both periods. Linear regression analyses were used to determine the significance of the relationships. Data were log transformed when necessary, in order to maximise linearity.

RESULTS

Environmental parameters and fish

The seasonal variation of the physical and chemical parameters and chlorophyll *a* of Lake Vela is presented in Figure 1.2. Daylight duration varied from a maximum of 15 hrs in June to a minimum of 9 hrs and 15 min in December. Rainfall varied seasonally and air temperatures were occasionally very high (>30ºC). Water temperatures were warm during the summer (around 25ºC) but they were mild during most part of the year (minimum 9.7ºC). Conductivity levels were more or less constant, while pH values were always slightly alkaline, increasing during the summer (up to 9.9) and decreasing due to rainfall. Water level varied from a minimum of 0.45 m at the end of the summer and a maximum of 1.5 m at the end of the most intense rainfall period (December). Lake Vela was highly turbid (Secchi depth $\leq$ 30 cm), except when it experienced a transient clear water phase from January onwards, with transparency levels ranging from 60 cm to 115 cm. Some environmental variables were measured simultaneously in M (macrophyte) and in P (pelagial) and a good correspondence was found between sites for dissolved oxygen, colour (CDOC), TN, TP, seston and chlorophyll *a*, in spite of fluctuations (Figure 1.2, right panel). High nutrient levels were found in Lake Vela: mean TN was 2.2 mg N L^{-1} (range: 0.62-3.6 mg N L^{-1}) and mean TP was 0.18 mg P L^{-1} (range: 0.081-0.34 mg P L^{-1}). The seasonal pattern exhibited by suspended particles (seston) confirmed the shift from a turbid (May-November) to a clear water phase (December-May). However, chlorophyll *a* did not show a pattern consistent with alternating phases, due to extreme variation. In fact, chl *a* reached its highest (296 µg L^{-1}) and lowest (18.2 µg L^{-1}) levels during the clear water phase.

The ichthyocenosis of Lake Vela was dominated in numbers by littoral mosquitofish and pumpkinseed (dominant in biomass). Juvenile largemouth bass (*Micropterus salmoides*) were represented from May to October, but planktivory was restricted to its early life stages (May and June). No juvenile bass were observed prior to May (fish data from April 2003 not shown here). The dynamics of the main planktivorous fish during the growing season is shown in Figure 1.3. Theoretical predation pressure on zooplankton (categorical variable FISH) was defined as a function of the presence of juvenile fish from April to October and extrapolated outside of this period based on water temperature, water level and daylight duration (Figure 1.3; see also Figure 1.2).

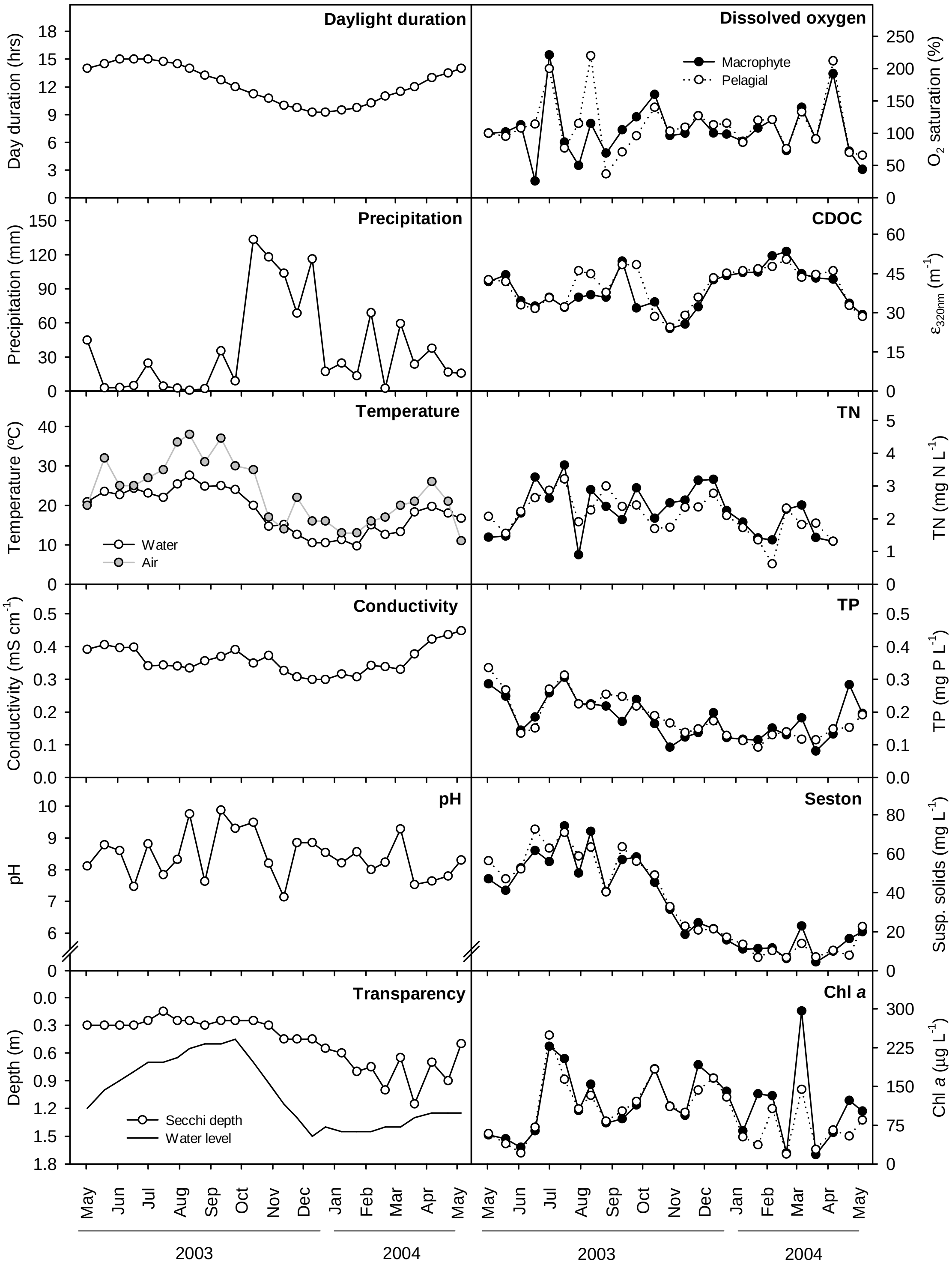

Figure 1.2 • Physical and chemical variables recorded during the study period in Lake Vela. The left panel represents variables concerning the whole lake and the right panel concerns variables that were measured at both sampling sites (macrophyte – black circles; pelagial – white circles). CDOC. TP and TN stand for coloured dissolved organic carbon, total phosphorous and total nitrogen, respectively.

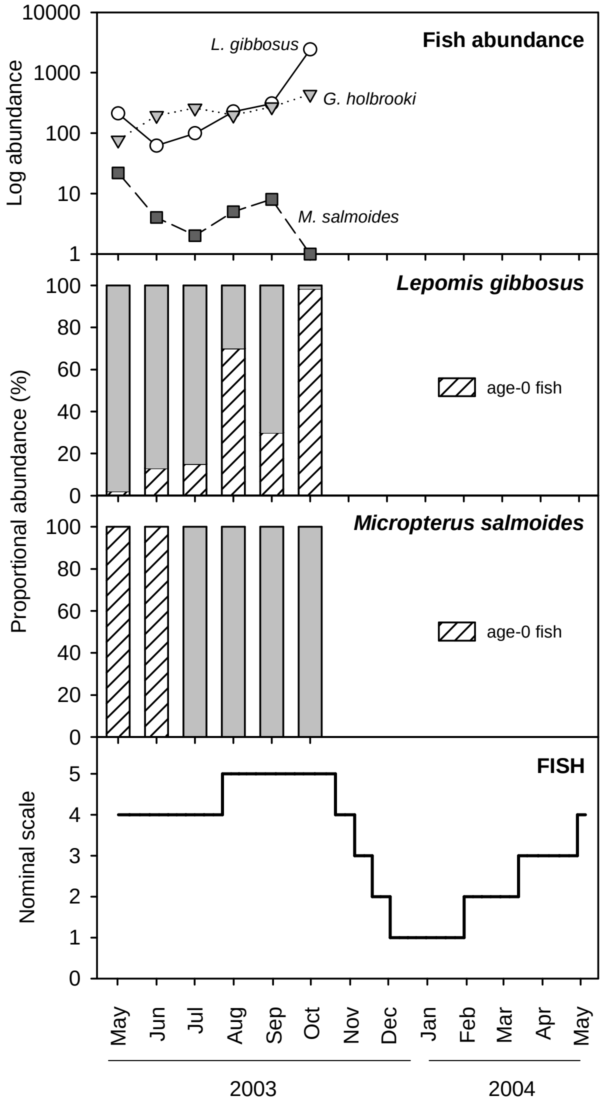

Figure 1.3 • Relative abundance and timing of recruitment of the main planktivorous fish taxa in Lake Vela. FISH is a dummy variable representing the level of predation pressure on zooplankton (see text for additional explanation).

Zooplankton community

The crustacean zooplankton of Lake Vela was not very diversified, being clearly dominated by small-bodied cladocerans (*Bosmina longirostris* and *Chydorus* sp.) and cyclopoid copepods and nauplii (Table 1.1). *Daphnia longispina* was also well represented, displaying seasonal fluctuations, which included absence periods (Figure 1.4). A pronounced seasonal dynamics was observed in the zooplankton assemblage (Figure 1.4). Nauplii and cyclopoid copepods were the exception, as they showed more or less constant densities during the year (except during early spring). *Bosmina* and *Chydorus* were present in high densities, mostly during fall and winter, while experiencing large fluctuations in spring (very low densities) and summer.

Table 1.1 • Zooplanktonic crustacean taxa observed in Lake Vela during the study period and corresponding abbreviations (taxa lacking abbreviation were excluded from the DCA analysis). Taxa are sorted in descending order of importance, in terms of average annual abundance.

Abbrev.	**Taxon**	**Mean abundance**[1]	**Frequency of occurrence**	
			Macrophyte	Pelagial
Bosm	*Bosmina longirostris*	++++	92 %	85 %
Chyd	*Chydorus* sp.	++++	96 %	92 %
Cycl	Copepoda cyclopoida	++++	100 %	100 %
Naup	Copepoda nauplii	++++	100 %	100 %
Daph	*Daphnia longispina*	+++	77 %	85 %
Moin	*Moina micrura*	++	31 %	31 %
Ceri	*Ceriodaphnia pulchella*	++	42 %	39 %
Alon	*Alona* spp.	+	54 %	46 %
Cala	Copepoda calanoida	o	31 %	23 %
Grap	*Graptoleberis testudinaria*	o	15 %	8 %
Simo	*Simocephalus vetulus*	o	15 %	4 %
Macr	*Macrothrix* sp.	o	8 %	4 %
Diap	*Diaphanosoma brachyura*	o	8 %	0 %
Leyd	*Leydigia leydigii*	o	8 %	4 %
-	*Ilyocryptus sordidus*	o	0 %	4 %
-	*Camptocercus rectirostris*	o	4 %	0 %
-	*Eurycercus lamellatus*	o	4 %	0 %
-	*Leydigia acanthocercoides*	o	4 %	4 %
-	*Oxyurella tenuicaudis*	o	0 %	4 %

[1] Abundance classes: o (< 0.1 ind L^{-1}), + (< 1 ind L^{-1}), ++ (< 10 ind L^{-1}), +++ (< 100 ind L^{-1}) and ++++ (100-1000 ind L^{-1}).

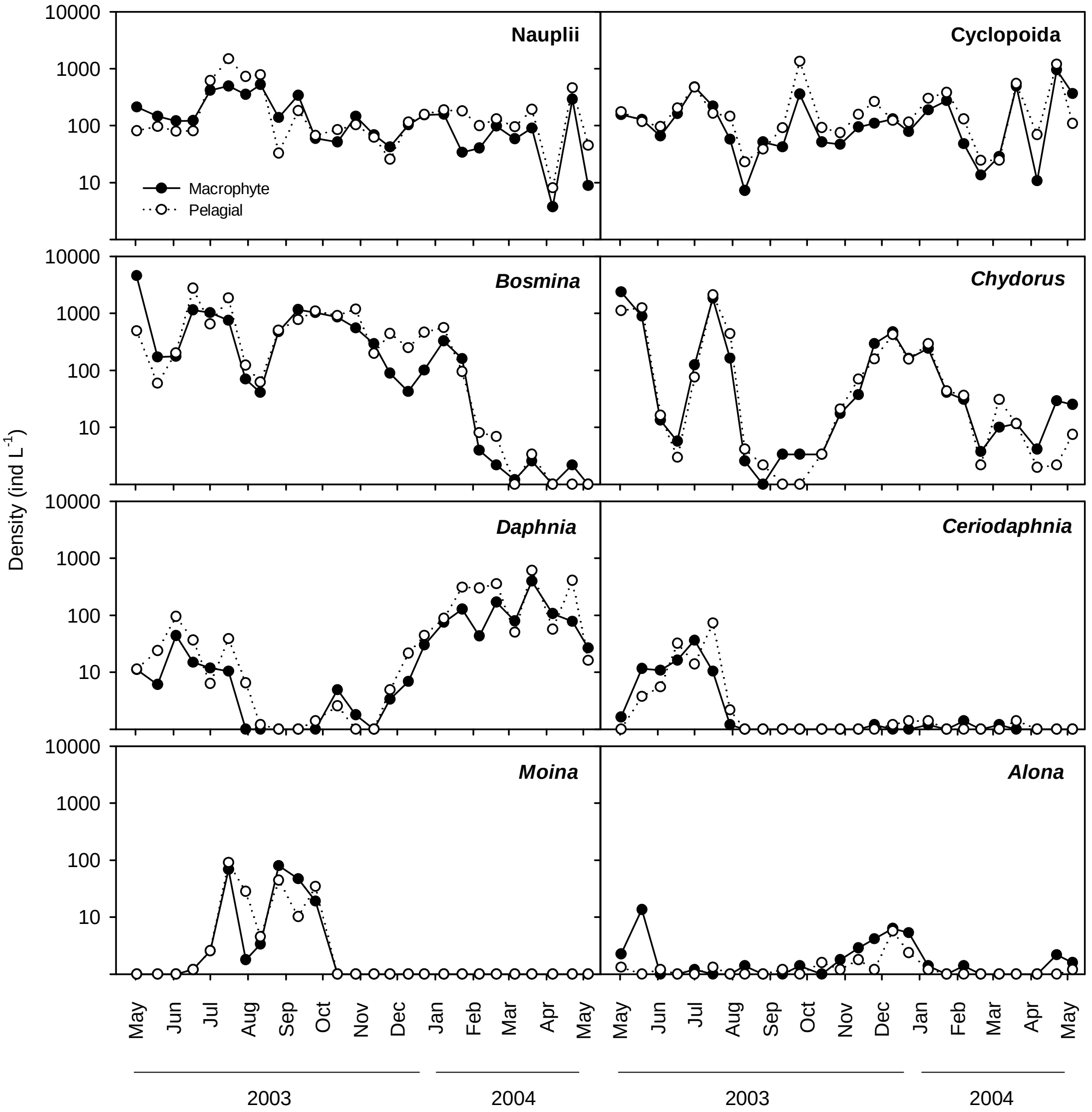

Figure 1.4 • Densities of the main zooplanktonic taxa and species richness observed in Lake Vela during the study period, in both sampling sites. A log scale was used to display densities, in order to facilitate the comprehension of the different orders of magnitude of the taxa.

Environment- and fish-zooplankton interactions

Most of the variation in water transparency (Table 1.2) was explained by seston (90%), followed by the densities of *Daphnia* (positive association) and small cladocerans (negative association). A weak relationship was found between chl *a* and transparency. In stepwise multiple regression (Table 1.2), the additional contribution of *Daphnia* density and water level was residual, when compared to seston. These results led us to focus on the underlying variation of seston, which was mostly (61%) due to variations in water level, although small cladocerans and *Daphnia* also contributed significantly (Table 1.3). In both cases (seston and transparency), daphniids and small-sized cladocerans displayed similar associations with the dependent variable, but in opposite directions. In fact, high *Daphnia* densities generally corresponded to low densities of small cladocerans – see Figure 1.4. Water level and small cladoceran densities together explained 78% of the variation in seston (Table 1.3). Variation in chl *a* was mostly unexplained, but *Daphnia* density explained a fraction (17%) of its total variation (Table 1.4). No relationships were found between chl *a* and TP, TN or seston.

Table 1.2 • Simple linear regressions and stepwise multiple regression of several abiotic and biotic variables on transparency (Secchi depth) (n = 26). The significance (*P*) and proportion of variation explained by the model (adjusted r^2) are shown for each regression.

Simple regressions

Predictors	Slope	r^2	P
Seston (log)	-0.74	0.90	0.000
Daphnia density (log)	0.24	0.61	0.000
Small cladocera density (log)*	-0.23	0.57	0.000
Water level (log)	1.11	0.47	0.000
TP (log)	-1.18	0.39	0.000
Chl *a* (log)	-0.43	0.17	0.002
TN	-0.19	0.17	0.027
CDOC	0.02	0.16	0.026

Stepwise multiple regression

Order entered	Predictors	r^2
1	Seston (log)	0.91
2	*Daphnia* density (log)	0.93
3	Water level (log)	0.95

* this included *Alona*, *Bosmina*, *Ceriodaphnia*, *Chydorus* and *Moina*

Table 1.3 • Simple linear regressions and stepwise multiple regression of several abiotic and biotic variables on log-transformed seston (n = 26). The significance (*P*) and proportion of variation explained by the model (adjusted r^2) are shown for each regression.

Simple regressions

Predictors	Slope	r^2	P
Water level (log)	-1.60	0.61	0.000
Small cladocera density (log)*	0.28	0.50	0.000
Daphnia density (log)	-0.28	0.49	0.000
Nauplii density (log)	0.29	0.12	0.047
Cyclopoida density (log)	--	0.00	NS

Stepwise multiple regression

Order entered	Predictors	r^2
1	Water level (log)	0.61
2	Small cladocera densities (log)	0.78

* this included *Alona*, *Bosmina*, *Ceriodaphnia*, *Chydorus* and *Moina*

Table 1.4 • Simple linear regressions and stepwise multiple regression of several abiotic and biotic variables on log-transformed chlorophyll *a* (n = 26). The significance (*P*) and proportion of variation explained by the model (adjusted r^2) are shown for each regression.

Simple regressions Predictors	Slope	r^2	P
Daphnia density (log)	-0.15	0.17	0.022
Seston (log)	--	0.10	NS
TN	--	0.08	NS
TP (log)	--	0.06	NS
Small cladocera density (log)*	--	0.06	NS
CDOC	--	0.04	NS
Water level (log)	--	0.01	NS

Stepwise multiple regression Order entered	Predictors	r^2
1	*Daphnia* density (log)	0.18

* this included *Alona*, *Bosmina*, *Ceriodaphnia*, *Chydorus* and *Moina*

DCA ordination resulted in low eigenvalues (see Figure 1.5) and relatively low lengths of gradient (1.9 and 1.0 for axes 1 and 2, respectively). Still, both axes (sample scores) revealed a seasonal component in the zooplankton assemblage (two-way ANOVA for month: axis 1 – $F_{11,28}$ = 28, P = 0.000; axis 2 – $F_{11,28}$ = 12, P = 0.000). No significant effects were found for site (axis 1 – $F_{1,28}$ = 0.10, P = 0.758; axis 2 – $F_{1,28}$ = 0.15, P = 0.698) or its interaction with month (axis 1 – $F_{11,28}$ = 0.08, P = 1.000; axis 2 – $F_{11,28}$ = 0.28, P = 0.985). The seasonal component of each axis was attributable to different features (or environmental gradients), as shown by correlation analyses (Table 1.5). The first dimension of the ordination was found to be strongly associated with transparency, water level, seston and also fish predation (FISH). Transparency and water level increased from the left (negative) to the right (positive) side of the diagram, while the opposite gradient was observed in seston and predatory pressure. A similar, but weaker, association was also recorded for temperature, TP, TN, chl *a* and pH, with these variables decreasing from the left to the right side of the biplot. Thus, DCA identified a clear association between high transparency (low seston), high water level, and reduced fish predation and a zooplankton assemblage with the presence of calanoid copepods, *Simocephalus*, *Graptoleberis*, and *Daphnia* (Figure 1.5). An assemblage characterised by the presence of *Bosmina* and, especially, *Moina* and *Macrothrix* was associated with low transparency (high seston), low water level and high fish predation (Figure 1.5). The second DCA dimension was less important to the ordination and its variation was mostly attributable to seasonal fluctuations in daylight duration and, to a lesser extent, temperature, conductivity and chl *a* (Table 1.5). Thus, taxa on the negative side of the axis (bottom) were most abundant during spring and/or summer, while taxa with positive scores (top) were mainly present in autumn and/or winter months (Figure 1.5). *Chydorus*, cyclopoid copepods and nauplii scored in the central zone of the diagram, apparently unaffected by the environmental framework of the lake (Figure 1.5).

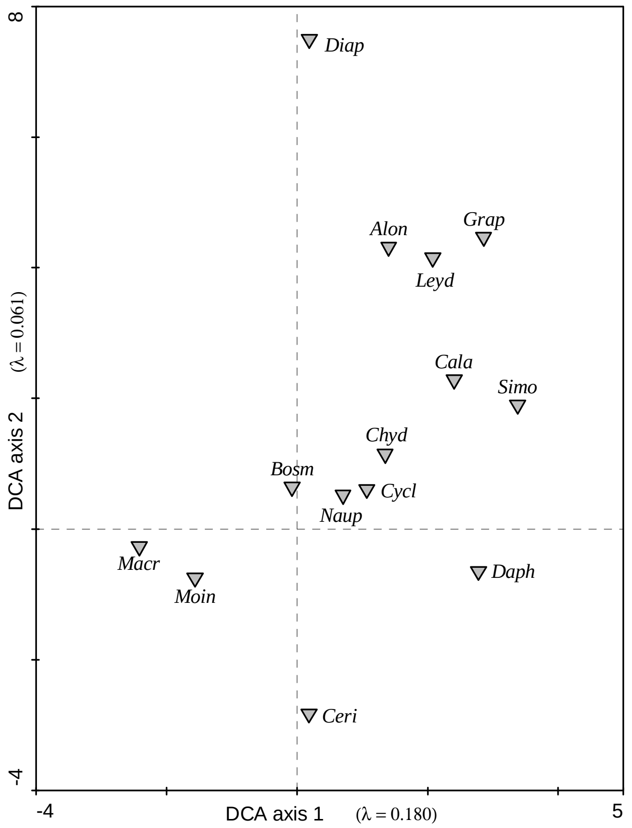

Figure 1.5 • DCA ordination biplot of species scores (total inertia = 0.456). See Table 1.1 for taxa abbreviations.

Given the large influence of FISH (a biotic variable) in the ordination, we tested its relationship with the (log) densities of the main zooplanktonic taxa and we found different responses between taxa. On one hand, no significant relationships (linear regression, $P > 0.05$) were found between FISH and the densities of adult copepods, nauplli or small-sized cladocerans (*Alona*, *Bosmina*, *Ceriodaphnia* and *Chydorus*). On the other hand, FISH could explain 29% and 36% of the variation in the densities of *Moina* (linear regression: $F_{1,24} = 11$; $P = 0.003$) and *Daphnia* (linear regression: $F_{1,24} = 15$; $P = 0.001$), respectively.

Their densities were inversely related to fish pressure, as revealed by the regression slopes (*Moina*: 0.25; *Daphnia*: -0.38).

Table 1.5 • Correlation (Pearson's *r*) between environmental variables (in alphabetical order) and sample scores for each DCA dimension. NS is shown for non-significant correlations ($P > 0.05$).

Variables	**axis 1**	**axis 2**
CDOC (log)	NS	NS
Chlorophyll *a* (log)	-0.33	0.32
Conductivity (log)	NS	-0.35
Daylight duration (log)	NS	-0.55
FISH	-0.63	NS
pH (log)	-0.29	NS
Seston (log)	-0.77	NS
Temperature (log)	-0.52	-0.39
TN (log)	-0.45	NS
TP (log)	-0.48	NS
Transparency (log)	0.80	NS
Water level (log)	0.79	NS

Daphnia dynamics

Most of the variation (49%) in the density of *D. longispina* could be attributable to seston (Table 1.6). Fish pressure (as seen above), chl *a* and temperature also contributed significantly to the total variation (the 4 variables together explained 65% of the variance in *Daphnia* density). In addition, *Daphnia* densities were positively correlated to transparency (Secchi depth) ($r = 0.776$; $P = 0.000$), enhancing their role as grazers and as evidenced in the DCA diagram (Figure 1.5). We sought additional insight on the body size distribution and reproductive condition of the *Daphnia* population during the study period (Figure 1.6). Due to the low densities or absence of *D. longispina* from mid-July to December, no data were available for this period. Figure 1.6 (A-C) shows the minimum, maximum and average body size of *Daphnia* in the pelagial and macrophyte sites. The pattern was similar for both sites: the period from May to July was more constant in terms of body size, while the period from December to May exhibited larger fluctuations. Additionally, and more important, daphnids from May-July were smaller that those from December-May, which was especially noticeable on the maximum body sizes recorded (see Figures 1.6 A-B).

Table 1.6 • Simple linear regressions and stepwise multiple regression of several abiotic and biotic variables on log-transformed *Daphnia* density (n = 26). The significance (*P*) and proportion of variation explained by the model (adjusted r^2) are shown for each regression.

Simple regressions

Predictors	Slope	r^2	*P*
Seston (log)	-1.75	0.49	0.000
FISH	-0.38	0.36	0.000
Chl *a* (log)	-1.41	0.22	0.000
TP (log)	-2.60	0.19	0.001
Temperature (log)	-2.72	0.17	0.001
CDOC (log)	3.83	0.14	0.004
TN (log)	-2.04	0.11	0.013

Stepwise multiple regression

Order entered	Predictors	r^2
1	Seston (log)	0.49
2	Chl *a* (log)	0.52
3	FISH	0.57
4	Temperature (log)	0.65

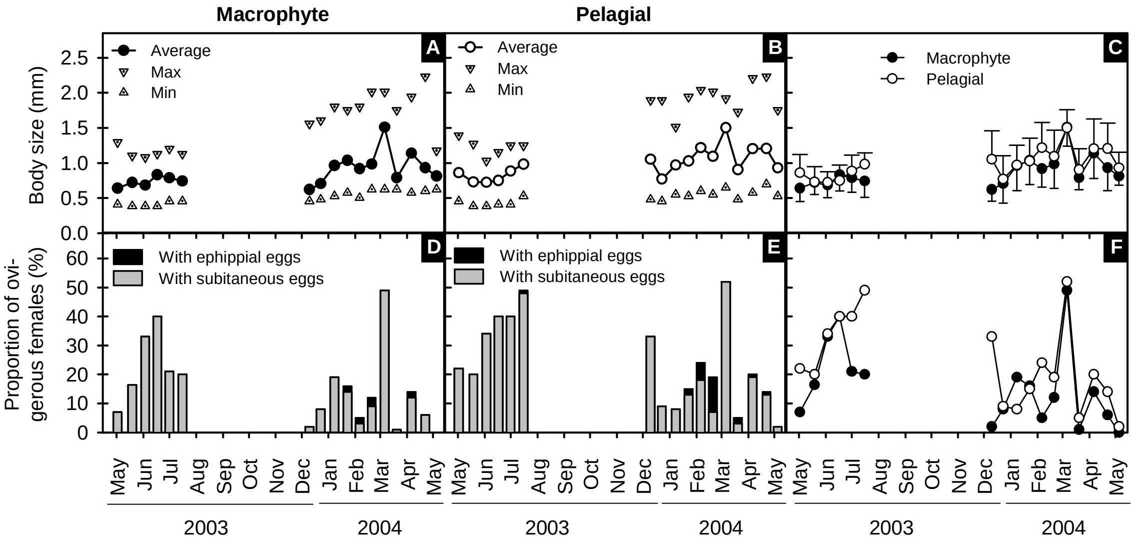

Figure 1.6 • Minimum (Min), maximum (Max) and average body size of *Daphnia longispina* recorded during the study period, at a macrophyte (A) and a pelagial (B) site in Lake Vela. For comparative purposes, the average body size (with respective SD) for both sites is also plotted together (C). Proportion of egg-bearing *Daphnia* females observed during the study period at a macrophyte (D) and a pelagial (E) site in Lake Vela. For comparative purposes, the total proportion of ovigerous females in both sites is also represented together (F). The discontinuity in the plots is due to low densities or absence of *Daphnia* from mid-July to December.

These observations lead us to look at these periods separately, an approach also supported by the fact that these two *Daphnia* peaks (see Figure 1.4) occurred under distinct transparency conditions (see Figures 1.2 and 1.4). Statistically, our interpretation on body size was supported by a significant effect of period (May-July *versus* December-May), which was independent of site (i.e., no significant period x site interaction; Table 1.7). This was true not only for maximum body size, but also for minimum and average body size, which were all found to be higher in December-May (Table 1.7). No differences in body size were found between sites (Table 1.7; Figure 1.6 C). Consistent with these results, an almost two-fold increase in size at maturity (SAM) was found from May-July to December-May, for both sites (Figure 1.7). The difference in adult body size between the two periods was so pronounced that the size distributions of ovigerous females barely overlapped (Figure 1.7). A significant site-independent effect of period was also obtained for the proportion of ovigerous females (Table 1.7). As seen in Figure 1.6 (D-E), a significant lower proportion of (subitaneous) egg-bearing females was recorded in December-May, comparatively to May-July. The large peak observed in March 6th represented an exception to this general pattern. Contrarily to the egg-bearing females, a significantly larger proportion of ephippium-bearing females occurred in December-May, when compared to May-July (Table 1.7; Figure 1.6). In both cases (egg- or ephippium-bearing females), no differences were found between sites (Table 1.7; Figure 1.6 F). Again, as for the body size data, the proportion of ovigerous females seemed to be more prone to fluctuations in the December-May period (Figure 1.6 D-E).

