PERSISTENCE, NICHE DIFFERENTATION AND ECOLOGICAL SIMILARITY IN TWO CRYPTIC ROTIFER SPECIES

CARMEN GABALDÓN TÉBAR
PhD THESIS 2015

VNIVERSITAT DE VALÈNCIA

VNIVERSITAT DE VALÈNCIA

Facultad de Ciencias Biológicas
Instituto Cavanilles de Biodiversidad y Biologia Evolutiva
Programa de doctorado de *Biodiversidad y Biología Evolutiva*

Persistence, niche differentiation and ecological similarity in two cryptic rotifers species

Carmen Gabaldón Tébar

2015

Directores:
Manuel Serra Galindo
María José Carmona Navarro
Javier Montero Pau

Tesis presentada por CARMEN GABALDÓN TÉBAR para optar al grado de Doctora en Ciencias Biológicas por la Universidad de Valencia.

Firmado: Carmen Gabaldón Tébar

Tesis dirigida por los Doctores en Ciencias Biológicas por la Universidad de Valencia, MANUEL SERRA GALINDO, MARÍA JOSÉ CARMONA NAVARRO y JAVIER MONTERO PAU.

Manuel Serra Galindo	Mª José Carmona Navarro	Javier Montero Pau
Catedrático de Ecología	Prof. Titular de Ecología	Doctor en Biología
Universidad de Valencia	Universidad de Valencia	Universidad de Valencia

Este trabajo ha sido financiado por una Beca-Contrato del Programa de Formación del Personal Investigador (BES2010-036384), concedida a CARMEN GABALDÓN TÉBAR, y por un proyecto de investigación (CGL2009-07364) ambos del Ministerio de Ciencia e Innovación (Gobierno de España).

A mis padres

Agradecimientos

Muchas han sido las personas que de una forma u otra me han ayudado, aconsejado y apoyado a lo largo de este camino, y gracias a ellas ha sido posible el desarrollo y la finalización de esta tesis doctoral. A todas ellas van dedicadas estas líneas de agradecimiento.

Especialmente quiero agradecer a Manuel Serra, María José Carmona y Javier Montero por la confianza depositada en mí para realizar este proyecto. Me siento infinitamente agradecida por todos los valiosos conocimientos que me han ofrecido, desde el primero hasta el último día, y por contagiarme el entusiasmo por trabajar en el mundo de la investigación. Han sabido enseñarme, aconsejarme y guiarme de la mejor forma posible. Además, no sólo han sido grandes directores de tesis sino que durante todos estos años me han brindado su amistad y a la vez que me han formado como investigadora también me han hecho crecer como persona.

Quiero agradecer a mis tutores de estancia África Gómez y Diego Fontaneto por acogerme y darme la oportunidad de trabajar en el *Departament to Biological Science, University of Hull*, Hull (Reino Unido) y en el *Consiglio Nazionale delle Ricerche*, *Istituto per lo Studio degli Ecosistemi, Verbania-Pallanza* (Italia), respectivamente. Gracias por contribuir en mi formación como investigadora, fueron dos estancias muy enriquecedoras, tanto en lo profesional como en lo personal.

Me siento muy afortunada por haber podido formar parte del grupo de Ecología Evolutiva donde he coincidido con compañeros que son inmejorables. Ana Tortajada, Nohe, Edu, Raquel, Lluís, Eva y Ana Hidalgo, muchas gracias por enseñarme, resolver dudas, compartir inquietudes y por todos los buenos momentos que han hecho tan agradable el día a día en el laboratorio.

Con mucho cariño quiero dar las gracias a Carla Olmo porque ha sido mi gran compañera en este recorrido, y sin ella estos años no hubieran sido tan maravillosos. Nos hemos apoyado la una en la otra, ella me ha comprendido como nadie

y hemos disfrutado de muchas, muchas conversaciones, risas, pintas, miedos…. ¡Gracias por estar ahí siempre!

A Luz, Ana, Marta, Ana Elena, Jose y Pepe, a todos ellos les debo grandes momentazos desde que nos conocimos haciendo la carrera y estoy segura que seguiremos viviendo muchos más. ¡Me encantan los momentos frikis! Nos unió nuestro entusiasmo por la biología y os habéis convertido en grandes amigos para mí. En especial Luz, con ella comparto una de las amistades más bonitas.

A mis chicas de Yecla 35, inolvidables esos años juntas. Todos los años de convivencia, de fiestas y cervecitas hicieron crecer grandes amistades que seguro serán para siempre. Vuestra amistad ha sido de vital importancia. ¡Gracias! Y en especial a Silbia Idoate, artista responsable de la portada.

Quiero agradecer también a mi gente de La Roda, que a pesar de que eso de los rotíferos sonaba muy a extraterrestre, habéis mostrado siempre interés en mis cosicas y me habéis prestado todo vuestro apoyo durante este tiempo y sobre todo en estos últimos meses. Gracias a Elisa, Juan, Carmen, Mª Reme… y en especial a Susana, por su incondicional amistad desde hace más de 25 años.

A Héctor, mil gracias por ser parte de mí, por esa mezcla de paciencia, alegría, dulzura y amor. ¡Qué sería yo sin ti!

Y por supuesto quiero agradecer a mis padres por TODO lo que han hecho: su esfuerzo para que yo pudiera cumplir mis sueños, la ilusión y confianza puesta siempre en mí y el apoyo en todas las decisiones que he tomado, algunas un poco locas. A mi *sister* Sara gracias por estar siempre tan loca y tan cerca. ¡Sois los mejores!

Persistence, niche differentiation and ecological similarity in two cryptic rotifer species

All that we can do, is to keep steadily in mind that each organic being is striving to increase in a geometrical ratio; that each at some period of its life, during some season of the year, during each generation or at intervals, has to struggle for life and to suffer great destruction. When we reflect on this struggle, we may console ourselves with the full belief, that the war of nature is not incessant, that no fear is felt, that death is generally prompt, and that the vigorous, the healthy, and the happy survive and multiply.

(Darwin 1859)

Contents

Resumen

La interacción entre especies competidoras y los procesos que intervienen y determinan el resultado de las dinámicas competitivas (i.e., coexistencia o exclusión) son temas fundamentales de estudio en ecología. La teoría establece que para que dos especies puedan persistir en el mismo hábitat y evitar la exclusión competitiva éstas deben tener diferentes requerimientos ecológicos (i.e., diferenciación de nicho). Sin embargo, la existencia de especies ecológicamente similares en simpatría pone en cuestión esta necesidad y ha abierto un debate en la comunidad científica. Este debate se ha visto intensificado por el descubrimiento en las últimas décadas de un gran número de especies crípticas (i.e., especies filogenéticamente próximas y con un alto grado de similitud morfológica) presentes en los mismos hábitats. En un principio se espera que las especies crípticas presenten un gran solapamiento de nicho ecológico, tanto por su similitud morfológica como por el hecho de que al ser

filogenéticamente próximas sus nichos hayan retenido aspectos ancestrales a lo largo del tiempo (i.e., retención de nicho). Como consecuencia, el estudio de los procesos que permiten la persistencia de especies simpátricas ecológicamente similares es un tema de gran relevancia.

La búsqueda de mecanismos –basados en particiones sutiles de nicho o no dependientes de una partición de nicho– que permitan la coexistencia estable de especies ecológicamente similares ha puesto de relieve la función de las fluctuaciones. Un ambiente fluctuante puede ocasionar que la exclusión competitiva se vea repetidamente desplazada, si la variación continua de las condiciones favorece a cada competidor en momentos distintos. Esto ha llevado a la formulación de mecanismos de coexistencia basados en fluctuaciones tales como el 'efecto de almacenamiento' (*'storage effect'*) que permiten explicar la persistencia de especies sin una marcada diferenciación de nicho. Más recientemente, se han propuesto mecanismos basados en compromisos (*'trade-offs'*) entre rasgos de la historia vital relacionados con la reproducción sexual y que permiten, al menos en teoría, la coexistencia de especies sin ningún tipo de diferenciación ecológica.

El complejo de especies crípticas *Brachionus plicatilis* constituye un buen modelo para explorar las implicaciones ecológicas y evolutivas de la persistencia en la misma localidad de especies ecológicamente similares. *B. plicatilis* y *B. manjavacas,* dos de las especies pertenecientes a este complejo, son virtualmente idénticas tanto en tamaño como en forma y, pese a que se espera que presenten un alto grado de solapamiento ecológico, estas especies cohabitan frecuentemente en lagos y lagunas de la península ibérica. Estos cuerpos de agua son salobres y están sujetos a importantes fluctuaciones ambientales de temperatura y salinidad, dos de los factores ecológicos abióticos más relevantes para estos rotíferos. Se sabe que estas dos especies presentan diferencias en sus preferencias de salinidad; *B. plicatilis* tiende a encontrarse en lagunas de salinidad baja, mientras que *B. manjavacas* suele estar presente en lagunas de salinidad alta. Se ha sugerido que la persistencia en simpatría de estas especies de rotíferos puede estar mediada por estas diferencias. Sin embargo, los rangos de salinidad en los que pueden crecer ambas especies solapan, lo que indica que en su persistencia deben de estar involucrados también otros factores. Otra característica de los hábitats de estas especies es que son ambientes con baja heterogeneidad espacial pero marcada heterogeneidad temporal. Las lagunas tienden a sufrir un fuerte estiaje que puede llevar a su desecación o a que alcancen valores de salinidad muy altos. Por tanto, las poblaciones de *B. plicatilis* y *B. manjavacas* dependen de la producción de huevos diapáusicos (i.e., formas de resistencia) para su supervivencia durante los periodos adversos. Estos rotíferos son partenogenéticos cíclicos que combinan en su ciclo vital la reproducción asexual con episodios de reproducción sexual, cuyo resultado son los huevos diapáusicos.

La presente tesis aborda la cuestión de cómo especies evolutivamente cercanas, y que por tanto cabe esperar tengan requerimientos ecológicos muy similares, son capaces de persistir en el mismo hábitat. El propósito principal es inferir mecanismos que puedan determinar el resultado de la dinámica competitiva, ya sea éste la coexistencia o la exclusión. Para ello, los objetivos en que se centra la tesis son: (1) caracterizar la diferenciación de nicho tanto biótico como abiótico de *B. plicatilis* y *B. manjavacas*; (2) explorar si estas especies difieren en rasgos relevantes de la historia vital relacionados con la reproducción sexual y la producción de huevos de diapausa; y (3) analizar las implicaciones de tales diferencias en el resultado de las dinámicas competitivas, así como en la persistencia de ambas especies en su hábitat natural.

La coexistencia de especies crípticas que pertenecen a un mismo complejo ha sido explicada en muchas ocasiones por una diferenciación de nicho biótico. En los rotíferos las diferencias en el tamaño corporal se han relacionado con diferencias en el uso de los recursos y en la vulnerabilidad a la depredación que podrían permitir la coexistencia de estas especies. Sin embargo, este tipo de diferencias son improbables entre *B. plicatilis* y *B. manjavacas* debido a su gran similitud morfológica. El Capítulo 2 de esta tesis trata de esclarecer, mediante experimentos en el laboratorio, si la sutil diferencia en el tamaño corporal de estas dos especies se traduce en una diferenciación ecológica en aspectos bióticos. El estudio se centra en tres características ecológicas clave relacionadas con el tamaño corporal: (1) la respuesta funcional, que se determinó mediante las tasas de aclaramiento de ambas especies de rotíferos en dos dietas de microalgas que difieren en forma, tamaño y movilidad; (2) la tolerancia de neonatos a diferentes periodos de ayuno, que se analizó mediante la comparación de las tasas de crecimiento y de reproducción mediante análisis de tablas de vida; y, por último, (3) la vulnerabilidad a la depredación por copépodos, que se estimó en experimentos de depredación utilizando neonatos de las dos especies de rotífero como presa. No se encontraron diferencias importantes entre *B. plicatilis* y *B. manjavacas* en estas características. Estos resultados demuestran un gran solapamiento en las dimensiones del nicho biótico de estas dos especies. De acuerdo con la 'hipótesis del límite de la similitud filogenética' (*'phylogenetic limiting similarity hypothesis'*), los rasgos ecológicos que influyen en la competencia se conservan a lo largo del linaje filogenético por lo que las especies cercanas tienden a ocupar nichos similares. La ausencia de diferencias en características del nicho biótico de *B. plicatilis* y *B. manjavacas* concuerda con esta hipótesis y sugiere que estos factores bióticos difícilmente explican la coexistencia de ambas especies en una misma laguna.

Puesto que *B. plicatilis* y *B. manjavacas* muestran diferentes preferencias para los factores abióticos, el Capítulo 3 tiene como objetivo determinar si existe una diferenciación de nicho entre las dos especies en relación con la temperatura y salinidad. Para ello se llevaron a cabo experimentos de laboratorio donde se determinaron las tasas de crecimiento poblacional, los niveles de reproducción sexual, y los patrones de eclosión y viabilidad de los huevos diapáusicos de ambas especies bajo diferentes condiciones de salinidad y temperatura. Las especies presentaron diferencias en sus tasas de crecimiento poblacional y en su inversión en reproducción sexual en respuesta a la salinidad. El incremento de la salinidad tuvo un efecto negativo en ambos parámetros, y afectó en mayor medida a *B. plicatilis*. Sin embargo, a bajas salinidades, la eficacia de *B. plicatilis* fue mayor que la de *B. manjavacas*. Además, se encontró que los patrones de eclosión y viabilidad de los huevos de diapausa difieren entre las dos especies: *B. plicatilis* produce huevos diapáusicos que permanecen viables durante más tiempo y siguen un patrón de eclosión extendido en el tiempo, mientras que los huevos de *B. manjavacas* se empiezan a degradar antes y eclosionan más tempranamente y de forma sincrónica. Los resultados muestran que estas especies presentan diferentes compromisos entre rasgos de su historia vital, que permiten una diferenciación ecológica en relación con factores abióticos y que esta divergencia ecológica entre especies no está acoplada a una diferenciación morfológica.

Con el propósito de estudiar con mayor profundidad la divergencia entre *B. plicatilis* y *B. manjavacas* en rasgos relacionados con la reproducción sexual, en el Capítulo 4 se realiza un estudio más detallado de los patrones de asignación reproductiva. En este capítulo se analizan los dos componentes del patrón de reproducción sexual (momento de inducción y cantidad de reproducción sexual). Para ello se realizaron bioensayos donde se estimó el umbral de densidad poblacional al que se induce la reproducción sexual, que es dependiente de la densidad en estos rotíferos. También se estimó la variación genética asociada a este rasgo. Además, se realizó un estudio comparativo mediante experimentos de tabla de vida de los rasgos de la historia vital de los tres tipos reproductivos de hembras que existen en el ciclo vital de estos rotíferos (hembras asexuales, hembras sexuales productoras de machos y hembras sexuales productoras de huevos de diapausa). Los resultados muestran diferencias dependientes de especie en los patrones de reproducción sexual. *B. plicatilis* tiene una asignación más temprana y mayor a la reproducción sexual que *B. manjavacas*. Además, la variabilidad genética en la propensión para reproducirse sexualmente fue mayor en *B. plicatilis*. Se observaron también diferencias entre las dos especies en los rasgos reproductivos de los tres tipos de hembras: las hembras sexuales productoras de machos de *B. manjavacas* producen sus descendientes antes y a una tasa diaria mayor que las correspondientes hembras de *B. plicatilis*; y las hembras productoras de huevos de diapausa de *B. manjavacas* invierten más recursos por huevo diapáusico que las hembras de *B. plicatilis*. Esta divergencia de estrategias entre especies que ocupan el mismo hábitat podría estar relacionada con su diferente tolerancia a la salinidad, que convierte el hábitat en más impredecible para *B. plicatilis* que para *B. manjavacas*.

En su conjunto, el estudio comparativo realizado en los capítulos 3 y 4 muestra por primera vez la existencia de divergencias relevantes en rasgos de historia vital relacionados con la reproducción sexual y la diapausa en dos especies de rotíferos tan similares. Estas diferencias podrían estar mediando la persistencia de ambos competidores en ambientes con salinidad variable en el tiempo. Los resultados sugieren que *B. plicatilis* presenta características típicas de una especie oportunista. Así, esta especie podría aprovechar los periodos efímeros de baja salinidad para crecer e invertir de forma temprana en la estación de crecimiento en reproducción sexual y producir huevos de diapausa que se acumularían en el sedimento. Esta mayor inversión en diapausa de *B. plicatilis* podría provocar su exclusión competitiva a corto plazo de la columna de agua, pero podría permitir su persistencia a largo plazo con *B. manjavacas* mediante los huevos de diapausa producidos.

Los resultados reseñados hasta aquí permiten elaborar predicciones sobre la dinámica conjunta de las dos especies cuando se les da la oportunidad de interaccionar. En el Capítulo 5 se estudian las dinámicas de competencia a largo plazo de ambas especies en sistemas experimentales. El efecto de las fluctuaciones en la salinidad se estudia comparando el resultado de las dinámicas competitivas en regímenes de salinidad constante y salinidad variable. Además, en el diseño experimental se simulan periodos desfavorables (p. ej., sequía), que se alternan con periodos favorables. Es decir, hay una alternancia de periodos de crecimiento poblacional activo en la columna de agua (estaciones de crecimiento) y periodos de diapausa. De esta forma se puede explorar la función de la inversión en diapausa en el resultado de la dinámica competitiva entre *B. plicatilis* y *B. manjavacas*. Los resultados indican que en regímenes de salinidad constante una u otra especie se extingue, dependiendo de si la salinidad es alta o baja. A salinidad constante alta *B. plicatilis* siempre es la especie excluida, mientras que a salinidad constante baja la excluida es *B. manjavacas*. Bajo regímenes de salinidad fluctuante, aunque también se da exclusión de una de las dos especies, la especie que resulta excluida varía para un mismo régimen. A pesar de que la persistencia conjunta de ambas especies no se consiguió en un régimen de salinidad fluctuante, los resultados muestran que –en comparación con un régimen de salinidad constante– el periodo de persistencia de ambas especies se ve prolongado por la fluctuación en salinidad. También se observa un efecto positivo, aunque leve, de la presencia de un banco de huevos diapáusicos en la persistencia conjunta de las dos especies. La exclusión competitiva en los sistemas experimentales contrasta con las observaciones de presencia conjunta de *B. plicatilis* y *B. manjavacas* en la naturaleza. Esto podría explicarse, en parte, por un efecto de la estocasticidad demográfica en nuestros microcosmos, aunque también podría deberse a que otros factores no relacionados con la salinidad u otros regímenes de salinidad diferentes a los ensayados puedan estar operando en la coexistencia de estas dos especies en la naturaleza.

El tipo de experimentos de competencia realizados en esta tesis, experimentos en los que se incluye el ciclo completo de las especies, es poco frecuente debido a las dificultades logísticas que entraña. Como alternativa, la modelización teórica es una herramienta valiosa para el análisis de las dinámicas de competencia a largo plazo. El estudio teórico de la dinámica competitiva de *B. plicatilis* y *B. manjavacas* que se presenta en el Capítulo 6 es un ejemplo de ello. En este capítulo se desarrolla un modelo teórico para el estudio del efecto de los rasgos de la historia vital y de la fluctuación en la salinidad en el resultado de la competencia entre *B. plicatilis* y *B. manjavacas*. Mediante el modelo se simulan escenarios ecológicos donde se combinan diferentes (1) longitudes de las estaciones de crecimiento, (2) niveles de inducción cruzada del sexo entre las especies (el sexo en estas especies es inducido por una señal química que puede no ser completamente específica de especie), y (3) regímenes de salinidad (constante y variable). Los resultados de las simulaciones apoyan las concludiones a las que apuntan los resultados empíricos, obtenidos en capítulos anteriores. Así, se observó un efecto positivo de la fluctuación de la salinidad en la persistencia de ambos competidores. Además, estos resultados muestran que los efectos de la duración de la estación de crecimiento y de la inducción cruzada del sexo difieren entre especies y afectan el resultado de la dinámica competitiva. El estudio realizado en este capítulo muestra que la construcción de modelos teóricos es valiosa en casos de estudio como el nuestro –la persistencia de especies con ciclos complejos en un ambiente complejo–, una vez que se han determinado empíricamente los valores de los parámetros relevantes.

Finalmente, en el Capítulo 7 se discuten de forma integrada las implicaciones de la similitud ecológica, la diferenciación de nicho, y de los rasgos del ciclo vital en la comprensión de la persistencia de especies competidoras similares y, por lo tanto, en el mantenimiento de la diversidad biológica. De manera global esta tesis revela la importancia ecológica y evolutiva de la inversión en reproducción sexual y diapausa, ya que ésta no sólo permite la supervivencia en condiciones ambientales adversas, sino que tiene implicaciones en la mediación de la persistencia de especies similares. Además, la aproximación empleada destaca que el estudio de la dinámica competitiva de especies con ciclos biológicos complejos requiere la consideración de todos los estadíos del ciclo, ya que la exclusión a corto plazo puede ser compatible con una coexistencia a largo plazo.

Chapter 1

General Introduction

Species persistence, sympatry and niche shifts

Explaining the maintenance of the wide species diversity in nature remains one of the main problems at the intersection of ecology and evolution (e.g., Chase and Myers 2011; Gravel et al. 2011; Violle et al. 2012), and it becomes specially challenging when dealing with ecologically similar species. Maintenance of diversity is commonly understood as the persistence of species in the same spatial region

(Chesson 2000). Species diversity increases through speciation events, but which factors allow a newly emerged species to persist in its habitat? According to the phylogenetic limiting similarity hypothesis (Webb 2000; Violle et al. 2011), phylogenetically closely related species retain the niche features, and are expected to have similar ecological requirements. Thus, sister species are especially likely to overlap their potential niches resulting in competition, with a risk of competitive exclusion (Gause 1934; Hardin 1960; MacArthur and Levins 1967). If speciation takes place by geographical barriers (allopatric speciation), the resulting species are isolated one from the other; this way, species do not interact between them. Since Mayr (Mayr 1942) stated this geographic view on speciation, allopatry has been considered the most common speciation mode (Coyne and Orr 2004). However, sympatric speciation might also occur. In these cases, the newly formed species can persist with the established species only if they develop ecological differences that prevent competitive elimination (Begon et al. 1996). Either from the early momemt when the new species appears (sympatric speciation) or when a secondary contact of the sister species occurs (allopatric speciation), persistence of phylogenetically-close species in a region or locality requires ecological divergence –i.e., the species must respond differently to their abiotic and biotic environments to survive (Leibold 1995; Chase and Leibold 2003). In this way, interspecific competition could decrease below intraspecific competition (MacArthur and Levins 1967; Chesson 2000; Adler et al. 2007), or alternatively each species could have a region of the niche where it is superior to its competitors, so that the realized niches are largely non-overlapping. A great number of possible factors promote niche partitioning of competing species as resource use, predation vulnerability, frequency-dependent predation, habitat heterogeneity and environmental fluctuations (Tilman 1982; Gendron 1987; Begon et al. 1996; Grover 1997; Amarasekare 2000; Chesson 2000; Tokeshi 2009).

Niche differentiation –regardless of whether it is the potential or the realized niche– involves trade-offs in the abilities of the species to interact with the environment (Chesson 2000; Siepielski and McPeek 2010; McPeek 2014). A trade-off occurs if an advantage when performing one function is linked to a disadvantage in performing another function (Stearns 1992). Many trade-offs occur in life-history traits, but the concept is applicable to any trait. Adaptation to a particular ecological condition occurs at the cost of being mal-adapted to other conditions. This prevents the evolution of 'Darwinian demons' –i.e. organisms that develop fast, do not age and reproduce continuously in any environment in which they are (Stearns 1992; Roff 1993). If trade-offs exist, neither species have higher values of all relevant fitness components, which makes the co-persistence possible. Relevant trade-offs for niche differentiation are the incapability to exploit several resources with high efficiency (Tilman 1982), the link between consumption efficiency and vulnerability to predation (Holt et al. 1994; Leibold 1996), or the constraints limiting the tolerance ranges to physical factors (Chesson and Huntly 1997). All these trade-offs work as evolutionary constrains for ecological specialization, so that several specialization trajectories can evolve for several-closely related species. The same applies to life-history traits, where trade-offs offer opportunities for divergence in the way of life of closely related species, especially in those organisms with complex life cycles. The *r/K* theory is the best-known case of the processes allowing that divergence (MacArthur and Wilson 1967; Pianka 1970), although other cases could occur if competitive performance in a life-history stage would trade off competitive-performance at a different stage.

Co-occurring competitors: stable coexistence vs. transient co-occurrence

Competitor co-occurrence is when species are found together regardless of if their persistence is permanent. It is a phenomenon not implying a specific dynamics or mechanism. When co-occurring competing species are protected from exclusion at a relevant ecological time scale, their persistence is permanent and stable, so those species 'coexist' in the same habitat, even if their population densities fluctuate and are not at equilibrium (Chesson 2000; Adler et al. 2007). Alternatively, competitor co-occurrence might be transient, i.e. some species are more or less slowly driven to exclusion by competitive interactions with other species (Leibold and McPeek 2006). Accordingly, the competing species regime can be stable or unstable, and different perspectives account for each option in this alternative.

By focusing on transient co-occurrence, the 'neutral theory' proposes that species similarities are key to explaining the high diversity of many natural communities. Neutral theory assumes that species in a guild are ecologically equivalent (i.e., they have similar fitness) regarding their probability of reproduction and death (Bell 2001; Hubbell 2001; Chave 2004). The species differences are not related to traits that affect their fitness or demography, and thus their interactions with the abiotic environment and other species. The only driver of species' relative abundances is random variation in births, deaths and dispersal. If there is not speciation, this 'random walk' (Hubbell 2001) would lead all but one species of the guild to the extinction (i.e., there is no mechanisms protecting from exclusion), but it might last so much that diversity would be maintained because new species arise. Alternatively, and focusing on (stable) coexistence, the competitor assemblage in natural communities can be explained considering that the differences between species drive the key processes in promoting their persistence. This approach invokes concepts as niche partition, habitat heterogeneity and environmental fluctuation (Begon et al. 1996; Chesson 2000; Tokeshi 2009). For that, it is required that each competing species in the system has the capability to recover from low densities in the presence of the other species (Chesson 2000). When focused in a rather isolated locality, two kind of stability may be differentiated: (1) stable coexistence at an equilibrium point and (2) fluctuation-dependent coexistence (Chesson 1994). Stable coexistence at equilibrium point –the only one considered in the tradional theory– involves that species densities tend to remain constant over the time. After any displacement, the system will return to the same equilibrium situation (Chesson and Case 1986). Resource partitioning, frequency-dependent predation and spatial heterogeneity are examples of coexistence at equilibrium point (Tilman 1982; Grover 1997). These mechanisms can act in presence of environmental fluctuation but it is not required. These mechanisms are known as 'fluctuation-independent mechanisms'. In contrast, the 'fluctuation-dependent coexistence' can only operate in the coexistence of competing species in environments in which conditions vary temporally (Chesson 2000). A non-equilibrium population density state is assumed in which the fluctuation prevents competitive exclusion. The first idea about the role of a varying environment in the co-persistence of competitors was exposed by Hutchinson (1961) when he hypothesized that phytoplankton diversity, which would be lost at competitive equilibrium, may be maintained by intermediated-frequency temporal fluctuation in the physical environment acting on a guild of species with differential specialization in relation to the fluctuating factor. In the last decades this idea has been developed and refined (Chesson and Huntly 1997; Chesson 2000; Chase and Leibold 2003). Now it is considered that just a fluctuating environment is not a sufficient condition for stable coexistence.

Cryptic species and their co-occurrence

Cryptic or sibling species are those species that have a great morphological similarity so that classical, morphologically-based taxonomy considered them a single species (Bickford et al. 2007; Pfenninger and Schwenk 2007). The number of cryptic species that have been identified within a morphological defined taxon has increased noticeably due to the use of molecular approaches (Gómez 2005; Kong and Li 2009). The cryptic species often differ in minor morphological features, but these differences only were observed once species were recognized by other methods (Knowlton 1993).

Cryptic species are distributed among all the animal taxa and its discovery has involved important implications for biodiversity, conservation and fundamental evolutionary questions (Knowlton 1993; Schonrogge et al. 2002). The occurrence of these species may be promoted if mate-recognition is not visual, as occurring in some species of insects, fogs, bats and birds as well as in many aquatic invertebrates (Narins 1983; Henry 1994; Cicero 1996; Gómez et al. 2002; Jones and Barlow 2003). This is so because morphological differences are not required for pre-mating reproductive isolation (i.e., the least costly isolation mechanism). Moreover, species should be under selection promoting morphological stasis (Knowlton 1993; Schonrogge et al. 2002).

Co-occurrence of cryptic species is not uncommon (Ortells et al. 2003; Leibold and McPeek 2006; Nicholls and Racey 2006; Wellborn and Cothran 2007). Given their remarkable morphological similitude and phylogenetic closeness, cryptic species are expected to have similar environmental requirements –i.e., to retain their niches and related ecological traits (Futuyma and Mitter 1996; Webb 2000; Violle et al. 2011). Consequently, cryptic species are expected to experience strong competitive interactions favoring the competitive exclusion (Hardin 1960). However, sympatric cryptic species may differ in morphologically-uncoupled traits (e.g., enzymatic machinery) that can differentially affect their response to environmental factors (e.g., water depth, salinity, temperature, substrata) to have evolved different optima for life-history trade-offs. However, it is unclear if these differences allow or promote their sympatric long-term persistence. Therefore, coexistence of cryptic species is an intriguing phenomenon that deserved scientific attention (Leibold and McPeek 2006).

Competitive dynamics, environmental fluctuation and life cycles

Species co-occurrence has been commonly explored by short-term studies. Then, the short-term evidence is often used to infer the long-term dynamics of competitors (Brown et al. 2001; Hastings 2004). However, in those cases in which the organisms face a fluctuating environment and have complex life cycles, the conclusion based on short-term observations may be misleading. For instance, in a fluctuating environment, active life-cycle stages of a competitor might disappear because the current environment favors their competitors. However, the species excluded from active competition might recover from resting life-cycle stages when the environment changes. In the long-term exclusion might not occur or even the species disfavored during a period might be the winner (Chesson and Huntly 1988; Chesson and Huntly 1989).

The role of some specific life-cycle stages in preventing competitive exclusion and allowing stable coexistence at no density equilibrium is stressed by the so-called 'storage effect' theory (Chesson and Huntly, 1989, 1988; Chesson, 2000). The crucial point is that some life-history stages work as a 'storage' because they are free of competition and are able to buffer population decline under adverse environment or competitive conditions (e.g., adults of many species, dormant seeds, cysts or other resting stages). In this way, competing species can persist together under stable regime a long-time scale if (i) a life cycle stage immune to competition exists, (ii) environment is advantageous to each species at different times allowing the recruitment of that stages, and (iii) the positive effect of the ambient is linked to an increase of the competition (i.e. covariance between environment and competition).

Sex and diapause: costs and effects on competitive outcome

Trade-offs involving reproduction are critical in shaping life histories, as conceptualized by 'the cost of reproduction'. This is a term used to define the compromise between (1) current reproduction and (2) survival and expected future reproduction (Reznick 1985; Stearns 1992). A general reason for the cost of reproduction is the allocation principle. Internal resources are limited, and they must be allocated between the conflicting demands of reproduction, and somatic growth and maintenance (Stearns 1992; Roff 1993).

In cyclical parthenogenetic organisms, which reproduce asexually and eventually sexually, there are some costs associated to the switch from clonal, asexual proliferation to sexual reproduction. First, sex incurs the standard 'twofold cost of sex' due to half of the reproductive effort is allocated to males which make no contribution to population growth (Maynard Smith 1971; Aparici et al. 1998; Innes and Singleton 2000; Aparici et al. 2002; Simon et al. 2002; Serra and Snell 2009; Carmona et al. 2009). Second, in two out of the three major cyclically parthenogenetic taxa (monogonont rotifers and cladocerans), the result of sexual reproduction involves production of diapausing eggs. These eggs are expensive because they have special features regarding energy stores and protection, so they require more resources than subitaneous eggs (Gilbert and Schröder 2004; Wang et al. 2005; Alekseev et al. 2007; Pauwels et al. 2007; Wu et al. 2008; Clark et al. 2012). Moreover, diapausing eggs have an obligate dormant period (Schwartz and Hebert 1987; Hagiwara and Hino 1989). This involves an additional cost because it increases the generation time. However, diapausing eggs allow population to survive unsuitable conditions (Pourriot and Snell 1983; Slusarczyk 1995; Alekseev and Lampert 2001), and are thought to be the main dispersal stage (Hairston and Kearns 2002; Louette and De Meester 2005). Therefore, the costs associated to sex and diapause cause a trade-off between investment into (1) current population growth by asexual (clonal) proliferation and (2) long-term population persistence throughout the sexually-produced diapausing eggs (Snell 1987; Serra and King 1999). This trade-off may affect the patterns of sexual reproduction and have an effect on the outcome of the competitive dynamics (Ciros-Pérez et al. 2002; Aranguiz-Acuña and Ramos-Jiliberto 2014). However, the role of the reproductive strategy on the persistence of competitive species has been poorly studied and has been limited to a few works (Zhang et al. 2004; Montero-Pau and Serra 2011; Aranguiz-Acuña and Ramos-Jiliberto 2014).

The cryptic species complex *Brachionus plicatilis*

General characteristics

Rotifers constitute a diverse phylum composed by more than 2000 species generally aquatic and free-living (Ricci and Balsamo 2000; Wallace et al. 2006; Wallace and Smith 2009; Wallace et al. 2015). Most of the rotifers are planktonic and constitute one of the major groups of zooplankton in continental waters (Makarewicz and Likens 1979; Pace and Orcutt Jr 1981). Among planktonic rotifers, in the subclass Monogononta, the best-known taxon is the *Brachionus plicatilis* species complex. This complex is composed for at least 14 species (Gómez 2005; Suatoni et al. 2006; Fontaneto et al. 2009; Malekzadeh-Viayeh et al. 2014). This species complex is widespread in brackish habitats and is one of the most common taxa in the world (Fontaneto et al. 2006). Species of the *B. plicatilis* complex are commonly used as models in ecological and physiological studies (e.g. (Carmona et al. 1989; Aparici et al. 1998; Lowe et al. 2005; Snell 2014), in aquaculture (Lubzens et al. 1989; Lubzens et al. 2001) and in ecotoxicology assessments (Snell and Carmona 1995; Del Valls et al. 1997; Snell and Joaquim-Justo 2007).

Brachionus species are passive filterers. Both the size of the body and the morphology of the 'corona' (i.e. a ciliated field at the anterior end of rotifers used in locomotion and food gathering) have been related to food-size preferences and feeding strategies (Edmondson 1965; Gilbert and Bogdan 1984; Rothhaupt 1990a; Ciros-Pérez et al. 2001a) raising the opportunity of niche differentiation regarding resource use. In fact, body size of some *Brachionus* species has been related to both the optimal particle size ingested and the width of the retention spectrum (Rothhaupt 1990b).

In natural habitats, rotifers are a common prey of vertebrate and invertebrate predators (e.g., Hurlbert et al. 1972; Starling and Rocha 1990; Schmid-Araya and Schmid 1995; Conde-Porcuna and Declerck 1998). On the one hand, planktonic rotifers are detected visually by aquatic vertebrate predators such as fish. Consequently the reactive distance of the predator is positively related to the prey size (Ware 1972; Ware 1973; Werner and Hall 1974; O'Brien et al. 1976). On the other hand, invertebrate predators as copepods detect rotifers by mechanoreception (Moore et al. 1999; Bundy and Vanderploeg 2002). The prey body size affects the mechanical disturbance created, and hence it affects the risk of being detected and predated (Kerfoot 1977). Species of the *B. plicatilis* complex lack any special structure of protection except a hard outer covering of chitin called 'lorica' (Stemberger and Gilbert 1987). Therefore, body size is also a crucial factor in determining their susceptibility to predation by invertebrate predators (Lapesa et al. 2002; Ciros-Pérez et al. 2004; Lapesa et al. 2004).

Life cycle

The species of genus *Brachionus* belong to monogonont rotifers, which are cyclically parthenogens. In the monogonont life cycle (Fig. 1.1), ameiotic parthenogenesis producing clonal females (asexual phase) is combined with occasional bouts of male production and sexual recombination resulting in diapausing eggs (sexual phase) (e.g., Gilbert 1974; Wallace and Smith 2009; Wallace et al. 2015). At temperate latitudes, rotifer populations are typically temporary, they are not continuously active in the water column; rather, annually re-colonize the water column (planktonic growing season). In a growing season, the active population is initiated by hatching of diapausing eggs from the sediment of the ponds and lakes where rotifers inhabit (Gilbert 1974; Pourriot and Snell 1983). The hatchlings are diploid asexual (amictic) females that produce genetically identical daughters by ameiotic parthenogenesis (e.g., Gilbert 1974; Pourriot and Snell 1983; Schröder 2005; Wallace et al. 2006). Typically, there is a period of exclusively asexual reproduction, followed by a period in which both sexual and asexual reproductions co-occur. In the genus *Brachionus* sexual reproduction is induced by a density-dependent chemical signal produced by the rotifers. The signal accumulates in the water column (Gilbert 1963; Carmona et al. 1993; Carmona et al. 1995; Stelzer and Snell 2003; Snell et al. 2006). This infochemical, which may be not completely species-specific (García-Roger et al. 2009) triggers asexual females to parthenogenetically produce sexual daughters as some fraction of their offspring (Fussmann et al. 2007). These sexual (mictic) females produce haploid eggs that develop into

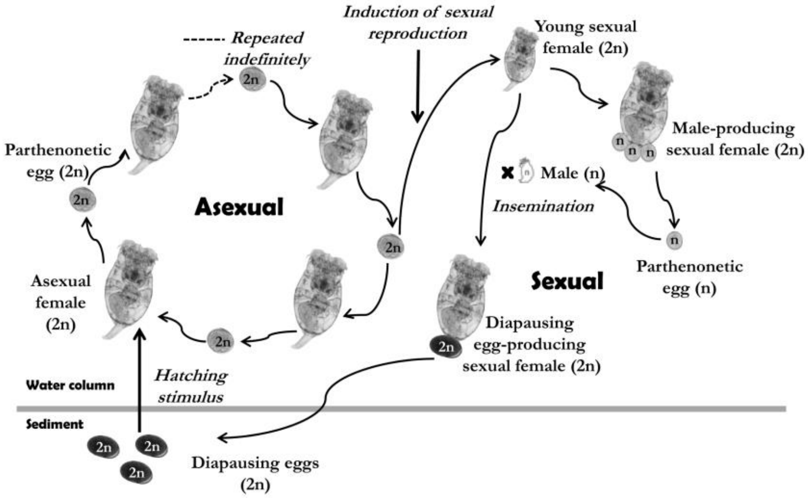


Figure 1.1. The typical life cycle of monogonot rotifers. Females reproduce indefinitely asexually; environmental cues trigger the production of sexual females. If a young sexual female is inseminated, she produces diapausing eggs, if unfertilized, she produces males.

either haploid males or, if inseminated while young, into encysted diploid embryos in an arrested stage of development called diapausing or resting eggs. Males are dwarf, do not feed and have shorter lifespan than females (King and Miracle 1980; Wallace et al. 2006). Diapausing eggs, which settle in the sediment, are able to resist desiccation and other adverse conditions, remaining viable for decades (Marcus et al. 1994; Kotani et al. 2001; García-Roger et al. 2006). After a variable period of dormancy (Hagiwara et al. 1989; Schröder 2005; Martínez-Ruiz and García-Roger 2014), and when suitable conditions resume in the water column, a fraction of diapausing eggs hatches and re-colonize the water column and a new growing season begins.

Different species of the *B. plicatilis* complex inhabit saline ponds in the Iberian Peninsula (Gómez et al. 2002; Gómez et al. 2007) (Fig. 1.2). Rotifers are exposed to wide and unpredictable environmental fluctuations due to the Mediterranean climate (Comín et al. 1992). Thus, their populations are only active in the water column during a period of the year (the so-called growing season) regardless the pond is permanent or temporary. Thus, diapausing eggs are the only way to cope adverse conditions until the next suitable growing season.

B. plicatilis and B. manjavacas as model organisms

B. plicatilis and *B. manjavacas* (Fig. 1.3) belong to the *B. plicatilis* cryptic species complex. The species in this complex of herbivorous filterers are commonly found in sympatry (Gómez et al. 2002; Gómez et al. 2007), so that they constitute a good ecological model to study evolutionary and ecological questions about the co-occurrence of closely related species. It has been suggested that these species diverged several million years ago (Gómez et al. 2002). The large genetic distance between them, the experimentally confirmed pre-mating reproductive isolation and the lack of evidence for hybridization in the field ensure their species status (Gómez and Snell 1996; Ortells et al. 2000; Suatoni et al. 2006).

These species have a remarkable morphological similitude. Despite *B. plicatilis* is on average 6% longer than *B. manjavacas* (Campillo et al. 2005), the only reliable taxonomic feature for species identification is the difference in the shape of satellites –i.e., small accessory pieces of the internal masticatory apparatus (Fontaneto et

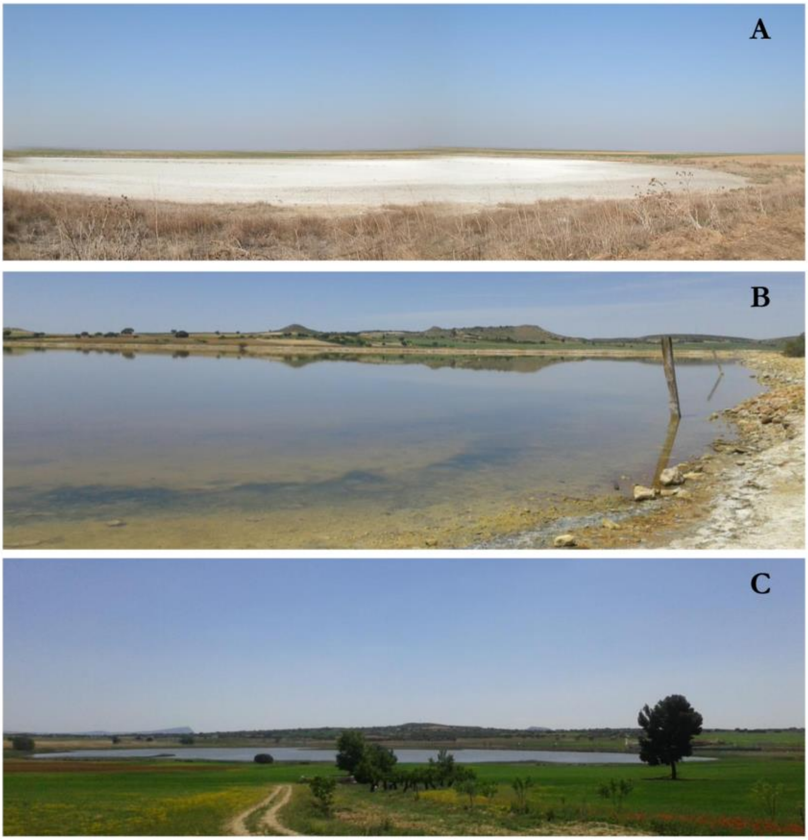


Figure 1.2. Some examples of natural habitats inhabited by species belonging to the *B. plicatilis* cryptic species complex in the Iberian Peninsula. (A) Balsa de Santed (Zaragoza, dry pond), (B) Pétrola (Albacete) and (C) Salobrejo (Albacete).

al. 2007). Nevertheless, the identification of specimens based on morphological features is not practicable. Consequently, molecular analyses are the most reliable way to discriminate both species (Campillo et al. 2005).

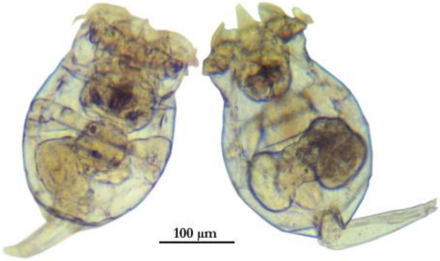


Figure 1.3. Microphotography of *B. plicatilis* (left) and *B. manjavacas*

B. plicatilis and *B. manjavacas* have been found co-occurring in the saline ponds of the Iberian Peninsula (Ortells et al. 2000; Gómez et al. 2002). They are likely present in the region since Pleistocene glaciations (Gómez et al. 2000; Gómez et al. 2007) and paleolimnological data suggest that they can co-occur in the same pond at least for decades (Montero-Pau et al. 2011). In agreement with these evidences, their co-occurrence is unlikely to be transient. However, the mechanisms promoting their coexistence remain unknown.

The ponds inhabited by *B. plicatilis* and *B. manjavacas* in the Iberian Peninsula are shallow, with low spatial heterogeneity but high temporal –e.g., seasonal– changes (Comín et al. 1992; Rodriguez-Puebla et al. 1998). These temporal changes make possible some seasonal specialization in relation to abiotic factors such as temperature and salinity, as well as to changes in their food and response-to-predation spectra. In fact, a differential adaptation has been evidenced for three species included in the *B. plicatilis* species complex: *B. plicatilis*, *B. ibericus* and *B. rotundiformis*. These species differ in their body size; being *B. plicatilis* 35% and 50% larger than *B. ibericus* and *B. rotundiformis* respectively, and *B. ibericus* being 23% larger than *B. rotundiformis* (Ciros-Pérez et al. 2001b). The three species co-occur in some ponds where they are involved in a seasonal succession in relation to temperature and salinity (Gómez et al. 1995; Gómez et al. 1997; Ortells et al. 2003). Additionally, differential use of resources might make possible their coexistence at an equilibrium point (Ciros-Pérez et al. 2001a). However, for the case of *B. plicatilis* and *B. manjavacas*, it is still unknown if their subtle differences in body size and morphology (see above) are enough for ecological differentiation. Laboratory experiments have shown that these two species have a wide overlap in their tolerance range to salinity, but differ in the response of the intrinsic growth rate to salinity (Montero-Pau et al. 2011). Consistently, despite they co-occur in some ponds, on average *B. plicatilis* tends to inhabit body waters with lower salinity than *B. manjavacas* (Gómez et al. 2007). Not surprisingly, coexistence of both species mediated by salinity fluctuation has been proposed (Montero-Pau et al. 2011).

Objectives and structure of this thesis

The present thesis aims to gain insight in how evolutionarily closely related species, which are expected to share similar ecological requirements, are able to persist in the same habitat. The main goal is to infer the mechanisms that can determine the two alternative outcomes of their competitive dynamics: either coexistence or exclusion. With this purpose, the rotifers *B. plicatilis* and *B. manjavacas* were used as model organisms. As described above, these rotifer species meet the conditions that make them excellent candidates to conduct this research. Belonging to the same cryptic species complex, they are phylogenetically closely related, and represent an extreme case of similar species. These two species are morphologically indistinguishable, have a complex life cycle which involves diapause, and commonly co-occur in spatially homogeneous but temporarily fluctuating habitats. Accordingly, this thesis focusses on the following objectives: (1) to empirically characterize the ecological similitudes and differences between *B. plicatilis* and *B. manjavacas* in relation to the most relevant abiotic factors (salinity and temperature) and biotic factors (resource use and predator vulnerability); (2) to empirically assess differences in their life-history traits regarding sexual reproduction and diapause; (3) to empirically explore the role of the salinity regime and the diapause on their long-term competitive outcome; and (4) to theoretically assess the effect of salinity regime, length of the growing season, and level of between-species crossed induction of sex on their competitive dynamics.

Taking into account these main objectives the thesis is organized in the following way:

Chapter 2 studies key ecological characteristics where body size could be relevant –functional response, tolerance to starvation and vulnerability to depredation– in order to assess if the subtle differences between *B. plicatilis* and *B. manjavacas* in body size and morphology translate into ecological differentiation (objective 1).

Niche overlap between *B. plicatilis* and *B. manjavacas* is estimated based on the studied features to quantify their ecological similitude in relation to biotic factors.

Chapter 3 characterizes the responses of *B. plicatilis* and *B. manjavacas* to abiotic factors (i.e., salinity and temperature) in terms of population growth, and patterns of sexual reproduction and diapause (objective 2). The investment in sexual reproduction and diapause, the viability and hatchability of diapausing eggs and the effect of salinity and temperature on several of these fitness components are analyzed in both species. Moreover, the contribution of these features to niche differentiation is estimated.

Chapter 4 analyses reproductive allocation patterns to gather more evidence on divergence of traits between these species (objective 2). Density thresholds for sex initiation –as a measure of investment in sex and diapause– are estimated and life histories of asexual females, unfertilized sexual females and fertilized sexual females in both species are compared. Differences in allocation among reproductive modes and differences in life-history traits between female reproductive types and species are tested.

