TESIS DOCTORAL

The role of waterfowl in the passive transport of aquatic organisms: from local processes to long-distance dispersal

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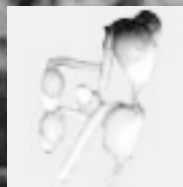
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The role of waterfowl in the
passive transport of aquatic organisms:
from local processes to long-distance dispersal

Memoria presentada por **Jordi Figuerola Borras**

para optar al grado de doctor en Biología

Director: Andy J. Green

Científico Titular, Departamento de Biológia Aplicada

Estación Biológica de Doñana, CSIC.

Tutor: Carlos Montes del Olmo

Catedrático de Ecología, Universidad Autónoma de Madrid

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Presentación de la tesis

La capacidad de dispersión de los organismos puede tener efectos sobre la diversidad genética, la riqueza y distribución de las especies y la estructura de las comunidades (Clobert 2001). En este sentido, las aguas continentales se pueden considerar como islas ecológicas rodeadas por un 'mar' de hábitats inadecuados para el desarrollo y dispersión de los organismos acuáticos. A pesar de este relativo aislamiento, muchos de estos organismos tienden a presentar áreas de distribución más extensas que sus parientes terrestres. Dejando de lado los posibles efectos de una asumida (que no demostrada) mayor uniformidad o estabilidad del ambiente en ecosistemas acuáticos y por tanto un tamponamiento de los efectos climáticos sobre la distribución de las especies (ver discusión en Reynolds 1998 y Santamaría 2002), es evidente que deben existir mecanismos que permitan la dispersión de los invertebrados y plantas acuáticas a través de las amplias zonas de hábitats inhóspitos, que les permitan colonizar y mantener cierto grado de flujo génico entre sus poblaciones. Si bien algunas especies de invertebrados presentan formas o estadíos de desarrollo capaces de dispersarse activamente (p.e. Bilton et al.2001), otras muchas especies carecen de esta capacidad de dispersión activa. En estos casos, la dispersión dependerá de la formación de estadíos de resistencia o de propágulos sexuales (efípios, semillas) o asexuales (estatoblastos, esporas). Los mecanismos por los cuales son dispersados estos propágulos han llamado la atención de los naturalistas desde hace mucho tiempo (p.e. Lyell 1832, Darwin 1859). Además de la lluvia y el viento, las aves acuáticas han sido consideradas como un importante vector para la dispersión de los organismos acuáticos. A pesar que en mucha de la literatura revisada se da por sentado y demostrada la relevancia de la dispersión por aves acuáticas, como veremos más adelante, son muy escasos los trabajos cuantitativos sobre este aspecto.

Por este motivo, el primer apartado de esta tesis se centra en revisar los estudios publicados hasta el momento sobre el papel de las aves en la dispersión pasiva de organismos acuáticos, presentando las evidencias disponibles e identificando algunas posibles lagunas en nuestro conocimiento

de los fenómenos de dispersión en ambientes acuáticos. Esta parte de la tesis está formada por dos artículos. En el primero se revisan la evidencia experimental de la resistencia de distintos propágulos a la ingestión por aves acuáticas, así como los trabajos cuantitativos que investigan la frecuencia de propágulos en las heces o adheridos externamente a las aves. En el segundo artículo se determinaron qué factores de la ecología de las aves podrían afectar la cantidad y calidad de dispersión que las mismas podrían proporcionar. De esta manera se sugirieron posibles factores morfológicos que podrían afectar los distintos componentes de la dispersión. También se revisó buena parte de la literatura existente sobre la dieta de las aves para extraer la información relevante sobre el consumo de propágulos y la presencia de propágulos intactos en los intestinos o las heces de las aves.

El segundo apartado de la tesis se centra en el análisis de la frecuencia de fenómenos de dispersión local en las Marismas del Guadalquivir (Doñana). Esta sección esta compuesta por dos manuscritos. El primero se centra en el transporte interno. Se analizó la abundancia de propágulos que habían resistido la ingestión en las heces de distintas especies de aves (patos y fochas) al principio y al final del invierno. Se determinó la variación interespecífica y estacional en la abundancia de distintos tipos de propágulos y se investigó el posible efecto de la morfología del pico de las aves sobre la abundancia de propágulos. En el segundo manuscrito se presenta una metodología para la recogida de propágulos adheridos al plumaje y se analiza la abundancia de propágulos adheridos a las patas y el plumaje de las aves capturadas en dos localidades de Doñana.

Una vez determinada que la frecuencia de transporte local es bastante elevada y permite por tanto abordar su estudio en el campo, en el siguiente apartado se analizaron los componentes de cantidad y calidad de dispersión por aves acuáticas sobre distintos organismos, centrándonos en particular en el efecto de la ingestión sobre la viabilidad de los propágulos. Esta sección esta compuesta por cinco manuscritos. El primero se centra en la dispersión de *Ruppia maritima* en Doñana. Se determina la variación interespecífica y estacional en la presencia de semillas, tasas de digestión y viabilidad de las semillas que sobreviven a la ingestión. Paralelamente se investiga la posible

relación entre la morfología interna de las aves y dos componentes de la calidad de dispersión: la proporción de semillas que sobrevive la ingestión y la posterior viabilidad de las semillas. En un segundo manuscrito se analizan los efectos de la ingestión por anátidas sobre la viabilidad y velocidad de germinación de semillas de *Potamogeton pectinatus*. Se intentaron simular las distintas fases de la digestión (tratamiento mecánico y químico) para determinar si alguna de las dos componentes era especialmente responsable de los cambios en el patrón de germinación. Una vez determinados los efectos sobre los patrones de germinación, en un tercer manuscrito analizamos los efectos a más largo plazo sobre el desarrollo de las plantas, comparando en una serie de tanques experimentales, el desarrollo de plantas de *Potamogeton pectinatus* provenientes de semillas ingeridas y no ingeridas por patos. Dado que en condiciones naturales, los efectos sobre la germinación pueden interactuar con otros factores naturales, se aborda en un cuarto manuscrito los posibles efectos de la ingestión por anátidas sobre la supervivencia de las plántulas de *Ruppia maritima* en el campo, y cómo este factor puede interactuar con los efectos de la herbivoría sobre la supervivencia de las mismas. El último manuscrito de este apartado se centra en el análisis de la resistencia de los estatoblastos de briozoo a la ingestión por aves acuáticas. Particularmente, nos centramos en *Cristatella mucedo* dado que es una especie para la cual se ha sugerido específicamente que las aves acuáticas son los vectores del flujo génico entre las poblaciones de este organismo distribuido por toda Europa (Freeland et al. 2000).

Una vez analizados algunos de los posibles efectos de la ingestión por las aves acuáticas sobre la viabilidad y desarrollo de los organismos acuáticos, en el cuarto apartado se analizan algunos de los efectos sobre los patrones de dispersión de organismos acuáticos que se pueden derivar del comportamiento de las aves. En primer lugar, se analiza cómo los patrones de alimentación justo antes de iniciar el vuelo migratorio pueden afectar las probabilidades de transporte a larga distancia. En un segundo manuscrito, se analiza la relación entre el flujo génico entre poblaciones de cuatro especies de invertebrados en toda Norteamérica y los movimientos migratorios de las

aves en ese continente. De esta manera se pretende determinar cómo la presencia de propágulos, documentada en secciones anteriores de esta tesis, se traduce en dispersión efectiva y flujo génico a larga distancia entre poblaciones de invertebrados acuáticos.

Por último, distintos factores pueden limitar la capacidad de establecimiento de los propágulos dispersados a largas distancias. Uno de esos factores puede ser la existencia de adaptación local. Es decir, que el propágulo dispersado pueda encontrar una serie de competidores mejor adaptados a las condiciones ambientales, y esto reduzca (o elimine) el flujo génico efectivo debido a la dispersión por aves acuáticas (ver p.e. De Meester et al. 2002). Este experimento se realizó usando tubérculos de *Potamogeton pectinatus* de distintas localidades de Europa, y cultivados en condiciones seminaturales en España, Holanda y Noruega. Si bien es cierto que las semillas (y no los tubérculos) son la parte de la planta con más posibilidades de ser dispersadas sobre largas distancias, el uso de tubérculos nos permitió validar la hipótesis de existencia de adaptación local, controlando por otros factores genéticos, gracias a la naturaleza clonal de los tubérculos.

En definitiva, las cinco partes que componen esta tesis intentan clarificar el papel de las aves acuáticas sobre la dispersión de organismos acuáticos analizando en primer lugar algunos de los factores que determinan su frecuencia a nivel local, el impacto sobre el desarrollo de los organismos, los factores que pueden afectar la frecuencia de transporte a larga distancia y por último, su impacto real sobre la estructura genética de los organismos acuáticos.

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Part 1

Synthesis of current knowledge on patterns
of bird mediated dispersal in aquatic environments

OPINION

Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies

JORDI FIGUEROLA and ANDY J. GREEN
Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. María Luisa s/n, Sevilla, Spain

SUMMARY

1. Inland wetlands constitute ecological islands of aquatic habitat often isolated by huge areas of non-suitable terrestrial habitats. Although most aquatic organisms lack the capacity to disperse by themselves to neighbouring catchments, many species present widespread distributions consistent with frequent dispersal by migratory waterbirds.
2. A literature review indicates that bird-mediated passive transport of propagules of aquatic invertebrates and plants is a frequent process in the field, at least at a local scale. Both endozoochory (internal transport) and ectozoochory (external transport) are important processes.
3. The characteristics of the dispersed and the disperser species that facilitate such transport remain largely uninvestigated, but a small propagule size tends to favour dispersal by both internal and external transport.
4. We review the information currently available on the processes of waterbird-mediated dispersal, establishing the limits of current knowledge and highlighting problems with research methods used in previous studies. We also identify studies required in the future to further our understanding of the role of such dispersal in aquatic ecology.

Keywords: dispersal capacity, seed dispersal, egg dispersal, habitat colonisation, community composition

Introduction

Continental wetlands usually consist of isolated units of temporary or permanently flooded areas in a 'sea' of habitat not suitable for aquatic organisms. Many aquatic plants and invertebrates lack the mobility necessary to travel directly from one catchment to another, to colonise new areas and to disperse to neighbouring water bodies in different catchments. Despite this apparent isolation of freshwater habitats, many aquatic plant and invertebrate species have widespread distributions (Good, 1953; Raven, 1963; Sculthorpe, 1967; Brown & Gibson, 1983; WCMC, 1998), spanning several continents in some cases. Although

some recent genetic studies have demonstrated that some 'species' are in fact morphologically similar complexes of sibling species (Hebert & Wilson, 1994), others have identified widely distributed species (Hebert & Finston, 1996). However, there are also numerous aquatic organisms with very restricted distributions (Frey, 1987; Stemberger, 1995). One major reason for such differences is that widespread species have a greater capacity to disperse and can thereby colonise new areas and maintain gene flow between different areas (Bohonak, 1999; Clobert *et al.*, 2001).

Passive dispersal capacity is a key trait explaining patterns of distribution and community composition of non-mobile organisms (Jenkins & Buikema, 1998). For example, Primack & Miao (1992) showed that limited dispersal capacity can restrict the distribution of many terrestrial species of plants. Similarly, despite the high potential for seed dispersal by water

Correspondence: J. Figuerola, Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. María Luisa s/n, E-41013 Sevilla, Spain. E-mail: jordi@ebd.csic.es

currents, the same occurs in *Zostera marina*, a marine macrophyte, 80% of seedlings were found less than 5 m away from the mother plant (Orth, Luckenbach & Moore, 1994). Dispersal capacity is also of vital importance in determining responses to climatic changes (Davis, 1989; Graham, Turner & Dale, 1990).

Although there is much information available about animal-mediated dispersal in terrestrial ecosystems (see reviews in Sorensen, 1986; Traveset, 1998), relatively little is known about the processes affecting long-distance dispersal between aquatic habitats, where waterbirds presumably are an important vector.

Many waterbirds undertake long migratory journeys from their breeding areas at extreme latitudes towards more temperate areas during the winter (del Hoyo, Elliott & Sargatal, 1992). Waterbirds have long been considered a major disperser of aquatic organisms, transporting plant and invertebrate propagules in their guts (endozoochory or internal dispersal) or attached to their bodies (ectozoochory or external dispersal) (Darwin, 1859; Ridley, 1930). However, most evidence for such dispersal by waterbirds is anecdotal and detailed quantitative investigations on the patterns of such transport are very scarce (see our review below). Recent indirect evidence supporting the role of long-distance dispersal by waterbirds comes from studies of the genetic population structure of different aquatic species. Most of these studies have concluded that geographical distance is usually unrelated to the genetic distance between populations (Hebert & Finston, 1996; Hollingsworth, Preston & Gornall, 1996; Vanoverbeke & DeMeester, 1997; Mader, van Vierssen & Schwenk, 1998; Freeland, Noble & Okamura, 2000). One exception is a study of the fennel pondweed *Potamogeton pectinatus* in Europe (Mader *et al.*, 1998). However, in this case the association between genetic and geographic distances was only significant for ponds not visited by swans during migration (considered the main potential disperser of *P. pectinatus* in the study area). The genetic distribution of *Daphnia laevis* reflects the major waterfowl (ducks, geese and swans) flyways in North America, with higher similarity between populations in a North-South than an East-West direction (Taylor, Finston & Hebert, 1998). Similarly, the genetic distribution of the bryozoan *Cristatella mucedo* in northern Europe follows the major waterfowl flyway in this region (Freeland *et al.*, 2000). However, the lack of association between genetic and geographic distances

in aquatic organisms does not constitute direct evidence supporting bird-mediated dispersal, but rather indicates that populations are not at equilibrium, or that colonisation events are independent of geographic distance. This may be because dispersal is more frequent in the direction of bird migration or between major stopover areas, but alternatively may be because loss of alleles as a result of drift is not balanced by their replacement by gene flow between populations in a region (McCauley, 1993).

In this paper we review the research conducted to date on waterbird-mediated dispersal of aquatic organisms, highlighting the series of processes involved and assessing the likely frequency of such dispersal in the field. We concentrate on quantitative and semiquantitative studies rather than anecdotal observations. We identify pitfalls and limitations of existing studies and methodologies and highlight some of the key questions that remain unanswered in this field. Finally, we identify some priorities for future research on the processes involved in the dispersal of aquatic organisms by birds. In this review, we do not deal with the question of how well a propagule is likely to compete to establish itself in an aquatic community once it has been dispersed by a waterbird (e.g. questions such as whether that propagule will be well adapted to the new environment, whether it can compete with conspecifics already present, or whether the arrival of a new genotype would be detectable given the size of existing seed or propagule banks in the wetland).

Internal transport step 1: do waterbirds consume propagules?

To facilitate internal dispersal, waterbirds first need to ingest propagules of aquatic organisms. Such ingestion may be voluntary when the propagules themselves constitute the foraging resource exploited by the animals, or involuntary when propagules are ingested with leaves, invertebrates or other preferred food items. The relative frequency of dispersal may be higher following involuntary digestion, as seed-eating birds can destroy most seeds ingested, whereas ingestion of seeds by consumers of leaves or insects may have less impact on their viability. Thus, in a given bird community, some species may be legitimate seed dispersers and others seed predators (Jordano, 1992; Traveset, 1998).

Diet studies of migratory waterfowl (Anatidae) have shown that they consume large quantities of propagules of aquatic organisms from a wide variety of species. This is especially the case for duck species (see, e.g. Cramp & Simmons, 1977; Thomas, 1982; Batt *et al.*, 1992). The filter-feeding method typically used by ducks is relatively unselective, and they typically ingest various classes of food items at a given time. It is thus difficult *a priori* to assess whether seeds and other propagules are ingested voluntarily or not.

Coots (*Fulica* sp.), flamingoes (*Phoenicopterus* sp.) and other groups of waterbirds also consume important quantities of seeds and other propagules (e.g. Cramp & Simmons, 1977). Waders (Charadridae and Scolopacidae) are a particularly interesting group because of their long-distance migrations and capacity for long-distance dispersal. Many species consume invertebrates that may contain resting eggs (Cramp & Simmons, 1983), and some species often ingest seeds of saltmarsh plants (Pérez-Hurtado, Goss-Custard & Garcia, 1997).

Diet studies of waterbirds generally have an autecological perspective focussing on the nutritional or habitat requirements of the waterbird species and, while giving information about the ingestion of propagules, they do not consider whether or not such propagules survive digestion. Most studies are carried out on oesophagus or gizzard contents, but even diet studies via faecal analyses do not consider how many propagules survive ingestion intact (e.g. Pérez-Hurtado *et al.*, 1997; Green & Selva 2000).

Internal transport step 2: can propagules survive digestion by waterbirds?

Various experiments on the survival of invertebrate and plant propagules after digestion by waterbirds have been carried out by feeding a large number of propagules to captive-reared ducks or waders (Table 1). Droppings were generally collected for a variable number of hours after consumption of the seeds (usually 24 h or less, but Proctor, 1968, included droppings collected at least 10 days after the feeding of the propagules), and the intact propagules were then cultured in the laboratory to assess their viability. These experiments have shown that a range of seeds, oospores, phytoplankton spores and crustacean eggs can survive digestion (see Table 1). For most of the species tested, some propagules survived digestion,

except for phytoplankton where, amongst seven algae species tested, only the spores of one diatom germinated after digestion (Atkinson, 1970, 1971).

Some of these studies compared the survival of different species after digestion to investigate which propagule characteristics favour internal transport by birds. These studies have produced contradictory results. De Vlaming & Proctor (1968) tested the resistance of seeds of 23 wetland plant species to digestion by mallard *Anas platyrhynchos* and killdeer *Charadrius vociferus*. They concluded that resistance to digestion is higher for small seeds with hard covers and is also affected by the characteristics of the disperser species (gizzard structure and retention time of seeds in the gut were proposed as the most important characteristics). In a similar study with mallard, Holt (1999) concluded that resistance to digestion was unrelated to size and lignin content in seeds of eight wetland plant species. However, she reported that the proportion of non-destroyed seeds germinating after ingestion by birds was positively associated with the size and lignin content of the seeds.