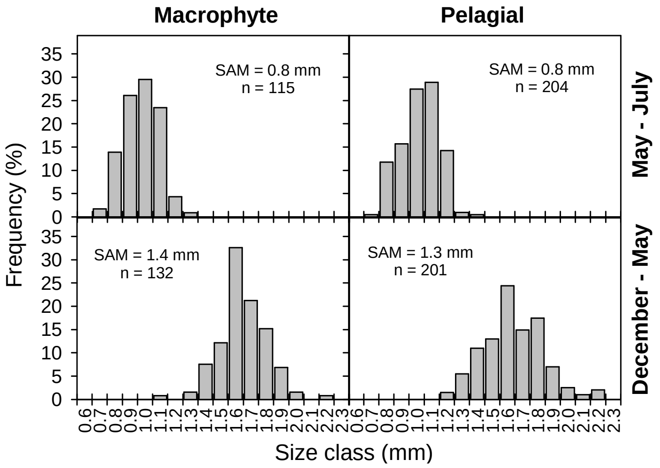

Figure 1.7 • Body size distribution of ovigerous *Daphnia longispina* at two different locations (macrophyte and pelagial) in Lake Vela and in two distinct periods (May-July and December-May). See text for additional information on the estimation of size at maturity (SAM).

Table 1.7 • Summary table of the two-way ANOVAs applied to the *Daphnia longispina* data on body size and proportion of ovigerous females. Significant *P* values (α=0.05) are shown in bold.

Parameter	Source of variation	d.f.	MS	*P*
Average body size	Period	1	0.429	**0.001**
	Site	1	0.095	0.109
	Period x site	1	0.004	0.732
	Residual	30	0.035	
Maximum body size	Period	1	3.429	**<0.001**
	Site	1	0.080	0.189
	Period x site	1	0.009	0.659
	Residual	30	0.044	
Minimum body size	Period	1	0.151	**<0.001**
	Site	1	0.000	0.909
	Period x site	1	0.001	0.549
	Residual	30	0.004	
Females with subitaneous eggs	Period	1	0.357	**0.002**
	Site	1	0.096	0.097
	Period x site	1	0.003	0.758
	Residual	30	0.033	
Females with ephippial eggs	Period	1	0.362	**0.029**
	Site	1	0.007	0.315
	Period x site	1	0.001	0.648
	Residual	30	0.007	

Regression analysis showed no relationship between average body size, proportion of ovigerous females or density of *Daphnia* and a set of pre-selected variables, in the May-July period (Figure 1.8). In the December-May period, however, three statistically significant linear relationships were detected: i) proportion of females with (subitaneous) eggs *versus* chlorophyll *a*; ii) proportion of females with ephippia *versus* density; iii) *Daphnia* density *versus* chlorophyll *a*.

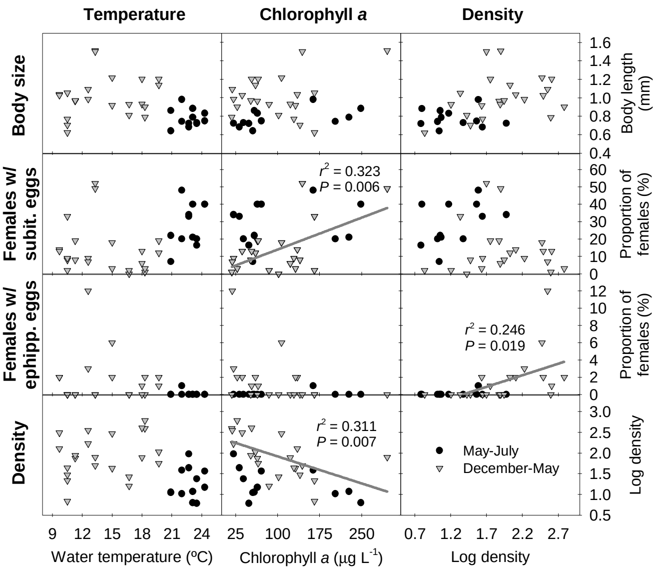

Figure 1.8 • Relationships between several variables recorded in *Daphnia longispina* (body size, proportion of ovigerous females and density) and some potential explanatory variables (temperature, chlorophyll *a* and *Daphnia* density) in two distinct periods. The regression lines (grey, Dec-May; black, May-Jul) and respective coefficients of determination (r^2) are shown only for statistically significant ($P \leq 0.05$) linear relationships.

DISCUSSION

Information on the abiotic and biotic environment of Lake Vela is scarce and discontinuous in time. The present study provides evidence of the present ecological condition of this small polymictic lake, which sustains a considerable amount of aquatic and terrestrial biodiversity. According to Wetzel (1983), Lake Vela can be viewed as eutrophic or hypereutrophic, concerning TN (annual average = 2200 mg m^{-3}), TP (180 mg m^{-3}), chlorophyll *a* (100 mg m^{-3}) and transparency (0.5 m) values. Correspondingly, Carlson's trophic state index (Carlson, 1977) was high for TP (range: 70-87), chlorophyll *a* (60-84) and transparency (58-87). Due to high nutrient availability, phytoplankton was chiefly top-down controlled (by grazers), as revealed by the absence of a significant TP-chl *a* relationship. The water from Lake Vela was also highly coloured (high CDOC) and, according to the model proposed by Williamson et al. (1999), it should be viewed as mixotrophic, as it is subjected to high amounts of nutrients (N and P) but also to allochthonous organic compounds, such as humic substances (Wetzel, 1983). High turbidity was also observed and the levels of suspended particles (seston) were found to be important to the overall response of the lake's limnology and zooplankton assemblage (see below).

The seasonal pattern observed for the zooplankton of Lake Vela was clearly associated with water level, seston and transparency, but also with fish predation pressure. In late spring / early summer (May-July), a *Bosmina-Chydorus-Daphnia-Ceriodaphnia* assemblage was recorded, under high seston, moderate predatory pressure from fish and progressively increasing chlorophyll *a* levels. During this period, *Daphnia* exhibited small body sizes and reduced SAM, which is concordant with size-selective predation from fish (Tessier et al., 1992; Gliwicz, 1994; Caramujo et al., 1997). Summer and fall represented a shift towards progressively smaller species, from a *Bosmina-Moina* (August-September) to a *Bosmina-Chydorus* (October-December) assemblage. During this high predation period, *Daphnia* was virtually absent and chlorophyll *a* levels were always high. We can not rule out the additional influence of other factors (such as cyanobacteria) in the summer absence of *Daphnia*, as suggested in the literature (Sommer et al., 1986; Abrantes et al., 2006a). As predatory pressure dropped with decreasing winter temperatures and seston was reduced due to an increase in water level, *Daphnia* populations reappeared and became dominant from January onwards, inducing a transient clear water phase (with the contribution of macrophyte-associated species, such as *Simocephalus*) and suppressing small-sized species, as predicted by the size-efficiency hypothesis (Brooks & Dodson, 1965; Hall et al., 1976). Compared to the May-July period, *Daphnia* exhibited larger body sizes and higher densities during late winter and early spring, but a lower proportion of egg-bearing females and a greater number of ephippium-carrying females. These features can be attributed to inter-specific competition for resources, as shown by significant resource-dependence (chlorophyll *a* vs. density, chlorophyll *a* vs. proportion of ovigerous females) and density-dependence relationships (density vs. proportion of ephippial females). In fact, when comparing both periods in terms of *Daphnia* body size and proportion of ovigerous females, it seems evident that the clear water period was more prone to fluctuations, probably as the result of more unstable (i.e. limiting) environmental conditions, particularly food. Absolute chlorophyll *a* levels suggest food is not a limiting factor in Lake Vela, but it is nevertheless true that chlorophyll *a* exhibited large fluctuations during the clear water phase, reaching its lowest values in the whole sampling period (in February and again in March). Similar outcomes, illustrating the role of food shortage in the *Daphnia* spring peak and fish predation in the summer have been widely reported in the literature, even in eutrophic conditions (Mills & Forney, 1983; Luecke et al., 1990; Boersma et al., 1996; Hülsmann, 2003).

Transparency was chiefly dependent on the reduction of seston, which, in turn, was dependent on water level rise (see regression analyses). In shallow lakes, winds cause substantial mixing in the water column (polymixis) and enhance sediment-water column interaction (Scheffer, 1998), a phenomenon intensified at low water. In these conditions, sediment resuspension easily occurs by wave and wind action, and is facilitated by benthivorous fish (Breukelaar et al., 1994; Scheffer et al., 2003), such as carp. Two additional reasons make Lake Vela especially subjected to high levels of suspended materials: i) the lakebed is coated by a thick layer of fine and easily-resuspended organic particles (Fernandes, 1999); ii) due to the slope of the piezometric surface towards the lake depression, most of its water drains from superficial fluxes (Fernandes, 1999; Abrantes et al., 2006b), which carry many dissolved and suspended materials from the surrounding area. However, the reduction of seston was insufficient to explain the transient spring clear water phase. Although seston displayed low levels from November onwards, the highest transparency values were only observed at high *Daphnia* densities (> 100 ind L^{-1}), which were recorded later, between February and April. To some extent, *D. longispina* also contributed to increased transparency by grazing on phytoplankton, as supported by the significant relationship between *Daphnia* density and chl *a*, and the two chl *a* minima observed in February and March. However, this control over zooplankton was irregular (see Figure 1.2), and large fluctuations in chl *a* may explain the low association between chl *a* and transparency. Lampert et al. (1986) have stressed out the role of grazers in the occurrence of clear water phases in eutrophic lakes, and have recognised that the increase in water clarity can occur just for a few days, in a transient and irregular pattern.

Regression analysis revealed that seston was the main driver of the *Daphnia* population, while fish predation contributed less, along with chl *a* and temperature. However, the dummy variable FISH was also correlated to seston (r = 0.74; P = 0.000). We therefore expect some bias in the regression. Nevertheless, Rellstab & Spaak (2007) observed detrimental effects in the fitness of *D. hyalina* exposed to concentrations of suspended particles above 25 mg L^{-1}. Seston in Lake Vela exceeded this level from May to November, when the *Daphnia* population was reduced to low levels or absent. However, other evidences (e.g. reduction in SAM, replacement of *Daphnia* by smaller cladocerans) seemed to point out to a top-down regulation of *Daphnia* by fish. Both factors may have contributed to the summer disappearance of *Daphnia* until its winter/spring peak. Further research is necessary to unveil the timing and relative contribution of seston and fish predation to the zooplankton dynamics in lakes exhibiting high turbidity. We emphasize that a clear water condition as the one shown in this study is not always observed in Lake Vela.

A few differences were observed between the seasonal succession pattern of Lake Vela and the one described by the PEG group (Sommer et al., 1986), which is widely accepted as the norm in northern temperate lakes. First, re-suspended particles contributed significantly to the zooplankton dynamics, although its interaction with fish predation must be clarified. Qualitatively, the sequence of mechanisms determining grazer abundance and community structure was similar, but different timings were perceptible. Abiotic factors (temperature or food shortage) did not suppress *Daphnia* populations during the winter. On the contrary, temperature presumably released large grazers from planktivory, allowing the formation of a winter/spring *Daphnia*-dominance phase until the advent of predation. Fish predation in Lake Vela seemed to be more prolonged in time than predicted in the PEG model, extending its influence from late spring to early winter. Large shoals of YOY pumpkinseed were present in September and October and their overall activity was not diminished by the mild autumnal temperatures, thus extending their temporal influence on zooplankton.

The prolonged importance of fish predation and the reduced role of abiotic constraints in the winter have already been stressed out by Saunders et al. (1999), who discussed this in the light of the pattern they observed in Lake Oglethorpe (U.S.A.) and other cases reported in the literature. They concluded that predator influence was much higher in warmer lakes, when comparing studies performed above (1-2 months year^{-1}) and below 40º latitude (3-7 months year^{-1}). Although Lake Vela (40º 5’ N) is geographically more close to the northern cluster of lakes, its alien fish community and high levels of suspended particles pose serious constraints to zooplankton, particularly to large grazers, such as *Daphnia*. Suspended particles pose an additional problem, as they also represent an additional source of P and N to phytoplanktonic production. The large influence of fish predation and suspended particles on zooplankton and transparency must be taken into account in order to promote adequate restoration programs in Lake Vela.

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Capítulo 2

Recrutamento e ecologia alimentar da ictiocenose

Compreendendo o sucesso dos peixes alienígenas de um lago Ibérico pouco profundo: padrões de recrutamento e ecologia alimentar

In: Castro B.B., Vingada J. & Gonçalves F. (submetido). Understanding the success of alien fish in an Iberian shallow lake: recruitment patterns and feeding ecology.

RESUMO

O objectivo do presente trabalho foi o de estudar a dinâmica populacional e a ecologia alimentar da comunidade de peixes alienígenas da Lagoa da Vela, um lago pouco profundo que se apresenta muito turvo, eutrófico e em perda de biodiversidade. Os principais *taxa* alienígenas (achigã – *Micropterus salmoides*, perca-sol – *Lepomis gibbosus*, e gambúzia – *Gambusia holbrooki*), que substituíram completamente a comunidade de ciprinídeos indígena, exercem uma forte pressão predatória sobre os níveis tróficos mais baixos. A amostragem foi conduzida mensalmente utilizando uma combinação de pesca eléctrica e redes de pesca (rede de mão e rede de cerco), durante a época de crescimento (Abril a Outubro). Adicionalmente, procedeu-se a uma campanha de amostragem adicional, em Janeiro, para complementar os dados da dieta. A ictiocenose da Lagoa da Vela revelou ser pouco diversificada e dominada por elevada abundância de peixes alienígenas de pequenas dimensões (perca-sol e gambúzia), exibindo recrutamento mais ou menos contínuo durante todo o verão. O achigã, a única espécie piscívora, desovou na primavera e os seus primeiros recrutas foram observados no início de Maio, crescendo rapidamente até Setembro. Foi observada uma mudança ontogenética radical na sua dieta, sendo os espécimes de menores dimensões (TL < 50 mm) essencialmente planctívoros, ao passo que os indivíduos maiores se tornaram piscívoros especializados. Infelizmente, o número de exemplares capturados foi demasiado pequeno para conclusões robustas. O zooplâncton foi uma categoria alimentar muito importante para a gambúzia e para a perca-sol (especialmente para os exemplares mais pequenos), apesar da sua dieta poder ser caracterizada como omnívora, dada a sua capacidade para explorar diferentes tipos de presas (sobretudo larvas, ninfas, pupas e imagos de insectos) de acordo com a ocorrência sazonal. A perca-sol passou por uma mudança de hábitos alimentares progressiva, que foi menos pronunciada na gambúzia. A componente bêntica (i.e. macroinvertebrados) foi bastante menos importante para estas espécies de peixes do que noutras populações ibéricas, provavelmente por causa do reduzido coberto de macrófitas da Lagoa da Vela. Ainda assim, a sobreposição dietária entre as três espécies foi globalmente baixa, o que poderá ser uma das razões do sucesso e coexistência de populações tão densas (sobretudo gambúzia e perca-sol).

PALAVRAS-CHAVE

Recrutamento, análise da dieta, peixes alienígenas, lago Mediterrânico pouco profundo

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O presente capítulo constitui um manuscrito submetido para publicação em revista internacional com arbitragem científica e é, por esse motivo, apresentado na língua inglesa.

Understanding the success of alien fish in an Iberian shallow lake: recruitment patterns and feeding ecology

ABSTRACT

The aim of this work was to study the population dynamics and feeding ecology of the alien fish assemblage of Lake Vela, a turbid shallow lake facing eutrophication and biodiversity loss. Main alien taxa (largemouth bass – *Micropterus salmoides*, pumpkinseed sunfish – *Lepomis gibbosus* and mosquitofish – *Gambusia holbrooki*) exert strong top-down effects on lower trophic levels and have completely replaced the native cyprinid assemblage. Fish were sampled every month using a combination of electrofishing and fish nets (dip and seine nets), during the growing season (April-October). Additional sampling took place in January to complement diet data. The fish community of Lake Vela was poorly diversified and dominated by very high numbers of small-sized alien fish (pumpkinseed and mosquitofish), displaying more or less continuous recruitment during the summer. Largemouth bass, the only piscivore species, spawned in spring and its first recruits were observed in early May, rapidly growing until September. A radical ontogenetic diet shift was observed in bass, with smaller specimens (TL < 50 mm) being essentially zooplanktivorous, while larger individuals became specialised piscivores, although this evidence is hampered by small sample size. Zooplankton was a very important food category for mosquitofish and pumpkinseed (especially smaller specimens), although their diet could be classified as omnivorous, since they explored several types of prey (chiefly insect larvae, nymphs, pupae and adults) according to their seasonal occurrence. Pumpkinseed experienced a progressive ontogenetic diet shift, which was much less pronounced in mosquitofish. The benthic niche (i.e. macroinvertebrates) was less important for all taxa than reported for other Iberian populations, probably because of low macrophyte coverage in Lake Vela. Still, diet overlap between the three taxa was low overall, which may be one of the reasons for the success and coexistence of such dense populations (particularly pumpkinseed and mosquitofish).

KEYWORDS

Recruitment, diet analysis, alien fish, Mediterranean shallow lake

INTRODUCTION

In southern Europe, the cyprinid zone (impoundments, lakes and low reaches of rivers) has been extremely vulnerable to alien fish species introduction (Almaça, 1995a). In Iberia, adventive species gradually formed well-established populations, with a variable degree of invasiveness, displacing the autochthonous cyprinids and contributing to biotic homogenisation (Almaça, 1995a; 1995b; Godinho & Ferreira, 1998b; 2000; Garcia-Berthou & Moreno-Amich, 2000; Blanco et al., 2003). This has not been subject of enough

attention from local authorities and populations, and the high degree of endemism of the Iberian fish fauna has been particularly neglected. According to Almaça (1995b), the successive introduction of alien species is the most serious conservation problem for Portuguese endemics. Inhabitants of inland waters include endemic omnivorous cyprinids from the genera *Barbus*, *Chondrostoma* and *Squalius* (formerly placed under *Leuciscus*), but also an array of introduced species, including widespread invaders such as pumpkinseed sunfish (*Lepomis gibbosus* (L.)), mosquitofish (*Gambusia holbrooki* (Girard)) carp (*Cyprinus carpio* L.) and largemouth bass (*Micropterus salmoides* (Lacépède)).

According to Almaça (1995a), one of the reasons for the success of alien species in Iberia is the absence of naturally-occurring predator fish in the indigenous freshwater fauna. This feature is in contrast with the piscivore-planktivore cascade of northern temperate lakes and may contribute to facilitating the establishment of invasive taxa at the expenses of indigenous species. Additionally, and depending on the case, the competitive capabilities and phenotypic plasticity of alien invasive species, in terms of resource exploitation and/or reproductive strategy, also account for their extraordinary success away from their original environment. Increased knowledge on the ecology and life-history of alien species in homogenised habitats (or where homogenisation is in progress) is therefore urgently needed.

With few exceptions (e.g. Blanco et al., 2003), most studies on the ecology of alien species in the Iberian Peninsula have neglected shallow lakes, focusing mainly on deep lakes, large reservoirs, or streams (Fernández-Delgado, 1989; Godinho & Ferreira, 1998a; 1998b; Cabral & Marques, 1999; García-Berthou & Moreno-Amich, 2000; Vila-Gispert & Moreno-Amich, 2000; Godinho & Ferreira, 2000; García-Berthou, 2002). Although rare in Iberia, shallow lakes constitute important pools of biodiversity, sustaining associated wildlife and providing unique ecological niches. However, these habitats usually face the threat of eutrophication and terrestrialisation, requiring adequate management. Their small dimensions, island-like nature and trophic status make shallow lakes ideal subjects for lake restoration programs (including fish manipulation with reintroduction of native species).

Bearing this in mind, we conducted a survey on the ichthyocenosis of a shallow polymictic lake (Lake Vela), which is facing eutrophication and biodiversity loss (due to homogenisation of the fish fauna). The main goals of this study were: 1) to evaluate the current status of the fish assemblage of Lake Vela and to assess its recent evolution by comparing it to the records of a previous work (see below; Ferreira, 1997); 2) to study the population dynamics of the main species (*L. gibbosus*, *G. holbrooki* and *M. salmoides*) and, more specifically, their recruitment patterns; 3) to assess the general feeding preferences (feeding strategy and prey importance) of the three alien fish, as well as underlying patterns in diet variation. Our attention was mainly focused on the alien planktivores because of their key role on the top-down regulation of the trophic cascade (e.g. Christofferson et al., 1993; Williams & Moss, 2003; Tátrai et al., 2003a; 2003b; Blanco et al., 2004), especially juvenile fish (Romare et al., 1999).

MATERIALS AND METHODS

Study site

Lake Vela is one of the few natural shallow lakes (average depth *circa* 1 m) existing in Portugal, being the largest (60-70 ha) of a cluster of small shallow lakes located within a coastal dune system (Quiaios, Portugal). Agriculture, livestock activities and, to a lesser extent, domestic runoffs from adjacent human settlements supply regular nutrient inputs to the lake, originating high primary productivity and turbidity. Occasional fish kills due to transient anoxic stress have occurred in recent years (for details, see Antunes et al., 2003; Abrantes et al., 2006). Continuous introductions of alien fish species have contributed to a loss of diversity in the ichthyocenosis, which has been aggravated since the invasion of pumpkinseed in the late 1990s (José Vingada, unpublished data). The only study (Ferreira, 1997) on the fish community of Lake Vela was conducted in 1993/94 and it already reported dominance of alien species (mainly *G. holbrooki* and *M. salmoides*) and low diversity (a total of eight taxa were observed). Nonetheless, the presence of indigenous taxa, such as *Cobitis* sp. (frequent), *Anguilla anguilla* and *Atherina boyeri* (rare), was still reported. Tench (*Tinca tinca*) was also observed, although it is still unclear whether this species is part of the indigenous Portuguese ichthyofauna (Almaça, 1995a).

Sampling procedures

Fish were collected monthly from April to October 2003 as a pooled sample from (at least) two different sampling points in Lake Vela. An additional sampling campaign was carried out in January 2004 (for collection of additional gut content data – see below).

A combination of seine nets and electrofishing (using portable gear and dip nets) was used, covering both open-water and vegetated areas, with an approximate constant sampling effort. The joint use of such methods underestimates the abundance of large evasive fish but it is particularly useful in collecting the smallest specimens (namely new recruits), particularly in structured environments (near the shore and in macrophyte beds). Eel (*A. anguilla*) was particularly evasive because of its burying abilities and greater resistance to the electric pulse. All captured fish were sorted by taxon and immediately stored on ice, and later frozen.

In the laboratory, all individuals were counted and weighed (as a pooled sample), after thawing. Length measurements (TL) were performed in *M. salmoides*, *G. holbrooki* and *L. gibbosus* specimens, with a precision of 0.5 mm. Sexual differentiation was only carried out for *G. holbrooki* (juveniles, males and females), based on external sexual dimorphism (anal fin morphology). Because it is difficult to distinguish between undifferentiated juveniles and small-sized females, we used 18 mm as the cut-off value between juvenile (TL < 18 mm) and female mosquitofish (Cabral & Marques, 1999; García-Berthou, 1999). An initial subsample of 50 specimens (or all, if n < 50) for each combination of month and species was selected for diet analysis (see below), comprehending all possible size classes (centrarchids: < 50 mm, 50-100 mm and > 100 mm; mosquitofish: < 20 mm, 20-40 mm and > 40 mm). These specimens were eviscerated and non-empty stomachs (centrarchids) or guts (mosquitofish) were preserved in 70% ethanol, until further processing was possible. Since mosquitofish lack a distinctive and individualised stomach, unlike pumpkinseed and largemouth bass, the anterior third of the gut was excised (Cabral et al., 1998).

For diet analysis, observations focused only on non-empty stomachs in which most food categories could be clearly identified (proportion of digested material < 50% total stomach content volume) to minimise bias from differential digestion of prey items (Hyslop, 1980; Amundsen et al., 1996). For bass, all stomachs were analysed, while a total of 25 stomachs were analysed in average – per month – for mosquitofish and pumpkinseed, except where impossible. Gut contents were examined under a dissecting microscope, where prey items were usually sorted to genus or family level. Prey abundance was determined by counting each food item, using subsampling if necessary (especially for microinvertebrates), while prey volume (relative to total stomach content volume) was visually estimated. Although zooplankton taxa present in the diet were individually enumerated, volume estimates were performed for zooplankton as a group, pooling all zooplankters.

Data analysis

Size structure of the main fish taxa was analysed with analysis of variance (ANOVA) to assess seasonal and sexual (only in mosquitofish) variation in fish length. We tested for trends (linear trend contrasts) in fish length across months using orthogonal polynomials (Quinn & Keough, 2002). Additional planned contrasts and treatment-contrast interactions (Quinn & Keough, 2002) were used to explore interactions between main factors, when applicable. Fish length was log-transformed when this improved homoscedascity and linearity. A χ^2 test was used to test for independence between sex ratio and sampling month in mosquitofish.

Prey-specific abundance (*sensu* Amundsen et al., 1996) and frequency of occurrence (FO) were used to estimate the dietary importance of each food category. For prey *i*, prey-specific abundance (%) is the number of individuals or volume of prey *i* divided by the total number or volume of all prey items in predators who have ingested prey *i*. Correspondingly, FO corresponds to the proportion of stomachs containing prey *i* (relative to the total number of examined stomachs). These parameters were used to describe feeding strategy for each fish species using the graphical method of Amundsen et al. (1996), which is a modification from the Costello (1990) method. The former method has corrected some of the drawbacks of Costello's bi-dimensional diagrams concerning the feeding strategy of predators, by using prey-specific abundance instead of percent abundance (*sensu* Hyslop, 1980 and Costello, 1990), because the latter measure underestimates the importance of variation in resource use among individuals of the population. Thus, feeding strategy analysis according to Amundsen et al. (1996) provides an analytical approach to assessing dietary niche width components: a) within-phenotype component, which concerns variation in resource use within each individual (e.g. broad diet vs. preferential prey); b) between-phenotype component, which concerns variation in resource use between individuals of the same population (e.g. large vs. small fish). Information on (absolute) percent abundance can also be represented in the feeding strategy diagrams as isolines, resulting from the product between frequency of occurrence and prey-specific abundance (see Amundsen et al., 1996).

Detrended correspondence analysis (DCA) was applied to relative abundance data (excluding rare food categories) in order to describe the main sources of diet variation, using each fish (stomach) as a sampling unit. DCA is an improved eigenvector ordination technique based on reciprocal (weighted) averaging (Gauch, 1982; ter Braak, 1995). This family of techniques is commonly used in community ecology (Gauch, 1982; ter Braak, 1995) and also for dietary data (Godinho et al., 1997; Godinho & Ferreira, 1998a; 1998b; García-Berthou, 1999; García-Berthou & Moreno-Amich, 2000; García-Berthou, 2001; 2002). Its purpose is to reduce a species (food category) x sample (fish) matrix to a few dimensions (the eigenvectors) that explain the highest proportion of total variation in the data, without *a priori* pooling food categories, size classes or explanatory factors. Similar food categories are thus grouped based on common occurrence and the ordination can be interpreted with external data (see ter Braak, 1995). In this study, the ordination axes were interpreted in terms of the potential sources of variation of the samples (fish length, sampling month and sex), by analysing the sample (fish) scores with correlation analysis and ANCOVA (using log fish length as the covariate). Additionally, the direction of maximum correlation between fish length and the first two ordination axes was determined by multiple regression of (log) fish length on the fish scores of the first two dimensions (ter Braak, 1995). The graphical output is an arrow beginning at the centroid of the plot and whose relative length is proportional to the importance of fish length (ontogeny) in the ordination diagram.

Mean relative abundance and mean relative volume of the main food categories were analysed with ANCOVA (using fish length as covariate) in order to assess the significance of fish length (ontogenic component) and sampling month (seasonal component) in diet variation. Mean relative abundance and volume were arcsine square-root (arcsin $\sqrt{x}$) transformed and fish length was log-transformed to improve homoscedascity and linearity. ANCOVA's homogeneity of slopes assumption was verified by graphical analysis and by testing the interaction between factor (month) and covariate (fish length) under a full model (Quinn & Keough, 2002).

For descriptive purposes, diet overlap was estimated for all combinations of species and functional groups (length classes) in each month, using a simple algorithm for overlap similarity (Schoener, 1968; Abrams, 1980):

$$\alpha = 1 - 0.5 \cdot \sum \left| p_{ia} - p_{ib} \right|,$$

where α is overlap similarity – Schoener's (1968) index of overlap – between species/functional groups *a* and *b*, while p_{ia} and p_{ib} represent the proportion of resource *i* relatively to the total resources used by species/functional groups *a* and *b*, respectively. The index varies between 0 (no overlap) and 1, and values greater than 0.6 are considered biologically significant (Wallace, 1981).

RESULTS

Ichthyocenosis status and size structure

A total of 5063 specimens were caught from April to October, heterogeneously distributed in five taxa: *Lepomis gibbosus* (introduced), *Gambusia holbrooki* (introduced), *Micropterus salmoides* (introduced), *Cyprinus carpio* (introduced) and *Atherina* sp. (native). Eel (*Anguilla anguilla*) was also reported in the area by fishermen, but no specimens were caught in this study due to avoidance of fishing gear (see Materials and Methods). In January, additional 110 specimens (48 *L. gibbosus* and 62 *G. holbrooki*) were caught for gut content analysis data. The ichthyocenosis of Lake Vela was markedly dominated by pumpkinseed (*L. gibbosus*) and mosquitofish (*G. holbrooki*) in terms of abundance, with pumpkinseed being dominant in terms of biomass (Figure 2.1). Nearly half of the captured specimens (2417) were YOY pumpkinseeds collected in October, when they were highly abundant near the shore. YOY largemouth bass were well represented during their growing season (May-September), while only two YOY carps were caught in August. Sand smelt (*Atherina* sp.) was present from August to September. Because large specimens (> 200 mm) of bass and carp were captured only sporadically throughout the sampling period, they were not taken into account in abundance and biomass estimates (Figure 2.1). On-site direct observation showed that occasional capture of larger specimens occurred in unstructured areas (pelagic), but most fish were caught in or nearby structured habitats. Mosquitofish, in particular, was extremely abundant near the shoreline (in very shallow water), amidst terrestrial vegetation and aquatic macrophytes.

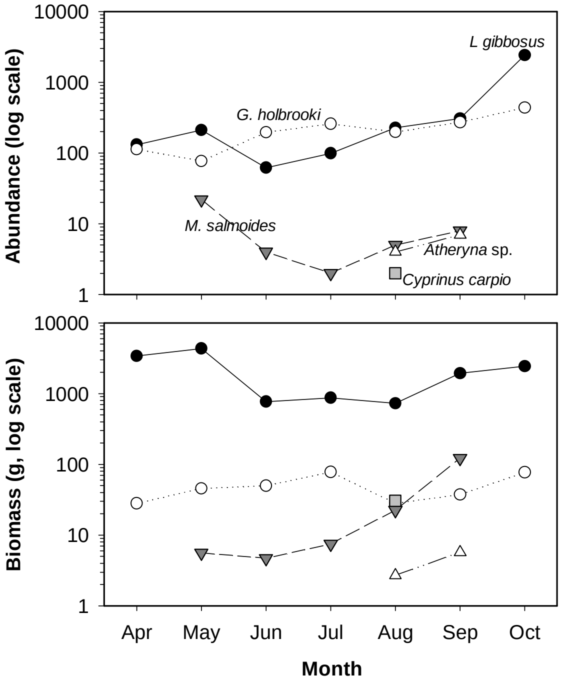

Figure 2.1 • Monthly variation in abundance (top) and biomass (bottom) of the fish species captured in Lake Vela.