Chapter 5 explores the long-term competitive dynamics –i.e., several growing seasons– of *B. plicatilis* and *B. manjavacas* (objective 3). The effect of regimes of constant and fluctuating salinity on the competitive dynamic is analyzed in experimental microcosms. Periods of habitat unsuitability are simulated in the laboratory. In this way, our experimental approach takes into account the whole rotifer life cycle and it includes the role of investment in diapause in the long-term competitive dynamics.

Chapter 6 theoretically explores the effect of the salinity fluctuation, the duration of the growing season, and the crossed induction of sex in the long-term competitive outcome of the two rotifer species by developing a simulation model (objective 4). Long-term competitive outcome is inferred on the basis of life-history traits of both species, which are estimated experimentally in the previous chapters. This approach facilitates the interpretation of some empirical results.

Finally, Chapter 7 discusses in general terms the main results of this thesis presented in the previous chapters, proposes prospective future research and sets out the most important conclusions.

References

Adler PB, Hillerislambers J, Levine JM (2007) A niche for neutrality. Ecol Lett 10:95–104.

Alekseev V, Lampert W (2001) Maternal control of resting-egg production in *Daphnia*. Nature 414:899–901.

Alekseev V, Ravera O, Stasio B de (2007) Introduction to diapause. In: Alekseev V, de Stasio B, Gilbert JJ (eds.) Diapause Aquat. Invertebr. Theory Hum. Use. Springer Netherlands, pp 3–10.

Amarasekare P (2000) Coexistence of competing parasitoids on a patchily distributed host: local vs. spatial mechanisms. Ecology 81:1286–1296.

Aparici E, Carmona MJ, Serra M (1998) Sex allocation in haplodiploid cyclical parthenogens with density-dependent proportion of males. Am Nat 152:652–7.

Aparici E, Carmona MJ, Serra M (2002) Evidence for an even sex allocation in haplodiploid cyclical parthenogens. J Evol Biol 15:65–73.

Aranguiz-Acuña A, Ramos-Jiliberto R (2014) Diapause may promote coexistence of zooplankton competitors. J Plankton Res 36:978–988.

Begon M, Mortimer M, Thompson DJ (1996) Interspecific competition. Popul. Ecol. A Unified Study Anim. Plants, Third Edit. pp 75–116

Bell G (2001) Neutral macroecology. Science 293: 2413–2418 .

Bickford D, Lohman DJ, Sodhi NS (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22:148–155.

Brown JH, Whitham TG, Morgan Ernest SK, Gehring CA (2001) Complex species interactions and the dynamics of ecological systems: long-term experiments. Science 293:643–50.

Bundy M, Vanderploeg H (2002) Detection and capture of inert particles by calanoid copepods: the role of the feeding current. J Plankton Res 24:215–223.

Campillo S, García-Roger EM, Martínez-Torres D, Serra M (2005) Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. Hydrobiologia 546:181–187.

Carmona M, Serra M, Miracle M (1993) Relationships between mixis in *Brachionus* plicatilis and preconditioning of culture medium by crowding. In: Gilbert JJ, Lubzens E, Miracle MR (eds) Rotifer Symp. VI. Springer Netherlands, Dordrecht, pp 145–152

Carmona MJ, Dimas-Flores N, García-Roger EM, Serra M (2009) Selection of low investment in sex in a cyclically parthenogenetic rotifer. J Evol Biol 22:1975–83.

Carmona MJ, Gómez A, Serra M (1995) Mictic patterns of the rotifer *Brachionus plicatilis* Müller in small ponds. Hydrobiologia 313/314:365–371.

Carmona MJ, Serra M, Miracle MR (1989) Protein patterns in rotifers: the timing of aging. Hydrobiologia 186-187:325–330.

Chase J, Leibold M (2003) Ecological niches: linking classical and contemporary approaches. University of Chicago Press.

Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. Philos Trans R Soc Lond B Biol Sci 366:2351–2363.

Chave J (2004) Neutral theory and community ecology. Ecol Lett 7:241–253.

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Chesson P (1994) Multispecies competition in variable environments. Theor Popul Biol 45:227–276.

Chesson P, Case T (1986) Overview: nonequilibrium community theories: chance, variability, history, and coexistence. Community Ecol 1:229–239.

Chesson P, Huntly N (1997) The roles of harsh and fluctuating conditions in the dynamics of ecological communities. Am Nat 150:519–553.

Chesson P, Huntly N (1988) Community consequences of life-history traits in a variable environment. Ann Zool Fennici 25:5–16.

Chesson P, Huntly N (1989) Short-term instabilities and long-term community dynamics. Trends Ecol Evol 4:293–8.

Cicero C (1996) Sibling species of titmice in the *Parus inornatus* complex (Aves: Paridae). University of California Press.

Ciros-Pérez J, Carmona M, Serra M (2002) Resource competition and patterns of sexual reproduction in sympatric sibling rotifer species. Oecologia 131:35–42.

Ciros-Pérez J, Carmona MJ, Lapesa S, Serra M (2004) Predation as a factor mediating resource competition among rotifer sibling species. Limonology Oceanogr 49:40–50.

Ciros-Pérez J, Carmona MJ, Serra M (2001a) Resource competition between sympatric sibling rotifer species. Limonology Oceanogr 46:1511–1523.

Ciros-Pérez J, Gómez A, Serra M (2001b) On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. J Plankton Res 23:1311–1328.

Clark MS, Denekamp NY, Thorne MAS, et al. (2012) Long-term survival of hydrated resting eggs from *Brachionus plicatilis*. PLoS One 7:e29365.

Comín F, Rodó X, Comín P (1992) Lake Gallocanta (Aragón, NE Spain): a paradigm of fluctuations at different scales of time. Limnetica 8:79–86.

Conde-Porcuna JM, Declerck S (1998) Regulation of rotifer species by invertebrate predators in a hypertrophic lake: selective predation on egg-bearing females and induction of morphological defences. J Plankton Res 20:605–618.

Coyne J, Orr H (2004) Speciation. Sinauer, Sunderland, MA

Edmondson W (1965) Reproductive rate of planktonic rotifers as related to food and temperature in nature. Ecol Monogr 35:61–111.

Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. Hydrobiologia 583:297–307.

Fontaneto D, Kaya M, Herniou EA, Barraclough TG (2009) Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. Mol Phylogenet Evol 53:182–189.

Fontaneto D, De Smet WH, Ricci C (2006) Rotifers in saltwater environments, re-evaluation of an inconspicuous taxon. J Mar Biol Assoc UK 86:623.

Fussmann GF, Kramer G, Labib M (2007) Incomplete induction of mixis in *Brachionus calyciflorus*: patterns of reproduction at the individual level. Hydrobiologia 593:111–119.

Futuyma DJ, Mitter C (1996) Insect-plant interactions: the evolution of component communities. Philos Trans R Soc London Ser B Biol Sci 351:1361–1366.

García-Roger EM, Carmona MJ, Serra M (2006) Patterns in rotifer diapausing egg banks: Density and viability. J Exp Mar Bio Ecol 336:198–210.

García-Roger EM, Dias N, Carmona MJ, Serra M (2009) Crossed induction of sex in sympatric congeneric rotifer populations. Limnol Oceanogr 54:1845–1854.

Gause G (1934) The struggle for existence. Williams and Wilkins, Baltimore.

Gendron R (1987) Models and mechanisms of frequency-dependent predation. Am Nat 603–623.

Gilbert JJ (1974) Dormancy in rotifers. Trans Am Microsc Soc 93:490–513.

Gilbert JJ, Schröder T (2004) Rotifers from diapausing, fertilized eggs: unique features and emergence. Limnol Oceanogr 49:1341–1354.

Gilbert JJ (1963) Mictic female production in the rotiferBrachionus calyciflorus. J Exp Zool 153:113–123.

Gilbert JJ, Bogdan KG (1984) Rotifer grazing: in situ studies on selectivity and rates. In: Meyer DG, Strickler JR (eds) Trophic Interact. Within Aquat. Ecosyst. Westview, Boulder, CO, pp 97–133

Gómez A (2005) Molecular Ecology of Rotifers: From Population Differentiation to Speciation. Hydrobiologia 546:83–99.

Gómez A, Carmona MJ, Serra M (1997) Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). Oecologia 111:350–356.

Gómez A, Carvalho GR, Lunt DH (2000) Phylogeography and regional endemism of a passively dispersing zooplankter: mtDNA variation of rotifer resting egg banks. Prooceedings R Soc B 267:2189–2197.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution (N Y) 56:1431–1444.

Gómez A, Snell TW (1996) Sibling species and cryptic speciation in the *Brachionus plicatilis* species complex (Rotifera). J Evol Biol 9:953–964.

Gómez A, Temprano M, Serra M (1995) Ecological genetics of a cyclical parthenogen in temporary habitats. J Evol Biol 8:601–622.

Gravel D, Guichard F, Hochberg ME (2011) Species coexistence in a variable world. Ecol Lett 14:828–839.

Grover J (1997) Resource competition. Chapman & Hall, London.

Hagiwara A, Hino A (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Hydrobiologia 186-187:415–421.

Hagiwara A, Lee C-S, Miyamoto G, Hino A (1989) Resting egg formation and hatching of the S-type rotifer *Brachionus plicatilis* at varying salinities. Mar Biol 103:327–332.

Hairston NG, Kearns CM (2002) Temporal dispersal: ecological and evolutionary aspects of zooplankton egg banks and the role of sediment mixing. Integr Comp Biol 42:481–91.

Hardin G (1960) The competitive exclusion principle. Science 131:1292–1297.

Hastings A (2004) Transients: the key to long-term ecological understanding? Trends Ecol Evol 19:39–45.

Henry CS (1994) Singing and cryptic speciation insects. Trends Ecol Evol 9:388–92.

Holt R, Grover J, Tilman D (1994) Simple rules for interspecific dominance in systems with exploitative and apparent competition. Am Nat 741–771.

Hubbell S (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ.

Hurlbert SH, Zedler J, Fairbanks D (1972) Ecosystem Alteration by Mosquitofish (Gambusia affinis) Predation. Science 175:639–641.

Hutchinson G (1961) The paradox of the plankton. Am Nat 95:37–145.

Innes DJ, Singleton DR (2000) Variation in allocation to sexual and asexual reproduction among clones of cyclically parthenogenetic *Daphnia pulex* (Crustacea : Cladocera). Biol J Linn Soc 71:771–787.

Jones G, Barlow K. (2003) Cryptic species of echolocating bats. In: Thomas J, Moss C, Vater M (eds) Echolocation Bats Dolphins. University of Chicago Press, pp 345–349.

Kerfoot WC (1977) Implications of copepod predation. Limnol Oceanogr 22:316–325.

King CE, Miracle MR (1980) A perspective on aging in rotifers. Hydrobiologia 73:13–19.

Knowlton N (1993) Sibling Species in the Sea. Annu Rev Ecol Syst 24:189–216.

Kong L, Li Q (2009) Genetic evidence for the existence of cryptic species in an endangered clam *Coelomactra antiquata*. Mar Biol 156:1507–1515.

Kotani T, Ozaki M, Matsuoka K, et al. (2001) Reproductive isolation among geographically and temporally isolated marine *Brachionus* strains. Hydrobiologia 283-290:446–447.

Lapesa S, Snell TW, Fields DM, Serra M (2002) Predatory interactions between a cyclopoid copepod and three sibling rotifer species. Freshw Biol 47:1685–1695.

Lapesa S, Snell TW, Fields DM, Serra M (2004) Selective feeding of *Arctodiaptomus salinus* (Copepoda, Calanoida) on co-occurring sibling rotifer species. Freshw Biol 49:1053–1061.

Leibold M (1996) A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. Am Nat 784–812.

Leibold M, McPeek M (2006) Coexistence of the niche and neutral perspectives in community ecology. Ecology 87:1399–1410.

Leibold MA (1995) The niche concept revisited: mechanistic models and community context. Ecology 76:1371.

Louette G, De Meester L (2005) High dispersal capacity of cladoceran zooplankton in newly founded communities. Ecology 86:353–359.

Lowe C, Kemp S, Montagnes D (2005) An interdisciplinary approach to assess the functional diversity of free-living microscopic eukaryotes. Aquat. Microb. Ecol. 41: 67-77.

Lubzens E, Tandler A, Minkoff G (1989) Rotifers as food in aquaculture. Hydrobiologia 186-187:387–400.

Lubzens E, Zmora O, Barr Y (2001) Biotechnology and aquaculture of rotifers. Rotifera IX 337–353.

MacArthur R, Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. Am Nat 101:377–385.

MacArthur R, Wilson E (1967) The theory of island biogeography. Princeton University Press, Princeton.

Makarewicz JC, Likens GE (1979) Structure and function of the zooplankton community of Mirror Lake, New Hampshire. Ecol Monogr 49:109–127.

Malekzadeh-Viayeh R, Pak-Tarmani R, Rostamkhani N, Fontaneto D (2014) Diversity of the rotifer *Brachionus plicatilis* species complex (Rotifera: Monogononta) in Iran through integrative taxonomy. Zool J Linn Soc 170:233–244.

Marcus NH, Lutz R, Burnett W, Cable P (1994) Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: Evidence of an egg bank. Limnol Oceanogr 39:154–158.

Martínez-Ruiz C, García-Roger E (2014) Being first increases the probability of long diapause in rotifer resting eggs. Hydrobiologia 1–11.

Maynard Smith J (1971) What use is sex? J Theor Biol 30:319–335.

Mayr E (1942) Systematics and the origin of species, from the viewpoint of a zoologist. Columbia University Press, New York.

McPeek MA (2014) Keystone and intraguild predation, intraspecific density dependence, and a guild of coexisting consumers. Am Nat 183:1–16.

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Moore PA, Fields DM, Yen J (1999) Physical constraints of chemoreception in foraging copepods. Limnol Oceanogr 44:166–177.

Narins P (1983) Divergence of acoustic communication systems of two sibling species of eleutherodactylid frogs. Copeia 4:1089-1090.

Nicholls B, Racey PA (2006) Contrasting home-range size and spatial partitioning in cryptic and sympatric pipistrelle bats. Behav Ecol Sociobiol 61:131–142.

O'Brien WJ, Slade NA, Vinyard GL (1976) Apparent size as the determinant of prey selection by bluegill sunfish (*Lepomis macrochirus*). Ecology 57:1304–1310.

Ortells R, Gómez A, Serra M (2003) Coexistence of cryptic rotifer species: ecological and genetic characterisation of *Brachionus plicatilis*. Freshw Biol 48:2194–2202.

Ortells R, Snell TW, Gómez A, Serra M (2000) Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. Arch für Hydrobiol 149:529–551.

Pace M, Orcutt Jr J (1981) The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnol Oceanogr 26:822–830.

Pauwels K, Stoks R, Verbiest A, De Meester L (2007) Biochemical adaptation for dormancy in subitaneous and dormant eggs of *Daphnia magna*. Hydrobiologia 594:91–96.

Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evol Biol 7:121.

Pianka ER (1970) On r- and K-Selection. Am Nat 104:592–597.

Pourriot R, Snell TW (1983) Resting eggs in rotifers. Hydrobiologia 104:213–224.

Reznick D (1985) Costs of reproduction: an evaluation of the empirical evidence. Oikos 257–267.

Ricci C, Balsamo M (2000) The biology and ecology of lotic rotifers and gastrotrichs. Freshw Biol 44:15–28.

Rodriguez-Puebla C, Encinas AH, Nieto S, Garmendia J (1998) Spatial and temporal patterns of annual precipitation variability over the Iberian Peninsula. Int J Climatol 18:299–316.

Roff D (1993) Evolution of life histories: theory and analysis. Chapman and Hall, New York

Rothhaupt KO (1990a) Differences in particle size-dependent feeding efficiencies of closely related rotifer species. Limnol Oceanogr 35:16–23.

Rothhaupt KO (1990b) Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. Limnol Oceanogr 35:24–32.

Schmid-Araya J, Schmid P (1995) Preliminary results on diet of stream invertebrate species: the meiofaunal assemblages. Jahresbericht Biol Stn Lunz 15:23–31.

Schonrogge K, Barr B, Wardlaw JC, et al. (2002) When rare species become endangered: cryptic speciation in myrmecophilous hoverflies. Biol J Linn Soc 75:291–300.

Schröder T (2005) Diapause in Monogonont Rotifers. Hydrobiologia 546:291–306.

Schwartz SS, Hebert PDN (1987) Methods for the activation of the resting eggs of *Daphnia*. Freshw Biol 17:373–379.

Serra M, King C (1999) Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. J Evol Biol 12:263–271.

Serra M, Snell T (2009) Sex Loss in Monogonont Rotifers. In: Schön I, Martens K, Dijk P (eds) Lost sex. Springer Netherlands, Dordrecht, pp 281–294

Siepielski AM, McPeek MA (2010) On the evidence for species coexistence: a critique of the coexistence program. Ecology 91:3153–3164.

Simon J-C, Rispe C, Sunnucks P (2002) Ecology and evolution of sex in aphids. Trends Ecol Evol 17:34–39.

Slusarczyk M (1995) Predator-induced diapause in *Daphnia.* Ecology 76:1008–1013.

Snell TW (1987) Sex, population dynamics and resting egg production in rotifers. Hydrobiologia 144:105–111.

Snell TW (2014) Rotifers as models for the biology of aging. Int Rev Hydrobiol 99:84–95.

Snell TW, Carmona MJ (1995) Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus.* Environ Toxicol Chem 14:415–420.

Snell TW, Garman BL (1986) Encounter probabilities between male and female rotifers. J Exp Mar Bio Ecol 97:221–230.

Snell TW, Joaquim-Justo C (2007) Workshop on rotifers in ecotoxicology. Hydrobiologia 593:227–232.

Snell TW, Kubanek J, Carter W, et al. (2006) A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). Mar Biol 149:763–773.

Starling FLRM, Rocha AJA (1990) Experimental study of the impacts of planktivorous fishes on plankton community and eutrophication of a tropical Brazilian reservoir. Hydrobiologia 200-201:581–591.

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford.

Stelzer CP, Snell TW (2003) Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. Limnol Oceanogr 48:939–943.

Stemberger RS, Gilbert JJ (1987) Defenses of planktonic rotifers against predators. In: Kerfoot W, Sih A (eds) Predation Direct Indirect Impacts Aquat. Communities. University Press of New England, Hanover, pp 227–239.

Suatoni E, Vicario S, Rice S, et al. (2006) An analysis of species boundaries and biogeographic patterns in a cryptic species complex: the rotifer–*Brachionus plicatilis*. Mol Phylogenet Evol 41:86–98.

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press, New Jersey.

Tokeshi M (2009) Species coexistence: ecological and evolutionary perspectives. Blackwell Science, Oxford.

Del Valls TÁ, Lubián LM, Forja JM, Gómez-Parra A (1997) Comparative ecotoxicity of interstitial waters in littoral ecosystems using Microtox® and the rotifer *Brachionus plicatilis*. Environ Toxicol Chem 16:2323–2332.

Violle C, Enquist BJ, McGill BJ, et al. (2012) The return of the variance: Intraspecific variability in community ecology. Trends Ecol Evol 27:244–252.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Wallace R, Smith H (2009) Rotifer. In: Likens GE (ed) Encycl. Inl. waters. Elsevier, Oxford, pp 689–703

Wallace R, Snell T, Ricci C, Nogrady T (2006) Rotifera 1: Biology, Ecology and Systematics. Backhuys Publishers, Leiden.

Wallace RL, Snell TW, Smith HA (2015) Rotifer. Vol. I, Chapter 13: Ecology and General Biology. In Thorp J, Covich A, (eds) Freshw. Invertebr. Elsevier.

Wang G, Jiang X, et al. (2005) Differences in the density, sinking rate and biochemical composition of *Centropages tenuiremis* (Copepoda: Calanoida) subitaneous and diapause eggs. Mar Ecol Prog Ser 288:165–171.

Ware DM (1972) Predation by Rainbow Trout (Salmo gairdneri): the Influence of Hunger, Prey Density, and Prey Size. J Fish Res Board Canada 29:1193–1201.

Ware DM (1973) Risk of Epibenthic Prey to Predation by Rainbow Trout (*Salmo gairdneri*). J Fish Res Board Canada 30:787–797.

Webb CO (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am Nat 156:145–155.

Wellborn GA, Cothran RD (2007) Niche diversity in crustacean cryptic species: complementarity in spatial distribution and predation risk. Oecologia 154:175–183.

Werner EE, Hall DJ (1974) Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55:1042–1052.

Wu L-S, Wang G-Z, Li S-J (2008) The differences in metabolic activity of subitaneous and diapause eggs in *Centropages tenuiremis*. J Plankton Res 31:223–231.

Zhang DY, Lin K, Hanski I (2004) Coexistence of cryptic species. Ecol Lett,7:165–169.

Chapter 2

Morphological similarity and ecological overlap in two rotifer species

Carmen Gabaldón, Javier Montero-Pau, Manuel Serra and
María José Carmona

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A.O.22085, 46071, Valencia, Spain

Published in PLoS One (2013), 8: e57087

Abstract

Co-occurrence of cryptic species raises theoretically relevant questions regarding their coexistence and ecological similarity. Given their great morphological similitude and close phylogenetic relationship (i.e., niche retention), these species will have similar ecological requirements and are expected to have strong competitive interactions. This raises the problem of finding the mechanisms that may explain the coexistence of cryptic species and challenges the conventional view of coexistence based on niche differentiation. The cryptic species complex of the rotifer *Brachionus plicatilis* is an excellent model to study these questions and to test hypotheses regarding ecological differentiation. Rotifer species within this complex are filtering zooplankters commonly found inhabiting the same ponds across the Iberian Peninsula and exhibit an extremely similar morphology−some of them being even virtually identical. Here, we explore whether subtle differences in body size and morphology translate into ecological differentiation by comparing two extremely morphologically similar species belonging to this complex: *B. plicatilis* and *B. manjavacas*. We focus on three key ecological features related to body size: (1) functional response, expressed by clearance rates; (2) tolerance to starvation, measured by growth and reproduction; and (3) vulnerability to copepod predation, measured by the number of preyed upon neonates. No major differences between *B. plicatilis* and *B. manjavacas* were found in the response to these features. Our results demonstrate the existence of a substantial niche overlap, suggesting that the subtle size differences between these two cryptic species are not sufficient to explain their coexistence. This lack of evidence for ecological differentiation in the studied biotic niche features is in agreement with the phylogenetic limiting similarity hypothesis but requires a mechanistic explanation of the coexistence of these species not based on differentiation related to biotic niche axes.

Key-words: Body size; *Brachionus*; coexistence; cryptic species; niche overlap; rotifer

Introduction

In the last decade, molecular approaches have revealed great biological diversity in the form of cryptic species (Gómez 2005; Pfenninger and Schwenk 2007; Kong and Li 2009). Co-occurrence of these species is common (Bickford et al. 2007) and raises important questions, especially in terms of their coexistence and ecological similarity. Given their great morphological similitude and close phylogenetic relationship, these species are expected to have similar environmental requirements (i.e., niche retention) (Futuyma and Mitter 1996; Webb 2000; Violle et al. 2011) and thus strong competitive interactions. Consequently, cryptic species are expected to be prone to competitive exclusion (i.e., the limiting similarity principle) (MacArthur and Levins 1967). The apparent lack of phenotypic and/or ecological differences between cryptic species raises the problem of finding the processes that may explain their co-occurrence and challenges the conventional view of coexistence (McPeek and Gomulkiewicz 2005; Leibold and McPeek 2006) because species coexistence has traditionally been explained by niche differentiation mechanisms (e.g., partitioning of resources, differential risk to enemies, temporal and spatial patchiness, and environmental fluctuations). However, there are alternative processes not based on biotic niche axis differentiation that could explain the co-occurrence of ecologically similar species, such as density-dependent life-history adjustments (Zhang and Hanski 1998; Montero-Pau and Serra 2011) or those invoked by neutral models (Hubbell 2001). In cryptic species, this differentiation either does not exist or is subtle. Although the degree of ecological differentiation needed for stable coexistence depends on the degree of fitness differences (i.e., the more similar their fitness, the less difference is required) (Chesson 2000), it is still unclear how subtle these ecological differences that promote coexistence can be. Thus, the study of co-occurring cryptic species can illuminate the existing mechanisms, aid in the discovery of new ones, and offer the opportunity to experimentally quantify concepts such as limiting similarity.

Body size affects life history, the ecological niche of an organism, and its interactions with other organisms. The impact of body size in determining the ecological niche is especially significant in aquatic systems (Werner and Gilliam 1984), as body size has implications for predation susceptibility and competitive ability. Many aquatic invertebrate predators detect their prey by mechanoreception (Moore et al. 1999; Bundy and Vanderploeg 2002); thus, the greater the size of the prey, the greater the mechanical disturbance created, and the greater the risk of being detected (Kerfoot 1977). Additionally, aquatic vertebrate predators such as fishes use visual orientation to capture prey so that the reactive distance of the predator is positively related to the prey size (Ware 1972; Ware 1973; Werner and Hall 1974; O'Brien et al. 1976). Body size also shapes the consumer niche because large prey is more difficult to catch and has longer handling times (Kerfoot 1977; Pastorok 1981; Roche 1987; Lapesa et al. 2002) or, in the case of filter-feeding zooplankton, because the size limit of the comestible particles is determined by the mesh width of the filtering apparatus (Gliwicz 1980). Thus, a higher similarity in predation vulnerability and consumer niche is expected among competing species with similar body size and morphology.

The objective of this study is to explore whether subtle differences in body size and morphology translate into ecological differentiation. To this end, we used two cryptic rotifers species belonging to the *Brachionus plicatilis* species complex (Gómez 2005), *B. plicatilis* and *B. manjavacas*, as a model. Both species are commonly found living in sympatry in the plankton of many bodies of salt water in the Iberian Peninsula (Gómez et al. 2002; Gómez et al. 2007). The rotifer communities of these habitats are poorly diversified, and populations are expected to be regulated by food availability and predation (Pitta et al. 1997). The ponds inhabited by *B. plicatilis* and *B. manjavacas* are shallow, with low spatial heterogeneity but a highly variable salinity regime (Rodriguez-Puebla et al. 1998), and it has been suggested that a differential response to salinity could mediate the stable coexistence of the rotifers (Montero-Pau et al. 2011). Both species are virtually morphologically identical. The only reliable feature for morphological identification is the shape of small accessory pieces of the internal masticatory apparatus (i.e., satellites) (Fontaneto et al. 2007). In addition, *B. plicatilis* is on average 6% longer than *B. manjavacas* (Campillo et al. 2005). In this study, we focus on three key ecological features (vulnerability to predation, food particle size preference, and starvation tolerance) where body size has been proven to be determinant in the *Brachionus* genus.

Species of the *B. plicatilis* complex lack any conspicuous escape response from predators or structures of protection such as spines, apart from the lorica, a hard outer covering of chitin (Stemberger and Gilbert 1987); thus, body size is a crucial factor in their susceptibility to predation by copepods (Lapesa et al. 2002; Ciros-Pérez et al. 2004; Lapesa et al. 2004). Rotifers are primarily passive filterers, and their diets are affected by the structure and size of their feeding structures. For example, when comparing the maximum size of particles ingested by a *B. plicatilis* species, Hino and Hirano (1980) concluded that the largest particle size that a rotifer is able to capture is dependent on its body size. This is most likely because the food groove, which is responsible for transporting the collected particles to the mouth using cilia, increases with body size, as has been demonstrated for gastropod larvae (Hansen 1991). Interestingly, the body size of some species of *B. plicatilis* is clearly related to both the optimal particle size and the width of the retention spectrum (Rothhaupt 1990a). In addition, for some rotifer species, feeding efficiencies increased with increasing dietary particle size (Rothhaupt 1990b; Rothhaupt 1990d), although that efficiency cannot be predicted solely by body size (Bogdan and Gilbert 1984; Ciros-Pérez et al. 2001), and other factors related to predator prey encounter rate need to be considered (Turner and Borkman 2005; Zhou et al. 2009). The ability to survive during periods of extreme resource limitation affects the competitive capability of a species. In some zooplankton groups, starvation resistance has been related to organism body size (Threlkeld 1976; DeMott 1989). Lapesa (2004) showed that the smallest of three studied *Brachionus* species was the least able to endure starvation.

The effect of slight morphological differentiation on the biotic dimensions of the niche of competing cryptic species has strong implications for fundamental problems in ecology such as limiting the similarity or the degree of ecological differentiation needed to promote coexistence. Previous studies have examined the differential susceptibility to predation and exploitative competition, including feeding strategies, of some species of the complex *B. plicatilis* (Lapesa et al. 2002; Ciros-Pérez et al. 2004; Lapesa et al. 2004). However, no study has addressed this question by comparing two extremely morphologically similar species belonging to this complex: *B. plicatilis* and *B. manjavacas*. The assumption seems to have been that subtle morphological differences do not allow such ecological differentiation; however, this assumption needs to be evaluated, especially in the framework of coexisting cryptic species. In this study, we address the differential ecological response between *B. plicatilis* and *B. manjavacas*. We study the following ecological features known to be affected by body size: (1) vulnerability to copepod predation, as measured by the number of preyed upon neonates; (2) functional response, as measured by the clearance rates of both species using two different algae species as food resources; and (3) tolerance to starvation, as measured by growth and reproduction. As the differences are expected to be subtle, methodological attention was given to the statistical power of the data analysis.

Material and Methods

Rotifer species

We used two species of cyclical parthenogenetic rotifers belonging to the *B. plicatilis* cryptic species complex: *B. plicatilis* and *B. manjavacas*. The reproductive cycle of rotifers of the genus *Brachionus* begins with the hatching of asexual females from diapausing eggs. Females reproduce by ameiotic parthenogenesis for several generations, producing clones. Sexual reproduction is density-dependent and induced by a chemical cue (Stelzer and Snell 2003; Snell et al. 2006). Then, asexual females begin producing sexual females that produce haploid oocytes that then develop into males if unfertilized or into diapausing eggs if fertilized.

Media and culture conditions

The rotifers were fed two species of microalgae, which differ in size and mobility: *Tetraselmis suecica* (Prasinophyceae, motile, ellipsoidal, equivalent spherical diameter, ESD= 9 μm; provided by the Collection of Marine Microalgae of the Instituto de Ciencias Marinas de Andalucía, Cádiz, Spain) and *Nannochloris atomus* (Chlorophyceae, non-motile, spherical, ESD= 2.5 μm; strain CCAP 251/7; provided by the Collection of Algae and Protozoa of the Scottish Association of Marine Sciences, Oban, Scotland). The microalgae species were individually cultured at 20.0±0.1ºC in an f/2 enriched saline water medium (Guillard and Ryther 1962) at 10 g L^{-1} salinity under constant aeration and illumination (35 μmol quanta m^{-2} s^{-1}). This salinity was selected because it is in the range of optimal values for the studied rotifer species in Salobrejo Lake (Montero-Pau et al. 2011). Saline water was created with commercial sea salt (Instant Ocean®; Aquarium Systems). The microalgae were maintained in exponentially growing, semi-continuous cultures (dilution rate: 0.5 day^{-1}) to provide food of constant quality during the experiments. Microalgae density was estimated by 750-nm wavelength light extinction using an absorption vs. density calibration curve. The equivalence to carbon content per microalgae cell was estimated using an elemental analyzer with thermal conductivity, EA 1108 CHNS-O (Fisons Instruments), using the flash combustion technique. Unless otherwise indicated, the rotifers were cultured under the same standard conditions of temperature, salinity and illumination as the microalgae.

Rotifer isolation and species identification

The rotifer clones used in the experiments were established from diapausing egg hatchlings. Sediment containing these eggs was collected in June 2010 with a Van Veen grab (Eijelkamp Agrisearch Equipment) from the upper sediment layer of Salobrejo Lake (Eastern Spain, 38º 54.765' N, 1º 28.275' O). The sediment samples were stored in the dark at 4°C for 30 days to ensure the completion of the obligate period of dormancy of the diapausing *Brachionus* eggs (Hagiwara and Hino 1989). Diapausing eggs of *B. plicatilis* and *B. manjavacas* were isolated from the sediment samples using a modified sucrose flotation technique (Gómez and Carvalho 2000). The eggs were then individually transferred to 96-well plates (NuncTM) containing 150 μL of 10-g L^{-1} saline water and induced to hatch under the following conditions: 25.0±0.1°C and constant illumination (150-170 μmol quanta m^{-2} s^{-1}). The eggs were checked every 24 h, and neonate females hatching from the eggs were isolated, fed with *T. suecica* (250,000 cells mL^{-1}, ≈ 33 mg C L^{-1}), and allowed to found clones by parthenogenetic proliferation.

Species identification of the clones was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of a fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) (Campillo et al. 2005). DNA was extracted from 5-7 females per clone using the HotSHOT method (Montero-Pau et al. 2008), and the mitochondrial COI fragment was amplified using PCR using the invertebrate universal primers LCO1490 and HCO2198 (Folmer et al. 1994) as described in Gómez et al. (2000). The RFLP analysis was performed with Kpn I and Pvu II endonucleases following Campillo et al. (2005).

Stock cultures of 25 clones from each rotifer species were maintained separately under standard conditions. Prior to the experiments, multiclonal pre-experimental populations of *B. plicatilis* and *B. manjavacas* were established under different experimental conditions (see below) by mixing approximately 25 females of each of the 25 clones (approximately 1 female mL^{-1} of each clone). These populations were cultured for three generations to reduce maternal effects (e.g., Stelzer and Snell 2006) and to acclimate the rotifers to the experimental conditions.

Clearance rates

The feeding behavior of *B. plicatilis* and *B. manjavacas* was studied by measuring their clearance rates in short-term feeding experiments in monoalgal cultures of *T. suecica* and *N. atomus* following Ciros-Pérez et al. (2001). Four rotifer multiclonal pre-experimental populations were established (2 rotifer species × 2 microalgae species). The rotifers were transferred from the pre-experimental cultures to the experimental food concentration 1 h before the experiments. For that purpose, these cultures were filtered through a 30 μm Nitex mesh, and the retained rotifers washed with saline water at 10 g L^{-1} to eliminate any remnants of algae. Afterwards, the rotifers were transferred to Petri dishes containing a culture medium with the experimental concentration of algae. The experiments were performed by pipetting 20 rotifers for *T. suecica* and 40 rotifers for *N. atomus* into Eppendorf® tubes with 1 mL algae culture at a concentration of 0.6 mg C L^{-1}. The tubes were kept for 1 hour in a centrifuge at a constant speed (6 rpm), at 20°C, and in darkness to avoid algal growth during the experiment. After 1 hour, the tubes were fixed with 20 μL of Lugol's solution. Ten replicates were performed for each rotifer-algae combination. Additionally, three tubes with *T. suecica* and three tubes with *N. atomus* without rotifers were used as controls and fixed immediately after inoculation with the algae. The experimental concentration of each algae species was 0.6 mg C L^{-1}, which corresponds to 3,140 cell mL^{-1} of *T. suecica* and 375,000 cell mL^{-1} of *N. atomus*. According to Ciros-Pérez et al. (2001), this concentration of food is below the incipient limiting level (ILL) for both *T. suecica* and *N. atomus*. The clearance rate remains constant below the ILL (Rigler 1961), a critical food concentration from which the clearance rate exponentially decreases (Rothhaupt 1990c). Below this level, filtration rates decrease linearly with decreasing food concentrations. However, to confirm that our experimental food concentration was below the ILL, three additional tubes for each rotifer-algae combination were prepared following the same procedure, except that the incubation was for 2 hours prior to fixation.

The algae were counted using an inverted Olympus® SZK10 microscope. A minimum of 800 cells were counted per sample to obtain a confidence interval of 7% (Lund et al. 1958). The clearance rates were calculated following Peters (1984):

$$CR = \frac{\ln C_0 - \ln C_t}{N * t},$$

where C_0 and C_t are the initial and final algae concentrations, N is the rotifer density, and t is the time in hours. For each microalgae species, the C_0 value was the average concentration of the three control tubes.

The data were independently analyzed for each algal species. To confirm whether the experimental algae concentration was under the ILL, a linear regression analysis (algae concentration vs. incubation time, i.e., 0, 1 or 2 h) was performed using R version 2.12.1 (R Foundation for Statistical Computing 2010). Student's t test was used to test for differences between *B. plicatilis* and *B. manjavacas* in CR for each alga. The highest and lowest values for each species were excluded from the analysis. Additionally, to test the power of our analysis, the minimum detectable statistically significant difference given the observed experimental variance was computed; the CR value of one species remained fixed while the mean CR value of the other species was gradually increased, without modifying the variance, until the difference between the two groups was statistically significant. These analyses were performed using SPSS version 9.0 (SPSS Inc., Chicago, Illinois).

Predation susceptibility

The relative susceptibility of *B. plicatilis* and *B. manjavacas* to predation by *Arctodiaptomus salinus* (Copepoda, Calanoida) was tested through differential predation experiments on the rotifer species. This copepod was selected as the predator because the adult stage feeds on small zooplankters including species of the *B. plicatilis* complex (Lapesa et al. 2004) and because *A. salinus* co-occurs with *B. plicatilis* and *B. manjavacas* (e.g., in Salobrejo Lake; (Lapesa et al. 2004); Montero-Pau, personal communication); thus, this copepod is a potential predator of both species and might play an important role in the coexistence of these two cryptic species if the predation were differential.

Diapausing eggs of *A. salinus* were isolated using the same sucrose flotation technique from the same sediment samples from which both rotifer species were obtained. The copepod eggs were incubated under standard conditions until they hatched. The nauplii were individually isolated in 24-well plates (NuncTM) and maintained on a mixed diet of *T. suecica* and *N. atomus*. The medium was renewed every 5-7 days, and the copepods reached the adult stage in approximately 3-4 weeks.

For the predation experiments, we selected rotifer neonates as prey from pre-experimental multiclonal populations (see above). *A. salinus* prefers small prey (Lapesa et al. 2004). Thus, by using neonates, predation is expected to be more efficient. We performed two predation experiments. In the first, we used only adult copepod females as predators, whereas we examined both sexes separately in the second to test for differential predation by adult females and males. The procedure in both experiments was the same. Adult copepods were individually placed in the wells of 24-well plates (NuncTM), with each well containing 1 mL of 10-g L^{-1} saline water without food. After 15-16 hours, 25 rotifer neonates were added per well. Both rotifer species were tested separately. Ten replicates plus three controls without copepods, to control for mortality due to other factors (i.e., the intrinsic mortality of rotifers), were completed for each rotifer species. The copepods and rotifers were incubated together for 24 hours. After that time, the copepods were removed, and the rotifers, including those in the controls, were fixed with Lugol's solution. The rotifers were counted under a Leica SZX2 stereomicroscope. The number of rotifers suffering predation was calculated as the difference between the initial and final counts in each well.

After checking for equal variances, the differences in the predation rate between the prey species in the first experiment were analyzed using Student's t test. The variances in the second experiment were not homogenous. Thus, a robust two-way ANOVA was applied to test for the effects of prey species and predator gender on predation. The power of our analysis and the minimum detectable statistically significant difference were computed. For each rotifer species, we randomly chose a surviving rotifer from one of the replicates and considered it as instead suffering predation, and then we statistically reanalyzed the simulated data. This process was repeated, accumulating randomly chosen individuals as suffering predation, until the difference between both species was significant. SPSS version 9.0 (SPSS Inc., Chicago, Illinois) was used to perform Student's t test, and R version 2.12.1 (R Foundation for Statistical Computing 2010) was used to perform the robust two-way ANOVA.

Tolerance to starvation

The response of *B. plicatilis* and *B. manjavacas* to different periods of starvation before the age of maturity was measured using a dynamic life table experiment. To establish the cohorts, aliquots of approximately 8 mL of pre-experimental multiclonal cultures were transferred into assay tubes and gently shaken to detach the eggs from the females (Tortajada et al. 2010). The detached eggs harboring female embryos (the gender of an embryo can easily be distinguished by egg size) were removed and isolated on plates in 10-g L^{-1} saline water. These eggs usually hatch in less than 4-5 hours. The neonate females hatched within 2 h were individually transferred into 1 mL of saline water in the wells of 24-well plates (NuncTM). For each rotifer species, the neonates were divided into five cohorts containing 25 females each and assigned to one of five fasting times of 0, 6, 12, 18 or 24 hours (2 rotifer species × 5 treatment of starvation= 10 cohorts). The species and starvation treatments were randomly distributed across the 24-well plates. After the corresponding hours of starvation, the females were fed *T. suecica* at a concentration of 100,000 cell mL^{-1} (≈ 13 mg C L^{-1}). The females were followed and monitored every 24 hours until all died. Daily, the survival and number of offspring produced were recorded, and the female was transferred to a new well of a 24-well plate (NuncTM) containing 1 mL of fresh medium with 100,000 cell mL^{-1} of *T. suecica*.

The lifespan (e_0), mean generation time (G_c), net reproduction rate (R_0), survival function (l_x, with x being age), and age-specific fecundity (m_x) of both rotifer species under each starvation treatment were calculated. Comparisons among the survival curves were performed using two non-parametric tests: a Log-rank test and a Breslow test (Cox and Oakes 1984). The former assumes equal importance of all observations, whereas the latter gives more weight to the initial part of the survival curve. These tests were performed using SPSS version 9.0 (SPSS Inc., Chicago, Illinois). In addition, potential intrinsic growth rates (i.e., the rate of increase that a population would have if no investment in sex occurred, r_{pot}) (Montero-Pau et al. unpublished manuscript) were obtained using the Euler-Lotka equation (e.g., Stearns 1992). The r_{pot} for a life-table experiment is obtained by exclusively considering the asexual fraction of the population. As births do not necessary occur at the moment of the observation, the estimated r_{pot} was improved by considering each time of observation (x) as the midpoint between this time and the next (Caswell 2006). ANCOVA was performed to analyze the effects of species and starvation on r_{pot}, e_0, G_c and R_0. The ANCOVA results of r_{pot} should be interpreted considering the intrinsic biases of the estimates of r_{pot} (McGraw and Caswell 1996; Caswell 2006). However, these ANCOVA results were supported by the r_{pot} confidence intervals. These analyses were performed using R version 2.12.1 (R Foundation for Statistical Computing 2010). The 95% confidence intervals of r_{pot} were obtained using bootstrap resampling (Caswell 2006) and corrected following the bias-corrected percentile method (Efron 1981; Efron and Tibshirani 1986). The bootstrapping and its correction were implemented in R version 2.12.1 (R Foundation for Statistical Computing 2010), and 10,000 randomizations were performed for each treatment and species.

Niche overlap

Interspecific biotic niche overlap was estimated based on the studied features using the analytical approach of Geange et al. (2011). This method can account for multiple niche axes, each characterized by different data types, and computes a unified analysis of niche overlap. We used the clearance rates, susceptibility to predation, and starvation tolerance data sets to calculate the biotic niche overlap between *B. plicatilis* and *B. manjavacas* along the following eight axes: (1) Clearance rate for *T. suecica* (continuous data); (2) Clearance rate for *N. atomus* (continuous data); (3) Susceptibility to predation by copepod females (binary data); (4) Susceptibility to predation by copepod males (binary data); (5) Potential intrinsic growth rate after 6 h of starvation (continuous data); (6) Potential intrinsic growth rate after 12 h of starvation (continuous data); (7) Potential intrinsic growth rate after 18 h of starvation (continuous data); and (8) Potential intrinsic growth rate after 24 h of starvation (continuous data). Before analysis, the clearance rates were log-transformed, and the r_{pot} values were corrected by subtraction from the r_{pot} values obtained in the 0 h starvation treatment (see above) to remove the constant (starvation independent) interspecific effect. Niche overlap indexes (NO) were calculated for each dimension following Geange et al. (2011). Then, a single unified niche overlap index (Geange et al. 2011) was obtained by averaging the niche overlap over each different axis *t* as follows:

$$NO = \frac{1}{T}\sum_{t=1}^{T} NO_t$$

where T is the number of dimensions, and NO ranges from 0 (disjoint niches) to 1 (total niche overlap).

To assess the statistical niche differences between species, null model permutation tests were performed to test whether both the niche overlap along each axis and the mean niche overlap were significantly lower than expected by chance (Gotelli and Graves 1996; Geange et al. 2011). Statistical null distributions (the distribution of the test statistic under the null hypothesis of no niche differentiation) were generated by calculating pseudo-values through randomly permuting species labels in the corresponding data set over 10,000 runs. The distribution of the average niche overlap for the null model was then computed. To correct for multiple comparisons, we performed a sequential Bonferroni adjustment (Quinn and Keough 2005).

The niche overlap calculations and associated null model tests were performed using R version 2.12.1 (R Foundation for Statistical Computing 2010) using the source code provided as supporting information in Geange et al. (2011).

Results

Functional response

The log concentration of both *T. suecica* and *N. atomus* decreased linearly ($R^2 > 0.68$) with increasing incubation feeding time for both rotifer species (Fig. 2.1), indicating that the experimental food concentrations were below the incipient limiting level (ILL) (Rigler 1961), the threshold food concentration up to which clearance rates remain constant. The averaged clearance rates for both rotifer species are shown in Table 2.1. When the rotifers were fed *T. suecica*, *B. plicatilis* presented a clearance rate that was on average 3.9% higher than that of *B. manjavacas*. In contrast, when they were fed *N. atomus*, *B. manjavacas* filtered 4.2% more than *B. plicatilis*. However, these differences were not statistically significant (Student's t test $P= 0.72$ for *T. suecica* and $P= 0.24$ for *N. atomus*). The power analysis demonstrated that, given our data variance, the difference between the average clearance rates of the two rotifer species would be need to

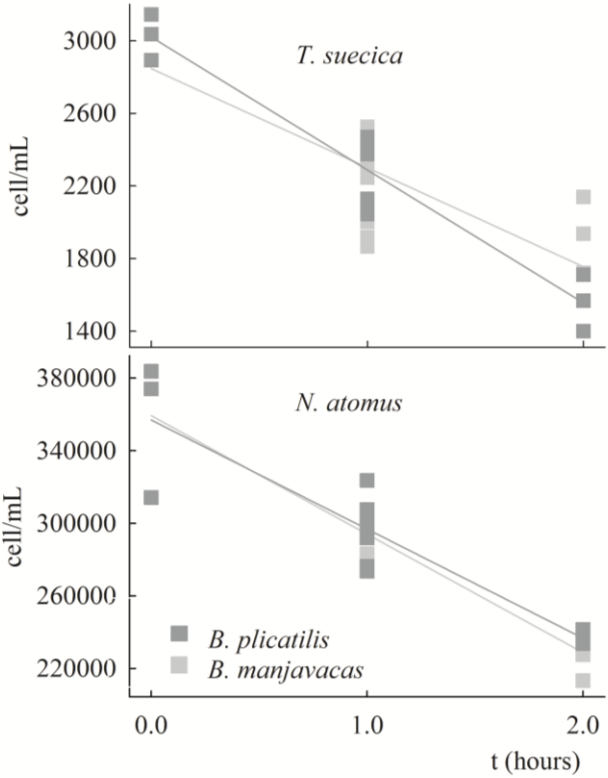


Figure 2.1. Concentration (cell mL^{-1}) of *T. suecica* and *N. atomus* for different incubation times with *B. plicatilis* and *B. manjavacas*.

be 30% to detect a statistically significant difference at the 5% significance level for rotifers fed *T. suecica*, whereas a 15% difference would be required when using *N. atomus* as food. Both *B. plicatilis* and *B. manjavacas* were three times more efficient when feeding on *T. suecica* than on *N. atomus*.

Table 2.1. Clearance rates (μL ind^{-1} h^{-1}) of *B. plicatilis* and *B. manjavacas* feeding on the microalgae *T. suecica* and *N. atomus*. Values are the means ± SE (sample size in brackets). Estimations based on 1 h observations.