The study of the propagule characteristics that facilitate survival after digestion is important in order to understand the factors that affect the colonisation and dispersal capacities of different aquatic organisms. However, various problems limit the usefulness of the experimental set-ups used so far to address this topic. First, the numbers of birds used in these studies were very low (Table 1) and sometimes not stated by the authors, making it hard to interpret the results obtained given the high intraspecific variability in the structure and size of the digestive organs of waterbirds (Kehoe, Ankney & Alisauskas, 1988). Secondly, various factors can affect the structure of digestive organs of captive waterbirds, including time in captivity (Clench & Mathias, 1995) and diet (Al-Dabbagh, Jiad & Waheed, 1987). This makes results difficult to compare between studies. Although we can be confident of results obtained by feeding different seed types simultaneously to the same individual bird, the percentage of seeds surviving digestion reported in different studies are not directly comparable, as differences in seed viability between studies could be because of either the characteristics of the seeds or the characteristics of the birds used in the experiment (even when either dispersed or disperser organisms belong to the same species).

Table 1 Summary of experiments carried out to test the viability of propagules after digestion by waterbirds. The number of individual birds used in the experiments is reported in brackets

Propagule species	Disperser species	Summary of main results	Reference
Eggs of two gastropods	*Anas platyrhynchos* (5) *Charadrius vociferus* (10)	A small proportion of the eggs of two species of gastropoda remained viable after digestion	Malone (1965b)
Artemia (Crustacea)	*Anas platyrhynchos* (5–20)	Some survived passage, eggs entering in the ceca are retained for much longer than expected from intestine length	Malone (1965a)
Artemia	*Tadorna tadorna* (2) *Phoenicopterus ruber* (2)	Hatching efficiency of cysts was unchanged by passage through *Ph. Ruber*, and reduced by *T. tadorna*	MacDonald (1980)
Twelve genera of crustaceans	*Anas platyrhynchos* (34)	Some species hatched after digestion, others did not	Proctor (1964)
Artemia, other invertebrates, algae and *Chara* (Characeae)	*Charadrius vociferus* *Anas platyrhynchos*	Survived digestion by *Ch.vociferus* better than by *A. platyrhynchos*	Proctor *et al.*, (1967)
Artemia and other invertebrates, algae and *Chara*	*Anas platyrhynchos*	All survived digestion	Proctor & Malone (1965)
Nitella (Characeae)	Duck (1)	Some germinated after digestion	Imahori (1954)
Riella americana (Riellaceae)	*Anas platyrhynchos* (3)	Germination and development 'similar' to undigested spores	Proctor (1961)
Six species of *Chara*	*Anas platyrhynchos* (3)	Some germinated after digestion	Proctor (1962)
Asterionella (Diatomea)	*Anas platyrhynchos* (4)	None germinated after digestion	Atkinson (1970)
Five diatomea, one Cyanophyta	*Anas platyrhynchos* (4)	Only one diatomea species germinated after digestion	Atkinson (1971)
Potamogeton natans (Potamogetonaceae)	*Anas platyrhynchos*	60% germinated after digestion	Guppy (1906)
Scirpus paludosus (Cyperaceae)	*Anas platyrhynchos* (1)	Some germinated after digestion	Low (1937)
Seeds of 23 wetland plant species	*Charadrius vociferus* (≥5)* *Anas platyrhynchos* (≥4)*	Small seeds and those with a hard cover have higher resistance to digestion. Retention time longer in *Ch. vociferus* than in *A. platyrhynchos*	De Vlaming & Proctor (1968)
Seeds of eight wetland plants species	*Anas platyrhynchos* (4–16)	Large seeds and those with a hard cover germinate better after digestion, no effects of these variables on resistance to digestion	Holt (1999)
Najas marina (Najadaceae)	*Anas platyrhynchos* (4)	Thirty per cent survived digestion, more than 30% germinated	Agami & Waisel (1986)
Seeds of 13 plant species	Twelve species of birds (2–5)	Retention times longer in waders that in ducks (up to 10 days)	Proctor (1968)
Seed of five plant species	Three species of ducks (5)	Viable seeds recovered for four of the seed species	Powers *et al.* (1978)

*Indicates that different types of propagules were tested in different individual birds.

There are also statistical problems with those studies that take each seed as the unit for analyses without accounting for the effects of individual ducks (the case for all studies cited in Table 1). This leads to pseudoreplication, as the resistance and viability of seeds digested by the same duck cannot be considered statistically independent observations. This can be solved by including a random factor controlling for individual-related effects on seed digestion and viability in the analyses (see Bennington & Thayne, 1994, for more information on random factors).

It is also difficult to compare different studies because the methods used to feed propagules to experimental birds differ widely between and within studies (force-feeding, mixed with leaves, grain or inside gelatinous pills, see, e.g. Proctor & Malone, 1965). The type of food ingested with the propagules can strongly influence the rate of passage through the digestive tract (Malone, 1965a). Furthermore, the conditions optimal for germination differ between propagule species. As Proctor (1964) recognised for crustacean eggs, the apparent differences in viability of different propagule species after digestion may merely reflect interspecific differences in requirements for germination or hatching. This problem can be solved by the use of control (undigested) propagules to determine the suitability of the germination procedures used (see, e.g. Agami & Waisel, 1986), and by making statistical comparisons of germination rates of digested and undigested propagules. To date, few studies have included such analyses, and none have separated the effects of digestion on the speed of propagule germination from its effects on long-term viability (see Traveset, 1998, for a review of this problem in studies of terrestrial organisms). For example, Agami & Waisel (1986) found that digestion by mallards accelerated germination of holly leaved naiad *Najas marina* seeds, but did not assess its effects on long-term viability.

There is evidence that waterbird species can differ widely in their potential for internal dispersal (see De Vlaming & Proctor, 1968), but the variables explaining such differences remain uninvestigated. Gizzard structure (as suggested by Proctor, Malone & De Vlaming, 1967), the nature of grit in the gizzard (Mateo, Guitart & Green, 2000) and intestine and caeca length (see Malone, 1965a) are variables that seem likely to influence survival of propagules and their retention time (see below) and that should be

considered in future studies. Fibrous diets in mallards lead to heavier gizzards (Miller, 1975; Kehoe *et al.*, 1988) more likely to crush propagules, and gizzards become heavier in periods of the annual cycle when waterbird diets are more plant-based (see review in Piersma, Koolhaas & Dekinga, 1993). Herbivorous and omnivorous waterbirds have heavier gizzards than carnivorous species (Kehoe & Ankney, 1985; Barnes & Thomas, 1987), and such differences are likely to influence the proportion of propagules passing undigested through the birds. The ingestion of *Artemia* eggs with grit (sand) reduced their survival of digestion (MacDonald, 1980).

Internal transport step 3: do propagules stay long enough in a waterbird to be dispersed over a long distance?

The maximum dispersal distance of a propagule by internal transport is limited by how long the propagule remains in the gut of a waterbird (retention time), and how far the bird is capable of flying during that period. There is little information about retention times of propagules in waterbirds, although studies of terrestrial birds suggest that retention time should be longer for smaller propagules (Levey & Grajal, 1991; Izhaki, Korine & Arad, 1995). Agami & Waisel (1986) reported retention times of 10–12 h for *N. marina* seeds in mallards, whilst Swanson & Bartonek (1970) found that some *Scirpus* seeds can be retained in the guts of blue-winged teal *Anas discors* for over 72 h, although their viability was not tested. Malone (1965a) investigated how foods consumed with the propagules influence retention time in Mallards. Retention time changed significantly with the food provided with eggs of the crustacean *Artemia salina*, with minimum and maximum retention time of viable eggs being twice as long when fed with corn (mean maximum retention of viable propagules of over 5 h) than when fed with a non-fibrous aquatic plant (*Elodea canadensis*).

Ducks fly at speeds of 60–78 km h^{-1}, and waders at speeds of 48–60 km h^{-1} (Welham, 1994). Green-winged teal *Anas crecca* have been known to migrate over 1200 km in less than 24 h (P. Clausen, pers. comm.). During migration, Pintail *A. acuta* often move about 1000 km between satellite locations at 72 h intervals (Miller *et al.*, 2000). Thus, it is clear that propagules can be dispersed long distances by

internal transport, although the frequency of such long-distance dispersal events remains unknown.

External transport: can propagules adhere to waterbirds?

Experiments on the potential for external transport of propagules by adhesion to the feathers, bills or legs of waterbirds are even scarcer than for internal transport. A number of anecdotal observations of organisms adhering to plumage have been reported (Maguire, 1959; Maguire, 1963; Swanson, 1984). Although a number of experiments to determine the characteristics of seeds that facilitate transport by adhesion have been performed for terrestrial organisms (Sorensen, 1986; Kiviniemi & Telenius, 1998), experimental evidence for aquatic organisms is scarce. The earliest study was performed by Darwin (1859), who removed the leg of a dead waterbird and placed it in a tank with pond snails. The snails crawled onto the bird foot and many stayed there when he removed the foot from the water and waved it around to simulate flight. Many years later, Segerstråle (1954) established that the amphipod *Gammarus lacustris* can adhere to the plumage of ducks for up to 2 h, and *Cercopagis* (Cladocera) also fouled to the plumage of a dead trial duck (Makarewicz *et al.*, 2001). In freshwater environments, exposure to desiccation during transport is likely to limit the potential of many aquatic organisms to disperse by adhesion. As yet no study has addressed how desiccation affects the viability of different kinds of propagules. Many seeds and resting eggs are likely to be highly resistant to desiccation (e.g. Bilton *et al.*, 2001). However, Frisch (2001) showed that the larval resting stages of two cyclopoid copepod species die within 24 h, even at 100% relative humidity, suggesting that desiccation limits the distance of dispersal of the larvae by waterbirds. There is anecdotal evidence that smaller propagules and those with hook-like structures are more likely to be attached to waterbird plumage. Statoblasts of bryozoans that have hooks have often been observed on moulted duck feathers (Okamura & Hatton-Ellis, 1995), and Vivian-Smith & Stiles (1994) described the characteristics of the seeds found adhered to waterfowl. Seeds and resting eggs that float are obviously more likely to become attached to plumage. However, we are not aware of any experiments testing how different propagule morphologies

influence their potential to remain attached to waterbirds.

With what frequency are propagules dispersed by waterbirds in the field?

The studies reviewed above show that waterbirds have the potential to transport propagules, but do not provide us with information about the frequency of waterbird-mediated dispersal in the field. Waterbirds are not only potentially important as agents of long-distance dispersal and of colonisation of new habitats, but also as agents of dispersal at a local scale. There are very few field studies of the frequency of transport (summarised in Table 2), but they are enough to show that waterbird-mediated transport occurs at a high frequency in the field both by internal and external transport, specially for plant seeds. For example, Powers, Noble & Chabreck (1978) reported 17 species of non-digested angiosperm seeds in the intestines of 51 hunted ducks, and Vivian-Smith & Stiles (1994) reported angiosperm seeds attached to the feathers and feet of 28 out of 36 brant geese *Branta bernicla* and ducks.

There are no studies to date that combine information on the quantities of propagules in faeces or attached externally to waterbirds with precise information on the movements of the birds; the distances over which the propagules were dispersed in these studies are thus open to conjecture.

Priorities for future research

Our review of published studies demonstrates that many organisms can potentially be dispersed by waterbirds, and that these processes are likely to be frequent enough to have a major impact on metapopulation dynamics and gene flow in many aquatic organisms, at least at a local scale. The relative importance of different vectors (waterbirds and other animals; this study, Lowcock & Murphy, 1990; wind, Brendonck & Riddoch, 1999; rain, Jenkins & Underwood, 1998; and man, Reise, Gollasch & Wolff, 1999) for dispersal of different propagule types at different scales needs to be addressed.

Further research is required from a range of disciplines to advance our understanding of how bird-mediated dispersal can shape the structure of aquatic communities. There are various questions

Table 2 Summary of studies analysing the potential frequency of dispersal by waterbirds in the field. The type of organisms dispersed, the prevalence of propagules in the field. The type of organisms dispersed, the prevalence of propagules (proportion of individuals or faecal samples carrying at least one intact propagule), and the number and species of disperser are listed

Propagule species	Prevalence of propagules	Disperser species	Reference
Studies on internal transport			
Four genera of Cladocera – Ostracoda	17%	18 ind. of ≥4 species of waterbirds	Proctor (1964)
Metazoans	0%	*Anas platyrhynchos*	Jenkins & Underwood (1998)
Two species of Amphipods	8%	12 ducks of 3 species	Daborn (1976)
Chara zeylanica and *Chara braunii* (Characeae)	34%	47 ind. of ≥ 7 species of waterbirds	Proctor (1962)
Seeds of 17 plant species	53%	51 ind. of 7 species of Anatidae	Powers *et al.* (1978)
Potamogeton sp. (Potamogetonaceae)	23%	13 *Anas platyrhynchos*	Guppy (1906)
Seeds of plants of ≥ 12 genera	38%	71 *Charadrius vociferus*	De Vlaming & Proctor (1968)
Seeds of plants of seven genera	46%	6 species of Anatidae	Holt (1999)
Fruits of five plants	79%	250 *Chloephaga picta* and *C. podiocephala*	Willson, Traveset & Sabag (1997)
Chlorophyte and Cyanophyta	present	96 ind. of 25 species of waterbirds	Proctor (1959)
Spores of *Riella* (Riellaceae)	present	waterbirds	Proctor (1961)
Algae	80%	20 ind. of 4 species of waterbirds	Atkinson (1972)
Studies on external transport			
Seeds ≥11 genera of wetland plants	78%	36 ind. of 4 species of Anatidae	Vivian-Smith & Stiles (1994)

relating both to the characteristics of the propagules that are dispersed (size, shape, hardness, resistance to desiccation) and to the characteristics of the disperser and their effects on the potential for dispersal.

A range of focussed experiments are needed to establish how the various characteristics of different propagules influence their potential to be transported internally (quantifying digestability, retention time, etc.) or externally (quantifying time spent attached to plumage, resistance to desiccation, etc.). A number of different genetic methods to estimate the tails of the propagules' dispersal distance curves are under active development (Cain, Milligan & Strand, 2000), opening promising opportunities to test the relation between propagule characteristics and dispersal capacity, and to investigate the frequency of long-distance dispersal phenomena in the field.

Research is required on how the diet and structure of digestive organs of waterbirds affects the proportion of propagules surviving digestion, and their retention time. The retention time of propagules in the digestive system determines the range of distances potentially travelled by the propagules via internal transport (Proctor, 1968). However, retention time may also have profound effects on the viability of digested propagules; for example, the propagules retained longest may have lower survival. Although some authors have suggested that longer retention times are because of variability in the passage through the intestines and that these may have little effect on the viability of propagules (Clench & Mathias, 1992), others have stated that variation in retention times was because of variation in the time spent in the gizzard, which is likely to lead to a strong effect of retention time upon viability (Proctor *et al.*, 1967). Thus there is a need for detailed experiments on the effects of retention time upon future viability of propagules.

Some species of passerine birds seem to initiate a period of fasting prior to the start of a migratory flight (Fransson, 1998). Piersma & Gill (1998) also reported a reduction in the size of the digestive organs of knots *Calidris canutus* during migration (see also Piersma & Lindström, 1997). If birds empty their digestive tracts prior to migration, this could reduce the potential for long-distance dispersal during migration. However, it seems unlikely that birds can completely empty their digestive organs, including the long caeca characteristic of waterfowl (Clench & Mathias, 1992, 1995).

Furthermore, evidence from terrestrial plants suggests that even low-frequency, long-distance dispersal events can have profound effects on patterns of colonisation and distribution (Cain, Damman & Muir, 1998), especially on the expected speed of range expansion (Higgins & Richardson, 1999).

No information is so far available on the characteristics of propagule shadows (microscale patterns in the dispersal movements of propagules via birds) in aquatic environments, or on the characteristics of the locations where propagules are redistributed by waterbirds in relation to the germination and growth requirements of the organisms. So far it is unknown if dispersal by waterbirds is directional, towards good (or bad) places for the growth of the organisms, or random (see Wenny & Levey, 1998, for an example of directional dispersal by bellbirds).

Satellite tracking is providing much new data on the long- and short-distance movements of waterbirds, although research to date has so far focussed on large swan and goose species that are likely to be less important for dispersal of aquatic organisms than smaller and more abundant ducks and waders (but see Miller *et al.*, 2000). The recoveries of ducks a couple of days after marking can also provide interesting information on the potential range of dispersal provided by waterfowl. Existing data are sufficient to show that long-distance movements by migratory waterbirds are not entirely confined to autumn and spring migration. Many waterfowl species undergo long-distance movements to moulting sites after breeding has been completed (Alerstam, 1990). After arriving at wintering sites, waterfowl and other waterbirds often undergo further mid-winter movements, e.g. related to spells of harsh weather (Ridgill & Fox, 1990) or to changes in water level in response to heavy rainfall (Simmons, Barnard & Jamieson, 1998). There generally appears to be a constant 'turnover' of individual waterbirds on wintering areas, and the only detailed study we are aware of found that the majority of green-winged teals *A. crecca* wintering in the Camargue stayed in the study area for less than 10 days (Pradel *et al.*, 1997).

Many waterbird species breeding at non-extreme latitudes are not strictly migratory but rather nomadic, making long-distance movements at any time of the year in response to spatial fluctuations in wetland availability (del Hoyo *et al.*, 1992; Kingsford & Porter, 1993). The frequent daily movements of all waterbirds from foraging to resting areas at any time of the year and the exploratory movements of juveniles can potentially result in the dispersal of plant or invertebrate species or genotypes over larger distances, through successive short-distance dispersal steps. Such bird movements at a local scale tend to occur without any particular direction (as opposed to the directionality of migration), and can be very frequent during the periods of maximum propagule productivity (Wilkinson, 1997).