Mean size of largemouth bass varied seasonally (1-way ANOVA: $F_{4,36}$ = 134, P = 0.000), increasing monotonically from May to September (linear trend contrast: $F_{1,36}$ = 403, P = 0.000). This species spawned in April (visual confirmation) and the first recruits were observed in early May. This synchronised new cohort exhibited fast growth and, by the end of the summer, most captured specimens were already above 100 mm (Figure 2.2).

In April, pumpkinseed showed a bimodal size structure, with the younger cohort progressively shifting the population’s mode to the left as they grew, from April to June. We observed nest-protection behaviour in late spring (May-June) and recruitment was continuously recorded from July to October (Figure 2.2). A progressive disappearance of larger size classes was recorded and, by the end of the summer, only YOY pumpkinseeds were caught (in high numbers near the shore). Consequently, mean size of pumpkinseed varied significantly between months (1-way ANOVA: $F_{6,1531}$ = 558, P = 0.000), decreasing monotonically from April to October (linear trend contrast: $F_{1,1531}$ = 2174, P = 0.000).

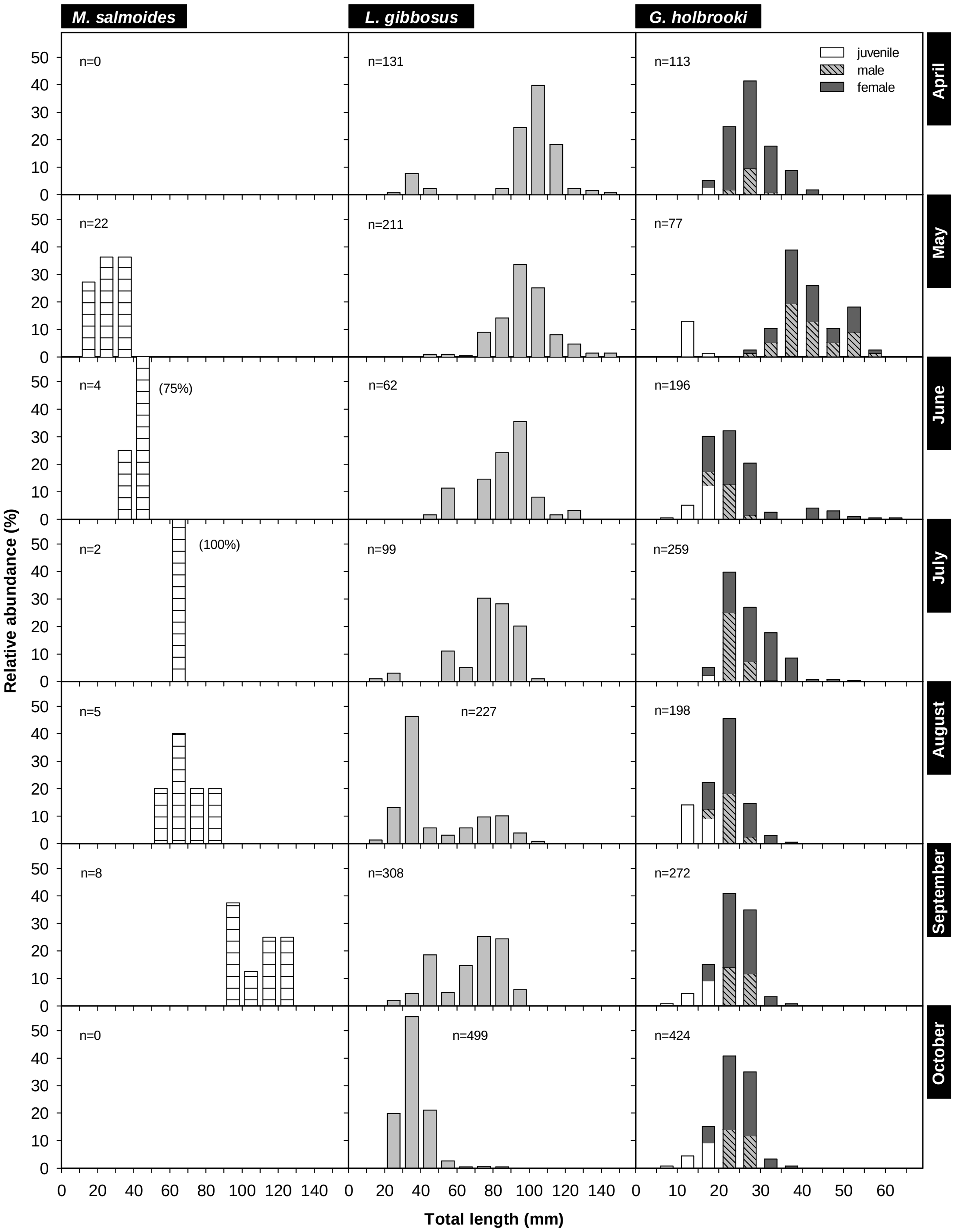

Figure 2.2 • Size structure of the most abundant fish species of Lake Vela, by month and by sex (only for *G. holbrooki*).

The larger portion of variance in mosquitofish length variation was due to sex (2-way ANOVA: $F_{2,1518} = 428$, $P = 0.000$), with female mosquitofish being overall larger than males (Figure 2.2). Seasonal variation in size structure was also recorded ($F_{6,1518} = 17.7$, P

= 0.000), but it was found to be sex-dependent (month x sex interaction: $F_{12,1518} = 23.7$, $P = 0.000$). Sexual dimorphism was shown to vary between months (male vs. female contrast x month interaction: $F_{1,1518} = 60.6$, $P = 0.000$) and linear trends in fish length were also found to vary with sex (male vs. female contrast x linear trend contrast: $F_{1,1518} = 60.6$, $P = 0.000$). The number of females was always higher than males (mean approximate ratio 3♀ : 1♂), but the absolute sex ratio (nº females/nº males) varied significantly with month (test of independence: $\chi^2_6 = 31.8$, $P = 0.000$), ranging from 1.75 (in May) to 6.86 (in April). Length-frequency histograms (Figure 2.2) revealed two recruitment periods (May-June and August-September), with a midsummer interval (July). This interval denoted cohort substitution, as the parental generation (larger fish) disappeared from July onwards and the new cohort (born in May) reproduced later in the summer.

Largemouth bass diet

Only 45 stomachs were analysed, comprising 6450 prey items. Overall, the most important food category for largemouth bass was zooplankton (see Figure 2.3), namely *Chydorus* but also Copepoda, *Bosmina* and *Daphnia*, followed by fish. Adult insects and larvae were less important (FO < 0.3), although in some cases giving a large contribution in terms of volume. A clear dichotomy was observable in the diet of bass (between-phenotype component of niche width), with a specialisation either on zooplankton or on fish (Figure 2.3). This dichotomy was directly related to fish length, given the clear ontogenetic shift observed (Figure 2.4), with smaller specimens (TL < 40 mm) being essentially zooplanktivorous and later becoming exclusive piscivores (TL > 60 mm). Adult insects and nymphs became important food categories in the transition between planktivory and piscivory. Piscivory in the sampled size classes of bass was almost exclusively centred on mosquitofish, the only exceptions being specimens beyond recognition due to digestion. Due to the low number of fish sampled and their rapid growth, any statistical analysis on seasonal and ontogenetic components of diet variation in largemouth bass would be biased. Still, when conjugating size structure (Figure 2.2) and ontogenetic variation in diet (Figure 2.4), it is possible to infer that planktivory in bass was intensive in May (and also July), while piscivory became important from August onwards.

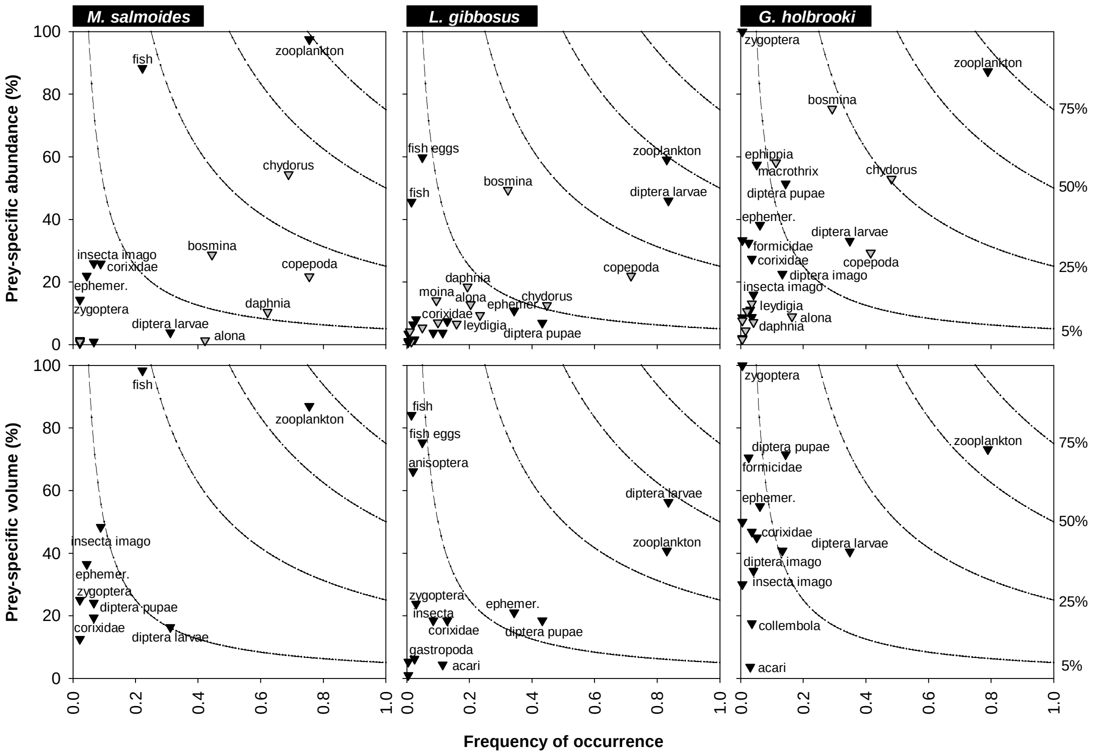

Figure 2.3 • Feeding strategy diagrams (according to Amundsen et al., 1996) for *M. salmoides* (n = 45), *L. gibbosus* (n = 201) and *G. holbrooki* (n = 195). Triangles represent different food items (only the main categories are labelled). On the abundance plots (top), microinvertebrates are depicted both as individual taxa (grey triangles) and as a pooled food category (zooplankton). Curves represent isolines (5-75%) of prey volume or abundance (see Amundsen et al., 1996).

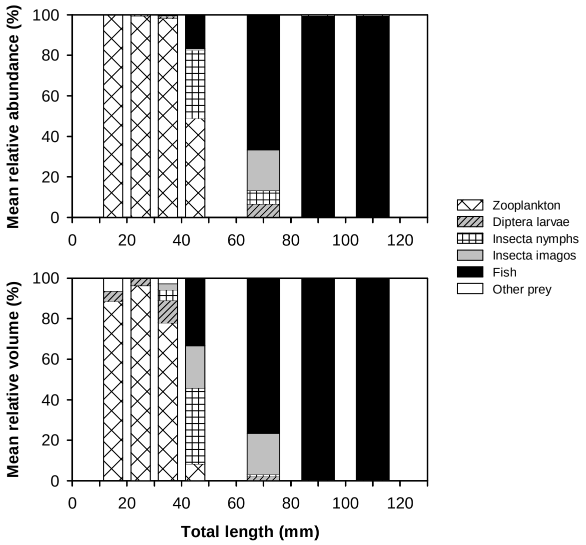

Figure 2.4 • Ontogenetic variation in the main food categories of largemouth bass (*M. salmoides*) diet. Data are presented as the proportion (%) of total number of prey (top panel) and stomach content volume (bottom panel), averaged for 10 mm (fish < 60 mm) and 20 mm (fish > 60 mm) length classes of bass.

Pumpkinseed diet

A total of 201 stomachs were analysed, distributed over 8 months and comprising 39 781 prey items. The most important food categories in pumpkinseed diet were, by far, zooplankton (especially copepods and *Bosmina*) and diptera larvae (Figure 2.3). The former gave a larger contribution in terms of abundance, while the latter were most relevant in terms of relative volume. Both categories were frequently found in pumpkinseed stomachs (FO > 0.8). Insect nymphs (particularly ephemeroptera) and diptera pupae were also of some importance in pumpkinseed diet, especially in terms of volume (Figure 2.3). Pumpkinseeds were mainly generalist (opportunistic) predators, with each individual profiting from a wide range of prey (i.e. reasonable within-phenotype component of niche width). The only exception to this generalist strategy was the high relative importance of large prey (odonata anisoptera nymphs and fish) and fish eggs, which was verified in a small percentage of individuals (FO < 0.05). This suggests some degree of feeding variation among individuals, as shown below.

The first two DCA axes were significantly correlated to fish length (axis 1: $r = -0.493$, $P = 0.000$; axis 2: $r = 0.388$, $P = 0.000$) and the direction of maximum correlation between the axes and fish length is shown in the diagram (Figure 2.5). This length gradient (arrow in biplot) demonstrated an ontogenetic diet shift in the diet of pumpkinseeds. Thus, smaller pumpkinseed fed mainly on small planktonic invertebrates (opposite direction to arrow), while larger pumpkinseed preferred larger prey, namely benthic macroinvertebrates and fish eggs (Figure 2.5). Besides ontogeny, there was also a seasonal component in diet variation, reflected in a significant effect of sampling month on fish scores of axis 1 (ANCOVA on month: $F_{7,191} = 23.9$, $P = 0.000$) and axis 2 (ANCOVA on month: $F_{7,191} = 51.0$, $P = 0.000$). As expected, fish length (covariate) was also significant for both axes (axis 1: $F_{1,191} = 57.7$, $P = 0.000$; axis 2: $F_{1,191} = 97.0$, $P = 0.000$), providing a larger contribution than month to the total variance of the models. Mean fish scores by month (Figure 2.5) showed some association with particular prey types which occurred in high abundances only in some months, such as *Daphnia* (April and January), *Alona* (May), *Bosmina* (October). August occupied a position relatively far from the centroid of the plot (arrow origin) due to the reduced planktivory observed during this month.

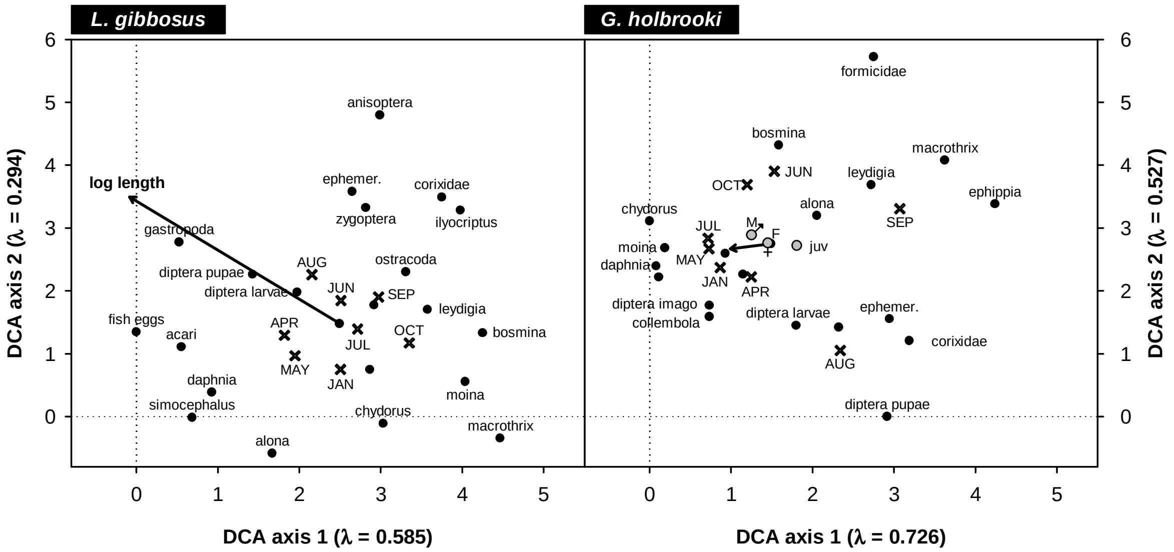

Figure 2.5 • Detrended correspondence analyses (DCA) of gut contents (prey relative abundance) of pumpkinseed (*L. gibbosus* - left) and mosquitofish (*G. holbrooki* - right). Prey category scores (●), mean fish scores by month (X), and mean fish scores by sex (only for mosquitofish: M, F and juvenile, in grey symbols) are shown for the first two ordination axes. Arrows begin at the centroid of the plot and represent the direction of maximum correlation between (log) fish length and the first two dimensions, for each fish species.

These evidences on diet variation were corroborated by analysing the main (pooled) food categories of pumpkinseed (see Figure 2.6 and Table 2.1). Figure 2.6 shows that zooplankton importance varied seasonally (e.g. reduced planktivory in August) and that smaller fish were more dependent on zooplankton, but the effects of month and fish length were not independent (significant interaction under full ANCOVA model, $P < 0.001$). Seasonal and ontogenetic variations in fish egg consumption were also observed, as this was only verified for fish TL > 100 mm and restricted from April to June. Again, however, these effects were statistically inter-dependent (significant interaction under full ANCOVA model, $P = 0.001$). The use of all other food categories varied seasonally and increased significantly with fish length (either in volume, abundance or both – see Table 2.1), except for insect larvae (no ontogenetic variation) and other prey (no seasonal variation). Diptera larvae and pupae were more important in August and April, but less important in October (Figure 2.6). Ephemeroptera nymphs were particularly important from July to September (in opposition to April and May), while other insect larvae (mostly corixidae and odonata) were only relevant in pumpkinseed diet in June and, to a lesser extent, in October (Figure 2.6).

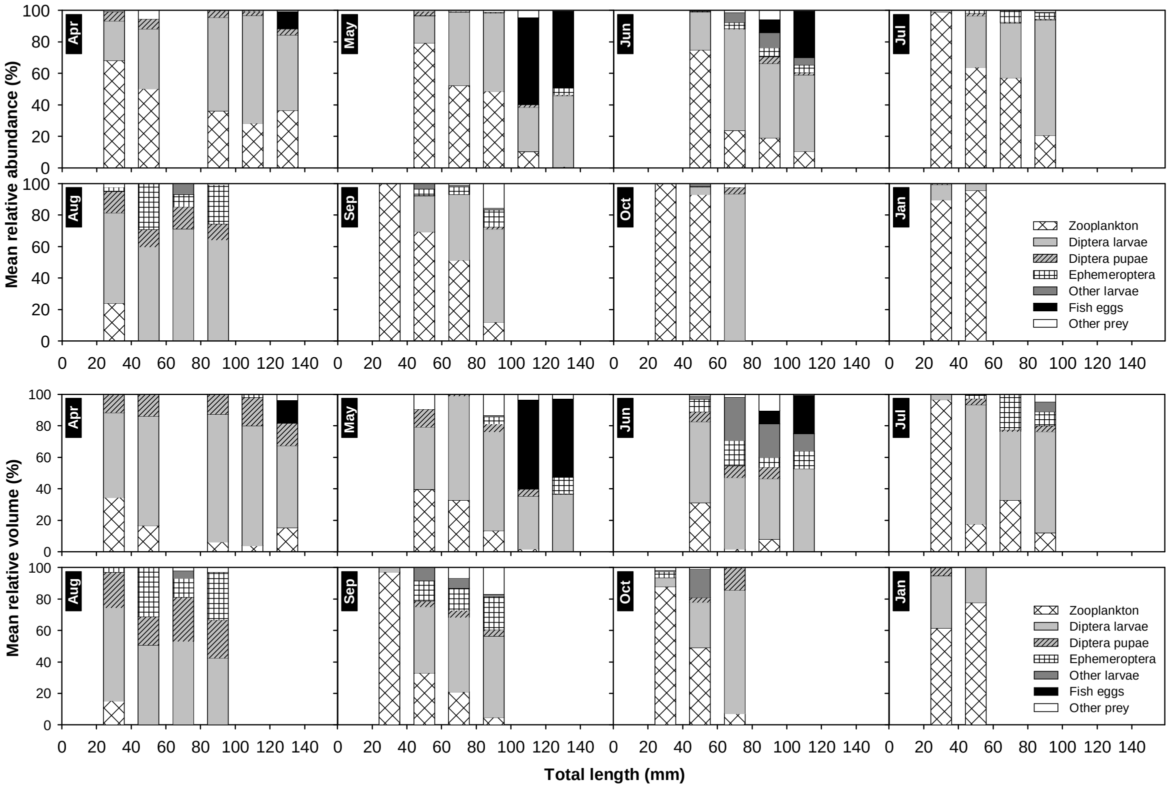

Figure 2.6 • Ontogenetic and seasonal variation in the main food categories of pumpkinseed (*L. gibbosus*) diet. Data are presented as the proportion (%) of total number of prey (top panel) and stomach content volume (bottom panel), averaged for 20 mm length classes of pumpkinseed.

Mosquitofish diet

We analysed 195 non-empty mosquitofish guts over the 8 months of sampling, comprising a total number of 10 889 prey items. Empty stomachs were frequent (unlike pumpkinseed and bass) but they were discarded for the analysis. The most important food category in mosquitofish diet was zooplankton (particularly *Bosmina*, *Chydorus* and copepods), both in volume and abundance (Figure 2.3). Diptera larvae were also important, although they were less frequently found in mosquitofish stomachs. Although foraging on zooplankton was frequent, mosquitofish displayed a broad resource use as a whole population. However, resource use in mosquitofish varied much between individuals (i.e. a high between-phenotype component of niche width), as demonstrated by high specific contributions (in abundance or volume) of rare (FO < 0.15) prey items, mostly insect imagos, nymphs or pupae (Figure 2.3).

Table 2.1 • Summary table of ANCOVAs applied to main food categories in pumpkinseed (*L. gibbosus*) diet. Fish length was used as covariate.

Food category	**Source**	**Relative abundance**		**Relative volume**	
		***F* statistic**	***P***	***F* statistic**	***P***
Zooplankton	Fish length Month	no homogeneity of slopes		no homogeneity of slopes	
Diptera larvae	Fish length	$F_{1,192}$ = 34.8	**0.000**	$F_{1,192}$ = 5.79	**0.017**
	Month	$F_{7,192}$ = 6.1	**0.000**	$F_{7,192}$ = 2.57	**0.015**
Diptera pupae	Fish length	$F_{1,192}$ = 2.82	0.095	$F_{1,192}$ = 5.34	**0.022**
	Month	$F_{7,192}$ = 14.1	**0.000**	$F_{7,192}$ = 10.3	**0.000**
Ephemeroptera	Fish length	$F_{1,192}$ = 21.8	**0.000**	$F_{1,192}$ = 15.9	**0.000**
	Month	$F_{7,192}$ = 13.2	**0.000**	$F_{7,192}$ = 10.0	**0.000**
Insecta larvae	Fish length	$F_{1,192}$ = 3.62	0.059	$F_{1,192}$ = 3.28	0.072
	Month	$F_{7,192}$ = 4.26	**0.000**	$F_{7,192}$ = 4.78	**0.000**
Fish eggs	Fish length Month	no homogeneity of slopes		no homogeneity of slopes	
Other prey	Fish length	$F_{1,192}$ = 3.73	0.055	$F_{1,192}$ = 6.87	**0.009**
	Month	$F_{7,192}$ = 0.70	0.672	$F_{7,192}$ = 1.23	0.286

The first DCA axis was significantly correlated to fish length (axis 1: r = -0.149, P = 0.038), unlike its second dimension (axis 2: r = -0.005, P = 0.942). The direction of maximum correlation between the axes and fish length is shown in the diagram, and it is almost coincident to the first DCA dimension (Figure 2.5). However, this weak (and marginally significant) ontogenetic gradient was shown to be overall unimportant in mosquitofish diet, as reflected in non-significant effects of fish length (covariate) on fish scores (ANCOVA on axis 1: $F_{1,169}$ = 1.49, P = 0.224; on axis 2: $F_{1,169}$ = 0.00, P = 0.988). We also found no significant diet variation that could be attributed to sex (axis 1: $F_{2,169}$ = 1.95, P = 0.145; axis 2: $F_{2,169}$ = 1.45, P = 0.236), although a marginally significant interaction between sex and sampling month was recorded in the second DCA dimension (axis 1: $F_{14,169}$ = 1.04, P = 0.415; axis 2: $F_{14,169}$ = 1385, P = 0.036). Not surprisingly, mean fish scores by sex (Figure 2.5) showed no association with any particular food category, being very close to the centroid of the plot. The largest contribution to the total variance in mosquitofish diet was due to sampling month (axis 1: $F_{7,169} = 45.3$, $P = 0.000$; axis 2: $F_{7,169} = 34.3$, $P = 0.000$). Consequently, mean fish scores by month showed a large scattering in the DCA biplot (Figure 2.5), displaying some association with particular prey types that occurred in noticeably high abundances in some months, such as *Chydorus* (July and May), diptera pupae (August), *Bosmina* (June and October) and *Macrothrix* (September).

These evidences on diet variation were corroborated by the analysis of main (pooled) food categories of mosquitofish (see Figure 2.7 and Table 2.2). Because differences in diet due to sex were found to be unimportant in the DCA, we excluded this factor from the analysis.

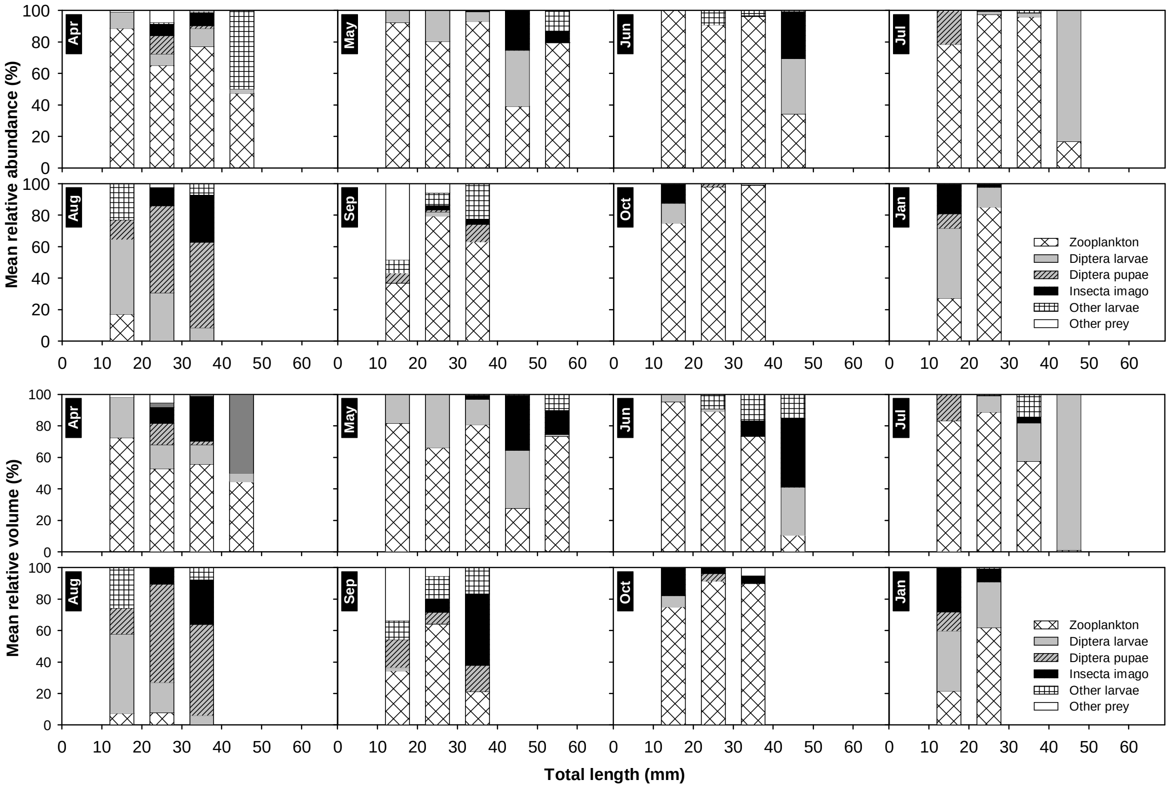

Figure 2.7 • Ontogenetic and seasonal variation in the main food categories of mosquitofish (*G. holbrooki*) diet. Data are presented as the proportion (%) of total number of prey (top panel) and gut content volume (bottom panel), averaged for 10 mm length classes of mosquitofish.

Figure 2.7 shows high variation in mosquitofish diet, both seasonally and ontogenetically, but trends were hard to interpret. As a consequence, significant interactions between fish length and month were recorded for diptera larvae, pupae and other prey category (significant interaction under full ANCOVA model, $P < 0.01$). The use of zooplankton was significantly less important in larger mosquitofish, while the use of adult insects increased with ontogeny (see Table 2.2 and Figure 2.7). In addition, the importance of zooplankton as prey also varied seasonally (Table 2.2), being noticeably reduced in August and attaining its maximum peak in October and July (Figure 2.7). No seasonal or ontogenetic variation was found on the use of insect larvae (Table 2.2).

Table 2.2 • Summary table of ANCOVAs applied to main food categories in mosquitofish (*G. holbrooki*) diet. Fish length was used as covariate.