Rotifer species	***T. suecica***	***N. atomus***
B. plicatilis	15.5 ±1.1 (11)	4.7±0.2 (8)
B. manjavacas	14.9±1.3 (11)	4.9±0.3 (10)

Predation by copepods

No rotifers died in the control replicates, so mortality was due to copepod predation. *A. salinus* females preyed 12% more on *B. plicatilis* than on *B. manjavacas* in the first experiment. In contrast, *B. manjavacas* was preyed upon on average 32% (female predators) and 41% (male predators) more than *B. plicatilis* in the second experiment. However, no statistically significant difference was found in either assay (Fig. 2.2; *P*= 0.642 and 0.287, for the first and second experiments, respectively). The results of the second experiment revealed a significant effect of copepod sex in the efficiency of predation (*P*= 0.003). The *A. salinus* females had four times higher predation efficiency than copepod males on both *B. plicatilis* and *B. manjavacas*. According to the power analysis, the difference between the predation efficacies of *A. salinus* on *B. plicatilis* and *B. manjavacas* must be at least 39% to be statistically significant at the 5% significance level in the first experiment (*P*= 0.045). At least a 49% difference between the predation efficiencies of *A. salinus* females and a 48% difference

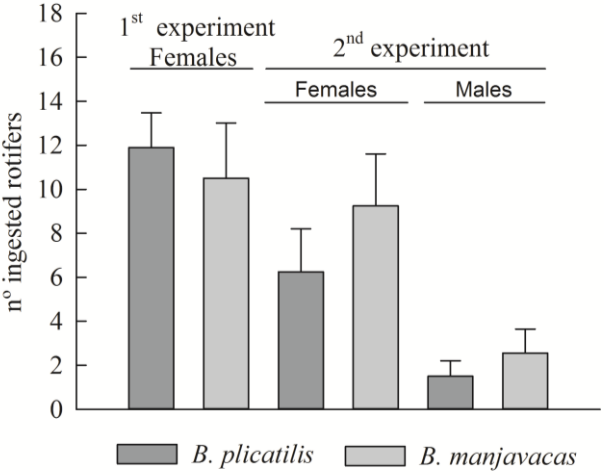


Figure 2.2. Average numbers of B. *plicatilis* and B. *manjavacas* consumed by *A. salinus* females and males in two predation experiments. Vertical bars are ± SE.

between the efficiencies of *A. salinus* males were needed in the second experiment (*P*= 0.049).

Tolerance to starvation

Fig. 2.3 shows the l_x and m_x curves of B. *plicatilis* and B. *manjavacas* females under different starvation times. Both non-parametric tests (log-rank and Breslow) failed to find statistically significant differences between the survival functions when all of the survival functions of B. *plicatilis* and B. *manjavacas* were globally compared (*P*= 0.065 and 0.1, respectively). Moreover, no differences were found in survival when the data for each starvation level were compared

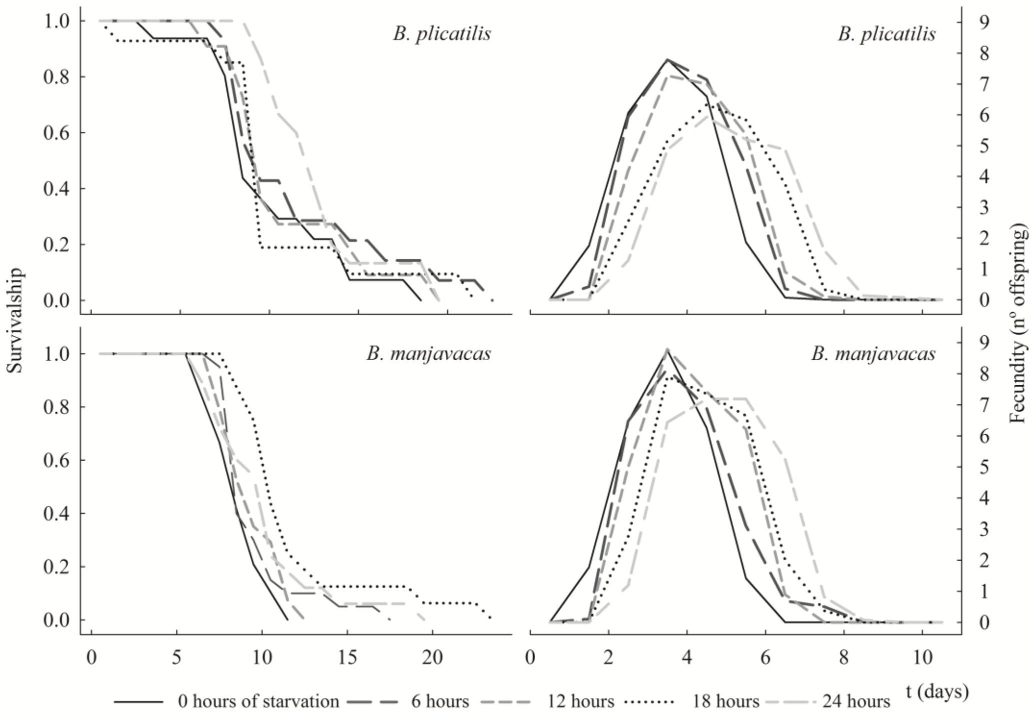


Figure 2.3. Survival and fecundity curves of *B. plicatilis* and *B. manjavacas* under different starvation times.

between species (all *P*> 0.12). The fecundity pattern of both rotifer species showed similar trends: maximum fecundity tended to decrease with starvation time, whereas the length of the reproductive period increased (Fig. 2.3). G_c also tended to be positively related to increasing starvation period (Fig. 2.4) and was dependent on species, with *B. plicatilis* having slightly higher values than *B. manjavacas* (*P*= 0.036).

However, the increasing effect of starvation on G_c was similar in both species, with no significant differences between the slopes of the regression lines relating G_c to starvation time (*P*= 0.369). The rotifer species did not show significantly different responses of e_0 (*P*= 0.07) or R_0 (*P*= 0.41) to starvation. Additionally, no significant differences were found in these parameters when both species were compared (*P*= 0.169 and 0.079, for e_0 and R_0, respectively).

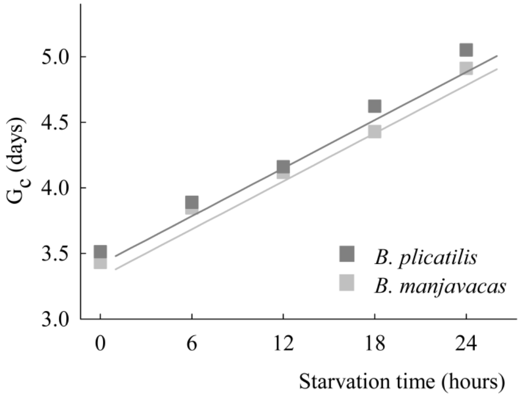


Figure 2.4. Mean generation times (G_c) of *B. plicatilis* and *B. manjavacas* under different starvation times. Regression lines are shown.

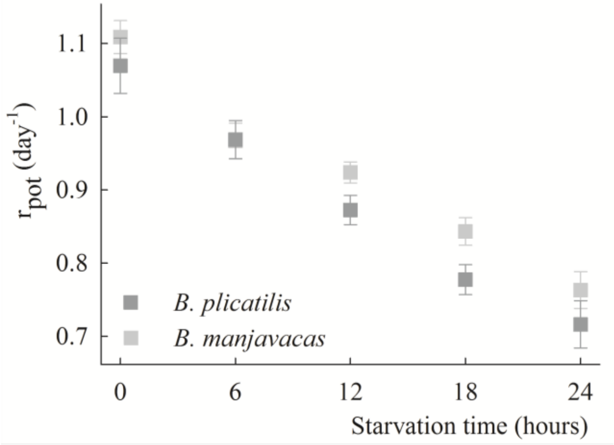


Figure 2.5. Response of the potential intrinsic growth rates (r_{pot}) of *B. plicatilis* and *B. manjavacas* to starvation time following birth. Vertical bars are the lower and upper 95% confidence intervals.

The potential intrinsic growth rates for starved *B. plicatilis* and *B. manjavacas* females under the different starvation treatments are shown in Fig. 2.5 ANCOVA revealed that, regardless of the species, increasing starvation period led to decreasing r_{pot} (P< 0.001). In addition, statistical analysis indicated an effect of species on r_{pot} values (P< 0.039); *B. manjavacas* had slightly higher values of r_{pot} than *B. plicatilis* for all treatments. However, both species responded similarly to starvation time (i.e., equal slopes, *P* for species-treatment interaction= 0.275).

Niche overlap

The niches of *B. plicatilis* and *B. manjavacas* were found to be very similar (mean niche overlap= 0.78, *P*= 0.162) as the overlap values for

Table 2.2. Niche overlap indexes (NO) between *B. plicatilis* and *B. manjavacas* for the analyzed niche axes. CR_{Ts} and CR_{Na}, clearance rates for *T. suecica* and *N. atomus*; D_f and D_m, predation by female and male copepods; r_{pot6}, r_{pot12}, r_{pot18} and r_{pot24}, potential intrinsic growth rate after 6, 12, 18 and 24 hours of starvation, respectively.

Niche Axis	**NO**	***P****
CR_{Ts}	0.798	0.450
CR_{Na}	0.852	0.906
D_f	0.954	0.166
D_m	0.958	0.118
r_{pot6}	0.755	0.345
r_{pot12}	0.707	0.282
r_{pot18}	0.673	0.210
r_{pot24}	0.546	**0.033**

* Axis with statistically different niches, significant at $P < 0.05$ as identified by a null model test, are indicated in bold. No value remained significant after sequential Bonferroni correction.

seven of the eight axes were high, and the analysis showed no significant differences between the two species (Table 2.2). Tolerance to 24 h fasting appeared to be a distinguishing factor between the species as it was the only axis indicating significantly different niches, but it did not remain significant after sequential Bonferroni correction. The most similar niches were associated with the axes related to predation by female and male copepods.

Discussion

Difference in body size is an important mean by which species avoid direct overlap in resource use and can have important effects on vulnerability to predation (Schoener 1974; Wilson 1975). Size influences interspecific ecological interactions and has been proven to be an important feature related to the biotic dimensions of the niches of cryptic species, for example, as in the case of bats or amphipods (Barlow et al. 1997; Wellborn and Cothran 2007). The primary objective of this paper was to examine the extent to which a subtle body size differentiation could cause differences in the biotic dimensions of the niche. In some species of the *B. plicatilis* complex, body size differences ranging from 23 to 50% have been shown to be associated with ecological differentiation (Rothhaupt 1990c; Stelzer 2001; Ciros-Pérez et al. 2004; Lapesa et al. 2004). However, in this study, no major differences in vulnerability to predation, food particle size preference, or starvation tolerance have been found between *B. plicatilis* and *B. manjavacas*, which are virtually identical in morphology but differ by 6% in body size.

Functional response

Body size is considered to be an important factor in determining trophic niche for filter-feeding organisms because the widths of their mouths limit the size of particles that they can ingest (Hino and Hirano 1984; Hansen 1991). In this study, we used two microalgae, *T. suecica* and *N. atomus*, which include a significant portion of the particle size preference range of *Brachionus* species (Rothhaupt 1990b;

Vadstein et al. 1993). Our results demonstrated that the clearance rates for both rotifer species are equal despite their difference in body size. The values reported here are within the range described by other authors (Rothhaupt 1990a; Rothhaupt 1990c; Ciros-Pérez et al. 2001) for *Brachionus* species. *T. suecica* was filtered three times faster than *N. atomus*, which is smaller and immobile. These data are in agreement with the optimal prey size reported for *Brachionus* species (Rothhaupt 1990c; Hansen et al. 1997). The clearance efficiencies of *B. plicatilis* for *T. suecica* were lower than recorded in previous studies (Ciros-Pérez et al. 2001). This discrepancy might be because we used rotifer neonate females in our experiments, whereas female age was not controlled in Ciros-Pérez et al. 2001). Neonates are smaller than adults, and because the size of the particles that they can ingest is limited by body size, their algae filtration rates are expected to be lower.

The body size difference between *B. plicatilis* and *B. manjavacas* does not appear to be sufficient to affect their clearance rates. Moreover, assuming Tilman's model (Tilman 1982) and assuming that a stable equilibrium point exists in the use of the remarkably different resources tested here, our estimation of the clearance rates implies that the resource supply should be in a sector representing less than 1.5% of the resource space. Thus, niche partitioning by differential use of these microalgae seems highly unlikely, contrary to what has been observed in other species of the rotifer complex whose differences in body size are greater (*B. plicatilis, B. ibericus* and *B. rotundiformis* sizes ranging from 23 to 50%) (Ciros-Pérez et al. 2001), so that their coexistence of could be explained by differential clearance rates. Our conclusion of lack of differential resource use between *B. manjavacas* and *B. plicatilis* was supported by results from niche overlap analysis.

There are factors in addition to body size that affect prey selection in filter-feeding organisms and could also shape the trophic niche. Selectivity has been linked to algal characteristics such as cell surface (Dumont 1977; Pourriot 1977), physiological conditions (Chotiyaputta and Hirayama 1978) and motility (Gilbert and Bogdan 1984). Actively moving prey−microalgae in this case−may increase their encounter rates with predators (Turner and Borkman 2005). The presence of cilia provides mobility to *T. suecica*, which, in addition to its larger size, may account for the higher grazing rates on these microalgae observed in our experiments for *B. plicatilis* and *B. manjavacas*.

Tolerance to starvation

In aquatic systems, resource quantity and quality can vary drastically over short periods of time, and episodes of severe resource scarcity are expected. The time scale of these phenomena may vary from hours, due to daily vertical migration (Hutchinson 1967), to days or weeks during seasonal change (Stewart and Wetzel 1986; Sommer 1989). Accordingly, the ability to withstand starvation is considered an important element in species persistence (Kirk 1997), and this ability may affect the competitive outcome between zooplankton species (Threlkeld 1976; Stemberger and Gilbert 1985; DeMott 1989; Rothhaupt 1990c; Schulze 1995; Kirk 1997). We found that the effect of food limitation on survival was similar in *B. plicatilis* and *B. manjavacas*. In accordance with previous studies on the response of *B. plicatilis* to starvation (Yoshinaga et al. 2003), *B. plicatilis* and *B. manjavacas* both altered their fecundity schedule under food-limited conditions. Starvation seems to cause a general delay in age-related reproductive traits in both species in our study: longer generation times, older age at maturity, and longer reproductive periods were recorded in our experiments. In contrast, the life expectancies and net reproduction rates of the two studied species were not affected by food limitation. The generation time and the potential growth rate are correlated. Thus, consistent with the effect of starvation on the generation time, the potential growth rate decreased linearly with increasing starvation time. *B. manjavacas* had slightly but consistently higher r_{pot} than *B. plicatilis* in all of the starvation treatments, although the differences were not statistically significant. Interestingly, both species are able to maintain positive growth rates after starving for one day, which is approximately 9% of their life expectancy. Starved newborn female rotifers are able to survive several days using egg reserves as their only source of energy (Yoshinaga et al. 2003; Gilbert 2004; Garcia-Roger 2005; Ricci and Perletti 2006). However, tolerance to this longer starvation time was the only axis suggesting niche segregation between the two species.

Predation by copepods

Most of the rotifers species co-occurring with predatory copepods have been described as prey of copepods (Williamson 1987; Conde-

Porcuna and Declerck 1998). In addition to prey size, vulnerability to predation also depends on morphological and behavioral features that could protect individuals from being successfully attacked (Gilbert and Williamson 1978; Stemberger 1982; Williamson 1987). In contrast with other rotifers, the *Brachionus* species studied here do not exhibit conspicuous features to avoid predation by copepods, except for a hard, chitinous lorica and a 'dead-man' behavioral response to attacks (Lapesa et al. 2002). Because *Brachionus* species are morphologically very similar, their size becomes relevant, with the highest susceptibility to predation being associated with the smallest sized species of the complex (Lapesa et al. 2002; Ciros-Pérez et al. 2004; Lapesa et al. 2004). Therefore, the morphological and size similarity between *B. plicatilis* and *B. manjavacas* provides an explanation for their very similar vulnerability to predation by both male and female copepods, the tested axes that seemed to contribute most to their high interspecific niche overlap. We recorded predation rates by *A. salinus* in the range of those obtained by Lapesa et al. (2004) when they studied predation of *A. salinus* on *B. plicatilis*. Our finding of sex-dependent predation efficiency in *A. salinus* is also consistent with the experimental data obtained by Ciros-Pérez et al. (2004) using the copepod *Diacyclops bicuspidatus odessanus* as predator. The higher efficiency of female copepods may be due to their larger size. Prey handling time is expected to be negatively related to predator-prey size ratio, and a higher handling time involves a lower predation efficiency (Roche 1990). Interestingly, although the difference was not statistically significant, the females and males of *A. salinus* preyed slightly more on the smaller species, *B. manjavacas*.

Ecological similarity

This study did not detect evidence that the cryptic species *B. plicatilis* and *B. manjavacas* differentiate their biotic niches by having different clearance rates, susceptibility to predation, or starvation tolerance. Three concerns could arise from this conclusion. (1) Generally, no test can rigorously demonstrate a lack of difference between data sets because that implies the acceptance of the null hypothesis. Thus, knowing the statistical power of the tests is critical to assessing the plausibility of the absence of differences. In our study, low statistical power might affect the predation experiment results, although we were able to detect significant differences for one factor (predator sex); this was likely because, although the sample size was small, our design controlled for prey age in the experiments, thus reducing the variability in intraspecific prey size (error variance). Overall, this suggests that even if statistical significance could be achieved by increasing the sample size, the differences would be minor and of low ecological significance. (2) Another consideration is whether phenotypic plasticity causing morphological divergence could contribute to the evolutionary differentiation of the biotic niche of these species, because of a differential response of their morphology to an environmental factor. However, evidence suggests that the morphology of *Brachionus* species either respond similarly or do not respond to changes in environmental factors, at least for temperature and salinity (Serra and Miracle 1987). Moreover, these species inhabit a spatially homogenous environment, so that individuals should experience similar developmental environments. In addition, we did not notice indications of character displacement when the species were grown together. (3) A third concern is whether unanalyzed niche axes could promote niche differentiation. We intentionally limited our study to those biotic axes related to resource use and predation vulnerability. However, efficiency in resource use might be dependent on physical environmental factors. Our hypothesis is that this dependence does occur in relation to salinity and temperature, which are critical mediating their long-term coexistence (see below).

The evidence for ecological similitude between the two cryptic species studied here is in agreement with the phylogenetic limiting similarity hypothesis, which predicts that phylogenetically closely related species are likely to possess ecological similarities (Wiens and Graham 2005; Losos 2008; Wiens et al. 2010; Violle et al. 2011). This hypothesis also assumes that a higher ecological similitude is likely to result in more frequent competitive exclusion. However, *B. plicatilis* and *B. manjavacas* co-occur in many lakes along the Iberian Peninsula, and their occurrence in the region has been traced back to the Pleistocene (Gómez et al. 2002; Gómez et al. 2007); thus, their coexistence is unlikely to be transient. The discovery of cryptic species co-occurring in the same habitat, particularly if the habitat is spatially homogeneous, raises new questions in explaining the coexistence of these species. Moreover, it challenges the limit of ecological differentiation needed to promote coexistence and favors the description of novel coexistence mechanisms not based on niche partitioning (Zhang et al. 2004; Montero-Pau and Serra 2011).

Differential adaptation to abiotic factors, such as water salinity and temperature, is known to affect the coexistence of the *B. plicatilis* species complex (Gómez et al. 1995; Gómez et al. 1997; Montero-Pau et al. 2011). For example, laboratory experiments show *B. plicatilis* grows better at lower salinities than *B. manjavacas,* although the salinity tolerance ranges of these two species largely overlap (Montero-Pau et al. 2011). Because of this evidence and the abundances recorded in the field, it has been proposed that their coexistence could be mediated by salinity fluctuations providing differential advantages to the two species in turn (Montero-Pau et al. 2011). However, mechanisms explaining stable coexistence that are not based on niche differentiation are also likely to be acting on the coexistence of *B. plicatilis* and *B. manjavacas*, as well as the other species belonging to the cryptic complex. As noted using modeling by Montero-Pau and Serra (2011), density-dependent investment in sex and diapause is able to mediate the stable coexistence of facultative sexuals with identical niches if the response to density is to some extent species-specific. In the genus *Brachionus*, sexual reproduction is density-dependent, and some level of differentiation in the chemical signal that induces sex seems to exist in sympatric populations of the *B. plicatilis* complex, including *B. plicatilis* and *B. manjavacas* (García-Roger et al. 2009). Different patterns of sexual investment have already been described in some populations of the *B. plicatilis* species complex (Carmona et al. 1995; Campillo et al. 2009). Sexual patterns of *B. plicatilis* and *B.*

manjavacas should be studied under different environmental conditions to explore whether a possible differential investment could act as an explanatory mechanism of their coexistence.

Despite only minor differences in morphology, closely related sympatric species can display different preferences for abiotic conditions, especially in relation to factors for which the adaptation to specific ranges is based on a physiological mechanism and only loosely related to morphology. If *B. plicatilis* and *B. manjavacas* differentially respond to abiotic factors so that those factors drive the outcome of competition, fluctuations in the abiotic environment could facilitate their coexistence. We propose as a hypothesis to be tested that the advantages that fluctuating salinity provides to each species are mediated not only through differential effects of salinity on population growth rate but also through differences in sex and diapause patterns.

Acknowledgments

We are grateful to L. Franch Gras for his help in the lab during the experiments. This work was founded by a grant from the Ministerio de Ciencia (CGL2009-07364) to MS. CG was supported by a fellowship from the Spanish Ministerio de Ciencia e Innovacio´n (BES2010-036384). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Barlow KE, Jones G, Barratt EM (1997) Can skull morphology be used to predict ecological relationships between bat species? A test using two cryptic species of pipistrelle. Proc Biol Sci 264:1695–700.

Bickford D, Lohman DJ, Sodhi NS (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22:148–155.

Bogdan KG, Gilbert JJ (1984) Body size and food size in freshwater zooplankton. Proc Natl Acad Sci U S A 81:6427–6431.

Bundy M, Vanderploeg H (2002) Detection and capture of inert particles by calanoid copepods: the role of the feeding current. J Plankton Res 24:215–223.

Campillo S, García-Roger EM, Carmona MJ, et al. (2009) Selection on life-history traits and genetic population divergence in rotifers. J Evol Biol 22:2542–2553.

Campillo S, García-Roger EM, Martínez-Torres D, Serra M (2005) Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. Hydrobiologia 546:181–187.

Carmona MJ, Gómez A, Serra M (1995) Mictic patterns of the rotifer *Brachionus plicatilis* Müller in small ponds. Hydrobiologia 313/314:365–371.

Caswell H (2006) Matrix Population Models. Encycl. Environmetrics. John Wiley & Sons, Ltd.

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Chotiyaputta C, Hirayama K (1978) Food selectivity of the rotifer *Brachionus plicatilis* feeding on phytoplankton. Mar Biol 45:105–111.

Ciros-Pérez J, Carmona MJ, Lapesa S, Serra M (2004) Predation as a factor mediating resource competition among rotifer sibling species. Limonology Oceanogr 49:40–50.

Ciros-Pérez J, Carmona MJ, Serra M (2001) Resource competition between sympatric sibling rotifer species. Limonology Oceanogr 46:1511–1523.

Conde-Porcuna JM, Declerck S (1998) Regulation of rotifer species by invertebrate predators in a hypertrophic lake: selective predation on egg-bearing females and induction of morphological defences. J Plankton Res 20:605–618.

Cox DR, Oakes D (1984) Analysis of survival data. Chapman & Hall, London.

DeMott WR (1989) The role of competition in zooplankton succession. In: Sommer U (ed) Plankt. Ecol. Springer-Verlag, Berlin.

Dumont HJ (1977) Biotic factors in the population dynamics of rotifers. Arch für Hydrobiol 8:98–122.

Efron B (1981) Nonparametric standard errors and confidence intervals. Can J Stat 9:139–158.

Efron B, Tibshirani R (1986) Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat Sci 1:54–75.

Folmer O, Black M, Hoeh W, et al. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299.

Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. Hydrobiologia 583:297–307.

Futuyma DJ, Mitter C (1996) Insect-plant interactions: the evolution of component communities. Philos Trans R Soc London Ser B Biol Sci 351:1361–1366.

Garcia-Roger EM (2005) Starvation tolerance of rotifers produced from parthenogenetic eggs and from diapausing eggs: a life table approach. J Plankton Res 28:257–265.

García-Roger EM, Dias N, Carmona MJ, Serra M (2009) Crossed induction of sex in sympatric congeneric rotifer populations. Limnol Oceanogr 54:1845–1854.

Geange SW, Pledger S, Burns KC, Shima JS (2011) A unified analysis of niche overlap incorporating data of different types. Methods Ecol Evol 2:175–184.

Gilbert JJ (2004) Females from resting eggs and parthenogenetic eggs in the rotifer *Brachionus calyciflorus*: lipid droplets, starvation resistance and reproduction. Freshw Biol 49:1505–1515.

Gilbert JJ, Bogdan KG (1984) Rotifer grazing: in situ studies on selectivity and rates. In: Meyer D.G, Strickler JR (ed) Trophic Interact. Within Aquat. Ecosyst. Westview, Boulder, CO, pp 97–133

Gilbert JJ, Williamson CE (1978) Predator-prey behavior and its effect on rotifer survival in associations of *Mesocyclops edax, Asplanchna girodi, Polyarthra vulgaris,* and *Keratella cochlearis*. Oecologia 37:13–22.

Gliwicz ZM (1980) Filtering rates, food size selection, and feeding rates in cladocerans-another aspect of interspecific competition in filter-feeding zooplankton. In: Kerfoot W (ed) Evol. Ecol. Zooplankt. communities. University Press of New England, Hanover London, pp 282–291

Gómez A (2005) Molecular ecology of rotifers: from population differentiation to speciation. Hydrobiologia 546:83–99.

Gómez A, Carmona MJ, Serra M (1997) Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). Oecologia 111:350–356.

Gómez A, Carvalho G (2000) Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. Mol Ecol 9:203–214.

Gómez A, Carvalho GR, Lunt DH (2000) Phylogeography and regional endemism of a passively dispersing zooplankter: mtDNA variation of rotifer resting egg banks. Prooceedings R Soc B 267:2189–2197.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution (N Y) 56:1431–1444.

Gómez A, Temprano M, Serra M (1995) Ecological genetics of a cyclical parthenogen in temporary habitats. J Evol Biol 8:601–622.

Gotelli N, Graves G (1996) Null models in ecology. Smithsonian Institute Press, Washington D.C.

Guillard RRL, Ryther JH (1962) Studies of marine diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Gran. Can J Microbiol 8:229–239.

Hagiwara A, Hino A (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Hydrobiologia 186-187:415–421.

Hansen B (1991) Feeding behaviour in larvae of the opisthobranch *Philine aperta* II. Food size spectra and particle selectivity in relation to larval behaviour and morphology of the velar structures. Mar Biol 111:263–270.

Hansen B, Wernberg-Møller T, Wittrup L (1997) Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J Exp Mar Bio Ecol 215:217–233.

Hino A, Hirano R (1980) Relationship between body size of the rotifer *Brachionus plicatilis* and the maximum size of particles ingested. Bull Japanese Soc Sci Fish 46:1217–1222.

Hino A, Hirano R (1984) Relationship between body size of the rotifer *Brachionus plicatilis* and the minimum size of particles ingested. Bull Japanese Soc Sci Fish 50:1139–1144.

Hubbell S (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ

Hutchinson G (1967) A treatise on limnology. Vol. II. Introduction to lake biology and the limnoplankton. John Wiley & Sons, New York.

Kerfoot WC (1977) Implications of copepod predation. Limnol Oceanogr 22:316–325.

Kirk KL (1997) Life-history responses to variable environments: starvation and reproduction in planktonic rotifers. Ecology 78:434–441.

Kong L, Li Q (2009) Genetic evidence for the existence of cryptic species in an endangered clam *Coelomactra antiquata.* Mar Biol 156:1507–1515.

Lapesa S (2004) Efecto de la depredación por invertebrados sobre poblaciones simpátricas de especies crípticas de rotíferos. Universidad de Valencia, Spain

Lapesa S, Snell TW, Fields DM, Serra M (2002) Predatory interactions between a cyclopoid copepod and three sibling rotifer species. Freshw Biol 47:1685–1695.

Lapesa S, Snell TW, Fields DM, Serra M (2004) Selective feeding of *Arctodiaptomus salinus* (Copepoda, Calanoida) on co-occurring sibling rotifer species. Freshw Biol 49:1053–1061.

Leibold M, McPeek M (2006) Coexistence of the niche and neutral perspectives in community ecology. Ecology 87:1399–1410.

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol Lett 11:995–1003.

Lund JWG, Kipling C, Cren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:143–170.

MacArthur R, Levins R (1967) The limiting similarity, convergence and divergence of coexisting species. Am Nat 101:377–385.

McGraw J, Caswell H (1996) Estimation of individual fitness from life-history data. Am Nat 147:47–64.

McPeek M, Gomulkiewicz R (2005) Assembling and depleting species richness in metacommunities: insights from ecology, population genetics and macroevolution. In: Holyoak M, Leibold MA, Holt RD (eds) Metacommunity Ecol. Emerg. views community Struct. Dyn. above local scale. University of Chicago Press, Chicago, Illinois, USA., pp 355– 373.

Montero-Pau J, Gómez A, Muñoz J (2008) Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. Limnol Oceanogr Methods 6:218–222.

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Moore PA, Fields DM, Yen J (1999) Physical constraints of chemoreception in foraging copepods. Limnol Oceanogr 44:166–177.

O'Brien WJ, Slade NA, Vinyard GL (1976) Apparent size as the determinant of prey selection by bluegill sunfish (*Lepomis macrochirus*). Ecology 57:1304–1310.

Pastorok RA (1981) Prey vulnerability and size selection by *Chaoborus* larvae. Ecology 62:1311–1324.

Peters RH (1984) Methods for the study of feeding, grazing and assimilation by zooplankton. In: Downing JA, Rigler FH (eds) A Man. methods Assess. Second. Product. fresh waters. Blackwell Scientific Publications, Oxford, pp 336–412

Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evol Biol 7:121.

Pitta P, Giannakourou A, Divanach P, Kentouri M (1997) Planktonic food web in marine mesocosms in the Eastern Mediterranean: bottom-up or top-down regulation? Hydrobiologia 363:97–105.

Pourriot R (1977) Food and feeding habits of Rotifera. Arch für Hydrobiol 8:243–260.

Quinn GP, Keough MJ (2005) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK

Ricci C, Perletti F (2006) Starve and survive: stress tolerance and life-history traits of a bdelloid rotifer. Funct Ecol 20:340–346.

Rigler FH (1961) The relation between concentration of food and feeding rate of *Daphnia magna* Straus. Can J Zool 39:857–868.

Roche K (1990) Some aspects of vulnerability to cyclopoid predation of zooplankton prey individuals. Hydrobiologia 198:153–162.

Roche KF (1987) Post-encounter vulnerability of some rotifer prey types to predation by the copepod *Acanthocyclops robustus*. Hydrobiologia 147:229–233.

Rodriguez-Puebla C, Encinas AH, Nieto S, Garmendia J (1998) Spatial and temporal patterns of annual precipitation variability over the Iberian Peninsula. Int J Climatol 18:299–316.

Rothhaupt KO (1990a) Changes of the functional responses of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. Limnol Oceanogr 35:24–32.

Rothhaupt KO (1990b) Resource competition of herbivorous zooplankton: a review of approaches and perspectives. Arch für Hydrobiol 118:1–29.

Rothhaupt KO (1990c) Differences in particle size-dependent feeding efficiencies of closely related rotifer species. Limnol Oceanogr 35:16–23.

Rothhaupt KO (1990d) Population growth rates of two closely related rotifer species: effects of food quantity, particle size, and nutritional quality. Freshw Biol 23:561–570.

Schoener TW (1974) Resource partitioning in ecological communities. Science 185:27–39.

Schulze PC (1995) Competition between crustacean zooplankton in continuous cultures. Limnol Oceanogr 40:33–45.

Serra M, Miracle MR (1987) Biometric variation in three strains of *Brachionus plicatilis* as a direct response to abiotic variables. Hydrobiologia 147:83–89.

Snell TW, Kubanek J, Carter W, et al. (2006) A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). Mar Biol 149:763–773.

Sommer U (1989) The role of competition for resources in phytoplankton succession. In: Sommer U (ed) Plankt. Ecol. succession Plankt. communities. Springer, Berlin, pp 57–106.

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford.

Stelzer CP (2001) Resource limitation and reproductive effort in a planktonic rotifer. Ecology 82:2521–2533.

Stelzer CP, Snell TW (2003) Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. Limnol Oceanogr 48:939–943.

Stelzer C-P, Snell TW (2006) Specificity of the crowding response in the *Brachionus plicatilis* species complex. Limnol Oceanogr 51:125–130.

Stemberger RS (1982) Mechanisms controlling selection and rates of predation on rotifers in *Cyclops bicuspidatus thomasi.* Ann Arbor, Michigan, EEUU, University of Michigan.

Stemberger RS, Gilbert JJ (1987) Rotifer threshold food concentrations and the size-efficiency hypothesis. Ecology 68:181–187.

Stemberger RS, Gilbert JJ (1985) Body size, food concentration, and population growth in planktonic rotifers. Ecology 66:1151–1159.

Stewart AJ, Wetzel RG (1986) Cryptophytes and other microflagellates as couplers in planktonic community dynamics. Arch fur Hydrobiol 106:1–19.

Threlkeld ST (1976) Starvation and the size structure of zooplankton communities. Freshw Biol 6:489–496.

Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton.

Tortajada AM, Carmona MJ, Serra M (2010) Effects of population outcrossing on rotifer fitness. BMC Evol Biol 10:312.

Turner JT, Borkman DG (2005) Impact of zooplankton grazing on *Alexandrium* blooms in the offshore Gulf of Maine. Deep Sea Res Part II Top Stud Oceanogr 52:2801–2816.

Vadstein O, Øie G, Olsen Y (1993) Particle size dependent feeding by the rotifer *Brachionus plicatilis*. Hydrobiologia 255-256:261–267.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Ware DM (1972) Predation by rainbow trout (*Salmo gairdneri*): the influence of hunger, prey density, and prey size. J Fish Res Board Canada 29:1193–1201.

Ware DM (1973) Risk of epibenthic prey to predation by rainbow trout (*Salmo gairdneri*). J Fish Res Board Canada 30:787–797.

Webb CO (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am Nat 156:145–155.

Wellborn GA, Cothran RD (2007) Niche diversity in crustacean cryptic species: complementarity in spatial distribution and predation risk. Oecologia 154:175–183.

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. Annu Rev Ecol Syst 15:393–425.

Werner EE, Hall DJ (1974) Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55:1042–1052.

Wiens JJ, Ackerly DD, Allen AP, et al. (2010) Niche conservatism as an emerging principle in ecology and conservation biology. Ecol Lett 13:1310–24.

Wiens JJ, Graham CH (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. Annu Rev Ecol Evol Syst 36:519–539.

Williamson CE (1987) Predator-prey interactions between omnivorous diaptomid copepods and rotifers: the role of prey morphology and behavior. Limnol Oceanogr 32:167–177.

Wilson D (1975) The adequacy of body size as a niche difference. Am Nat 109: 769–784.

Yoshinaga T, Hagiwara A, Tsukamoto K (2003) Life history response and age-specific tolerance to starvation in *Brachionus plicatilis* O.F. Müller (Rotifera). J Exp Mar Bio Ecol 287:261–271.

Zhang DY, Hanski I (1998) Sexual reproduction and stable coexistence of identical competitors. J Theor Biol 193:465–473.

Zhang DY, Lin K, Hanski I (2004) Coexistence of cryptic species. Ecol Lett 7:165–169.

Zhou W, Tang X, Qiao X, et al. (2009) Ingestion of *Brachionus plicatilis* under different microalgae conditions. Chinese J Oceanol Limnol 27:473–479.

Chapter 3

Life-history traits, abiotic environment and coexistence: the case of two cryptic rotifer species

Carmen Gabaldón, Manuel Serra, María José Carmona and Javier Montero-Pau

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A.O.22085, 46071, Valencia, Spain

Published in Journal of Experimental Marine Biology and Ecology (2015), 10.1016/j.jembe.2015.01.016

Abstract

Trade-offs are important in life-history evolution and coexistence of competitors. However, how alternative life-history optima might promote competitor coexistence has received little attention. The rotifers *B. plicatilis* and *B. manjavacas* are two cryptic species with a considerable ecological overlap in relation to biotic factors. These species often co-occur in temporal brackish ponds characterised by environmental fluctuations. In these rotifers, sexual reproduction results in diapausing stages, which are able to survive through adverse conditions but constrain current population growth. In the present work, the differential responses of both cryptic rotifer species to varying salinities and temperatures in terms of population growth, sexual reproduction and diapause patterns (i.e., investment, viability and hatchability of diapausing eggs) were investigated to assess their possible roles in species coexistence. *Brachionus plicatilis* and *B. manjavacas* showed differential responses to salinity and temperature and differed consistently in traits involved in diapause. The niche overlap between *B. plicatilis* and *B. manjavacas* decreased dramatically when the temperature and salinity response niche axes were added to a number of previously studied biotic niche axes. The results suggest that *B. plicatilis* exploits ephemeral and favourable conditions (low salinity) to invest in diapause, which would accelerate its exclusion from the water column but promote its long-term persistence. The findings show that cryptic species may evolve different compromises among life-history traits, allowing ecological divergence, and that ecological divergence may be uncoupled from morphological divergence for some factors in the abiotic environment.

Key-words: *Brachionus*; Diapause; Niche differentiation; Trade-off.

Introduction

How competing species co-occur is a fundamental question in ecology. Co-occurrence of competitors in the same habitat may be transient, i.e., some species are slowly being driven to exclusion by the interactions with other species (Leibold and McPeek 2006). However, species also may co-occur in the same habitat because each species is protected from exclusion, so that they show stable coexistence, even if their population densities fluctuate and are not at equilibrium (Chesson 2000; Adler et al. 2007). Trade-offs among competitor capabilities are a necessary, but nor sufficient condition for their stable coexistence (Chesson 2000; Siepielski and McPeek 2010; McPeek 2014). A trade-off occurs whenever a trait that confers an advantage when performing one function is linked to a disadvantage in performing another function (Stearns 1992). Trade-offs can result in niche differentiation if neither species presents higher values of all relevant fitness components; the advantages that one species may present over others are offset by compensating disadvantages; for instance, being an efficient consumer of a kind of resources compromises being an efficient consumer of other resources. In absence of trade-offs, one of the species could achieve higher performance than their competitor in all features, and then becoming the superior competitor. Additionally, stable coexistence is favoured by fitness equivalence among competitors (Chesson 2000). If competitors have similar fitness, a small stabilizing niche difference allows stable coexistence (Adler et al. 2007). Therefore, the analysis of trade-offs and niche differentiation of competitors are needed steps in the study of stable coexistence, and can provide cues on what other conditions need to be investigated in order to demonstrate that a stable coexistence mechanism is acting. There are several potential trade-offs that can promote coexistence between species, such as trade-offs between growth capacity and low-resource tolerance (Angert et al. 2009), between competitive ability and predator susceptibility (Wellborn 2002) or dispersal ability (Lei and Hanski 1998), or between the efficiencies of using different resources (Tilman 1982).

Relevance of trade-off analysis in ecology and evolution is not limited to the study of competition, as this analysis is crucial in the evolutionary theory of life history. Trade-offs involving reproduction shape life histories, and are expected to evolve in response to a suit of environmental features. Traits such as age at maturity, clutch size and the period of parental care are thought to evolve as a result of trade-offs between fecundity and survival or somatic growth (Stearns 1992). These adaptive changes in life history traits can have implications on niche differentiation. However, with the exception of classical conceptual framework of r- and K-selection, the connection between interspecific competition and those trades-offs on which life history theory focuses on is relatively poorly understood.

The effects of life-history trade-offs on competition are particularly interesting in the case of cryptic species (i.e., closely related species with high morphological similarity), as life-history traits may evolve uncoupled from morphology, they may promote niche differentiation and even they open the possibility of coexistence mechanisms not based on niche differentiation (Zhang et al. 2004; Montero-Pau and Serra 2011). Additionally, life-history analysis is useful to understand why a competitor becomes dominant and how the species respond to the competitive dynamics. Coexistence of cryptic species is a challenging and puzzling phenomenon (Leibold and McPeek 2006) as they are expected to present high ecological overlap (i.e., niche conservatism) (Violle et al. 2011) given their lack of morphological differences and phylogenetic relatedness.

In the present work, the response of two cyclically parthenogenetic cryptic species to their physical environment was investigated. The model organisms are two rotifer species –*B. plicatilis* and *B. manjavacas*– belonging to the cryptic *Brachionus plicatilis* complex. This study differs from prior studies performed with other species of this complex in one important respect: *B. manjavacas* and *B. plicatilis* cannot be identified on a morphologically basis (Campillo et al. 2005; Fontaneto et al. 2007), whereas other species of the complex show differences in shape and size (Ciros-Pérez et al. 2001b). Interestingly, in this last case niche differentiation has been demonstrated in both biotic (Ciros-Pérez et al. 2001a; Ciros-Pérez et al. 2004) and abiotic factors (Gómez et al. 1997).

Rotifers are one of the major zooplanktonic groups found in continental waters and represent a key component of the trophic webs of these habitats. *B. plicatilis* and *B. manjavacas* show high ecological overlap for biotic niche axes (Gabaldón et al. 2013). The two species co-occur in brackish ponds across the Iberian Peninsula (Gómez et al. 2002; Gómez et al. 2007), likely showing stable dynamics (Montero-Pau et al. 2011). In temperate latitudes, the ponds where they inhabit are unsuitable for periods of varying predictability (Ortells et al. 2000), either because the pond dries or because seasonal conditions results in their exclusion from the water column. In these rotifers, re-colonisation of the water column takes place via the hatching of diapausing eggs stored in the sediment (Gilbert 1974). *Brachionus* species, like cladocerans, aphids and others rotifers, are cyclical parthenogens (De Meester et al. 2004). Asexual diploid females hatch from diapausing eggs, and after an initial phase of clonal propagation, sexual reproduction is induced by a density-dependent infochemical produced by the rotifers themselves (Carmona et al. 1993; Stelzer and Snell 2003; Kubanek and Snell 2008), although asexual reproduction does not completely cease. In the sexual phase, sexual females produce haploid eggs that develop into males if unfertilised or into diploid diapausing female embryos, referred to as diapausing or resting eggs, if fertilised. Diapausing eggs are embryos in an arrested stage of development that are able to survive through adverse environmental conditions, such as drought and high or low temperature for several years or decades.

Thus, diapause is a key feature for the survival of these organisms, and encompasses different life history trade-offs. First, diapausing egg production involves diversion of resources from current, asexual proliferation to resting stages and future growth (e.g., Serra and King 1999). Diapause is costly in these animals because it involves the two-fold cost of sex (Maynard Smith 1971), diapausing eggs require more resources than subitaneous, asexual eggs (Gilbert and Schröder 2004; Alekseev et al. 2007; Clark et al. 2012), and because diapausing eggs imply an obvious generation time increase. Thus, if an organism starts producing diapausing stages too early, before the end of the suitable period for growing, it will miss opportunities for its descendants to proliferate, while if too late, it will incur in the risk of dying before producing any diapausing stages. Second, diapausing egg survival, diapause duration, and hatching success are dependent on the allocation of resources into the diapausing egg, hence compromising the number of diapausing eggs produced or the timing –with more or less resources in the environment– when these eggs are produced. Third, diapause cessation implies risks for the hatchlings due to unpredictable suitability of the water column, but opens opportunities for fast proliferation. Thus, how to pattern the diapausing egg hatching implies a trade-off. Interestingly, both diapausing egg production (e.g., sex initiation without cessation of asexual proliferation in rotifers (Fussmann et al. 2007) and diapause termination are expected to be spread over time as bet-hedging strategy in unpredictably fluctuating environments (Cohen and Levin 1987; Ellner 1997).

Diapausing eggs and processes involved in diapause are relevant to intraspecific competition in different ways. On one hand, competition dynamics in temporarily active populations could be better assessed focusing on diapausing egg production, since exclusion during the period of activity does not necessarily mean long-term exclusion. On the other hand, diapausing eggs are protected against competition, and such as stage is needed if competing species are differentially favoured during different periods in a fluctuating environment (Chesson 2000). Finally, investment in diapause decreases the current rate of population proliferation and may affect the population dynamics of competing species during their growing season (Montero-Pau and Serra 2011). Thus, a complex interplay occurs because diapause, being a response to abiotic or biotic, predictable or unpredictable environmental fluctuations, has consequences for the competitive dynamics and long-term competitor coexistence.

It has been suggested that coexistence of *B. plicatilis* and *B. manjavacas* is possible partly due to salinity fluctuations (Montero-Pau et al. 2011). The brackish ponds on the Iberian Peninsula where these cryptic species co-occur are characterised by environmental fluctuations (Comín et al. 1992). In the case of *B. plicatilis* and *B. manjavacas,* salinity is the only ecological factor known to differentially affect these species: *B. manjavacas* grows better at higher salinities (Montero-Pau et al. 2011). However, previous work has not thoroughly investigated how cryptic species with virtually identical biotic niches respond to major abiotic factors. Specifically, it is unknown, whether differential response to salinity and trade-offs resulting from a key life-cycle stage –namely, diapause– occurs, which is most relevant to understand how the long-term co-occurrence of these species is maintained.

The objective in the present work was to test if *B. plicatilis* and *B. manjavacas* differentiate their niches in relation to two major abiotic factors: salinity and temperature. The focus was on their effect on current growth rates, sexual reproduction and diapause patterns. Three experiments were performed to explore differences in (1) population growth rates and sexual reproduction ratio under different salinities and temperatures, (2) hatching proportion and hatching pattern of diapausing eggs produced and hatched under three salinities, and (3) degradation pattern of diapausing eggs produced under the same three salinities. The aim was to assess how these two important abiotic variables affect niche differentiation of *B. plicatilis* and *B. manjavacas* in ways that may allow for the rotifer species to co-occurrence.

Materials and Methods

Rotifer isolation, species identification and culture conditions

The experiments were carried out with *B. plicatilis* and *B. manjavacas* clones founded from hatchlings from diapausing eggs. Because rotifers can grow asexually, clonal lines can be established starting from a single female. Diapausing eggs were isolated from sediment samples collected in June 2010 with a Van Veen grab (Eijelkamp Agrisearch Equipment) from the upper sediment layer of Salobrejo Lake (Eastern Spain: 38° 54.765' N, 1° 28.275' W). Samples were taken in different points of the lake and thoroughly mixed. Sediment was stored in darkness at 4°C during three months before diapausing egg isolation and hatching induction. Hatchlings were identified at species level by PCR-RFLP (for isolation and identification details, see Gabaldón et al. 2013). For each rotifer species, 25 clonal lines were established. The clones were maintained in stock cultures at 10 g

L^{-1} salinity, at 20ºC and weekly fed the unicellular microalga *Tetraselmis suecica* (see Gabaldón et al. 2013 for details).

The pre-experimental and experimental rotifer culture media were the same as described for the stock cultures, but salinity was fixed at the indicated experimental values (see below). In the third experiment, in which rotifers were grown under several different salinity conditions, inert *T. suecica* was used as food. Using inert algae avoids the problem of growing *T. suecica* at salinities outside of its optimum and ensures a constant quality of the food provided in the different salinity treatments. Inert algae have been used to grow efficiently *Brachionus* (Yamasaki et al. 1989; Lubzens et al. 1995). To obtain inert algae, cultures of *T. suecica* were grown at 27.5 g L^{-1}salinity and 20° C, which are intermediate conditions within the range of experimental conditions; and then were concentrated by centrifugation for 11 min at 1,500 r.c.f. (relative centrifugal force) and maintained at -80° C until use.

Effect of salinity on the hatching patterns of diapausing eggs

Differences between *B. plicatilis* and *B. manjavacas* in the hatching success of diapausing egg were tested in relation to salinity. The hatching of diapausing eggs produced at three different salinities (salinity for diapausing egg formation; 8, 16 and 24 g L^{-1}) and induced to hatch at the same three salinities (salinity for diapausing egg hatching) was recorded for the two species (18 experimental combinations: 3 egg-producing salinities × 3 egg-hatching salinities × 2 species).

Obtaining sufficient diapausing eggs for the experiments required the culture of rotifers at a high density in larger volumes than used for the stock cultures. For this purpose, the clonal rotifer stock cultures were gradually increased in volume. First, each stock culture was transferred to 250 mL of culture medium with 12 g L^{-1} salinity and grown at 20° C, under constant illumination and agitation in an orbital incubator shaker at low speed (50 rpm). A salinity of 12 g L^{-1} was selected because this was an intermediate value among the three experimental salinities. After 7 days, six pre-experimental multiclonal populations (2 species × 3 salinities for egg formation) were established by combining females from the 25 stock clones. About 10,000-15,000 individuals from each clone were transferred to 2 L of fresh culture medium with salinities of 8, 16 and 24 g L^{-1}. After three days of acclimation, the pre-experimental populations were transferred to 5 L of culture medium to establish experimental populations under the corresponding conditions. As diapausing eggs tend to sink, to avoid accidental transfer of diapausing eggs, only the upper part of the culture was transferred. The experimental cultures were allowed to grow and produce diapausing eggs for 10 days. Then, the diapausing eggs accumulated at the bottom of the cultures were isolated. This collection of eggs was split to perform two experiments: the experiment described in this subsection and the experiment described in the next subsection.