Much more information is required before we can understand the relative importance of the capacity for bird-mediated dispersal in structuring the composition of aquatic invertebrate and plant communities in comparison to other forces operating at a local scale, such as interspecific competition (MacIsaac & Gilbert, 1991) or resource constraints (Lampert, 1985). Likewise, much more information is needed before we can understand the role of bird-mediated dispersal in determining gene flow between populations of a given aquatic invertebrate or plant species, in comparison with other forces such as intraspecific competition, local adaptation and priority effects (see Husband & Barrett, 1996; Jenkins & Buikema, 1998). The main conclusion of our review is that the available information demonstrates that transport of aquatic organisms by waterbirds is frequent in the field, but that almost no information is available on which features of the disperser and dispersed organisms affect such processes, and how these influence the frequency of dispersal. Fortunately, dispersal processes are frequent enough at a local scale to facilitate further direct research of such processes in the field. To advance our knowledge of patterns of bird-mediated dispersal in aquatic environments, we recommend an approach combining quantitative analyses of the frequency of transport in the field at a local scale with experiments to test specific hypotheses on the effects of different parameters on dispersal capacity. Great care must be taken in field studies of natural dispersal vectors to separate their role from that of human vectors, which have greatly accelerated dispersal of some taxa (Reise *et al.*, 1999; Bilton *et al.*, 2001).

Acknowledgments

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ELSEVIER

Implications of waterbird ecology for the dispersal of aquatic organisms

Andy J. Green *, Jordi Figuerola, Marta I. Sánchez

Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. Maria Luisa s/n, 41013 Sevilla, Spain

Abstract

In this paper, we review some potential implications of waterbird ecology for their role as dispersers of aquatic plants and invertebrates. We focus particularly on internal transport (endozoochory) by the Anatidae (mainly ducks) and shorebirds, groups especially important for dispersal processes owing to their abundance, migratory habitats and diets. We conduct a literature review to assess the seasonal patterns shown by Anatidae in consumption of seeds and plankton, the interspecific patterns in such consumption (including the effects of body size, bill morphology, etc.), and differences in habitat use (e.g., shoreline vs. open water specialists) and migration patterns between species (e.g., true migrants vs. nomads). We show that many shorebirds are important consumers of seeds as well as plankton, and suggest that their role in plant dispersal has been underestimated. This review confirms that Anatidae, shorebirds and other waterbirds have great potential as dispersers of aquatic organisms, but illustrates how closely related, sympatric bird species can have very different roles in dispersal of specific aquatic organisms. Furthermore, great spatial and temporal variation is likely in dispersal patterns realized by a given bird population. We present evidence suggesting that northbound dispersal of aquatic propagules by endozoochory during spring migration is a frequent process in the northern hemisphere. Much more systematic fieldwork and reanalysis of the existing data sets (e.g., from diet studies) are needed before the relative roles of various waterbird species as dispersers can be fully assessed. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Waterfowl; Shorebirds; Diet; Habitat use; Endozoochory

1. Introduction

There is substantial evidence from both field and laboratory studies that waterbirds disperse the propagules of many aquatic organisms, either externally (ectozoochory) by adhering to their feathers, feet or bill, or internally (endozoochory) via the digestive tract (see Figuerola and Green, 2002a; Charalambidou and Santamaría, 2002 for reviews). All waterbird (sensu Rose and Scott, 1997) groups (including shorebirds, rails, ibises, flamingos, etc.) are likely to be important for dispersal of propagules. Even the various groups of fish-eating birds are likely to be secondary dispersers of seeds, ephippia and other propagules found within their fish prey (Mellors, 1975).

In a recent paper (Figuerola and Green, 2002a), we reviewed direct evidence that waterbirds can disperse propagules both externally and internally. We do not repeat that evidence in this paper, though we do cite some additional evidence that has recently come to our attention. Here, we will focus on the potential implications of some aspects of waterbird ecology for the dispersal of aquatic plants and invertebrates. We do not consider the important role of the functioning of the alimentary canal reviewed by Charalambidou and Santamaría (2002). We also try to minimize overlap with Clausen et al. (2002) who present a critical view of the potential for dispersal of submerged plants (Zosteraceae, Potamogetonaceae and Ruppiaceae) by Anatidae (especially geese and swans) in northern Europe, discussing various essential steps to effective long-distance dispersal. In contrast to Clausen et al. (2002), we do not limit our scope to this region, nor to this group of plants, nor to dispersal events exceeding 300 km (shorter dispersal events are also of great ecological importance). In addition, we include information on all kinds of aquatic plants and invertebrates in our review, and even include terrestrial plants. Many waterbird species are likely to play an important role in long-distance dispersal of terrestrial plants, as has been clearly demonstrated for yellow-legged gulls, *Larus cachinnans* (Nogales et al., 2001). Even in northern

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* Corresponding author.
  E-mail address: andy@ebd.csic.es (A.J. Green).

Europe, Anatidae are likely to have an important role in the dispersal of terrestrial seeds abundant in temporarily flooded grasslands (see, e.g., Thomas, 1982).

The Anatidae (ducks, geese and swans) are of particular importance for dispersal of other aquatic organisms because of their abundance, widespread distribution across the world's wetlands, as well as their tendency to show long-distance movements (del Hoyo et al., 1992). Furthermore, the importance of plant seeds and aquatic invertebrates in the diet of most Anatidae species makes them vectors for dispersal by internal as well as external transport (see Gaevskaya, 1966 for a review of the plant seeds consumed by various Anatidae and coot, *Fulica atra*). Owing largely to their importance as a hunting resource, a great deal of research has been conducted on Anatidae ecology, though this research has been focused largely on migratory species in the northern hemisphere (Baldassarre and Bolen, 1994). Almost nothing is known about the ecology of many of the large number of tropical and southern hemisphere species (Green, 1996), though we review the existing information below.

Very little has been published about the role of shorebirds in dispersal in the field. However, migratory shorebirds are likely to be particularly important for long-distance dispersal, because they are abundant (Rose and Scott, 1997) and often make non-stop flights of 4000 km or more (e.g., Piersma, 1987; Kvist et al., 2001). Unlike Anatidae, many shorebirds expel propagules in pellets as well as in faeces, potentially diversifying both retention time and propagule viability.

Most existing literature on waterbird ecology is based largely on an autecological perspective focusing on the nutritional or habitat requirements of the individual species and the implications for its management. The remaining literature tends to deal with the ecology of the waterbird community without considering its influence on other aquatic communities, e.g., via dispersal. The extensive literature on Anatidae diet usually provides no information as to whether or not seeds consumed survive digestion, or as to whether cladocerans or other invertebrates consumed contain resting eggs or not. Unfortunately, many papers even fail to distinguish between plant seeds and other parts of the plant, the objective being simply to identify those species that act as food plants for bird species of interest. However, our aim is to review the implications that much of this literature has for the role of waterbirds as dispersers of aquatic organisms via internal and external transport of propagules. We pay special attention to the dabbling ducks (tribe Anatini) and pochards (tribe Aythyini; see del Hoyo et al., 1992), which are the most abundant and most studied Anatidae on inland wetlands, as well as reviewing information on the nomadic, seed-eating whistling ducks (subfamily Dendrocygninae). We also review the literature on shorebirds.

2. Do shorebirds carry propagules?

A surprisingly large proportion of shorebird species, including many long-distance migrants, have been recorded consuming seeds (Table 1). After recording undigested seeds in guts, several authors (e.g., Alexander et al., 1996) have suggested that seeds are ingested to act as grit for breaking down animal food, though others (e.g., Davis and Smith, 1998) assume that they are consumed because of their high energy content, and some species are clearly seed or berry specialists. Saltmarsh seeds (mainly Chenopodaceae) were found in 13–44% of droppings or pellets of five of eight shorebird species wintering in the Cádiz Bay, Spain (Pérez-Hurtado et al., 1997). In nearby Doñana, invertebrate eggs and saltmarsh seeds are carried externally by shorebirds (J. Figuerola and A.J. Green, unpublished). Phalaropes predate and can disperse ephippial *Daphnia* (Dodson and Egger, 1980).

Seeds of at least 122 genera of 48 families have been observed in the stomachs of common snipe (*Gallinago gallinago*) (Mueller, 1999). Some 31 species of seeds (mainly Poaceae and Chenopodiaceae) were found in 80 faecal samples of the plains-wanderer (*Pedionomus torquatus*) in Australia (Baker-Gabb, 1988). Seeds were abundant throughout the year, though *Atriplex* spp. was consumed mainly in autumn and winter.

Widgeongrass (*Ruppia maritima*) seeds were found in the faeces of knot (*Calidris canutus*) and curlew sandpiper

Table 1
Summary of the proportion of shorebird species from various families and subfamilies within major geographical regions that have been recorded as including seeds in their diet. We used a conservative approach, listing as 'not consuming seeds' also those species for which plant material is reported without explicitly specifying seeds

	Western Palaearctic[a]		Australasia[b]		North America[c]	
	Yes	No	Yes	No	Yes	No
PEDIONOMIDAE	–	–	1	0	–	–
ROSTRATULIDAE	1	0	1	0	–	–
JACANIDAE	–	–	1	0	1	0
BURHINIDAE	1	1	1	1	–	–
HAEMATOPODIDAE	0	1	0	5	0	2
RECURVIROSTRIDAE	2	0	3	1	2	0
DROMADIDAE	0	1	–	–	–	–
CHARADRIIDAE						
Charadriinae	5	5	8	5	7	2
Vanellinae	3	2	3	0	–	–
SCOLOPACIDAE						
Gallinagoninae	3	0	2	3	1	0
Scolopacinae	1	0	–	–	–	–
Tringinae	8	5	3	4	5	2
Arenariinae	1	0	0	1	0	1
Limnodrominae	–	–	0	1	1	0
Calidrinae	9	0	8	1	7	2
Phalaropodinae	2	0	–	–	2	0
GLAREOLIDAE	1	3	1	1	–	–

[a] Data from Cramp and Simmons (1983).
[b] Data from Marchant and Higgins (1993), Higgins and Davies (1996).
[c] Data from Poole and Gill (1992–2000).

(*Calidris ferruginea*) (as well as whistling ducks) at coastal lagoons in Ghana during October–November (Ntiamoa-Baidu et al., 1998). The seeds found in knot faeces were intact, but deposited in unsuitable habitat for *Ruppia* (T. Piersma, personal communication). The hooded plover (*Thinornis rubricollis*) also consumes *Ruppia* seeds (Marchant and Higgins, 1993).

Shorebirds have often been shown to consume seeds during migration. Baldassarre and Fischer (1984) found seeds to be important in the diet of five of nine wader species studied on autumn migration in September in the Texas Playa Lakes, comprising 19–37% of the aggregate percent gullet volume and occurring in 38–100% of individuals. Seeds included *Polygonum* spp. and *Scirpus* sp. Davis and Smith (1998) compared the oesophagus contents of American avocets (*Recurvirostra americana*), long-billed dowitchers (*Limnodromus scolopaceus*), least sandpipers (*Calidris minutilla*) and western sandpipers (*C. mauri*) on the Playa Lakes, and in total found more seeds on autumn (37–69% of birds) than on spring (8–30%) migration in all four species. However, *Amaranthus* and *Eleocharis* seeds were more abundant in spring in some species. Seeds (mainly *Potamogeton* and *Scirpus*) were found in 59–89% of gizzards of five species on autumn migration through Saskatchewan (Alexander et al., 1996).

Taris and Bressac-Vaquer (1987) found a major seasonal shift in seed consumption by black-tailed godwits (*Limosa limosa*) migrating through the Camargue. In spring 1986, none of 11 gizzards studied contained *P. pectinatus* seeds, yet they were the most abundant item in autumn, 1986 (268 seeds distributed between 10 of 14 gizzards studied). Small numbers of *Scirpus mucronatus*, *Alisma plantago-aquatica*, *Echinochloa crus-galli* and unidentified seeds were found in spring and small numbers of *S. maritimus*, *S. litoralis* and unidentified seeds were found in autumn. Black-tailed godwits wintering in Senegal fed almost exclusively on rice grain, with small amounts of natural seeds (Tréca 1984).

3. How do Anatidae morphology and microhabitat use influence transport of propagules?

The relative importance of plant material and invertebrates in the diet of Anatidae varies greatly according to species (Cramp and Simmons, 1983; Krapu and Reinecke, 1992; Marchant and Higgins, 1993; Baldassarre and Bolen, 1994; Higgins and Davies, 1996; Poole and Gill, 1992–2000). Some important sexual differences in diet occur (Krapu and Reinecke, 1992) which, owing to differences in movement behaviour between sexes (Baldassarre and Bolen, 1994), may translate into various sexual roles in dispersal. However, dietary differences between species are generally much larger than intraspecific sexual differences.

Body mass ranges over more than an order of magnitude in the Anatidae and, overall, there is a weak trend for larger species to feed on larger prey items (including seeds and invertebrates). However, the radiation in bill morphology and associated foraging methods is more important as a predictor of ingestion rates of various items than body size itself. Bill size and morphology show only a weak relationship with body size (Kehoe and Thomas, 1987; Kooloos et al., 1989; Nudds et al., 1994; Green et al., 2001). Within the dabbling ducks, considerable attention has been paid to the importance of the variation in the density of the filtering lamellae within the bill and their influence on the size of invertebrates or seeds consumed by each species. Among northern hemisphere ducks, lamellar density is particularly high in the northern shoveller (*Anas clypeata*) and particularly low in the mallard (*A. platyrhynchos*) (Nudds et al., 1994). All four of the world's shoveller species plus the pink-eared duck (*Malacorhynchus membranaceus*) are well adapted to feeding on zooplankton (del Hoyo et al., 1992), and thus are likely to be particularly important as vectors of resting eggs that can resist digestion. The same is true of filter-feeding flamingos (Zweers et al., 1995).

High lamellar densities enable ducks to filter smaller particles (Crome, 1985), leading to negative correlations between lamellar densities and invertebrate prey size and seed size in several field studies (Thomas, 1982; Nudds and Bowlby, 1984; Nudds, 1992; Nummi, 1993; Tamisier and Dehorter, 1999). However, ducks are highly plastic in their feeding behaviour and show great flexibility in their size selection in relation to food abundance. Denser lamellae appear to reduce the costs of filtering small items, but increase that of filtering larger items, and may often increase the variance in the size of items taken rather than decrease the average size (see, e.g., seed sizes recorded in various ducks by Thomas, 1982). Thus, in various studies, lamellar differences do not explain interspecific differences in size selection (see Mateo et al., 2000). Ducks have mechanisms of feeding on items smaller than the interlamellar gap (Kooloos et al., 1989; Gaston, 1992), though these mechanisms remain poorly understood. Correlations between lamellar densities and food size have only been established for north-temperate dabbling ducks and it remains unclear whether or not such relationships occur in other Anatidae (e.g., diving ducks) and in other parts of the world.

The densities of various plant and invertebrate propagules in wetlands tend to vary greatly from the shoreline to offshore, open microhabitats (with depth, nature of vegetation, etc.). The distribution of aquatic plant seed banks in relation to depth varies between plant species and wetland type (Pederson and van der Valk, 1984; Bonis et al., 1995), but in large wetlands with deeper, open centres both seeds and waterbirds tend to be concentrated around the shoreline facilitating the consumption and adherence of seeds.

Propagule distribution tends to be highly patchy (e.g., floating propagules become highly concentrated along the shoreline facing prevalent winds), but distribution patterns are highly specific to each propagule species and vary greatly over time. *Artemia* eggs concentrate into large

scums along the shoreline of salines where flamingos and shelduck (*Tadorna tadorna*) feed on them (MacDonald, 1980; Walmsley and Moser, 1981). There is evidence that dabbling duck species with fine lamellae spend more time in offshore, open habitats, whereas those with coarse lamellae spend more time in shoreline habitats (Nudds et al., 1994; Green, 1998a), a pattern likely to influence the propagules that may adhere to each species externally, as well as those that are ingested. The chances of external transport are obviously related to the use by waterbirds of habitats where adherent propagules or other transportees are abundant. Rogers and Korschgen (1966) comment on how *Gammarus* were frequently seen clinging to the belly feathers of preening lesser scaup (*Aythya affinis*) in lakes where these amphipods were abundant.

However, duck body size also has a strong influence on microhabitat use, larger dabbling species tending to feed at greater depths (Green, 1998b; Nudds et al., 2000). The depth distribution of propagules varies greatly between seasons (e.g. as propagules are produced near the water surface and later incorporated into the propagule bank in the sediments). It is thus no surprise that feeding behaviour of dabbling ducks also shows a marked seasonal shift, with feeding occurring at greater depths during the non-breeding/wintering period (Thomas, 1980, 1982; DuBowy, 1988). Eurasian teal (*Anas crecca*), mallard and pintail (*A. acuta*) wintering in western France switched to relatively deeper feeding methods (i.e., more neck dipping and upending sensu Green, 1998b) as the winter progressed, probably because of food depletion in shallow areas that permit higher intake rates (Guillemain and Fritz, 2002). In general, diving ducks feed more in offshore, deeper habitats than dabbling ducks (Pöysä, 1983a; Nudds, 1992).

4. Factors influencing selection and consumption of propagules by Anatidae

Though considerable work has been done on how the nutritional quality of leaves of various species influences foraging decisions by grazing Anatidae, much less has been done on the influence of nutritional quality on selection of seed types by waterfowl, but the evidence available suggests that this is important. Thus breeding white-faced whistling ducks (*Dendrocygna viduata*) and red-billed teal (*Anas eythrorhyncha*) fed mainly on terrestrial graminoid *Panicum schinzii* seeds which have a particularly high fat content, whereas ducklings of the former species fed mainly on *Amaranthus* seeds with a particularly high crude protein content as appropriate for growth (Petrie, 1996; Petrie and Rogers, 1996). However, ducks also consume poisonous castor beans in lethal doses (Jensen and Allen, 1981), suggesting that they have a limited capacity to assess nutritional quality of various seeds. Another factor favouring seed selection is the ease with which they can be digested (obviously this reduces dispersal potential; see also

Charalambidou and Santamaría, 2002). Such ease may explain the strong selection shown for water lily (Nympheaceae) seeds in several duck studies (Tréca, 1981a). Experiments suggest that seeds of *Nyphaea*, *Nuphar* and *Nymphoides* water lilies do not survive digestion by ducks or coots, though *Nymphoides peltata* seeds seem to be well adapted for external transport (Smits et al., 1989).