Food category	Source	Relative abundance		Relative volume	
		***F* statistic**	***P***	***F* statistic**	***P***
Zooplankton	Fish length	$F_{1,186} = 4.33$	**0.039**	$F_{1,186} = 8.20$	**0.005**
	Month	$F_{7,186} = 22.6$	**0.000**	$F_{7,186} = 16.1$	**0.000**
Diptera larvae	Fish length Month	no homogeneity of slopes		no homogeneity of slopes	
Diptera pupae	Fish length Month	no homogeneity of slopes		no homogeneity of slopes	
Insecta imago	Fish length	$F_{1,186} = 11.2$	**0.001**	$F_{1,186} = 19.7$	**0.000**
	Month	$F_{7,186} = 1.58$	0.145	$F_{7,186} = 1.61$	0.136
Insecta larvae	Fish length	$F_{1,186} = 0.76$	0.385	$F_{1,186} = 1.27$	0.262
	Month	$F_{7,186} = 1.77$	0.097	$F_{7,186} = 1.94$	0.065
Other prey	Fish length Month	no homogeneity of slopes		no homogeneity of slopes	

Diet overlap

Based on diet composition and variation, groups of size classes within species (functional groups) were distinguished (see Table 2.3). Schoener overlap indices, calculated for inter-and intra-specific pairs of functional groups, varied between 0 and 0.93 (Table 2.3). There was inconsistency in overlap values, due to strong seasonal variation, but values were overall low (Table 2.3). High intra-specific overlap was observed in some cases among pumpkinseed and mosquitofish, the latter case being consistent in spring. In pumpkinseed, occasional high overlap values were more noticeable between the larger size classes, while smaller pumpkinseed seemed to explore different resources (except in August). Low values of α were found between mosquitofish and pumpkinseed functional groups (Table 2.3), except in some cases involving small pumpkinseed (LG1) and small- and medium-sized mosquitofish (GH1 and GH2). Diet overlap between pumpkinseed and largemouth bass and mosquitofish and bass was low, except in May (Table 2.3), when YOY bass used similar resources (zooplankton) to mosquitofish.

Table 2.3 • Diet overlap (Schoener's index, α) calculated for all combinations of species and functional groups (size classes) in each month. Functional groups for pumpkinseed (LG) and mosquitofish (GH) are LG1 (< 60 mm), LG2 (60-100 mm), LG3 (> 100 mm), and GH1 (< 20 mm), GH2 (20-40 mm), GH3 (> 40 mm), respectively. MS stands for largemouth bass. Values in bold represent biologically significant overlaps ($\alpha > 0.6$).

Combination		Apr	May	Jun	Jul	Aug	Sep	Oct	Jan
LG-LG	LG1-LG2	0.40	0.60	0.41	0.53	**0.83**	0.54	0.03	
	LG1-LG3	0.38	0.26	0.27					
	LG2-LG3	**0.93**	0.41	**0.65**					
GH-GH	GH1-GH2	**0.68**	**0.88**	**0.86**	0.34	0.40	0.41	0.36	0.50
	GH1-GH3		**0.68**	0.34	0.00				
	GH2-GH3	0.50	**0.70**	0.34	0.03				
LG-GH	LG1-GH1	**0.64**	0.54	0.47	**0.70**	**0.72**	0.14	0.38	0.36
	LG1-GH2	**0.69**	0.49	0.45	0.40	0.38	0.24	**0.93**	0.46
	LG1-GH3	0.50	0.50	0.59	0.23				
	LG2-GH1	0.20	0.39	0.07	0.29	**0.74**	0.173	0.13	
	LG2-GH2	0.22	0.39	0.09	0.14	0.39	0.16	0.00	
	LG2-GH3	0.10	0.35	0.39	**0.64**				
	LG3-GH1	0.18	0.15	0.10					
	LG3-GH2	0.19	0.17	0.06					
	LG3-GH3	0.08	0.24	0.36					
LG-MS	LG1-MS		0.37	0.24		0.07	0.00		
	LG2-MS		0.30	0.23		0.11	0.07		
	LG3-MS		0.05	0.09					
GH-MS	GH1-MS		**0.61**	0.09		0.07	0.00		
	GH2-MS		**0.69**	0.13		0.09	0.00		
	GH3-MS		0.56	0.07					

DISCUSSION

Ichthyocenosis status

The ichthyocenosis of Lake Vela was poorly diversified (6 taxa) and exhibited marked dominance of alien omnivorous species (chiefly *L. gibbosus* and *G. holbrooki*). This high degree of homogenisation has heightened the prevalence of small-sized omnivores and low abundance of piscivores, which represent structural features usually assigned to fish assemblages from warmer areas (Blanco et al., 2003). Lake Vela represents an extreme scenario and is therefore a potential candidate for restoration and progressive re-introductions of native fish, especially because of its small size (70 ha). However, information on its current ichthyocenosis status was lacking until now. This study has shown a marked dominance of the bass-pumpkinseed assemblage in Lake Vela, with very high abundances of the latter. Carp is more frequent in the deeper zones of the lake (Ferreira, 1997), while mosquitofish was restricted to the shallow protected margins. The use of littoral electrofishing coupled with seine nets was chosen because fish distribution in Lake Vela had shown to be extremely heterogeneous (Ferreira, 1997), and most catches were performed in structured habitats. However, the method clearly underestimated largemouth bass and carp populations, due to the larger size of their adults and evasiveness.

In comparison to the only study carried out so far in Lake Vela (Ferreira, 1997), a reduction in biodiversity was observed. This was associated with the introduction and proliferation of the invasive pumpkinseed (*L. gibbosus*) – presence not recorded in Ferreira's study – although there is not enough information to conclude if this was the cause for local extinction of the few resilient native species (such as *Cobitis* sp.). Alien fish species pose a major concern to the conservation status of Iberian freshwater fish assemblages, which are unique because of their high degree of endemicity (Elvira, 1995; Almaça, 1995b). Small lakes and warm monomictic reservoirs (particularly in the southern part of Portugal) are especially prone to proliferation of a dominant bass-pumpkinseed (Centrarchidae) assemblage (Godinho & Ferreira, 1994; García-Berthou & Moreno-Amich, 2000; García-Berthou, 2002).

Largemouth bass ecology

The absence of other piscivores in Iberian freshwaters favoured the introduction of largemouth bass (*M. salmoides*) as gamefish in the 1950s. In Portugal, well-established populations exist for a long time in the quiet waters of lakes and reservoirs, particularly in the centre and south of the country (Godinho & Ferreira, 1994; Almaça, 1995a). In Lake Vela, bass produced a more or less synchronised YOY cohort in early May, which continuously grew until September (at least). García-Berthou's (2002) study also reported reproduction in May and rapid juvenile growth during the summer in Lake Banyoles (Spain). Lorenzoni et al. (2002) showed that reproduction in bass from Lake Trasimeno (Italy) was concentrated in a brief period from May (mostly) to June, when water temperatures were closer to 20º C or above.

In spite of the low number of individuals sampled, our study showed that largemouth bass experienced an ontogenetic diet shift to progressively larger prey until becoming an exclusive piscivore, as shown for other Iberian populations (Godinho & Ferreira, 1994; Godinho et al., 1997; García-Berthou, 2002). In Lake Vela, zooplanktivory was particularly important in smaller bass (< 40 mm), while insects (adults, nymphs and larvae) were only important in the summer (July-August), during the transition to piscivory. Other studies in Iberia have shown a much higher use of macroinvertebrates in bass diet, namely shrimp (*Atyaephyra)* and insect nymphs (Godinho & Ferreira, 1994; Godinho et al., 1997; García-Berthou, 2002). This is probably related to the high zooplanktonic productivity of Lake Vela and low importance of macroinvertebrates other than diptera larvae (unpublished results from our team). Resource availability is likely to be the main factor behind the high heterogeneity in bass diet reported in Iberia (Rodríguez, 1989; Godinho & Ferreira, 1994; Godinho et al., 1997; García-Berthou, 2002), which strengthens the idea of Godinho et al. (1997) that bass forage on whichever prey is most frequent and abundant. The shift to exclusive piscivory (main prey mosquitofish) observed in Lake Vela can be considered precocious. This could be due to bias resulting from reduced sample size or a consequence of the extremely high availability of mosquitofish near the shore. Further data are needed for additional inference on piscivory in bass, including stomach content data from larger specimens (> 120 mm).

Pumpkinseed sunfish ecology

Pumkinseed introduction in Lake Vela is recent (less than 10 years), but the fish assemblage is now dominated by this centrarchid. One of the reasons for its success is the multi-spawner strategy and early maturity of Iberian populations (Vila-Gispert & Moreno-Amich, 1998; 2000; Gutiérrez-Estrada et al., 2000). In Lake Vela, the pumkinseed population was mainly dominated by small-sized specimens, as a result from continuous recruitment from July to October. Progressive disappearance of larger size classes could be

related to the migration of larger specimens to deeper waters (García-Berthou & Moreno-Amich, 2000).

Pumpkinseed is essentially an omnivorous feeder, adapted to benthic feeding, with a known preference on macroinvertebrates, although zooplankton is also an important prey, particularly in the smallest specimens (Godinho et al., 1997; Godinho & Ferreira, 1998a; García-Berthou & Moreno-Amich, 2000). Our results confirmed this and also showed a strong seasonal variation in the use of macroinvertebrates, which were most relevant during the summer (depending on the specific taxon, see results). Diptera larvae, in particular, constituted a frequent and important prey item, which is a common feature of other European populations (Rodríguez, 1989; Godinho et al., 1997; Godinho & Ferreira, 1998a; Wolfram-Wais et al., 1999). Still, most pumpkinseed explored numerous food resources, displaying a reasonable within-phenotype variation in diet, which has also been observed in other Iberian populations (Rodríguez, 1989; Godinho et al., 1997; Godinho & Ferreira, 1998a). In its natural range of distribution (North America), the adults are viewed as moluscivores (feeding mostly on snails), but this feature seems to be much less important in Iberian populations (Godinho et al., 1997; García-Berthou & Moreno-Amich, 2000), including Lake Vela. Instead, large fish foraged on fish eggs (chiefly in May) in considerable amounts (number and volume), as also reported by García-Berthou & Moreno-Amich (2000) and Copp et al. (2002). As these authors, we suspect egg consumption to be intra-specific (i.e. cannibalism). Unlike the population studied by Copp et al. (2002), piscivory in Lake Vela was residual, probably because other resources were available for pumpkinseed in Lake Vela.

Mosquitofish ecology

Ferreira (1997) had already shown that mosquitofish concentrated in very high densities near the shoreline in Lake Vela, amidst vegetation. This is a useful defence mechanism from bass predation (see above), as bass forages less efficiently in structured habitats (see e.g. Godinho & Ferreira, 1994). Due to its small size and susceptibility to predation, mosquitofish is usually restricted to vegetated zones and/or shallow habitats, such as marshes, rice paddies or irrigation channels (Fernández-Delgado, 1989; Cabral & Marques, 1999; García-Berthou, 1999). In Lake Vela, an extremely abundant population dominated by females (except in May) displayed substantial sexual activity throughout spring and summer. As shown by other authors (Fernández-Delgado, 1989; Fernández-Delgado & Rossomanno, 1997), mosquitofish produced two generations during the reproductive period. Most of the parental generation disappeared after the spring reproduction period (Fernández-Delgado, 1989; Fernández-Delgado & Rossomanno, 1997; Cabral & Marques, 1999) and some of their progeny (early spring cohort) gave birth to a late summer generation.

This small-sized Poeciliidae is native to eastern USA and Mexico and it primarily feeds on zooplankton (chiefly cladocerans) and insect larvae and adults (Rodríguez, 1989; Cabral et al., 1998; García-Berthou, 1999; Blanco et al., 2004). Unlike Cabral et al. (1998), we found no significant diet variation due to sex. Mosquitofish diet varied seasonally and only a weak ontogenetic diet shift was observed, corroborating García-Berthou's (1999) observations. Although littoral zooplankton was the main food category (both in abundance and volume), a large variety of other prey (omnivory) was consumed (as shown by Rodríguez, 1989; García-Berthou, 1999), including benthic larvae (mostly dipterans), terrestrial insects and aquatic neuston (chiefly diptera pupae and imagos). Substantial variation among individual fish was recorded, resulting in a high between-phenotype component of niche width. The large seasonal component in diet variation suggests a high degree of opportunist feeding, with mosquitofish foraging on the most available resource in each month.

Resource exploitation in alien taxa

Largemouth bass, pumpkinseed and mosquitofish displayed substantial variation in their diet, suggesting both ontogenetic shifts and opportunistic feeding. The latter could not be totally confirmed because of the lack of a contemporaneous resource matrix, but the high seasonal variation of mosquitofish and pumpkinseed diet strongly suggests so. Water column niche was very important (planktivory) and diversified in all taxa, while the benthic niche was chiefly represented by diptera larvae (most other macroinvertebrates were found on an occasional basis). The opposite (low importance of planktivory) has been reported in lotic habitats (Godinho et al., 1997; Godinho & Ferreira, 1998a). The low diversity of the benthic environment is the likely result of low macrophyte coverage in Lake Vela, which is a consequence of very high turbidity. In spite of high degree of planktivory and poor benthic resources, most combinations between functional groups revealed low diet overlap. Despite opportunistic feeding, there was no consistent evidence for resource competition among these taxa, although some combinations between YOY pumpkinseed and mosquitofish showed high overlap indices. The less noticeable ontogenetic diet shift in mosquitofish enhanced resource overlap between intra-specific functional groups during the spring reproduction period (when two cohorts coexisted).

The overall low diet overlap between the most abundant fish species in Lake Vela is one of the factors contributing to the success and coexistence of these alien taxa. Other factors include omnivory and ontogenetic diet shifts (observed in pumpkinseed and bass), which allow a broader resource use, reducing inter- and intra-specific competition, thus enhancing growth rates and fecundity. As a result, as seen above, the production of numerous offspring during the reproductive period allows the maintenance of large populations, which exert strong top-down effects on lower trophic levels. The current study could not evaluate the impact of piscivory by bass on mosquitofish and pumpkinseed populations.

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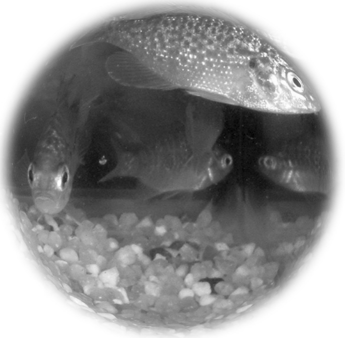
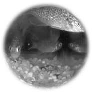


Capítulo 3

Dinâmica sazonal da planctivoria na ictiocenose



Planctivoria em peixes alienígenas e implicações para as interacções tróficas de um lago Mediterrânico pouco profundo

In: Castro B.B. & Gonçalves F. (submetido). Planktivory in non-indigenous fish and implications for trophic interactions in a Mediterranean shallow lake.

RESUMO

À semelhança de outros lagos Mediterrânicos, a Lagoa da Vela exibe um estado avançado de homogeneização da sua fauna piscícola (dominância marcada de espécies alienígenas). Todavia, tem sido dada pouca importância ao impacto de uma comunidade exótica nos níveis tróficos inferiores dos lagos pouco profundos e eutróficos. Neste estudo, a zooplanctivoria na ictiocenose omnívora da Lagoa da Vela foi examinada de Abril a Outubro de 2003 (e também em Janeiro de 2004). Durante este período, a ictiocenose caracterizou-se por elevada abundância de peixes juvenis, que consumiram primordialmente zooplâncton. Os adultos de gambúzia (*Gambusia holbrooki*) e perca-sol (*Lepomis gibbosus*) recorreram às mesmas presas que os seus juvenis, apesar da planctivoria ser menos importante nos peixes de maiores dimensões. Com o recurso à análise multivariável, foi possível atribuir a maior parte da variabilidade na planctivoria à disponibilidade das presas, na medida em que ambas as espécies de peixes ingeriram os zooplanctontes mais abundantes em cada mês. Apesar deste comportamento oportunista, a gambúzia exibiu selecção positiva relativamente a cladóceros litorais, enquanto a perca-sol foi selectiva com respeito a *Alona* e *Daphnia*. Na ausência de planctívoros mais eficientes, a perca-sol é agora a principal espécie planctívora na Lagoa da Vela e o predador principal de *Daphnia*. Todavia, devido às baixas densidades de *Daphnia* na maior parte do ano, este cladócero esteve virtualmente ausente da dieta da perca-sol de Junho a Outubro. Durante este período, a perca-sol explorou predominantemente presas de menores dimensões. A predação sobre *Daphnia* na primavera deverá ter contribuído para o colapso dos dafníideos em Maio, mas a sua ausência durante o Verão e Outono não pode ser explicada sem o recurso a outras variáveis.

PALAVRAS-CHAVE

Interacções tróficas, planctivoria, peixes alienígenas, lago Mediterrânico pouco profundo, disponibilidade de recursos

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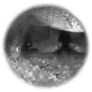
O presente capítulo constitui um manuscrito submetido para publicação em revista internacional com arbitragem científica e é, por esse motivo, apresentado na língua inglesa.



Planktivory in non-indigenous fish and implications for trophic interactions in a Mediterranean shallow lake

ABSTRACT

Like other Mediterranean lakes, Lake Vela displays an advanced state of fish fauna homogenisation (marked dominance of alien species), but the impact of such an assemblage on the lower trophic levels of shallow eutrophic lakes has been overlooked. In this study, zooplanktivory in the omnivorous fish assemblage of Lake Vela was examined from April to October 2003 (and also in January 2004). During this period, the ichthyocenosis was characterised by high abundances of juvenile fish, which strongly depended on zooplankton. Adult mosquitofish (*Gambusia holbrooki*) and pumpkinseed (*Lepomis gibbosus*) foraged on the same zooplanktonic prey than juveniles, although planktivory was less important in larger fish. Using canonical correspondence analysis, it was possible to attribute most of the variability in planktivory to resource availability, as both fish foraged on the most abundant prey in each month. Albeit this opportunistic behaviour, mosquitofish was found to be positively selective towards small-sized littoral cladocerans, while pumpkinseed displayed positive selection towards *Alona* and *Daphnia*. In the absence of more efficient planktivores, pumpkinseed is now the main planktivore in Lake Vela and the main predator of *Daphnia*. However, due to low densities of *Daphnia* during most of the study period, *Daphnia* was virtually absent from the diet of pumpkinseed from June to October, a period during which this benthi-planktivore foraged chiefly on less-rewarding prey (small-sized cladocerans and cyclopoid copepods). Predation upon *Daphnia* in spring may contribute to daphniid collapse in May, but their absence during summer and autumn cannot be explained without additional influence of undetermined factors.

KEYWORDS

Food-web interactions, planktivory, alien fish, Mediterranean shallow lake, resource availability

INTRODUCTION

Most literature available on freshwater trophic cascades in Europe concerns northern temperate lakes, where the structuring role of the pelagic fish assemblage on lower trophic levels has been studied in detail (see e.g. Christofferson et al., 1993; Jacobsen et al., 1997; Romare et al., 1999; Eklov & VanKooten, 2001; Romare & Hansson, 2003). At these latitudes, pike (*Esox lucius*) and/or perch (*Perca* spp.) usually represent the main piscivores, while roach (*Rutilus rutilus*) and juvenile perch make up the planktivore assemblage (Luecke et al., 1990; Jacobsen et al., 1997; Romare et al., 1999; Eklov & VanKooten, 2001; Irz et al., 2002; Romare & Hansson, 2003). However, little information is available on trophic interactions in Mediterranean lakes, and especially in Iberia, where fish communities of inland waters exhibit strong dominance of omnivorous cyprinids (such as *Barbus* spp., *Chondrostoma* spp. and *Squalius* spp.) and absence or low abundance of piscivores (Almaça, 1995; Blanco et al., 2003). Moreover, in most Iberian habitats where water current is low and aquatic vegetation is present, small-sized omnivorous invaders (chiefly mosquitofish and pumpkinseed) have become the predominant fish species, displacing indigenous fauna and reducing biodiversity. The impact of such a fish assemblage on the lower trophic levels of Mediterranean lakes has been overlooked, particularly in shallow polymictic lakes, where eutrophication is a serious threat to biodiversity and ecosystem function.

Planktivores are known to be size-selective, preferring larger, more rewarding prey (Hall et al., 1976; Gliwicz, 1994), but they must also adapt to dynamic fluctuations in the density thresholds of their prey (Gliwicz, 2001; 2002; Gliwicz et al., 2004; Scheuerell et al., 2005). Flexible foraging behaviour (Roseman et al., 1996; Scheuerell et al., 2005) allows predators to explore diverse food resources without compromising growth rates. The importance of planktivory in freshwater ecosystems is well illustrated by the structuring role of planktivores in trophic cascading phenomena (e.g. Christofferson et al., 1993; Williams & Moss, 2003; Tátrai et al., 2003a; 2003b; Blanco et al., 2004), especially YOY fish (Post et al., 1997; Romare et al., 1999). In Lake Vela (Portugal), cascading effects were evident in 2001 when an abnormal *Daphnia*-dominance phase was recorded during the whole summer, after a large fish kill at the end of May, leading to effective control of summer phytoplankton (Antunes et al., 2003; Abrantes et al., 2006). Lake Vela displays a poorly diversified fish community, dominated by alien taxa, which exert strong predatory pressure on zooplankton. Non-natives pumpkinseed (*Lepomis gibbosus* (L.)) and mosquitofish (*Gambusia holbrooki* Girard), in particular, are present in very high abundances. Diet analysis of these taxa revealed a generalist (omnivorous) diet (Castro et al., submitted manuscript[1]), making both species little dependent of a few food categories. This allows them to attain high growth rates and reduced mortality, as a result of a phenotypically plastic exploitation of resources (detritus, benthic fauna and zooplankton). Although more important in the diet of smaller specimens, zooplanktivory was substantial in all life stages of pumpkinseed and mosquitofish. The use of zooplankton was also

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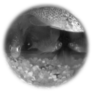
[1] CAPÍTULO 2 da tese



important in the diet of juvenile (YOY) largemouth bass (*Micropterus salmoides* (Lacépède)).

Here, we present a detailed analysis on the use of zooplankton in the diet of the main planktivorous fish from Lake Vela. The main goals of the study were: 1) to identify the main sources of variation (ontogeny vs. resource availability) in the diet of YOY bass, pumpkinseed and mosquitofish; 2) to assess the relative importance of the main zooplanktonic taxa in the diet and to detect patterns of prey selection (electivity); 3) to improve our understanding of fish-zooplankton interactions in shallow eutrophic lakes facing fish fauna homogenisation.

MATERIALS AND METHODS

Fish sampling and gut content analysis

Fish were collected monthly from April to October 2003 as a pooled sample from (at least) two different sampling points in Lake Vela. In order to make our data set more comprehensive, we also captured fish later in January (winter). A combination of seine nets and electrofishing (using portable gear and dip nets) was used, covering both open-water and vegetated areas, with an approximate constant sampling effort. The joint use of such methods underestimates large evasive fish but it is particularly useful in collecting smaller specimens (including new recruits), especially in structured environments (near the shore and in macrophyte beds). All captured fish were sorted by taxon and immediately stored on ice, and later frozen.

In the laboratory, all individuals were counted after thawing, and length measurements (TL) were performed with a precision of 0.5 mm. An initial subsample of 50 specimens (or all, if n < 50), comprehending all possible size classes for each combination of month and species, was selected for gut content inspection. These specimens were eviscerated and non-empty stomachs were preserved in 70% ethanol, until further processing was possible. Since mosquitofish lack a distinctive and individualised stomach, the anterior third of the gut was excised (Cabral et al., 1998). Enumeration of gut contents was only carried out on non-empty stomachs in which most food categories could be clearly identified (proportion of digested material < 50% total stomach content volume) to minimise bias due to differential digestion of prey items (Hyslop, 1980; Amundsen et al., 1996). All bass stomachs were analysed, while a total of 25 stomachs per month were analysed in average for mosquitofish and pumpkinseed. Gut contents were sorted to genus or family level and counted, under a dissecting microscope. If necessary, subsampling was used for very abundant contents. Volume of the total zooplankton (pooling all taxa) relative to total stomach content volume was visually estimated.

Zooplankton sampling

Zooplankton was sampled fortnightly from April 2003 to January 2004 as a pooled composite sample from two different sampling points in Lake Vela: a vegetation-free site and near a macrophyte (*Nymphaea alba*) patch. Composite water samples were collected at each site with a vertical Van Dorn bottle (sample volume: 16-25.6 L) and concentrated with a 55-µm-mesh plankton net. Each sample was immediately preserved in sucrose-saturated 4% formalin, stained with Bengal rose, and stored until further examination. In the laboratory, cladocerans were identified to the genus/species level and copepods were separated into nauplii and copepodites + adults (cyclopoid or calanoid). Counting by subsampling was used for the most abundant taxa, but the whole sample was usually screened in order to enumerate large organisms (particularly *Daphnia*). Zooplankton abundance data were expressed as densities (ind L^{-1}).

Data analysis

Correlation was used to assess the relationship between zooplankton ingestion and fish size (Pearson's r was used for abundance data, while Spearman's r_s was utilised for percent volume). Log transformation of data was used when this improved linearity.

Canonical correspondence analysis (CCA; ter Braak, 1986) was used to describe the main sources of diet variation in each fish species. This family of multivariate techniques has already been used in the analysis of dietary data (Godinho et al., 1997; Godinho & Ferreira, 1998; García-Berthou, 1999; García-Berthou & Moreno-Amich, 2000; García-Berthou, 2001; 2002). The purpose of CCA is two-fold: it reduces a species x sample matrix to a few dimensions (the eigenvectors) that explain the highest proportion of total variation in the data while, at the same time, it extracts "environmental" gradients that enable the researcher to ascertain and quantify relationships between species data and explanatory variables (ter Braak, 1986; 1995). In our study, CCA was applied to prey (zooplankton) abundance data, log transformed, using each fish (stomach) as a sampling unit. Rare prey items were down-weighted to minimise their influence in the analysis (ter Braak, 1995). Two environmental matrices were taken into account as potential sources of variation in planktivory: 1) a resource matrix, comprising the densities of the zooplanktonic taxa recorded in the lake; 2) a (log) fish size matrix. The statistical significance of the model was tested using a Monte-Carlo (unrestricted) permutation test. A CCA-derived variation partitioning technique (Borcard et al., 1992; Økland & Eilersten, 1994) was used to quantify the variation explained by resource availability and fish size. This was performed by partialling out (as covariables - see ter Braak, 1988) each of the subsets of explanatory variables at a time and comparing the percentage of variance explained by the partial CCAs with the one obtained with the global CCA model (resource availability + fish size).

The preferences of fish for different zooplankton species were estimated for each predator and prey item using Vanderploeg & Scavia's relativised electivity index (E^*), following Lechowicz (1982):

$$E_i^* = \frac{W_i - (1/n)}{W_i + (1/n)}, \text{ with } W_i = \frac{r_i/p_i}{\sum r_i/p_i},$$

where r_i is the percent share (proportional abundance) of prey i in the diet, p_i is the percent share of prey i in the environment, and n is the number of prey types included in the analysis. The index ranges from -1 (negative selection) to 1 (positive selection), with values close to zero indicating neutral selectivity (see also Lechowicz, 1982; Gliwicz et al., 2004; Alcaraz & Garcia-Berthou, 2006). Spearman correlation coefficient (r_s) was used to assess the relationship between electivity and fish size (TL). To test whether electivity significantly deviated from 0 (i.e. neutral selection), a one-sample sign test was applied to the most important prey taxa. The use of this simple non-parametric method was justified by the non-normal and/or highly skewed distribution of the data. A significance level (α) of 0.05 was used.

RESULTS

The fish community of Lake Vela was dominated in numbers by pumpkinseed sunfish (*L. gibbosus*), mosquitofish (*G. holbrooki*) and largemouth bass (*M. salmoides*). These three alien fish species displayed a high degree of planktivory, depending on their ontogeny. Bass reproduced early in spring and the new cohort grew fast until the end of the summer (Figure 3.1). Planktivory was restricted to its very early life stages (TL < 50 mm), which corresponded to the period from May to June (Table 3.1). Figure 3.2 clearly shows the ontogenetic diet shift in bass, which became exclusive piscivores at TL > 60 mm. Because planktivory was restricted in time, and due to the low number of bass specimens caught, we decided not to analyse in further detail the planktivory data in this species.

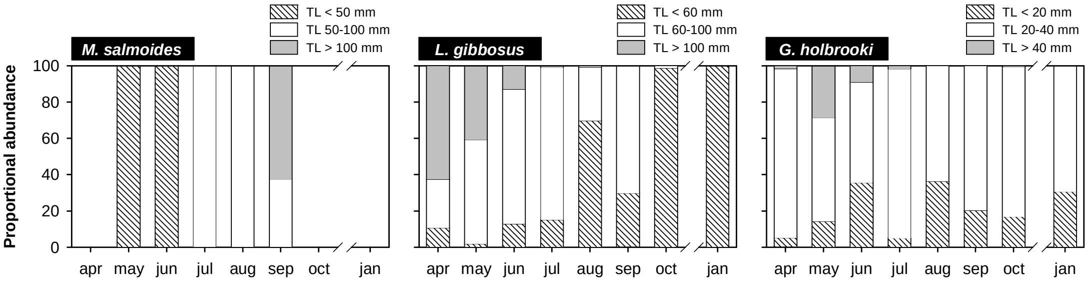


Figure 3.1 • Monthly variation in size structure of the main planktivorous fish captured in Lake Vela.

Table 3.1 • Planktivory in YOY largemouth bass in Lake Vela from April to January. The contribution of zooplankton to total diet is shown as mean abundance relative to total stomach contents (%A) and percent occurrence (%O). Results are presented as percent composition in terms of numerical abundance. ND stands for no data.

Month	Contribution of zooplankton to total diet		Mean percent composition within zooplankton component				
			Daphnia	*Bosmina*	*Chydorus*	Other Cladocera	Copepoda
	%O	%A					
Apr	-	-					
May	100	99	8	18	55	1	18
Jun	75	61	10	8	0	1	81
Jul	ND	ND					
Aug	0	0	-	-	-	-	-
Sep	0	0	-	-	-	-	-
Oct	ND	ND					
Jan	ND	ND					

A high proportion of younger specimens was observed for pumpkinseed and mosquitofish throughout the summer, while larger specimens were only caught in spring (Figure 3.1). Dominance in numbers of YOY individuals was particularly noticeable in fall and winter, for pumpkinseed. As a whole (pooling all zooplanktonic taxa), zooplankton was the most important food category for both species, in terms of abundance. The use of zooplankton in the diet of pumpkinseed and mosquitofish decreased significantly with size

(Figure 3.2), and this was more pronounced in the former species. Smaller-sized pumpkinseeds were therefore more dependent on zooplankton, with its contribution to total stomach contents decreasing in terms of number and relative volume. Large mosquitofish ate more zooplankton (in numbers), but their relative contribution (volume) to the diet decreased only slightly with ontogeny (Figure 3.2).