In the first experiment, the eggs were kept at the same salinity as during egg formation, in the dark and at 4°C for one month to ensure the completion of the required period of egg dormancy

(Hagiwara and Hino 1989). After this period, diapausing eggs were individually isolated in 96-multiwell dishes (Nunc^{TM}) and induced to hatch in water with salinities of 8, 16 or 24 g L^{-1} at 25° C under constant illumination. For each of the 18 experimental combinations (2 species × 3 salinities for egg formation × 3 salinities for egg hatching), 40-60 eggs were assayed (total: 960 eggs). Diapausing eggs were checked every 24 hours, and hatchings were recorded for 17 days.

The effects of the species, salinity for diapausing egg formation and salinity for diapausing egg hatching on the proportion of diapausing eggs that hatched were analysed using a generalised linear model (GLM) with binomial errors and a logit link function. The goodness of fit of the model was assessed with a Chi-square test. These analyses were carried out using SPSS statistical software (version 19.0, IBM Corp R 2010). The effects of the species, salinity for egg formation and salinity for egg hatching on the timing of diapausing egg hatching were tested using a robust three-way ANOVA, performed with R statistical software, v. 2.12.1 (R Development Core Team, 2010), using the functions of the WRS package (Wilcox RR 2010). Robust ANOVA was used due to the lack of homogeneity of variances.

Effect of salinity on diapausing egg deterioration

The deterioration of the diapausing *B. plicatilis* and *B. manjavacas* eggs produced at three different salinities (8, 16 and 24 g L^{-1}) was monitored for one year. Diapausing eggs were exposed to outdoor temperature conditions to mimic the conditions that the eggs experience in lake sediments.

Diapausing eggs from the two species produced at three salinities and collected as described in the previous subsection were used. For each experimental combination (2 species × 3 salinities for egg formation), 384 eggs were individually transferred to 96-multiwell dishes (NuncTM) containing 200 μL of 30 g L^{-1} saline water and maintained in wet chambers outdoors in the dark. High salinity and darkness were used to prevent egg hatching (Pourriot and Snell, 1983). The diapausing eggs were checked weekly for the first month, then monthly during the next 5 months and, finally, in the 9th and 12th months (11 observations in total). As light is a cue that induces hatching (Hagiwara et al. 1985) special care was taken during monitoring to minimise the exposure of the eggs to light. During each assessment, the diapausing eggs were classified according to their deterioration status, based on the proportion of the egg occupied by the embryo: Type I if the proportion occupied by the embryo is more than 100%; Type II if the proportion is more than 75% but less than 100%; Type III if the proportion is between 50 and 75%; Type IV if the proportion is between 25 and 50%; and Type V if the proportion is less than 25% (García-Roger et al., 2005). The relative contribution of each egg type to hatchlings found by García-Roger et al. (2005) was: Type I= 78.5 %, Type II= 20.4 %, Type III= 1.1%, Type IV and V= 0.0 %. In order to have a reasonable number of observations per category, in the subsequent statistical analyses these five egg types were grouped into viable (Type I and II) and degraded (Type III, IV and V).

The effects of the species and the salinities on the degradation status of the diapausing eggs were tested using a generalised linear model (GLM) with a multinomial distribution and logit link function. The goodness of fit of the model was assessed with a Chi-square test. The survival functions (l_x) for diapausing eggs were estimated for each rotifer species and salinity for egg formation in order to analyse the time to diapausing egg degradation. Here, survival means that the egg conserved the Type I or II status (i.e., viable eggs). Diapausing eggs that remain without degrading at the end of the experiment were treated as censored data. Despite the high salinity and dark conditions imposed on eggs, it was observed that some diapausing eggs hatched during the experiment (see the Results section), so these data were also considered censored. To compare survival curves between the (1) species and (2) the salinity treatments within each species, two non-parametric tests (log-rank and Breslow tests) were performed. The log-rank test assigns equal importance to all observations, while the Breslow test gives more weight to the initial part of the survival curve (Cox and Oakes, 1984). Statistical analyses were carried out using SPSS statistical software (version 19.0, IBM Corp R 2010).

To test whether the hatching and degradation rates were related, Pearson's correlation coefficients between the two variables were calculated for each species. Additionally, the effects of the rotifer species and hatching rate on the degradation rate of the diapausing eggs were tested via ANCOVA. These statistical analyses were carried out using R statistical software, v. 2.12.1 (R Development Core Team 2010).

Effects of salinity and temperature on growth rates

The combined effects of seven different salinities (5, 10, 20, 30, 40, 50 and 60 g L^{-1}) and three temperatures (15, 20 and 25° C) on both the intrinsic population growth rate and sexual investment (i.e., the proportion of females reproducing sexually) in the *B. plicatilis* and *B. manjavacas* populations were studied. Pre-experimental multiclonal populations were established by combining females from the 25 stock clones in 250 mL flasks (approx. 1 female mL^{-1} per clone). In the first step, these populations were grown for one week under intermediate conditions of salinity (27.5 g L^{-1}) and temperature (20° C), with constant illumination to obtain a high rotifer density. In the second step, these multiclonal populations were used to initiate pre-experimental cultures at the experimental salinity and temperature conditions by transferring 200 rotifers that were randomly selected from the multiclonal populations to 100 mL flasks (42 cultures: 2 species × 3 temperatures × 7 salinities). These pre-experimental cultures were fed inert algae *T. suecica* to a density of 250,000 cells mL^{-1} every three days. The rotifers were allowed to grow for 10 days (approx. 3 generations) for acclimatisation. Then, experimental cultures were initiated by randomly transferring 20 females from each pre-experimental culture to Petri dishes with 50 mL of medium containing 100,000 cells mL^{-1} of inert algae (same salinity and temperature as in the pre-experimental conditions). Three replicate

Petri dishes were included in the experiment (126 cultures: 2 rotifer species × 3 temperatures × 7 salinities × 3 replicates). Pre-experimental and experimental multiclonal cultures fed with inert microalgae were maintained under constant agitation in an orbital shaker (50 rpm) in the dark to avoid algal degradation. Both pre-experimental and experimental food concentrations were chosen to be above the daily clearance rate of rotifers during the period of culture, so food was not a limiting factor.

After 4 days of growth, the cultures were fixed with 0.3 % Lugol solution, and rotifers were counted. The following data were recorded: (1) the number of non-ovigerous females; (2) the number of ovigerous females, classified based on the types of eggs they were carrying (Carmona et al., 1995) as asexual, unfertilised sexual or fertilised sexual; (3) the number of eggs (asexual, unfertilised sexual and fertilised sexual) both carried and detached; and (4) the number of males. Using these counts, the observed growth rate (r_{obs}) and sexual reproduction ratio (i.e., the fraction of sexual females to total females produced) at different salinities and temperatures was calculated. Population growth rate was calculated as $r_{obs} = \ln (N_t / N_0) / t$, where N_t and N_0 are the number of females at the beginning and after 4 days of incubation, respectively, and t is the time in days. The sexual ratio was calculated as the proportion of ovigerous females that were sexual.

Additionally, the potential growth rate (r_{pot}) was calculated (Montero-Pau et al. 2014). The r_{pot} is defined as the growth rate that a population would have if all of its females were reproducing asexually.

r_{pot} is a performance measure that brings to a single metric the investment in current population growth and in sex and diapause. This makes possible to compare the efficiency of converting resources into offspring and survival (Montero-Pau et al. 2014), instead the current proliferation rate. This relevance is that it neutralizes the effect of the diapausing investment strategy, which might have evolved by a suit of factors not related to the physiological efficiency of a genotype in the abiotic conditions of interest.

A robust three-way ANOVA was carried out to analyse the effects of the temperatures, salinities and rotifer species on r_{pot}. To test whether the investment in sex, i.e., the sexual reproduction ratio, responded to population density differently depending on species, an ANCOVA was performed independently for each experimental temperature. Both analyses were performed with R statistical software, v. 2.12.1 (R Development Core Team 2010), using the functions of the WRS package (Wilcox RR 2010) for the robust three-way ANOVA.

Niche overlap

Interspecific abiotic niche overlap was estimated based on the studied life-history traits using the analytical approach of Geange et al. (2011). This method allows the consideration of multiple niche axes, each of which is characterised by a different type of data, and computes a unified niche overlap analysis. The hatching success, degradation ratio and r_{pot} datasets were used to calculate the abiotic niche overlap between *B. plicatilis* and *B. manjavacas* along the 42 axes detailed in Fig. 3.5. Following Geange et al. (2011), niche overlap indexes (*NO*) were calculated for each dimension, and a single unified niche overlap index was then obtained by averaging the niche overlap along each axis. *NO* ranges from 0 (disjoint niches) to 1 (complete niche overlap). For the unified niche overlap index, data on eight biotic axes from a previous study by Gabaldón et al. (2013) related to clearance rates, susceptibility to predation and ability to withstand starvation were included.

To assess the statistical niche differences between species, null model permutation tests were performed to test whether the niche overlap along each axis and the mean niche overlap were significantly lower than expected by chance (Gotelli and Graves 1996; Geange et al. 2011). To correct for multiple comparisons, a sequential Bonferroni adjustment (Quinn and Keough 2005) was performed. The niche overlap calculations and associated null model tests were performed using R version 2.12.1 (R Development Core Team 2010) with the source code provided as supporting information in Geange et al. (2011).

Results

Effect of salinity on the hatching patterns of diapausing eggs

Diapausing *B. manjavacas* eggs showed a relatively synchronous pattern of hatching, with most of the hatchings occurring in the first 6 days of the experiment (Fig. 3.1). In contrast, the diapausing eggs of *B.*

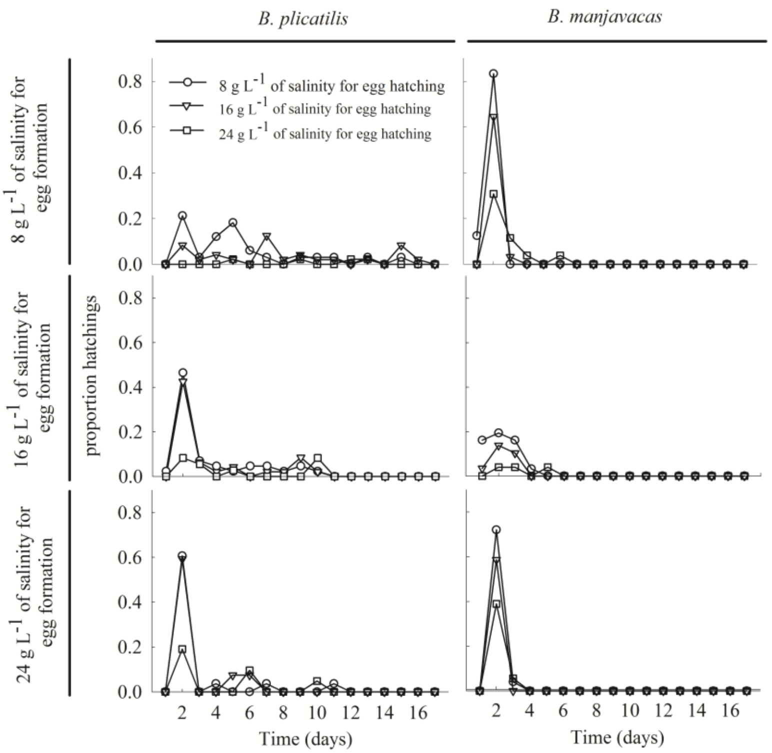


Figure 3.1. The hatching dynamics of the diapausing eggs of B. *plicatilis* and B. *manjavacas* formed and hatched at 8, 16 and 24 g L^{-1} of salinity.

plicatilis continued hatching for up to 12-17 days of incubation. For both rotifer species, the synchrony of the hatching pattern increased with an increase in the salinity for egg formation. Mean time to hatching for diapausing eggs of both species (Table 3.1) showed statistically significant differences (Table 3.2), the values being 4.08 days for B. *plicatilis* and 1.6 days for B. *manjavacas*. Both salinity for egg formation and salinity for egg hatching were statistically significant as

Table 3.1. The mean time to hatching (days) and proportion of hatching (%) of diapausing eggs of *B. plicatilis* and *B. manjavacas* formed and hatched under 8, 16 and 24 g L^{-1} of salinity.

		Salinity for diapausing egg hatching (g L^{-1})					
		8		16		24	
Salinity for diapausing egg formation (g L^{-1})	**Species**	Mean time to hatching (days)	Hatching proportion (%)	Mean time to hatching (days)	Hatching proportion (%)	Mean time to hatching (days)	Hatching proportion (%)
8	*B. plicatilis*	4.85	78.79	7.38	52.08	9.25	8.89
	B. manjavacas	1.37	95.83	1.55	67.74	2.19	50.00
16	*B. plicatilis*	3.07	81.82	3.26	70.21	4.72	27.03
	B. manjavacas	1.62	54.84	1.75	30.00	2.83	12.00
24	*B. plicatilis*	2.30	71.43	2.20	74.07	3.79	33.33
	B. manjavacas	1.55	76.00	1.50	58.62	1.63	44.44

well as the interaction of species with both salinity for egg formation and salinity for egg hatching. A consistent trend was that hatching was delayed with an increased salinity for diapausing egg hatching, with the exception of eggs formed at the highest salinity, which showed a rather flat response (Table 3.1). High salinities during egg formation decreased significantly the time to hatching in *B. plicatilis*. Interactions between salinity for egg formation and salinity for egg hatching and the triple interaction were not statistically significant.

GLM analysis showed that the hatching proportion (Table 3.1) was significantly affected by the salinity for diapausing egg formation and the salinity for hatching, as well as by species × salinity for egg formation and species × salinity for egg hatching interactions (Table 3.2). However, statistical differences were not found between species, and both the interaction of salinity for egg formation × salinity for egg hatching and the triple interaction were not significant. Globally, the proportion of hatching of both species was higher at lower salinities for hatching. However, the salinity for diapausing egg formation had a species-specific effect. *B. plicatilis* presented a higher hatching proportion at higher salinity for egg formation, except when salinity for egg hatching was 8 g L^{-1}, in which case the hatching proportion at 24 g L^{-1} for egg formation was lower than at 16 g L^{-1}. Instead, *B. manjavacas* presented its highest hatching proportion at lowest tested salinity for egg formation.

Table 3.2. *P*-values after three-way robust ANOVA on the mean time to hatching (days) and after GLM analysis on the proportion of hatching (%) of diapausing eggs of *B. plicatilis* and *B. manjavacas* formed and hatched under 8, 16 and 24 g L^{-1} of salinity.

	Mean time to hatching	Hatching proportion
Species	<0.001	0.794
Salinity for diapausing egg formation	<0.001	0.004
Salinity for diapausing egg hatching	0.002	<0.001
Species × Salinity for diapausing egg formation	0.001	<0.001
Species × Salinity for diapausing egg hatching	0.022	0.027
Salinity for diapausing egg formation × Salinity for diapausing egg hatching	0.14	0.169
Species × Salinity for diapausing egg formation × Salinity for diapausing egg hatching	0.1	0.989

Effect of salinity on diapausing egg deterioration

Diapausing *B. manjavacas* eggs began to degrade during the first week of incubation, earlier than those of *B. plicatilis*, which did not show a notable deterioration until the 180 days of incubation (Fig. 3.2). Once deterioration began, the diapausing eggs of both species began to

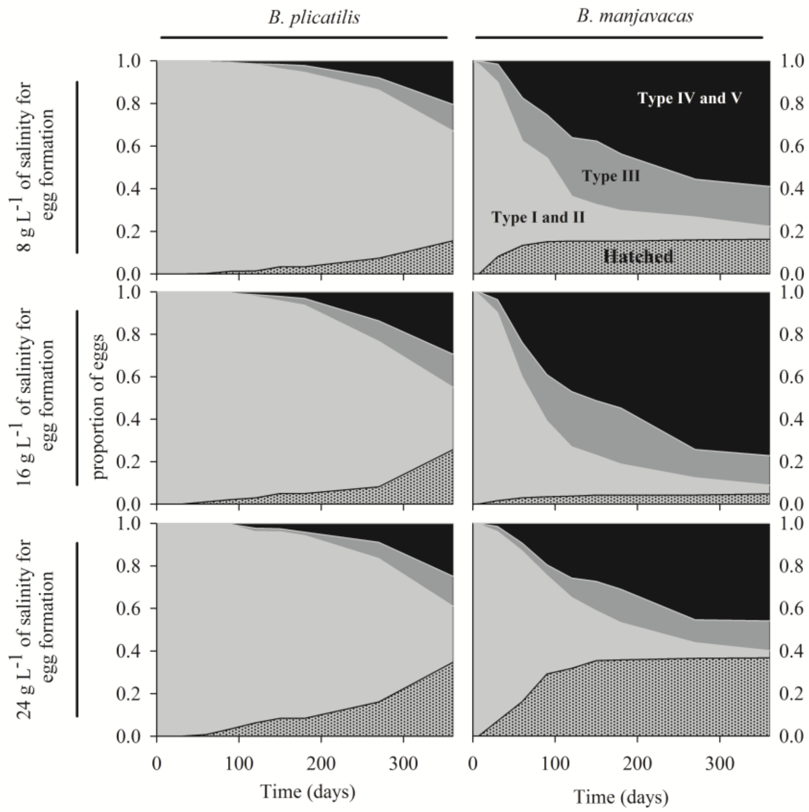


Figure 3.2. Cumulative proportions of the different diapausing egg status of *B. plicatilis* and *B. manjavacas* along one year of exposure to natural conditions. Unhatched eggs were classified into five types (I-V) according to their degradation following the embryo-size criterion (García-Roger et al., 2005). The relative contribution of each egg type to hatchlings found by García-Roger et al. (2005) was: Type I= 78.5 %, Type II= 20.4 %, Type III= 1.1%, Type IV and V= 0.0 %.

degrade at a high rate. According to the GLM analysis, the deterioration response is dependent upon the interaction between the species and salinity for diapausing egg formation ($P < 0.001$). The survival curves of diapausing *B. plicatilis* and *B. manjavacas* eggs formed under different salinity conditions differed remarkably between the species, and the log-rank and Breslow tests showed this difference to be statistically significant ($P < 0.001$). Both non-parametric tests performed independently for each species also revealed that salinityfor egg formation affected the survival schedule of diapausing eggs ($P = 0.011$ and 0.010 for *B. plicatilis* and $P = 0.001$ and 0.007 for *B. manjavacas*; log-rank and Breslow test, respectively).

Although diapausing eggs were incubated in the dark to prevent the induction of hatching, 520 of the 2295 eggs included in these experiments hatched during the time course of the experiment (one year). No hatching of *B. plicatilis* eggs occurred within the first two months of incubation, with most of the hatchings for this species occurring after 180 days. In contrast, diapausing *B. manjavacas* eggs only hatched in the first 90 days of the experiment. For *B. plicatilis* the mean time to hatching (245, 261 and 241days for 8, 16 and 24 g L^{-1}, respectively) was nearly coincident with the mean deterioration time (260, 257 and 259 days for 8, 16 and 24 g L^{-1}, respectively). This coincidence was not as clear for *B. manjavacas* (mean times to hatching were 146, 82 and 69 days and the mean deterioration times were 89, 89 and 124 days for 8, 16 and 24 g L^{-1}, respectively). For both rotifer species, there was a correlation between the daily hatching rate (hatchings per egg per day) and deterioration rate (eggs deteriorated

Table 3.3. *P*-values after ANCOVA on degradation rate of diapausing egg of *B. plicatilis* and *B. manjavacas* produced under 8, 16 and 24 g L^{-1} of salinity and using egg hatching rate as a covariate. Data were collected during one year of monitoring.

	P
Species	0.001
Hatching rate	<0.001
Species × Hatching rate	0.49

per egg per day). The Pearson's correlation coefficients were 0.89 and 0.64 for *B. plicatilis* and *B. manjavacas*, respectively ($df = 28$, $P < 0.0001$ for both species). According to the ANCOVA with the hatching rate as a covariate, the deterioration rate was dependent on the species, and the correlation between the hatching and deterioration rates was confirmed, although this analysis did not detect a significant effect of interaction with the species factor on this correlation (Table 3.3).

Effect of salinity and temperature on growth rates

The observed growth rates (r_{obs}) and sexual reproduction ratios of the *B. plicatilis* and *B. manjavacas* differed when populations grown under the different experimental salinity and temperature combinations (Fig. 3.3). Within the tested ranges, temperature had a positive effect, and salinity tended to have a negative effect on r_{obs}. At 15° C, the r_{obs} values for both species decreased approximately linearly with salinity, while at the other tested temperatures, r_{obs} tended to peak at an intermediate salinity, but in the low part of the tested range. Regardless of the

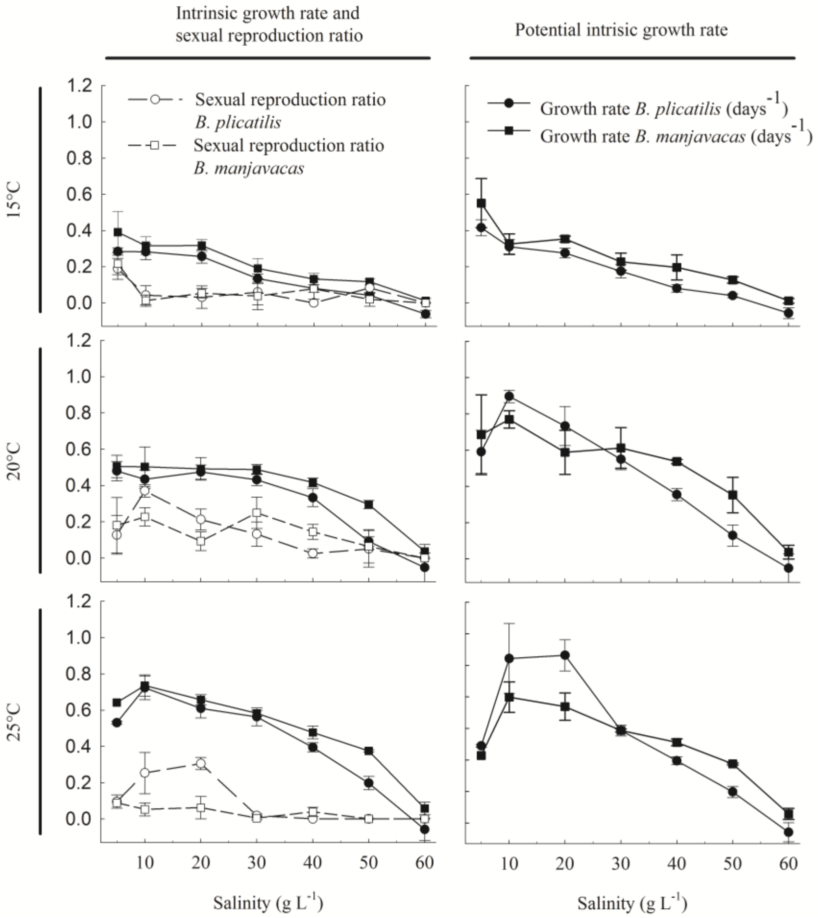


Figure 3.3. Growth rates and sexual reproduction ratios of the *B. plicatilis* and *B. manjavacas* populations grown under seven salinities (5, 10, 15, 20, 30, 40, 50 and 60 g L^{-1}) and three temperatures (15, 20 and 25º C). Left panels: observed intrinsic growth rate and sexual reproduction ratio. Right panels: potential intrinsic growth rates. Vertical bars are the lower and upper 95% confidence intervals.

global effects of these abiotic conditions, *B. manjavacas* always exhibited slightly higher growth rates than *B. plicatilis*. The mean sexual reproduction ratios were between 0 and 0.13 for both rotifers. In the range of 10-20 g L^{-1}, where the two species showed very close r_{obs} values, *B. plicatilis* exhibited higher sexual reproduction ratios than *B. manjavacas* at 20 and 25°C. *B. plicatilis* consistently exhibited a higher r_{pot} than *B. manjavacas* within this salinity and temperature intervals (Fig. 3.3), and this difference became greater as the temperature increased. Robust ANOVA showed that the species, temperature and salinity as well as their double and triple interactions had an effect on r_{pot} (all $P < 0.02$).

A positive relationship between sexual reproduction ratio and population densities of both rotifer species was observed, with *B. plicatilis* tending to present higher sexual reproduction ratios than *B.manjavacas* (Fig. 3.4). According to the ANCOVAs for the sexual reproduction ratios applied separately to each temperature (covariate: population density), the sexual reproduction ratio exhibited the

Table 3.4. *P*-values of ANCOVAs on sexual reproduction ratio of *B. plicatilis* and *B. manjavacas* growing under 15, 20 and 25ºC using population density as a covariate.

	Temperature (ºC)		
	15	20	25
Species	0.913	0.967	0.002
Population density	0.003	<0.001	<0.001
Species × Population density	0.912	0.598	<0.001

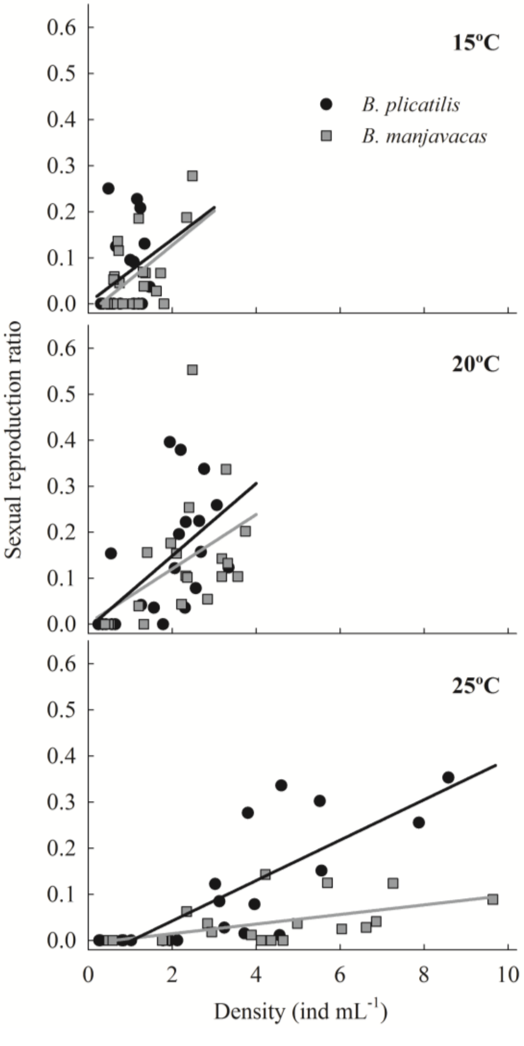


Figure 3.4. The relationship between the sexual reproduction ratio and the population densities of *B. plicatilis* and *B. manjavacas* rotifer species, separately for the three experimental temperatures (data for the different salinities are not distinguished). Lines are linear regressions relating sexual reproduction ratios to population densities for each species and temperature.

expected significant relationship with population density (Table 3.4). At 15 and 20º C, the species had no significant effect on the sexual reproduction ratio or the relationship between sexual reproduction and population density. In contrast, at 25º C, *B. plicatilis* exhibited a significantly higher sexual reproduction ratio than *B. manjavacas,* with the former species showing a significantly stronger relationship between the sexual reproduction ratio and population density.

Niche overlap

The niches of *B. plicatilis* and *B. manjavacas* were found to be different (mean niche overlap= 0.51, $P < 0.001$) according to the niche overlap indexes (*NO*) between both species for abiotic axes studied in the present work and the biotic axes studied in Gabaldón et al. (2013) (Fig. 3.5). After sequential Bonferroni correction, the highest *NO* values were those associated with the hatching proportion axes, except for the hatching proportion of diapausing eggs formed at 8 g L^{-1} and hatched at 24 g L^{-1} and those both formed and hatched at a concentration of 16 g L^{-1}. Additionally, the *NO* values showed that *B. plicatilis* and *B. manjavacas* had similar niches regarding the axes for diapausing egg hatching timing, except for the three axes corresponding to the hatching timing of eggs formed at 8 g L^{-1}. The *NO* values from the diapausing egg degradation axes showed niche differentiation between the two species. For axes associated with r_{pot}, the *NO* values varied and were dependent on temperature: at higher temperatures, more of the niche axes showed low overlap between species.

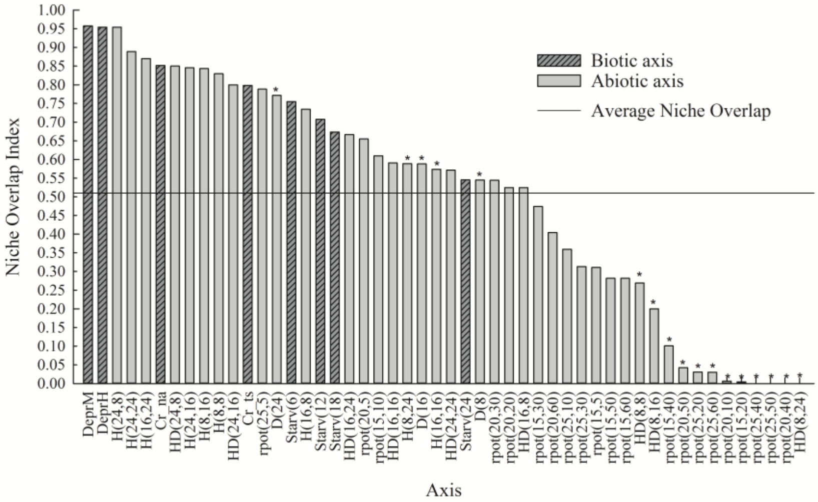


Figure 3.5. Niche overlap indexes (*NO*) between *B. plicatilis* and *B. manjavacas*. From this study: *H(I,J)* is based on the hatching fate of each single diapausing egg (binary data), and *I* the salinity for egg formation and *J* the salinity for egg hatching; *HD(I,J)* is based on the hatching day of each single diapausing egg (categorical data; *I* and *J* as above); *D(I)* is based on the degradation status of each diapausing egg after one year of maintenance (categorical data; *I* as above); *rpot(I,J)* is based on the potential intrinsic growth rate at temperature *I* and salinity *J* (continuous data). From (Gabaldón et al., 2013): Cr_ts and Cr_na are based on the clearance rate for *T. suecica* and *Nannochloris atomus*, respectively (continuous data); DeprF are DeprM are based on the susceptibility to predation by copepod females and by copepod males, respectively (binary data); Starv(*I*) is the potential intrinsic growth rate after starvation, and *I* the hours of starvation (continuous data). Axis with statistically different niches, as identified by null model tests (*P* <0.001), after the Bonferroni correction are indicated by asterisks

Discussion

Co-occurrence of cryptic species is a common phenomenon (Bickford et al. 2007), and the rotifers *B. plicatilis* and *B. manjavacas* are a good example (Gómez et al. 2007). In these highly similar rotifers, the niche differentiation based on biotic factors (predation and resources) is low (Gabaldón et al. 2013), and their morphological similarity therefore translates into ecological similarity for these niche axes. Thus, the physical environmental factors addressed in the present study (temperature and salinity) were good candidates for playing a role in niche differentiation. In fact, differentiation between *B. plicatilis* and *B. manjavacas* based on salinity has been previously suggested by experimental and field observations (Montero-Pau et al. 2011). The fluctuations of temperature and salinity in the natural habitats where these species co-occur are significant (Comín et al. 1992), and each species might therefore have the opportunity to be competitively dominant in a different period. In this work, the genotypes of *B. plicatilis* and *B. manjavacas* used were from the same pond to avoid a possible confounding effect due to local adaptation (Campillo et al. 2010). It was observed that these populations responded differentially to salinity and temperature in terms of several fitness components. Moreover, they consistently differed regarding life-history traits involved in diapause, from the investment in sex to the hatching of diapausing eggs. Differentiations of this kind have been invoked as mechanisms allowing stable species coexistence (Chesson 2000; Montero-Pau and Serra 2011). They are expected to work as stabilizing processes on the competition of these species, which on the other hand, show similar fitness, so that even weak stabilization might be sufficient to allow stable coexistence if it is actually occurring in the wild.

Some of the observed patterns are shared by the two species to some extent. First, our results show that the diapausing eggs of these species undergo significant levels of short-term deterioration. For instance, 37.6-94.2 % eggs deteriorated in less than one year. The high ratio of deteriorated diapausing eggs detected in our experiment for these species is in agreement with the values found in the sediment egg bank of their habitat, Salobrejo Lake, where 88% of the eggs were identified as deteriorated (E.M. Garcia-Roger, personal communication). The clones studied here inhabit shallow ponds, which may dry out, so that our experimental conditions mimic the natural conditions at least of some dry years. This means that survival through an adverse period, from a growing season to a future time, might require a large investment in the production of diapausing eggs and, hence, in sexual reproduction. The correlation between hatching and deterioration rates was clear for *B. plicatilis* and suggests that deterioration is more likely to occur during embryonic development once diapausing egg hatching is induced and embryonic developmental arrest ends. The disruption of diapause could be associated with an increase in egg coat permeability, as observed in other taxonomic groups (e.g., Perry et al. 1983), which might make the embryo more vulnerable. Moreover, diapausing eggs are the result of sexual reproduction, and during the development of the embryo, new gene combinations are expressed for the first time in the individual. Thus, possible deleterious effects of these combinations would occur at this time. Inbreeding depression has been found to be relatively stronger in terms of diapausing egg viability (Tortajada et al. 2009), which determines the hatching of diapausing eggs, the first step of the asexual proliferation phase in the rotifer lifecycle. Our data also indicate how costly sexual reproduction can be, as sex can reduce the finite growth rate (i.e., exp (r)) of clonal proliferation by up to 38.2 %.

Second, significant differences in diapausing egg quality (degradability and hatchability) related to salinity conditions during diapausing egg production were found. In rotifers, the sexual phase of the life cycle, which is the phase involved in diapausing egg production, can be more sensitive to environmental change than the asexual phase (Snell and Carmona 1995; Snell et al. 1998). In agreement with this observation, our findings emphasise that the life-history traits involved in diapause are the most relevant to understanding the success of populations and their long-term persistence.

Third, regardless the two studied species have been reported to be active in the water column at salinities above 45 g L^{-1} salinity (Montero-Pau et al. 2011), salinity has an adverse effect on the growth of these animals, except close to the mesohaline range. The growth rate, investment in sex and diapausing egg hatching decline with salinity in the range explored in the present study. One caveat regarding hatching is that salinity might act as a cue rather than a constraining physical condition. If, as frequently observed (Gómez et al. 1995; Montero-Pau et al. 2011), salinity tends to increase during the growing season, low salinity might promote exit from diapause because it indicates that a suitable season has begun (e.g., heavy rainfall has refilled the lake), even if the optimal salinity occurring in that season is not the initial salinity. Nevertheless, the negative effect of salinity on performance is not surprising. Similar to many other invertebrates dwelling in saline lakes, both studied rotifer species are likely osmoregulators (Lowe et al. 2005), which entails metabolic costs. In fact, saline inland lakes, even being 47 % of all lakes (Wetzel 2001), show low species richness, which is typical in adverse conditions.

In contrast to these shared tendencies, the cryptic species *B. plicatilis* and *B. manjavacas* showed remarkable differences in their life-history traits related to diapause. First, *B. plicatilis* tended to shows a greater propensity for sexual reproduction, although its higher investment in sexual reproduction in response to population density was only statistically significant at 25 ºC. As suggested by the differences between potential and actual growth rates, this pattern likely implies incurring in a cost by decreasing asexual proliferation. Such a high investment is expectable to evolve in response to environment uncertainty (Serra and King 1999; Fussmann et al. 2003) or with growing seasons that are short due to, for example, early deterioration of the physical environment or competitive exclusion. Second, the females of this species produce diapausing eggs that show lower degradation rates. Diapausing eggs are more costly than subitaneous eggs (Gilbert and Schröder 2004; Alekseev et al. 2007; Clark et al. 2012). If this extra cost is related to keeping viability, then it is expectable more resistant diapausing eggs to be associated to fewer egg productions or to produce them at better environmental conditions, likely occurring early in the growing season. As the latter causes decreased environment exploitation, both cases imply a cost. Third, the diapausing eggs of B. *plicatilis* present a hatching pattern that is extended in time. This last feature has been proposed to be an opportunistic strategy associated with unpredictable environments (Pourriot and Snell 1983). It is reasonable to infer that if eggs have a large time window in which to hatch, then this strategy will require diapausing eggs to be viable for long periods with low degradation rates. All these features fit in what is expectable for an opportunistic species.

In another study on B. *plicatilis* and B. *manjavacas*, Montero-Pau et al. (2011) observed a subtle differential effect of salinity on population growth and differences in the temporal distribution of these species in the water column, this temporal distribution being differentially associated with changes in salinity. Consequently, these authors suggested that salinity is a factor allowing these species to differentiate their niches. Accordingly, our robust ANOVA results showed that the potential growth rate of B. *plicatilis* was found to be more negatively affected by salinity than that of B. *manjavacas* (species × salinity interaction statistically significant) and that temperature significantly interacts with salinity in determining the growth rate. Additionally, high salinity conditions during diapausing egg formation appear to have a greater effect on the egg-hatching pattern of *B. plicatilis* than on that of *B. manjavacas*. In agreement with this observation, a dramatic decrease of mean niche overlap between these species was found when temperature and salinity were added to a number of biotic factors as axes for computing the *NO* index (from *NO*= 0.78 to *NO*= 0.51 (Gabaldón et al. 2013)). However, on the basis of the observed growth rates, there is no range of temperature and salinity combinations under which *B. plicatilis* grows faster than *B. manjavacas*, although these rates were very similar at the lowest tested salinity. Nevertheless, at the two highest temperatures tested, the higher performance of *B. plicatilis* at low salinity is clear when the investment in sex is taken into account and the comparison between species is based on the potential growth rates (r_{pot}). In other words, if neither species invests in sex, then *B. plicatilis* will exhibit higher clonal proliferation rates than *B. manjavacas* at low salinities, whereas the opposite pattern will occur at high salinities.

Our results show that the trade-off between diapause and current population growth is relevant in these species. *B. plicatilis* consistently showed a slightly lower observed growth rate (r_{obs}) than *B. manjavacas* at low salinity. Thus, the prediction is that, in absence of sex and under a hypothetical scenario of constant low salinity, *B. plicatilis,* which has a higher performance (higher r_{pot}), would be able to exclude *B. manjavacas*. Notice that a difference in growth rate of 0.1 days^{-1} would result in a two-fold population density difference between species in a week. By contrast, when the demographic cost of the higher investment in sex of *B. plicatilis* is considered, the expectation is that *B. manjavacas* would have an opportunity to increase according with the mechanism modelled in Montero-Pau and Serra (2011). Nevertheless, these assumptions are unlikely; the populations studied here inhabit in a highly variable environment, and they do always invest in sex. The inland pond where they were isolated, similar to many other ponds in the same region of the Iberian Peninsula, floods and dries out relatively regularly, hence determining the hydroperiod (length of the inundation phase) and causing variable salinity. Frequently, rainfall occurs in the region at only a few times, seldom in summer, and in large amounts (Domínguez-Castro et al. 2008). In dry years, the salinity is high for long periods, such as the period studied by Montero-Pau et al. (2011), and especially during the hot season due to evaporation, while in years that are relatively wet, a period of low, but increasing salinity from the cold to the hot season, is expected to occur. This period of low salinity offers a window of opportunity for *B. plicatilis*, given its higher performance at low salinities. However, as suggested by our findings *B. plicatilis*, rather than proliferating at higher rates than *B. manjavacas* would use its higher performance during the low salinity periods to produce diapausing eggs, which would then be stored in the sediment. These eggs appear to be suited to resist long diapause periods and to bet-hedge their hatching as a way to explore and take advantage of suitable low-salinity periods, an adaptive strategy if periods of low salinity will be scarce. In summary, *B. plicatilis* would represent an in-time fugitive species in this type of ecological system, and in the trade-off between leaving diapause synchronically (i.e., trusting the cues for habitat suitability), or extending diapausing egg hatching at a lower rate over the growing season, the latter was selected for.

Given the strong similarity between *B. plicatilis* and *B. manjavacas*, is expected that they share major biological features in a greater extent than co-generic species. Life-history divergence has been previously documented within genus (Reznick et al. 2000). It is likely that the evolution of the resource allocation patterns shaping life histories are not strongly internally constrained because such evolution implies a diversion of resources into different sinks, rather than an increase of the resources acquired or the acquisition of a new function. The crucial idea here is that all relevant fitness components cannot be maximised simultaneously and that increasing one component results in a trade-off with other components. Thus, different species with very similar biology may evolve different compromises among life-history traits if they match with different niches. Hence, a special attention should be paid to life histories when studying the ecological divergence of closely related species.

According to phylogeographic studies, *B. plicatilis* and *B. manjavacas* have co-occurred in this region of the Iberian Peninsula since the last glaciation, 2.5 million years ago (Gómez et al. 2000; Gómez et al. 2007), and paleolimonological data suggest that these species can co-occur in the same pond for at least decades (Montero-Pau et al. 2011). As these two rotifers show very similar performances, long-lasting transient exclusion of the inferior competitor or random walks cannot be ruled out. However, stable coexistence based on niche temporal differentiation is a plausible explanation. If so, this coexistence might be based on time fluctuations or in spatial variation, although phylogeographic studies suggest that effective migration between ponds is rather low (De Meester et al. 2002; Gómez et al. 2007). A storage effect (Chesson 2000) might be functioning because (1) the physical environment experienced by these species fluctuates;(2) these species are specialised for this environment; and (3) the variance of the diapausing egg densities in the sediment layers shows the expected signatures if the rare species is relatively free of competition, i.e., higher variance in the diapausing egg recruitment of the rare species (Montero-Pau et al. 2011). Moreover, density-dependent investment in sex, rather than mediating coexistence in the water-column (Montero-Pau and Serra 2011), appears to function as a way to effectively exploit short, unpredictable, beneficial periods. This would reinforce the storage effect by producing a stage of the lifecycle free of competition, i.e., the diapausing egg stage. Accumulative evidence makes this hypothesis a strong candidate to explain the long-lasting co-occurrence of these very similar species, and thus worthy of further investigation. This study has shown that salinity and temperature do decrease dramatically niche overlap, but other unstudied factors may work similarly.

Classical theories of niche differentiation have stressed the correlation between the niche and morphology (e.g., Fenchel 1975). However, the consistent discovery of cryptic species challenges this classical view, creating an opportunity for the development of new stable coexistence mechanisms and for the use of these species as model organisms to test the limits of the ecological theory. It would be interesting to investigate if cryptic species are prone to differentiate their niches on abiotic factors, rather than on biotic ones, more related to resources acquisition and anti-predator defence, and evolving different life history options for traded-off traits. Cryptic species provide a perfect benchmark for identifying adaptations that might offer opportunities for niche differentiation associated with low morphological divergence and for studying the effects of life history traits on ecological features, without other confounding effects.

Acknowledgements

This work was funded by a grant from the former Spanish Ministerio de Ciencia e Innovación (CGL2009-07364), co-financed with FEDER funds (EU). C.G. was supported by a fellowship from the Spanish Ministerio de Ciencia e Innovación (BES2010-036384). We thank to anonymous referee for the careful reading of our manuscript and the supportive and valuable comments.

References

Adler PB, Hillerislambers J, Levine JM (2007) A niche for neutrality. Ecol Lett 10:95–104.

Alekseev V, Ravera O, Stasio B de (2007) Introduction to diapause. In: Alekseev V, de Stasio B, Gilbert JJ (eds.) Diapause Aquat. Invertebr. Theory Hum. Use. Springer Netherlands, pp 3–10.

Angert AL, Huxman TE, Chesson P, Venable DL (2009) Functional tradeoffs determine species coexistence via the storage effect. Proc Natl Acad Sci U S A 106:11641–5.

Bickford D, Lohman DJ, Sodhi NS (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22:148–155.

Campillo S, García-Roger EM, Carmona MJ, Serra M (2010) Local adaptation in rotifer populations. Evol Ecol 25:933–947.

Campillo S, García-Roger EM, Martínez-Torres D, Serra M (2005) Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. Hydrobiologia 546:181–187.

Carmona M, Serra M, Miracle M (1993) Relationships between mixis in *Brachionus plicatilis* and preconditioning of culture medium by crowding. In: Gilbert JJ, Lubzens E, Miracle MR (eds) Rotifer Symp. VI. Springer Netherlands, Dordrecht, pp 145–152

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Ciros-Pérez J, Carmona MJ, Lapesa S, Serra M (2004) Predation as a factor mediating resource competition among rotifer sibling species. Limonology Oceanogr 49:40–50.

Ciros-Pérez J, Carmona MJ, Serra M (2001a) Resource competition between sympatric sibling rotifer species. Limonology Oceanogr 46:1511–1523.

Ciros-Pérez J, Gómez A, Serra M (2001b) On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. J Plankton Res 23:1311–1328.

Clark MS, Denekamp NY, Thorne MAS, et al. (2012) Long-term survival of hydrated resting eggs from *Brachionus plicatilis*. PLoS One 7:e29365.

Cohen D, Levin S (1987) The interaction between dispersal and dormancy strategies in varying and heterogeneous environments. In: Teramoto E, Yumaguti M (eds) Math. Top. Popul. Biol. Morphog. Neurosci. Springer Berlin Heidelberg, pp 110–122.

Comín F, Rodó X, Comín P (1992) Lake Gallocanta (Aragón, NE Spain): a paradigm of fluctuations at different scales of time. Limnetica 8:79–86.

Domínguez-Castro F, Santisteban JI, Barriendos M, Mediavilla R (2008) Reconstruction of drought episodes for central Spain from rogation ceremonies recorded at the Toledo Cathedral from 1506 to 1900: A methodological approach. Glob Planet Change 63:230–242.

Ellner S (1997) You bet your life: life-history strategies in fluctuating environments. Case Stud. Math. Model. Ecol. Physiol. cell Biol. Prentice Hall, Upper Saddle River, New Jersey, pp 3–24.

Fenchel T (1975) Character displacement and coexistence in mud snails (Hydrobiidae). Oecologia 20:19–32.

Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. Hydrobiologia 583:297–307.

Fussmann GF, Ellner SP, Hairston NG (2003) Evolution as a critical component of plankton dynamics. Proc Biol Sci 270:1015–22.

Fussmann GF, Kramer G, Labib M (2007) Incomplete induction of mixis in *Brachionus calyciflorus*: patterns of reproduction at the individual level. Hydrobiologia 593:111–119.

Gabaldón C, Montero-Pau J, Serra M, Carmona MJ (2013) Morphological similarity and ecological overlap in two rotifer species. PLoS One 8:e57087.

García-Roger EM, Carmona MJ, Serra M (2005) Deterioration patterns in diapausing egg banks of *Brachionus* (Muller, 1786) rotifer species. J Exp Mar Bio Ecol 314:149–161.

Geange SW, Pledger S, Burns KC, Shima JS (2011) A unified analysis of niche overlap incorporating data of different types. Methods Ecol Evol 2:175–184.

Gilbert J (1974) Dormancy in rotifers. Trans Am Microsc Soc 93:490–513.

Gilbert J, Schröder T (2004) Rotifers from diapausing, fertilized eggs: unique features and emergence. Limnol Oceanogr 49:1341–1354.

Gómez A, Carmona MJ, Serra M (1997) Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). Oecologia 111:350–356.

Gómez A, Carvalho G, Lunt DH (2000) Phylogeography and regional endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting egg banks. Proc R Soc B Biol Sci 267:2189–2197.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution (N Y) 56:1431–1444.

Gómez A, Temprano M, Serra M (1995) Ecological genetics of a cyclical parthenogen in temporary habitats. J Evol Biol 8:601–622.

Gotelli N, Graves G (1996) Null models in ecology. Smithsonian Institute Press, Washington D.C.

Hagiwara A, Hino A (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer Brachionus plicatilis. Hydrobiologia 186-187:415–421.

Hagiwara A, Hino A, Hirano R (1985) Combined effects of environmental conditions on the hatching of fertilized eggs of the rotifer *Brachionus plicatilis* collected from an outdoor pond. Nippon Suisan Gakkaishi 51:755–758.

IBM Corp R (2010) IBM SPSS Statistics for Windows, Version 19.0.

Kubanek J, Snell T (2008) Quorum sensing in rotifers. Chem Commun among Bact ASM Press 453–461.

Lei G, Hanski I (1998) Spatial dynamics of two competing specialist parasitoids in a host metapopulation. J Anim Ecol 67:422–433.

Leibold M, McPeek M (2006) Coexistence of the niche and neutral perspectives in community ecology. Ecology 87:1399–1410.