There is much less information about the factors determining the ingestion of animal propagules. Regrettably, almost no authors make a distinction between propagules and other animal tissues in diet studies. For example, while *Daphnia* ephippia are frequently consumed by many duck species, it is unclear to what extent they are consumed from the propagule bank in the sediments, or consumed while inside ephippial *Daphnia*. We expect both mechanisms to be important. Though *Daphnia* are mentioned in many studies of duck diet (e.g., Rogers and Korschgen, 1966; Swanson, 1977; DuBowy, 1997), no mention is made of the presence or the absence of ephippia (except for Sánchez et al., 2000). However, ephippial *Daphnia* were more likely to be consumed by red phalaropes (*Phalaropus fulicarius*) than non-ephippial *Daphnia* (Dodson and Egger, 1980), a positive selection also observed by fish predators (Mellors, 1975).

5. When are propagules likely to be carried by Anatidae?

Clausen et al. (2002) assume that submerged macrophyte seeds are consumed directly off the plant, yet ducks also consume seeds from the sediments favouring dispersal long after the seeds have been shed from the mother plant. Anatidae may rapidly deplete seeds when taken directly from the plants (e.g., *Salicornia* seeds, Van Eerden, 1984; Summers et al., 1993; Potamogeton seeds, Santamaria, unpubl.; Clausen et al., 2002), and the availability of such seeds may vary greatly from year to year (because of changes in the environmental conditions or relative abundance of various plant species). In comparison, availability of seeds from banks in the sediments is likely to be less variable between years (e.g., Bonis et al., 1995), though fluctuations in water depth have a major influence on their availability to dabbling ducks and waders (Pöysä, 1983b; Gray and Bolen, 1987; Ntiamoa-Baidu et al., 1998). Furthermore, depletion of seeds from banks in the sediments by Anatidae is slower and less efficient. Even in the shallow areas most used by feeding ducks, Gray and Bolen (1987) found only marginally significant seed bank depletion between September and April. In temporary marshes in the Camargue, France, Bonis et al. (1995) found no measurable depletion by birds. Thus there is likely to be less potential for seasonal and annual variation in dispersal of seeds consumed from sediments than from seed heads on the plant. However, in Doñana we have recorded a 53% reduction in the density of widgeongrass *Ruppia maritima*

seeds in sediments from September to May ($P = 0.007$) because of consumption by ducks, other waterbirds and potentially also fish. There is no consistent change between duck species in the area in the numbers of intact seeds defecated between autumn and spring (authors, unpublished), suggesting similar potential for southward and northward dispersal (contrary to Clausen et al., 2002).

In this section, we consider seasonal changes in dispersal potential by reviewing studies of Anatidae diet (mainly data on the contents of the gullet and gizzard). Our review is not totally comprehensive, especially given the enormous quantity of grey literature on waterfowl diet. It is very difficult to predict dispersal potential based on such diet studies for several reasons. First, the proportion of propagules surviving digestion is itself affected by changes in diet composition. Research in captivity suggests that seeds mixed with a mainly animal diet can survive digestion better than those incorporated in a purely seed diet (Charalambidou and Santamaría, 2002, I. Charalambidou, unpublished). We provide here a unique example of how seasonal differences in the consumption rates of various seeds are *not* reflected by a change in dispersal potential. *Ruppia* and *Salicornia* seeds were much more abundant in marbled teal (*Marmaronetta angustirostris*) diet at Sidi Moussa, Morocco in October than in May, whereas Ranunculus seeds were much more abundant in May (A.J. Green and M.I. Sánchez, unpublished). However, when the numbers of intact, apparently viable seeds found in faeces are compared, there is only a significant seasonal effect in the case of the Ranunculus seeds (Fig. 1). This illustrates how diet switches do not necessarily translate into changes in dispersal potential.

Furthermore, diet studies show that seeds of a given type are often present in small numbers but in a large proportion of the bird population (e.g., Cyperaceae seeds in garganey,

Anas querquedula, Tréca, 1981a), whereas seeds of other types are found in large numbers but only in a small number of birds (e.g., *Echinocloa* seeds in garganey, Tréca, 1981a). Without affecting the overall proportion of a given seed type in the diet of the bird population, these two distribution patterns have very different implications for dispersal. When a seed type is carried by more birds, there is more chance that one bird will move the seed a long distance to a suitable habitat. However, ingestion of the same seed in large quantities may increase survival of digestion in some situations (authors, unpublished). Tamisier (1971) commented that relatively more seeds survived digestion (i.e., remained intact in the rectum) by Eurasian teal as their overall ingestion rate increased.

Seasonal diet switches are inevitable in seasonal environments, and there is a tendency in migratory duck species in the northern hemisphere to feed relatively more on seeds rich in carbohydrates during the autumn and winter periods and relatively more on invertebrates during the breeding season and in the immediate postbreeding period when the flightless moult occurs (DuBowy, 1988; Hohman et al., 1992; Krapu and Reinecke, 1992; Baldassarre and Bolen, 1994), but there are many exceptions to this, some of which are covered in the following review.

Though considerable information is available on seasonal differences in the importance of invertebrates in duck diets, we are only aware of one published study providing information on seasonal differences in the ingestion of invertebrate resting eggs. *Artemia* cysts were present in the faeces of shelduck wintering in the Camargue from October to February, being present in 20% (October) to 98% (December) of samples (Walmsley and Moser, 1981).

Sánchez et al. (2000) found cladoceran ephippia in the upper guts of 21 of 68 stifftails (*Oxyura* spp.) in Spain and they were present in birds sampled throughout the year with no clear seasonal trend (authors, unpublished). *Daphnia* ephippia were found in 42% of marbled teal faecal samples collected at Sidi Moussa, Morocco in October, and 32% of samples in May, a non-significant difference (A.J. Green and M.I. Sánchez, unpublished). Likewise, there was no seasonal change in the numbers of intact ephippia per sample (Fig. 1).

5.1. Studies of north-temperate ducks on migration

Despite the wealth of studies of migratory ducks in North America and Europe, relatively few studies have been made of the diet of ducks at passage sites. However, several studies give an insight into the relative potential for southward movements of propagules during autumn migration and northward movements during spring migration.

Aquatic seeds (from seven genera) were found in 82% of the gullets of buffleheads (*Bucephala albeola*) and lesser scaup on spring migration through California, USA, constituting 34% and 23% of aggregate volume, respectively
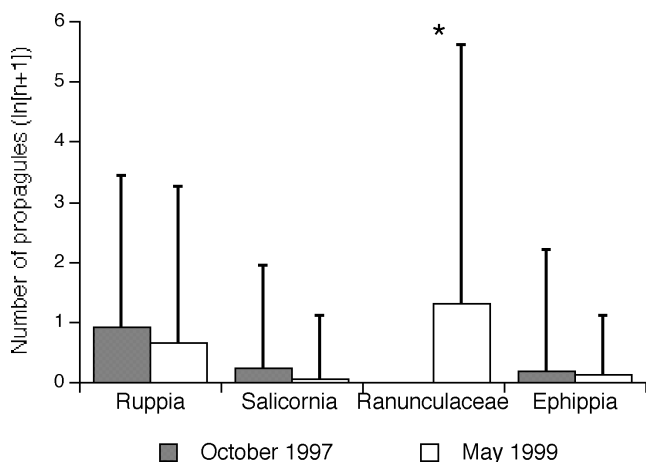
Fig. 1. Seasonal variation in the presence of propagules in faeces of marbled teal (*Marmaronetta angustirostris*) collected at Sidi Moussa-Oualidia in Morocco, showing means and range after transforming ($\log_e [n + 1]$, thus means are similar to geometric means) the numbers of intact seeds and ephippia per faecal sample. Ephippia were from *Daphnia* spp. The seasonal effect was only significant in the case of Ranunculus seeds (Mann–Whitney U-test; $n = 19, 28$; $U = 152$; $P < 0.002$).

(Gammonley and Heitmeyer, 1990). The most abundant seeds were *P. pectinatus* (45% of bufflehead gullets, 36% of scaup), *Scirpus robustus* (64% and 27%) and *Polygonum lapathifolium* (45% and 54%). These species are generally considered invertebrate specialists, yet their capacity for northward dispersal of seeds could be important. Lesser scaup on autumn migration through Illinois were consuming much less plant material, though *Potamogeton* and *Scirpus* seeds were present in 26–27% of gizzards (Rogers and Korschgen, 1966). In scaup migrating through northwestern Minnesota, seeds had a similar frequency in gullets in spring (eight genera, 42%) and autumn (five genera, 26% for immatures, 50% for adults), but *Potamogeton* seeds were more abundant in spring (21%) than in autumn (6% immatures, 14% adults; Afton et al., 1991).

Blue-winged teal (*Anas discors*) on spring migration through Missouri consumed seeds from 28 genera, totalling 35% of aggregate gullet mass and with 55–100% of gullets containing spike rush (*Eleocharis*) seeds and 55–70% containing floating primrose willow (*Ludwigia repens*), rice-cut grass (*Leersia oryzoides*) or *Panicum* grass seeds (Taylor, 1978). In California, 26% of aggregate gut content dry mass in unpaired male cinnamon teal *A. cyanoptera* on spring migration was made of seeds of 11 genera (including widgeongrass; Hohman and Ankney, 1994). In New Mexico, oesophagi of females on spring migration contained 19% *Scirpus* and 4% *Polygonum* seeds (by aggregate dry mass), whereas immatures on autumn migration contained 25% *Scirpus*, 10% *Polygonum* and 40% *Echinochloa* (Gammonley, 1996).

The gullet contents of migratory mallards from Missouri during October–December were dominated by seeds of wild millet (*Echinochloa* spp.), nodding smartweed (*Polygonum lapathifolium*), rice-cut grass, arrowhead (*Sagittaria latifolia*) and Pennsylvania smartweed (*P. pensylvanicum*) (Gruenhagen and Fredrickson, 1990). Apparently intact seeds were found in the large intestine or cloaca of 10 of 20 mallard, four of 10 green-winged teal (*Anas carolinensis*) and two of six blue-winged teal collected during October–November in Wisconsin (Montaba, 1971). Seeds included Pennsylvania smartweed, *Polygonum persicaria*, *P. punctuatum*, *Scirpus validus*, *Potamogeton natans*, *Eleocharis elliptica* and rice-cut grass.

Pintail using vernal pools in California have been reported to feed on moist-soil seeds (mainly *Crypsis*, *Echinochloa*, *Polygonum* and *Eleocharis*) during both autumn and spring migration periods, but to feed on emergent seeds (mainly *Scirpus acutus* and *S. maritimus*) in midwinter (Silveira, 1998).

Gill (1974) suggested that ducks and geese internally disperse slough grass (*Beckmannia syzigachne*) seeds on both autumn and spring migration in Canada, but that spring migration is the most important in explaining its northerly distribution.

5.2. Studies of north-temperate ducks on wintering grounds

Many studies allow us to compare diet at a given wintering site during autumn/early winter and late winter/spring, when long-distance dispersal is most likely southwards and northwards, respectively. In the San Joaquin Valley, California, swamp timothy (*Heleochloa schoenoides*) seeds were present in 56% of northern pintail oesophagi sampled from September to November but only 15% of birds sampled from December to February, and curly dock (*Rumex crispus*) seeds decreased from 33% of birds to 9%. By contrast, the presence of nodding smartweed (*Polygonum lapathifolium*) seeds increased from 16% of birds to 28% (Connelly and Chesemore, 1980). In the Central Valley, California, swamp timothy and barnyardgrass (*Echinochloa crusgalli*) seeds became progressively less abundant in the oesophagi of pintails from October to February, but alkali bulrush (*Scirpus paludosus*) and sprangletop (*Leptochloa* spp.) seeds became progressively more important from December onwards. Seasonal trends for a given plant species were not so marked in the diet of green-winged teal collected in the same area, but overall seeds became progressively less important in the diets of both ducks as the winter proceeded (Euliss and Harris, 1987). Pintail and green-winged teal were reported to concentrate feeding on ammania (*Ammania coccinea*) and barnyardgrass seeds when they floated and concentrated on pond surfaces in early winter, though pintail later switched to feeding on barnyardgrass seeds concentrated on the pond bottoms (Euliss and Harris, 1987).

Consumption of water lily (*Nymphaea odorata*) seeds by ring-necked ducks (*Aythya collaris*) reduced from 29% of the total food dry weight in the gullet during October–December to 15% during January–March (Hoppe et al., 1986), though these seeds may not be able to survive digestion. By contrast, Jeske et al. (1993) found water lily seeds to be relatively less important in the diet of this species during November–December than during late winter.

In northern Europe, brent geese (*Branta bernicla*), Eurasian wigeon (*Anas penelope*) and other Anatidae feed extensively on *Salicornia europaea* seeds and leaves in autumn shortly after the seed crops ripen, and consume many fewer seeds in late winter (Van Eerden, 1984; Summers et al., 1993). Greylag geese (*Anser anser*) wintering in Doñana feed mainly on *Scirpus* tubers (Amat, 1995), but c. 20% of faeces contained intact *S. maritimus* seeds in January (authors, unpublished), a time when the geese regularly move hundreds of kilometres to alternative wintering sites (Nilsson et al., 1999). Giant Canada geese (*Branta canadensis maxima*) about to start spring migration in Minnesota were consuming some grass seeds of the millet tribe Paniceae (McLandress and Raveling, 1981).

In the Camargue, seeds were much more important in the diet of Eurasian coot during August–October (when they constituted 43% of prey items) than during November–
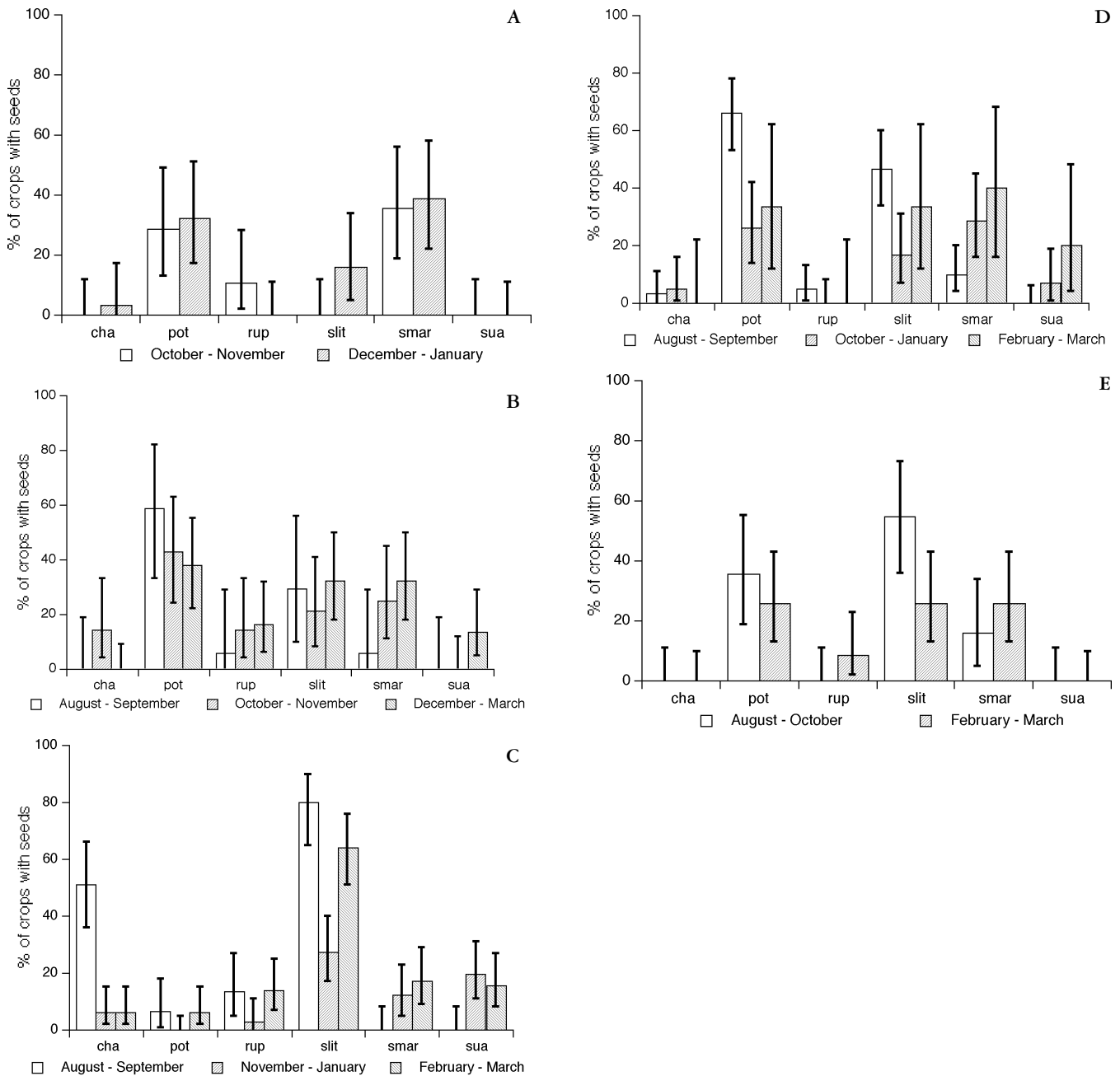
Fig. 2. Frequency of diaspores (i.e., percentage of individual ducks in which each seed or oospore type was recorded), in the crops of various duck species collected in the Camargue, France, at various times of the autumn and winter, showing 95% confidence intervals. A: northern pintail (*Anas acuta*) (*n* = 59), B: northern shoveller (*Anas clypeata*) (*n* = 82), C: Eurasian teal (*Anas crecca*) (*n* = 175), D: mallard (*Anas platyrhynchos*) (*n* = 119) and E: garganey (*Anas querquedula*) (*n* = 66). Diaspores shown are cha = *Chara* sp. (large size), pot = *Potamogeton pectinatus*, rup = *Ruppia maritima*, slit = *Scirpus litoralis*, smar = *Scirpus maritimus*, sua = *Suaeda maritima*. Data from Pirot (1981) show only a selection of the most important diaspores in duck diets (with the permission of the author). Birds were collected from 1964 to 1966 and from 1979 to 1981; hence, there is a confounding year effect.