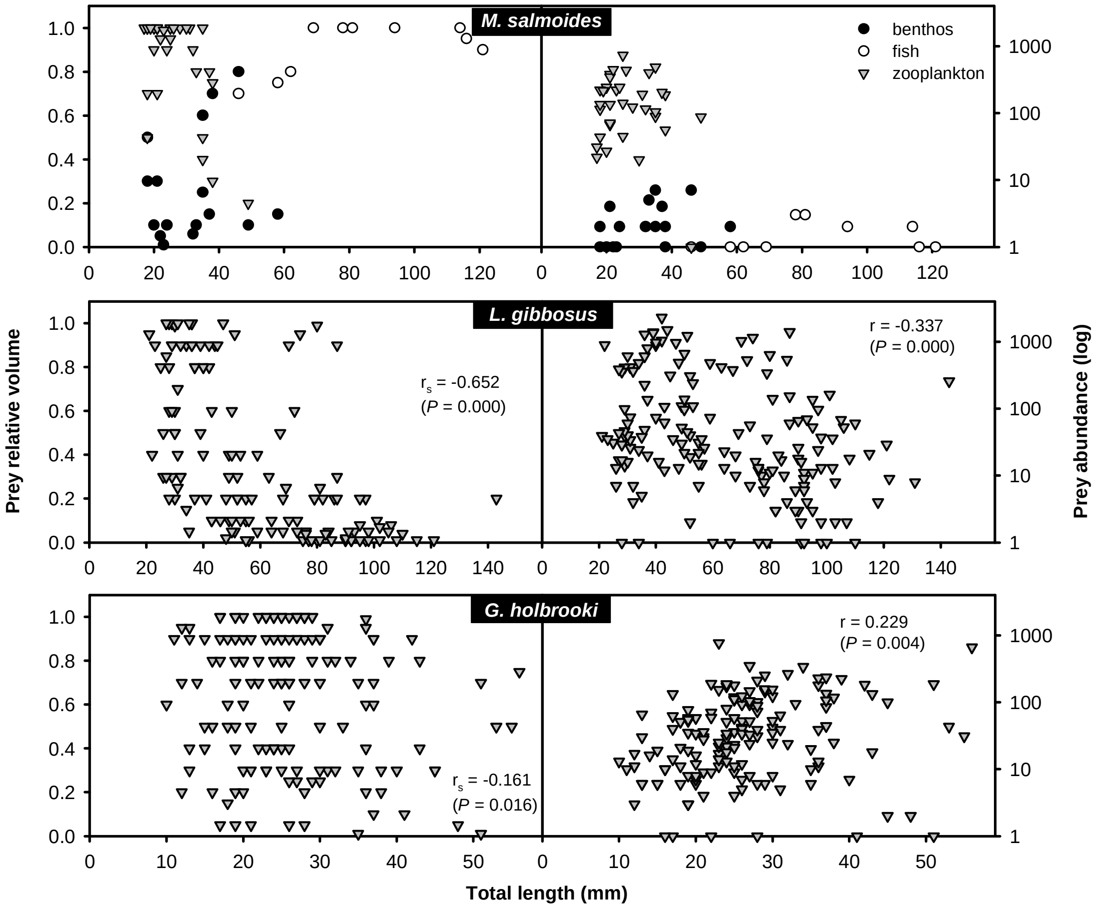


Figure 3.2 • Ontogenetic variation in planktivory (pooling all zooplankton taxa) of largemouth bass (*M. salmoides*), pumpkinseed (*L. gibbosus*) and mosquitofish (*G. holbrooki*).

Planktivory in pumpkinseed

With the exception of August, planktivory in pumpkinseed was always very frequent (%O > 67%), as shown in Table 3.2. Additionally, in the case of YOY specimens (TL < 60 mm), zooplankton was the single most important food category (%A > 63%). There was substantial seasonal variation in the proportion of each zooplanktonic species consumed (Table 3.2). For all size classes of pumpkinseed, planktivory was mainly focused on small-sized cladocerans (mainly *Bosmina longirostris*, *Chydorus* spp., *Alona* spp., *Moina micrura* and *Macrothrix* sp.) and cyclopoid copepods. *Daphnia longispina* was overall unimportant, except in April and only in the larger size classes (TL > 60 mm), where it was the dominant item in the zooplanktonic diet component. This suggests a strong dependence on resource availability (Figure 3.3), which was confirmed by the CCA analysis (see below).

Table 3.2 • Planktivory in pumpkinseed sunfish in Lake Vela from April to January. The contribution of zooplankton to total diet is shown as mean abundance relative to total stomach contents (%A) and percent occurrence (%O). Results are presented as percent composition in terms of numerical abundance. ND stands for no data.

Month	Contribution of zooplankton to total diet		Mean percent composition within zooplankton component				
			Daphnia	*Bosmina*	*Chydorus*	Other Cladocera	Copepoda
< 60 mm	%O	%A					
Apr	100	63	0	0	9	23	68
May	100	79	0	0	34	58	8
Jun	100	73	0	56	8	15	21
Jul	100	79	1	2	38	28	31
Aug	29	15	0	15	0	56	29
Sep	100	70	0	48	0	18	34
Oct	100	96	0	93	2	1	4
Jan	100	90	14	2	22	1	61
60-100 mm	%O	%A					
Apr	100	36	79	0	0	1	20
May	100	49	0	1	30	40	29
Jun	80	14	0	42	8	16	34
Jul	94	34	0	1	21	31	47
Aug	7	<0.1	0	0	100	0	0
Sep	80	34	0	16	0	14	70
Oct	0	0	-	-	-	-	-
Jan	ND	ND					
> 100 mm	%O	%A					
Apr	100	31	71	0	0	2	27
May	71	8	2	0	26	40	32
Jun	67	10	0	0	50	0	50
Jul	ND	ND					
Aug	ND	ND					
Sep	ND	ND					
Oct	ND	ND					
Jan	ND	ND					

Zooplankton density in Lake Vela and fish size together explained 55.3% of the total variation (total inertia 2.056) of planktivory in pumpkinseed (CCA: Monte-Carlo test, $P < 0.05$). Using partial CCAs, we estimated the contribution of zooplankton density (resource availability) and fish size to be 49.7% (sum of all canonical eigenvalues = 1.021) and 1.9% (sum of all canonical eigenvalues = 0.039), respectively. Both models were significant (Monte-Carlo test, $P < 0.05$) and the remaining portion of variation (3.7%) resulted from the intersection of both sets of explanatory variables.

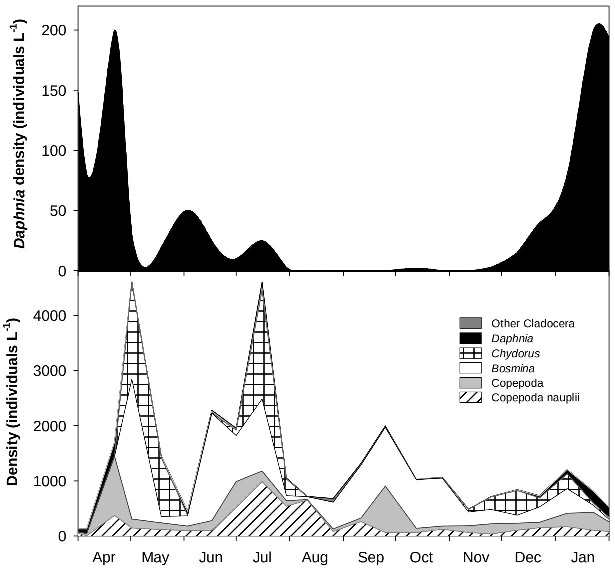


Figure 3.3 • Seasonal variation in the relative contribution (abundance) of the main zooplanktonic taxa (with emphasis on *Daphnia* – top pannel) of Lake Vela during the fish sampling campaign. Note the different scales in YY axes.

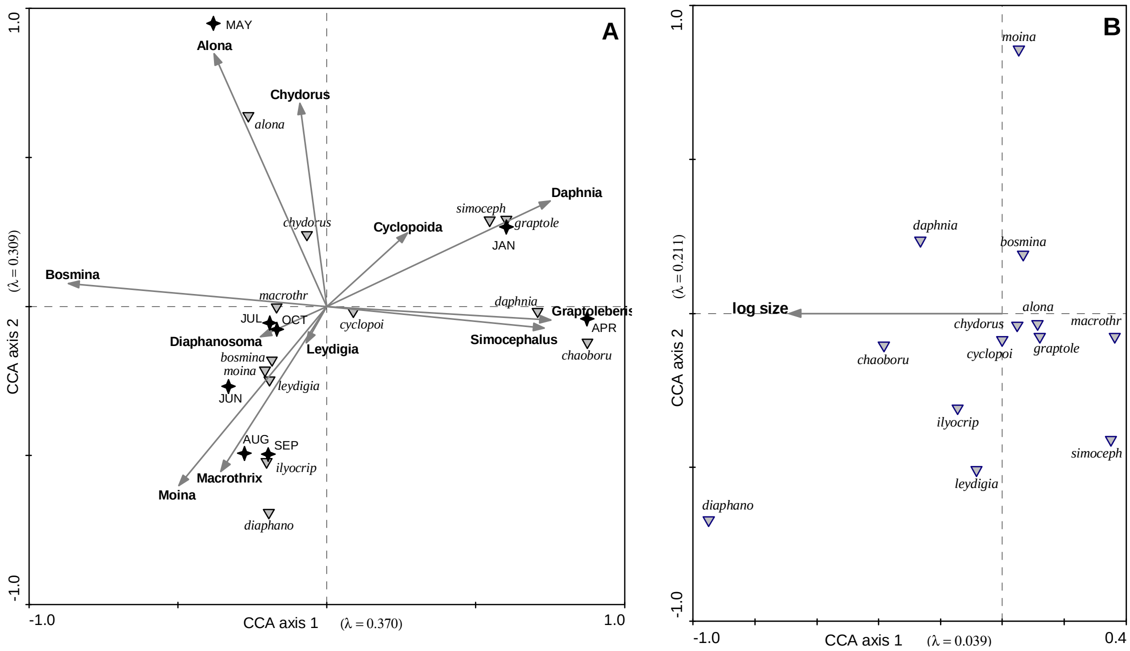


Figure 3.4 • Partial CCA ordination biplots of stomach contents (log prey abundance) from pumpkinseed sunfish. Diagrams display variation in planktivory due to: A) resource availability (lake zooplankton density); B) fish size. Triangles and stars represent zooplanktonic prey scores and mean fish scores by month, respectively. Arrows represent gradients extracted by the multivariate procedure.

Figure 3.4A shows that, overall, pumpkinseed fed on the most abundant microinvertebrates available, since all species scores were located on the positive side of its respective resource gradient (arrows in biplot). This was, however, more pronounced in some taxa (e.g. *Daphnia*, *Alona*) than others (e.g. Cyclopoida, *Macrothrix*). The CCA analysis also identified a seasonal component in planktivory, with fish feeding on *Alona* (chiefly) and *Chydorus* in May, while *Daphnia* provided substantial contributions to the diet in April and January (Figure 3.4A and Table 3.2). During summer and fall, planktivory was variable but no prey item was found to be predominant overall. Mean fish scores by season clearly illustrate the seasonal variation in planktivory (Figure 3.4A). Although fish size contributed modestly to the total diet variation, a moderate ontogenetic shift in planktivory can be observed in Figure 3.4B. Prey preferred by larger fish are situated on the left side of the diagram (e.g. *Daphnia*), while species on the right side of the diagram (e.g. *Macrothrix*, *Alona*, *Bosmina*) were consumed chiefly by smaller specimens.

Planktivory in mosquitofish

Like pumpkinseed, mosquitofish foraged substantially less on zooplankton in August (Table 3.3), but in the remaining months planktivory was very frequent (%O > 67%), although less in the largest specimens (TL > 40 mm). Albeit substantial seasonal fluctuations, zooplankton was almost always the single most important food category (%A > 50%). There was also substantial seasonal variation in the proportion of each zooplanktonic species consumed (Table 3.3). These fluctuations suggest a dependence on resource availability (Figure 3.3), which was confirmed by the CCA analysis (see next paragraph). For all size classes of mosquitofish, planktivory was almost exclusively focused on small-sized cladocerans (mainly *Bosmina longisrostris*, *Chydorus* spp. and *Macrothrix* sp.) and cyclopoid copepods. *Daphnia* was virtually absent from mosquitofish diet.

Zooplankton density in Lake Vela and fish size together explained 39.8% of the total variation (total inertia 3.573) of planktivory in mosquitofish (CCA: Monte-Carlo test, $P < 0.05$). The contribution of zooplankton density (resource availability) was significantly (partial CCA: Monte-Carlo test, $P < 0.05$) related to variation in the planktivory data, explaining 38.7% of the total variation (sum of all canonical eigenvalues = 1.383). The remaining 1.1% was attributable to fish size (0.4%) and to the intersection (0.7%) of both sets of explanatory variables (fish size and resource availability). The contribution of fish size to the total inertia (sum of all canonical eigenvalues = 0.013) was found to be non-significant (partial CCA: Monte Carlo test, $P > 0.05$).

Table 3.3 • Planktivory in mosquitofish in Lake Vela from April to January. The contribution of zooplankton to total diet is shown as mean abundance relative to total stomach contents (%A) and percent occurrence (%O). Results are presented as percent composition in terms of numerical abundance. ND stands for no data.

Month	Contribution of zooplankton to total diet		Mean percent composition within zooplankton component					
			Daphnia	*Bosmina*	*Chydorus*	*Macrothrix*	Other Cladocera	Copepoda
< 20 mm	%O	%A						
Apr	100	85	2	0	39	0	1	58
May	100	92	0	0	79	0	15	6
Jun	100	100	0	83	14	0	0	3
Jul	100	84	0	16	41	0	14	29
Aug	22	15	0	0	0	100	0	0
Sep	67	37	0	16	7	66	3	8
Oct	83	79	0	36	32	3	0	29
Jan	75	35	0	0	78	0	0	22
20-40 mm	%O	%A						
Apr	84	70	3	0	7	0	6	84
May	100	88	3	0	81	0	2	14
Jun	93	92	0	97	0	0	0	3
Jul	100	97	0	1	90	0	3	6
Aug	0	0	-	-	-	-	-	-
Sep	88	76	0	16	0	79	2	3
Oct	100	99	0	95	4	0	0	1
Jan	100	85	0	0	67	0	8	23
> 40 mm	%O	%A						
Apr	50	47	0	0	0	0	0	100
May	78	62	0	0	77	0	23	0
Jun	50	34	0	100	0	0	0	0
Jul	50	17	0	0	0	0	100	0
Aug	ND	ND						
Sep	ND	ND						
Oct	ND	ND						
Jan	ND	ND						

As shown in Figure 3.5, mosquitofish fed on the most abundant microinvertebrates available, since all species scores were located on the positive side of its respective resource gradient (arrows in biplot), except for *Moina*. This was particularly pronounced in *Macrothrix*, which was the preferred food item in August and September (Table 3.3 and Figure 3.5). The CCA analysis thus identified a seasonal component in planktivory, most evident in August and September and also in June and October. The latter case was mostly due to the relative contribution of *Bosmina* to the diet in these months (Table 3.3 and Figure 3.5). As shown by plotting mean fish scores by season (Figure 3.5), no distinct variation in planktivory could be identified between April, May, July and January, since mosquitofish foraged chiefly on *Chydorus* and/or copepods during this period (Table 3.3).

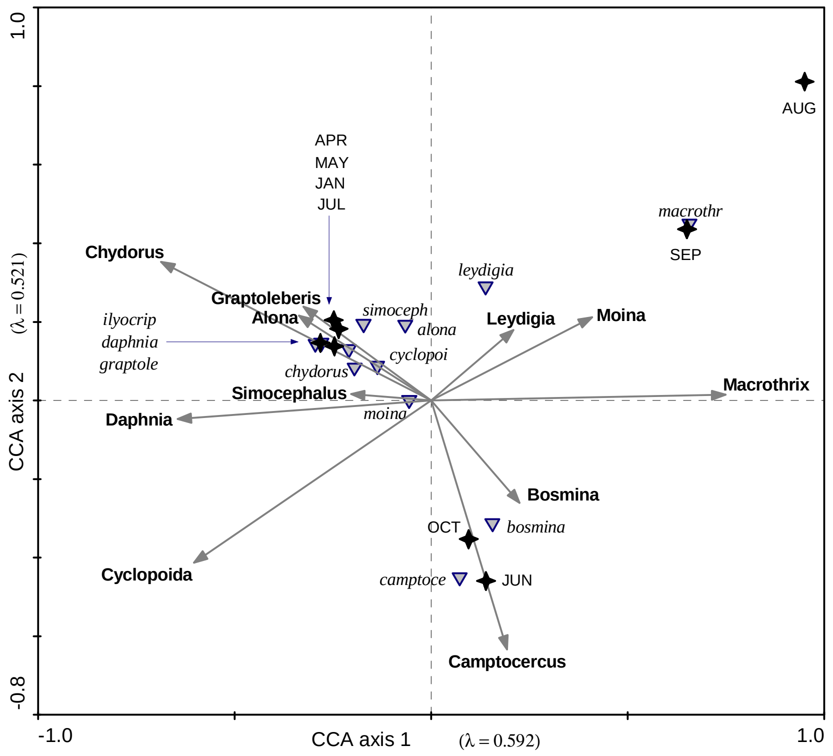


Figure 3.5 • Partial CCA ordination biplot of gut contents (log prey abundance) from mosquitofish. Diagram displays variation in planktivory due to resource availability (lake zooplankton density). Triangles and stars represent zooplanktonic prey scores and mean fish scores by month, respectively. Arrows represent gradients of resource availability.

Prey selection (electivity)

Electivity was only computed for mosquitofish and pumpkinseed, where it was found to be overall independent of fish size (Figures 3.6 and 3.7). However, negative significant correlations where found when prey categories where absent from the diet (E_i^* = -1) in larger specimens, as in the case of *Bosmina* and *Chydorus* in pumpkinseed (Figure 3.6), and *Macrothrix* in mosquitofish (Figure 3.7). A positive significant correlation was observed between the electivity in *Alona* and mosquitofish size (Figure 3.7). The absence of prey categories in the diet had a large influence in their mean electivity index, resulting in negative selection for all zooplankton taxa (Figure 3.8). However, a substantially different pattern was produced when all individual fish data points of value -1 (prey category absent in diet) were discarded (Figure 3.8). We believe this latter scenario to be ecologically sounder, since the absence of a particular prey item could reflect the influence of other sources of variation (resource patchiness or ontogenetic diet shifts, as shown above), rather than actual prey selectivity. Thus, neutral selection (sign test, $P > 0.05$; H_0: $E_i^* = 0$) was found for most prey categories, when guts where prey categories were absent were not accounted for. However, pumpkinseed was found to be positively selective (sign test, $P < 0.05$) for *Alona* and *Daphnia*, while mosquitofish displayed significant positive selection for *Alona*, *Chydorus* and *Macrothrix*.

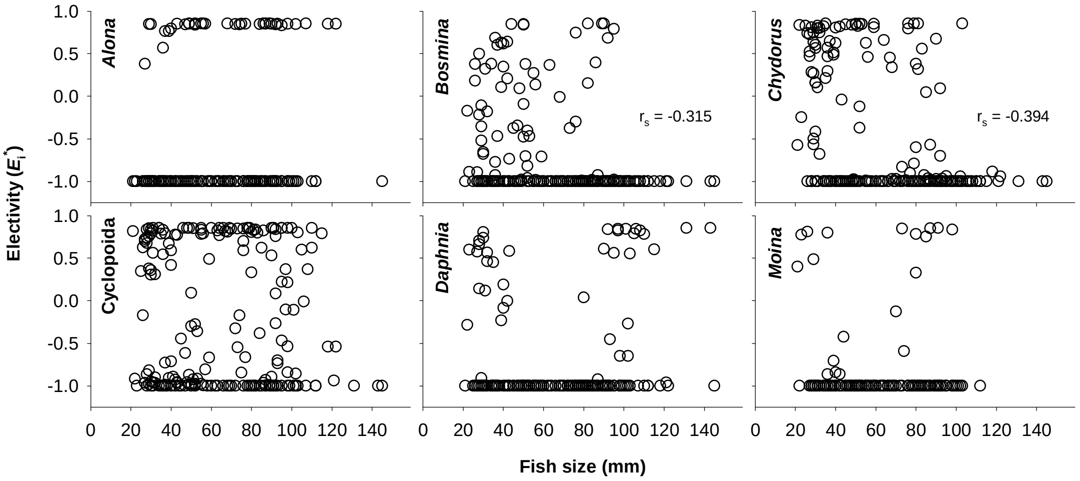


Figure 3.6 • Relationship between fish size (TL) and food selectivity (electivity, E_i^*) in individual pumpkinseed (n = 201 for each major prey category). Only significant ($P \leq 0.05$) correlations are shown.

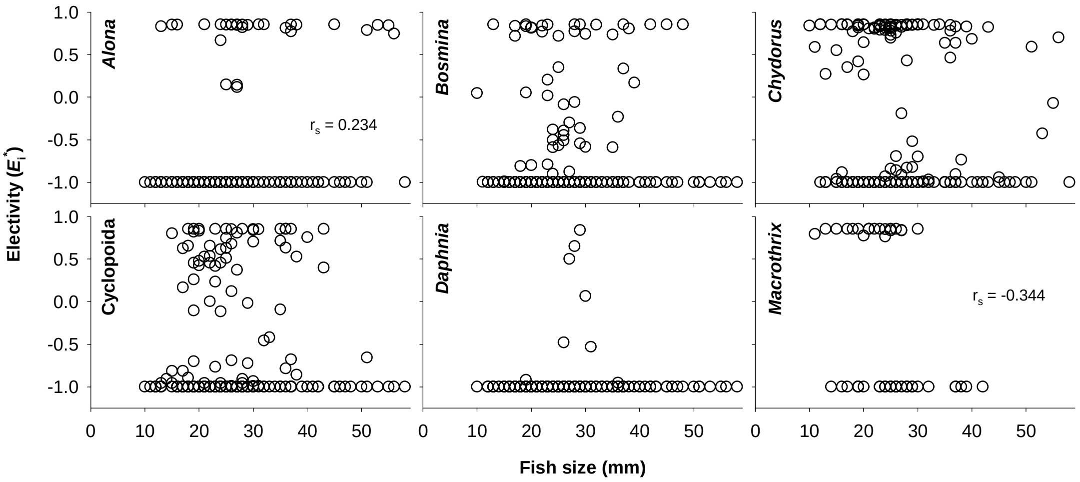


Figure 3.7 • Relationship between fish size (TL) and food selectivity (electivity, E_i^*) in individual mosquitofish (n = 195 for each major prey category). Only significant ($P \leq 0.05$) correlations are shown.

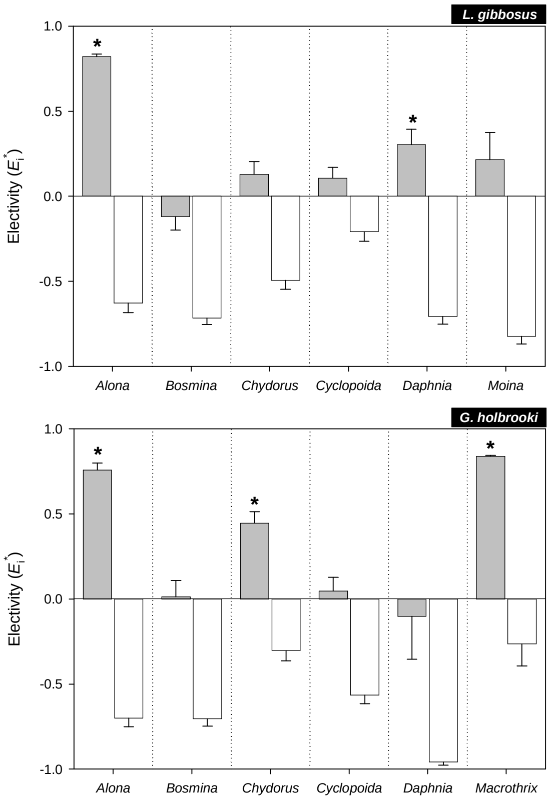


Figure 3.8 • Electivity (E_i^*) values (mean ± SE) for the major prey categories in pumpkinseed (top) and mosquitofish (bottom), averaged for the entire sampling period (April-January). White and grey bars represent the selectivity index when computed with and without accounting for guts where prey category was absent ($E_i^* = -1$), respectively. Significant deviations from 0 are marked with * (sign test, $P \leq 0.05$).

DISCUSSION

Although more important in the diet of smaller specimens, zooplanktivory was substantial in all life stages of pumpkinseed and mosquitofish (see below). Largemouth bass was also planktivorous during the early stages of its life, maximising resource use by shifting from a benthi-planktivorous phase (May-June) to piscivory (from July onwards).

The CCA showed that a large portion of variation in the use of zooplankton in pumpkinseed and mosquitofish diet could be explained by the resource matrix alone (zooplankton abundance in the environment). Still, a large fraction of variation remained unexplained, which could be attributable to random variation among individuals and unknown factors. The fraction of variation explained by resource availability was larger for pumpkinseed than for mosquitofish. In both diets (mosquitofish and pumpkinseed), the strong dependence on resource availability confirmed these taxa as opportunistic feeders, foraging on the most frequent and abundant prey. The CCA diagrams confirmed that the seasonal variation in planktivory was mostly a reflex of the availability of prey in the environment. Although the use of zooplankton (as a whole) in the diet was found to decrease with fish length, the use of zooplanktonic taxa was shown to be little dependent on fish size. No substantial ontogenetic shifts were found in either mosquitofish or pumpkinseed, although the latter displayed some degree of heterogeneity in the use of some taxa (e.g. *Daphnia*) between larger and smaller specimens.

Planktivory in pumpkinseed was higher than what had been previously recorded outside of its natural range of occurrence (Godinho et al., 1997; Wolfram-Wais et al., 1999; García-Berthou & Moreno-Amich, 2000). Pumpkinseed is usually viewed as being adapted to benthic feeding, foraging on macroinvertebrates (Robinson et al., 1993). Although frequent, zooplanktivory usually is not very significant, except in the smallest specimens (Godinho et al., 1997; García-Berthou & Moreno-Amich, 2000). However, Robinson et al. (1993) showed that the degree of zooplanktivory increases in the absence of more efficient zooplanktivores, such as bluegill sunfish or roach (see also discussion by García-Berthou & Moreno-Amich, 2000). When abundant, large cladocerans (such as *Daphnia*) seem to be the preferred zooplankton prey of pumpkinseed (García-Berthou & Moreno-Amich, 2000). In Lake Vela, substantial consumption of *D. longispina* was observed only in April and January, when daphniids were abundant (> 100 ind L^{-1}). Moreover, pumpkinseed exhibited positive selection for *Daphnia*, confirming it as a preferential prey (along with *Alona* spp.). Due to overall low densities of *Daphnia* in Lake Vela, however, planktivory in pumpkinseed consisted mainly of smaller cladocerans and cyclopoid copepods during summer and fall.

In spite of its name and reputation, most literature on mosquitofish diet in Mediterranean countries (Cabral et al., 1998; García-Berthou, 1999; Margaritora et al., 2001; Blanco et al., 2004) has portrayed this species as being planktivorous, foraging chiefly on littoral microinvertebrates, such as cladocerans and copepods. This was also observed in the present study, where mosquitofish exhibited positive selection for littoral cladocerans, namely *Alona*, *Chydorus* and *Macrothrix*. Most planktivorous fish usually display a strong preference for larger prey (i.e. daphniids), but we found very few mosquitofish foraging on *Daphnia* or *Simocephalus* (littoral taxon). García-Berthou (1999) also found overall reduced importance of *Daphnia* in mosquitofish diet. There are two possible alternative or complementary explanations for this: a) *Daphnia* is less abundant in the littoral micro-habitat occupied by mosquitofish; b) most mosquitofish size classes are gape-limited relatively to large cladocerans. The coexistence of mosquitofish in mesocosms with large *Daphnia carinata* (Matveev et al., 2000) seems to support the latter hypothesis.

Based on what has been discussed so far, it is clear that the impact of mosquitofish and pumpkinseed on the lower trophic levels of Lake Vela is different. Furthermore, mosquitofish is restricted to the margins of the lake or to structured environments, such as macrophyte patches. Several studies (Hurlbert et al., 1972; Margaritora et al., 2001; Nagdali & Gupta, 2002) have shown that the impact of mosquitofish can be very large, with its presence or absence producing distinct lake states (trophic cascade). However, these studies were conducted in ponds or artificial mesocosms, where mosquitofish was the only fish species present. In Lake Vela, as in many other shallow lakes, the small-sized mosquitofish competes with and is preyed upon other fish species, which confine its occurrence to shallow channels, lake margins or sites with dense macrophyte coverage (Fernández-Delgado, 1989; Cabral & Marques, 1999; García-Berthou, 1999). Although marginal overall, the impact of mosquitofish on lake zooplankton is not unimportant, since its littoral foraging habits may nullify the refuge effect of macrophytes as a predator-avoidance strategy of zooplankton (Burks et al., 2002; Castro et al., 2007).

We also carried out sporadic gut inspections in carp (not shown here) and we observed, albeit the low number of captures, a predominance of detritus and seeds in the diet and residual planktivory. Planktivory in carp is uncommon in lakes, since they are not efficient planktivores (see discussion in García-Berthou, 2001). Since planktivory in mosquitofish and largemouth bass is restricted in space (lake margins) and time (May-June), respectively, pumpkinseed can therefore be considered the main planktivore in Lake Vela. Furthermore, pumpkinseed was the only fish species to forage extensively on *D. longispina* (mostly in April), a key taxon in controlling phytoplankton growth and water transparency in Lake Vela (Castro & Gonçalves, submitted manuscript[2]; Antunes et al., 2003; Abrantes et al., 2006). Our results have shown that predation on *Daphnia* was not restricted to YOY fish and that larger pumpkinseeds seemed more interested in *Daphnia* than smaller size classes. When *Daphnia* was less abundant, however, pumpkinseed shifted to smaller cladocerans and copepods, as shown for other planktivores (Roseman et al.,

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[2] CAPÍTULO 1 da tese

1996; Gliwicz, 2001; 2002; Gliwicz et al., 2004; Scheuerell et al., 2005). This is not surprising, given the extremely high abundances of small-sized cladocerans and cyclopoid copepods in Lake Vela (see Figure 3.3), which reduce the fish perception of larger prey below a certain density threshold (see theoretical insight in Gliwicz, 2001; Gliwicz et al., 2004). Pumpkinseed seems to be able to dynamically shift food resource use (benthos, large and small zooplankton) without compromising its growth rates or reproductive success in Lake Vela (Castro et al., submitted manuscript), thus confirming flexible foraging behaviour (*vide* Roseman et al., 1996; Gliwicz, 2002; Scheuerell et al., 2005). Not all species remain unaffected by the collapse of *Daphnia* in the lake (e.g. Prout et al., 1990; Roseman et al., 1996).