Lowe CD, Kemp SJ, Bates AD, Montagnes DJS (2005) Evidence that the rotifer *Brachionus plicatilis* is not an osmoconformer. Mar Biol 146:923–929.

Lubzens E, Gibson O, Zmora O, Sukenik A (1995) Potential advantages of frozen algae (Nannochloropsis sp.) for rotifer (Brachionus plicatilis) culture. Aquaculture 133:295–309.

Maynard Smith J (1971) What use is sex? J Theor Biol 30:319–335.

McPeek MA (2014) Keystone and intraguild predation, intraspecific density dependence, and a guild of coexisting consumers. Am Nat 183:1–16.

De Meester L, Gómez A, Okamura B, Schwenk K (2002) The Monopolization Hypothesis and the dispersal–gene flow paradox in aquatic organisms. Acta Oecologica 23:121–135.

De Meester L, Gómez A, Simon J (2004) Evolutionary and ecological genetics of cyclical parthenogens. In: Moya A, Font E (eds) Evol. from Mol. to Ecosyst. Oxford University Press, Oxford, UK, pp 122–134.

Montero-Pau J, Gabaldón C, Carmona MJ, Serra M (2014) Measuring the potential for growth in populations investing in diapause. Ecol Modell 272:76–83.

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Ortells R, Snell TW, Gómez A, Serra M (2000) Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. Arch für Hydrobiol 149:529–551.

Perry R, Clarke A, Hennessy J, Beane J (1983) The role of trehalose in the hatching mechanism of *Heterodera goettingiana*. Nematologica 29:323–334.

Pourriot R, Snell TW (1983) Resting eggs in rotifers. Hydrobiologia 104:213–224.

Quinn GP, Keough MJ (2005) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput., Vienna, Austria.

Reznick D, Nunney L, Tessier A (2000) Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol Evol 15:421–425.

Serra M, King C (1999) Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. J Evol Biol 12:263–271.

Siepielski AM, McPeek MA (2010) On the evidence for species coexistence: a critique of the coexistence program. Ecology 91:3153–3164.

Snell TW, Carmona MJ (1995) Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. Environ Toxicol Chem 14:415–420.

Snell TW, Serra M, Carmona MJ (1998) Toxicity and sexual reproduction in rotifers: Reduced resting production and heterozygosity loss. In: ForbesVE (ed) Genet. Ecotoxicol. Taylor & Francis, Philadelphia, PA, USA, pp 169–185.

Stearns S (1992) The evolution of life histories. Oxford University Press, Oxford.

Stelzer CP, Snell TW (2003) Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. Limnol Oceanogr 48:939–943.

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press, New Jersey.

Tortajada AM, Carmona MJ, Serra M (2009) Does haplodiploidy purge inbreeding depression in rotifer populations? PLoS One 4:e8195.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Wellborn G (2002) Trade-off between competitive ability and antipredator adaptation in a freshwater amphipod species complex. Ecology 83:129–136.

Wetzel R (2001) Limnology: lake and river ecosystems. Academic Press, London.

Wilcox RR SF (2010) WRS: A compiled package of R.R. Wilcox' robust statistics functions. R package version 0 12.1.

Yamasaki S, Tanabe K, Hirata H (1989) Efficiency of chilled and frozen *Nannochloropsis sp.*(marine Chlorella) for culture of rotifer. Mem Fac Fish Kagoshima Univ 38:77–82.

Zhang DY, Lin K, Hanski I (2004) Coexistence of cryptic species. Ecol Lett 7:165–169.

Chapter 4

Allocation patterns in modes of reproduction in two facultatively sexual cryptic rotifer species

Carmen Gabaldón and María José Carmona

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A.O.22085, 46071, Valencia, Spain

Published in Journal of Plankton Research, (2015), 10.1093/plankt/fbv012

Abstract

Many zooplankters rely on diapausing stages to survive unsuitable conditions in time-varying habitats. In facultative sexual rotifers, reproductive effort allocated to the sexually produced diapausing eggs is at the expense of the subitaneous parthenogenetic eggs, generating a trade-off between current and future population growth. The timing and the amount of sex (the sexual pattern) affect diapausing-egg production. This switch to sex is complex because the reproductive mode is separated in distinct females: asexual (female-producing), unfertilized sexual (male-producing) and fertilized sexual (diapause-egg-producing). We studied sexual patterns and life-history variation of these females in two cryptic species (*Brachionus plicatilis* and *Brachionus manjavacas*) co-occurring in Spanish ponds. Results revealed species-specific differences in sexual pattern; *B. plicatilis* had an earlier and higher sex allocation. Female types allocated resources differently among life-history traits, revealing relationships between lifespan and reproductive traits that demonstrate the cost of reproduction. Sexual females reproductive traits showed between-species differences. *Brachionus manjavacas* unfertilized females produced more sons earlier and at a greater daily rate than *B. plicatilis* ones. Moreover, *B. manjavacas* fertilized females had higher relative allocation per diapausing egg than those of *B. plicatilis*. We relate these differences to the environmental uncertainty faced by each species, and discuss their implications for competitive outcome.

Key-words: density threshold for sex initiation; life-table parameters; female reproductive types; competition; diapausing eggs

Introduction

The production of diapausing eggs is a widespread life-history adaptation among zooplankters, such as cladocerans, rotifers and copepods (Hairston et al. 1995; Gilbert and Schröder 2004; Alekseev et al. 2007). Diapausing eggs enable survival during unsuitable environmental conditions caused by abiotic (e.g., drought; extreme salinity; extreme temperature) and/or biotic factors (e.g., occurrence of competitors, predators, etc.) (Pourriot and Snell 1983; Slusarczyk 1995; Alekseev and Lampert 2001). Additionally, diapausing eggs facilitate temporal and spatial dispersion (Hairston and Kearns 2002; Louette and De Meester 2005). However, the production of diapausing eggs is expected to have higher costs than the production of subitaneous eggs. First, diapausing eggs have special features regarding energy stores and protection that make them energetically costly (Gilbert and Schröder 2004; Alekseev et al. 2007; Clark et al. 2012). This agrees with the fact that, generally, whereas subitaneous eggs are produced at relatively high per-capita rates, only a few diapausing eggs are produced (Gilbert 1993; Gilbert 2004; Fink et al. 2011). Secondly, diapause implies an obligate dormant period during which development does not resume, even under favourable conditions (Schwartz and Hebert 1987; Hagiwara and Hino 1989; Marcus and Lutz 1998). This delayed hatching implies that there is no contribution to the immediate population growth. Thirdly, in the case of monogonont rotifers and most cladocerans, which are cyclical parthenogens, sex is required for the production of diapausing eggs. Because this requires the participation of males, females accrue the 'twofold cost of sex' (Maynard Smith 1971). Consequently, the switch from subitaneous reproduction to the production of diapausing eggs is associated with higher costs and depresses population growth rate.

Optimizing the trade-off between current population growth and investment in sex and diapause is especially relevant for rotifer populations inhabiting temperate zone habitats. In these habitats, water bodies show strong and random temporal environmental variability (Quintana Pou et al. 2006; Zacharias et al. 2007). Because diapause is the only way to survive unsuitable conditions between growing seasons under this selective regime, optimally timing the switch to sexual reproduction and balancing resource allocation between the two modes of reproduction are expected to be selected to maximize diapausing egg production (Serra and Carmona 1993; Serra et al. 2004). The number of diapausing eggs produced at the end of a growing season is a between-year fitness measure (Serra and King 1999; Campillo et al. 2010). Rotifer populations, such as those in the common genus, *Brachionus*, are temporary and annually colonize the water column (during the planktonic growing period) through the hatching of diapausing eggs located in sediment. The hatchlings are asexual females that produce subitaneous eggs parthenogenetically, which hatch into other genetically identical females. This asexual mode of reproduction, which can continue for many generations resulting in an exponential growth of clonal lineages, co-occurs with episodes of sexual reproduction. Sex initiates when environmental factors trigger asexual females to produce some sexual-female offspring (e.g., Gilbert 1974; Schröder 2005). Sexual females produce meiotic haploid eggs that develop into either dwarf males or, if females are fertilized while young, into diapausing eggs. After an obligatory dormant period of varying duration (Hagiwara and Hino 1989; Martínez-Ruiz and García-Roger 2014), diapausing eggs hatch into asexual females and begin a new growing season once suitable water column conditions are present.

The switch between the two reproductive modes is density dependent in the genus *Brachionus*. Sex is induced by an infochemical (Snell et al. 2006) produced by females and released into the medium (Gilbert 1963; Stelzer and Snell 2003). The concentration of this infochemical increases with population density and triggers sexual reproduction when reaching a threshold. This density threshold for the initiation of sex allows the characterization of the patterns of sexual reproduction because it determines when sex occurs in the growing season of a rotifer population (Serra and King 1999; Carmona et al. 2010). Thus, estimating this threshold has ecological and evolutionary importance due to the large impact that the timing of sex has on fitness in cyclical parthenogenetic rotifers (Aparici et al. 1996; Serra et al. 2004). Differences in timing of and allocation to sex can affect competitive interactions and sympatric species coexistence (Montero-Pau and Serra 2011), as well as clonal selection, genetic diversity and adaptive traits (Gómez and Carvalho 2000; Ortells et al. 2006; Serra and Snell 2009).

In a sexually reproducing rotifer population, three different types of females can be found: (i) asexual females that produce parthenogenetic eggs that hatch into females, (ii) unfertilized sexual females that produce parthenogenetic meiotic eggs that hatch into males and (iii) fertilized sexual females that generate diapausing eggs. These three types of females are morphologically indistinguishable, except for their mode of reproduction. This lack of morphological difference makes it possible to analyze how different reproductive allocation behaviours relate to life-history traits without the interference of other phenotypic variation (King 1970). Several studies have addressed life-history traits of asexual and unfertilized sexual females in different rotifer species (King 1970; Pourriot 1977; Carmona and Serra 1991; Pourriot and Rougier 1991; Snell and Carmona 1995; Gribble and Welch 2013; Smith and Snell 2014), but only a few have included fertilized sexual females (revised by Gilbert, 1993; Snell, 2014; Xi et al., 2001). These studies showed that female reproductive types differ in their age schedules of reproduction and survival and that there is interspecific variation in these allocation patterns. However, despite the fact that the occurrence of cryptic species complexes is very common among rotifers (Gómez et al. 2002; Walsh et al. 2009; Fontaneto et al. 2010), no study on whether this divergence among female types is consistent among these closely related species has yet been conducted.

The cryptic rotifer species *Brachionus plicatilis* and *Brachionus manjavacas* live in sympatry in brackish inland ponds in Spain (Gómez et al. 2002; Gómez et al. 2007). Because of their striking morphological similarity and large ecological overlap (Gabaldón et al. 2013), competition is expected to be high. Previous records have shown that these species differ in their tolerance to salinity and in traits relating to sexual reproduction and diapause (Gabaldón et al. 2015). These differences may play an important role in their coexistence. In the present study, to gather more evidence on these species' trait divergence, we thoroughly investigated patterns of sexual reproduction by estimating sexual reproduction ratios and density thresholds for the initiation of sex in both species. We also compared life histories of asexual, unfertilized sexual and fertilized sexual females. The aim of this study was to gain insight in the divergence of reproductive allocation patterns in these two species, their relationships with the biotic and abiotic environment and the implications for species coexistence.

Material and Methods

Isolation, identification and culture of rotifers clones of *B. plicatilis* and *B. manjavacas* were obtained by asexual proliferation of individual females hatched from diapausing eggs isolated from sediments of Salobrejo Lake (Eastern Spain; 38854.7650N, 1828.2750W) (Gabaldón et al. 2013). Species identification of each clone was performed by the RFLP-PCR method (Gabaldón et al. 2013). Stock cultures of 20 clones per species were individually maintained at 10 g L^{-1} salinity and 20°C temperatures. Both rotifer species co-occur under these conditions in the field (Montero-Pau et al. 2011). Culture medium was f/2-enriched saline water (Guillard and Ryther 1962) prepared with commercial sea salt (Instant Ocean®; Aquarium Systems). Culture medium contained 2×10^5 cells mL^{-1} of the microalga *Tetraselmis suecica* (ICMAN Collection) as food for rotifers.

Microalgae were maintained in exponential growth phase and constant illumination. Unless otherwise indicated, pre-experimental and experimental rotifer culture media and conditions were the same as for stock cultures (hereafter, 'standard conditions').

Estimation of density threshold for sex initiation

Genetic variation in the propensity to reproduce sexually in *B. plicatilis* and *B. manjavacas* was studied by conducting 96 bioassays (2 species × 16 clones × 3 replicates) to estimate the density threshold for sex initiation following the procedure of Carmona et al., (2009). Before bioassays, rotifer clones were pre-cultured under standard conditions at a low population density and medium renewal for three generations to control for any effect the maternal environmental conditions may have on the reproductive type of the offspring (Snell et al. 2006). To do so, three independent sublines (one per replicate) for each clone of both species were created. From every clone stock culture, one egg-carrying asexual female was individually transferred to a dish with 40 mL of culture medium. After 24 h, one newborn female (F_1) was transferred to a new dish where it proliferated asexually for 48 h. Then, one second-generation newborn female (F_2) was transferred to a new dish to produce a third generation, from which one newborn female (F_3) was used in the bioassay. Each experimental newborn was individually isolated in a dish with 15 mL of culture medium, allowed to grow and reproduce, and monitored every 12 h until the first male was observed. Then, the culture was fixed with 0.3% Lugol's solution and the population density estimated (Aparici et al. 2001).

The effects of species and clone on the log-transformed population density at first male appearance were tested by a nested robust ANOVA using R statistical software v. 3.1.1 (Wang et al. 2010). The genetic variation of this trait was measured using broad-sense heritability (H^2), which is the appropriate measure for clonal organisms (Lynch and Walsh 1998). H^2 (i.e., the ratio of the among-clone variance to the total within- and among-clone variance) was estimated from the genetic (i.e., between clones) component of the variance for each species following the procedure for clonal organisms (Pfrender and Lynch 2000). For these estimations, a one-way ANOVA was performed using SPSS statistic software v. 19.0 (IBM Corp 2010).

Life-history trait analysis

Dynamic life-table experiments were performed to compare life-history traits of the three female reproductive types in both rotifer species. Female cohorts of each species were established from multiclonal populations created by mixing 50 females of each of the 20 clones in 1 L of culture medium. These populations were grown under standard conditions. To obtain the three female types, males were allowed to fertilize newborn females (insemination is possible for only a few hours after birth) (Snell and Childress 1987). The multiclonal populations were grown in continuous flow culture conditions in a chemostat with a dilution rate of 0.15 per day and regularly monitored. Once sexual reproduction was observed, culture aliquots were gently shaken to detach extruded eggs from the females

(Tortajada et al. 2010). Only detached eggs in an advanced stage of development were selected. Eggs containing male embryos were identified by their smaller size. Several wells were set, each containing 100 eggs with female embryos and 100 eggs with male embryos. Preliminary experiments showed that there was a lower number of fertilized females in *B. manjavacas*, so for this species a higher number of wells were set. Eggs usually hatch in, 4-5 h and mating was allowed for 48 h (Tortajada et al. 2010). Afterwards, cohort females were individually isolated in 24-well plates (NuncTM) containing 1 mL of medium and kept under standard conditions in darkness. At maturity, each cohort female was classified as (i) asexual if giving rise to daughters, (ii) unfertilized sexual, if giving rise to sons or (iii) fertilized sexual, if producing diapausing eggs. When female type was identified, the group of asexual females was reduced to 400 females by random elimination of surplus females to reduce experimental effort. Survival and the numbers of offspring or diapausing eggs produced were recorded daily for the entire lifespan of each cohort female. At the time of each observation, the surviving parental females were transferred to wells with fresh medium. The sexual reproduction ratio (i.e., the mixis ratio) of each species was calculated as the proportion of sexual females in the initial cohort (including the eliminated asexual females). From life-table experiment records, the following were calculated individually for each female: (i) the lifespan and the reproductive lifespan (i.e., timing between first and last offspring produced), (ii) the lifetime reproductive output, (iii) the mean daily fecundity (i.e., lifetime reproductive output divided by reproductive lifespan) and (iv) the generation time (i.e., weighted average age at birth of the offspring). These individual parameters were averaged for the asexual, unfertilized sexual and fertilized sexual cohorts of *B. plicatilis* and *B. manjavacas*. Additionally, the age-specific survival (l_x) functions of each female type for both species were computed by pooling individual values. Survival curves were compared using nonparametric tests: log-rank and Breslow tests (Cox and Oakes 1984). We performed two-way robust ANOVAs using the 'robust' package (Wang et al. 2010) in R statistical software v. 3.1.1 (R Core Team 2014) to compare the lifespan, generation time, lifetime reproductive output and mean total daily fecundity between *B. plicatilis* and *B. manjavacas* and between female reproductive types. Post hoc Games–Howell tests (Games et al. 1981) for pairwise multiple comparisons were performed using SPSS statistic software v. 19.0 (IBM Corp 2010).

Following Snell and King (1977), we used partial correlation analysis to test the relationships between lifespan and life-history traits related to timing, amount and rate of offspring produced by all females and by females grouped by reproductive type (SPSS v. 19.0, IBM Corp, 2010). For each comparison between lifespan and a given reproductive variable, partial correlation coefficients were calculated while controlling for the effect of the two remaining reproductive variables.

To compare reproductive allocation of female reproductive types between species, we calculated the ratios of both female and male offspring (produced by asexual and unfertilized asexual females, respectively) to diapausing eggs produced by fertilized sexual females. The 95% confidence intervals for these ratios were obtained using bootstrap resampling (Caswell 2006) and corrected following the bias-corrected percentile method (Efron 1981), implemented in R version 3.1.1(R Core Team 2014). A total of 1000 randomizations were performed for each ratio and species.

The intrinsic growth rates (*r*) of *B. plicatilis* and *B. manjavacas* were calculated from life-table data by solving Euler–Lotka's equation (e.g., Stearns 1992). Additionally, the potential intrinsic growth rates (r_{pot}) were calculated for these species following the method of Montero-Pau et al., (2014). The r_{pot} is the growth rate of a population entirely composed of asexual females and is considered a measure of performance. The 95% confidence intervals for intrinsic growth rates were obtained by bootstrapping the life-table dataset using the procedure described above.

Results

In general, population density at first male appearance was lower in B. plicatilis clones than in *B. manjavacas* clones, ranging from 0.55 to 7.15 females mL^{-1} for *B. plicatilis* (average 2.91 females mL^{-1}) and from 1.22 to 22.64 females mL^{-1} for *B. manjavacas* (average 8.24 females mL^{-1}) (Fig. 4.1). Statistical analyses confirmed differences in density at first male appearance between species ($P< 0.001$) and among clones within species ($P< 0.001$). H^2 estimates of this parameter were high (0.51) for *B. plicatilis* and intermediate (0.29) for *B. manjavacas*. These heritability differences were due to differences in the amount of

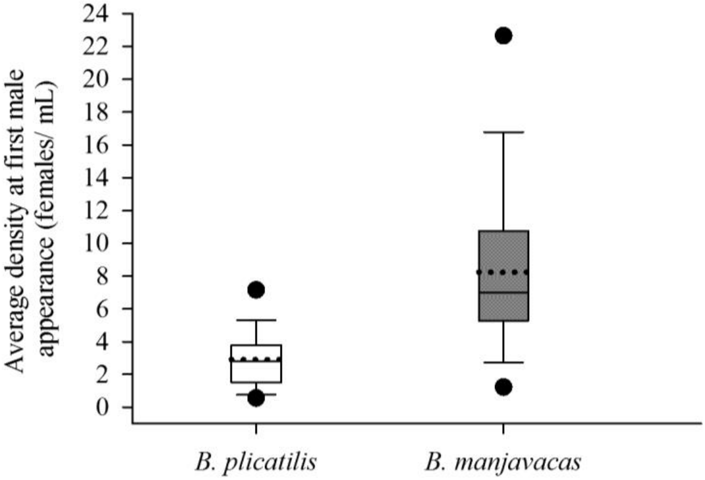


Figure 4.1. Box-and-whisker plot of clonal averages of population density at first male appearance in B. *plicatilis* and B. *manjavacas*. The bottom of the box is the 25th percentile and the top is the 75th percentile. Median (straight line) and average (dotted line) values are shown. The whiskers extend to the 10th and 90th percentiles; observations outside this range are plotted as circles.

genetic variance (0.11 for B. *plicatilis* and 0.04 for B. *manjavacas*, whereas environmental variance was ~0.10 for both species).

We analysed the life histories of 400 asexual, 126 unfertilized sexual and 62 fertilized sexual females of B. *plicatilis*, and 399 asexual, 111 unfertilized sexual and 17 fertilized sexual females of B. *manjavacas*. Age-specific survival (l_x) and mean lifespan for the three female reproductive types of both species are shown in Figs 4.2 and 4.3. Survivorship followed type I curves (Pearl 1928), but different trends were found. Both female type and species affected lifespan; the

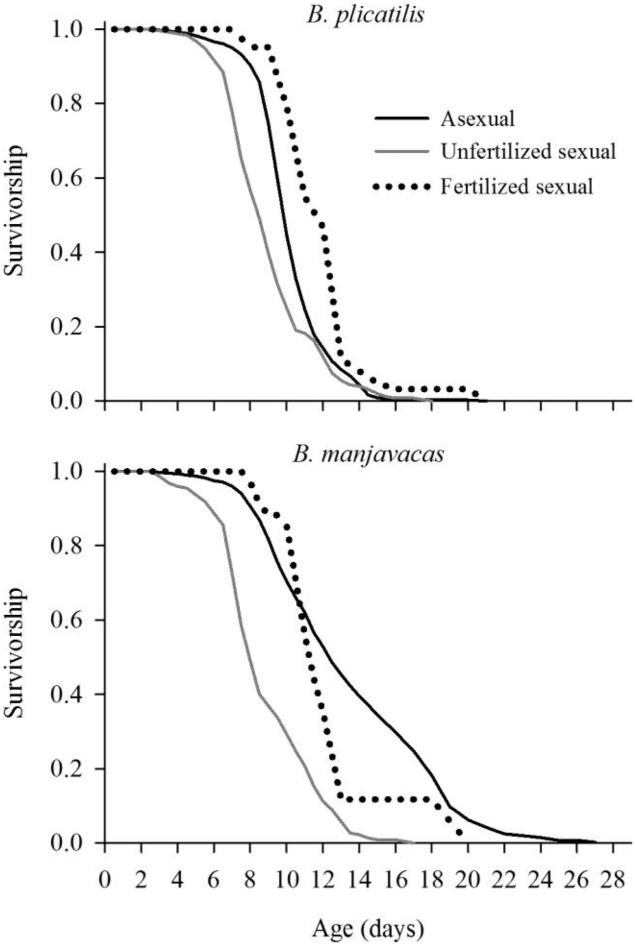


Figure 4.2. Age-specific survivorship (l_x) curves of the three female reproductive types of *B. plicatilis* and *B. manjavacas*.

effects of both factors and their interaction were highly significant (two-way robust ANOVA, $P< 0.001$). Survival curves of the three female types were significantly different in both species ($P< 0.001$ log-rank and Breslow tests). Fertilized sexual females had higher survival rates at middle age classes (Fig. 4.2) and longer lifespans, on average (Fig. 4.3A), than did unfertilized females of either species. Fertilized sexual females also had significantly longer mean lifespans than asexual females in *B. plicatilis*, but not in *B. manjavacas*. Between-species comparison of survival curves of each female reproductive type was only significantly different between asexual females (log-rank and Breslow tests; $P < 0.001$ for asexual and $P > 0.540$ for unfertilized and fertilized sexual). Asexual females of *B. manjavacas* had higher survival at both middle and old age classes and, consequently, a longer mean lifespan than asexual females of *B. plicatilis* (Fig. 4.3A). In contrast, unfertilized and fertilized sexual females had remarkably similar survivorship patterns and lifespan across species ($P > 0.54$ for both tests). In both species, female fertilization status affected lifespan; unfertilized sexual females had shorter lifespans than fertilized ones. Unfertilized sexual females also had shorter lifespans than asexual females, although this difference was marginally significant in *B. plicatilis*.

Reproductive female type had a statistically significant effect on lifetime reproductive output, generation time, mean total daily fecundity and reproductive lifespan; however, species only had a significant effect on the latter three parameters (two-way robust ANOVA, $P < 0.001$, Fig. 4.3B–E). The interaction effect of the three parameters was significant ($P < 0.001$), indicating that the species effect differed among female types. In both species, unfertilized sexual females produced their offspring earlier than both asexual and fertilized sexual females (Fig. 4.3B). In *B. plicatilis*, asexual females reproduced earlier than fertilized sexual females, whereas in *B.*

manjavacas the opposite pattern was found. These differences between generation times were statistically significant, except between unfertilized and fertilized sexual females of *B. manjavacas*. Post hoc comparison of each female type between species showed that unfertilized *B. plicatilis* females produced male offspring significantly later than did *B. manjavacas*, whereas the production of female offspring by asexual females followed the opposite pattern. In both species, asexual females had the highest lifetime reproductive output, and fertilized females had the lowest (Fig. 4.3C); these differences were statistically significant except for asexual and unfertilized sexual females of *B. manjavacas*. When comparing this parameter between species for each female type, the only statistically significant difference detected was that unfertilized sexual females of *B. manjavacas* produced higher numbers of males than *B. plicatilis* ones. Both species had the same general trend in the mean total daily fecundity: unfertilized sexual females had significantly greater daily fecundity than asexual females, which in turn had higher daily fecundity than fertilized sexual females.

However, unfertilized sexual females of *B. manjavacas* had significantly higher total mean daily fecundity than *B. plicatilis* females. This pattern reversed when comparing the daily fecundities of asexual females of both species (Fig. 4.3D). Reproductive lifespan was shorter in sexual than asexual females of both species, but fertilization status had no effect on this parameter (Fig. 4.3E). Asexual and unfertilized sexual females of *B. manjavacas* had higher reproductive lifespans than those of *B. plicatilis*.

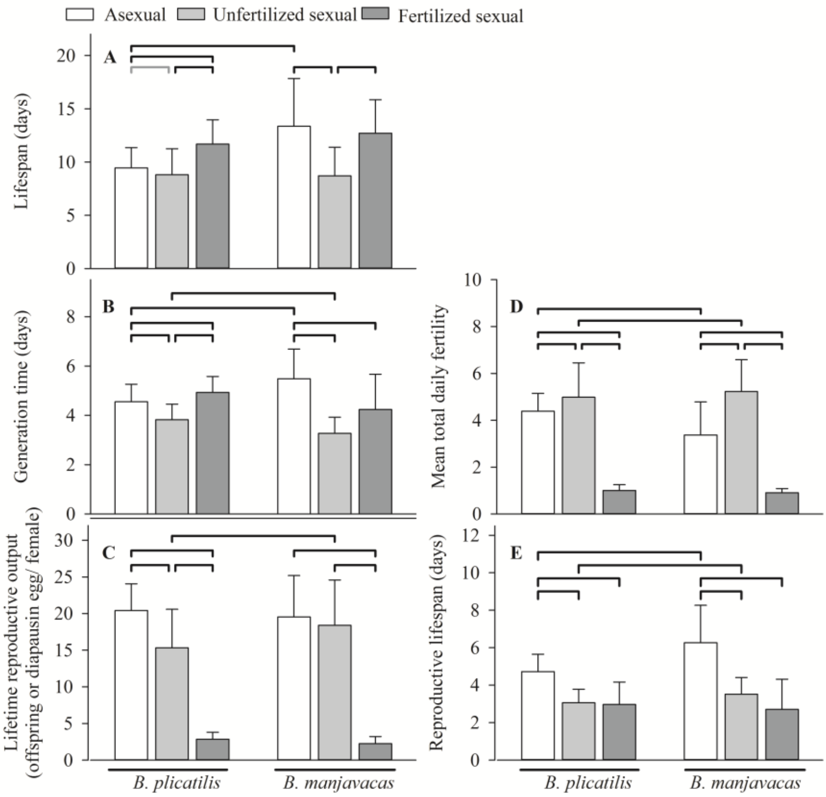


Figure 4.3. Lifespan (**A**), generation time (**B**), lifetime reproductive output (**C**), mean daily fertility (**D**) and reproductive lifespan (**E**) of the three female reproductive types of B. plicatilis and B. manjavacas. Vertical bars are the standard deviation of the mean. Horizontal lines in black and grey connect values that are statistically significant (*P*< 0.05) and marginally significant (*P*= 0.08), respectively, after pairwise multiple comparisons performed by a post hoc Games–Howell test.

Partial correlations (Table 4.1) revealed significant relationships between lifespan and reproductive traits for all females. This result holds for the three female types, with the exception of lifespan and generation time for fertilized females (the group with the smallest sample size). The positive, moderate partial correlation of lifespan and generation time indicates that females producing their offspring earlier tend to have shorter lifespans. This relationship was not a mere consequence of short-lived females being unable to complete reproduction, as 81% of females had a post- reproductive lifespan. The negative partial correlations between mean daily fecundity and lifespan, albeit weak, indicate that higher rates of offspring production during reproductive lifespans are associated with shorter overall lifespans. The lifetime reproductive output and the lifespan showed a weak and positive partial correlation. Likely, this relationship is due to the extension of the reproductive period in long-lived females and not to a higher rate of reproduction during the reproductive period, which is consistent with (i) the negative relationships found between mean daily fecundity and lifespan and (ii) the significant positive simple correlation between lifetime reproductive output and reproductive lifespan ($r = 0.45$; $P < 0.0001$). Longer-lived females had higher fecundity, but lower rates of offspring production during their reproductive period.

Fertilized sexual *B. plicatilis* females produced up to five diapausing eggs and fertilized sexual *B. manjavacas* females produced up to four diapausing eggs, with an average of 2.8 diapausing eggs for *B. plicatilis* and 2.2 diapausing eggs for *B. manjavacas*. Although this

Table 4.1. Partial correlation coefficients (and their probabilities) of three reproductive traits, with the lifespan of both all females and females grouped by reproductive type. In the calculation of each coefficient, the effects of the two remaining reproductive variables are partialled out.

	Total females	Asexual females	Unfertilized sexual females	Fertilized sexual females
Generation time	0.49 (**$P<$ 0.0001**)	0.51 (**$P<$ 0.0001**)	0.42 (**$P<$ 0.0001**)	-0,02 ($P=$ 0.8750)
Lifetime reproductive output	0.15 (**$P<$ 0.0001**)	0.20 (**$P<$ 0.0001**)	0.22 (**$P<$ 0.0100**)	0.25 (**$P=$ 0.0280**)
Mean daily fecundity	-0.23 (**$P=$ 0.0010**)	-0.36 (**$P<$ 0.0001**)	-0.20 (**$P=$ 0.0020**)	-0.32 (**$P=$ 0.0040**)

difference was not statistically significant (Fig. 4.3), there were differences between species in allocation to diapausing-egg production relative to subitaneous reproduction: *B. manjavacas* had ahigher relative resource allocation per diapausing egg (when compared with subitaneous reproduction) than did *B. plicatilis* (Fig. 4.4). To produce one diapausing egg, *B. manjavacas* requires a resource allocation equivalent to that used to produce 8.5 daughters or 8.2 sons by parthenogenetic reproduction. The resources used to produce one diapausing egg was slightly lower for *B. plicatilis*, equivalent to that used to produce 7.3 daughters or 5.5 sons by parthenogenetic reproduction.

The intrinsic population growth rate of both species was similar. However, when considering the potential values of this

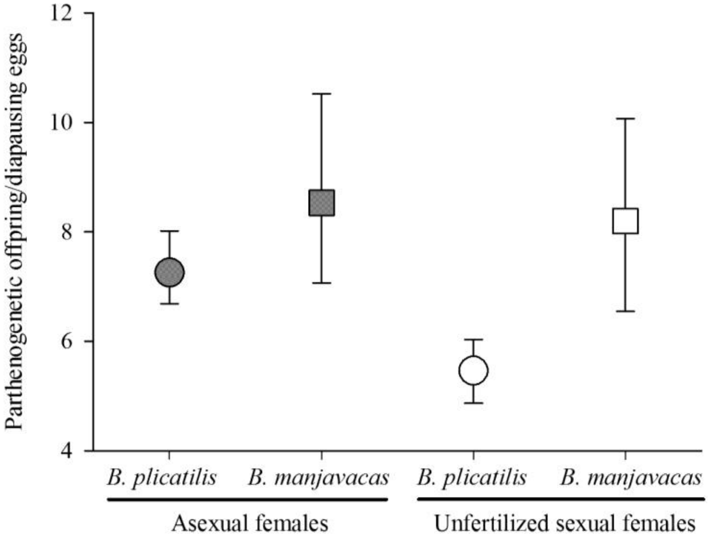


Figure 4.4. Ratios of parthenogenetic offspring (daughters produced by asexual females or sons produced by unfertilized sexual females) to diapausing eggs (produced by fertilized sexual females) of *B. plicatilis* and *B. manjavacas*. Error bars show 95% confidence intervals.

parameter (i.e., in the absence of sexual reproduction), *B. plicatilis* had a higher performance than *B. manjavacas* (Fig. 4.5). This finding agrees with the estimated values of the mixis ratios: 0.371±0.037 for *B. plicatilis* and 0.085±0.013 for *B. manjavacas*.

Discussion

Sexual reproduction pattern (i.e., investment in sex during the growing season) is a key feature of the cyclically parthenogenetic rotifer life cycle (Schröder 2005) and has two major components: the timing of sex and the proportional allocation to sexual reproduction,

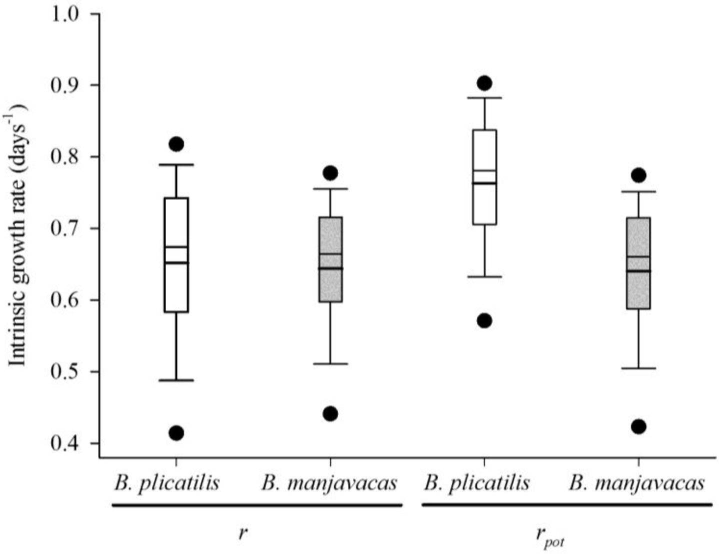


Figure 4.5. Comparison between observed (*r*) and potential (r_{pot}) intrinsic growth rates of *B. plicatilis* and *B. manjavacas*. Median (thin line) and average (thick line) values are shown. The whiskers extend to the 10th and 90th percentiles; observations outside this range are plotted as circles.

the mixis ratio (Serra and Carmona 1993; Serra and King 1999). In rotifer species where population density induces sexual reproduction, timing of sex can be estimated by the density threshold for sex initiation (Serra et al. 2004; Serra and Snell 2009). A low-density threshold will cause early sex initiation and high investment in sex, as this would lead to a lower rate of asexual proliferation during the growing period (Serra et al. 2004; Carmona et al. 2009). In the present study, despite among-clone variation within species, *B. plicatilis* showed consistently lower density thresholds for sex initiation than did *B. manjavacas*. Heritability analysis revealed the existence of genetic variation for this trait in the populations of these cryptic species co-occurring in Salobrejo Lake. Genetic variation in propensity to sex in *B. manjavacas* is reported here for the first time, but H^2 values from both species fall within the range of those already recorded in *B. plicatilis* populations (Aparici et al. 2001; Carmona et al. 2009). Our pre-experimental procedure was designed to reduce phenotypic differences due to environmental differences among mothers (Lynch and Ennis 1983; Schwaegerle et al. 2000). Notwithstanding, this procedure ensures that clonal heritabilities were not upwardly biased by environmental maternal effects because these effects did not contribute to the between-clone component of variance in the final analysis (Pfrender and Lynch 2000). Consequently, it is reasonable to interpret our heritability values as providing meaningful insights into the potential for adaptive evolution in natural populations of *B. plicatilis* and *B. manjavacas* (Weigensberg and Roff 1996). The finding of a substantial amount of genetic variation in sex propensity is particularly relevant, given the effect of timing of sex on individual fitness (reviewed in Serra et al. 2004; Gilbert 2010). These results also suggest that the fraction of phenotypic variation in the propensity to initiate sex that could be attributable to heritable variation is higher in *B. plicatilis* than in *B. manjavacas*. Therefore, these species may differ in this trait in terms of their evolutionary potential to respond to selection.

The second relevant component of the sexual reproduction pattern, the mixis ratio, was four times higher in *B. plicatilis* than in *B. manjavacas*, consistent with previous studies of Salobrejo populations

(García-Roger et al. 2009; Gabaldón et al. 2015). This difference can also account for the divergent sexual reproduction patterns of these species. Because sex and diapause are linked in the monogonont life cycle, sexual females do not contribute to current population growth. Thus, the higher the mixis ratio, the greater the decrease in population growth rate (Snell 1987). In fact, the mixis ratio quantifies the within growing season cost of sex in cyclical parthenogenetic rotifers (Serra and Snell 2009; Carmona et al. 2009). According to our observed and potential intrinsic growth rates, sexually reproducing *B. plicatilis* could decrease their population growth rate 1.6 times more than *B. manjavacas*, incurring a higher cost of sex. However, despite this cost, sexual females contribute to long-term population persistence by producing diapausing eggs, which leads to the trade-off between current and future population growth. Both components, timing of sex and mixis ratio, have been studied in terms of the optimal patterns that maximize diapausing-egg production in relation to habitat features (e.g., Serra and King, 1999; Serra et al., 2004). For instance, earlier sex and higher mixis ratios, the pattern found in *B. plicatilis* has been reported in rotifer populations undergoing more environmental risk and uncertainty during their growing seasons (Carmona et al. 1995; Campillo et al. 2009; Gilbert and Diéguez 2010).

In our sexually reproducing populations of *B. plicatilis* and *B. manjavacas*, as in many other rotifer species, asexual females produced asexual and sexual daughters. As typically reported (Schröder 2005), mixis ratio was lower than 0.5 in both species. This incomplete sex induction is a conservative bet-hedging strategy that allows the maintenance of several female reproductive modes within the population (Gilbert 2003; Fussmann et al. 2007). Hence, diapausing eggs are produced concurrently with population proliferation by asexual reproduction, as long as the environment remains suitable (Carmona et al. 1995; Serra and King 1999).

Our study shows that, in both species, the three female reproductive types allocate resources differently among life-history traits. These females are morphologically indistinguishable, but differ in the eggs and offspring they produce. Both asexual and unfertilized sexual females produce subitaneous eggs similar in appearance, although unfertilized female eggs that hatch into males are much smaller. Contrary to what might be expected from the trade-off between size and number of offspring (e.g., Stelzer, 2005), unfertilized sexual females tended to show lower lifetime reproductive output than asexual females, at least in *B. plicatilis*. Although the relative reproductive output of these two types of females varies among rotifer species (King 1970), previous studies on the *B. plicatilis* complex also reported a lower production of sons by unfertilized sexual females (Carmona and Serra 1991; Gribble and Welch 2013). Assuming that egg and offspring size properly reflect resource provisioning, the higher fecundity of asexual females implies that unfertilized sexual females allocated fewer resources to offspring production than did asexual females. However, unfertilized females produced their offspring earlier than asexual females and had a larger mean daily fecundity, which can be interpreted as a direct result of the lower cost of producing males because of their significantly smaller size (Carmona and Serra 1991). By allocating fewer resources per descendant, unfertilized sexual females can produce offspring sooner and at a higher rate than asexual females. Because their reproductive lifespan is shorter, unfertilized sexual females consequently invest the resources allocated to reproduction over a shorter period of time than asexual females. Hence, unfertilized sexual females had a higher reproductive effort at earlier ages. This age-specific reproductive allocation could account for the shortness of their lifespan due to a physiological trade-off. This interpretation agrees with our finding that lifespan was positively correlated with generation time and negatively correlated with mean daily fecundity in these females. Our results are consistent with the interpretation that a high rate of male production will increase the fitness of unfertilized females (Epp and Lewis Jr 1979; Carmona and Serra 1991). It will be adaptive for these females to produce male offspring as quickly as possible, to increase the probability that their sons will find a mate and lead to the production of diapausing eggs (Carmona and Serra 1991; Gribble and Welch 2013).

A second important difference in the pattern of resource allocation is that fertilized sexual females had a much smaller lifetime reproductive output and mean daily fecundity than the other two types of females. This is a general tendency in cyclical parthenogenetic rotifers (Xi et al. 2001; Snell 2014), supported by the fact that diapausing eggs are more costly to produce than subitaneous eggs. Not only are diapausing eggs bigger and structurally more complex, but they also contain more energy-storing molecules and protective compounds than subitaneous eggs (Gilbert 2004; Clark et al. 2012). These costly adaptive traits would result in a diminished fertility of fertilized females. We found that fertilization increased the lifespan of sexual females, which coincides with the only two previous reports on the *B. plicatilis* complex (Lubzens and Zmora 2003; Snell 2014). These changes in the life-history pattern of fertilized females could occur because the optimal allocation strategy for sexual females differs according to their mating status. For instance, late onset of reproduction and the more spread reproductive investment over reproductive period might be a way to conserve resources and survive longer, thus maximising a female's diapausing-egg output. Overall, the different resource patterns of *B. plicatilis* and *B. manjavacas* females revealed relationships between lifespan and reproductive traits related to timing, amount and rate of offspring production, providing evidence of the trade-off between reproduction and future survival. The cost of reproduction in rotifers has been addressed using a variety of approaches (revised in Stelzer, 2005), but empirical data differentiating between rotifer female reproductive types have been missed.

Our primary focus was to detect differences in reproductive allocation between *B. plicatilis* and *B. manjavacas*. The relative fecundity, lifespan and rate of offspring production of sexual and asexual females may differ considerably among rotifer species (Gilbert 1993). In the present study, significant differences between species were found to mainly affect reproductive traits of sexual females.

Unfertilized sexual *B. manjavacas* females produced significantly more sons, and at an earlier age and greater daily rate, than did unfertilized sexual *B. plicatilis* females. On the other hand, fertilized *B. manjavacas* females tended to produce a lower maximum and a lower average number of diapausing eggs than fertilized *B. plicatilis* females. This difference was not statistically significant, most likely due to the smaller sample size of this group. However, the observed tendency is consistent with the higher relative resource allocation per *B. manjavacas* diapausing egg; more costly eggs might result in a lower fecundity of fertilized females of this species. Few studies have explicitly examined fertilized females in the genus *Brachionus* (Pourriot and Rieunier 1973; Xi et al. 2001; Gilbert 2010), and only two investigated species in the *B. plicatilis* complex. Interestingly, it was found that *B. manjavacas* (Snell 2014) produced a lower average number of diapausing eggs than *B. plicatilis* (Lubzens and Zmora 2003).

The differences in allocation to reproductive modes and life-history traits between female reproductive types of these cryptic species are striking. The studied *B. plicatilis* and *B. manjavacas* populations co-occur in the same habitat in Salobrejo Lake (Montero-Pau et al. 2011). This inland shallow brackish pond, similar to many other ponds in the western Mediterranean region (see Quintana Pou et al., 2006 for references; Walsh et al. 2014), has highly variable water inputs that cause sharp fluctuations in water level and physicochemical and biological composition. Randomness in these abiotic and biotic factors determines the variance in the growing season length of the rotifer populations and, hence, the uncertainty these populations must cope. The sexual reproduction pattern of *B. plicatilis*, which places a high investment into sex, is the pattern that is expected in highly uncertain or ephemeral environments. This strategy protects against unpredictably short growing seasons by ensuring diapausing-egg production (Serra and King 1999; Carmona et al. 2009; Franch-Gras et al. 2014).

The divergent strategies of these two cryptic species occupying the same habitat might be related to their tolerance of salinity. The range of *B. manjavacas* is wider and includes higher salinities than that of *B. plicatilis* (Montero-Pau et al. 2011; Gabaldón et al. 2015). Accordingly, *B. plicatilis* occurs in Salobrejo during low-range salinity periods (Montero-Pau et al. 2011). Salinity exhibits large seasonal and interannual variability in Mediterranean shallow lakes (e.g., Comín et al. 1992) and is very sensitive to water inputs and evaporation (Coops et al. 2003). Typically, these habitats have a much greater inter-annual variation in rainfall than in evaporation rates, the latter showing a marked seasonality (Vicente et al. 2006; Beklioğlu 2007). In other words, whereas periods of high-salinity predictably occur annually each summer, low-salinity periods associated with rainfall episodes will vary more randomly. Hence, we suggest that Salobrejo Lake might be a more uncertain habitat for *B. plicatilis* than for *B. manjavacas*, which could account for their different patterns of sexual reproduction observed in our experiment. Further monitoring of populations of these species in Salobrejo Lake might provide valuable information in this respect.

Diapausing-egg production is dependent upon the number of sexual females in the population. The higher the population density and mixis ratio at the onset of sexual reproduction, the more diapausing eggs will be deposited in the sediment bank. Assuming that population size increases during the growing season, *B. manjavacas* (which has a higher density threshold) will initiate sex later and at higher population density than *B. plicatilis*. However, the mixis ratio of *B. plicatilis* quadruples that of *B. manjavacas*; in addition to the tendency in fecundity of *B. plicatilis* fertilized females, this might act to partially equalize the diapausing-egg output of both species. Despite providing a greater potential of producing diapausing eggs, late onset of sex risks the possibility of resource shortage before diapausing eggs can be produced (Gilbert 2010). This could be particularly critical for *B. manjavacas* due to its higher relative resource allocation per diapausing egg, which suggest a possible adaptive explanation for the differences in reproductive traits of unfertilized sexual females we found between species. This suite of traits could allow *B. manjavacas* to complete diapausing-egg production sooner than *B. plicatilis*. On the other hand, timing of sex is expected to affect not only the number of diapausing eggs but also their diversity. Since clonal selection erodes genetic diversity during the growing season (Gómez and Carvalho 2000; Ortells et al. 2006) diapausing eggs produced early in the growing season will be more diverse than those produced later in the growing season. This expectation coincides with the higher genetic variation in the propensity to sex within the *B. plicatilis* population (i.e., the one initiating sex earlier), as revealed in our heritability analysis.

The presence of competitors can also affect the length of the period that a rotifer population will grow in the water column. An early onset of sex may ensure diapausing-egg production if the population is prone to competitive exclusion. *B. plicatilis* only performs better than *B. manjavacas* at low salinity and has been characterized as a species that opportunistically exploits salinity favourable periods by engaging in sex and producing diapausing eggs (Gabaldón et al. 2015).The higher investment in sex found in the present study implies a trade-off with the ability of *B. plicatilis* to offset competitively induced mortality through rapid asexual proliferation (King and Serra 1998). Consequently, its sexual pattern might accelerate *B. plicatilis* exclusion from the active planktonic growth phase. Accordingly, higher investment in sex by the inferior competitor has been reported to have this accelerating effect on exclusion in pairwise competition experiments between *B. plicatilis* and two other species in the cryptic species complex (Ciros-Pérez et al. 2002). Other recent studies have stressed the relationships between sexual reproduction and interspecific competition outcome in zooplankters (Aranguiz-Acuña and Ramos-Jiliberto 2014; Montero-Pau and Serra 2011; Gabaldón et al., unpublished data), showing that investment in sex and diapausing eggs can lead to different short-term outcomes. However, in fluctuating environments, the long-term competitive dynamics can strongly rely on the diapausing eggs recruited to the sediment (Montero-Pau and Serra 2011); diapausing eggs are insensitive to competition and may allow the persistence of competitors that otherwise would not coexist (i.e., storage effect;

Chesson, 2000). Hence, patterns of allocation to sexual reproduction by determining diapausing egg production can have both short-term and long-term consequences on competitive dynamics. This study, by expanding our knowledge of species-specific differences in reproductive traits, contributes to the increasing knowledge that life-history variation shaped by biotic and abiotic environmental factors has the potential to substantially alter the outcomes of interspecific interactions.

Acknowledgements

We thank S. Pérez for contributing to preliminary experiments and R. Ortells and E.M. García-Roger for their valuable comments and suggestions.

Funding

This work was supported in part by Spanish Ministry of Economy and Competitiveness, co-financed by FEDER funds [CGL2012-30779 toM.J.C., BES2010-036384 to C.G.].