February (less than 8% of food items, Allouche and Tamisier, 1984). Major seasonal variations have been found in Eurasian teal (Tamisier, 1971) that consumed relatively large amounts of *Suaeda* seeds during November–January, and relatively large amounts of charophyte oospores and *Scirpus* seeds during August–October and also during February–March. Pirot (1981) provides fascinating data on the

ingestion rates of various plant seeds by five different dabbling ducks at different stages of the autumn and winter period in the Camargue, one of Europe's most important wintering sites (Fig. 2).

These data refer to the proportions of birds carrying each seed type in their crops, and provide no information on the abundance of those present (shovellers fed principally on

invertebrates, whereas the other four species fed principally on seeds; see Tamisier and Dehorter, 1999 for quantitative data). In general, there are marked differences between duck species in the relative importance of various seeds (e.g., note the greater importance of *Chara* and *S. litoralis* and the lesser importance of *P. pectinatus* in the teal). Within species, there are significant differences between seasons in the importance of various seeds, but few seasonal trends are consistent between species. *Scirpus maritimus* is the seed showing the most marked trend, growing in frequency as the winter period goes on in all five species. In contrast, the closely related *S. litoralis* tends to be more abundant during August–September than during October–January (highly significant for teal and mallard).

M. Guillemain (unpublished) found that mallard and Eurasian teal wintering in western France began the winter by feeding on relatively large seeds at shallow depths (likely to provide higher rates of energetic intake), then increased their niche separation through the winter with mallard feeding on large seeds but at progressively greater depths, and teal remaining in shallow areas but switching to smaller seeds. However, this seasonal shift in seed size was not recorded in the Camargue (Fig. 2).

5.3. Studies of north-temperate ducks on breeding sites

Some studies allow us to compare diets at the beginning of the breeding season (when birds are still arriving and some continue on northward) with the end of the summer (when some birds are moving southwards on autumn migration, or potentially northwards on moult migration).

Blue-winged teal on breeding grounds in Saskatchewan fed on seeds from 13 genera, with *Sparganium* consumed mainly during May–June after arrival, *Scirpus* and *Nuphar* consumed mainly during August–September before leaving and *Eleocharis* and *Carex* important from May to September (Dirschl, 1969). Lesser scaup on breeding grounds in Saskatchewan fed on seeds from 11 genera, with *Nuphar* seeds being a major diet component during August–September just after they ripened and *Sparganium* consumed mainly during July–September (Dirschl, 1969).

5.4. Studies of ducks outside north-temperate regions

Outside the north-temperate climatic regions (where most research on Anatidae has been concentrated), there is by no means a general rule that ducks consume more invertebrates and fewer seeds during the breeding seasons. For example, Petrie and Rogers (1996) found breeding white-faced whistling ducks in South Africa to feed almost exclusively on seeds, especially the terrestrial graminoid *Panicum schinzii*, which is abundant in newly flooded ephemeral wetlands. In the same region, Petrie (1996) found red-billed teal feeding mainly on *P. schinzii* seeds during

both the breeding and postbreeding periods. Both duck species regularly consumed aquatic *Scirpus* and *Polygonum* seeds in small quantities during the breeding season. The diet of non-breeding white-faced whistling ducks (in a different part of South Africa) from early winter to spring was dominated by aquatic seeds, especially *Scirpus brachyceras* at one site and *Nymphaea* sp. and *Polygonum lapathifolium* at another, with no consistent trends as the season progressed (Petrie and Rogers, 1997a).

Tréca (1981a, b, 1986) provides a fascinating comparison of seasonal variation in the diets of the migratory garganey and the tropical white-faced and fulvous (*D. bicolor*) whistling ducks in the Senegal Delta. The whistling ducks are present in the delta all year round, but are capable of long-distance movements across Africa (Scott and Rose, 1996). As well as rice, all three species consumed diaspores of *Nymphea*, Cyperaceae (*Scirpus* and *Picreus*), Graminaceae (*Echinochloa colona* and *Panicum laetum*), Gentianaceae (*Limnanthemum senegalense*) and *Chara*. However, there were major differences between duck species; for example, garganey consumed much more Cyperaceae and *Chara*. Strong seasonal patterns were observed within each duck species, with garganey feeding relatively more on *Echinocloa* during October–November after arrival, on *Chara* in March before leaving, on Cyperaceae in December and February and on *Nymphea* in January. In contrast, white-faced whistling ducks fed relatively more on *Echinocloa* from March to October, on *Limnanthemum* from November to December, on *Nymphea* from November to March and on *Chara* in November and January. *D. bicolor* fed relatively more on *Echinocloa* from June to September, on *Limnanthemum* from January to April, on *Nymphea* from September to February and on *Chara* in March.

In semi-arid and arid environments, various seed types are dominant in Anatidae diets during the wet and dry seasons, largely due to the differences in vegetation types between ephemeral and more permanent wetlands. In Australia (Marchant and Higgins, 1993), the wandering whistling duck (*D. arcuata*) feeds more on grass seeds in the wet season and more on *Nymphoides* and *Polygonum* seeds in the dry season. The magpie goose (*Anseranas semipalmata*) also feeds on grass seeds in the wet season and on sedge rhizomes in the dry season. For the grey teal (*Anas gracilis*) in coastal Queensland, Australia, seeds of both freshwater and brackish plants were more abundant in the wet season, with invertebrates more important in the dry season.

6. How far are propagules carried, and in which direction?

Clausen et al. (2002) review the speed and timing of long-distance movements by Anatidae. All waterbirds also make local movements (e.g., between feeding and roosting or nesting sites) throughout the annual cycle and can thus readily move propagules between various wetlands that are

nearby, but have separate catchments. Thus even such local movements can have a major role in the dispersal of aquatic organisms that cannot move between catchments via flotation, fish or other means. Ducks and geese often fly tens of kilometres between feeding and roosting sites (see, e.g., Tamisier and Dehorter, 1999, for movements of wintering ducks and coots within the Camargue). Wintering ducks in western France (Guillemain et al., 2002) and elsewhere disperse locally at night. In addition, northern migratory ducks rarely remain in the same area for several months once they reach a wintering site. Many species show high mobility during the winter, making regular movements between various wetlands (Pradel et al., 1997), partly in response to cold weather (Ridgill and Fox, 1990). In Mediterranean and semi-arid regions, bird concentrations regularly shift location by tens or hundreds of kilometres at any time of the year in response to rainfall. As an extreme example of foraging movements, flamingos breeding at Fuente de Piedra, Spain, fly up to 360 km to feed at various wetlands before returning to feed their chicks (Amat et al., 2001).

Long-distance movements of propagules are not only confined to those waterbird species with cyclical and predictable north–south migrations to and from more extreme latitudes. Many species show long-distance nomadic movements in response to the flooding and drought cycles of temporary wetlands in arid and semi-arid environments (Kingsford and Porter, 1993; Simmons et al., 1998; Kingsford et al., 1999). Among classic migratory species, there are great differences between species and even between populations in migratory behaviour. Thus, among western Palaearctic ducks, pintail and garganey make particularly long movements, whereas mallards are particularly sedentary but with great variation between and within mallard populations (del Hoyo et al., 1992; Scott and Rose, 1996). As well as long-distance movements between breeding and wintering grounds, many migratory Anatidae species make long movements upon completing breeding to sites more suitable for the flightless moult completed before winter migration begins (Hohman et al., 1992; see Clausen et al., 2002).

An extreme case of nomadic long-distance movements is shown by pink-eared duck (*Malacorhynchos membranaceus*) (Kingsford, 1996), which feeds principally on invertebrates but also on seeds (Marchant and Higgins, 1993). Sizeable flocks of over 1000 ducks were seldom recorded at the same wetland more than once in a 12-year period of annual surveys. Such nomadic dispersal patterns between ephemeral wetlands may have major effects on the genetic structure of dispersed plants and animals, but would be much harder to demonstrate than the effects of a directional north–south migration pattern, as there are no defined flyways.

Major switches in habitat use are usually associated with long-distance movements by waterbirds, and Clausen et al. (2002) discuss how this can hinder dispersal. Ducks show a tendency to winter on larger, more open wetlands and breed on smaller wetlands with more luxuriant vegetation, though the details are species-specific. Heitmeyer and Vohs (1984) found major differences in the way eight dabbling duck species distributed on small wetlands of different types (lakes, rivers, open and vegetated marshes) while on migration through Oklahoma. Seasonal differences were also found within species; for example, green-winged teal selected lakes in autumn, but marshes in spring. However, great spatial and temporal variation is often found in habitat use for a given waterbird species. Even when a duck undergoes a major shift in wetland size or maximum depth after a migration journey, there are often plant or invertebrate species that can survive in both sites (especially around the shoreline) and be dispersed between them.

At intermediate latitudes, classical migratory duck species overlap with other species with greater nomadic tendencies and each group may disperse propagules in different directions. For example, marbled teal (which shows both migratory and nomadic tendencies) in Spain often move northeast in late summer/autumn (Navarro and Robledano, 1995) when classical migratory species breeding further north are moving in the opposite direction (Navarro and Robledano, 1995; Green and Navarro, 1997). Diet studies (A.J. Green and M.I. Sánchez, unpublished) and captive experiments (Figuerola and Green, 2002b) strongly suggest that marbled teal internally transport viable *Ruppia* and *Scirpus* seeds between suitable habitats during these northerly movements.

Throughout the world, the creation of reservoirs and other artificial wetlands has led to major changes in migratory movements of waterbirds (e.g. Švažas et al., 2001) with consequent implications for dispersal that are yet to be understood. In South Africa, Petrie and Rogers (1997b) suggest that the creation of irrigation ponds has caused whistling ducks "to winter close to breeding areas and be more fixed and predictable in their annual movements (i.e., more migratory and less nomadic, dispersive, facultative")".

Waterfowl undergo high mortality rates during the winter period (Baldassarre and Bolen, 1994; Krementz et al., 1997) and the numbers of waterfowl migrating south are much higher than those returning north. This factor will tend to make long-distance dispersal more frequent southwards than northwards.

7. Conclusions

The literature on waterbird ecology suggests that Anatidae, shorebirds and other waterbirds have an important role in the population and community ecology of aquatic invertebrates and plants by acting as vectors of passive dispersal. Our review confirms that Anatidae and shorebirds have great potential as dispersers of aquatic organisms, but shows that there are certainly to be great differences

between closely related, sympatric bird species in their roles in dispersal of specific aquatic organisms. Furthermore, there is likely to be great spatial and temporal variation (i.e., a great deal of noise) in dispersal patterns realized by a given bird population.

Though seed consumption by north-temperate ducks is generally higher during the migration and wintering periods than during the breeding season, the above review shows that the seasonal patterns recorded in autumn and spring or in early and late winter are not consistent. In a given study, some seeds are consumed more during autumn or early winter and others are consumed more during late winter or spring. These patterns also show major variation between study sites and probably also years, as has been described in bird-mediated dispersal within terrestrial ecosystems (Herrera, 1998).

Though many duck species have quite marked migratory patterns, long-distance movements of propagules by them are likely to be highly unpredictable. The consumption and adhesion of propagules by and to ducks is certain to be subject to great temporal and spatial variation, even within a given wetland complex, in relation to changes in the distribution of propagules and of birds, water depth fluctuations (which change the availability of propagules in the sediments, especially to dabbling ducks) and changes in the availability of other food items (invertebrates, agricultural waste grain, etc.). The survival by propagules of digestion is also certain to fluctuate enormously (Figuerola and Green, 2002a; Charalambidou and Santamaría, 2002), making it difficult to draw conclusions from diet studies reviewed above in which the state of propagules after digestion is not addressed.

Previous authors have usually focused on the bird species without paying attention to their role as dispersers, and there is relatively little detailed information from field studies that allows us to identify patterns in dispersal. Much more fieldwork or reanalysis of the existing data sets (e.g., more detailed analysis of gut contents) is needed before the relative importance of different waterbird species in the dispersal of specific plants or invertebrates can be accurately assessed, or before any seasonal trends in dispersal can be firmly established.

In the few studies allowing seasonal diet comparisons, they are confounded by year effects (i.e., birds are collected over different years, then combined). There is an acute lack of studies comparing diet at the same site over different parts of the same annual cycle (e.g., autumn with the following spring). Thus, the observed seasonal patterns reported above may be strongly biased by differences between years (in seed production, water levels, etc.). There is a particular need for studies of faecal contents that compare the number of viable propagules defecated by birds at a given location at different parts of a single annual cycle (especially comparing autumn and spring migration periods). Faecal analysis has rarely been used to study duck diet (Green and Selva, 2000), largely owing to the difficulty in assessing the relative proportions of various items at ingestion, yet this method is much more suitable for studies of dispersal capacity than for studies of gut contents. There is an urgent need for more work about how and when waterbirds ingest invertebrate propagules, and particularly on spatial and temporal variation in external transport of both plant and animal propagules (about which we have said little owing to the acute shortage of available data).

The relative importance of moult migration, winter movements and autumn/spring migration in long-distance dispersal of plants and invertebrates is currently open to speculation and is one of many subjects for future research. We suggest that Clausen et al. (2002) underestimate the potential for dispersal of seeds northwards in temperate environments, especially owing to the consumption of seeds from sediments. We have cited several studies documenting consumption of *Potamogeton* and *Ruppia* seeds by ducks on spring migration or in late winter. Our own unpublished data demonstrate high rates of consumption of widgeongrass *R. maritima* seeds by spring migrants in Doñana, as well as defecation of viable seeds. The data we have presented on marbled teal (Fig. 1) show that consumption and dispersal of *Ruppia* seeds are not restricted to the late summer/autumn period when seeds are produced.

We encourage waterbird biologists to make a contribution to furthering understanding of dispersal processes by using birds captured or collected for other studies. Studies conceived to address an aspect of the ecology of bird species could often be easily adapted to address the role of that bird in the wider aquatic community. For example, conventional diet studies can be easily extended to record data on the presence and state (i.e., intact or not) of invertebrate and plant propagules. The screening of birds captured for ringing programmes for externally attached propagules is straightforward and would provide invaluable information about external transport, which could be integrated with the ringing recovery data to assess the directionality of dispersal. Birds collected for diet studies could also be inspected externally for propagules and the inspection of lower gut as well as upper gut contents would clarify which propagules apparently survive ingestion intact, complementing information on ingestion. Where birds are not collected, faeces samples could often be taken and the viability of propagules could be assessed (e.g. birds often defecate when collected for ringing).

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Part 2

How frequent is bird mediated transport in the field?

How frequent is external transport of seeds and invertebrate eggs by waterbirds? A study in Doñana, SW Spain

Jordi Figuerola and Andy J. Green

Estación Biológica de Doñana, CSIC, Spain

Abstract: Dispersal of aquatic organisms by birds has long been assumed to be an important process, but quantitative studies of its frequency are scarce. We determined the presence of plant and invertebrate propagules adhering to the plumage or feet of 4 7 waterbirds of 6 species (2 ducks, 2 waders and 2 rallids) trapped during the spring migration period in two localities in Doñana, south-west Spain. The percentage of waterbirds transporting propagules was high, with large differences between sites in the proportion of individuals carrying propagules (35% and 100% respectively) and the numbers and types of propagules carried. Seeds of at least 15 plant species, eggs of at least 6 invertebrate species and at least one alga were encountered, with each bird carrying up to 12 different types. Seeds tended to be attached to the plumage, and invertebrate eggs to the feet. The efficiency of protocols for removing propagules from birds varied between bird and propagule species. External transport of propagules by waterbirds seems a frequent process at least at a local scale and is likely to facilitate the rapid colonisation of new or temporary wetlands, and maintain gene flow between populations.

Introduction

The transport of plant and invertebrate propagules by adhesion has long been considered a significant mode of dispersal in aquatic environments (DARWIN 1859, RIDLEY 1930). DARWIN (1859) provided some experimental evidence of the possibility of dispersal of pond snails by adhesion. Many years later, SEGERSTRÄLE (1954), showed that the amphipod *Gammarus lacustris* can adhere to the plumage of ducks for up to two hours. More recently, MAKAREWICZ et al. (2001) observed that *Cercopagis pergoi* (Cladocera) can adhere to duck plumage.

Although there are a number of anecdot al observations of propagules, plant fragments or invertebrates adhering to waterbirds (reviewed in Maguire 1963), quantitative analyses of the frequency of external transport are very scarce (see review by FIGUEROLA & GREEN 2002). In the only such study to date, VIVIAN-SMITH & STILES (1994) reported seeds adhering to the feathers and feet of 28 out of 36 geese and ducks, suggesting that external transport may be an important process, at least in some areas.

Here we present information on the identity and frequency of propagules adhering to waterbirds in Doñana (south-west Spain). This is the first study to consider spatial variation in the external presence of propagules in birds using different parts of a wetland complex, and to address variation between waterbird species. This is also the first study to look for differences between propagules attached to feet, and those to plumage. Finally, this is the first study to test the efficacy of protocols for detecting attached propagules, and to test for differences between waterbird species in this efficacy.