Daphnia grazing represents a fundamental mechanism in shallow lakes (Scheffer, 1998) and we (Castro & Gonçalves, submitted manuscript) have shown that its population dynamics mediates two distinct water states in Lake Vela. Thus, *Daphnia* population usually collapses in late spring and eventually disappears in midsummer (July-August). The timing (April-May) of planktivory upon *Daphnia* by pumpkinseed and YOY largemouth bass suggests they may have contributed to the population collapse (see Figure 3.3). This joint effect of adult and YOY fish has also been pointed out as the most likely reason for the decline of copepods in Lake Krankesjön (Hansson et al., 2007). After the collapse of *D. longispina* in May, low densities were observed and no recovery in *Daphnia* was observed throughout the summer, as typically observed in other temperate lakes and attributable to fish-induced mortality (e.g. Luecke et al., 1990; Boersma et al., 1996). However, virtually no *Daphnia* were found from June to October in fish stomachs from Lake Vela (but they were still observed in the lake in June and July). This result suggests that other factors besides predation also contribute to the suppression of *Daphnia* during the summer and autumn. Clearly, additional research is required to understand the interactions between zooplankton and the alien omnivorous assemblage, particularly pumpkinseed sunfish.

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Capítulo 4

Selecção de *habitat* em crustáceos zooplanctónicos

Selecção de *habitat* e distribuição diária dos crustáceos zooplanctónicos de um lago Mediterrânico pouco profundo, durante as fases de água turva e água transparente

In: Castro B.B., Marques S.M. & Gonçalves F. (2007). Habitat selection and diel distribution of the crustacean zooplankton from a shallow Mediterranean lake during the turbid and clear water phases. *Freshwater Biology* 52(3):421-433.

RESUMO

O presente trabalho pretendeu estudar a variação diária dos padrões de distribuição vertical e horizontal dos crustáceos zooplanctónicos de um pequeno lago eutrófico e polimíctico. Para tal, foram levados a cabo programas diários de amostragem durante três dias consecutivos, ao longo de um transepto horizontal que incluiu estações na zona pelágica e na zona litoral (presença da macrófita *Nymphaea alba*). Dado que a transparência desempenha um papel crucial no risco colocado pelos predadores visuais, as campanhas de amostragem foram conduzidas em duas fases distintas (água turva e água transparente). Na fase de água turva, a maioria dos *taxa* zooplanctónicos exibiram uma distribuição homogénea ao longo dos eixos verticais e horizontais, durante os três dias consecutivos. A única excepção foi observada nos náuplios de copépodes, que revelaram um padrão vertical heterogéneo, possivelmente como resposta a predadores invertebrados. Na fase de maior transparência, a maioria dos zooplanctontes exibiu selecção de *habitat*. Verticalmente, a resposta geral consistiu num padrão de migração vertical, apesar da reduzida profundidade (1.6 m), enquanto que horizontalmente foram observadas densidades zooplanctónicas mais elevadas na zona pelágica, independentemente da altura do dia. Este resultado contradiz o papel das macrófitas enquanto refúgio para os zooplanctontes. Os padrões horizontais e verticais observados nesta fase foram particularmente conspícuos para os espécimes de maiores dimensões, sugerindo que o risco de predação colocado pelos peixes foi o principal factor por detrás da heterogeneidade espacial na Primavera. Deste modo, as diferenças observadas entre as duas campanhas de amostragem parecem ser mediadas pelo risco de predação, que está directamente relacionado com o grau de transparência da água. Em suma, o zooplâncton da Lagoa da Vela exibiu uma distribuição vertical heterogénea para minimizar a elevada pressão predatória exercida pela ictiocenose exótica. Horizontalmente, contudo, as macrófitas aquáticas não proporcionaram um refúgio adequado para os zooplanctontes, possivelmente devido às distintas características ecológicas da comunidade de peixes.

PALAVRAS-CHAVE

Zooplâncton dulçaquícola, distribuição espacial, migração, estados alternativos de transparência

O presente capítulo constitui um manuscrito publicado em revista internacional com arbitragem científica e é, por esse motivo, apresentado na língua inglesa.

Habitat selection and diel distribution of the crustacean zooplankton from a shallow Mediterranean lake during the turbid and clear water phases

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ABSTRACT

The fish fauna of many shallow Mediterranean Lakes is dominated by small-sized alien omnivores, with potential implications for the fish-zooplankton interactions still largely unknown. Here we studied diel variation in the vertical and horizontal distribution of the crustacean plankton in Lake Vela, a shallow polymictic and eutrophic lake. Diel sampling was carried out on three consecutive days along a horizontal transept, including an open-water station and a macrophyte (*Nymphaea alba*) bed. Since transparency is a key determinant of the predation risk posed by fish, the zooplankton sampling campaigns were conducted in both the turbid (autumn) and clear water (spring) phases. In the turbid phase, most taxa were homogeneously distributed along the vertical and horizontal axes in the three consecutive days. The only exception was for copepod nauplii, which showed vertical heterogeneity, possibly as a response to invertebrate predators. In the clear water phase, most zooplankton taxa displayed habitat selection. Vertically, the general response consisted of a daily vertical migration (DVM), despite the limited depth (1.6 m). Horizontally, zooplankters showed an overall preference for the pelagic zone, independent of the time of the day. Such evidence is contrary to the postulated role of macrophytes as an anti-predator refuge for the zooplankton. These vertical (DVM) and horizontal (macrophyte-avoidance) patterns were particularly conspicuous for large *Daphnia*, suggesting that predation risk from size-selective predators (fish) was the main factor behind the spatial heterogeneity of zooplankton in the spring. Thus, the difference in the zooplankton spatial distribution pattern and habitat selection among seasons (turbid and clear water phases) seems to be mediated the predation risk from fish, which is directly related to water transparency. The zooplankton in Lake Vela displays anti-predator behaviour that minimises predation from fish. We hypothesise that, due to the distinct fish community of shallow Mediterranean lakes, aquatic macrophytes may not provide adequate refuge to zooplankters, as seen in northern temperate lakes.

KEYWORDS

Freshwater zooplankton, spatial distribution, patchiness, migration, alternative states

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INTRODUCTION

The distribution of aquatic organisms, and particularly plankton, has long been known to be heterogeneous. Spatial heterogeneity is a common feature in all ecosystems and is the result of many interacting physical and biological processes (Pinel-Alloul, 1995; Folt & Burns, 1999; Hülsmann et al., 1999; Thackeray et al., 2004). Physical processes,

such as wind-induced turbulence or currents (Thackeray et al., 2004), seem to be the main drivers of large-scale heterogeneity. As the spatial scale decreases, the importance of biological processes increases and may overlap that of the physical processes (Folt & Burns, 1999). The formation of swarms or aggregations in plankton is considered to be a consequence of the search for food (Jensen et al., 2001; Roozen & Lürling, 2001) and mates (Folt & Burns, 1999), and to predator-avoidance (Kvam & Kleiven, 1995; Roozen & Lürling, 2001). Predatory pressure, in particular, has received considerable attention as a major driving force of zooplankton patchiness. Examples of predator-induced aggregations, resulting in spatial patterns of freshwater zooplankton, include avoidance of the shore (Romare & Hansson, 2003; Pinel-Alloul et al., 2004), diel vertical migration (Gilbert & Hampton, 2001; Han & Straskraba, 2001; Hembre & Megard, 2003; Ringelberg & Van Gool, 2003) and diel horizontal migration (Kvam & Kleiven, 1995; Lauridsen & Buenk, 1996; Burks et al., 2002; Wojtal et al., 2003).

Diel vertical migration (DVM) is one of the best-studied antipredator defences of zooplankton. The most usual pattern of DVM is for a population of zooplankton to dwell in deeper waters during the day and then to rise towards the warm surface waters for a few hours at night, thus avoiding visually-oriented predators during the day (Dodson, 1990; Han & Straskraba, 2001). This behavioural pattern is triggered by light changes at dusk and dawn (Ringelberg, 1999; Ringelberg & Van Gool, 2003), and is related to light penetration of the water column (Dodson, 1990; Alonso et al., 2004). The phototactic reactions leading to DVM are strongly enhanced by predator signals, particularly fish kairomones (Loose & Dawidowicz, 1994; Ringelberg, 1999; Ringelberg & Van Gool, 2003). In shallow lakes, however, there may be no hypolimnetic refuge available (Burks et al., 2002). Han and Straskraba (2001) suggested that aggregation near the sediment during the day may be used as a defence strategy in shallow lakes, as confirmed in the field by Jeppesen et al. (2002). Numerous studies have also shown that large zooplankters often aggregate in vegetation stands, swimming out only at night to the adjacent open water (Kvam & Kleiven, 1995; Lauridsen & Buenk, 1996; Masson et al., 2001; Wojtal et al., 2003). This phenomenon – diel horizontal migration (DHM; Burks et al., 2002) – depends on the effectiveness of macrophytes as a refuge, which seems to result from the balance between predator pressure and macrophyte density (Schriver et al., 1995; Jeppesen et al., 1998; Perrow et al., 1999; Burks et al., 2002). In addition, macrophyte stands also

represent a potential hazard for the zooplankton, which can suffer severe predation losses to macrophyte-associated biota (Perrow et al., 1999; Hülsmann et al., 1999; Masson et al., 2001). This is particularly important in the case where planktivorous fish (especially juveniles) also use macrophytes as a refuge from piscivores (Burks et al., 2002; Romare et al., 2003; Romare & Hansson, 2003).

Predation gradients are usually coupled with marked physical gradients, both vertically (epilimnion/hypolimnion) and horizontally (near-shore/offshore). In shallow polymictic lakes, however, such gradients are less well defined, due to the poorly marked separation between littoral and pelagic zones and to the constant mixing of the water column. Furthermore, in shallow Mediterranean lakes, the fish community is dominated by omnivorous fish (Blanco et al., 2003) that prey heavily on zooplankton, including in littoral areas (García-Berthou, 1999; García-Berthou & Moreno-Amich, 2000), thus precluding the littoral refuge effect of aquatic vegetation. Therefore, the spatial heterogeneity of zooplankton in such lakes may not comply with what has been observed in northern temperate lakes, where most of the studies on DVM and DHM have been conducted.

Given the distinctive characteristics of shallow Mediterranean lakes, we conducted a study on the spatial distribution patterns of the zooplankton from Lake Vela (Portugal) under contrasting conditions (turbid vs. clear water phase). The main aim was to assess if zooplankters exhibited antipredator behaviour, such as habitat selection and/or diel migration pattern, as a response to predation pressure from fish. More specifically, we intended: i) to determine whether DVM is constrained in this shallow and polymictic lake; ii) to assess if macrophytes provide refuge for zooplankters against littoral-foraging predators. To do so, we performed 3-day diel sampling cycles at littoral (macrophyte bed) and pelagic sampling sites at two depths (near the surface and near the bottom). The study was conducted during the turbid and the clear water phases, since these two alternative states represent contrasting conditions in terms of water transparency and fish predation pressure.

MATERIALS AND METHODS

Site description

Lake Vela is a small (maximum floodable area 70 ha), shallow (0.9 m average depth; 2.4 m maximum depth) and polymictic water body in the centre of Portugal. It is

threatened by eutrophication and exotic species introductions, as a consequence of human activities. It is the largest lake of a system of small lakes located within a maritime pine forest. Although emergent macrophytes (especially *Cladium mariscus* (L.) and *Nymphaea alba* L.) occur in some patches near the shoreline, 80-90% of the flooded lakebed has no macrophytes. This is probably due to high turbidity, which has been increasing for the past two decades. A few patches of submerged macrophytes develop during the clear water phase in spring and some persist during the summer. Large fluctuations in phytoplankton have been observed and frequent Cyanobacteria blooms have also been recorded (Antunes et al., 2003). *Daphnia* cf. *longispina* (O.F. Müller) (*D. galeata* x *hyalina* complex), *Bosmina longirostris* (O.F. Müller), *Ceriodaphnia pulchella* G.O. Sars, chydorids and cyclopoid copepods are the dominant crustacean taxa in Lake Vela and the most significant grazers (particularly *Daphnia*). Due to very high densities of planktivorous fish, predator pressure on zooplankton is substantial. The fish community has changed radically, due to repeated introductions of alien species, which have displaced native cyprinids (*Barbus* spp., *Chondrostoma* spp. and *Squalius* spp.). At the present, the dominant fish species are all non-indigenous: omnivores/planktivores – pumpkinseed sunfish (*Lepomis gibbosus* (L.)) and mosquitofish (*Gambusia holbrooki* Girard); benthivores – carp (*Cyprinus carpio* L.); piscivore – largemouth bass (*Micropterus salmoides* (Lacépède)). Eel (*Anguilla anguilla* (L.)) is the only native species still present in large numbers. Both mosquitofish and pumpkinseed exert a strong, though seasonally variable, predatory pressure on zooplankton (Cabral et al., 1998; García-Berthou, 1999; García-Berthou & Moreno-Amich, 2000). Pumpkinseed now comprises more than 50% of the fish community by numbers. The displacement of the indigenous cyprinid community by such an exotic assemblage has been a common trend in Mediterranean lowland lakes (Almaça, 1995; Blanco et al., 2003).

Sampling strategy

Sampling was carried out twice: in November, during the annual turbid water phase, and in early spring (late March) in the clear water phase. These timings were chosen to accommodate a potential seasonal component of the spatial distribution pattern of the crustacean plankton. In November the macrophytes are senescent and the water is turbid, while March is the beginning of its growing period (low turbidity). Furthermore, these two

periods have different plankton assemblages, with November being dominated by small-sized species while a *Daphnia* is dominant in March. The summer was excluded from our sampling because *Daphnia*, which is the largest filter feeder and the most susceptible taxon to predation by fish, is usually absent or decline during this period.

Diel samples were collected over three consecutive days at two depths (near the bottom and near the surface). Samples were taken at two contiguous sites (M and P): M was a littoral sampling station, situated at the edge of a large bed of *Nymphaea alba*, while P was in the adjacent (20-30-m distant from M) pelagic zone (closer to the centre of the lake). A small boat was used to minimise sediment resuspension. Composite zooplankton samples were collected with a transparent Van Dorn water sampler (horizontal) and poured through a 55-µm-mesh plankton net, until a volume of 16L was filtered. Collection was performed as quickly as possible to prevent possible avoidance by larger specimens. This procedure was repeated near the surface and close to the bottom, at three distinct sub-areas within each site. Each sample was immediately preserved in sucrose-saturated 4% formalin, stained with Bengal rose, and stored until further examination.

In November, daily sampling was carried out 5-6 hours before and after sunset, roughly corresponding to noon (day) and midnight (night), respectively. In March, sampling took place at a finer temporal resolution (every four hours: 8h, 12h, 16h, 20h, 24h, 4h). Sampling at 8h and 20h corresponded roughly to sunrise and sunset, respectively.

Sample processing and analysis

Zooplankton samples were sorted and counted under a stereozoom microscope. Cladocerans were identified to genus and cyclopoid copepods were separated into nauplii and copepods (copepodites + adults). Counting by subsampling was used for the most abundant taxa, with at least 100 individuals of the dominant zooplankton species being counted and the total number of counts always being higher than 400. When necessary, the whole sample was screened in order to count large organisms (especially *Daphnia*).

In spring (March), an additional parameter was quantified: in order to assess the distribution of the different size classes of *Daphnia*, their average body size in each combination of treatments (site x depth x sampling time x day) was estimated by measuring a random subsample of 50 individuals in each replicate sample. Size at maturity (SAM) was determined according to Caramujo et al. (1997), at each combination of site

and depth (pooling sampling times). The SAM estimate was the same for all combinations (1.5 mm) and this value was used in order to estimate the proportion of adult *Daphnia* in each replicate sample.

Statistical analysis

Data were analysed using a repeated measures (RM) ANOVA, with the sampled sub-areas within each site as the subjects. The analyses were carried out for each day independently, as a means to simplify the statistical design (by reducing the number of factors and interactions) but also to assess if the distribution patterns observed were consistent for the three days. Since this artificial separation was decided merely for statistical convenience, the associated significance level of the family-wise Type I error was adjusted using the Dunn-Sidak procedure (Quinn & Keough, 2002), for a total of three comparisons (one per day) for each parameter. A significance level of 0.017 was therefore used in all analyses. The between-subjects factors of the RM ANOVA were site (P vs. M) and depth (surface vs. bottom), while the within-subject factor was time of the day (day vs. night). The density data matrix from the spring campaign was reduced to the temporal resolution of that of the autumn campaign (noon and midnight sampling), so that the statistical conclusions could be compared between both surveys. Additionally, a RM ANOVA was also applied to the high temporal resolution data, using time of the day (day vs. night) and hour (8h vs. 12h vs. 16h and 20h vs. 24h vs. 4h) as within-subjects factors. The data on the size distribution of *Daphnia* (body size and proportion of adults) were analysed using the latter procedure alone, since no comparison between spring and autumn was required. Because of deviations to multisample sphericity (measured by ε), the degrees of freedom were adjusted in the RM ANOVA procedure for the high resolution data, using either the Greenhouse-Geisser (if $\varepsilon<0.75$) or the Huynh-Feldt (if $\varepsilon>0.75$) estimate (Quinn & Keough, 2002). Prior to all statistical analyses, the zooplankton density data were log-transformed, $\log(x+1)$, and the proportion of adults (*Daphnia*) was arcsine square-root transformed, $\arcsin(\sqrt{x})$, in order to comply with sample normality.

RESULTS

The physical and chemical environment of Lake Vela was homogeneous across depth, day period and site during the both sampling campaigns. Chlorophyll *a* (chl *a*) was

the most variable parameter, exhibiting large fluctuations (particularly between days), although with no consistent spatial or temporal pattern. Given the lack of patterns, only the range of the parameters recorded during this study is shown (Table 4.1). Water depth was similar at both sites and varied between 1.0 m (autumn) and 1.6 m (spring). The temperature was still quite high in November (unusual for the season), exceeding those observed in March. The two sampling periods represented two different phases in the lake: in November, the lake was characterised by low transparency and high concentrations of TP and chl *a*, while in March the water was less turbid and chl *a* lower (Table 4.1).

The zooplankton community also differed between the two seasons. In November, copepod nauplii were the most abundant crustacean zooplankters (range 186-1148 L^{-1}), followed by *Chydorus* (69-825 L^{-1}). *Bosmina* (74-391 L^{-1}) and cyclopoid copepods (41-343 L^{-1}) were also common, while *Daphnia* were scarce (1.5-13 L^{-1}). Other taxa (*Ceriodaphnia*, *Moina*, *Iliocryptus*, *Alona*, and predator *Chaoborus*) were observed in very low numbers (< 2 L^{-1}). In March, the crustacean zooplankton was less dense overall and less diverse, being mainly dominated by cyclopoid copepods (range 94-695 L^{-1}) and *Daphnia* (31-245 L^{-1}). *Chydorus* was also present in low numbers (0.5-42 L^{-1}). A few copepod nauplii were observed, as well as other cladocerans (*Bosmina*, *Simocephalus*).

Table 4.1 • Range of physical and chemical parameters measured during the two sampling campaigns.

Parameter	November	March
Water depth (m)	1.0	1.6
Secchi depth (m)	0.30	0.75
Water temperature (ºC)	19 – 22	12 – 16
TP (mg P L^{-1})	0.31 – 0.38	0.13 – 0.15
Chlorophyll *a* (µg L^{-1})	107 – 267	30 – 89

Turbid water phase (autumn)

The weather remained calm and sunny with a few clouds for the three consecutive days. The distribution patterns of the most abundant zooplanktonic taxa are presented in Figure 4.1. No consistent statistical differences among days were found for copepod nauplii (Table 4.2), although a significant overall effect of sampling depth was observed on days 2 and 3. However, there were often significant interactions between depth and site or time of the day, suggesting that the daily vertical pattern differed between M and P. In fact, the density of nauplii was always higher at the bottom in M, while in P density was higher at the bottom during the day but similar to surface density at night (Figure 4.1). This is consistent with a daily vertical migration. No difference in the overall average density was found between sites (Table 4.2).

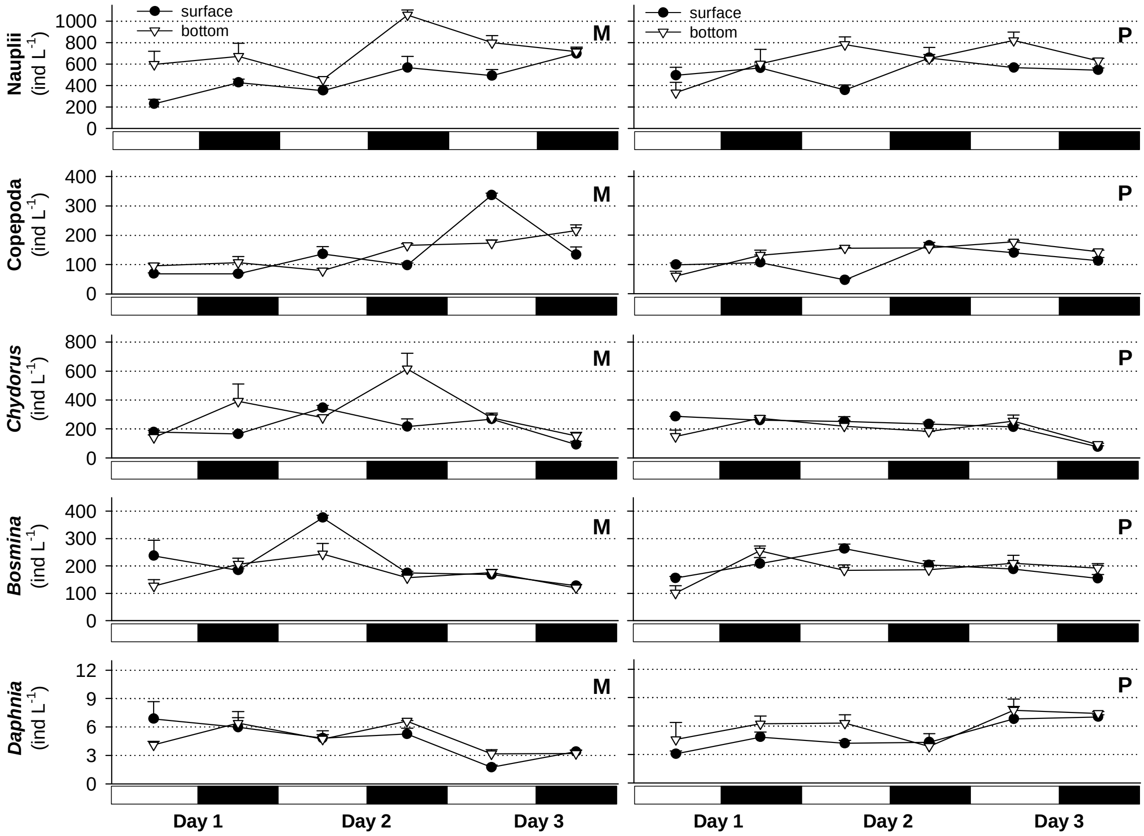

Figure 4.1 • Diel variation of mean (±SE) zooplankton densities during the autumn campaign (turbid water phase). M (macrophyte) and P (pelagic) represent adjacent sites, which were sampled at two depths. The bars on the x-axis represent day (white) and night (black) sampling periods.

Table 4.2 • Summary table of the RM ANOVA applied to the distribution data of the zooplankton sampled in the autumn campaign (turbid water phase). Significant values are indicated in bold (the level of significance used was 0.017 – see text for further explanation). Time stands for time of the day (day vs. night).
(*see next page*)

	RM ANOVA		Day 1			Day 2			Day 3	
	Source of variation	**df**	**MS**	***P***	**df**	**MS**	***P***	**df**	**MS**	***P***
Nauplii density	Between subjects									
	Site	1	0.008	0.430	1	0.003	0.597	1	0.003	0.259
	Depth	1	0.066	0.048	1	0.201	**0.003**	1	0.072	**0.000**
	Site x depth	1	0.255	**0.003**	1	0.002	0.663	1	0.000	0.977
	Residual	8	0.012		8	0.011		8	0.002	
	Within subjects									
	Time	1	0.158	**0.013**	1	0.209	**0.000**	1	0.000	0.802
	Time x site	1	0.001	0.844	1	0.055	**0.012**	1	0.021	**0.014**
	Time x depth	1	0.000	0.907	1	0.014	0.141	1	0.033	**0.005**
	Time x site x depth	1	0.059	0.090	1	0.105	**0.002**	1	0.004	0.198
	Residual	8	0.016		8	0.005		8	0.002	
Copepod density	Between subjects									
	Site	1	0.018	0.066	1	0.001	0.564	1	0.143	**0.000**
	Depth	1	0.014	0.098	1	0.091	**0.001**	1	0.006	0.130
	Site x depth	1	0.078	**0.002**	1	0.091	**0.001**	1	0.031	**0.005**
	Residual	8	0.032		8	0.003		8	0.002	
	Within subjects									
	Time	1	0.060	0.028	1	0.202	**0.000**	1	0.091	**0.001**
	Time x site	1	0.039	0.062	1	0.046	**0.014**	1	0.006	0.207
	Time x depth	1	0.048	0.044	1	0.002	0.488	1	0.093	**0.001**
	Time x site x depth	1	0.027	0.112	1	0.369	**0.000**	1	0.092	**0.001**
	Residual	8	0.008		8	0.005		8	0.003	
***Chydorus* density**	Between subjects									
	Site	1	0.029	0.115	1	0.187	**0.007**	1	0.067	0.052
	Depth	1	0.000	0.901	1	0.016	0.324	1	0.050	0.085
	Site x depth	1	0.105	**0.010**	1	0.112	0.023	1	0.002	0.672
	Residual	8	0.009		8	0.014		8	0.013	
	Within subjects									
	Time	1	0.150	**0.001**	1	0.000	0.947	1	0.967	**0.000**
	Time x site	1	0.010	0.217	1	0.013	0.285	1	0.008	0.116
	Time x depth	1	0.233	**0.000**	1	0.104	**0.013**	1	0.018	0.028
	Time x site x depth	1	0.008	0.251	1	0.139	**0.006**	1	0.012	0.058
	Residual	8	0.005		8	0.010		8	0.020	
***Bosmina* density**	Between subjects									
	Site	1	0.005	0.524	1	0.006	0.188	1	0.059	**0.011**
	Depth	1	0.039	0.087	1	0.076	**0.001**	1	0.006	0.337
	Site x depth	1	0.004	0.534	1	0.001	0.574	1	0.009	0.243
	Residual	8	0.010		8	0.025		8	0.005	
	Within subjects									
	Time	1	0.167	**0.004**	1	0.142	**0.001**	1	0.061	**0.003**
	Time x site	1	0.056	0.051	1	0.062	**0.013**	1	0.011	0.106
	Time x depth	1	0.135	**0.008**	1	0.027	0.068	1	0.000	0.990
	Time x site x depth	1	0.000	0.901	1	0.000	0.788	1	0.942	0.360
	Residual	8	0.011		8	0.049		8		
***Daphnia* density**	Between subjects									
	Site	1	0.043	0.058	1	0.019	0.127	1	0.646	**0.000**
	Depth	1	0.001	0.721	1	0.016	0.158	1	0.019	0.122
	Site x depth	1	0.051	0.043	1	0.000	0.815	1	0.003	0.521
	Residual	8	0.009		8	0.007		8	0.006	
	Within subjects									
	Time	1	0.056	0.023	1	0.000	0.917	1	0.019	0.057
	Time x site	1	0.011	0.252	1	0.042	0.040	1	0.015	0.088
	Time x depth	1	0.013	0.215	1	0.002	0.584	1	0.020	0.053
	Time x site x depth	1	0.023	0.111	1	0.026	0.090	1	0.010	0.141
	Residual	8	0.007		8	0.007		8	0.004	

Adult copepods and copepodites showed no regular distribution pattern (Figure 4.1; Table 4.2). On most days, there was a significant interaction between the three factors (time of the day x depth x site), but no obvious trend was visible (Figure 4.1). No difference in the overall average density was found between sites, except on day 3 (Table 4.2), when density was higher at M.

No consistent statistical differences among days were found for *Chydorus* or *Bosmina* (Table 4.2), confirmed by the lack of a consistent pattern of habitat selection patterns (Figure 4.1). No differences in the overall average densities were found between sites, except in day 2 for *Chydorus* (M>P) and day 3 for *Bosmina* (P>M) (Table 4.2). Data on the distribution pattern of *Daphnia* were more consistent (i.e., statistically more coherent) than for the other taxa (Table 4.2). No significant differences were found between site, depth, time of the day or in their interactions, suggesting a homogeneous distribution pattern along the vertical, horizontal and diurnal gradients. The only exception was recorded on day 3, where a significant overall effect of site was observed, with *Daphnia* density being higher in P than in M (Figure 4.1).

Clear water phase (spring)

In general, the weather was calm and partially sunny, except on the afternoon of day 3, when there was strong wind and heavy rain. The distribution patterns of the most abundant zooplanktonic taxa are presented in Figure 4.2. For copepods, a significant effect of time of the day was found on all days (Table 4.3), as seen by an increase in copepod density during the night, which was more noticeable at P. A similar pattern was observed in *Chydorus*, with a consistent significance of time of the day and time x site interaction over the three days (Table 4.3). This site-dependent effect of time of the day is visible in Figure 4.2, because there was an increase in density at night, which was more noticeable and consistent at P. No consistent statistical difference between sites was recorded in the overall density of copepods or chydorids, although copepods exhibited higher overall density at P.