References

Alekseev V, Lampert W (2001) Maternal control of resting-egg production in *Daphnia*. Nature 414:899–901.

Alekseev V, Ravera O, Stasio B de (2007) Introduction to diapause. Diapause Aquat. Invertebr. Theory Hum. Use. Springer Netherlands, pp 3–10.

Aparici E, Carmona M, Serra M (2001) Variability for mixis initiation in *Brachionus plicatilis*. In: Sanoamuang L, Segers H, Shiel RJ, Gulati RD (eds) Rotifera IX. Springer Netherlands, Dordrecht, pp 45–50

Aparici E, Carmona MJ, Serra M (1996) Polymorphism in bisexual reproductive patterns of cyclical parthenogens. A simulation approach using a rotifer growth model. Ecol Modell 88:133–142.

Aranguiz-Acuña A, Ramos-Jiliberto R (2014) Diapause may promote coexistence of zooplankton competitors. J Plankton Res 36:978–988.

Beklioğlu M (2007) Role of hydrology, nutrients and fish in interaction with global climate change in effecting ecology of shallow lakes in Turkey. Proc. Int. Congr. River Basin Manag. pp 583–595

Campillo S, García-Roger EM, Carmona MJ, et al. (2009) Selection on life-history traits and genetic population divergence in rotifers. J Evol Biol 22:2542–2553.

Campillo S, García-Roger EM, Carmona MJ, Serra M (2010) Local adaptation in rotifer populations. Evol Ecol 25:933–947.

Carmona M, Serra M (1991) Comparative total protein and demographic patterns of mictic and amictic female rotifers. Verh Internat Verein Limnol 24:2754–2759.

Carmona MJ, Dimas-Flores N, García-Roger EM, Serra M (2009) Selection of low investment in sex in a cyclically parthenogenetic rotifer. J Evol Biol 22:1975–83.

Carmona MJ, Dimas-Flores N, Montero-Pau J, Serra M (2010) Effect of experimental methodology on estimation of density at sex initiation in cyclically parthenogenetic rotifers. Hydrobiologia 662:131–139.

Carmona MJ, Gómez A, Serra M (1995) Mictic patterns of the rotifer Brachionus plicatilis Müller in small ponds. Hydrobiologia 313/314:365–371.

Caswell H (2006) Matrix Population Models. Encycl. Environmetrics

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Ciros-Pérez J, Carmona M, Serra M (2002) Resource competition and patterns of sexual reproduction in sympatric sibling rotifer species. Oecologia 131:35–42.

Clark MS, Denekamp NY, Thorne MAS, et al. (2012) Long-term survival of hydrated resting eggs from *Brachionus plicatilis*. PLoS One 7:e29365.

Comín F, Rodó X, Comín P (1992) Lake Gallocanta (Aragón, NE Spain): a paradigm of fluctuations at different scales of time. Limnetica 8:79–86.

Coops H, Beklioglu M, Crisman TL (2003) The role of water-level fluctuations in shallow lake ecosystems – workshop conclusions. Hydrobiologia 506-509:23–27.

Cox DR, Oakes D (1984) Analysis of survival data. CRC Press.

Efron B (1981) Nonparametric standard errors and confidence intervals. Can J Stat 9:139–158.

Epp R, Lewis Jr W (1979) Sexual dimorphism in *Brachionus plicatilis* (Rotifera): evolutionary and adaptive significance. Evolution (N Y) 919–928.

Fink P, Pflitsch C, Marin K (2011) Dietary essential amino acids affect the reproduction of the keystone herbivore *Daphnia pulex*. PLoS One 6:e28498.

Fontaneto D, Iakovenko N, Eyres I, et al. (2010) Cryptic diversity in the genus *Adineta* Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. Hydrobiologia 662:27–33.

Franch-Gras L, Montero-Pau J, Serra M (2014) The effect of environmental uncertainty and diapause investment on the occurrence of specialist and generalist species. Int Rev Hydrobiol 99:125–132.

Fussmann GF, Kramer G, Labib M (2007) Incomplete induction of mixis in Brachionus calyciflorus: patterns of reproduction at the individual level. Hydrobiologia 593:111–119.

Gabaldón C, Montero-Pau J, Serra M, Carmona MJ (2013) Morphological similarity and ecological overlap in two rotifer species. PLoS One 8:e57087.

Gabaldón C, Serra M, Carmona MJ, Montero-Pau J (2015) Life-history traits, abiotic environment and coexistence: The case of two cryptic rotifer species. J Exp Mar Bio Ecol 465:142–152.

Games PA, Keselman HJ, Rogan JC (1981) Simultaneous pairwise multiple comparison procedures for means when sample sizes are unequal. Psychol Bull 90:594–598.

García-Roger EM, Dias N, Carmona MJ, Serra M (2009) Crossed induction of sex in sympatric congeneric rotifer populations. Limnol Oceanogr 54:1845–1854.

Gilbert J (1993) Rotifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of invertebrates, vol VI, part A. Asexual propagation and reproductive strategies. Oxford and IBH. Publishing Company, New Delhi, pp 231–263.

Gilbert J (1974) Dormancy in rotifers. Trans Am Microsc Soc 93:490–513.

Gilbert J, Schröder T (2004) Rotifers from diapausing, fertilized eggs: unique features and emergence. Limnol Oceanogr 49:1341–1354.

Gilbert JJ (2004) Females from resting eggs and parthenogenetic eggs in the rotifer *Brachionus calyciflorus*: lipid droplets, starvation resistance and reproduction. Freshw Biol 49:1505–1515.

Gilbert JJ (1963) Mictic female production in the rotifer *Brachionus calyciflorus*. J Exp Zool 153:113–123.

Gilbert JJ (2003) Environmental and endogenous control of sexuality in a rotifer life cycle: developmental and population biology. Evol Dev 5:19–24.

Gilbert JJ (2010) Effect of food concentration on the production and viability of resting eggs of the rotifer *Brachionus*: implications for the timing of sexual reproduction. Freshw Biol 55:2437–2446.

Gilbert JJ, Diéguez MC (2010) Low crowding threshold for induction of sexual reproduction and diapause in a Patagonian rotifer. Freshw Biol 55:1705–1718.

Gómez A, Carvalho G (2000) Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. Mol Ecol 9:203–214.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution 56:1431–1444.

Gribble KE, Welch DBM (2013) Life-span extension by caloric restriction is determined by type and level of food reduction and by reproductive mode in *Brachionus manjavacas* (Rotifera). J Gerontol A Biol Sci Med Sci 68:349–58.

Guillard RRL, Ryther JH (1962) Studies of marine diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Gran. Can J Microbiol 8:229–239.

Hagiwara A, Hino A (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Hydrobiologia 186-187:415–421.

Hairston NG, Brunt RA Van, Kearns CM, Engstrom DR (1995) Age and survivorship of diapausing eggs in a sediment egg bank. Ecology 76:1706.

Hairston NG, Kearns CM (2002) Temporal dispersal: ecological and evolutionary aspects of zooplankton egg banks and the role of sediment mixing. Integr Comp Biol 42:481–91.

IBM Corp (2010) IBM SPSS Statistics for Windows, Version 19.0.

King C (1970) Comparative survivorship and fecundity of mictic and amictic female rotifers. Physiol Zool 43:206–212.

King CE, Serra M (1998) Seasonal variation as a determinant of population structure in rotifers reproducing by cyclical parthenogenesis. Hydrobiologia 387-388:361–372.

Louette G, De Meester L (2005) High dispersal capacity of cladoceran zooplankton in newly founded communities. Ecology 86:353–359.

Lubzens E, Zmora O (2003) Production and nutritional value of rotifers. In: Stottrup JG M LA (ed) Live Feed. Mar. Aquac. Blackwell Publishing, Oxford, pp 300–303.

Lynch M, Ennis R (1983) Resource availability, maternal effects, and longevity. Exp Gerontol 18:147–165.

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland.

Marcus NH, Lutz R V. (1998) Longevity of subitaneous and diapause eggs of *Centropages hamatus* (Copepoda: Calanoida) from the northern Gulf of Mexico. Mar Biol 131:249–257.

Martínez-Ruiz C, García-Roger E (2014) Being first increases the probability of long diapause in rotifer resting eggs. Hydrobiologia 1–11.

Maynard Smith J (1971) What use is sex? J Theor Biol 30:319–335.

Montero-Pau J, Gabaldón C, Carmona MJ, Serra M (2014) Measuring the potential for growth in populations investing in diapause. Ecol Modell 272:76–83.

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Ortells R, Gómez A, Serra M (2006) Effects of duration of the planktonic phase on rotifer genetic diversity. Arch für Hydrobiol 167:203–216.

Pearl R (1928) The rate of living. University Press, London.

Pfrender ME, Lynch M (2000) Quantitative genetic variation in *Daphnia*: temporal changes in genetic architecture. Evolution 54:1502–1509.

Pourriot R (1977) Food and feeding habits of Rotifera. Arch für Hydrobiol 8:243–260.

Pourriot R, Rieunier M (1973) Recherches sur la biologie des rotifères III. — Fécondité et durée de vie comparées chez les femelles amictiques et mictiques de quelques espèces. Ann Limnol 9:241–258.

Pourriot R, Rougier C (1991) Volumetric importance of the eggs in planktonic rotifers. Ann Limnol - Int J Limnol 27:15–24.

Pourriot R, Snell TW (1983) Resting eggs in rotifers. Hydrobiologia 104:213–224.

Quintana Pou X, Boix Masafret D, Badosa i Salvador A, et al. (2006) Community structure in mediterranean shallow lentic ecosystems: size-based vs. taxon-based approaches. Limnetica 25:303–320.

R Core Team (2014) R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria.

Schröder T (2005) Diapause in monogonont rotifers. Hydrobiologia 546:291–306.

Schwaegerle KE, McIntyre H, Swingley C (2000) Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. Evolution 54:452–461.

Schwartz SS, Hebert PDN (1987) Methods for the activation of the resting eggs of *Daphnia*. Freshw Biol 17:373–379.

Serra M, Carmona M (1993) Mixis strategies and resting eeg production of rotifers living in temporally-varying habitats. Rotifer Symp. VI. Springer Netherlands, pp 117–126.

Serra M, King C (1999) Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. J Evol Biol 12:263–271.

Serra M, Snell T (2009) Sex Loss in Monogonont Rotifers. In: Schön I, Martens K, Dijk P (eds) Lost sex. Springer Netherlands, Dordrecht, pp 281–294.

Serra M, Snell T, King C (2004) The timing of sex in cyclically parthenogenetic rotifers. In: Moya A, Font E (eds) Evol. from Mol. to Ecosyst. Oxford University Press, New York, pp 135–146.

Slusarczyk M (1995) Predator-induced diapause in daphnia. Ecology 76:1008.

Smith HA, Snell TW (2014) Differential evolution of lifespan and fecundity between asexual and sexual females in a benign environment. Int Rev Hydrobiol 99:117–124.

Snell TW (2014) Rotifers as models for the biology of aging. Int Rev Hydrobiol 99:84–95.

Snell TW (1987) Sex, population dynamics and resting egg production in rotifers. Hydrobiologia 144:105–111.

Snell TW, Carmona MJ (1995) Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. Environ Toxicol Chem 14:415–420.

Snell TW, Childress M (1987) Aging and loss of fertility in male and female *Brachionus plicatilis* (Rotifera). Int J Invertebr Reprod Dev 12:103–110.

Snell TW, King C (1977) Lifespan and fecundity patterns in rotifers: the cost of reproduction. Evolution 882–890.

Snell TW, Kubanek J, Carter W, et al. (2006) A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). Mar Biol 149:763–773.

Stearns S (1992) The evolution of life histories (POD). Oxford University Press, Oxford.

Stelzer C (2005) Evolution of rotifer life histories. In: Herzig A, Gulati RD, Jersabek CD, May L (eds) Rotifera X. Springer-Verlag, Berlin/Heidelberg, pp 335–346.

Stelzer CP, Snell TW (2003) Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. Limnol Oceanogr 48:939–943.

Tortajada AM, Carmona MJ, Serra M (2010) Effects of population outcrossing on rotifer fitness. BMC Evol Biol 10:312.

Vicente I, Moreno-Ostos E, Amores V, et al. (2006) Low predictability in the dynamics of shallow lakes: Implications for their management and restoration. Wetlands 26:928–938.

Walsh E, Schröder T, Wallace R, Rico-Martinez R (2009) Cryptic speciation in *Lecane bulla* (Monogononta: Rotifera) in Chihuahuan Desert waters. Verh Int Ver Theor Angew Limnol 30:1046–1050.

Walsh EJ, Smith HA, Wallace RL (2014) Rotifers of temporary waters. Int Rev Hydrobiol 99:3–19.

Wang J, Zamar R, Marazzi A, et al. (2010) Robust: Insightful robust library.

Weigensberg I, Roff D (1996) Natural heritabilities: can they be reliably estimated in the laboratory? Evolution 50: 2149–2157.

Xi Y, Huang X, Jin H (2001) Life history characteristics of three types of females in *Brachionus calyciflorus* Pallas (Rotifera) fed different algae. Rotifera IX. Springer Netherlands, pp 95–98

Zacharias I, Dimitriou E, Dekker A, Dorsman E (2007) Overview of temporary ponds in the Mediterranean region: threats, management and conservation issues. J Environ Biol 28:1–9.

Chapter 5

Long-Term competitive dynamics of two cryptic rotifer species: diapause and fluctuating conditions

Carmen Gabaldón, María José Carmona, Javier Montero-Pau and Manuel Serra

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A.O.22085, 46071, Valencia, Spain

Published in PLoS One, (2015), 10(4): e0124406

Abstract

Life-history traits may have an important role in promoting species coexistence. However, the complexity of certain life cycles makes it difficult to draw conclusions about the conditions for coexistence or exclusion based on the study of short-term competitive dynamics. *Brachionus plicatilis* and *B. manjavacas* are two cryptic rotifer species co-occurring in many lakes on the Iberian Peninsula. They have a complex life cycle in which cyclical parthenogenesis occurs with diapausing stages being the result of sexual reproduction. *B. plicatilis* and *B. manjavacas*are identical in morphology and size, their biotic niches are broadly overlapping, and they have similar competitive abilities. However, the species differ in life-history traits involving sexual reproduction and diapause, and respond differently to salinity and temperature. As in the case of certain other species that are extremely similar in morphology, a fluctuating environment are considered to be important for their coexistence. We studied the long-term competitive dynamics of *B. plicatilis* and *B. manjavacas* under different salinity regimes (constant and fluctuating). Moreover, we focused on the dynamics of the diapausing egg bank to explore how the outcome of the entire life cycle of these rotifers can work to mediate stable coexistence. We demonstrated that these species do not coexist under constant-salinity environment, as the outcome of competition is affected by the level of salinity –at low salinity, *B. plicatilis* excluded *B. manjavacas*, and the opposite outcome occurred at high salinity. Competitive dynamics under fluctuating salinity showed that the dominance of one species over the other also tended to fluctuate. The duration of co-occurrence of these species was favoured by salinity fluctuation and perhaps by the existence of a diapausing egg bank. Stable coexistence was not found in our system, which suggests that other factors or other salinity fluctuation patterns might act as stabilizing processes in the wild.

Key-words: *Brachionus*; complex life-cycle; diapausing egg bank; salinity

Introduction

Competitive theory predicts that the strength of competition involving two or more species is greater between closely related species than between distantly related species (Darwin 1859). According to the phylogenetic limiting similarity hypothesis (Violle et al. 2011), cryptic species –which have a close phylogenetic relationship and high morphological similarity– are expected to have similar ecological requirements (i.e.,, niche conservatism) (Wiens and Donoghue 2004; Losos 2008; Wiens et al. 2010; Violle et al. 2011) and, hence, to experience stronger competition and competitive exclusion (Hardin 1960). However, there are many examples of co-occurrence of cryptic species (e.g., Hebert et al. 2004; Stuart et al. 2006; Murray et al. 2007; King et al. 2008). Coexistence may be mediated by subtle niche differentiation, e.g., differential susceptibility to predation and/or resource partitioning (Ciros-Pérez et al. 2004; Nicholls and Racey 2006; Wellborn and Cothran 2007). In certain cases, this niche differentiation is based on small morphological differences that were neglected due to the past taxonomic status of the species (e.g., Ciros-Pérez et al. 2001). Moreover, coexistence of cryptic species would be favoured as a result of their shared features if these features translate into similar fitness. Similar fitness (an equalizing process) implies that weak stabilizing processes (niche differentiation) can be sufficient for stable coexistence (Chesson 2000; Adler et al. 2007).

The potential role of the organism's life cycle in promoting the coexistence of competitors has been demonstrated (Loreau and

Ebenhöh 1994; Moll and Brown 2008). For instance, investment in diapause by a superior competitor may provide an opportunity for coexistence to inferior ones (Montero-Pau and Serra 2011). Moreover, the occurrence of life cycle stages relatively free of competition –as are diapausing stages– is a necessary condition for the storage effect, a stable coexistence process based on environmental fluctuations (Chesson and Huntly 1989; Loreau and Ebenhöh 1994; Chesson 2000; Moll and Brown 2008; Montero-Pau and Serra 2011). A fluctuating environment may allow species coexistence if conditions favour different species at different times, indefinitely delaying the outcome of competitive exclusion.

In complex life cycles, the study of competitive dynamics and conditions for coexistence or exclusion becomes more difficult as fitness components relate to fitness in a complex way. This makes it difficult to predict the outcome of competition solely from observations of short-term dynamics such as somatic growth in plants or clonal proliferation in cyclical parthenogens. This problem might particularly affect congeneric species because their life cycle strategies may diverge (Fournet et al. 2000; Reznick et al. 2000).

Cyclically parthenogenetic rotifers have complex life cycles involving sexual and asexual reproduction. This is the case of *Brachionus plicatilis* and *B. manjavacas*, two cryptic species that belong to the *B. plicatilis* species complex. Their species status is guaranteed by prezygotic reproductive isolation which has been confirmed by mating experiments as well as by the lack of evidence for hybridization in field studies (Gómez and Snell 1996; Ortells et al.

2000). These two species co-occur in salt ponds of the Iberian Peninsula (Gómez et al. 2002; Gómez et al. 2007), with marked salinity fluctuations. In temperate climates, rotifer populations are active in the water column during a fraction of the year (the so-called growing season) even in permanent ponds. Thus, they periodically re-colonize the water column from banks of diapausing eggs, which are the product of sexual reproduction. During the growing season, their populations can fluctuate due to episodic investment in sex or due to exposure to substantial environmental variation. *B. plicatilis* and *B. manjavacas* have identical morphology and size (Campillo et al. 2005; Fontaneto et al. 2007) and consistently do not show ecological differentiation in biotic niche axes (Gabaldón et al. 2013). Hence, because these species inhabit a rather spatially homogeneous habitat, classical niche differentiation mechanisms that operate independently of environmental fluctuations (Tilman 1982; Chesson 2000), such as resource partitioning or differential susceptibility to predation, are unlikely to influence their coexistence (Gabaldón et al. 2013).

B. plicatilis and *B. manjavacas* differ in life-history traits (Gabaldón et al. 2015), such as their growth response to salinity and temperature (Montero-Pau et al. 2011), and their patterns of investment in diapause and hatching of diapausing eggs (Gabaldón et al. 2015). Single-species cultures have shown that the salinity tolerance ranges of the two species largely overlap (Montero-Pau et al. 2011). However, if salinity is low, *B. plicatilis* shows better performance and invests more in the production of diapausing eggs than *B. manjavacas*, even at the cost of decreasing its current proliferation rate.

Diapausing eggs of *B. plicatilis* also show a greater viability than those of *B. manjavacas*. Therefore, although *B. manjavacas* is predicted to be a better competitor (i.e., can tolerate a wide range of salinities), a growing season of low salinity might offer an opportunity window for *B. plicatilis* to produce the diapausing eggs needed for its persistence.

Previous empirical studies have analysed the importance of competition among coexisting cryptic species of zooplankters and the conditions to avoid exclusion (e.g., Seitz 1980; Hebert 1982; Spaak 1994; Boersma 1995; Ciros-Pérez et al. 2002; Ortells et al. 2003; Ciros-Pérez et al. 2004; Montero-Pau et al. 2011). Several of these studies emphasise the importance of changing environments and of disturbance for the maintenance of coexistence (Hebert 1982; Spaak 1994; Ciros-Pérez et al. 2001a; Ciros-Pérez et al. 2002; Ciros-Pérez et al. 2004). However, empirical evidence on the implications of fluctuations is still scarce. When dealing with changing environments, zooplankters have to face fluctuations of different magnitude scales: those fluctuations leading alternation between suitable and unsuitable periods, and those fluctuations causing variation in conditions when the habitat is suitable. The long-term competitive output will rely on the rates of diapausing stages production under a range of suitable conditions, as diapausing stages are the way to survive during unsuitable periods. The objective of the present work was to study experimentally the long-term competitive outcome of *B. plicatilis* and *B. manjavacas*. According with the habitat characteristics of these species we explored the effect of salinity fluctuations, comparing constant salinity and different patterns of time-varying salinity. In our experiments, periods of habitat unsuitability were simulated experimentally. In this way, investment in diapause had a role on the competitive experimental dynamics. Hence, it included the whole life cycle of these rotifers mimicking what is expected to occur in nature. Like in the wild, each suitable period the active population was initiated by the hatchlings of diapausing eggs produced in previous active growth periods (i.e., growing seasons). Two conditions for diapause duration were simulated. In one, no diapausing egg was allowed to survive longer that a single unsuitable period. Similarly to the terminology used for plant seed banks, this pattern was called 'without diapausing egg bank'. In the other condition, called 'with diapausing egg bank', survival of diapausing eggs for longer than a single unsuitable period might occur (Hairston Jr 2000). Our approach to the experimental study of zooplankton is novel. As far as we are concerned, it is the first time that competitive dynamic of two zooplanktonic species is explored not only during the growth of the active populations in the water column but also taking into account the effect of the production of diapausing eggs which remain in the sediment and are able to hatch in next growing seasons. Notice also that a putative stable species coexistence could rely on diapause.

We are interested in testing the following points. First, we hypothesise that the dynamics of competition depends on salinity; specifically the expectation is that low salinity favours *B. plicatilis* whereas high salinity is advantageous to *B. manjavacas*. This hypothesis is based on previous results on single-species cultures, and, if accepted, it would show the predictive power of single-species studies on the outcome of competition. Second, we want to know whether the divergence in diapause investment of these species (Gabaldón and Carmona 2015; Gabaldón et al. 2015), makes possible stable coexistence or, at least, delays exclusion. Third, we hypothesise that fluctuating salinity affects the competitive outcome, we expect coexistence or, at least, an extremely long time to exclusion.

Materials and Methods

Rotifer clone foundation and maintenance

Diapausing eggs were isolated from sediment samples collected in Salobrejo Lake (Eastern Spain: 38° 54.765' N, 1° 28.275' W) as described in Gabaldón et al. (2013). Permission for the field work was issued by the Junta de Comunidades de Castilla-La Mancha, Spain (Consejería de Agricultura y Medio Ambiente).Clones of *B. plicatilis* and *B. manjavacas* were established by asexual proliferation of the hatchlings from these diapausing eggs. Each clonal line was identified to the species level by PCR-RFLP (Gabaldón et al. 2013). For each rotifer species, 25 clones were founded and kept individually as stock cultures in 15 mL flasks at 25°C and a salinity of 10 g L^{-1}. Each week, one-half of each stock culture was renovated with fresh medium. This medium was f/2-enriched saline water (Guillard and Ryther 1962) prepared with commercial sea salt (Instant Ocean; Aquarium Systems) in which the microalga *Tetraselmis suecica* (Collection of Marine Microalgae of Marine Sciences Institute from Andalusia, Spain) was grown as a source of food for the rotifers. Microalgae were grown at 10 g L^{-1} salinity, 25°C, and constant aeration and illumination (PAR:

approx. 35 µmol photons m^{-2} s^{-1}). The pre-experimental and experimental rotifer culture media were the same as described for the stock cultures, except for salinity (see below).

Experiment 1: Interspecific competition and different salinities

Experimental populations of *B. plicatilis* and *B. manjavacas* were grown in competition for 24 day cycles in the lab. Field observations show that such short growing seasons can be common and are enough to observe both species in the water column in Salobrejo pond (Montero-Pau et al. 2011). Moreover, due to the short generation time of *Brachionus* females, this period is enough for diapausing egg hatching, to reach population density for sexual reproduction initiation, and to produce diapausing eggs (e.g., Hairston Jr 2000). The successive growing seasons were restarted from the hatchlings of diapausing eggs produced in the previous season, mimicking the process that occurs in a natural pond after desiccation and refilling. The experiment lasted for six cycles (growing seasons) or less if one species was not detected in the diapausing eggs produced in two consecutive cycles or no diapausing egg was observed after a cycle.

These competition experiments were performed under five different salinity regimes: (1) 10 g L^{-1} constant salinity (all growing seasons at 10 g L^{-1}); (2) 40 g L^{-1} constant salinity (all growing seasons at 40 g L^{-1}); (3) 10–40 g L^{-1} alternating salinity (first growing season at 10 g L^{-1}); (4) 40–10 g L^{-1} alternating salinity (first growing season at 40 g L^{-1}); and (5) 10 to 40 g L^{-1} increasing salinity within each growing season. Thus, the experiment consisted of 15 cultures (five salinity regimes × three replicates).

Before the experiments, pre-experimental cultures of each clonal line were started from the stock cultures and grown in culture medium at 10 and 40 g L^{-1} in 75 mL flasks (2 rotifer species × 2 salinities × 25 clonal lines = 100 pre-experimental cultures). Each clonal line was reared in exponential growth for at least three generations to control for maternal effects (e.g., Stelzer and Snell 2006) and to acclimate the rotifers to the experimental conditions. Juvenile females from these pre-experimental cultures were used to initiate experimental cultures. For each experimental culture (i.e., salinity regime and replicate), 10 juvenile females were randomly selected from each of the 25 clones of the two rotifer species and inoculated in 500 mL of culture medium containing 250,000 cells mL^{-1} of *T. suecica*. Experimental cultures were kept in 2000 mL plastic containers at 25°C in darkness and under constant agitation (60 rpm). Darkness was selected in order to avoid algae proliferation. Every six days, 500 mL of fresh culture medium containing 250,000 cells mL^{-1} of *T. suecica* at 10 or 40 g L^{-1} was added to each experimental culture. Thus, three feeding events within growing season were performed, and the final volume at the end of the growing season was 2000 mL. In the case of the fifth salinity regime (10 to 40 g L^{-1} increasing salinity), the salinity was increased in each feeding event, first at 20 g L^{-1}, then at 30 g L^{-1} and finally at 40 g L^{-1}.

At the end of every 24 day cycle (i.e., growing season), rotifer populations were filtered through 30 μm Nitex mesh, and the diapausing eggs produced were collected and isolated in Petri dishes that were exposed at 25°C to allow water evaporation. Dried diapausing eggs were kept at 4°C and in the dark for 28 days to ensure the completion of the obligate period of dormancy of *Brachionus* diapausing eggs (Hagiwara and Hino 1989). Then, one-half of these diapausing eggs were allocated to species identification, and the other half of the eggs were used to restart the following growing season. Immediately before the restart of a new growing season, the dried diapausing eggs were rehydrated and incubated for 72 hours at 25°C and constant illumination to induce hatching (Minkoff et al. 1983; Hagiwara et al. 1985). To improve hatching (Gabaldón et al. 2015), induction salinity conditions were set at 5 g L^{-1} for those eggs starting the next growing season at 10 g L^{-1} and at 20 g L^{-1} for those starting at 40 g L^{-1}. All hatchlings were used to initiate the new growing season. Because *B. plicatilis* has an extended diapausing egg hatching pattern (Gabaldón et al. 2015), the remaining unhatched diapausing eggs were monitored daily, and the new hatchlings were successively added to the experimental cultures during the first six days of each growing season (i.e., until the cultures were fed for the second time). The diapausing eggs remaining unhatched after this time were discarded.

Species identification of the harvested eggs at the end of each growing season was performed by PCR-RFLP as detailed in Gabaldón et al. (2013). In this case, DNA was extracted from individual diapausing eggs. For each replicate, up to 100 diapausing eggs were identified if possible. This identification allowed the proportion of diapausing eggs produced by *B. plicatilis* and *B. manjavacas* during the competition dynamics to be determined. For each regime, the analyses were performed until we determined that all the identified individuals belonged to the same species during two consecutive growing seasons. This information allowed us to assume that the other species had been excluded.

Experiment 2: Interspecific competition, fluctuating salinity, and diapausing egg bank

We used a design similar to that in Experiment 1 to test the effect of salinity fluctuations on the competitive dynamics between *B. plicatilis* and *B. manjavacas* under two different conditions for diapause duration (1) 'without diapausing egg bank' as in Experiment 1 and (2) 'with diapausing egg bank'. In this second experimental condition, in contrast to Experiment 1, the successive growing cycles were started with all of the diapausing eggs harvested in the previous season (i.e., non-hatched diapausing eggs were not discarded but were inoculated in the culture). As a result, recruitment from the diapausing eggs might occur at any time during the 24 days of a growing season (i.e., delayed hatching can take place). Additionally, the harvested eggs in a growing season might have not been produced in that growing season (i.e., eggs that could persist in diapause for longer than a single growing season are included). These are the conditions that characterize a diapausing egg bank (Hairston Jr 2000), as new clones can originate from diapausing eggs produced in any of the previous growing seasons. Three replicates of each condition were used (2 conditions × 3 replicates = 6 experimental cultures). Experimental culture populations were founded with hatchlings from diapausing eggs formed during Experiment 1 after single-species cultures had been achieved. For each replicate, 500 diapausing eggs of each species were mixed in a Petri dish with 50 mL of saline water at 10 g L^{-1} and allowed to hatch for 72 hours at 25°C and constant illumination.We selected the salinity regime of 10-40 g L^{-1} alternating salinity because Experiment 1 showed that a salinity fluctuation starting at low salinity favours *B. plicatilis*, which was commonly inferior competitor. The experiment ended when one of the two species was not detected in the diapausing eggs produced after two consecutive growing seasons.

To avoid fluctuations in food supply, each growing season was carried out in a chemostat with 950 mL of culture medium and a dilution rate of 0.15 day^{-1}. The inflow rotifer culture médium consisted of *T. suecica* continuously cultured in f/2 medium (see above) at 25°C and constant illumination.

During each growing season, the effluent volume from each experimental rotifer culture was recovered in a container with a filter of 30 µm Nitex mesh. This mesh size ensured that individuals and eggs were retained. Every 1 or 2 days, the material retained on the filters was transferred to Petri dishes with saline water, and the diapausing eggs were counted. These eggs were then dehydrated and kept at 4°C in darkness until the start of the next growing season. After 24 days (the duration of the growing season), the rotifer populations were filtered, and diapausing eggs were isolated, dehydrated and kept at 4°C in the dark for 28 days.

As in the first experiment, the next growing season was initiated after 28 days of diapause from hydrated diapausing eggs from the previous growing season, and one-half of the eggs were allowed to hatch. To obtain a successful hatching, the hatching salinity was 10 g L^{-1} if the next season was at 10 g L^{-1} and 20 g L^{-1} if it was at 40 g L^{-1}. The other half of the diapausing eggs were used for species identification based on PCR-RFLP. At least 100 diapausing eggs per growing season were identified.

Statistical analysis

Heterogeneity among replicates within a cycle and salinity condition was tested by chi-square test (counts of diapausing eggs identified as *B. plicatilis* and *B. manjavacas* × replicate). The association between the competitive output (winner species) in Experiment 1 and salinity regime (10 g L^{-1} constant, 40 g L^{-1} constant, fluctuating salinity) was tested by a chi-square test with Yate's correction for continuity. This analysis could not be performed for Experiment 2 due to low count number. In these tests, performed with R statistical software v. 2.12.1 (R Core Team 2014), *P*s were compute by Monte Carlo simulation.

In order to assess the effect of salinity on the diapausing egg production of competitors, we computed a rate of diapausing egg increase (log of diapausing eggs produced/eggs inoculated per growing season) for each replicate where both species were presented. The arithmetic difference between the rates of the competitors was used as the dependent variable in an ANOVA with salinity (10 g L^{-1}

vs. 40 g L^{-1}) as factor. These analyses were carried out using SPSS statistical software (IBM Corp 2010).

Results

In Experiment 1, the diapausing egg number averaged 1870 per growing period and replicate (n = 50; range: 0–3,970) (Fig. 5.1). The total number of diapausing eggs identified as *B. plicatilis* or *B. manjavacas* via molecular methods was 3,738. We followed an average of 3.27 cycles per replicate. This relatively low number of cycles was due primarily to the absence of one of the species in the diapausing eggs produced during two consecutive growing seasons. However, one of the replicates at 10-40 g L^{-1} alternating salinity was lost in its fourth cycle due to unknown causes after a 100% frequency of *B. plicatilis* frequency had been achieved, and another replicate maintained at the same conditions did not produce diapausing eggs. The proportion of *B. plicatilis* in the diapausing eggs produced after each growing season in response to the five salinity fluctuation regimes (Experiment 1) is shown in Fig. 5.2. If the salinity regime was constant throughout the growing seasons, one of the species was excluded in all replicates in a consistent way. At a lower salinity, *B. manjavacas* was excluded; at a high salinity, *B. plicatilis* was excluded. *B. plicatilis* was also excluded under a fluctuating-salinity regime in which the salinity of the first growing season was high (40-10 g L^{-1} treatment).

In the fluctuating salinity regime starting at low salinity and in the regime of increasing salinity during the growing season, the same

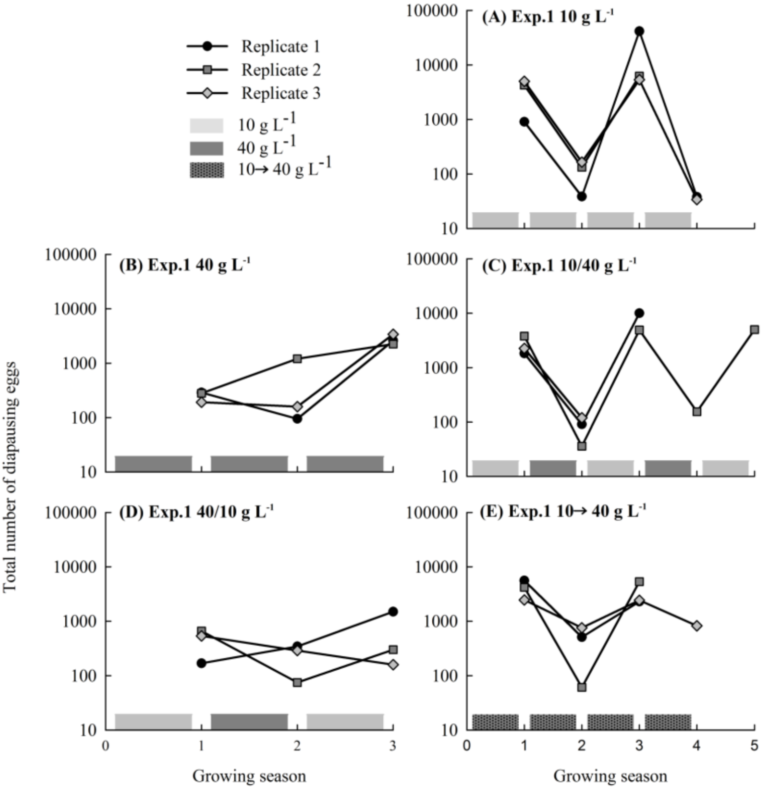


Figure 5.1. Total number of diapausing eggs produced during each growing season in response to the salinity fluctuation regime in both experiments. Experiment 1 (A) 10 g L^{-1} constant salinity (all growing seasons at 10 g L^{-1}); (B) 40 g L^{-1} constant salinity (all growing seasons at 40 g L^{-1}); (C) 10–40 g L^{-1} alternating salinity (the first growing season at 10 g L^{-1} followed by the second growing season at 40 g L^{-1}, and then again at 10 g L^{-1}, and so on); (D) 40–10 g L^{-1} alternating salinity (the same as (C) but starting at 40 g L^{-1}); and (E) 10 to 40 g L^{-1} increasing salinity (each growing season started at 10 g L^{-1}, but the salinity was gradually increased during the growing season until it reached 40 g L^{-1}); and Experiment 2 (F-G) 10–40 g L^{-1} alternating salinity regime in two conditions: without and with diapausing egg bank.

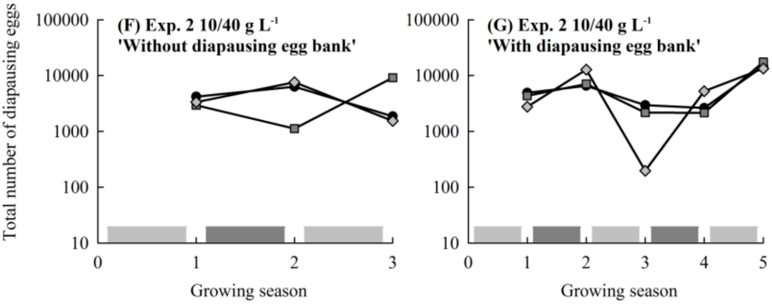


Figure 5.1. Continued.

species was not consistently excluded in the three replicates. In certain cases, we found that a species became extinct even after its competitor was extinct. In these cases, extinction occurred after a substantial decrease in the production of diapausing eggs. The competitive output was significantly associated to the salinity regime ($X^2 = 6.96$; *d.f.* = 2; $P = 0.03$).

In Experiment 2, the diapausing egg number averaged 5,717 per growing season and replicate (n = 24; range: 197-7,457) (Fig. 5.1). A total of 2,601 diapausing eggs were identified by molecular methods as *B. plicatilis* or *B. manjavacas*, and an average of four cycles per replicate were followed. The population densities were noticeably higher. No anomalies (e.g., a lack of diapausing egg production) occurred. Fig. 5.3 shows the proportion of *B. plicatilis* in the diapausing eggs after each growing season in response to a fluctuating salinity regime in the two conditions tested (with and without a diapausing egg bank). *B. plicatilis* was excluded by *B. manjavacas* in all replicates of both conditions. However, in one of the replicates of the

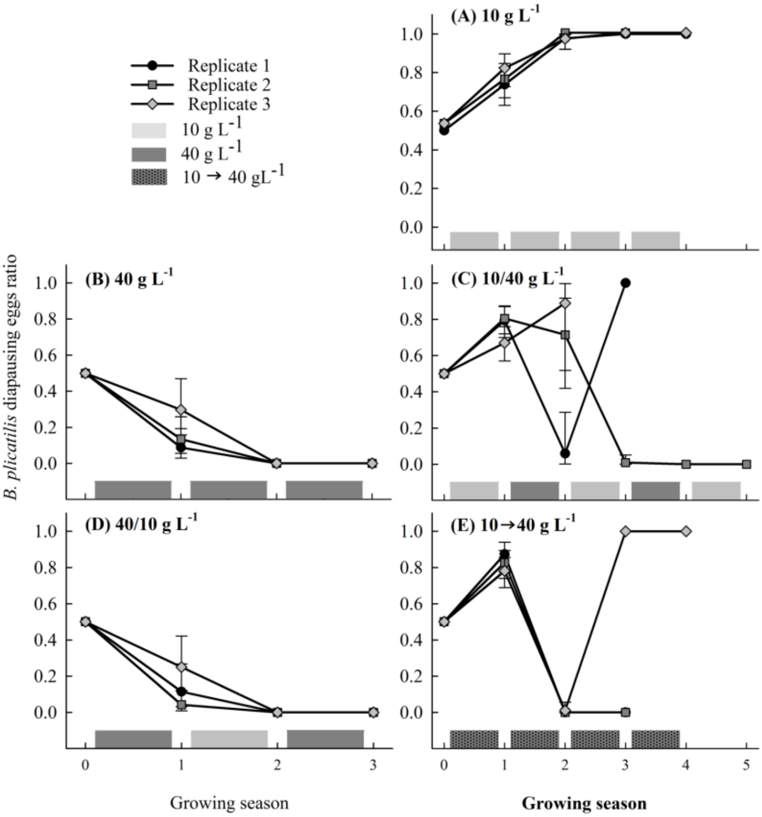


Figure 5.2. *B. plicatilis* diapausing egg ratio in the egg bank produced during each growing season inresponse to the salinity fluctuation regime in Experiment 1. (A) 10 g L^{-1} constant salinity (all growing seasons at 10 g L^{-1}); (B) 40 g L^{-1} constant salinity (all growing seasons at 40 g L^{-1}); (C) 10–40 g L^{-1} alternating salinity (the first growing season at 10 g L^{-1} followed by the second growing season at 40 g L^{-1}, and then again at 10 g L^{-1}, and so on); (D) 40–10 g L^{-1} alternating salinity (the same as (C) but starting at 40 g L^{-1}); and (E) 10 to 40 g L^{-1} increasing salinity (each growing season started at 10 g L^{-1}, but the salinity was gradually increased during the growing season until it reached 40 g L^{-1}). Vertical bars are ± SE.

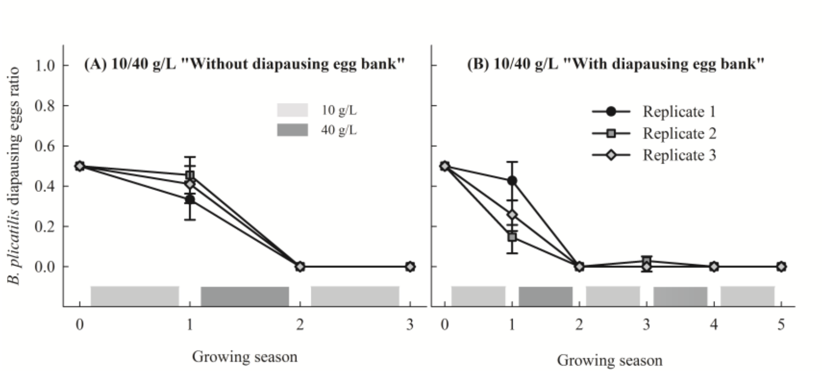


Figure 5.3. *B. plicatilis* diapausing egg ratio in the egg bank harvested after each growing season in Experiment 2. Rotifer populations were grown under a 10–40 g L^{-1} alternating salinity regime in two conditions (A) without diapausing egg bank and (B) with diapausing egg bank. Vertical bars are ± SE.

condition 'with diapausing egg bank' *B. plicatilis* was able to recover – 62 diapausing eggs were observed– in the third growing season, which corresponded to one of the low salinity periods (10 g L^{-1}) within the alternating salinity regime.

The replicates in each growing season, were frequently heterogeneous in their proportion of diapausing eggs belonging to each species. According to chi-squared test, heterogeneity was significant ($P< 0.05$) in seven out of the thirteen cases where the test could be applied.

The difference (*B. manjavacas* minus *B. plicatilis*) in the log rate of increase of diapausing eggs per growing season average 1.48 and 3.2 at 40 g L^{-1} salinity in Experiment 1 and 2 respectively, showing an advantage for *B. manjavacas*. The corresponding averages were -0.91 and -1.18 at 10 g L^{-1}. The effect of salinity on this difference was significant ($F = 21.8$; *d.f.* = 1,29; $P < 0.01$).

Discussion

B. plicatilis and *B. manjavacas* are a well-documented instance of the long-term coexistence of cryptic species (Gómez et al. 2002; Gómez et al. 2007), which is a more common phenomenon than previously thought. These species also provide a good example of how close phylogenetic relationship, especially if associated with an almost total morphological similarity, translates into niche overlap, particularly on the biotic axis (Gabaldón et al. 2013). However, the two species physiologically respond differently to temperatura and salinity (Gabaldón et al. 2015), two factors with low spatial variation but with large temporal fluctuation in their habitat (Comín et al. 1992). Additionally, each species has evolved different patterns for the trade-off involved in their complex life cycles. *B. plicatilis*, as an opportunistic species, invests earlier in diapause, producing diapausing eggs with a higher viability and a more extended hatching pattern than *B. manjavacas* (Gabaldón et al. 2015). In this study, we have proved that salinity affects the competitive outcome of *B. plicatilis* and *B. manjavacas*. The observed outcomes in constant salinity support our prediction made from single-species cultures where species performance was assessed (Montero-Pau et al. 2011; Gabaldón et al. 2015): at low constant salinity *B. manjavacas* is excluded whereas at high constant salinity *B. plicatilis* is excluded.

Competition experiments between zooplankters have commonly focused on the dynamics of interactions in the water column. In contrast, our experiment integrated the complete life cycle of the competitors from clonal propagation to sex initiation and the production of diapausing eggs, and from the production to the hatching of these eggs. This approach allowed us to explore whether exclusion in the water column implies competitive exclusion as a result of the long-term dynamics. Note that the merely observation of exclusión in the water column is not conclusive, as the excluded competitor could have sufficient time to produce a large number of diapausing eggs before its exclusion in the water column. As expected, our results show that exclusion occurs rapidly in constant salinity, in 2–3 growing seasons, despite the similar performance of both species when they grow without competitor. Interestingly, the prediction that *B. plicatilis* was superior to *B. manjavacas* at low salinity has been demonstrated in our experiments. This prediction was based not only on the former species growth rate, but also on its potential to invest in diapause at this salinity level. Previous works have reported that the potential growth rate (r_{pot}; (Montero-Pau et al. 2014), Gabaldón and Carmona 2015; Gabaldón et al. 2015), which is a measure of performance that takes into account the growth rate and the diversion of resources to diapause, was higher in *B. plicatilis* than in *B. manjavacas* at low salinity. Therefore, our results support that the higher performance of *B. plicatilis* at low salinity is used to produce diapausing eggs at the cost of decreasing its rate of asexual proliferation, i.e., its within-growing season growth rate. This strategy would cause the demographic dominance of *B. plicatilis* at low salinity in a sequence of growing seasons. Our results suggest that the dynamics of competition in the wáter column, if based solely on the rates of proliferation, could not be a good predictor of the longterm output because the competitor that was in the process of being excluded from the wáter column might be contributing more diapausing eggs in the sediment bank.

Under fluctuating salinity, our results showed that the dominance of one species over the other also tended to fluctuate. The fluctuations in the relative frequencies of the species were consistent with the species-specific preferences in terms of salinity: at low salinity, the recovery of *B. plicatilis* was more probable than at high salinity, which is consistent with the effect of salinity showed by ANOVA. Moreover, Experiment 1 showed that, on average, salinity fluctuations tended to delay exclusion by 0.6 growing seasons. Salinity fluctuation within a growing season had not a noticeably different effect when compared to salinity fluctuation among growing seasons. Under fluctuating conditions, *B. manjavacas* tended to be the persistent species. This finding is consistent with the observation that its performance was superior to that of *B. plicatilis* at intermediate fixed salinities in single-species cultures (Montero-Pau et al. 2011; Gabaldón et al. 2015). Although exclusión was observed in all the replicates, the persistent species varied among replicates within the same fluctuation regime. This heterogeneity is not surprising given the similar fitness of both species (Gabaldón et al. 2015).

The saline ponds where these rotifers co-occur in the Iberian Peninsula are habitats characterised by substantial salinity fluctuations within and between growing seasons (Comín et al. 1992; Montero-Pau et al. 2011). Hence, coexistence or, at least, an extremely long time to exclusion might be expected in our fluctuating experimental conditions, but these outcomes did not occur. The lack of long co-occurrence periods in our experiments can be a result of several factors. First, co-occurrence in the wild might be maintained by spatial, among-pond heterogeneity. Second, the fluctuation regime in our experiments might not be the same process that stabilizes coexistence in the wild. Other factors (correlated or not correlated with salinity) or other salinity fluctuation patterns might act as stabilizing processes. However, this seems to be unlikely because, the experimental salinity does have an effect on the species excluded and because the experimental salinity fluctuation range was observed in the natural habitats where the species occur (Montero-Pau et al. 2011; Gabaldón et al. 2015) and both species tolerate the extreme values in this range. Third, demographic stochasticity can play a role in preventing coexistence. Notice that the number of diapausing eggs fluctuated strongly over growing seasons, the number being rather low in some of them. This explanation is suggested by the heterogeneity among replicates and the variation in the outcome of competition in two of the experimental conditions. Additionally, we found a few replicates in which a population became extinct after the other species had already been excluded. Moreover, variation among replicates was higher in Experiment 1, where pulses in the food supply and the corresponding changes in population densities were expected to increase intrinsic stochasticity. Contrasting to experimental populations, natural rotifer populations are composed of extremely large numbers of individuals. For instance, a density up to 8 females mL^{-1} has been reported for the *B. plicatilis* population of Salobrejo pond (Montero-Pau et al. 2011) which considering the pond dimensions (Montes and Martino 1987) allow to roughly estimate a population size of 230×10^9 individuals for this species. Hence natural populations will have much lower demographic stochasticity. If so, small volume experiments could yield exclusion for conditions allowing coexistence in nature.