Methods

Birds were captured at two localities, Veta la Palma (6º14´ W, 36º 57´ N) and Cañada de los Pájaros (6º09´ W, 37º14´ N), approximately 20 km apart within the extensive wetlands found in Doñana, SW Spain. Veta la Palma consists of a complex of c. 40 brackish ponds managed for fish farming, with

a total area of 3,200 ha. It is used by many waterbirds during the winter (monthly aerial waterbird counts during the study were: January 2000, 50,709, February 26,435 and March 16,745 individuals, Estación Biológica de Doñana, unpublished data). Submerged vegetation is dominated by wigeongrass, *Ruppia maritima*, with much smaller amounts of sago pondweed, *Potamogeton pectinatus*, and the shores and islands are covered with saltmarsh vegetation, especially *Arthrocnemum* and *Suaeda*, together with small amounts of *Phragmites* and other emergent plants . Cañada de los Pájaros consists of a small, freshwater pond (c.5 ha) resulting from the restoration of an abandoned gravel pit. The area is managed as a recreational wetland used by several hundred wintering waterbirds attracted by artificial feed. There is no visible submerged or emergent vegetation, and the steep banks are covered with terrestial plants (chiefly Gramineae). One baited walk-in-trap was operated in each of the two localities between 28 January and 15 March 2000 (nine capture occasions in Veta la Palma, and two capture occasions in Cañada de los Pajaros) . The Veta la Palma trap was a 4 m square covered with poultry mesh, and funnels to allow the entrance of birds (see Wainwright 1957 and Figure 157 in Bub 1991) . Rice was used to attract the birds into the traps. The trap at Cañada de los Pajaros was of the same design but larger (apr. 10 m x 4 m side). At Veta la Palma the trap was situated in a shallow unvegetated area of marsh of 10 cm depth. The trap was opnened at 1800 h and emptied at 1000 h the following morning. At Cañada de los Pajaros the trap was set on dry land, and was opened at 2100 h and emptied at 1000 h the next morning. In both cases, birds entered the trap during hours of darkness. Captured birds were removed from the traps, their feet (up to the ankle) were cleaned in a plastic cup with distilled water, and they were then held individually for less than one hour in cloth bags until further processing.

Each bird was held over a plastic tray and brushed for three minutes with a soft shoe-brush. All the particles in the plastic tray were transferred to a plastic vial and transported together with the cloth bag to the laboratory for posterior examination and identification under the dissection microscope. The cloth bags were brushed in the laboratory to recover propagules that were

shed from birds while in the bag. The water used to clean the feet was filtered through a 0.04 mm sieve and the material retained in the sieve examined under a binocular m icroscope (x25). There was a size limit for the type of propagules detectable in our study , thus organisms such as rotifers were too small to be detected by the methods used. For some individuals only samples from the feet or feather were obtained, due to insufficient cloth bags for storing samples. To prevent contamination from previous captures, cloth bags were not reused. Identification of propagules was based on CAMPREDON et al. (1982) and ALONSO (1996).

To evaluate the performance of our brushing method for recovering propagules from the plumage, we conducted tests using captive ducks at the Wildlife Recovery Centre in Doñana National Park. Ten seeds of different plant species or ephippia of *Daphnia magna* were embedded manually in the plumage by one person. A second observer, unaware of the distribution of the seeds, applied the above protocol, and the number of propagules recovered after three minutes was counted.

Data were analysed using general linea r modelling (GENMOD procedure, SAS INSTITUTE 1997), with a binomial error distribution, due to the nature of the response variable (presence/absence of propagules, or number of propagules recovered/number used in the test, see CRAWLEY 1993). The dispersion of the model was adjusted to one by scaling the model with the square root of the ratio deviance/degrees of freedom (SAS INSTITUTE 1997). For abundance data we used a negative binomial error distribution, which is more adequate for count data. Correlation between continuous variables was tested using Spearman Rank Correlations and differences in the frequency and abundance of propagules on the feet and/or the plumage were tested with Chi-Square and Wilcoxon signed-rank tests.

Results

The tests in captivity showed that the method for recovering the seeds and ephippia from plumage was highly effective. After three minutes of brushing, we recovered between 72 and 96% of the propagules, depending on the

propagule type and duck species (see Table 1). The efficiency of the method varied between species and was less efficient in the mallard than in the teal (factor species: $\chi^2 = 7.16$, 2 d.f ., P = 0.03, post-hoc contrast $\chi^2 = 6.92$, 1 d.f., P = 0.009), and for *Ruppia* seeds compared to the other propagules tested (factor propagule, $\chi^2 = 11.48$, 3 d.f., P = 0.009, P < 0.04 for all the contrasts involving *Ruppia*). No significant interaction between propagule and duck species was detected ($\chi^2 = 7.03$, 6 d.f ., P = 0.32).

In our field studies, propagules were more frequent in the plumage of birds from Veta la Palma (85 % of 20 individuals examined) than from Cañada de los Pájaros (45 % of 22 individuals, $\chi^2 = 7.14$, 1 d.f., P = 0.007). Birds captured in Veta la Palma also presented a higher number and diversity of propagules than birds captured in Cañada de los Pájaros (see Fig. 1). Restricting the analyses to species captured in both localities showed that differences in frequency and abundance of propagules varied locally (presence: $F_{1,29} = 9.18$, P = 0.00 5; abundance: $\chi^2 = 2 5.34$, P < 0.0001), but not between bird species (presence: $F_{1,29} = 0.90$, P = 0. 35; abundance: $\chi^2 = 0.06$, P = 0. 81). In Veta la Palma, seeds of saltmarsh species and cladoceran ephippia predominated. In the Cañada, seeds of terrestrial plants dominated, although with a lower overall frequency than in Veta la Palma.

From the analysis with propagules found on the feet, the same pattern emerged. Propagules were more frequent in Veta la Palma (100% of 16 individuals examined) than in Cañada de los Pájaros (1 5% of 2 6 individuals, $\chi^2 = 2 8.43$, P < 0.0001). The diversity of propagules was also higher in Veta la Palma (see Fig. 2). When analysing data for species captured in both localities, both frequency and abundance of propagules varied between sites (presence: $F_{1,34} = 68.47$, P < 0.0001; abundance: $\chi^2 = 42.42$, P < 0.0001), but not between species (presence: $F_{1,34} = 0.59$, P = 0.45; abundance: $\chi^2 = 0.06$, P < 0.80).

Overall, all individual birds sampled at Veta la Palma and 35% of individuals at Cañada de los Pájaros were carrying propagules externally. The diversity of propagules in the feet and the plumage was positively correlated for Veta la Palma samples ($R_S = 0.52$, N = 15, P < 0.05), but not in Cañada de los Pájaros ($\chi^2 = 0.75$, 1 d.f ., N = 22, P = 0.39). The

characteristics of the propagules adhering to the plumage or the feet differed. A similar proportion of individuals carried invertebrate propagules attached to the feathers or the feet (χ^2 = 1.00, 1 d.f., P = 0.32). However, more individuals carried seeds in their plumage than on their feet (χ^2 = 4.00, 1 d.f ., P = 0.04). Invertebrate propagules were more abundant in feet samples (Wilcoxon signed-rank paired test = 66, P < 0.001), whereas seeds were more abundant in the plumage (W = 63, P = 0.02, see Table   2).

Discussion

Like those of Vivian-Smith & Stiles (1994), our results indicate that adhesion of propagules to waterbirds is common in the field, and thus that external transport can be a frequent process, at least at a local scale (i.e. within a wetland complex). The potential for propagules to be transported externally may be limited by their capacity to adhere to birds, but also by their capacity to resist desiccation. Although we have not tested propagule viability, all the seeds found had external covers allowing survival outside the water. Desiccation favours hatching of *Daphnia magna* ephippia just after rehydration (Doma 1979), and the propagules of many other invertebrates can resist desiccation (Bilton et al. 2001). Consequently, the viability of the propagules found by us in the plumage is not likely to have been strongly reduced during transport by birds.

The local differences in presence of propagules on the waterbirds were consistent between different bird species and can be explained by at least three different factors. First, the substratum in Veta la Palma consists of sticky mud, whereas in Cañada de los Pájaros it is sandy and less sticky. The mud in Veta la Palma may have facilitated the adhesion of propagules to the feathers and feet of the birds. Second, the shoreline in Veta   la Palma is densely vegetated, whereas shoreline vegetation is scarcer in the Cañada, where the artificial pond is surrounded by terrestrial vegetation. This probably resulted in more contact between waterbirds and vegetation in Veta la Palma, translating into higher rates of adhesion of propagules. Third, propagules may be scarcer in the Cañada, a relatively simpler environment. This illustrates a

possible drawback of extrapolating from data obtained from artificial wetlands with a low diversity , because bird-mediated dispersal may be less frequent in these systems.

The small size of many invertebrate prop agules, in combination with the presence of mud, could explain their higher presence in feet samples. Many of the recovered seeds have hairs or serrations that could have facilitated their adhesion to feathers (e.g. *Salicornia*, *Arthrocnemum*), and explain their greater abundance in plumage than in feet samples. Interestingly, seeds apparently lacking any adaptation for dispersal by adhesion (as identified by SORENSON 1986) were also recovered, although at a much lower frequency than in the droppings of ducks in the same population (e.g. *Ruppia*, see FIGUEROLA et al. 2001).

Within the limited range of waterbird species examined by us, no interspecific differences in the frequency or abundance of propagules adhered was found, although analyses of a more diverse avian sample   may produce contrasting results. We suggest that future studies of external transport should include tests of the efficiency of the protocols used to remove propagules. Our tests show that the efficiency varies between bird and propagule species, and such biases may potentially influence the apparent differences in abundance of different propagules attached to different bird species. However, these biases are not likely to affect the main conclusions of our study. We also suggest that future studies include tests of the effects of trapping methods on the abundance of adhered propagules . We can not rule out the possibility that some propagules bec ame detached or attached to birds between entering our traps and being screened. Experiments adhering propagules to birds then placing them in the trap overnight to determine propagule loss inside the trap, or examining the presence of propagules in birds emptied of propagules and kept inside the trap overnight would give relevant information to to quantify propagule detachment-attachment inside the traps.

The capacity for external long-distance transport depends largely on the migratory behaviour of the waterbird concerned. Amongst the species included in this study, the waders migrate most and the moorhens the least

(CRAMP & SIMMONS 1977, 1983). In any case, the mobility of all the bird species sampled is high enough to facilitate the dispersal of many aquatic organisms between different wetland complexes or catchments. The implications of external transport for population dynamics and community diversity in aquatic communities clearly merits further research.

Acknowledgements

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Table 1. Mean±s.e. of number of propagules recovered from tests performed on captive ducks to assess the efficiency of our protocol for collecting propagules from waterbird plumage. 10 propagules from each propagule species were inserted in the plumage of each individual bird before starting the brushing protocol (see methods).

	Mallard *Anas platyrhynchos*	Eurasian teal *Anas crecca*	Red-crested pochard *Netta rufina*
Daphnia magna ephippia	8.67 ± 0.41	9.57 ± 0.43	9.40 ± 0.60
Potamogeton pectinatus	8.78 ± 0.22	9.00 ± 0.38	9.20 ± 0.58
Ruppia maritima	7.11 ± 0.59	9.14 ± 0.46	7.20 ± 0.97
Scirpus maritimus	8.11 ± 0.61	8.71 ± 0.57	9.40 ± 0.40
Number of ducks tested	9	7	5

Table 2. Number of individuals examined of each waterbird species in each wetland, range of the number of propagules found on each bird, number of individual birds with propagules (in brackets) and geometric mean (in italics) of the number of propagules found on each bird carrying at least one propagule. Results are presented separately for plumage and feet samples. Geometric means were provided because this can be a best estimate of the central tendency for right skewed count data (see Sokal & Rohlf 1995). Means are not given when there was no difference in the number of propagules found on different individuals. Unid.=Unidentified, Ind.=Individuals

		Ephippia *Daphnia magna*	Ephippia unid.	Invertebrate egg unid.	*Arthrocnemum*	*Chenopodium*	*Phalarix arundinacea*	*Ruppia*	*Salicornia*	*Scirpus littoralis*	*Scirpus maritimus*	Unid. seeds	*Chara* oospores	Algae	Ind.examined	Ind.without propagules
Veta la Palma																
Anas platyrhynchos (mallard)	Plumage	1-5 (4) *2.11*	1-6 (5) *2.93*	1-3 (3) *1.44*	1-8 (8) *2.28*	-	-	1 (2)	1-2 (2) *1.41*	-	1 (1)	1-2 (4) *1.19*	-	-	10	1
	Feet	1 (2)	1-8 (7) *3.50*	1-164 (10) *16.96*	1-4 (3) *1.59*	-	-	1 (1)	2 (1)	-	-	-	1 (2)	-	10	0
Fulica atra (coot)	Plumage	1 (1)	1-3 (4) *1.73*	3 (1)	1-3 (3) *1.44*	-	1 (1)	-	1 (2)	-	-	1-2 (2) *1.41*	-	-	5	1
	Feet	1-5 (3) *1.71*	2-20 (4) *5.18*	4-101 (6) *15.57*	1-10 (3) *2.71*	1 (2)	-	3 (1)	2 (1)	1 (1)	-	1 (1)	1 (1)	-	6	0
Recurvirostra avosetta (avocet)	Plumage	-	1 (1)	-	-	-	-	-	-	-	-	-	-	-	1	0
Tringa totanus (redshank)	Plumage	-	1-2 (3) *1.26*	1-2 (2) *1.41*	-	-	-	-	-	-	-	1 (1)	-	-	4	1
Cañada de los Pájaros																
Anas platyrhynchos (mallard)	Plumage	-	-	-	-	-	-	-	-	-	-	1 (1)	-	-	7	6
	Feet	-	-	-	-	-	-	-	-	-	-	-	-	3 (1)	10	9
Anas strepera (gadwall)	Plumage	-	-	-	-	-	-	-	-	-	-	1 (1)	-	-	2	1
	Feet	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
Fulica atra (coot)	Plumage	-	1 (2)	-	-	-	-	-	-	-	-	1 (3)	-	-	10	7
	Feet	-	-	1 (2)	-	-	-	-	-	-	-	-	-	-	11	9
Gallinula chloropus (moorhen)	Plumage	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
	Feet	-	-	-	-	-	-	-	-	-	-	1 (1)	-	-	3	2

Fig. 1. Number of types of propagules found attached to the plumage of individual waterbirds at two wetlands in Doñana.
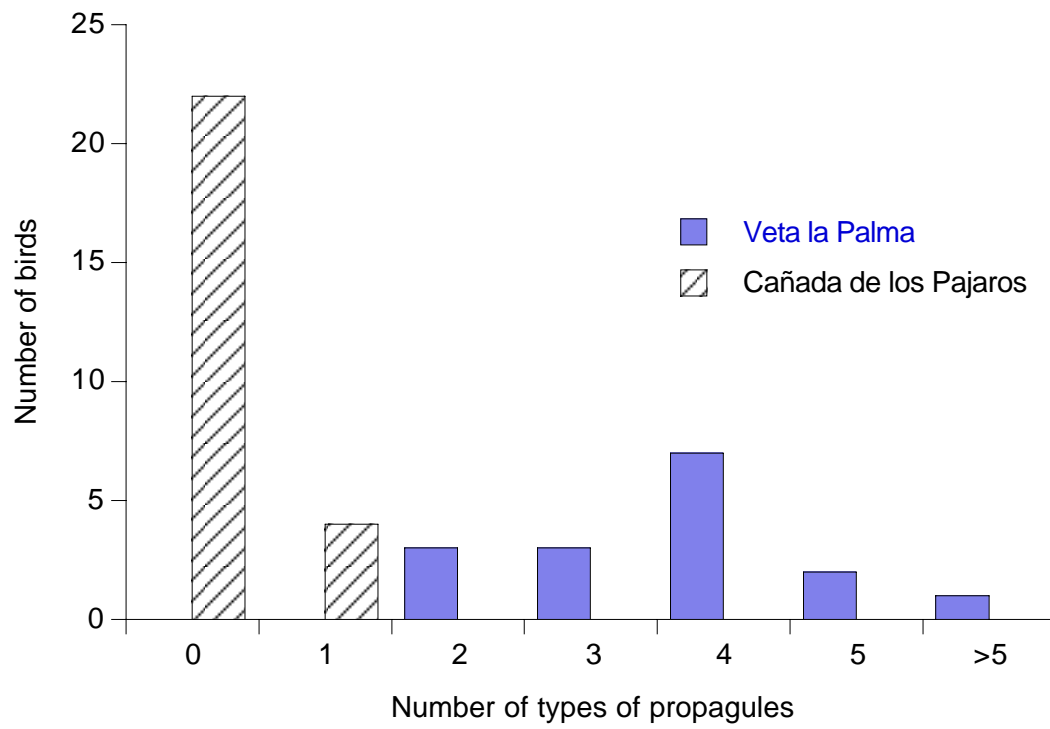
Fig. 2. Number of types of propagules found attached to the feet of individual waterbirds at two wetlands in Doñana.

Passive internal transport of aquatic organisms by waterfowl in Doñana, south-west Spain

Jordi Figuerola[1], Andy J. Green[1] and Luis Santamaria[2]

1 Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. De María Luisa s/n, E-41013 Sevilla, Spain.

2 Netherlands Institute of Ecology, Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands.

Author for correspondence: Jordi Figuerola, Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. De María Luisa s/n, E-41013 Sevilla, Spain. E-mail: jordi@ebd.csic.es. Fax: 34 95 4621125.

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Submitted

Summary

1. Waterbirds may play an important role in the maintenance of aquatic ecosystem biodiversity by transporting plants and invertebrate propagules between different wetlands. However, quantitative data on the frequency of such transport are scarce. We present the most detailed study of internal transport by waterbirds to date.

2. We quantified the number of intact seeds and invertebrate eggs in 386 faecal samples from 11 migratory waterfowl species (10 ducks and coot), collected from the Doñana marshes in south-west Spain, from 3 November to 3 December 1998 (when birds were arriving from further north), and 22-25 February 1999 (when birds were leaving Doñana).

3. Seeds of at least 7 plant genera, and invertebrate eggs (ephippia of 2 crustacean genera, statoblasts of bryozoa and Corixidae eggs) occurred in 65.6% of the faecal samples in early winter and 67.8% in late winter. The abundance of different propagule types varied between waterfowl species in a seasonal and species specific manner, probably owing to differences in foraging strategies, bill and gut morphology, and seasonal shifts in propagule availability or distribution. Lamellar density was positively correlated with the abundance of intact propagules.

4. In early winter, *Fulica atra* and *Anas clypeata* were the most important disperser of propagules overall, and *Anas platyrhynchos* the least important. In late winter, *Anas clypeata* was the most important disperser, and *Anas crecca* and *Fulica atra* the least.