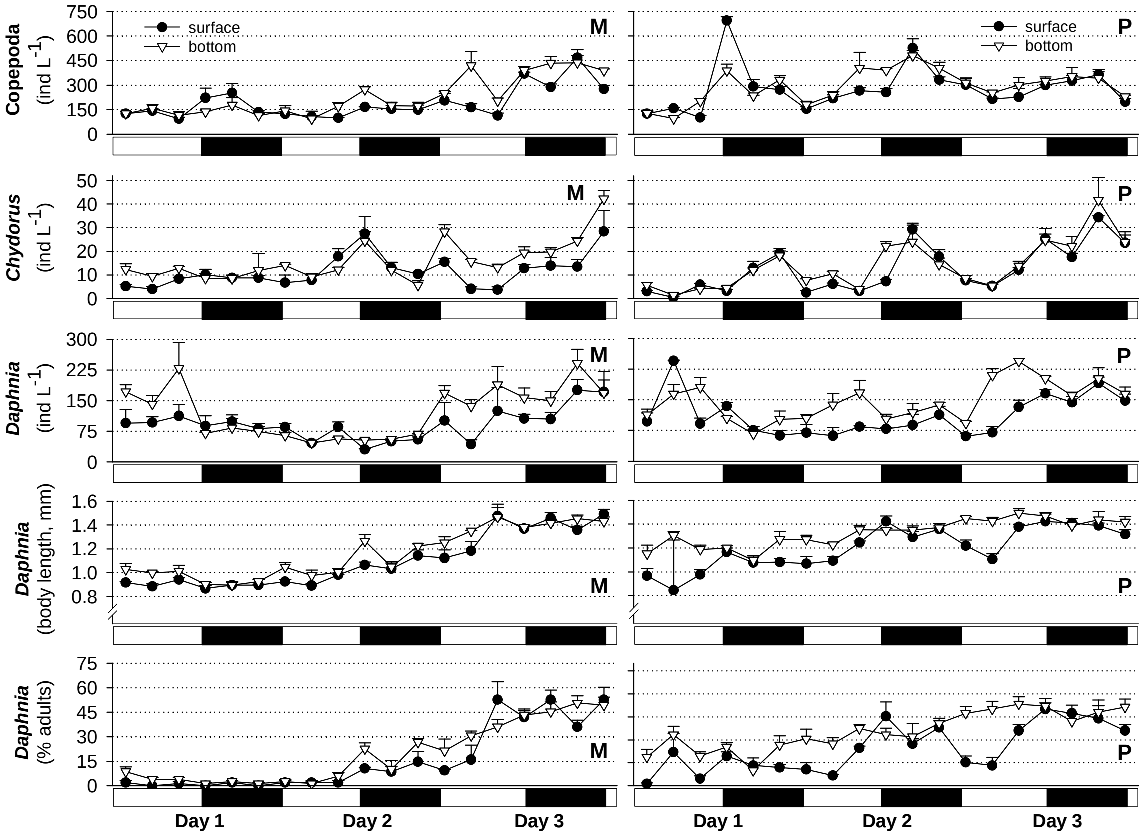

Figure 4.2 • Diel variation of mean (±SE) zooplankton densities and *Daphnia* size-structure during the spring campaign (clear water phase). M (macrophyte) and P (pelagic) represent adjacent sites, which were sampled at two depths. The bars on the x-axis represent day (white) and night (black) sampling periods.

For *Daphnia*, no consistent significant effects were found for any factor (Table 4.3), although there was a significant effect of site on days 2 and 3. In fact, *Daphnia* density was slightly higher at in site P than at site M (Figure 4.2). Time of the day x depth interaction was only significant at day 3 (Table 4.3). In general terms, the analysis of the low and high temporal resolution data (see Methods – statistical analysis) produced a similar statistical output, with *Daphnia* being an exception, due to a consistent effect of depth on the high resolution, but not in the low resolution data. We chose not to present the significance table for the high resolution data, since it supports similar conclusions overall but does not allow comparison between the two surveys (spring and autumn).

Table 4.3 • Summary table of the RM ANOVA applied to the distribution data of the zooplankton sampled in the spring campaign (clear water phase). Significant values are indicated in bold (the level of significance used was 0.017 – see text for further explanation). Time stands for time of the day (day vs. night).

	RM ANOVA	**Day 1**			**Day 2**			**Day 3**		
	Source of variation	**df**	**MS**	***P***	**df**	**MS**	***P***	**df**	**MS**	***P***
Copepod density	Between subjects									
	Site	1	0.003	0.687	1	1.135	**0.000**	1	0.005	0.293
	Depth	1	0.072	0.084	1	0.000	0.871	1	0.167	**0.000**
	Site x depth	1	0.018	0.350	1	0.000	0.903	1	0.086	**0.002**
	Residual	7	0.018		8	0.009		8	0.004	
	Within subjects									
	Time	1	0.301	**0.000**	1	0.499	**0.001**	1	0.132	**0.012**
	Time x site	1	0.060	0.018	1	0.019	0.328	1	0.000	0.860
	Time x depth	1	0.004	0.452	1	0.000	0.929	1	0.025	0.192
	Time x site x depth	1	0.027	0.080	1	0.010	0.483	1	0.010	0.388
	Residual	7	0.006		8	0.018		8	0.013	
***Chydorus* density**	Between subjects									
	Site	1	0.236	**0.002**	1	0.126	0.092	1	0.012	0.386
	Depth	1	0.077	0.030	1	0.002	0.815	1	0.228	**0.004**
	Site x depth	1	0.008	0.403	1	0.000	0.915	1	0.132	**0.016**
	Residual	7	0.011		8	0.035		8	0.115	
	Within subjects									
	Time	1	1.389	**0.000**	1	0.540	**0.000**	1	0.920	**0.000**
	Time x site	1	0.745	**0.001**	1	0.129	**0.017**	1	0.089	**0.012**
	Time x depth	1	0.137	0.062	1	0.082	0.043	1	0.028	0.108
	Time x site x depth	1	0.002	0.794	1	0.019	0.278	1	0.075	0.018
	Residual	7	0.028		8	0.014		8	0.009	
***Daphnia* density**	Between subjects									
	Site	1	0.020	0.232	1	0.408	**0.004**	1	0.126	**0.007**
	Depth	1	0.005	0.526	1	0.116	0.062	1	0.540	**0.000**
	Site x depth	1	0.025	0.190	1	0.085	0.101	1	0.007	0.432
	Residual	7	0.012		8	0.025		8	0.010	
	Within subjects									
	Time	1	0.471	**0.002**	1	0.018	0.252	1	0.154	0.020
	Time x site	1	0.167	0.022	1	0.000	0.897	1	0.019	0.333
	Time x depth	1	0.003	0.717	1	0.014	0.310	1	0.236	**0.007**
	Time x site x depth	1	0.057	0.131	1	0.025	0.181	1	0.003	0.704
	Residual	7	0.020		8	0.012		8	0.018	

A consistently significant effect of depth on the body length and the proportion of adults of *Daphnia* was observed over the three days (Table 4.4), with larger daphniids near the bottom (Figure 4.2). This pattern was more conspicuous at P, although no significant depth x site interaction was found. Significant differences were observed for both parameters (body length and proportion of adults) between sites (P>M), except on day 3.

Table 4.4 • Summary table of the RM ANOVA applied to the size distribution data of *Daphnia* during the spring campaign (clear water phase). Significant values are indicated in bold (the level of significance used was 0.017 – see text for further explanation). Time stands for time of the day (day vs. night).

	RM ANOVA		**Day 1**			**Day 2**			**Day 3**	
	Source of variation	**df**	**MS**	***P***[1]	**df**	**MS**	***P***[1]	**df**	**MS**	***P***[1]
***Daphnia* body length**	Between subjects									
	Site	1	0.582	**0.001**	1	0.977	**0.000**	1	0.011	0.278
	Depth	1	0.263	**0.013**	1	0.118	**0.002**	1	0.147	**0.003**
	Site x depth	1	0.070	0.139	1	0.001	0.724	1	0.019	0.161
	Residual	8	0.026		8	0.006		8	0.008	
	Within subjects									
	Time	1	0.001	0.911	1	0.422	**0.000**	1	0.134	**0.011**
	Time x site	1	0.091	0.163	1	0.001	0.658	1	0.002	0.690
	Time x depth	1	0.089	0.168	1	0.018	0.030	1	0.083	0.033
	Time x site x depth	1	0.018	0.513	1	0.033	**0.008**	1	0.017	0.282
	Residual (time)	8	0.039		8	0.003		8	0.013	
	Hour	2	0.014	0.625	2	0.057	**0.000**	2	0.069	**0.002**
	Hour x site	2	0.003	0.861	2	0.004	0.330	2	0.032	0.030
	Hour x depth	2	0.008	0.733	2	0.005	0.277	2	0.003	0.627
	Hour x site x depth	2	0.014	0.619	2	0.008	0.135	2	0.001	0.888
	Residual (hour)	16	0.041		16	0.004		16	0.007	
	Time x hour	2	0.004	0.768	2	0.009	0.159	2	0.076	**0.000**
	Time x hour x site	2	0.018	0.537	2	0.015	0.058	2	0.005	0.258
	Time x hour x depth	2	0.046	0.326	2	0.002	0.585	2	0.032	**0.003**
	Time x hour x site x depth	2	0.024	0.480	2	0.012	0.100	2	0.001	0.752
	Residual (time x hour)	16	0.042		16	0.004		16	0.004	
***Daphnia* %adults**	Between subjects									
	Site	1	1.443	**0.000**	1	1.666	**0.000**	1	0.059	0.043
	Depth	1	0.370	**0.001**	1	0.069	**0.007**	1	0.194	**0.002**
	Site x depth	1	0.011	0.376	1	0.002	0.704	1	0.060	0.041
	Residual	8	0.013		8	0.013		8	0.010	
	Within subjects									
	Time	1	0.000	0.909	1	0.763	**0.000**	1	0.488	**0.000**
	Time x site	1	0.061	0.123	1	0.036	0.040	1	0.022	0.226
	Time x depth	1	0.096	0.062	1	0.023	0.088	1	0.139	**0.011**
	Time x site x depth	1	0.010	0.497	1	0.142	**0.001**	1	0.092	0.028
	Residual (time)	8	0.020		8	0.006		8	0.013	
	Hour	2	0.008	0.392	2	0.183	**0.008**	2	0.083	**0.014**
	Hour x site	2	0.004	0.601	2	0.001	0.873	2	0.028	0.185
	Hour x depth	2	0.011	0.269	2	0.003	0.774	2	0.014	0.404
	Hour x site x depth	2	0.007	0.418	2	0.028	0.260	2	0.000	0.991
	Residual (hour)	16	0.008		16	0.019		16	0.015	
	Time x hour	2	0.024	0.277	2	0.013	0.439	2	0.124	**0.000**
	Time x hour x site	2	0.240	**0.003**	2	0.012	0.447	2	0.010	0.353
	Time x hour x depth	2	0.020	0.333	2	0.004	0.783	2	0.088	**0.002**
	Time x hour x site x depth	2	0.005	0.666	2	0.014	0.399	2	0.008	0.426
	Residual (time x hour)	16	0.018		16	0.015		16	0.009	

[1] after adjustment of the df's (see text for further explanation)

DISCUSSION

No physical and chemical gradients were identified across site, depth or sampling time in either autumn or spring. This suggests that Lake Vela is frequently mixed,

confirming it as a polymictic lake. The absence of physical and chemical gradients that provide directional cues presumably constrains habitat selection by the zooplankton while the efficiency of any vertical refuge must be limited in such a shallow lake. Hence, the overall inconsistency in the distribution patterns of the zooplankton, particularly across the vertical axis, was not surprising. Nevertheless, some small-scale patterns were recorded. There were two main problems with the density data: first, densities along the horizontal and vertical axes were fairly similar, possibly as the result of the continuous mixing of the shallow water column; secondly, zooplankton differed markedly between replicate samples, indicating micro-scale patchiness. In part, variability in the zooplankton density data may be due to the limitations of classical sampling methodologies in assessing spatial patchiness. The use of zooplankton traps (Wojtal et al., 2003) and particularly of acoustic surveys (Hembre & Megard, 2003) may help to resolve such fine patterns.

In autumn, no consistent patterns were observed in the spatial distribution of most zooplankton taxa, either horizontal or vertical, except for copepod nauplii. The distribution pattern of nauplii in the pelagic site (P) suggests daily vertical migration pattern (DVM), with a higher density near the bottom during the day and a more uniform distribution at night. The classical DVM pattern implies a higher density near the surface at night (Dodson, 1990; Han & Straskraba, 2001), but the homogeneous depth distribution at night presumably results from the absence of vertical environmental gradients (temperature, food), which is typical in shallow lakes (Gilbert & Hampton, 2001). Since nauplii are not very susceptible to fish predation, due to their small dimensions, we interpret these findings as a potential defence against invertebrate predators, as seen by Gilbert & Hampton (2001). The only pelagic invertebrate predator we found was *Chaoborus* sp., which has been shown to be a more efficient predator in the light, thus inducing diurnal swarming in zooplankton Kvam & Kleiven (1995). In the macrophyte bed (M), nauplii preferred the bottom layer rather than migrating, which could be a response against macrophyte-associated invertebrate predators foraging below the lily pads.

In the spring, the densities of copepods and chydorids was greater by night at both sites (though more obvious at P), independently of depth. The most reasonable explanation is that these taxa migrate vertically, aggregating near the bottom by day and rising to the surface at dusk, attaining a more homogeneous distribution at night (as observed by Jeppesen et al., 2002). We failed to detect aggregation near the bottom, because we could

not sample the first few centimetres above the sediment, where zooplankton have been seen during the day (Jeppesen et al., 2002). Data on *Daphnia* density did not reveal a vertical migration, although a preference for the deeper layer by day was apparent. The spatial pattern of the body size distribution of daphniids showed that large individuals aggregated near the bottom. The small-scale daily vertical migration by copepods and chydorids, and the vertical habitat selection exhibited by adult *Daphnia*, has been reported in the few studies conducted in shallow lakes (Gilbert & Hampton, 2001; Jeppesen et al., 2002). Fish predation risk is the most likely trigger of vertical heterogeneity in our study, especially considering that larger and more susceptible daphniids avoided the surface by day.

In recent years, really clear water has not been seen in Lake Vela, in spite of mild and/or transient clear water phases such as the one shown here. This presumably accounts for the reduced success of submerged macrophytes. Nevertheless, the differences observed between autumn and spring still suggest that transparency was a major factor dictating the vulnerability of zooplankton to visual predators (such as fish), as found by other authors (e.g. Dodson, 1990; Alonso et al., 2004). We only found vertical heterogeneity in zooplankton, as a defence against fish, in spring, when transparency was higher; however, predation pressure was higher in autumn, because of high temperatures and the presence of summer-born (YOY) fish. This confirms the need of a light gradient (Ringelberg & Van Gool, 2003) to induce vertical heterogeneity, which does not seem to occur in turbid phases or in permanently turbid lakes, despite intense predatory pressure.

Overall, copepod density in spring was consistently higher in the pelagic (P), suggesting the avoidance of macrophytes. The mean body length of *Daphnia*, and the proportion of adults, was also greater in the pelagic samples on two of the three sampling days in spring. This apparent “shore-avoidance” behaviour of copepods and large daphniids could be due to the repellence of littoral macrophytes, which is well documented in the literature (e.g. Lauridsen & Lodge, 1996; Burks et al., 2000; Burks et al., 2001). In addition, there is also evidence that the lower abundance of zooplankton in the littoral is related to higher predation by fish in these areas (Hülsmann et al., 1999). In fact, we frequently observed small fish swimming within the protection of the water lily leaves and field studies have shown that the mosquitofish is strictly littoral (Cabral et al., 1998; García-Berthou, 1999) while small pumpkinseed are also mainly littoral (García-Berthou

& Moreno-Amich, 2000). Data on *Daphnia* suggest that larger specimens were less common in macrophytes, which could indicate size-selective predation in macrophyte beds. This is a typical indicator of fish predation (Gliwicz, 1994). The suppression of large zooplankton in macrophytes has been termed ”fish-mediated shore-avoidance” (Lauridsen et al., 2001), which is contrary to the idea that macrophytes provide a refuge for zooplankton against fish predators, as reported for most studies in temperate lakes (Kvam & Kleiven, 1995; Lauridsen & Buenk, 1996; Jeppesen et al., 1998; Masson et al., 2001; Burks et al., 2002; Wojtal et al., 2003). Burks et al. (2002) had already hypothesized that DHM might not be an efficient mechanism in lakes where littoral planktivores (namely mosquitofish) thrive. This hypothesis has been supported for some subtropical and tropical systems (Burks et al., 2002; Meerhoff et al., 2006), and our results suggest that the zooplankton from Mediterranean shallow lakes may also experience higher predation risks in littoral macrophytes.

The spring campaign showed horizontal habitat selection, especially by large daphniids, while no patterns were observed in the autumn. As previously discussed, behavioural anti-predator defences were heightened in the clear water phase, presumably due to the presence of a vertical light gradient. However, transparency is not a reasonable argument to explain the difference observed in the horizontal spatial patterns between spring and autumn. These differences are dependent on macrophyte-associated factors, such as the production of allellopathical substances and, more importantly, the foraging ability of invertebrate or vertebrate predators within the macrophytes. Such macrophyte-associated factors are likely to have a strong seasonal component related to factors other than transparency (e.g. macrophyte cover; see Schriver et al., 1995; Burks et al., 2001).

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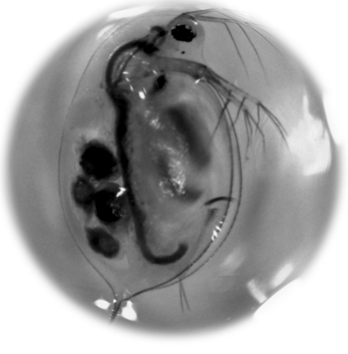

Capítulo 5

Respostas populacionais de *Daphnia* a predadores

Respostas populacionais de *Daphnia longispina* a sinais químicos exsudados por *Gambusia holbrooki* e *Lepomis gibbosus*

In: Castro B.B., Consciência S., Gonçalves F. (*in press*). Life history responses of *Daphnia longispina* to mosquitofish (*Gambusia holbrooki*) and pumpkinseed (*Lepomis gibbosus*) kairomones. Aceite para publicação nos *Proceedings of the VII*th *International Symposium on Cladocera* que serão publicados na publicação periódica *Hydrobiologia*.

RESUMO

No presente estudo foi avaliado o efeito de concentrações crescentes de sinais químicos de duas espécies de peixes (*Gambusia holbrooki* e *Lepomis gibbosus*) sobre respostas populacionais de *Daphnia longispina*. Estas duas espécies de peixes representam os planctívoros mais abundantes de muitos lagos pouco profundos do SO Europa, onde a ictiofauna indígena foi substituída por uma ictiocenose exótica. Os resultados demonstraram uma resposta idêntica de *D. longispina* a ambos os predadores: os sinais químicos de peixes estimularam a produção de mais neonatos de *Daphnia*, o que se traduziu numa mais elevada taxa de crescimento populacional (*r*), relativamente a um controlo sem a presença de peixes. A presença de peixes induziu igualmente uma reprodução mais precoce dos dafníideos, um menor tamanho corporal das primíparas, e a produção de neonatos de menores dimensões. Foram encontradas correlações significativas entre o tamanho corporal das primíparas e o dos neonatos com a densidade do predador, para ambas as espécies de peixes. Estes resultados estão de acordo com a "resposta positiva" observada por outros autores, que representa um mecanismo de defesa que permite fazer face às perdas causadas pela predação. A redução de tamanho corporal das primíparas e dos neonatos, mediada por sinais químicos, é uma resposta adaptativa à predação selectiva dos peixes sobre os indivíduos de maiores dimensões. Apesar da introdução da perca-sol (*L. gibbosus*) ser recente no lago de origem do clone de *D. longispina* utilizado nas experiências, a sua presença produziu efeitos similares aos induzidos por *G. holbrooki* nas respostas populacionais de *Daphnia*. Estes resultados são contrários à ideia de um sinal químico específico de cada espécie de peixe e suportam a hipótese de um sinal químico geral.

PALAVRAS-CHAVE

Daphnia, respostas populacionais, sinais químicos de peixes, *Gambusia holbrooki*, *Lepomis gibbosus*

O presente capítulo constitui um manuscrito em publicação em revista internacional com arbitragem científica e é, por esse motivo, apresentado na língua inglesa.

Life history responses of *Daphnia longispina* to mosquitofish (*Gambusia holbrooki*) and pumpkinseed (*Lepomis gibbosus*) kairomones

ABSTRACT

In the present study the effect of chemical cues from two fish species (mosquitofish and pumpkinseed), at different concentrations, was tested in life history experiments with *Daphnia longispina*. The two fish species used represent the most abundant planktivores of many Mediterranean shallow lakes (SW Europe), where the indigenous fish communities have been replaced by such exotic assemblages. Results have shown a similar response of *D. longispina* to both fish species: kairomones stimulated daphniids to produce more offspring, which resulted in higher fitness (*r*), relatively to a fishless control. Fish presence also induced an earlier first reproduction, a smaller size at maturity of daphniids, and the production of smaller-sized neonates. Significant correlations with fish concentration (indirect measure of fish kairomone concentration) were found for size at maturity and neonate size, for both fish species. These results are in accordance to the "positive response" observed by other authors, which represents a defence mechanism to face losses caused by fish predators. The chemically-mediated size reduction of mature females and neonates is an adaptive response to the size-selective predation exerted by fish. Pumpkinseed introduction is very recent in the lake of origin of the daphniids used in the experiments and its kairomone produced similar effects to mosquitofish in the life history of *D. longispina*. These results are contrary to the existence of a species-specific cue and support the hypothesis of a general fish kairomone.

KEYWORDS

Daphnia, life history responses, fish kairomones, *Gambusia holbrooki*, *Lepomis gibbosus*

INTRODUCTION

Daphniids react to predators by increasing their fecundity (life history shifts) and/or by reducing their mortality (morphological and behavioural defences), thus increasing their fitness under predation regimes. These phenotypic responses are chemically induced (see review by Lass & Spaak, 2003) and seem to represent a compromise between the least costly and the most effective antipredator defence strategy (Boersma et al., 1998; De Meester & Weider, 1999; Sakwinska & Dawidowicz, 2005). Many examples of chemically-induced antipredator defences, for both invertebrate and vertebrate (fish) predators, are present in the literature. We will focus on vertebrate predators, as they seem to be the main driving force in shaping the zooplanktonic communities in shallow lakes, by exerting a strong top-down control (Jeppesen et al., 1997).

Morphological alterations in response to fish chemical cues include the induction of longer tail spines (Spaak & Boersma, 1997; Stabell et al., 2003) and the modification of body (Weber & Vesela, 2002) and head shape (Weber & Vesela, 2002; Stabell et al., 2003). The reduction of body size (Engelmayer, 1995; De Meester & Weider, 1999; Sakwinska, 2000; Weber, 2003; Sakwinska & Dawidowicz, 2005) is also frequently observed in *Daphnia* exposed to fish chemical cues or kairomones. Antipredator behavioural responses allow daphniids to react to predator presence within a short period of time (De Meester & Cousyn, 1997; Lass & Spaak, 2003) and include avoidance or escape abilities (Pijanowska & Kowalczewski, 1997; Roozen & Lürling, 2001), changes in phototactic behaviour (De Meester & Cousyn, 1997; Van Gool & Ringelberg, 2002; Michels & De Meester, 2004), depth selection (Dodson, 1988; De Meester & Cousyn, 1997; Weber & van Noordwijk, 2002), changes in swimming speed (Pijanowska & Kowalczewski, 1997; Weber & van Noordwijk, 2002), and aggregation or swarming formation (Pijanowska & Kowalczewski, 1997; Roozen & Lürling, 2001). Ultimately, these simple predator-induced behaviours constitute the basis of more complex migratory phenomena, such as diel vertical (DVM) or diel horizontal (DHM) migration (Lass & Spaak, 2003).

Life history shifts in the presence of fish predators have received considerable attention in the recent literature. However, *Daphnia* life history responses to fish chemical cues are still somewhat contradictory. Negative responses, i.e. reduced fecundity or delayed reproduction, have been observed by some authors (Burks et al., 2000; Hanazato et al., 2001). However, the most usual response pattern is an early maturation (decrease in age and size at maturity), as well as a production of larger clutches of smaller neonates, resulting in higher fitness (*r*) (e.g. Engelmayer, 1995; Reede, 1997; Sakwinska, 2000; Weber, 2003; Hülsmann et al., 2004). Size and age at maturity are considered to be the most affected traits (Hülsmann et al., 2004) and this scenario clearly represents an adaptive response to the positive size-selective predation exerted by fish. However, no effects of fish kairomones on size or age at maturity of *Daphnia* were found in the studies by Sakwinska (1998) and Michels and De Meester (2004), for example. Weber (2003) considered that the heterogeneity in the life history responses of *Daphnia* to different fish species poses new challenges when considering the effects of each specific planktivore assemblage on the zooplankton populations.

Bearing this in mind, a life history experiment was performed to assess the responses of *Daphnia longispina* to chemical cues exuded by its main predators in shallow Lake Vela. This is a shallow polymictic lake, where daphniids undergo very high predation pressures from two invasive fish species: mosquitofish (*Gambusia holbrooki*) and pumpkinseed sunfish (*Lepomis gibbosus*). In fact, pumpkinseed is presently the most relevant planktivore/omnivore in many other Mediterranean shallow lakes (García-Berthou & Moreno-Amich, 2000; Blanco et al., 2003), having displaced the indigenous cyprinid community. In Portugal, the pumpkinseed invasion is much more recent than that of mosquitofish (Almaça, 1995), especially in lentic systems. In Lake Vela, pumpkinseed was introduced in the late 1990s and, since then, it has become the dominant taxon (J. Vingada, personal communication). Mosquitofish probably exists in Lake Vela since the early 1900s, as a result of introductions related to malaria control programs. Diet analysis of these two fish species in Lake Vela confirmed a high degree of planktivory, but they do not rely exclusively on zooplankton as food items (Castro et al., submitted manuscript[1]). The impact of pumpkinseed on zooplankton is higher than that of mosquitofish, mainly because the latter is restricted to the littoral and is partially gape-limited in respect to *Daphnia* as a prey item. The aims of this study were: 1) to assess how *D. longispina* responds to the presence of predators cues from mosquitofish and pumkinseed; 2) to verify if this effect is linearly dependent on the concentration of fish chemical cue; and 3) to determine if these two fish species shift the life history of *D. longispina* differently, given their distinctive features and introduction timings.

MATERIALS AND METHODS

Test organisms

Monoclonal cultures of *D. longispina* (clone EV20, *sensu* Antunes et al., 2003) were established from an ephippial egg coming from the eutrophic Lake Vela. This lake sustains large and seasonal populations of *Daphnia*, which are subjected to very high predatory pressure from fish, particularly pumpkinseed. Bulk group cultures of synchronous single-cohort daphniids have been asexually reared for several generations under a 16:8 hr light:dark cycle and a temperature of 20 ± 2ºC. Organisms were maintained in ASTM (American Society for Testing and Materials, USA) synthetic hardwater medium

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[1] CAPÍTULO 2 da tese

enriched with a standard organic additive (*Ascophyllum nodosum* extract; Baird et al., 1989). Medium was renewed every Monday, Wednesday and Friday. Food (*Selenastrum capricornutum*, presently *Pseudokirchneriella subcapitata*) was added daily at a final concentration of 1.5 x 10^5 cells mL^{-1}. Offspring from the first two clutches were discarded and neonates from the 3rd-5th clutch were used either in the life table experiments or for renewing the group cultures.

The actual taxonomic position of *D. longispina* records in Portugal is unclear, due to high morphological variation and interspecific hybridisation within the species complex (*D. longispina, D. galeata* and *D. cucullata*) (Schwenk et al., 1998; Giessler et al., 1999; Billiones et al., 2004). The ITS-RFLP pattern (Billiones et al., 2004) of the clone used in these experiments was concordant to that of *D. longispina* (Petrusek et al., 2005).

Fish-conditioned water

Mosquitofish (*Gambusia holbrooki*) and pumpkinseed sunfish (*Lepomis gibbosus*) were caught one week before the experiments in Lake Vela. Fish-conditioned water was obtained by maintaining either 5 juvenile *L. gibbosus* (average size: 45 mm TL; average wet weight: 1.1 g) or 10 adult *G. holbrooki* (average size: 35 mm TL; average wet weight: 0.34 g) in 10-L aquaria for 24 hrs in ASTM hard water medium. Fish concentrations (fish L^{-1}) in the aquaria were established in order to obtain approximate fish loads (biomass per L) between both species. A third aquarium was prepared containing only culture medium, without any fish (i.e. kairomone-free). Each day, 2 L of water (i.e. 20%) from each aquarium were removed, filtered through a 1.2-μm glass fibre filter, and used in the life table experiments with *D. longispina* (see below). The aquaria were then refilled with fresh medium. Prior to these procedures, fish were transferred to a smaller aquarium, where they were allowed to feed for 5-10 minutes on live *D. longispina* and chironomids, simulating their normal diet in the field (Castro et al., submitted manuscript). A separate feeding aquarium is important in order to minimise the potential influence of chemical cues released by crushed *Daphnia* (Pijanowska & Kowalczewski, 1997; Slusarczyk & Rygielska, 2004). Fish were returned to their original aquarium after medium transfer was completed. Oxygen (WTW portable oxygen meter) and ammonia (Nessler method) levels in the aquaria were monitored continuously for the duration of the experiment, since changes in these levels could be responsible for negative effects on the life history responses of *D. longispina*, working as confounding factors.

Life table experiments

Experiments were conducted for 14 days under the same temperature and photoperiod regimes as described for the rearing procedures (see above). Life table experiments were initiated with first-instar daphniids (<24-hr-old neonates). A semi-static design was employed, using 10 individual animals randomly assigned to each experimental treatment: no fish or control (CTL), *Gambusia*-conditioned water (0.20, 0.50, 1.0 fish L^{-1}) and *Lepomis*-conditioned water (0.10, 0.25, 0.50 fish L^{-1}), resulting in 7 (treatments) x 10 (replicates) = 70 jars with individual daphniids. Water from the fishless aquarium (i.e. kairomone-free) was used as the control and as the dilution water for fish kairomone treatments. Test media (50 mL per organism) were renewed on a daily basis to maintain kairomone concentration as high as possible, because the breakdown of these signals seems to occur in a few hours (Dodson, 1988). Organic additive (see rearing procedures under *Test organisms*) and food (1.5×10^5 cells mL^{-1}) were supplied daily, concurrently with medium renewal. Animals were daily checked for mortality and reproductive state and, when neonates were released, they were immediately counted and discarded. The following parameters were registered: total number of offspring per female, total number of broods per female and age at first reproduction (AFR). Body size measurements were performed on the test organisms (mothers) on the day they released the first batch of eggs in the brood pouch (size at maturity) and at the end of the experiment (body length at day 14). Additionally, body size measurements on the neonates from the first clutch were also performed (neonate size). Survival and fecundity estimates were used to compute the intrinsic rate of population increase (*r*), also called per capita rate of increase (Meyer et al., 1986). This demographic parameter was iterated from the Euler-Lotka equation:

$$1 = \sum_{x=0}^{n} e^{-rx} \, l_x m_x \ ,$$

where *r* is the rate of population increase (day^{-1}), x is the age class in days (0…n), l_x is the probability of surviving to age x, and m_x is the fecundity at age x. Replicate pseudo values for *r* were generated using the jack-knifing technique described by Meyer et al. (1986), thus enabling the use of statistical tests for analysing differences in the rate of population increase between experimental treatments.