Experiment 2 incorporates a diapausing egg bank which involves the opportunity of delayed hatchlings of diapausing eggs produced in previous seasons to take part in the competitive dynamics. In nature, not all diapausing eggs produced in a growing season hatch when the conditions are favourable for population growth, instead they accumulate in the sediment forming diapausing egg banks (Kotani et al. 2001; García-Roger et al. 2006). The recovery of the outcompeted *B. plicatilis* only happened in one of the replicates carried out 'with diapausing egg bank'. This constitutes a weak evidence of the buffering effect of the diapausing egg bank, but it suggests that exclusion of *B. plicatilis* is more difficult when a bank is present as occur in natural habitats. Interestingly, this strategy agrees with the fact that diapausing eggs of *B. plicatilis* have lower degradation rates than those of *B. manjavacas* (Gabaldón et al. 2015), allowing them to overcome longer unsuitable periods.

This study is the first to empirically address the competitive dynamics of the cryptic species *B. plicatilis* and *B. manjavacas* by growing the two species together. It is also the first pair-wise competition experiment in rotifers that includes the whole life cycle. We have demonstrated that these competitors are unable to coexist in a constant environment. Their competitive capabilities are similar, and the result of the competitive dynamics between the two species is dependent on the salinity regime. We found that *B. plicatilis* has higher productivity in terms of diapausing eggs produced when it grows at 10 g L^{-1}, showing a faster growth at low salinity. The timespan of co-occurrence of these competing species increases due to salinity fluctuation and perhaps due to the existence of a diapausing egg bank. However, none of these effects is dramatic, and stable coexistence has not been found.We suggest that demographic stochasticity, which is also associated with fluctuations and is important in small scale experiments, is obscuring a stabilizing the effect of fluctuations based on niche differentiation.

Acknowledgments

This work was funded by a grant from the former Spanish Ministerio de Ciencia e Innovación (CGL2009-07364), co-financed with FEDER funds (EU). C.G. was supported by a fellowship from the Spanish Ministerio de Ciencia e Innovación (BES2010-036384).

References

Adler PB, Hillerislambers J, Levine JM (2007) A niche for neutrality. Ecol Lett 10:95–104.

Boersma M (1995) Competition in natural populations of *Daphnia*. Oecologia 103:309–318.

Campillo S, García-Roger EM, Martínez-Torres D, Serra M (2005) Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. Hydrobiologia 546:181–187.

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Chesson P, Huntly N (1989) Short-term instabilities and long-term community dynamics. Trends Ecol Evol 4:293–8.

Ciros-Pérez J, Carmona M, Serra M (2002) Resource competition and patterns of sexual reproduction in sympatric sibling rotifer species. Oecologia 131:35–42.

Ciros-Pérez J, Carmona MJ, Lapesa S, Serra M (2004) Predation as a factor mediating resource competition among rotifer sibling species. Limonology Oceanogr 49:40–50.

Ciros-Pérez J, Carmona MJ, Serra M (2001a) Resource competition between sympatric sibling rotifer species. Limonology Oceanogr 46:1511–1523.

Ciros-Pérez J, Gómez A, Serra M (2001b) On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. J Plankton Res 23:1311–1328.

Comín F, Rodó X, Comín P (1992) Lake Gallocanta (Aragón, NE Spain): a paradigm of fluctuations at different scales of time. Limnetica 8:79–86.

Darwin C (1859) On the Origin of Species. Murray, London.

Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. Hydrobiologia 583:297–307.

Fournet S, Stapel JO, Kacem N, et al. (2000) Life history comparison between two competitive *Aleochara* species in the cabbage root fly, *Delia radicum*: implications for their use in biological control. Entomol Exp Appl 96:205–211.

Gabaldón C, Carmona MJ (2015) Allocation patterns in modes of reproduction in two facultatively sexual cryptic rotifer species. J Plankton Res. 37: 429-440.

Gabaldón C, Montero-Pau J, Serra M, Carmona MJ (2013) Morphological similarity and ecological overlap in two rotifer species. PLoS One 8:e57087.

Gabaldón C, Serra M, Carmona MJ, Montero-Pau J (2015) Life-history traits, abiotic environment and coexistence: The case of two cryptic rotifer species. J Exp Mar Bio Ecol 465:142–152.

García-Roger EM, Carmona MJ, Serra M (2006) Patterns in rotifer diapausing egg banks: Density and viability. J Exp Mar Bio Ecol 336:198–210.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution 56:1431–1444.

Gómez A, Snell TW (1996) Sibling species and cryptic speciation in the *Brachionus plicatilis* species complex (Rotifera). J Evol Biol 9:953–964.

Guillard RRL, Ryther JH (1962) Studies of marine diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Gran. Can J Microbiol 8:229–239.

Hagiwara A, Hino A (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Hydrobiologia 186-187:415–421.

Hagiwara A, Hino A, Hirano R (1985) Combined effects of environmental conditions on the hatching of fertilized eggs of the rotifer *Brachionus plicatilis* collected from an outdoor pond. Nippon Suisan Gakkaishi 51:755–758.

Hairston Jr N (2000) Diapause and dormancy. In: Levin SA (ed) Encycl. Biodivers. Academic Press, New York, pp 79–84.

Hardin G (1960) The competitive exclusion principle. Science 131:1292–1297.

Hebert P (1982) Competition in zooplankton communities. Ann. Zool. Fennici. pp 349–356.

Hebert PDN, Penton EH, Burns JM, et al. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proc Natl Acad Sci U S A 101:14812–7.

IBM Corp (2010) IBM SPSS Statistics for Windows, Version 19.0.

King RA, Tibble AL, Symondson WOC (2008) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. Mol Ecol 17:4684–98.

Kotani T, Ozaki M, Matsuoka K, et al. (2001) Reproductive isolation among geographically and temporally isolated marine *Brachionus* strains. Hydrobiologia 283-290:446–447.

Loreau M, Ebenhöh W (1994) Competitive exclusion and coexistence of species with complex life cycles. Theor Popul Biol 46:58–77.

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol Lett 11:995–1003.

Minkoff G, Lubzens E, Kahan D (1983) Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. Hydrobiologia 104:61–69.

Moll JD, Brown JS (2008) Competition and coexistence with multiple life-history stages. Am Nat 171:839–43.

Montero-Pau J, Gabaldón C, Carmona MJ, Serra M (2014) Measuring the potential for growth in populations investing in diapause. Ecol Modell 272:76–83.

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Montes C, Martino P (1987) Las lagunas saladas españolas. Semin. sobre bases científicas para la protección los Humed. España. Real Acad. Ciencias Exactas, Físicas y Nat.

Murray TE, Fitzpatrick Ú, Brown MJF, Paxton RJ (2007) Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. Conserv Genet 9:653–666.

Nicholls B, Racey PA (2006) Habitat selection as a mechanism of resource partitioning in two cryptic bat species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. Ecography (Cop) 29:697–708.

Ortells R, Gómez A, Serra M (2003) Coexistence of cryptic rotifer species: ecological and genetic characterisation of *Brachionus plicatilis*. Freshw Biol 48:2194–2202.

Ortells R, Snell TW, Gómez A, Serra M (2000) Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. Arch für Hydrobiol 149:529–551.

R Core Team (2014) R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria.

Reznick D, Nunney L, Tessier A (2000) Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol Evol 15:421–425.

Seitz A (1980) The coexistence of three species of *Daphnia* in the Klostersee. Oecologia 45:117–130.

Spaak P (1994) Genetical ecology of a coexisting *Daphnia* hybrid species complex. University of Utrecht, The Netherlands.

Stelzer C-P, Snell TW (2006) Specificity of the crowding response in the Brachionus plicatilis species complex. Limnol Oceanogr 51:125–130.

Stuart BL, Inger RF, Voris HK (2006) High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. Biol Lett 2:470–4.

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press, Princeton.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Wellborn GA, Cothran RD (2007) Niche diversity in crustacean cryptic species: complementarity in spatial distribution and predation risk. Oecologia 154:175–183.

Wiens JJ, Ackerly DD, Allen AP, et al. (2010) Niche conservatism as an emerging principle in ecology and conservation biology. Ecol Lett 13:1310–24.

Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. Trends Ecol Evol 19:639–644.

Chapter 6

Life-history variation, environmental fluctuations and competition in ecologically similar species: modeling the case of rotifers

Carmen Gabaldón, María José Carmona, Javier Montero-Pau
and Manuel Serra

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, A.O.22085, 46071, Valencia, Spain

Accepted in Journal of Plankton Research

Abstract

Competition for resources can lead to species exclusion. However, this exclusion may be avoided if species present differential adaptation to physical environment. Empirical studies on competition became difficult when species are phylogenetically close and have complex life-cycles. This is the case of *B. plicatilis* and *B. manjavacas*, two cryptic rotifer species differing in their salinity niches and in life-history traits related to sex and diapause. These differences have been suggested to promote the stable co-occurrence observed in natural populations of these species. However, in a previous empirical study the outcome of competition between both species was always exclusion. Here, we theoretically explored the effect of complex life-history traits and salinity fluctuations on the long-term competitive output of *B. plicatilis* and *B. manjavacas*. We developed a model and simulated ecological scenarios combining different growing period lengths, levels of crossed induction of sex between species and salinity regimes. Results showed that a fluctuating salinity regime, an intermediate length of the growing seasons and a low level of crossed induction of sex are essential conditions to take into account to explain coexistence.

Key-words: competitive dynamic, diapause, growing season, crossed induction to sex, long-term coexistence

Introduction

Competition between species belonging to the same trophic level has been a common interest in ecological studies. Interspecific competition for the same limiting resources can lead to exclusion of some species, which is relevant to understand the geographic ranges of species and the limits of species richness in a locality. However, exclusion might be delayed if species have similar competitive abilities (Hubbell 2001) or avoided by a spatial separation if similar species present partially different preferences (Leibold 1991). Moreover, species may also differ in their adaptation to physical environment and succeed temporarily or even coexist synchronically if this environment fluctuates (Chesson and Huntly 1997; Lampert et al. 1997; Chesson 2000). Finally, coexistence of competing species may be based on species differences in life-history trait trade-offs instead of on niche differentiation (Lei and Hanski 1998; Wellborn 2002; Angert et al. 2009; Montero-Pau and Serra 2011).

Studies about interspecific competition of closely related species have increased in the last decades (Bickford et al. 2007). These species constitute an especially interesting case as they are expected to share similar ecological requirements (Futuyma and Mitter 1996; Webb 2000; Violle et al. 2011) and hence to experience a strong competition for resources. The closer the phylogenetic relationship between species is, the more likely competitive exclusion occurs (MacArthur and Wilson 1967; Violle et al. 2011). Cryptic species constitute a limit case; these are species phylogenetically closely related and so similar morphologically that traditionally have been considered as a single species (Bickford et al. 2007; Pfenninger and Schwenk 2007). Cryptic species are more common than previously thought and coexistence of species belonging to the same cryptic species complex has been demonstrated in both terrestrial and aquatic habitats (Bickford et al. 2007). Cryptic diversity has been discovered in the three major groups of zooplankters: copepods, cladocerans and rotifers (see reviews in Hebert 1998).

Brachionus plicatilis is a well-known rotifer cryptic species complex constituted by several species which commonly co-occur in Spanish ponds (Ortells et al. 2000; Gómez et al. 2002). Coexistence among some of the species of this complex having the greatest level of morphological differentiation (Ciros-Pérez et al. 2001b) has been explained as a result of seasonal succession (Gómez et al. 1995; Gómez et al. 1997; Ortells et al. 2003), and both differential use of resources and susceptibility to predation (Ciros-Pérez et al. 2001a; Lapesa et al. 2002; Ciros-Pérez et al. 2004). However, co-occurrence of other species of the complex like *B. plicatilis* and *B. manjavacas*, which are virtually identical in morphology and size (Campillo et al. 2005; Fontaneto et al. 2007), seems to be unlikely mediated by a different resource use and/or vulnerability to predation, as morphological similarity correlates with niche similarity in biotic axes (Gabaldón et al. 2013). Long-term co-occurrence of *B. plicatilis* and *B. manjavacas* has been suggested to be possible by both the salinity fluctuations and the temporality of their habitat based on their partially different response to salinity and diapause strategies (Montero-Pau et al. 2011; Gabaldón et al. 2015b). These two species have co-occurred in inland salt lakes of Eastern Spain since the Pleistocene (Gómez et al. 2002; Gómez et al. 2007). These habitats are commonly temporary (Comín et al. 1992; Rodriguez-Puebla et al. 1998) as they dry up and flood more or less regularly following rain and temperature patterns. Thus, salinity and hydroperiod (duration of the flooding) differ within and between years depending on the amount of precipitation.

Rotifers, as other zooplankters, face the recurrent unsuitability of their habitat by producing diapausing stages (McPeek and Kalisz 1998). *Brachionus* species are cyclical parthenogens (Wallace et al. 2006), which means that they proliferate asexually forming clones of females, but sexual reproduction is episodically induced in the population. In *Brachionus*, sex is induced by a density-dependent chemical signal released by the rotifers that accumulates in the environment during population growing. This infochemical is not completely species-specific for *B. plicatilis* and *B. manjavacas* and some levels of crossed induction of sex occur (García-Roger et al. 2009) despite these two species are reproductively isolated (Gómez and Snell 1996; Ortells et al. 2000; Suatoni et al. 2006). Once sex induction takes place, asexual females (amictic) begin to produce sexual (mictic) daughters, but asexual reproduction continues occurring concurrently. Sexual females produce either encased diapausing embryos or males, depending on whether they have been fertilized or not. These diapausing eggs, which are the product of sexual reproduction, sink to the bottom sediment of the pond where accumulate forming an egg bank. These eggs are able to remain in the sediment from years to decades (Kotani et al. 2001; García-Roger et al. 2006). When environmental conditions became suitable a fraction of the diapausing eggs hatch into asexual females that restart the cycle.

Diapause is a relevant fitness component in rotifers' life-cycle that allows to survive unsuitable periods, and to overcome habitat unpredictability (Pourriot and Snell 1983). Because diapausing egg production involves a great cost (Aparici et al. 1996; Gilbert and Schröder 2004; Clark et al. 2012), the timing of production and hatching of these resting stages is critical (Ellner 1997). An early investment in sex, and hence in diapause stages, is expected when environmental unpredictability is high, which would increase the likelihood of producing diapausing stages during the growing season (Carmona et al. 1995; Campillo et al. 2009; Gilbert and Diéguez 2010). *B. plicatilis* has been shown to have an earlier and higher investment in diapause than *B. manjavacas* (Gabaldón and Carmona 2015; Gabaldón et al. 2015b). Moreover, the diapausing eggs of the former had lower degradation rates and a hatching pattern extended in time (Gabaldón et al. 2015b). After these traits, *B. plicatilis* has been regarded as more opportunistic species than *B. manjavacas.* These differences have been suggested to have implications in their competitive dynamics and hence in their long-term coexistence. Accordingly, it might be expected that the length of the growing season could have relevant effects on the competitive outcome.

Studies on niche differentiation and life-history traits have showed that these two cryptic species differ in traits related to diapause (Gabaldón and Carmona 2015; Gabaldón et al. 2015b) and in their response to salinity (Montero-Pau et al. 2011; Gabaldón et al. 2015b). Hence, their response to the major abiotic factors is well known, showing that *B. plicatilis* has its highest performance at lower salinity that *B. manjavacas*. In a previous empirical study (Gabaldón et al. 2015a), *B. plicatilis* and *B. manjavacas* were grown together under several salinity regimes for rows of suitable and unsuitable periods in order to know their long-term persistence. This study confirmed the differential preferences regarding salinity observed by Gabaldón et al. (2015b). However, the competitive outcome was always the exclusion of one or the other species, depending on the salinity regime. This results contrast with observations of co-occurrence in the wild (Gómez et al. 2002; Gómez et al. 2007; Montero-Pau and Serra 2011).

The aim of the present work was to theoretically address the effect of complex life-history traits on the competitive output. Despite our study system could be regarded as a specific one, it has the advantage that has been thoroughly empirically characterized, which allows us to include the most relevant complexities in our study. These are: the complete life cycle of these rotifer species which includes phases of active growth and others of diapause; the species interactions in relation to both exploitative competition and crossed induction of sex; the succession of habitat suitable and unsuitable periods; and the changes in salinity.

Accordingly, we built a model to explore the long-term dynamics of these two cryptic rotifer species with similar niches inhabiting a temporary aquatic system. The simulated scenarios were the combinations of (1) different salinity regimes: constant salinity, fluctuating salinity between growing season and fluctuating salinity within growing season; (2) a range of growing season lengths and (3) different levels of crossed induction of sex. Our focus is to integrate available empirical data with complex features to explore stable coexistence of both rotifer species. This brings the opportunity to understand how empirical data estimated in the laboratory fit observations performed in the wild, and to explain disagreements between laboratory and field studies.

Methods

Model

The model is composed of two consumer rotifer species, which compete for a microalga as single resource. The model assumes rotifer habitat to be suitable temporarily, so that a row of suitable periods, called 'growing seasons' (i.e., the period of time in which a rotifer population is active in the water column), each one followed by an unsuitable period (e.g., drought), was simulated. The effect of growing season length (ω) was explored by performing simulations differing in ω. In each growing season, the active population is initiated by hatchlings from diapausing eggs produced in previous seasons. Not all diapausing eggs hatch necessarily in the ongoing growing season and they can survive several years. Thus, a diapausing egg bank in the lake sediment may exist. During the growing season, females proliferate asexually, compete by resources, and produce sexual offspring and diapausing eggs. This proliferation is assumed to be dependent on salinity, which could change within or among growing seasons following predictable patterns (see below).

Population dynamics in the water column (i.e., within growing season) was described by a modification of the model proposed by Serra and King (1999) where asexual (A_i) and sexual (S_i) female densities are modeled for two competing species (i =1, 2) as follow:

$$\frac{dA_i}{dt} = b_{i,o}(R)(1-m_i)A_i - q_{i,o}A_i + h_iD_i \qquad \text{(Eq. 1a)}$$

$$\frac{dS_i}{dt} = b_{i,o}(R)m_iA_i - q_{i,o}S_i \qquad \text{(Eq. 1b)}$$

here, *b* is the species-specific birth rate and is dependent on both the level of explicitly modeled resource (*R*) and the salinity (*o*), *q* is the species-specific death rate assumed to be density-independent but salinity-dependent, *m* is the mixis ratio or proportion of sexual females in the offspring of an asexual female and is a measure of investment in sexual reproduction, *D* is the diapausing egg density (see below) and *h* is the hatching rate of these eggs. It is assumed that *m* is dependent on the species density and *h* on the time from the starting of the growing season, in a species-specific way. For notation simplicity, dependence of *m* and *h* on other variables is dropped in Eq. 1. Note that sexual females, *S*, do not contribute with births to the active population (i.e., to dA/dt or dS/dt). Sexual females only contribute to the diapausing egg bank (see below) either indirectly (through males) or directly producing diapausing eggs. Male density is not modeled because males do not eat, and because it is expected that half sexual females produce males and half sexual females produce diapausing eggs (Aparici et al. 1998; Aparici et al. 2002). Thus, S determines the diapausing egg production. Both birth and death rates are considered to be equal for asexual and sexual females because these females are morphologically indistinguishable and they are assumed to be functionally equivalents except for their reproductive mode.

Resource ingestion rate is assumed to follow a Monod function,

$$I_i(R) = \frac{I_i^* R}{R + K} \qquad \text{(Eq. 2a)}$$

where I^* is the maximum ingestion rate, and K is the Monod constant; i.e., the resource concentration at which ingestion rate is half the maximum. We assumed the Monod constant being independent on the consumer species (Gabaldón et al. 2013). As above, the subscript i refers to the species. The birth rate is assumed to be proportional to the resource ingestion rate (I); that is, consumed resources are allocated to consumer population growth with a given conversion efficiency. We express the birth rate using b_i^* as a parameter:

$$b_{i,o}(R) = \frac{b_{i,o}^* R}{R + K} \qquad \text{(Eq. 2b)}$$

Notice that $b_{i,o}^*$ is the conversion efficiency times I_i^*.

The concentration of resource available in the medium tends to decrease depending on the ingestion rate and the number of individuals of both species, but the habitat supplies resources at a constant rate, R_s:

$$\frac{dR}{dt} = R_s - \sum (A_i + S_i) I_i(R) \qquad \text{(Eq. 3)}$$

The dynamics of the diapausing egg bank within the growing season is modelled as:

$$\frac{dD_i}{dt} = p_i S_i - (d_i + h_i) D_i \qquad \text{(Eq. 4)}$$

where p is the rate of diapausing egg production per sexual female and d is the death rate of these eggs.

The dynamics of the diapausing egg bank (D) during an adverse period is described by the equation:

$$D_i(0, t+1) = s_i D_i(\omega, t) \qquad \text{(Eq. 5)}$$

where the time at the two relevant time scales, within- and between-growing seasons, is explicitly stated. Here 0 and ω are respectively the start and the end of the growing season, t is the growing season number and s is the proportion of diapausing eggs surviving the adverse period.

Parameterization: fixed parameters

Table 6.1 shows the set of parameters whose values were keeping constant in all the simulations. Values of the rest of parameters were varied in order to know whether they affected the competition output. For some model parameters lacking of empirical estimations, we made derivations from other traits. Birth rates were derived from the growth and death rates estimated in Gabaldón et al. (2015b), as $b = r - q$. Resource-dependent birth rate and the algae ingestion rate are assumed to be proportional (see above) and to follow Monod equations. The corresponding Monod constant (K), which is common to both rates, was derived using the relationship $K = K_C - R(0)$ (mathematical proof in Appendix A), where K_C is the Monod constant for the Monod function relating the growth rate (r) to the resource level (R). We used the K_C estimate for *B. plicatilis* in Ciros-Pérez et al. (2001a). $R(0)$ is the resource level for zero growth and it was estimated using the maximum growth rate and the K_C reported in Ciros-Pérez et al. (2001a), and the death rate estimated in Gabaldón et al. (2015b). All data the corresponded to the rotifer species *Brachionus plicatilis* (see Appendix 6.A). To derive the maximum ingestion rates, we used the relationship $I^* = C_r^* K$, where K is the Monod constant in eq. 2 (see above) and C_r^* is the maximum clearance rate. The corresponding rates were estimated in Gabaldón et al. (2013). The relationship we used was derived from the definition of the clearance rate as the resource ingestion rate divided by the resource concentration. ($C_r(R) = I(R)/R$). According to Eq. 2a, $C_r(R)$ decreases with R, having a maximum at $R = 0$, so that $C_r^* = I^*/K$. The daily rate of diapausing egg production per sexual female (*p*) for each species was calculated from the net reproduction rate (*NRR*) (i.e., the average number of diapausing eggs produced per fertilized sexual female) and the longevity (*LON*) estimated in Gabaldón and Carmona (2015). Assuming that half of the sexual females are fertilized (Aparici et al. 1998; Aparici et al. 2002), $p = 0.5\ NRR/LON$. Resource supply and the duration of the adverse periods –which were needed to estimate diapausing egg survival in these periods– were chosen to allow comparison with the experiments performed in Gabaldón et al. (2015a).

Parameters explored

In order to gain insight into the effect of the salinity regime promoting the coexistence of both rotifer species, their long-term dynamics growing alone and together were explored under different salinity regimes. Moreover, a range of growing season lengths (ω) was studied, as well as different degrees of crossed induction of sex (Table 6.2). In total 858 different scenarios were explored (13 different salinity regimes × 6 growing season lengths × 11 values of crossed induction of sex) if the competitive dynamics were on focus, and 156 different scenarios were explored when the dynamics for each single rotifer species growing alone were simulated (2 rotifer species × 13 different salinity regimes × 6 growing season lengths; crossed induction of sex, dropped). By comparing the single-rotifer species *vs.* the two-rotifer species dynamics it was possible to find out whether lack of persistence was due to competition.

Table 6.1. Model parameters that were fixed in the simulations and their assumed values.

Symbol	Parameter	Value	
$b^*_{1,10}$	Maximum birth rate of *B. plicatilis* at 10 g L^{-1} salinity	0.9251 day^{-1}	(1)
$b^*_{2,10}$	Maximum birth rate of *B. manjavacas* at 10 g L^{-1} salinity	1.1657 day^{-1}	(1)
$b^*_{1,20}$	Maximum birth rate of *B. plicatilis* at 20 g L^{-1} salinity	1.0358 day^{-1}	(1)
$b^*_{2,20}$	Maximum birth rate of *B. manjavacas* at 20 g L^{-1} salinity	1.2254 day^{-1}	(1)
$b^*_{1,30}$	Maximum birth rate of *B. plicatilis* at 30 g L^{-1} salinity	1.2893 day^{-1}	(1)
$b^*_{2,30}$	Maximum birth rate of *B. manjavacas* at 30 g L^{-1} salinity	1.2152 day^{-1}	(1)
$b^*_{1,40}$	Maximum birth rate of *B. plicatilis* at 40 g L^{-1} salinity	0.8916 day^{-1}	(1)
$b^*_{2,40}$	Maximum birth rate of *B. manjavacas* at 40 g L^{-1} salinity	0.9248 day^{-1}	(1)
$q_{1,10}$	Death rate of *B. plicatilis* at 10 g L^{-1} salinity	0.20189 day^{-1}	(2)
$q_{2,10}$	Death rate of *B. manjavacas* at 10 g L^{-1} salinity	0.42978 day^{-1}	(2)
$q_{1,20}$	Death rate of *B. plicatilis* at 20 g L^{-1} salinity	0.425918 day^{-1}	(2)
$q_{2,20}$	Death rate of *B. manjavacas* at 20 g L^{-1} salinity	0.567559 day^{-1}	(2)
$q_{1,30}$	Death rate of *B. plicatilis* at 30 g L^{-1} salinity	0.726335 day^{-1}	(2)
$q_{2,30}$	Death rate of *B. manjavacas* at 30 g L^{-1} salinity	0.632440 day^{-1}	(2)
$q_{1,40}$	Death rate of *B. plicatilis* at 40 g L^{-1} salinity	0.4966 (day^{-1})	(2)
$q_{2,40}$	Death rate of *B. manjavacas* at 40 g L^{-1} salinity	0.4480 (day^{-1})	(2)

Table 6.1. Continued.

Symbol	Parameter	Value	
m_1	Mixis ratio, B. *plicatilis*	0 if $Ne_1 < 2.9$ ind·mL^{-1}; otherwise, 0.0346 Ne_1. Maximum value 0.1.	(3)
m_2	Mixis ratio, B. *manjavacas*	0 if $Ne_2 < 8.2$ ind·mL^{-1}; otherwise, 0.0093 Ne_2. Maximum value 0.1	(3)
Rs	Resource supply	30000 cells mL^{-1} day^{-1}	(4)
I^*	Maximum ingestion rate (consumer-independent)	0.2615 cells ind^{-1} day^{-1}	(5)
K	Monod constant for the ingestion and birth rates (consumer-independent)	736.8 cells mL^{-1}	(6)
p_1	Rate of diapausing egg production, B. *plicatilis*	0.1199 eggs per female^{-1} day^{-1}	(7)
p_2	Rate of diapausing egg production, B. *manjavacas*	0.0865 eggs per female^{-1} day^{-1}	(7)
d_1	Death rate of diapausing eggs, B. *plicatilis* (growing season)	0.002 days^{-1}	(8)
d_2	Death rate of diapausing eggs, B. *manjavacas* (growing season)	0.006 days^{-1}	(8)
s_1	Proportion of diapausing eggs surviving the adverse period, B. *plicatilis*	0.047	(9)
s_2	Proportion of diapausing eggs surviving the adverse period, B. *manjavacas*	0.135	(9)

Table 6.1. Continued.

Symbol	Parameter	Value	
h_1	Time-dependent diapausing egg hatching rate, *B. plicatilis* (t = 0-16 days)	0.0026, 0.2955, 0.0267, 0.0296, 0.0436, 0.0307, 0.0288, 0.0073, 0.0251, 0.0252, 0.0097, 0.0025, 0.0082, 0.0000, 0.0126, 0.0023, 0.0000 hatchings day^{-1}	(10)
h_2	Time-dependent diapausing egg hatching rate, *B. manjavacas* (t = 0-16 days)	0.0356, 0.4281, 0.0609, 0.0079, 0.0044, 0.0043, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000 hatchings day^{-1}	(11)

(1) Derived from the intrinsic growth and death rates estimated empirically in Gabaldón et al. (2015b).

(2) Gabaldón et al. (2015b).

(3) This defines a truncated linear relationship between mixis ratio and the effective density, Ne. $Ne_i = N_i + f_{i,j} N_j$ where N is the species density, the subscripts *i* and *j* refer species, and $f_{i,j}$ is a factor from 0 to 1 accounting for the crossed induction of sex. Values estimated in Gabaldón et al., (2015b). For each species, a different density threshold for sex initiation was assumed accordingly to Gabaldón and Carmona (2015).

(4) Chosen in order to compare simulation to experimental set up in Gabaldón et al. (2015a).

Table 6.1. Continued.

(5) Derived from clearance rate estimated empirically in Gabaldón et al., (2013) and the Monod constant (see parametrization section).

(6) Derived from Ciros-Pérez et al. (2001a) (see parametrization section).

(7) Derived from Gabaldón and Carmona (2015) (see parametrization section).

(8) Gabaldón et al. (2015b).

(9) As (7) and assuming a length of the adverse period chosen to compare simulations to experimental set up in Gabaldón et al. (2015a).

(10) Gabaldón et al. (2015b). Diapausing egg hatching rate is zero after 16 days since the beginning of the growing season.

Simulations: initial conditions and time explored

Simulations were run for 1000 growing seasons. At the starting of any growing season, the resource level was fixed at 2×10^5 cells mL^{-1}, and population density ($A+S$) was assumed to be zero, so that diapausing egg hatching was required to initiate population dynamics in the water column. The only exception was the first growing season where no previous diapausing egg reservoir was assumed, and populations started with 1 asexual female mL^{-1} per rotifer species. Data recorded were (1) density of diapausing eggs in the bank for each species (zero diapausing eggs at the end of a growing season means exclusion of the species); (2) the day of the exclusion if it occurs; and (3) the population density of each species over time within a growing season, which was only recorded in specific cases of coexistence to explore if species exclusion took place during the growing season.

Table 6.2. Parameters varied among simulations

Symbol	Parameter/Condition	Tested values
Salinity regime		
N	Salinity kept constant at N;	10, 20, 30 & 40 gL^{-1}
N1-N2	Cyclical oscillation of salinity N1 a season, N2 the next, N1 the next and so on.	10-20, 10-30, 10-40, 20-10, 30-10, 40-10
N1toN2	Salinity increase at constant rate, from N1 to N2, within each growing season	10to20, 10to30, 10to40
Other parameters		
ϖ	Growing season length	10, 18, 24, 50, 100 and 250 d
f	Crossed induction to sex	0 (absence of crossed induction), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 (complete crossed induction)

Analysis

Our assumed model is deterministic. However, as a number of conditions were simulated, we analysed the relevance of different factors mimicking statistical analysis. Differences on the competitive outcome (i.e., in the number of cases of coexistence or competitive exclusion) between constant and fluctuating salinity regimes were assessed by a chi-square test with Yate's correction for continuity. For those scenarios were competitive exclusion occurred, robust ANOVAs were applied on the time (i.e., growing season) for exclusion. In a first ANOVA the following salinity fluctuation regimes were compared: (a) among-growing season fluctuations with the first growing season at 10 g L^{-1} salinity, (b) among-growing season fluctuations with the first growing season at salinity >10 g L^{-1} and (c) within-growing season fluctuation, which starts at 10 g L^{-1} salinity. Moreover, two different ANOVAs were carried out to assess the effect of crossed induction of sex on the average growing cycle at which *B. plicatilis* and *B. manjavacas* were excluded. Chi-square tests with Yate's correction for continuity were also performed to assess (1) the differences in the competitive outcome (i.e., the number of cases of persistence) between absence of and complete crossed induction of sex for each species, and (2) the effect of the hydroperiod length on the competitive outcome (i.e., *B. manjavacas* exclusion, *B. plicatilis* exclusion or species coexistence). All the tests were performed with R statistical software v. 3.1.1 (R Core Team 2014).

Results

The effect of competition is evident when the number of scenarios in which one and/or the other species persisted is compared between single rotifer species and two-rotifer species dynamics, the later for the two extreme values of the crossed induction of sex (i.e., absent

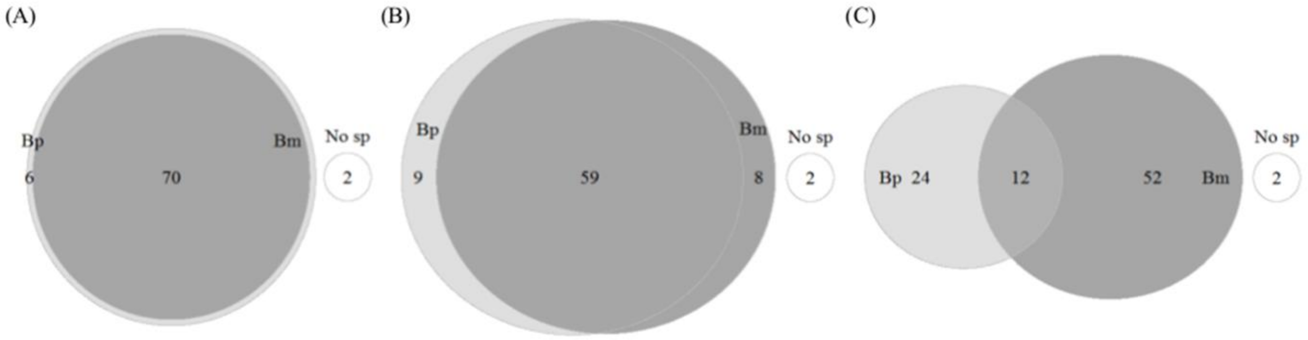


Figure 6.1. Venn diagrams representing the number of cases in which *B. plicatilis* (Bp) and/or *B. manjavacas* (Bm) persisted in (A) single species dynamics; (B) competitive dynamics without crossed induction of sex ($f = 0$) and (C) competitive dynamics with complete crossed induction of sex ($f = 1$).

and complete) (Fig. 6.1). *B. plicatilis* and *B. manjavacas* were able to persist in most of the simulated scenarios when growing alone, but competitive displacement happened when they were on interaction. This displacement was much stronger when there was a lack of specificity in the signal inducing sex (i.e., complete crossed induction of sex). The outcomes of all the simulations for the two-rotifer species dynamics showed that in 22 out of the 858 scenarios both species were excluded (Appendix 6.B). These scenarios were those with 30 or 40 gL^{-1} constant salinity and the shortest tested hydroperiod (10 days). Not surprisingly, results from single-rotifer species dynamics showed that in these salinity and hydroperiod conditions neither species could persist if they were growing alone (Appendix 6.B). Coexistence was found in 365 scenarios, whereas extinction of one of the species occurred in 471 scenarios –*B. plicatilis* became extinct in 332 and *B. manjavacas* in 139. *B. plicatilis* could persist if growing alone in all the salinity and hydroperiod conditions corresponding to these 332 cases where it was excluded. Thus, although its exclusion in some of the salinity and hydroperiod conditions might be dependent on the assumed value for the crossed induction of sex, these exclusions were caused by the presence of the competitor *B. manjavacas*. From the 139 scenarios in which *B. manjavacas* is the excluded species, nine are cases without crossed induction of sex, and in six out of these nine cases *B. manjavacas* could not persist growing alone. Surprisingly, a facilitation case was found. At 30-10 g L^{-1} salinity and 10-days hydroperiod *B. manjavacas* could not persist if growing alone but coexisted with *B. plicatilis* when

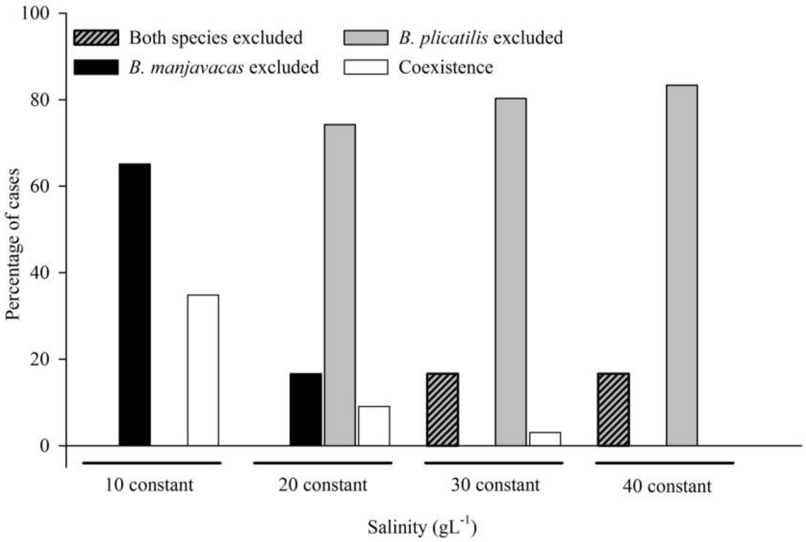


Figure 6.2. Percentage of cases for the four possible competitive outputs after simulations in each constant salinity regime.

crossed induction of sex ranged 0.1-0.5. According to simulation results, in a very large number of scenarios where only one of the pecies persisted (471= 332 resulting in *B. plicatilis* exclusion + 139 in *B. manjavacas* exclusion); the exclusion was due to the competitive interaction between the two species.

Coexistence of *B. plicatilis* and *B. manjavacas* was rarely found at constant salinity regimens (Fig. 6.2). At 10 g L^{-1} salinity, *B. plicatilis* was able to exclude *B. manjavacas* except in those scenarios with extended hydroperiod (ω =100 and 250 days) where coexistence occurred. In contrast, at the two higher salinities (i.e., 30 and 40 g L^{-1}) *B. manjavacas* excluded *B. plicatilis* in all simulations, except in those with the shortest hydroperiod where neither of the two species persisted. At

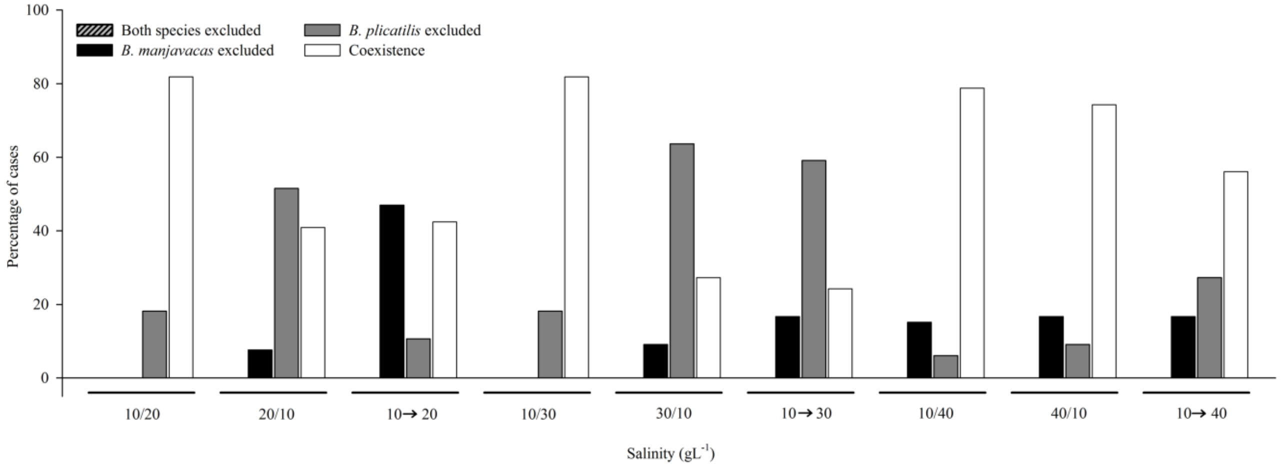


Figure 6.3. Percentage of cases of the four possible competitive outputs after simulations in each fluctuating salinity regime. The values separated by a slash are the pair of salinity values corresponding to two consecutive growing seasons, the pair being a repetitive oscillation in the simulations. Notice that the initial salinity varied. The values separated by an arrow are the salinity ends of a linear within-growing season increase.

constant 20 g L^{-1} salinity, exclusion was dependent on hydroperiod length: *B. plicatilis* was excluded by *B. manjavacas* when the hydroperiod was long, but this outcome reversed with short hydroperiods. Coexistence at 20 g L^{-1} constant salinity was only possible when there was not crossed induction of sex between species.

Fluctuation in salinity favored coexistence (Fig. 6.3). The percentage of cases where both species persist together was higher under fluctuating salinity regime (56.4%) than under constant salinity regime (11.7%) (X^2= 149.8, *df* = 1, $P < 0.001$). The highest percentages of coexistence were found in regimes with varying salinities between 10 and 20 g L^{-1} (i.e., 10-20, 20-10 and 10 to 20). The percentage of cases in which both rotifer species coexisted was 80% for this range of salinity fluctuation, whereas it was 50% and 40% for intermediate and high levels of fluctuation respectively (i.e., with varying salinities between 10 and 30 g L^{-1} and with varying salinities between 10 and 40 g L^{-1}, respectively). Moreover, the highest percentages of coexistence occurred at salinity regimes starting at 10 g L^{-1} and varying between growing seasons (Fig. 6.3). This initial salinity condition was the one favoring *B. plicatilis*. When coexistence was not found, an effect of the scheme of salinity fluctuation regime on the time for exclusion was observed (robust ANOVA for each species, both $P < 0.001$). Exclusion tended to occur later when the fluctuating regime started at the lowest salinity (data not shown).

Hydroperiod length also affected the competitive outcome (X^2 = 128.2, *df* = 4, $P < 0.001$) in a way suggesting species-dependence (Fig. 6.4). At short and intermediate hydroperiods *B. manjavacas* had a

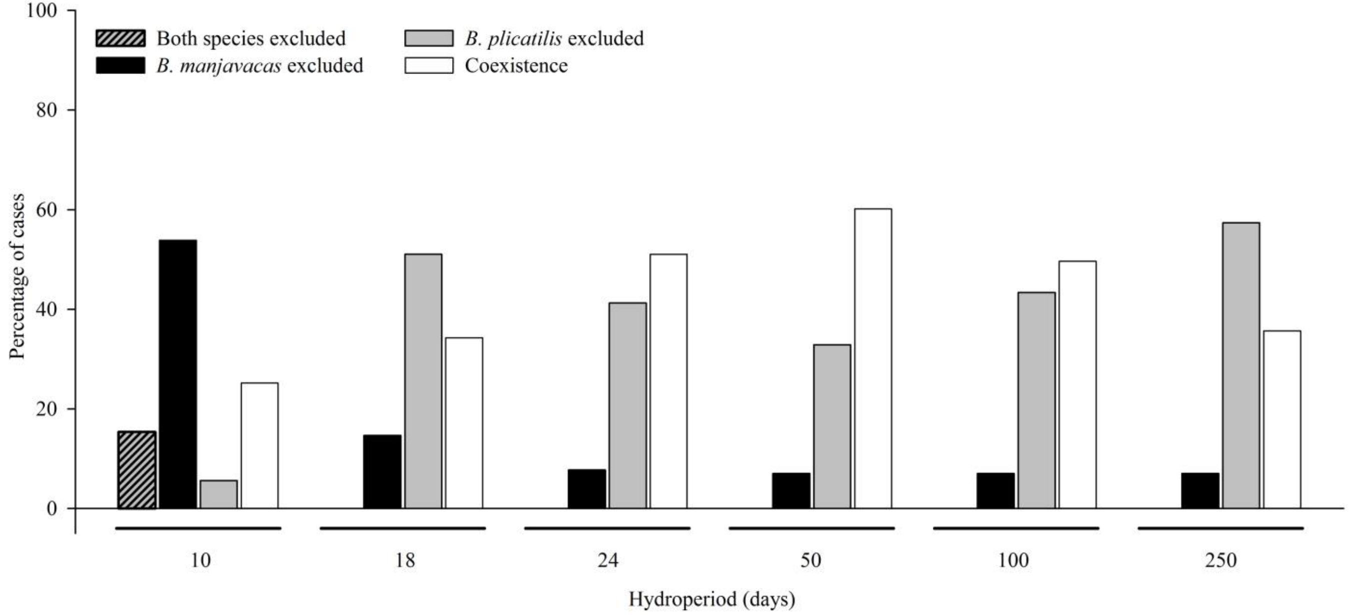


Figure 6.4. Percentage of cases of the four possible persistence outputs after competitive simulation for different duration of hydroperiod.

higher tendency to be excluded, increasing its competitive superiority at long hydroperiods. The opposite pattern (i.e., the observed competitive superiority of *B. plicatilis*) was observed at the shortest hydroperiod. As stated above, the shortest hydroperiod negatively affected to both species when salinity was constant and high (i.e., at 30 and 40 g L^{-1}) resulting in exclusion of both species. The most favorable scenario for the coexistence was a hydroperiod of 50-days. Moreover, the hydroperiod length effect was different for the two schemes of salinity fluctuation regime. While coexistence did not occur at long hydroperiods for among-growing-season fluctuation, *B. manjavacas* being favored, coexistence was promoted by long hydroperiods when within-growing-season fluctuation was assumed.

Crossed induction of sex affected rotifer species persistence, coexistence being promoted at low values of this parameter (Fig. 6.5). There were a few cases where crossed induction of sex did cause coexistence (see above). *B. plicatilis*, the species more negatively affected by that crossed induction, showed a significant four-fold increase in its percentage of exclusion when crossed induction of sex was complete as compared to the absence of crossed induction ($X^2 = 19.8$, $df = 1$, $P < 0.001$). Instead the percentage of cases in which *B. manjavacas* was excluded did not statistically differ between these two conditions of crossed induction of sex, ($X^2 = 2.3$, $df = 1$, $P = 0.133$). There was not statistical effect of the crossed induction values on the average growing season at with exclusion occurred for neither species (robust ANOVA for each species, both $P > 0.112$).

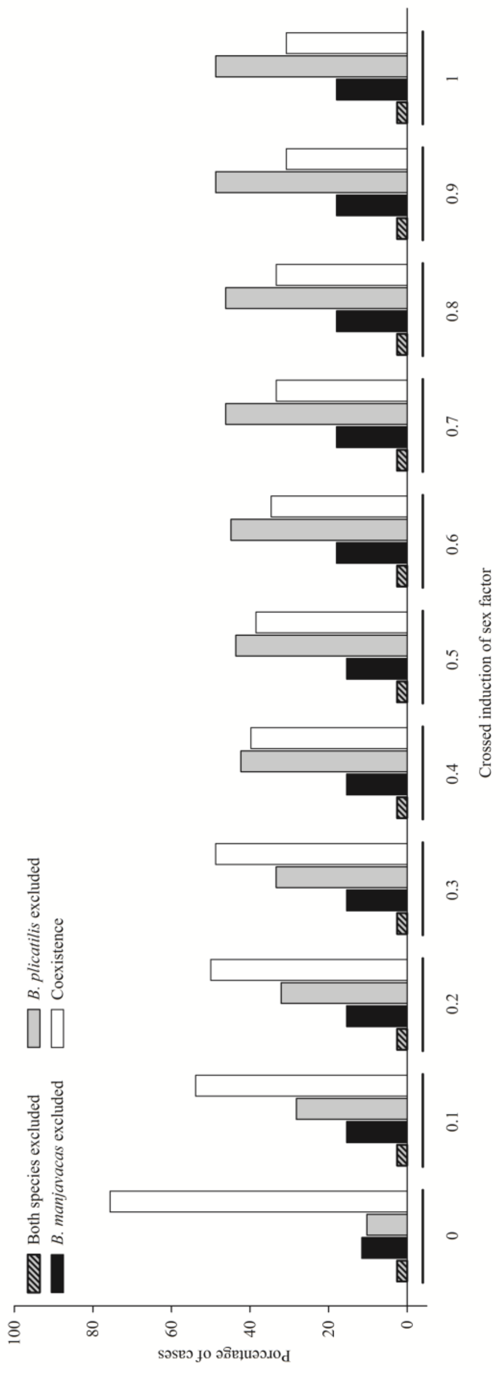


Figure 6.5. Percentage of the four possible persistence outputs after competitive simulation for different values of crossed induction of sex.