5. Our results confirm that waterfowl play an important role in the dispersal of organisms in aquatic environments by internal transport. Transport can occur even when propagule production and waterfowl movements do not overlap in time, as long as a propagule bank accesible to waterbirds exists.

Introduction

Inland wetlands often constitute isolated habitats, yet many species of aquatic invertebrates and plants lacking active means of dispersal are found over huge geographical areas. Since Darwin (1859) the possibility that waterbirds transport seeds and invertebrates or their eggs has often been recognised (see also Salisbury 1970; Galatowitsch & van der Valk 1996). Experiments in captivity suggest that waterbirds can transport seeds or invertebrate eggs both internally (via survival of digestion), or externally (see Figuerola & Green 2002 for review). However, there is a need for much more quantitative information on the occurrence of waterfowl mediated transport in the field (Figuerola & Green 2002 ; Green, Figuerola & Sánchez 2002).

This scarcity of data fo r aquatic ecosystems contrasts with the abundance of studies on the dispersal of plants by animals in terrestrial habitats (see reviews in Janzen 1983 ; Traveset 1998). Current understanding of dispersal processes is thus biased towards terrestrial ecosystems and processes operating in wetlands are poorly understood.

Recently, Clausen et al. (2002), argued that the long distance dispersal of submerged macrophyte seeds by waterfowl was unlikely for several reasons such as the apparent lack of temporal mismatch between propagule production and waterfowl movements, and suggested that any transport would be unidirectional occurring from north to south during autumn migration but not from south to north during spring migration.

Within the dabbling ducks, considerable attention has been paid to the importance of the variation in the density of the filtering lamellae within the bill and their influence of the size of invertebrates or seeds consumed by each species. Recently, Green et al. (2002) speculated that species with a high lamellar density can be particularly important as vectors of small resting eggs that can resist digestion. High lamellar densities enable ducks to filter smaller particles (Crome 1985), leading to negative correlations between lamellar densities and invertebrate prey size and seed size in several field studies (Thomas 1982; Nudds & Bowlby 1984; Nudds 1992; Nummi 1993; Tamisier & Dehorter 1999, but see Mateo, Guitart & Green 2000). However, ducks are

highly plastic in their feeding behaviour and show great flexibility in their size selection in relation to food abundance. Denser lamellae appear to reduce the costs of filtering small items, but increase that of filtering larger items, and may often increase the variance in the size of items taken rather than decrease the average size (see e.g. seed sizes recorded in different ducks by Thomas 1982).

In this paper we study the frequencies of plant and invertebrate propagules in the faeces of 10 ducks species and *Fulica atra* (Table 1) in one of Europe's most important wetlands. We test the validity of two of the potential limitations to long distance dispersal by waterfowl proposed by Clausen et al. (2002): 1) propagules are only transported at the time of their production, and 2) no propagules are transported during the northwards spring migration period. To our knowledge, ours is the first field study that analyses variation between waterfowl species or tests for differences between seasons in the abundances of intact propagules in waterfowl faeces. We also investigate the relevance of bill morphology for explaining the interspecific differences observed.

Material and methods

The study was conducted in Veta la Palma, a transformed marsh in Doñana (south-west Spain), one of the most important areas for wintering waterbirds in Europe (Scott & Rose 1996). Over a million waterbirds visit Doñana in a typical winter, many of them continuing further south into Africa. Veta la Palma is largely managed for extensive fish farming with ca. 40 brackish, rectangular ponds and c. 100 ha surface each (total surface 3,125 ha), and also includes 4,442 ha of untransformed, temporary marshes. Some islands are present in most ponds, and are used by roosting waterfowl during the day. During the study (winter 1998-99), most of the ducks wintering in Doñana concentrated in these ponds due to the scarcity of natural, seasonal habitats during a relatively dry winter. Waterbirds were counted monthly during the study as part of Doñana Biological Station's monitoring program (Table 1).

Fresh droppings were collected from islands where waterfowl roosted. The islands selected to collect the samples were ideal for pinpointing the position of the birds, being mainly bare mud interspersed with irregular patches of natural vegetation. With binoculars and a telescope we mapped the position of each individual using natural vegetation or small wooden stakes placed on each island as reference points. A first set of droppings was collected between 3 November and 3 December 1998, and a second set between 22 and 25 February 1999, referred to from hereon as early and late winter samples. These sampling periods overlapped with the autumn (southwards) and spring (northwards) waterfowl migrations.

Faecal samples (one dropping per sample) were sto red in individual tubes in the refrigerator until analysis. Given the large number of birds at the study site (see Table 1), samples were likely to be from different individuals. Faeces were washed successively in a 0.5 mm sieve (large enough to retain most seeds and large *Daphnia* ephippia) and a 0.04 mm sieve (to retain small propagules such as the oogonia of charophytes and resting eggs of zooplankton). The items remaining in the 0 .5 mm sieve were examined immediately under a dissecting microscope. Items in the 0 .04 mm sieve were fixed in 70^0 alcohol, to be examined later under the dissection microscope. Here we only present data for seeds and eggs that retained an intact cover and hardness and were considered to have survived the passage through the digestive tube. Taxonomic determination of retrieved seeds followed Cirujano (1986) for *Ruppia maritima*, following cultivation of germinated seeds; Castroviejo et al. (1986) for *Rannunculus*, also after cultivation; Campredon et al. (1982) for *Arthrocnemum* and *Salicornia* seeds and Characeae oogonia, and Alonso (1996) for crustacean ephippia.

We provide data on the presence of apparently viable propagules in the droppings rather than the results of germination assays , thus our data overestimate true viability. However, germination requirements are unknown for most of the species found in this study, and this makes germination rate an underestimate of viability after ingestion (see Brock & Casanova 1991 for a similar problem when estimating seed bank size). Furthermore, examination

of items retained in the 0 .04 mm sieve was very time consuming and fixation of the samples was required to prevent degradation of the samples.

Data on waterfowl lamellar density was obtained from Mateo et al. (2000). Since no information was available for *Marmaronetta angustirostris* we measured lamellar density from collection skins of 6 males and 6 females held in the collection of the Estación Biólogica de Doñana. The distance between 20 consecutive lamelas frontal to the posterior end of the nostril was measured (see Mateo et al. 2000) and expressed as number of lamellas/cm.

Statistical analyses were restricted to bird species with at least five faecal samples examined in a given season. Data were analysed using general lineal modelling in the SAS package (v8.2, SAS Institute 2000), with a negative binomial error distribution and a log link due to the nature of the response variable analysed (count data with a dispersion much greater than expected for a Poisson distribution, see Bliss & Fisher 1953). Spearman Rank correlation was used to test the relationship between mean abundance of propagules in faeces and the mean lamellar density of each duck species. This analysis was restricted to early winter because only 4 duck species provided five or more samples in late winter.

Results

Intact propagules were found in 258 of the 386 faecal samples examined (66.9% overall, 65.6% of the 218 early winter and 67.8% of the 183 late winter samples). Non-destroyed seeds from at least seven different plant genera and Characeae oogonia were recovered from the faeces, together with ephippia of *Daphnia magna* and other Cladocera, statoblasts of Bryozoa, and eggs of corixids and other invertebrates (Table 1).

Overall propagule abundance varied both betwe en waterfowl species (χ^2_8=124.21, p<0.0001, Figure 1) and seasons (χ^2_1=23.35, p<0.0001). A significant interaction was detected between these two factors (χ^2_3=100.84, p<0.0001). The number of propagules in *Anas acuta* droppings was similar in early and late winter (χ^2_1=2.57, p=0.11). Faeces of *Anas clypeata* and *Anas platyrhynchos* contained more propagules in late than in early winter

(χ^2_1>37.67, p<0.0001), but the opposite occurred in *Fulica atra* (χ^2_1=61.75, p<0.0001). In early winter *Fulica atra* and *Anas clypeata* were the species with more and *Anas platyrhynchos* the species with least propagules (see Figure 2). This pattern changed slightly by late winter, when *Anas clypeata* was the species with most propagules and *Anas crecca* and *Fulica atra* the ones with least. Lamellar density was positively correlated with the abundance of intact propagules in the faece (r $_s$=0.82, p=0.02, Figure 4).

In the case of plants, apparently viable seeds of *Ruppia* sp. were the most prevalent propagule, found in 19.8% of samples in early winter and 31.6% in late winter. All or most of these seeds were *R. maritima*, the dominant submerged vegetation in the study site (identification was confirmed by the cultivation of 30 germinated seeds). Important differences in the abundance of *Ruppia* seeds between waterfowl species were detected (χ^2_8=71.76, p<0.0001). A trend for seasonal differences in the abundance of seeds (χ^2_1=3.89, p=0.05), and a species-season interaction were detected (χ^2_3=48.02, p<0.0001). These were the result of species specific patterns of seasonal variation, with an increase in the number of seeds in late winter *Anas platyrhynchos* faeces (χ^2_1=40.06, p<0.0001), and a similar trend for *Anas acuta* (χ^2_1=3.21, p=0.07), but the opposite pattern for *Fulica atra* (χ^2_1=15.21, p<0.0001). No seasonal differences occurred for *Anas clypeata* (χ^2_1=1.43, p=0.23).

In early winter, *Anas clypeata* showing the highest and *Anas platyrhynchos* the lowest abundance of intact *Ruppia maritima* seeds (see Figures 2 and 3a). In late winter, *Anas clypeata* remained the species with greatest abundance of seeds, and no seeds were found in the faeces of *Anas crecca*.

The statistical models for *Salicornia* seeds had problems of convergence probably due to the reduced variability in all the late winter samples. To solve this problem we removed *Anas crecca* from the analysis, because it was the only species sampled in late winter without data for early winter. This analysis with a reduced number of waterfowl species identified interspecific differences in the abundance of *Salicornia* seeds (χ^2_7=20.07, p=0.005, Figure 3b). Seeds were less abundant in late winter faeces

(χ^2_1=4.05, p=0.04). There was a significant interaction between season and waterfowl species (χ^2_3=9.27, p=0.03). While *Anas acuta*, *Anas platyrhynchos* and *Fulicat atra* showed fewer seeds in late winter (χ^2>4.08, p<0.04), no seasonal change in the abundance of seeds occurred in *Anas clypeata* faeces (χ^2=1.84, p=0.18). In early winter, Marbled Teal *Marmaronetta angustirostris* had most seeds and *Anas clypeata* the least seeds in the faeces (Figure 2). In late winter *Anas clypeata* was the species with most seeds and *Anas acuta* the least.

The patterns for Characeae oogonia (Figure 3c), ephippia (Figure 3d), eggs of Corixidae (Figure 3e), and non-identified invertebrate eggs (Figure 3f) were very similar to each other and for simplicity here we only present statistical analysis for eggs of Corixidae. Waterfowl species differed in the abundance of eggs in the faeces (χ^2_8=96.06, p<0.0001), which also varied seasonally (χ^2_1=29.92, p<0.0001). A significant interaction between both factors occurred (χ^2_3=29.92, p<0.0001). No seasonal differences occurred in the abundance of corixid eggs in the droppings of *Anas platyrhynchos* and *Fulica atra* (χ^2_1<2.79, p>0.10). However, in the faeces of *Anas acuta*, corixid eggs were more abundant in early winter (χ^2_1=4.64, p=0.03), and the opposite was true for *Anas clypeata* (χ^2_1=36.41, p<0.0001). In early winter, *Anas clypeata* was the species with most and *Marmaronetta angustirostris* the species with least corixid eggs in faeces (Figure 2). In late winter, *Anas clypeata* remained the species with most corixid eggs, but *Anas acuta* had the least.

Discussion

Ours is the most extensive study to date of the internal transport of aquatic organisms by waterbirds and our results suggest that waterfowl disperse large quantities of aquatic organisms internally. We found a high prevalence of undigested, apparently viable propagules during both early and late winter. The species dispersed include submerged macrophytes (*Ruppia maritima*), salt marsh plants (*Salicornia* sp. and *Arthrocnemum* sp.), bulrush (*Scirpus* sp.), amphibious plants (*Rannunculus scleratus*), and eggs of

corixids, cladocerans (*Daphnia magna* and other species) and bryozoa. Experimental evidence showing that propagules are viable after gut passage is available for most of these groups, with the notable exception of corixid eggs (see reviews in Figuerola & Green 2002 ; Charalambridou & Santamaria 2002). The time the ingested seeds are retained in the digestive tube of ducks often exceeds 24 hours (Swanson & Bartonek 1970; Holt 1999; authors unpublished data). Consequently, given the abundance and frequent daily movements of the migratory waterfowl in our study, the potential for dispersal is high, al least at a local scale (e.g. between the ponds in the study area and to other wetlands in Doñana). There is also a great potential for long distance dispersal given the long distance movements made by waterfowl passing through Doñana during autumn and spring migrations (Scott & Rose 1996). Other waterbirds at Doñana, such as waders and flamingos also consume propagules, and have capacity to transport them long distances (Figuerola & Green 2002, Green et al. 2002).

Many authors have considered the potential for dispersal of seeds to be higher in autumn than in spring (e.g. Holt 1999 and Clausen et al. 2002 for aquatic habitats and Izhaki & Safriel 1985 for terrestrial ecosystems), because plants and invertebrates tend to produce resistance propagules in autumn in seasonal northern hemisphere environments. However, despite the fact that many of the propagules found in our study are only produced in late summer or autumn (e.g. *Ruppia* and *Salicornia*), our results refute this hypothesis, given that patterns of seasonal variation in the abundance of propagules in the faeces changed with the dispersor and dispersed species. Some waterfowl species disperse more propagules in early winter, but others do so in late winter.

The traditionally assumed pattern of temporal variation in propagule transport was only supported for *Salicornia* seeds, which were much more abundant in the droppings collected in early than late winter. Such a difference is probably related to the fact that these seeds were enclosed in the stems of the plant, and are primarily ingested by birds feeding on these stems in autumn (van Eerden 1984, Summers et al. 1993, Green et al. 2002). Thus most of the seeds we recovered in droppings were covered by

fragments of digested stems. This could favour the ingestion of the seeds, and enhance their passage though the digestive tube, particularly because *Salicornia* sp. seeds lack the hard cover present in many of the other types of seeds recovered from faeces (e.g. *Ruppia maritima* and *Arthrocnemum* sp.). In late winter, no fresh *Salicornia* leaves are available for feeding, and the number of ingested seeds was reduced, although intact seeds were still found in late winter samples.

The observed seasonal changes in the prevalence of intact propagules in different waterfowl species are likely to be related to major changes in diets and foraging methods typically observed between seasons (Batt et al. 1992; Baldassarre & Bolen 1994; Green et al. 2002). While pecking was the predominant feeding method in *Fulica atra*, duck species can also forage using the filter feeding strategy. The fact that seasonal patterns of variation in the abudance of propagules differed between the waterfowl species studied indicates that these variability is not only explained by changes in the abundance of propagules. Other factors like changes in habitat use, the diet or in the digestive treatment provided to food could be also involved. Individuals feeding on seeds or other hard foods present more developed gizzards that destroy many of the propagules ingested (see review in Piersma, Koolhass & Dekinga 1993). However, animals (or individuals) feeding on more soft prey develop smaller gizzards, that will allow more propagules to pass through undamaged.

These seasonal (but diet related) changes in the structure of the digestive organs could explain the seasonal changes in the presence of undamaged propagules in these duck species in spring. This is illustrated by a study of the diet of Marbled Teal in Morocco. Although *Ruppia* and *Salicornia* seeds were consumed much more in October and Rannunculaceae seeds in May, the number of intact seeds in faeces did not differed seasonally for *Ruppia* or *Salicornia* (Green et al. 2002). Recently, we have shown that the proportion of *Ruppia* seeds destroyed during digestion changes seasonally for some duck species (Figuerola et al., submitted) and the same is probably true for the other propagules found in the present study.

Local dispersal by waterfowl can influence the population structure of many plants and invertebrates but may also have important effects on the seed-propagule bank dynamics. Seed bank studies tend to ignore the role that movements by birds may play in these dynamics (e.g. Grillas et al. 1993 ; González 1999), and on temporal patterns of dispersal. Our results indicate that waterfowl consume and move an important number of propagules even 5 months after propagule production (see also Green et al. 2002).

The widespread comsumption of propagules in the sediments by waterbirds may result in major differences in the temporal patterns of seed dispersal by aquatic and terrestrial birds. In the terrestrial communities studied until now, birds tend to deliberately ingest seeds to consume the surrounding fruit, or directly consume part of the seeds (see Stiles 2000 ; Wilson & Traveset 2000). However, waterbirds consume the seeds and propagules present in the water column and upper sediment layer either intentionally or unintentionally, e.g. while searching for invertebrates. While some of the seeds will be destroyed during digestion, others will pass the digestive tube without damage.

Lamellar density appeared to explain some of the interspecific differences in the abundance of propagules in faeces, at least in early winter. At least two not mutually exclusive factors can explain such relationship. First, species with denser lamellae are more effective in retaining small size particles (Crome 1985; Nudds et al. 2000), and more propagules are likely to be ingested by the birds. Second, lamellae density may be correlated with other characteristics of gut structure, since species with denser lamellae are likely to feed more on invertebrates and less on plants. In this case species with denser lamellae may destroy fewer propagules during digestion. We cannot separate the relative contribution of these two processes to our results. Furthermore, in the light of the great seasonal (and probably local) variability in the abundance of propagules, further research is necessary to confirm the validity of the relationship between lamellae structure and quantity of dispersed propagules.

The importance of dispersal limitation vs local processes in the structuring of communities is an important topic in community ecology (e.g.

Shurin 2000; Shurin et al. 2000). Our results suggest that dispersal limitation is not a constraining factor for the distribution of plant and invertebrate species found in our study, at least in areas with abundant waterfowl populations (supporting Shurin 2000 and Shurin et al. 2000, but contrary to Dobson 1992). Capacity to survive ingestion by waterbirds can be an important parameter to consider when discussing possible causes of interspecific differences in the ubiquity of aquatic organisms.