Statistical analysis

To test the significance of the effect of fish (i.e. kairomone) presence and concentration on the life history of the daphniids, a one-way ANOVA was employed to each of the life history parameters, followed by a Dunnett test (post-hoc comparisons against the control group). A repeated measures ANOVA was used to test for differences between the experimental treatments in the number of offspring produced in the first three clutches (using clutch as the repeated measurement). Whenever an interaction between clutch and fish concentration was found, a test for simple main effects of fish concentration within each clutch was carried out using the within-cells error term as the denominator for the F test (Quinn & Keough, 2002). Occasionally, deviations from homogeneity of variances or normality were detected, but we assumed the ANOVAs to be robust to these deviations. Experimental treatments for each fish species were analysed independently. The Pearson product moment coefficient was used as a correlation measure between the life history parameters and fish concentration (log-transformed), as a way to assess whether the effect of fish kairomones on the life history of *D. longispina* was concentration-dependent. A fixed significance level (α) of 0.05 was used in all analyses.

RESULTS

During the course of the experiment, ammonia levels were gradually increasing over time. Although there were differences between treatments, ammonia (NH_4^+) concentrations were still fairly low: CTL – 0.04-0.08 mg L^{-1}; mosquitofish-conditioned water – 0.16-0.38 mg L^{-1}; pumpkinseed-conditioned water – 0.33-0.85 mg L^{-1}.

Significant effects of fish cue concentration were observed in all of the *D. longispina* life-history parameters for both pumpkinseed and mosquitofish. A similar pattern of response was obtained for the two fish species (Figures 5.1 and 5.2). Size at maturity was significantly reduced for all of the tested mosquitofish concentrations (ANOVA: $F = 10.2$; d.f. = 3, 35; $P < 0.001$) and for the two highest pumpkinseed concentrations (ANOVA: $F = 6.63$; d.f. = 3, 36; $P = 0.001$). Neonate body size (from the first clutch) was also significantly reduced. For mosquitofish kairomone, a significant reduction was found for the 0.50 and 1.0 fish L^{-1} concentrations (ANOVA: $F = 5.25$; d.f. = 3, 35; $P = 0.004$) and for pumpkinseed kairomone this was observed at the highest concentration – 0.50 fish L^{-1} (ANOVA: $F = 9.59$; d.f. = 3, 36; $P < 0.001$). Body length at day 14 was significantly affected by mosquitofish cue (ANOVA: $F = 3.17$; d.f. = 3, 35; $P = 0.036$), but no significant differences were observed in relation to the control (see Figure 5.1). In the pumpkinseed treatments, body length revealed to be significantly stimulated (ANOVA: $F = 4.02$; d.f. = 3, 35; $P = 0.015$) in the lowest concentrations (0.10 and 0.25 fish L^{-1}). Earlier reproduction was observed as a consequence of fish presence, as seen by a significant depression of age at first reproduction in all kairomone concentrations, for both mosquitofish (ANOVA: $F = 11.5$; d.f. = 3, 35; $P < 0.001$) and pumpkinseed (ANOVA: $F = 16.0$; d.f. = 3, 36; $P < 0.001$). In contrast, the total number of offspring produced by female *D. longispina* was significantly increased by both fish kairomones (mosquitofish – ANOVA: $F = 34.5$; d.f. = 3, 35; $P < 0.001$; pumpkinseed – ANOVA: $F = 128$; d.f. = 3, 35; $P < 0.001$), in all tested concentrations. A similar response was found in the number of broods, with mosquitofish (ANOVA: $F = 90.7$; d.f. = 3, 35; $P < 0.001$) and pumpkinseed (ANOVA: $F = 6.55 \times 10^4$; d.f. = 3, 35; $P < 0.001$) cues causing a significant enhancement.

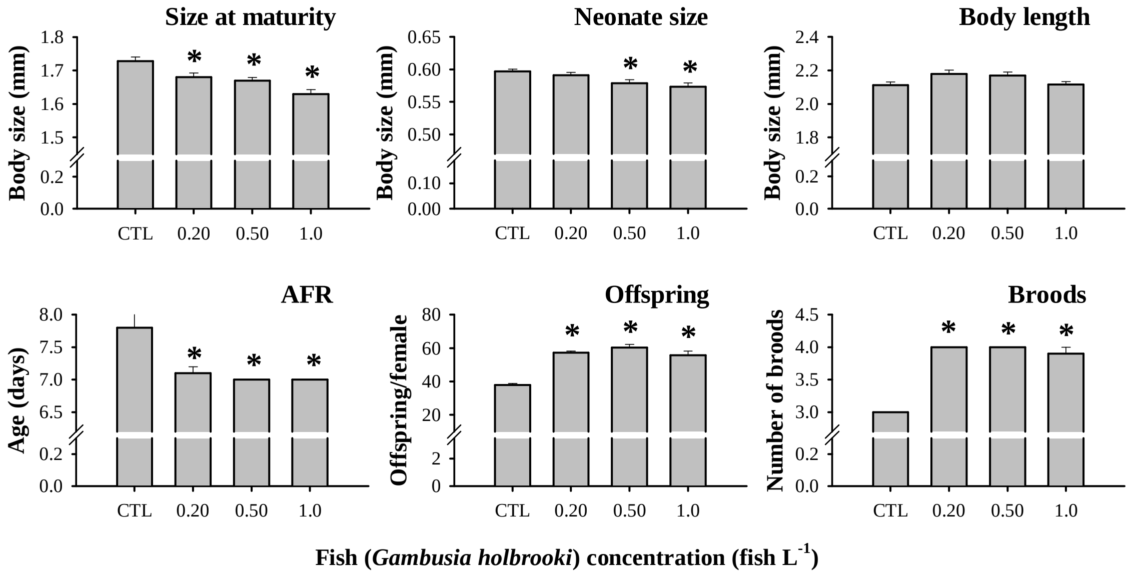

Figure 5.1 • Mean life history responses of *Daphnia longispina* exposed to several concentrations of mosquitofish (*G. holbrooki*) kairomones: size at maturity, neonate size, body length at day 14, age at first reproduction (AFR), total number of offspring per female and total number of broods per female. Error bars represent SE. Asterisks (*) stand for statistically significant differences relatively to the kairomone-free control (CTL).

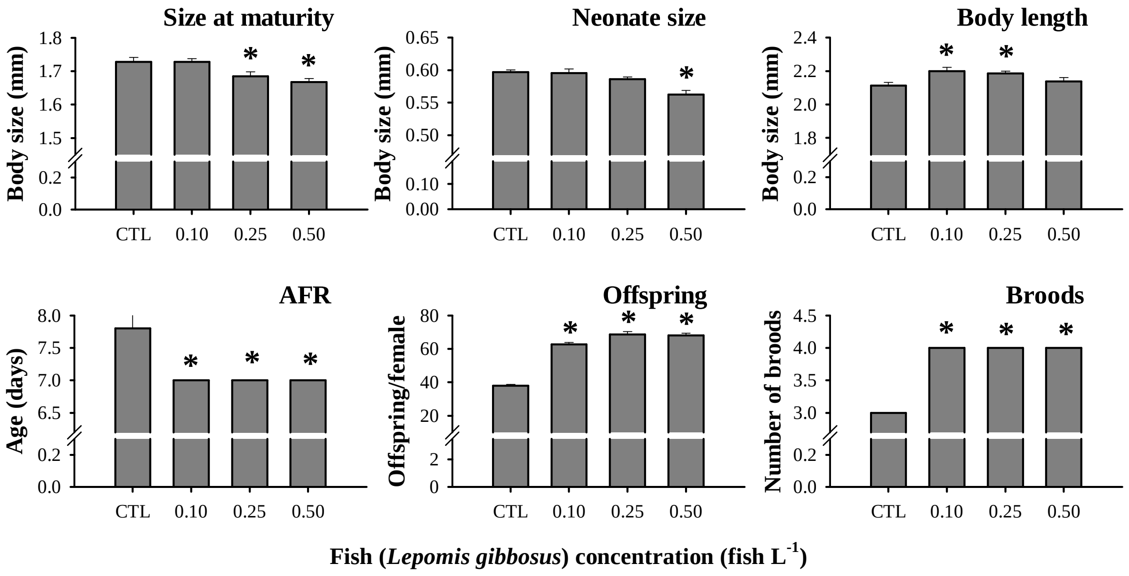

Figure 5.2 • Mean life history responses of *Daphnia longispina* exposed to several concentrations of pumpkinseed (*L. gibbosus*) kairomones: size at maturity, neonate size, body length at day 14, age at first reproduction (AFR), total number of offspring per female and total number of broods per female. Error bars represent SE. Asterisks (*) stand for statistically significant differences relatively to the kairomone-free control (CTL).

Earlier reproduction and higher fecundity in the presence of fish kairomones lead to significantly higher rates of population increase (*r*), both for mosquitofish (ANOVA: F = 7.14; d.f. = 3, 36; $P < 0.001$) and for pumpkinseed (ANOVA: F = 44.3; d.f. = 3, 36; $P <$ 0.001), in all concentrations (Figure 5.3).

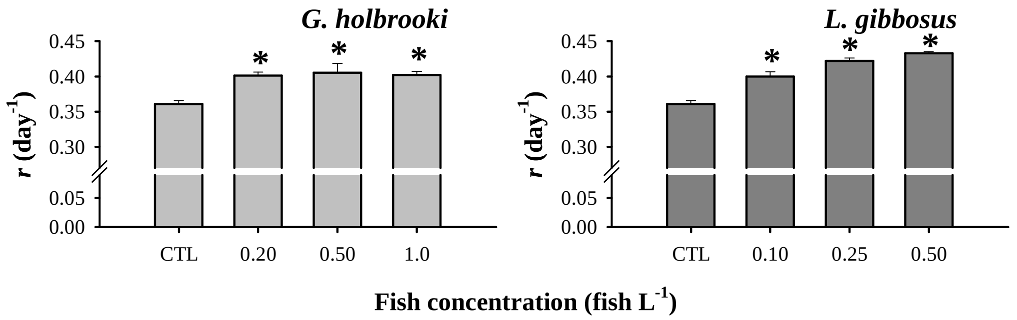

Figure 5.3 • Rate of population increase (*r*) of *Daphnia longispina* exposed to several concentrations of mosquitofish (*G. holbrooki*) and pumpkinseed (*L. gibbosus*) kairomones. Error bars represent SE. Asterisks (*) stand for statistically significant differences relatively to the kairomone-free control (CTL).

Since the development of the daphniids was enhanced by kairomones (as observed by earlier reproduction), a fourth clutch was usually produced in the experimental treatments, but not in the control. To assess if the observed differences in fecundity (Figures 5.1 and 5.2) were due to the production of this additional clutch, the fecundity data for the first three clutches alone were analysed. No differences were found between the mosquitofish concentrations, pooling the first three clutches, but the *P* value was marginal (Table 5.1). Significant differences were found for pumpkinseed concentration, and a significant interaction between clutch and concentration was observed (Table 5.1). Simple main effects testing showed that fish kairomone significantly enhanced neonate production only in the second and third clutch (Figure 5.4).

Table 5.1 • Summary of the repeated measures ANOVAs applied to the fecundity data (number of offspring produced in the first three clutches) of *Daphnia longispina* exposed to fish kairomones.

Source of variation	***Gambusia holbrooki***			***Lepomis gibbosus***		
	F	**d.f.**	***P***	***F***	**d.f.**	***P***
Between subjects						
concentration	2.68	3, 35	0.062	17.1	3, 35	<0.001
Within subjects						
clutch	542	2, 70	<0.001	514	2, 70	<0.001
clutch x concentration	1.39	6, 70	0.230	3.08	6, 70	0.010

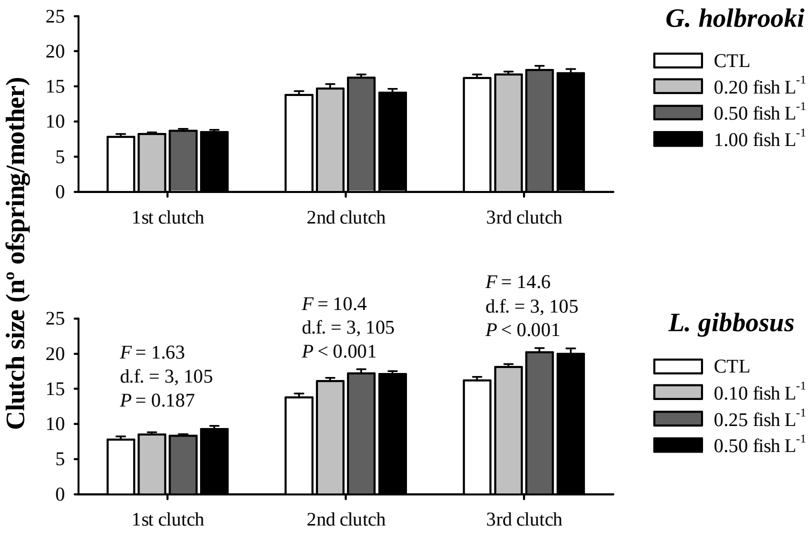

Figure 5.4 • Fecundity data (number of offspring released per female in the first three clutches) of *Daphnia longispina* exposed to several concentrations of mosquitofish (*G. holbrooki*) and pumpkinseed (*L. gibbosus*) kairomones. Control (CTL) consists of a kairomone-free treatment. Significance is indicated where simple main effects tests were conducted for each clutch (see text for further explanation).

Significant negative correlations between the life history parameters and fish concentration (log-transformed) were found for size at maturity and neonate size (Table 5.2). This means that as kairomone concentration rises, daphniids mature at an increasingly smaller size and produce increasingly smaller neonates.

Table 5.2 • Correlation coefficients (Pearson product moment) and associated significance between the life history parameters of *Daphnia longispina* exposed to fish kairomones and log fish concentration. NS stands for statistically non significant.

Parameter	***Gambusia holbrooki***		***Lepomis gibbosus***	
	Correlation	***P***	**Correlation**	***P***
Size at maturity	-0.97	0.03	-0.95	0.05
Neonate size	-0.98	0.02	-0.96	0.04
Body length	-0.09	NS	0.07	NS
AFR	-0.78	NS	-0.69	NS
Offspring	0.65	NS	0.78	NS
Broods	0.65	NS	0.69	NS
r	0.72	NS	0.92	NS

DISCUSSION

Exposure to fish kairomones resulted in earlier maturity and higher fecundity of *D. longispina*, which lead to higher fitness (*r*). These results are in accordance to the “positive response” (Hanazato et al., 2001) observed by other authors (e.g. Engelmayer, 1995; Reede, 1997; Sakwinska, 2000; Weber, 2003; Hülsmann et al., 2004), which represents an adaptive mechanism to face losses caused by fish predators. Additionally, daphniids exposed to fish cues in the present study attained a smaller size at maturity and produced smaller-sized offspring. This chemically-mediated body size reduction of mature females and neonates is an adaptive response to the positive size-selective predation exerted by fish, as reported by other authors (Engelmayer, 1995; De Meester & Weider, 1999; Sakwinska, 2000; Weber, 2003; Sakwinska & Dawidowicz, 2005; von Elert & Stibor, 2006). This is further enhanced by the fact that mother and neonate body size parameters (except body length at day 14) were affected in a concentration-dependent manner (see Table 5.2). Although exposure to kairomone also affected other life history parameters, this effect did not seem to be related with concentration. Fish presence alone seemed to be sufficient to induce certain plastic traits, independently of the strength (i.e. concentration) of the kairomone.

Higher NH_4^+ concentrations were observed in the fish treatments in comparison to the control, especially in pumpkinseed-conditioned water. However, the observed levels were not very high (< 1.0 mg L^{-1}). Lurling et al. (2003) showed that such ammonia levels do not produce effects on the life history of *Daphnia* comparable to those induced by conspecifics. However, they do recognise that ammonia may alter “negatively” the life history responses of *Daphnia*. This could lead to false “negative” responses of daphniids exposed to fish-conditioned water in the literature. Some other “negative” responses (see e.g. Hanazato et al., 2001), on the other hand, could result as a consequence of metabolic costs associated to the induction of behavioural or morphological defences in response to fish presence. In the present study, no visible alterations in *D. longispina* morphology were observed at the tested concentrations.

Body length at day 14 gave an unexpected and contradictory result. Growth seemed to be stimulated in the kairomone treatments in relation to the control, even after a smaller size at maturity of these experimental animals had been observed. This may be a consequence of the early maturation of daphniids under predator presence: they accelerate their development in order to mature at an earlier age and at a smaller size and consequently, they achieve a higher number of adult instars in the same period of time, because they start reproducing earlier in the presence of predators. This lead to the production of an additional brood in animals exposed to kairomones. We hypothesize that, for the same adult instar, daphniids under fish threat will present a smaller body size. However, daphniids at the end of our experiment were not in the same adult instar (as seen by the number of broods produced). To avoid this experimental bias, a moulting log should have been kept (by recording exuviae) or daily measures on body size should have been taken. It seems reasonable to conclude that this apparent adult growth stimulation is an indirect effect of fish kairomones. A larger adult body length seems to be counterproductive, making *Daphnia* more vulnerable to visually-oriented predators, unlike what was observed for other traits (size at maturity, neonate size). At this age, however, body length is probably unimportant because daphniids are very conspicuous (> 2 mm) and are unlikely to be encountered under actual fish predation regimes.

Although the general response to kairomones was similar between the two fish species used, daphniids exposed to pumpkinseed kairomone seemed to invest more in neonate production (see Figure 5.4). Weber (2003) also observed small discrepancies between the responses of *Daphnia galeata* clones to two different fish kairomones. This author interpreted these findings as an argument in favour of one of two hypotheses: i) the existence of several species-specific fish kairomones; ii) the existence of a chemical mixture of substances, rather than an isolated compound, thus increasing the complexity of its effects. The existence of species-specific kairomones could imply that *Daphnia* would not be responsive to kairomones from recently introduced fish, such as pumpkinseed. This is not the case in our study. Furthermore, Von Elert & Stibor (2006) have shown that the chemical nature of the kairomone is similar between some fish species, being comprised by a mixture of substances. The results of Stabell et al. (2003) gave a strong indication that fish kairomones may be latent alarm cues from *Daphnia*, resulting from the digestion of conspecifics by fish. Even when an exotic fish species was used by these authors, morphological alterations were observed in *Daphnia* only when the predator had been previously fed daphniids. The findings of Slusarczyk and Rygielska (2004) further supported this and showed that fish faeces were the primary source of kairomones, which were able to induce diapause in *Daphnia*. These findings support the theory of a universal chemical cue (or mixture of chemical cues) that results from the activation of a latent alarm signal of preyed *Daphnia* in the fish digestive tract. Slight differences between fish species (such as for mosquitofish and pumpkinseed in the present study) are therefore more likely to be explained as a result of variability in fish behaviour and feeding habits/metabolism (e.g. gut passage time), which may be important in defining the strength of the kairomone. Recent findings (von Elert & Stibor, 2006) suggest that the effects of fish cues may be even more complex to interpret, as shown by the differential induction of *Daphnia* anti-predator defences (behaviour and life history) by different fractions of a kairomone produced by *Leuciscus idus*.

To our knowledge, this is the first study reporting the effects of these two fish species on the life history responses of *Daphnia*. Mosquitofish has long been present in southern Europe, but pumpkinseed was only recently introduced in Lake Vela. However, their presence shifted the life history parameters of *D. longispina* in the same direction. In fact, pumpkinseed kairomone enhanced the fecundity of *D. longispina* more drastically than mosquitofish (see Figure 5.4). These results contribute to the idea of a non-specific fish kairomone, leading to an adaptive “positive” response of daphniids to substantial mortality due to size-selective predation by fish. The experimental design used here could not test whether the kairomone resulted from preyed *Daphnia*, because a treatment where no daphniids were fed to the fish is missing. Nonetheless, results clearly show the adaptive value of chemical communication in predator-prey signalling systems, which is maintained even with a recently introduced predator.

Inducible defences are an example of the importance of predation as a selective force, driving the evolution of life history, behavioural and morphological traits of the prey. In *Daphnia*, these traits present considerable phenotypic plasticity, which allows daphniids to adapt to changes in their environment. Chemical signals, such as fish kairomones, play a vital role as cues for daphniids to undergo adaptive life history shifts. Still, the ecological significance of kairomone-induced life history responses is complex. On one hand, there seems to be a compromise between different types of inducible defences (Boersma et al., 1998; De Meester & Weider, 1999; Sakwinska & Dawidowicz, 2005). On the other hand, the interpretation of the effects of fish kairomones on the life history traits of cladocerans is complicated by its interaction with additional factors, such as food level (Reede, 1997; Weber, 2001; Hülsmann et al., 2004) and temperature (Sakwinska, 1998). Further research should focus on these interactions and also on the direct and indirect costs associated with induced life history shifts, such as the production of smaller neonates (indirect effect), which are less resistant to starvation (Gliwicz & Guisande, 1992) and to contaminant exposure (Enserink et al., 1990), for example. There is also experimental evidence that the presence of predators directly reduces the tolerance of cladocerans to food shortage (Weber, 2001; Hanazato et al., 2001) and contaminants (Rose et al., 2001).

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Considerações finais

Os trabalhos de natureza essencialmente descritiva em Ecologia, embora fundamentais para a compreensão dos fenómenos naturais, têm sido progressivamente secundarizados nas publicações científicas mais prestigiadas (excepto se integrarem séries temporais longas, *i.e.* de vários anos). Por norma, este tipo de estudos carece de hipóteses concretas à partida e não permite o controlo das variáveis, o que diminui a sua relevância e originalidade. Os estudos de natureza experimental, por outro lado, têm uma maior receptividade na comunidade científica (consoante a originalidade, naturalmente) e integram todas as fases do método científico. Todavia, em algumas circunstâncias, as provas empíricas proporcionadas pelos estudos descritivos representam informação original, contribuindo com novo conhecimento para a temática em questão. Este cenário verifica-se, por exemplo, na ausência de informação prévia sobre a região/*habitat* estudado ou no caso de se utilizarem ferramentas inovadoras. Os lagos pouco profundos da Península Ibérica enquadram-se no primeiro caso, como resultado da conjugação da reduzida implementação histórica da Limnologia na Península Ibérica (Casado & Montes, 1992) e da pouca atenção recebida pelos lagos pouco profundos (comparativamente aos lagos profundos e estratificados) até há umas décadas atrás (Scheffer, 1998). Adicionalmente, e no caso concreto do local de estudo (Lagoa da Vela), o interesse é ainda reforçado pelo estado extremo de homogeneização da sua fauna piscícola, que apresenta reduzida diversidade e dominância de espécies exóticas como a perca-sol (*Lepomis gibbosus*), o achigã (*Micropterus salmoides*), a gambúzia (*Gambusia holbrooki*) e a carpa (*Cyprinus carpio*). A problemática do impacto das espécies alienígenas representa uma preocupação global e é particularmente grave no caso das espécies de peixes de água doce (Cambray, 2003; Garcia-Berthou et al., 2005).

Pelos motivos acima indicados, a presente dissertação de doutoramento teve que, em parte, desenvolver uma componente descritiva. Esta opção foi tomada face ao conhecimento limitado sobre as interacções entre a comunidade zooplanctónica e a ictiocenose alienígena. As evidências históricas e as publicações anteriores acerca da ecologia da Lagoa da Vela (e.g. Antunes et al., 2003; Abrantes et al., 2006a) sugeriam um papel estruturante da comunidade piscícola actual sobre a dinâmica do zooplâncton e da Lagoa como um todo. No entanto, nunca nenhum estudo havia integrado informação de ambas as componentes (peixes e zooplâncton). Mais, desconhece-se outros estudos na Europa que tenham abordado com alguma profundidade esta problemática tendo como

base uma ictiocenose com dominância das espécies aqui relatadas, que representam também um problema de conservação da natureza, sobretudo no sul da Europa. Os dados resultantes da primeira parte do trabalho (CAPÍTULOS 1-3) permitiram a formulação de hipóteses, sustentadas pela observação das interacções entre os diversos níveis tróficos da Lagoa da Vela, sobretudo o elo peixes-zooplâncton. Algumas das questões levantadas foram testadas no âmbito desta dissertação, utilizando uma abordagem de cariz experimental (CAPÍTULOS 4-5). Como em todos os trabalhos de investigação, o presente estudo deixa ainda outras questões e desafios a serem respondidos no futuro.

Para além do interesse científico em compreender como funcionam as interacções tróficas que regulam o funcionamento deste ecossistema modificado, a relevância deste estudo estende-se ainda para matérias de natureza mais aplicada, como a conservação da fauna e gestão da eutrofização. Mais, a evidente degradação da qualidade da água e da biodiversidade das lagoas de Quiaios (Lagoa da Vela incluída) compromete não só o valor ecológico da área, mas também a qualidade dos recursos hídricos para consumo humano (fins domésticos e agrícolas). Numa lagoa vizinha (Lagoa das Braças), por exemplo, existe um ponto de captação de água que abastece parcialmente o concelho da Figueira da Foz. A par deste estudo, uma outra tese de doutoramento (Abrantes, em preparação) está a ser produzida também com a Lagoa da Vela como objecto de estudo, mas abordando as fontes de contaminação difusa e os seus potenciais impactos (e.g. Abrantes et al., 2006b).

Do ponto de vista ecológico, no que foi possível demonstrar ao longo dos cinco capítulos desta dissertação, a Lagoa da Vela apresentou-se como um sistema controlado sobretudo por mecanismos de *top-down*. Tal não é surpreendente dada a elevada disponibilidade de nutrientes para o crescimento fitoplanctónico, que minimiza a sua influência (controlo *bottom-up*). Em lagos pouco profundos e eutróficos é comum a pressão predatória exercida pelos peixes ver a sua importância aumentada enquanto factor modelador dos níveis tróficos inferiores (Jeppesen et al., 1997). Na ausência de peixes planctívoros indígenas, a pressão predatória sobre o zooplâncton da Lagoa da Vela é exercida por planctívoros pouco eficientes, como é o caso da perca-sol (sobretudo), da gambúzia e dos juvenis de achigã. Estas espécies alienígenas exibem uma plasticidade assinalável na utilização dos recursos alimentares disponíveis, alternando oportunistamente entre as componentes planctónica e bêntica (sobretudo larvas de dípteros), sem aparente comprometimento do seu sucesso na lagoa. Os adultos de perca-sol destacaram-se pelo seu impacto negativo em *Daphnia* durante a Primavera. Quando liberto da pressão predatória pelas baixas temperaturas, o zooplâncton (sobretudo *Daphnia*) contribuiu significativamente para a ocorrência de uma fase de água transparente em Fevereiro e Março, controlando o crescimento fitoplanctónico de forma irregular e temporária. Esta fase de água transparente nem sempre se tem verificado na Lagoa da Vela nos últimos anos e, no futuro, é necessário compreender as flutuações inter-anuais da condição da lagoa, inerentemente ligadas às variações no recrutamento dos peixes e às flutuações hidrológicas.

Durante a maior parte do ano, a Lagoa da Vela apresentou elevada turbidez e dominância de zooplanctontes de pequenas dimensões, incapazes de controlar a produção fitoplanctónica. Contudo, o papel das partículas em suspensão para a transparência da lagoa também revelou ser determinante, o que vem ao encontro das sugestões anteriores de afundamento da lagoa (como medida de mitigação), de forma a incrementar o nível da água (Pereira, 1997; Fernandes, 1999). O controlo da transparência é vital na gestão de lagos eutróficos pouco profundos, já que um estado de água transparente permite o crescimento de macrófitas que, por sua vez, reforçam essa mesma transparência através de diversos mecanismos de histerese (Scheffer et al., 1993; Scheffer & Jeppesen, 1998; Scheffer, 1998), como, por exemplo, a fixação dos sedimentos, a produção de substâncias inibidoras do crescimento fitoplanctónico (alelopatia), ou o consumo de nutrientes (competindo com o fitoplâncton). É nesse pressuposto que assenta a biomanipulação (Moss, 1992; Perrow et al., 1997; Scheffer, 1998), uma eco-ferramenta cuja eficácia depende do conhecimento acerca das interacções tróficas entre peixes piscívoros-planctívoros e peixes planctívoros-zooplâncton.

Nos lagos pouco profundos, a ausência do refúgio hipolimnético (em profundidade) torna o zooplâncton particularmente susceptível à predação. Contudo, em determinadas condições, as macrófitas podem providenciar refúgio adequado para os zooplanctontes destes sistemas (Jeppesen et al., 1998; Burks et al., 2002). Ficou aqui demonstrado que este efeito de refúgio não se verifica para as manchas de nenúfar, na Lagoa da Vela. Esta observação coincidiu com a utilização primordial das áreas estruturadas (vegetação terrestre em decaimento, macrófitas aquáticas, etc.) pelos peixes de menores dimensões, sobretudo gambúzia (exclusivamente litoral) e juvenis de perca-sol. Perante as reduzidas hipóteses em escapar aos predadores, alguns zooplanctontes exibem agregações junto ao leito da lagoa durante o dia. A migração vertical do zooplâncton é um mecanismo vital na sobrevivência das populações zooplanctónicas nos lagos profundos e no oceano (Lampert, 1989; Ringelberg, 1995), na medida em que minimiza o impacto enorme da predação, apesar dos potenciais custos metabólicos do refúgio em profundidade (Dawidowicz & Loose, 1992; Loose & Dawidowicz, 1994). Outra estratégia para reduzir a mortalidade associada a peixes selectivos passa por minimizar o tamanho corporal e maximizar a produção de descendência. Os dafníideos são particularmente susceptíveis à predação, sobretudo a partir do momento da maturação, em que se tornam demasiado conspícuos (Taylor & Gabriel, 1992; Gliwicz, 1994). *Daphnia longispina* isolada da Lagoa da Vela exibiu plasticidade fenotípica na presença quer de perca-sol, quer de gambúzia, confirmando que a minimização do tamanho corporal e a maximização da produção de neonatos na presença de predadores selectivos continuam estratégias válidas na presença de espécies exóticas.

Enquanto ciência, a Ecologia dedica-se ao estudo dos processos, mecanismos e inter-relações entre organismos e interacções com todos os aspectos (vivos e não vivos) do ambiente que os rodeia. Dentre estes aspectos, a predação emerge como determinante do funcionamento dos ecossistemas, sobretudo desde que alguns trabalhos pioneiros (Hrbácek et al., 1961; Brooks & Dodson, 1965; Hurlbert et al., 1972) despoletaram essa escola de pensamento. A introdução de espécies predadoras afecta os equilíbrios existentes e pode gerar mudanças ambientais com consequências imprevisíveis. A compreensão, previsão e mitigação de impactos da mudança ambiental (como a globalização dos recursos piscícolas de água doce) reside no aprofundado conhecimento dos ecossistemas e das interacções entre os organismos, incluindo casos extremos como o da Lagoa da Vela.

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