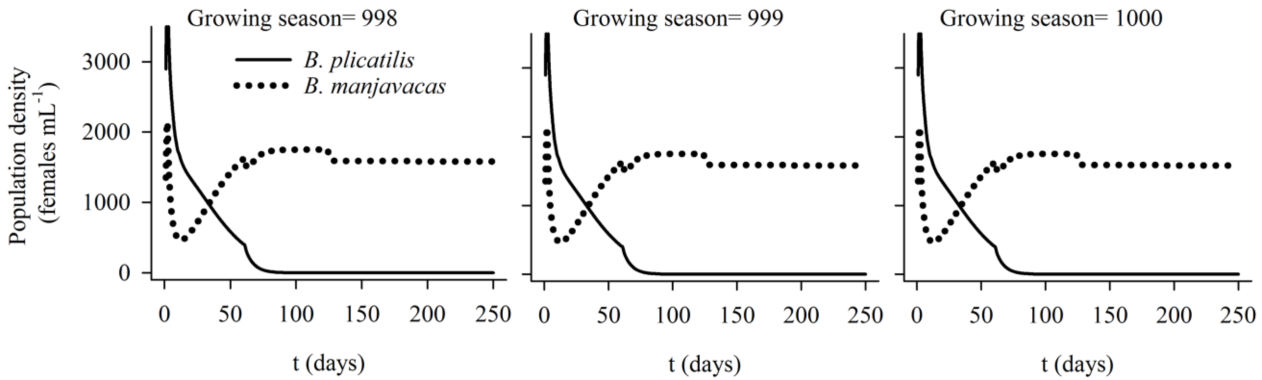


Figure 6.6. Population density of *B. plicatilis* and *B. manjavacas* during the last three cycles of the competitive dynamics simulated under a salinity fluctuating regime of 10 to 40 g L^{-1} and a hydroperiod of 250 days.

In general, the cases of long-term coexistence, which was assessed at the diapause stage, corresponded to cases where both species coexist within growing season along the complete hydroperiod. However, at the longest hydroperiods (i.e., 100 and 250 days) *B. plicatilis* was excluded from the water column within each growing season, but was able to produce enough diapausing eggs to allow its long-term coexistence (see Fig. 6.6 as an instance).

Discussion

B. plicatilis and *B. manjavacas* are a case study of coexistence and exclusion of species with remarkable morphological similarity. These two cryptic species have co-occurred in Easter Spain since the Pleistocene (Gómez et al. 2002; Gómez et al. 2007). They co-occur frequently in inland, shallow saline ponds, where spatial niche separation is unlikely, at least for long periods (Montero-Pau et al. 2011). However, an experimental system allowing their coexistence has not been found (Gabaldón et al. 2015a). After a series of studies, it is known that they have similar competitive abilities (Gabaldón et al. 2015a), they almost completely overlap in the biotic axis of their niches (Gabaldón et al. 2013), and they experience a strong exploitative competition (Gabaldón et al. 2015a). Consequently, their coexistence in a non-fluctuating, spatially homogeneous environment is rather difficult. However, these species differ in their response to salinity and temperature, as well as in life-history traits regarding timing and investment in sex and diapause (Montero-Pau et al. 2011; Gabaldón and Carmona 2015; Gabaldón et al. 2015b). Because their habitat shows strong seasonality and random temporal fluctuations (Comín et al. 1992), it has been hypothesized that a fluctuating regime of salinity is essential for their stable coexistence (Montero-Pau et al. 2011; Gabaldón et al. 2015b). Our theoretical model included a high number of parameters estimated for these species describing their life-history traits, their consumer behavior and their response to the physical environment. Competitive dynamics yielded the four possible qualitative outcomes –i.e., no species persists, one or the other species persists, and the two species persist–, which shows that the parametric space explored in our model and the fixed parameters assumed are not biased to find a given result. We also checked that the within growing season population densities are in the range of the observed values for these species (e.g., Carmona et al. 1995; Carmona et al. 2009; Montero-Pau et al. 2011). Most interestingly, we found that (1) a fluctuating salinity regime, (2) an intermediate length of the growing seasons and (3) a low level of crossed induction of sex are essential conditions to take into account to explain coexistence.

Pre-interactive and post-interactive niches

A first remarkable result was that these rotifers, even being fast colonizers, might be jeopardized by short growing seasons (e.g., very ephemeral ponds; 10 days in our simulations). The life cycle cannot be successfully completed and recruitment of diapausing eggs is too low. It has been reported that sexual reproduction could be very sensitive to non-optimal conditions (Snell and Carmona 1995; Snell et al. 1998). Interestingly, some differences between species were working. On one hand, under short hydroperiods B. *plicatilis* failed to persist at high salinity. Despite being an early-sex species, B. *plicatilis* performs better at low salinity (Gabaldón and Carmona 2015; Gabaldón et al. 2015b), which implies that at high salinity it was unable to proliferate enough to reach the population density to induce sex. On the other hand, B. *manjavacas,* which is a late-sex species, failed regardless the salinity, –suggesting that it had not enough time to produce diapausing eggs. This delay of B. *manjavacas* to initiate sex seems to make it more sensitive to salinity fluctuation, and is consistent with the idea that B. *plicatilis* is a more opportunistic species (Gabaldón et al. 2015b).

Most of the simulated physical environments allowed persistence of both species if growing alone (91%), implicating a high potential overlap between their pre-interactive abiotic niches. However, our model showed a strong reduction of niche overlap due to competitive exclusion when both species were interacting. For the set of conditions simulated, the reduction was not even; B. *plicatilis* having a higher reduction than B. *manjavacas*. B. *plicatilis* was favored by low constant salinity, and had its private niche at low salinity and relatively short hydroperiods; while the opposite held for B. *manjavacas*. This is consistent with the model assumptions, which in turn are based on experimental observations. The added information in the present study is that, when all the experimental observations were implemented in a rather complex life cycle, the dynamics fitted in the basic signatures previously observed in single species experiments.

Exclusion vs. coexistence

Surprisingly, we found that species coexistence is possible at constant salinity. That coexistence was observed (1) at the lowest constant salinity and long growing seasons for any value of crossed induction of sex, and (2) at medium-low constant salinity and very low crossed induction of sex. At these salinities, the species performances for clonal proliferation are very similar (Montero-Pau et al. 2011; Gabaldón et al. 2015b). This suggests that an equalizing mechanism exists, and that a weak stabilizing mechanism might be sufficient to allow stable coexistence (Adler et al. 2007). A possible explanation for the cases of coexistence at constant salinity is that formulated by Montero-Pau and Serra (2011), consisting in a negative effect of the investment in diapause on the competitive capability of the best-performing species. Allocation of resources in sex and diapause might cause demographic fluctuations allowing coexistence of opportunistic and non-opportunistic species. This effect is expected to be more important when crossed induction of sex is lower. If so, the differential patterns for sex induction of these two rotifer species might have a role on coexistence.

Salinity fluctuation dramatically promotes competitor coexistence, in line with the theory of the 'storage effect'. This theory states that fluctuations in the physical environment, when combined with species specialization regarding that environment and a life-cycle stage relatively free of competition (e.g., diapausing stages), are an important mechanism for coexistence of species with identical resource use (Chesson 2000). Most likely, in our model simulations we were observing the predictions of the storage effect theory, but combined with other mechanisms (e.g., Montero-Pau and Serra 2011). Crossed induction of sex generally acted as an antagonistic factor, making coexistence difficult. This is expectable because, if crossed induction of sex occurs, the species with a high population density can drive the species with low density excluded from the water column. Crossed induction of sex has been demonstrated in the species of the *B. plicatilis* complex, and different levels of species-specificity have been detected (Stelzer and Snell 2006; García-Roger et al. 2009). According to our results, *B. plicatilis* is more negatively affected by crossed induction of sex. This effect may be explained by the fact that *B. plicatilis* invests early in sex during the growing season, when its population density is low (Gabaldón and Carmona 2015; Gabaldón et al. 2015b). If it also responds to infochemicals released by *B. manjavacas*, *B. plicatilis* is likely to induce sex at too low population densities, which reduces both the number of sexual females and the chances of encounters among males and females resulting in a lower production of diapausing eggs (Serra and Carmona 1993; Serra et al. 2004).

Hydroperiod length had a non-monotonous effect on coexistence, this being more common at intermediate lengths. As expected, hydroperiod length favored *B. manjavacas*, the least opportunistic, the best competitor of both species, whereas it was detrimental for *B. plicatilis*. The corresponding patterns of sex and diapausing egg production explain well these contrasting effects.

Thus, not surprisingly, the performance of these species was more even at intermediate hydroperiod lengths.

Modeled and experimental dynamics

Contrarily to what we observed in our model simulations, microcosm competition experiments between *B. plicatilis* and *B. manjavacas* failed to find such coexistence, despite these experiments mimicked salinity fluctuations and rows of growing seasons each one followed by an adverse period (Gabaldón et al. 2015a). Nevertheless, this disagreement between experimental and theoretical results is partial. First, both experimental and modeled populations are unable to coexist under the most extreme constant salinity regimes (i.e., 10 and 40 g/L), and the same consistence was found for a regime of strong salinity fluctuation within a growing season (i.e., 10 to 40 regime). Second, the model –in some scenarios– and the experiments agree that, if fluctuating, the salinity at the onset of the dynamics can be important. When the salinity at the onset is the highest, *B. plicatilis* is the species more likely excluded. However, these coincidences do not dismiss the striking disagreement regarding coexistence, particularly because coexistence was found in simulations assuming both hydroperiod and regimes for salinity fluctuation almost identical to those set in the experiments with contrasting results.

A factor that may help to explain the disagreement between modeled and experimental populations is the importance of crossed induction of sex on the competitive interaction between species, and if this effect is symmetrical. Notice that intraspecific variation in the capability for that crossed induction exists (García-Roger et al. 2009). However, as these species co-occur in the wild (Montero-Pau et al. 2011), we favor the model results over the experimental ones. An important difference between microcosms on the one hand, and model and nature, on the other, is the role of demographic stochasticity. Demographic stochasticity, which was not included in the model, is not expected to be a strong factor in the wild, given the typical rotifer population sizes. For instance, it can be estimated that population size of *B. plicatilis* in Salobrejo pond is 2.3×10^{11} individuals (Montes and Martino 1987; Montero-Pau et al. 2011). However demographic stochasticity could be important in experimental systems, due to the culture volume. Similarly, diapausing egg banks are huge in the wild and in the model, but much smaller in the experimental populations. Hence, some of the observed extinctions in the experimental cultures could be random.

Our study has shown that case studies can be addressed theoretically after intense experimental studies so that almost all relevant parameters are known or a sound conjecture on their value can be performed. Our model did not intend to be general, but one focused on a specific case study. However, this allowed us to build a rich-detail model, which has been able to represent a system with species with complex life cycles living in a complex environment. Our simulations showed that this model clarifies how intrinsic and extrinsic complexities have to be combined in order to gain insight into the factors allowing species persistence in a competitive scenario.

Funding

This work was supported by Spanish Ministry of Economy and Competitiveness with a grant co-financed by FEDER funds to M.S. [CGL2009-07364] and a fellowship to C.G. [BES2010-036384].

References

Adler PB, Hillerislambers J, Levine JM (2007) A niche for neutrality. Ecol Lett 10:95–104.

Angert AL, Huxman TE, Chesson P, Venable DL (2009) Functional tradeoffs determine species coexistence via the storage effect. Proc Natl Acad Sci U S A 106:11641–5.

Aparici E, Carmona MJ, Serra M (1998) Sex allocation in haplodiploid cyclical parthenogens with density-dependent proportion of males. Am Nat 152:652–7.

Aparici E, Carmona MJ, Serra M (2002) Evidence for an even sex allocation in haplodiploid cyclical parthenogens. J Evol Biol 15:65–73.

Aparici E, Carmona MJ, Serra M (1996) Polymorphism in bisexual reproductive patterns of cyclical parthenogens. A simulation approach using a rotifer growth model. Ecol Modell 88:133–142.

Bickford D, Lohman DJ, Sodhi NS (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22:148–155.

Campillo S, García-Roger EM, Carmona MJ, et al. (2009) Selection on life-history traits and genetic population divergence in rotifers. J Evol Biol 22:2542–2553.

Campillo S, García-Roger EM, Martínez-Torres D, Serra M (2005) Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. Hydrobiologia 546:181–187.

Carmona MJ, Dimas-Flores N, García-Roger EM, Serra M (2009) Selection of low investment in sex in a cyclically parthenogenetic rotifer. J Evol Biol 22:1975–83.

Carmona MJ, Gómez A, Serra M (1995) Mictic patterns of the rotifer Brachionus plicatilis Müller in small ponds. Hydrobiologia 313/314:365–371.

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Chesson P, Huntly N (1997) The roles of harsh and fluctuating conditions in the dynamics of ecological communities. Am Nat 150:519–553.

Ciros-Pérez J, Carmona MJ, Lapesa S, Serra M (2004) Predation as a factor mediating resource competition among rotifer sibling species. Limonology Oceanogr 49:40–50.

Ciros-Pérez J, Carmona MJ, Serra M (2001a) Resource competition between sympatric sibling rotifer species. Limonology Oceanogr 46:1511–1523.

Ciros-Pérez J, Gómez A, Serra M (2001b) On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. J Plankton Res 23:1311–1328.

Clark MS, Denekamp NY, Thorne MAS, et al. (2012) Long-term survival of hydrated resting eggs from *Brachionus plicatilis*. PLoS One 7:e29365.

Comín F, Rodó X, Comín P (1992) Lake Gallocanta (Aragón, NE Spain): a paradigm of fluctuations at different scales of time. Limnetica 8:79–86.

Ellner S (1997) You bet your life: life-history strategies in fluctuating environments. Case Stud. Math. Model. Ecol. Physiol. cell Biol. Prentice Hall, Upper Saddle River, New Jersey, pp 3–24.

Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. Hydrobiologia 583:297–307.

Futuyma DJ, Mitter C (1996) Insect-plant interactions: the evolution of component communities. Philos Trans R Soc London Ser B Biol Sci 351:1361–1366.

Gabaldón C, Carmona MJ (2015) Allocation patterns in modes of reproduction in two facultatively sexual cryptic rotifer species. J Plankton Res. 37: 429-440.

Gabaldón C, Carmona MJ, Montero-Pau J, Serra M (2015a) Long-term competitive dynamics of two cryptic rotifer species: Diapause and fluctuating conditions. PLoS One 10:e0124406.

Gabaldón C, Montero-Pau J, Serra M, Carmona MJ (2013) Morphological similarity and ecological overlap in two rotifer species. PLoS One 8:e57087.

Gabaldón C, Serra M, Carmona MJ, Montero-Pau J (2015b) Life-history traits, abiotic environment and coexistence: The case of two cryptic rotifer species. J Exp Mar Bio Ecol 465:142–152.

García-Roger EM, Carmona MJ, Serra M (2006) Patterns in rotifer diapausing egg banks: Density and viability. J Exp Mar Bio Ecol 336:198–210.

García-Roger EM, Dias N, Carmona MJ, Serra M (2009) Crossed induction of sex in sympatric congeneric rotifer populations. Limnol Oceanogr 54:1845–1854.

Gilbert J, Schröder T (2004) Rotifers from diapausing, fertilized eggs: unique features and emergence. Limnol Oceanogr 49:1341–1354.

Gilbert JJ, Diéguez MC (2010) Low crowding threshold for induction of sexual reproduction and diapause in a Patagonian rotifer. Freshw Biol 55:1705–1718.

Gómez A, Carmona MJ, Serra M (1997) Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). Oecologia 111:350–356.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution 56:1431–1444.

Gómez A, Snell TW (1996) Sibling species and cryptic speciation in the *Brachionus plicatilis* species complex (Rotifera). J Evol Biol 9:953–964.

Gómez A, Temprano M, Serra M (1995) Ecological genetics of a cyclical parthenogen in temporary habitats. J Evol Biol 8:601–622.

Hebert PDN (1998) Variable environments and evolutionary diversification in inland waters. In: Carvalho GR (ed) Adv. Mol. Ecol. NATO Science Series, IOS Press, Amsterdam, pp 267–290.

Hubbell S (2001) The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton.

Kotani T, Ozaki M, Matsuoka K, et al. (2001) Reproductive isolation among geographically and temporally isolated marine *Brachionus* strains. Hydrobiologia 283-290:446–447.

Lampert W, Sommer U, Haney J (1997) Limnoecology: the ecology of lakes and streams. Oxford University Press, New York.

Lapesa S, Snell TW, Fields DM, Serra M (2002) Predatory interactions between a cyclopoid copepod and three sibling rotifer species. Freshw Biol 47:1685–1695.

Lei G, Hanski I (1998) Spatial dynamics of two competing specialist parasitoids in a host metapopulation. J Anim Ecol 67:422–433.

Leibold MA (1991) Trophic interactions and habitat segregation between competing Daphnia species. Oecologia 86:510–520.

MacArthur R, Wilson E (1967) The theory of island biogeography. Princeton University Press, Princeton.

McPeek M, Kalisz S (1998) On the joint evolution of dispersal and dormancy in metapopulations. Ergebnisse der Limnol 52:33–51.

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Montes C, Martino P (1987) Las lagunas saladas españolas. Semin. sobre bases científicas para la protección los Humed. España. Real Acad. Ciencias Exactas, Físicas y Nat.

Ortells R, Gómez A, Serra M (2003) Coexistence of cryptic rotifer species: ecological and genetic characterisation of *Brachionus plicatilis*. Freshw Biol 48:2194–2202.

Ortells R, Snell TW, Gómez A, Serra M (2000) Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. Arch für Hydrobiol 149:529–551.

Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evol Biol 7:121.

Pourriot R, Snell TW (1983) Resting eggs in rotifers. Hydrobiologia 104:213–224.

R Core Team (2014) R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria.

Rodriguez-Puebla C, Encinas AH, Nieto S, Garmendia J (1998) Spatial and temporal patterns of annual precipitation variability over the Iberian Peninsula. Int J Climatol 18:299–316.

Serra M, Carmona M (1993) Mixis strategies and resting eeg production of rotifers living in temporally-varying habitats. Rotifer Symp. VI. Springer Netherlands, pp 117–126.

Serra M, King C (1999) Optimal rates of bisexual reproduction in cyclical parthenogens with density-dependent growth. J Evol Biol 12:263–271.

Serra M, Snell T, King C (2004) The timing of sex in cyclically parthenogenetic rotifers. In: Moya A, Font E (eds) Evol. from Mol. to Ecosyst. Oxford University Press, New York, pp 135–146.

Snell T, Serra M, Carmona M (1998) Toxicity and sexual reproduction in rotifers: reduced resting egg production and heterozygosity loss. Genet. Ecotoxicol. pp 169–185.

Snell TW, Carmona MJ (1995) Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. Environ Toxicol Chem 14:415–420.

Stelzer C-P, Snell TW (2006) Specificity of the crowding response in the Brachionus plicatilis species complex. Limnol Oceanogr 51:125–130.

Suatoni E, Vicario S, Rice S, et al. (2006) An analysis of species boundaries and biogeographic patterns in a cryptic species complex: the rotifer –*Brachionus plicatilis*. Mol Phylogenet Evol 41:86–98.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Wallace R, Snell T, Ricci C, Nogrady T (2006) Rotifera 1: Biology, Ecology and Systematics. Backhuys Publishers, Leiden.

Webb CO (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am Nat 156:145–155.

Wellborn G (2002) Trade-off between competitive ability and antipredator adaptation in a freshwater amphipod species complex. Ecology 83:129–136.

Chapter 7

Final remarks and conclusions

How biological diversity is generated and maintained is a fundamental question in ecology and evolutionary biology (Pressey et al. 2003; Chase and Myers 2011). Traditionally, it has been considered that plant diversity is more difficult to explain than animal diversity (Hutchinson 1959; Huston 1979), as plants use the same resources. However, the discovery in the last two decades of similar animal species, being cryptic species an extreme case (Losos 2008; Violle et al. 2011), with similar ecological niches has challenged this classical idea (McPeek and Gomulkiewicz 2005; Leibold and McPeek 2006).

Niche retention (or niche conservatism) is strong among congeneric species, which makes more difficult to explain their persistence and contribution to diversity, in the Earth and particularly in single locations. Not surprisingly, some ideas initially developed to explain plant coexistence have been applied to explain the diversity of ecologically similar animals. This is the case of the mechanistic Tilman's models (Tilman 1982) initially focused on both plants and phytoplankton species (Tilman 1982; Sommer 1989; Huisman et al. 1999), but afterwards applied to, for example, rodents, snails or zooplankton (Abramsky and Rosenzweig 1984; Rothhaupt 1988; Boraas et al. 1990; Kreutzer and Lampert 1999; Byers 2000; Ciros-Pérez et al. 2001a). Particularly in rotifers, Rothhaupt (1988), Boraas et al. (1990) and Ciros-Pérez et al. (2001a) have predicted the competitive outcome of congeneric species using these models. Additionally, theoreticians have performed a long lasting effort to delineate new mechanisms making compatible the expected competition between similar animal species and their observed co-occurrence, and hence biodiversity maintenance (Shmida and Ellner 1984; Zhang and Hanski 1998; Chesson 2000; Montero-Pau and Serra 2011).

But, what 'similar' species means? Usually the first information collected on a species is its morphological description and sampling location. Thus, 'similar' usually means 'morphologically similar' and 'ecologically similar', the latter being stated frequently in gross terms (e.g., habitat sharing), and more rarely in a more technical way (e.g., overlap of their pre-interactive niches). The species studied in this thesis fit in this conception of 'being similar'. Firstly, *B. plicatilis* and *B. manjavacas* are two rotifer species almost morphologically identical (Campillo et al. 2005; Fontaneto et al. 2007). Secondly, both rotifer species co-occur in a number of shallow, saline ponds in the Iberian Peninsula (Gómez et al. 2002; Gómez et al. 2007; Montero-Pau et al. 2011). How theses similarities are relevant to predict competition? Firstly, ecological similarity provides opportunities to interact by sharing the distribution ranges. This is the case of our model species, which, as proved in this thesis overlap their ecological tolerance ranges (salinity, temperature), resource use (food intake) and geographical distribution. Secondly, morphological similarity is related to (1) resource use and (2) similar vulnerability to the same predators (Chapter II). However, morphological similarity and apparent complete biotic overlap may not necessarily lead to strong competitive interaction. For example, a differential physiological tolerance of closely related consumers to chemical defenses in their resources might be decoupled from a morphological divergence (DeMott 1999). This is suggested by differential tolerance to toxicants between congeneric species (Sturmbauer and Opadiya 1999; Umina and Hoffmann 1999; Mcgovern and Hellberg 2003). As another instance, different behaviors can cause differences in the vulnerability to predation without differentiation in morphology (Losey and Denno 1998). In our study case, we found that the low morphological differentiation between *B. plicatilis* and *B. manjavacas* translates into very low niche differentiation regarding biotic axes (Chapter II). This contrasts with previous reports on other species belonging to the same cryptic complex. This discrepancy is likely resulting from the fact that in the latter case, species present differences in body size that are involved in their differential resource use and vulnerability to predators (Ciros-Pérez et al. 2001a; Ciros-Pérez et al. 2001b; Ciros-Pérez et al. 2004; Lapesa et al. 2004). Therefore, the critical challenge to understand co-occurrence of 'similar' species is to identify in what way, if any, these species differ. If these differences exist, then there are chances that trade-offs between their performances could emerge enabling their stable coexistence (Chesson 2000).

One of the major changes in ecology during the last decades of the previous century was the transition from an equilibrium, spatially homogeneous approach for the population and community dynamics to a new paradigm that, besides equilibrium, includes the role of disturbances, environmental fluctuations and spatial heterogeneity in shaping critical ecological features –as the population density, the species richness or the species composition (Hutchinson 1961; Connell 1978; Holt 1984). This conceptual revolution has affected many areas of ecology, and not surprisingly has had effects on the ideas to explain the co-persistence of similar species. For instance, trade-offs between different aspects of species performance combined with habitat heterogeneity may cause each competitor being superior in a locality or microhabitat, and then species co-occurrence even at small spatial scale, could be explained by source-sink models (Levin 1974; Shmida and Ellner 1984; Holt 1993). Moreover, species in a guild could be ecologically equivalent, with similar fitness in their complete ecological spectrum, so that their dynamics would follow a slow random walk to extinction of all species except one (Hubbell 2001). None of these two explanations seems to be likely for the two species studied in this thesis. Firstly, the co-occurrence of *B. plicatilis* and *B. manjavacas* is suggested to be ancient, since the last glaciations in the Pleistocene (Gómez et al. 2000; Gómez et al. 2007), so a transient co-occurrence seems to be an unlikely explanation. Secondly, the ponds these species inhabit are shallow, lacking spatial heterogeneity, which makes improbable a co-occurrence mediated by habitat heterogeneity. Moreover, both rotifer species have been observed together in the water column (Montero-Pau et al. 2011).

A coexistence mechanism that is increasingly gaining attention is the so-called 'storage effect' (Chesson 2000). This mechanism states that competitive exclusion of species may be avoided by the combination of fluctuations in the physical environment, a differential ecological specialization regarding that environment, and the investment in a life cycle stage relatively free of competition (e.g., diapausing stages). Our model study seems to fit in this explanatory mechanism because the physical environment experienced by *B. plicatilis* and *B. manjavacas* fluctuates (Comín et al. 1992; Rodriguez-Puebla et al. 1998); there is evidence of specialization of both species to one of the main abiotic factors in this environment (i.e., salinity; Montero-Pau et al. 2011; Chapter III) and their reproductive cycle involves the investment in persistent long-lived diapausing eggs. As we have discussed, the differential response to salinity suggests that a fluctuating environment may allow long-term co-occurrence of both species. In fact, we have proved that under a constant environment one or the other species is consistently excluded from the competitive dynamics (Chapter V). However, the experimental competitive dynamics we performed in microcosms failed to found coexistence (Chapter V). A reasonable explanation for species exclusion in the microcosms under a fluctuating environment, even when a diapausing egg bank was present, is the effect of the demographic stochasticity. Laboratory rotifer population and diapausing egg bank sizes were small, in contrast with their sizes in natural habitats. For instance, *B. plicatilis* population size is estimated to be 2.3×10^{11} individuals in Salobrejo pond (Montes and Martino 1987; Montero-Pau et al. 2011), where demographic stochasticity is unlikely to have important effects. Accordingly, the size of the experimental population has to be taken into account in future studies on competitive dynamics. Performing experiments in mesocosms instead of microcosms could prevent species exclusion caused by demographic stochasticity.

Our results show that the studied system, apart from qualitatively fitting in the assumptions of the 'storage effect' theory, also points out a possible role of differentiation in life-history traits in promoting species coexistence. The 'storage effect' theory remarks the relevance of life-history traits in competitive dynamics (Chesson and Huntly 1988; Chesson and Huntly 1989; Chesson 2000). Notice that organism's life history is not the response to a single factor or process, namely competition, but is envisaged as the result of a suit of environmental factors acting as selective pressures on vital traits – such as survival, growth, age and size maturity, age-specific fertility, rates related to resting stages, etc.– that directly affect fitness and are linked by trade-offs (Stearns 1992; Roff 1993). Here we stress that the 'storage effect' theory is not based on divergence in life-history traits. The relevance of both competition and environmental fluctuations in life-history evolution was recognized early with the theory of *r/K* -selection (MacArthur and Wilson 1967; Pianka 1970). However, apart from *r/K*-selection theory, the role of divergence on life-history traits on species coexistence has received little attention. Some theoretical models (Zhang and Hanski 1998; Montero-Pau and Serra 2011) have suggested that the cost of males and reproduction could also promote coexistence of competitors with complex life cycles. The stable coexistence mechanism proposed by Montero–Pau and Serra (2011) is based on the negative effect that investment in sexual reproduction and diapause will cause on the competitive capability of the best performing species, which creates an opportunity for the inferior species to grow and reproduce. The present thesis demonstrates that *B. plicatilis* and *B. manjavacas* differently invest in sex and diapause (Chapters III and IV). However, the higher investment in sex of *B. plicatilis*, rather than acting as a mechanism mediating coexistence in the water-column –the one suggested by Montero-Pau and Serra (2011)–, seems to work as a way for this opportunistic species to effectively exploit short and unpredictable favorable periods (i.e., low salinity) and to produce diapausing eggs (Chapter III). Few experimental studies have previously addressed the effects of sexual reproduction patterns and investment in diapause in the competitive outcome of zooplankton species. A high investment in diapause has been found to accelerate short-term –i.e., in the water column– competitive exclusion (Ciros-Pérez et al. 2002; Li and Niu 2014; Chapter VI) or promote coexistence (Aranguiz-Acuña and Ramos-Jiliberto 2014). It has been suggested that these different competitive outcomes could be related with the phylogenetic relatedness of the species and hence with the strength of competition (Aranguiz-Acuña and Ramos-Jiliberto 2014). Obviously, further research is needed to clarify this question. In our study system, *B. plicatilis* might bet on diapause investment at the expense of being excluded from the water column in the short-term by *B. manjavacas*. Thus, long-term persistence of this former species may be possible through the diapausing eggs produced. In fact, the persistence of *B. plicatilis* and *B. manjavacas* was prolonged when a diapausing egg bank was included in the long-term competitive dynamics (Chapter V). Moreover, *B. plicatilis* and *B. manjavacas* not only differ in patterns of sexual reproduction (timing of and proportional allocation to sex), but also in patterns of diapause (viability and hatchability strategies of their diapausing eggs; Chapters III and IV). In short, the impact of the 'storage effect' to promote coexistence might be reinforced by divergent patterns of investment in sex and diapause: the effect of salinity fluctuation is enhanced by the coupling of the species growth cycles to it.

Overall, this thesis highlights the importance of considering the whole life cycle in competition studies, especially if the competing species have complex life cycles. Conclusions about the conditions for coexistence or exclusion cannot be drawn solely from the study of short-term competitive dynamics. On the one hand, short-term exclusion of the active stages of a competitor is compatible with this species being the long-term winner if it allocates more resting propagules than its competitor. On the other hand, short-term reciprocal exclusion of active stages is compatible with long-term coexistence.

Besides the achievements and the gaps in knowledge stressed above, some other questions remain open, as whether coexistence of these critically similar cryptic species can be found in controlled but fluctuating laboratory conditions. The role of demographic stochasticity on species persistence needs additional attention, other salinity fluctuation patterns should also be experimentally investigated, as well as fluctuations of other ecological factors as temperature, pH or oxygen concentration.

This thesis has demonstrated the potential of combining different approaches –i.e., empirical and theoretical studies– in competition studies between species with complex life histories. As a whole, this thesis presents a complete description of the main factors that have a role mediating the observed coexistence of two highly similar rotifer species in the wild.

Conclusions

The main conclusions derived from this thesis are enumerated below:

1. *B. plicatilis* and *B. manjavacas* have a wide biotic niche overlap. The two species have similar resource use and susceptibility to

predation by copepods. In contrast to other species of the *B. plicatilis* cryptic species complex, these biotic factors are unlikely to act in the stable coexistence of *B. plicatilis* and *B. manjavacas*. In this case, similar morphology translates into similar biotic niches.

2. The two studied species respond differently to salinity and temperature in terms of both the intrinsic growth rate and the investment in sexual reproduction. *B. manjavacas* grows better than *B. plicatilis* in a wide range of salinity, but the latter has higher performance than *B. manjavacas* at the lower salinity values explored. *B. plicatilis* uses its high performance at low salinity to produce sexual, diapausing eggs, so that its performance does not result in a high rate of clonal proliferation. Differences in relation to these abiotic factors dramatically enhance niche differentiation between these species.
3. These cryptic species differ in life-history traits related to sex and diapause. At the level of detail achieved in this thesis, this is a novel result in zooplankton studies including species so similar. *B. plicatilis* induces sexual reproduction at lower population density and makes a higher proportional allocation; hence it invests more in sex and produces the diapausing eggs earlier than *B. manjavacas*. The diapause pattern also differs between species. If compared to *B. manjavacas*, the diapausing eggs of *B. plicatilis* survive longer and have an extended hatching pattern.

4. The three reproductive types of females –asexual, unfertilized sexual and fertilized sexual females– allocate resources differently among life-history traits, revealing relationships between lifespan and reproductive traits that demonstrate the cost of reproduction. Reproductive traits of sexual females are different between species. *B. manjavacas* unfertilized sexual females produced more sons earlier and at a greater rate than *B. plicatilis* ones. Moreover, *B. manjavacas* fertilized sexual females had higher relative allocation per diapausing egg than those of *B. plicatilis*.
5. Differentiation in sex and diapause traits suggests that *B. plicatilis* behaves as an opportunistic species which takes advantage of favorable low salinity conditions to invest in sex and produce diapausing eggs. These characteristics of *B. plicatilis* fit to what is expected to evolve in an unpredictable environment. Since *B. plicatilis* is more negatively affected by high salinity, likely variation in salinity makes the habitat more uncertain for *B. plicatilis* than for *B. manjavacas* which grows and invests in sex in a wider range of salinity.
6. It is shown that the long-term outcome of the competition is reasonably predicted from a thoroughly study of these species in isolation. This conclusion was possible by integrating the whole life cycle in the competitive dynamics, a novel approach in zooplankton studies.
7. *B. plicatilis* and *B. manjavacas* have similar competitive abilities and the outcome of the competitive dynamics depends on the

salinity regime (i.e., salinity values and fluctuation patterns). Results prove that their coexistence is unlikely at constant salinity. Moreover, accordingly to predictions from single-species studies, when salinity is low, *B. plicatilis* is favored and *B. manjavacas* is excluded; the opposite holds when salinity is high.

8. Laboratory population dynamics suggests that the persistence of both species is most likely under a fluctuating salinity regime that starts at low salinity.
9. Competitive exclusion tends to be delayed by the existence of diapausing egg banks. Despite this evidence is preliminary, it seems that a longer co-persistence of *B. plicatilis* and *B. manjavacas* is found when a diapausing egg bank is presented across growing seasons than when diapausing eggs are allowed to survive only a single unsuitable period.
10. Simulations of the long-term competitive dynamics, using the developed theoretical model, show that a low level of crossed induction of sex and intermediated lengths of the growing seasons promote the coexistence of *B. plicatilis* and *B. manjavacas*. The effect of these factors is different for each species in accordance with their different strategies of investment in sex and diapause.
11. The findings on differentiation in life-history traits suggest the hypothesis that they play a role in the long-term coexistence of these cryptic species. *B. plicatilis* would tend to be excluded in the water column by *B. manjavacas*, the superior competitor,

but at low salinity the higher performance of *B. plicatilis* would allow it to invest in sex and produce diapausing eggs, which would take place early in the growing season and at the expense of reducing its population density and competitive ability. Nevertheless, with this production *B. plicatilis* would achieve its long-term persistence.

12. Theoretical and empirical results stress the idea that investment to diapause is not only a way to cope with environmental unsuitable conditions but it also might act as a component of the coexistence mechanism. Co-occurrence of *B. plicatilis* and *B. manjavacas* must be understood as a phenomenon stabilized by processes working in the long-term, rather than by mechanisms acting only in single growing seasons, i.e., in the water column, and at short-term scale.

References

Abramsky Z, Rosenzweig ML (1984) Tilman's predicted productivity-diversity relationship shown by desert rodents. Nature 309:150–151.

Aranguiz-Acuña A, Ramos-Jiliberto R (2014) Diapause may promote coexistence of zooplankton competitors. J Plankton Res 36:978–988.

Boraas ME, Seale DB, Horton JB (1990) Resource competition between two rotifer species (*Brachionus rubens* and *B. calyciflorus*): An experimental test of a mechanistic model. J Plankton Res 12:77–87.

Byers JE (2000) Competition between two estuarine snails: Implications for invasions of exotic species. Ecology 81:1225–1239.

Campillo S, García-Roger EM, Martínez-Torres D, Serra M (2005) Morphological stasis of two species belonging to the L-morphotype in the *Brachionus plicatilis* species complex. Hydrobiologia 546:181–187.

Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. Philos Trans R Soc Lond B Biol Sci 366:2351–2363.

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366.

Chesson P, Huntly N (1989) Short-term instabilities and long-term community dynamics. Trends Ecol Evol 4:293–8.

Chesson P, Huntly N (1988) Community consequences of life-history traits in a variable environment. Ann Zool Fennici 25:5–16.

Ciros-Pérez J, Carmona M, Serra M (2002) Resource competition and patterns of sexual reproduction in sympatric sibling rotifer species. Oecologia 131:35–42.

Ciros-Pérez J, Carmona MJ, Lapesa S, Serra M (2004) Predation as a factor mediating resource competition among rotifer sibling species. Limonology Oceanogr 49:40–50.

Ciros-Pérez J, Carmona MJ, Serra M (2001a) Resource competition between sympatric sibling rotifer species. Limonology Oceanogr 46:1511–1523.

Ciros-Pérez J, Gómez A, Serra M (2001b) On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. J Plankton Res 23:1311–1328.

Comín F, Rodó X, Comín P (1992) Lake Gallocanta (Aragón, NE Spain): a paradigm of fluctuations at different scales of time. Limnetica 8:79–86.

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302–1310.

DeMott WR (1999) Foraging strategies and growth inhibition in five daphnids feeding on mixtures of a toxic cyanobacterium and a green alga. Freshw Biol 42:263–274.

Fontaneto D, Giordani I, Melone G, Serra M (2007) Disentangling the morphological stasis in two rotifer species of the *Brachionus plicatilis* species complex. Hydrobiologia 583:297–307.

Gómez A, Carvalho G, Lunt DH (2000) Phylogeography and regional endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting egg banks. Proc R Soc B Biol Sci 267:2189–2197.

Gómez A, Montero-Pau J, Lunt DH, Serra M (2007) Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. Mol Ecol 16:3228–3240.

Gómez A, Serra M, Carvalho G, Lunt DH (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). Evolution (N Y) 56:1431–1444.

Holt R (1984) Spatial heterogeneity, indirect interactions, and the coexistence of prey species. Am Nat 123:377–406.

Holt R (1993) Ecology at the mesoscale: the influence of regional processes on local communities. In: Ricklefs RE, Schluter D (eds) Species Divers. Ecol. communities Hist. Geogr. Perspect. University of Chicago Press, Chicago, pp 77–88.

Hubbell S (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton.

Huisman J, Jonker R, Zonneveld C, Weissing F (1999) Competition for light between phytoplankton species: experimental tests of mechanistic theory. Ecology 80:211–222.

Huston M (1979) A general hypothesis of species diversity. Am Nat 113:81–101.

Hutchinson G (1959) Homage to Santa Rosalia or why are there so many kinds of animals? Am Nat 93:145–159.

Hutchinson G (1961) The paradox of the plankton. Am Nat 95:37–145.

Kreutzer C, Lampert W (1999) Exploitative competition in differently sized *Daphnia* species: a mechanistic explanation. Ecology 80:2348–2357.

Lapesa S, Snell TW, Fields DM, Serra M (2004) Selective feeding of *Arctodiaptomus salinus* (Copepoda, Calanoida) on co-occurring sibling rotifer species. Freshw Biol 49:1053–1061.

Leibold M, McPeek M (2006) Coexistence of the niche and neutral perspectives in community ecology. Ecology 87:1399–1410.

Levin S (1974) Dispersion and population interactions. Am Nat 108:207–228.

Li C, Niu C (2014) Effects of sexual reproduction of the inferior competitor Brachionus calyciflorus on its fitness against *Brachionus angularis*. Chinese J Oceanol Limnol 33:356–363.

Losey JE, Denno RF (1998) Interspecific variation in the escape responses of aphids: Effect on risk of predation from foliar-foraging and ground-foraging predators. Oecologia 115:245–252.

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol Lett 11:995–1003.

MacArthur R, Wilson E (1967) The theory of island biogeography. Princeton University Press, Princeton.

Mcgovern T, Hellberg M (2003) Cryptic species, cryptic endosymbionts, and geographical variation in chemical defences in the bryozoan *Bugula neritina*. Mol. Ecol. 12: 1207–1215.

McPeek M, Gomulkiewicz R (2005) Assembling and depleting species richness in metacommunities: insights from ecology, population genetics and macroevolution. In: Holyoak M, Leibold MA, Holt RD (eds) Metacommunity Ecol. Emerg. views community Struct. Dyn. above local scale. University of Chicago Press, Chicago, Illinois, USA., pp 355– 373

Montero-Pau J, Ramos-Rodríguez E, Serra M, Gómez A (2011) Long-term coexistence of rotifer cryptic species. PLoS One 6:e21530.

Montero-Pau J, Serra M (2011) Life-cycle switching and coexistence of species with no niche differentiation. PLoS One 6:e20314.

Montes C, Martino P (1987) Las lagunas saladas españolas. Semin. sobre bases científicas para la protección los Humed. España. Real Acad. Ciencias Exactas, Físicas y Nat.

Pianka ER (1970) On r- and K-Selection. Am Nat 104:592–597.

Pressey RL, Cowling RM, Rouget M (2003) Formulating conservation targets for biodiversity pattern and process in the Cape Floristic Region, South Africa. Biol Conserv 112:99–127.

Rodriguez-Puebla C, Encinas AH, Nieto S, Garmendia J (1998) Spatial and temporal patterns of annual precipitation variability over the Iberian Peninsula. Int J Climatol 18:299–316.

Roff D (1993) Evolution of life histories: theory and analysis. Chapman and Hall, New York

Rothhaupt KO (1988) Mechanistic resource competition theory applied to laboratory experiments with zooplankton. Nature 333:660–662.

Shmida A, Ellner S (1984) Coexistence of plant species with similar niches. Vegetatio 58:29–55.

Sommer U (1989) The role of competition for resources in phytoplankton succession. In: Sommer U (ed) Plankt. Ecol. succession Plankt. communities. Springer, Berlin, pp 57–106.

Stearns S (1992) The evolution of life histories. Oxford University Press, Oxford.

Sturmbauer C, Opadiya G (1999) Mitochondrial DNA reveals cryptic oligochaete species differing in cadmium resistance. Mol. Biol. Evol. 16:967–974.

Tilman D (1982) Resource Competition and Community Structure. Princeton University Press, Princeton.

Umina PA, Hoffmann AA (1999) Tolerance of cryptic species of blue oat mites (*Penthaleus spp.*) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. Anim Prod Sci 39:621–628.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Zhang DY, Hanski I (1998) Sexual reproduction and stable coexistence of identical competitors. J Theor Biol 193:465–473.

Appendix 6.A.

Derivation of the parameter relationships needed to estimate *K*

1. Derivation of the relationship *K* = *Kc* – R*(0).*

Variation of the rotifer growth rate (*r*) as depending on the resource level (*R*) can be modelled using the Monod equation (Ciros-Pérez et al., 2001a) as:

$$r(R) = \frac{r^*\big(R - R(0)\big)}{R + K_c - R\,(0)}$$

where r^* is the maximum growth rate, R(0) is the resource concentration where growth rate is zero (i.e., birth rate equals death rate), and *Kc* is the relevant Monod constant. Hereafter, we will assume that the effect of the resource level on the growth rate is through the birth rate, so that the mortality rate (*q*) is resource-independent; that is *r(*R*)* = *b(*R*)* - *q*. From this, and as birth rate without resources is zero, it follows that *r (0)* = *b(0)* – *q* = –*q*. Thus,

$$q = -r(0) = \frac{r^* R(0)}{K_c - R(0)} \qquad \text{Eq. a1}$$

and

$$b(R) = r(R) - q = \frac{r^*\big(R - R(0)\big)}{R + K_c - R\,(0)} + \frac{r^* R(0)}{K_c - R(0)}$$

This equation with some algebra and recalling that *r* = *b** – *q* (where *b** is the maximum birth rate) yields:

$$b(R) = \frac{b^*R}{R + K_c - R\,(0)} + \frac{r^*R(0)}{K_c - R(0)} - \frac{r^*R\,(0)}{R + K_c - R\,(0)} - \frac{qR}{R + K_c - R\,(0)}$$

A little additional algebra shows that in this equation the three last additive terms sum to zero, so that

$$b(R) = \frac{b^*R}{R + K_c - R\,(0)}$$

Comparing this equation with Eq. 2b, and keeping in mind that appropriate subscripts are required to term the parameter dependence on species and conditions, we find

$$K = K_c - R(0)$$

Notice that Eq. a1 allows estimating $R(0)$ if q, r^* and K_c are known.

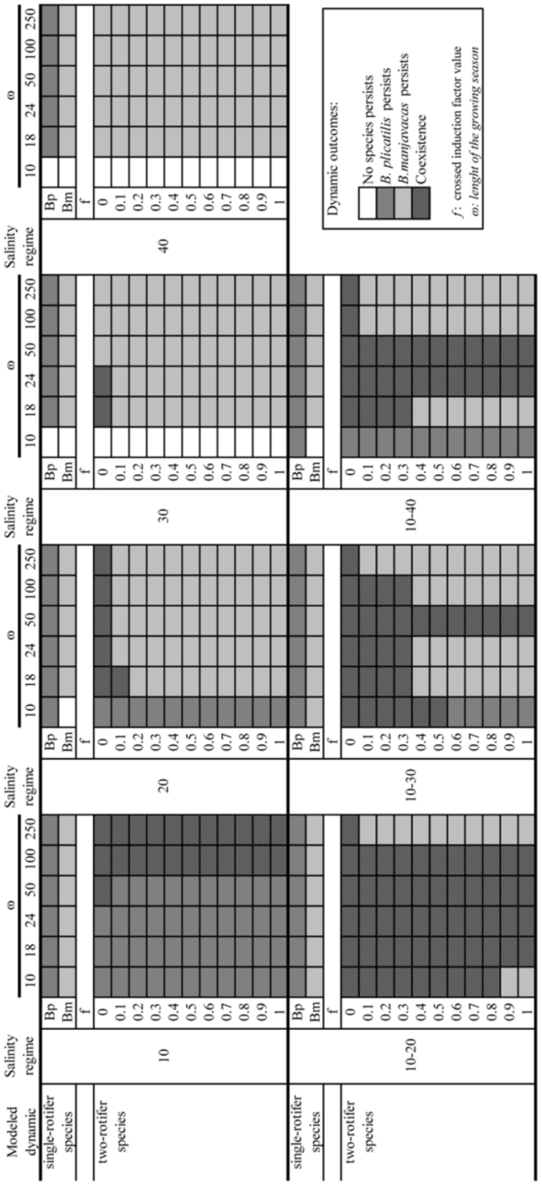
2. Primitive estimations used:

$r^* = 0.81\ \mathrm{d}^{-1}$ (Ciros-Pérez et al., 2001a).

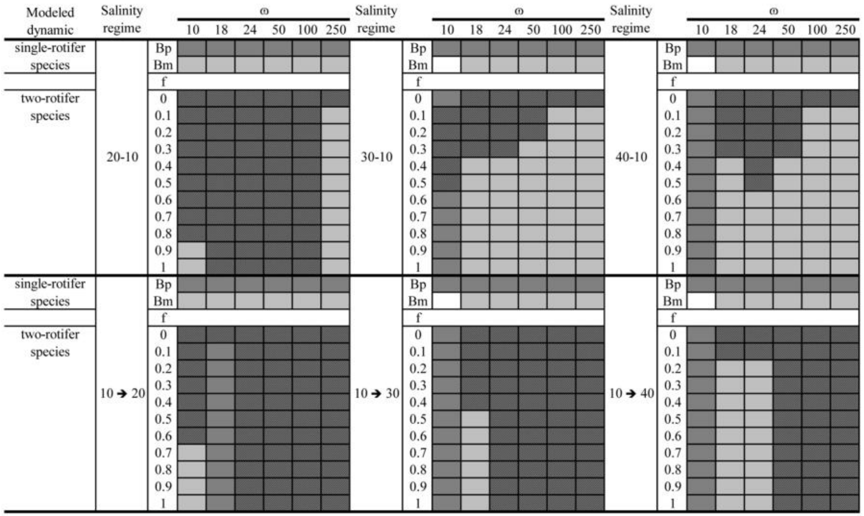
$K_c = 0.20$ mg C L^{-1} (Ciros-Pérez et al., 2001a), which is 1053 cells mL^{-1} (each microalga *Tetraselmis suecica,* which is used as source of food for rotifers and is the resource assumed in the model, has 19 10^{-8} mg C (unpublished data).

$q = 0.32\ \mathrm{d}^{-1}$ (Gabaldón et al., 2015).

Appendix 6.B.



Appendix 6.B. Competitive dynamic outcomes of *B. plicatilis* and *B. manjavacas* simulated (1) growing alone each species under 78 different scenarios (13 salinity regimes × 6 growing season lengths) and (2) under 858 different scenarios (13 salinity regimes × 6 growing season lengths × 11 values of crossed induction of sex) after 1000 growing cycles.



Appendix 6.B. Continued.

VNIVERSITAT
DE VALÈNCIA