In conclusion, waterfowl act as major dispersors of plant and invertebrate propagules in the Doñana marshes by internal transport. This adds to evidence that waterfowl in Doñana transport propagules externally (Figuerola & Green, submitted). Such dispersal occurs throughout the winter, with different patterns according to the characteristics of the dispersor and dispersed species. The overall movements of propagules in different directions will depend on a complex interaction between propagule characteristics, the abundance and behaviour of each dispersor species and the survival and establishment probabilities of propagules transported in each season (see De Meester et al. 2002, Green et al. 2002). Given the potential for interspecific, seasonal and regional variations in all such parameters it is hard or impossible to quantify the potential for long-distance dispersal of a given propagule type in different directions. For example, we expect great variation between years in Doñana in the potential for dispersal of different propagules, as there are major changes in the location and size of flooded areas (due largely to rainfall fluctuations) and the relative abundance of propagules within them (Montes et al. 1998). In addition, there is major variation in the nature of waterfowl movements within and beyond Doñana from one winter to the next, resulting in a great uncertainty in the interactions in these kind of systems.

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Early winter	Droppings examined	Droppings with propagules	Unidentified leguminosa	*Scirpus* sp.	*Arthrocnemum* sp.	*Ranunculus scleratus*	Unidentified seeds	*Daphnia magna* ephippia	Ephippia of other cladocera	Bryozoa statoblasts	Birds counted
Anas acuta Northern Pintail	16	10	0.4±1.8 (1)	0.8±3.3 (1)	-	-	0.3±0.7 (2)	0.1±0.3 (1)	0.1±0.3 (1)	-	41345
Anas clypeata Northern Shoveler	24	16	0.5±1.3 (6)	-	0.1±0.2 (1)	-	0.5±1.1 (5)	0.1±0.5 (1)	1.7±4.3 (8)	-	90141
Anas crecca Eurasian Teal	4	1	-	-	-	-	-	-	-	-	68172
Anas penelope Wigeon	12	8	-	-	-	-	-	-	0.1±0.3 (1)	-	21145
Anas.platyrhynchos Mallard	36	14	-	0.1±0.2 (1)	0.1±0.4 (1)	0.1±0.3 (2)	-	0.1±0.2 (1)	0.1±0.2 (1)	0.1±0.2 (1)	58365
Anas strepera Gadwall	17	12	-	-	0.1±0.3 (1)	-	0.2±0.4 (2)	-	-	-	3465
Aythya ferina Pochard	15	10	-	-	-	-	-	-	0.1±0.3 (1)	-	10470
Fulica atra Coot	85	64	-	-	0.2±0.6 (5)	-	0.3±1.0 (13)	0.1±0.4 (6)	2.3±4.6 (28)	0.1±0.2 (2)	14390
Marmaronetta angustirostris Marbled Teal	7	6	5.3±13.6 (2)	-	0.2±0.4 (1)	0.3±0.5 (2)	-	-	-	-	436
Netta rufina Red-crested pochard	2	2	-	-	3.0±4.3 (1)	-	-	-	-	-	1452
Late winter											
Anas acuta	28	25	0.1±0.4 (4)	-	-	-	0.3±0.7 (5)	-	0.3±0.6 (4)	-	2498
Anas clypeata	14	14	-	0.1±0.3 (1)	0.3±0.6 (2)	-	6.8±12.6 (8)	0.9±1.6 (4)	19.3±34.1 (10)	-	8902
Anas crecca	20	10	-	-	0.1±0.3 (1)	-	-	-	0.4±1.4 (3)	-	1593
Anas penelope	4	3	-	-	-	-	0.8±1.5 (1)	0.3±0.5(1)	-	-	480
Anas platyrhynchos	51	40	0.1±0.4 (4)	3.1±0.2 (2)	0.4±1.5(5)	-	0.4±1.1 (8)	-	0.2±0.6 (3)	-	14443
Fulica atra	61	29	-	-	0.2±1.0 (3)	-	0.2±0.7 (6)	-	0.2±0.5 (7)	-	8496
Netta rufina	2	0	-	-	-	-	-	-	-	-	25
Tadorna tardorna Shelduck	3	3	-	-	0.3±0.6 (1)	-	-	1.0±1.8 (1)	37.7±37.6 (3)	-	217

Table 1. Number of faecal samples examined for each waterfowl species in early and late winter in Veta la Palma (Doñana), mean number of intact propagules (±s.d.) and number of faeces with apparently viable propagules of each type (in brackets). The number of birds of each species counted in census of Veta la Palma coinciding with samples collection are shown in the last column. Data included in Figure 3 have been omitted from the table, except for crustacea ephippia that are presented separately here for the distinct taxa.

Figure 1. Abundance of propagules (mean+sd) in the faeces of ducks sampled in early and late winter, plotted on a logarithmic scale. Only data for species with at least five samples collected in a given season are shown. Numbers over each bar indicate the number of faecal samples in which propagules were present and the number of samples examined.
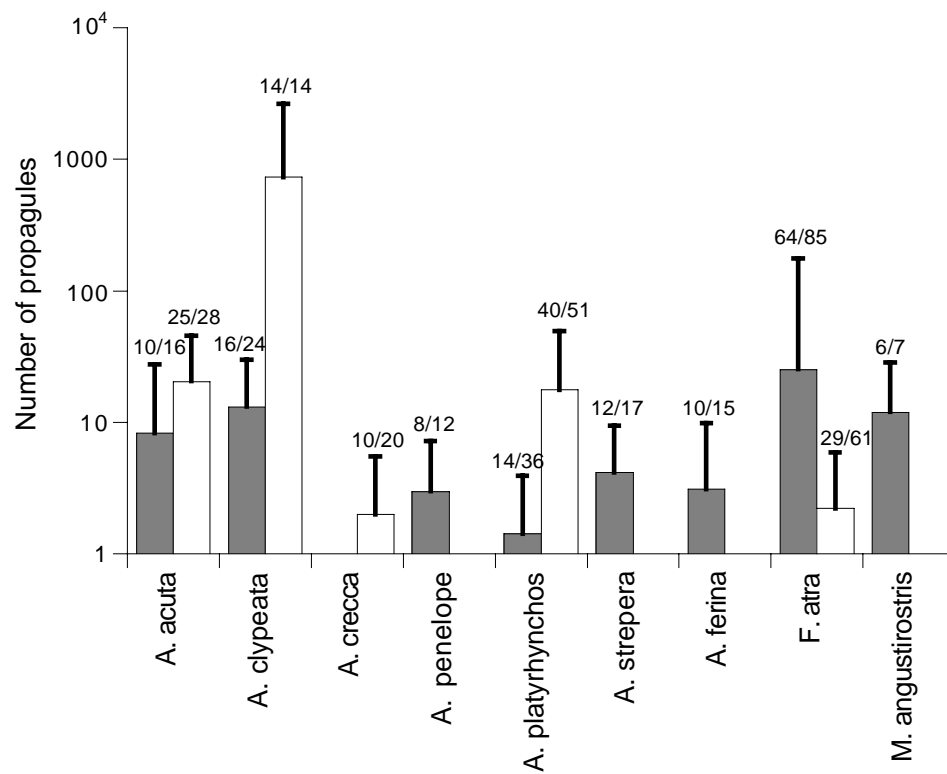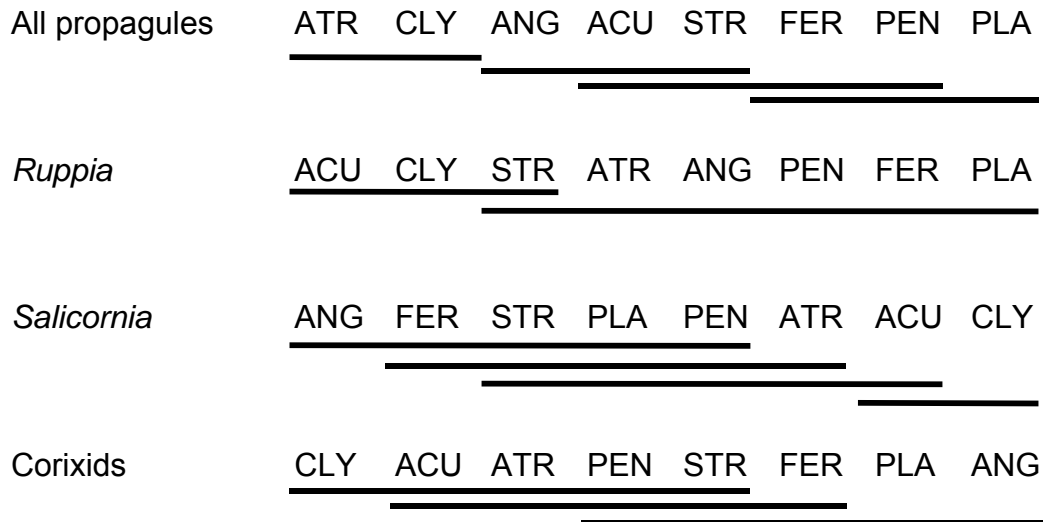
Figure 2. Relative abundance of propagules in the faeces of each species and season. Species are ranked from higher (on the left) to lower abundance of propagules in their faeces and codes correspond to the first three letters of the specific latin name. Differences between species and seasons were tested using the Wald Chi-square test (see methods).   Groups of species not presenting significant differences in the contrasts between them are joined by a horizontal line.

Early winter

All propagules   ATR   CLY   ANG   ACU   STR   FER   PEN   PLA

Ruppia   ACU   CLY   STR   ATR   ANG   PEN   FER   PLA

Salicornia   ANG   FER   STR   PLA   PEN   ATR   ACU   CLY

Corixids   CLY   ACU   ATR   PEN   STR   FER   PLA   ANG

Late winter

All propagules   CLY   ACU   PLA   ATR   CRE

Ruppia   ACU   PLA   CLY   ATR   CRE

Salicornia   CLY   ATR   PLA   ACU

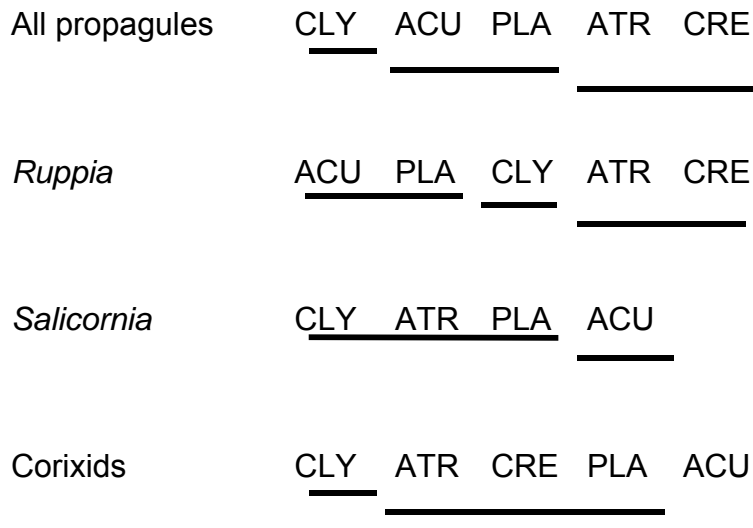Corixids   CLY   ATR   CRE   PLA   ACU

Figure 3. Abundance of propagules of different species or categories (mean+sd) in the faeces of ducks sampled in early and late winter. Only data for species with at least five samples collected in a given season are shown. Numbers over each bar indicate the number of samples in which propagules are present and the number of samples examined. a. *Ruppia* seeds, b. *Salicornia* seeds, c. characeae oogonia, d. ephippia of crustacea, e. eggs of corixids, f. non identified invertebrate eggs.
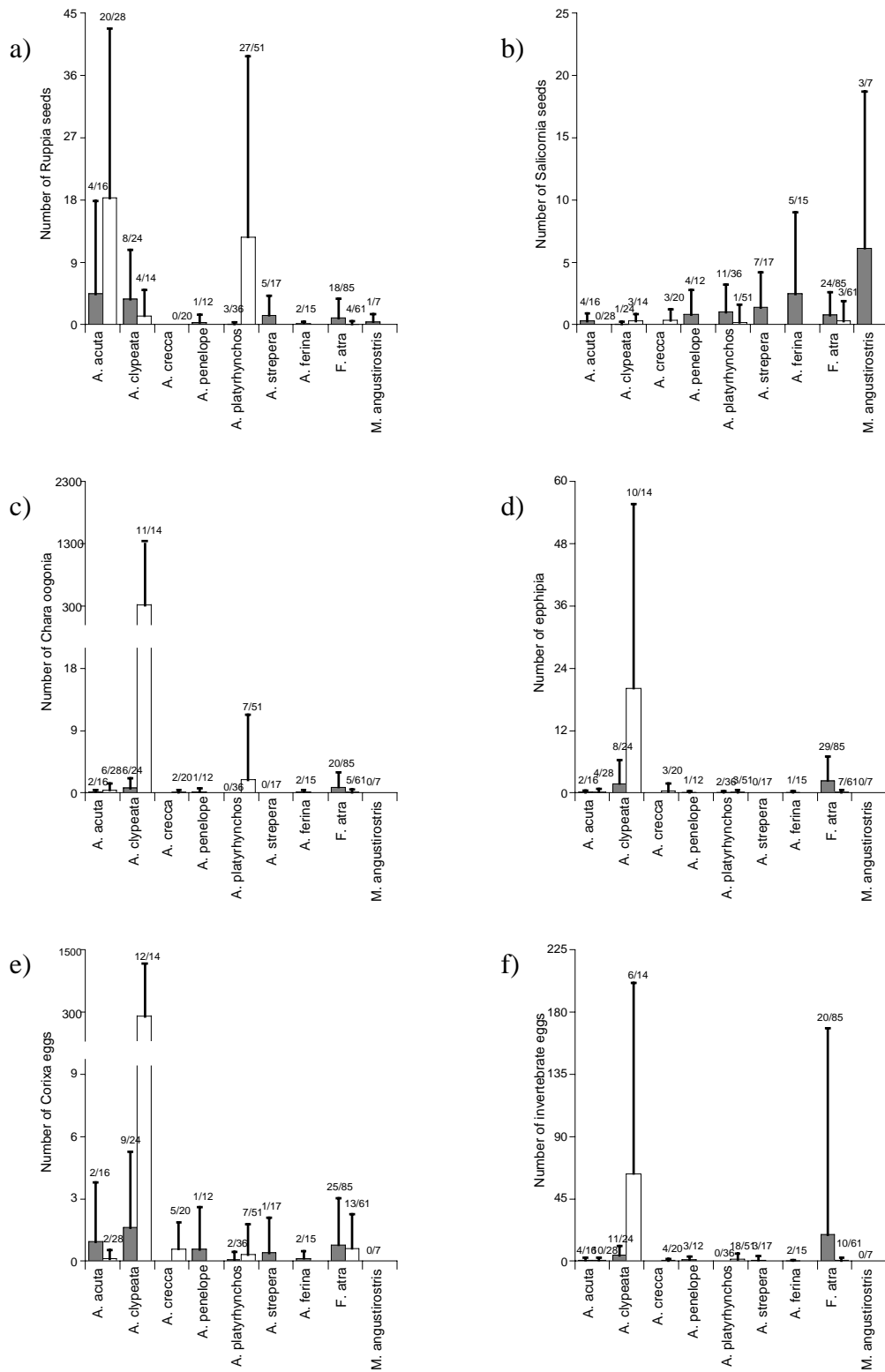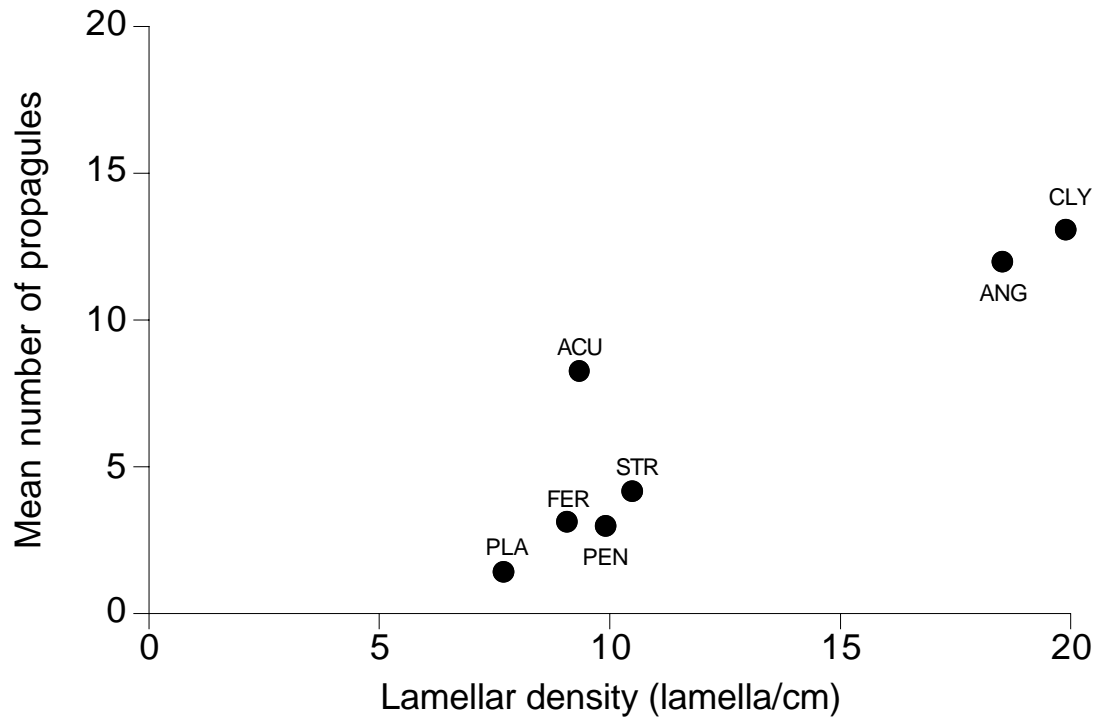
Figure 4. Mean number of intact propagules recovered in the faeces of 7 duck species in early winter in Doñana in relation to bill structure estimated as lamellar density (lamella/cm).

Part 3

Quantity and quality components of internal transport by birds. Effects of ingestion on propagule viability

Comparative dispersal effectiveness of wigeongrass seeds by waterfowl wintering in south-west Spain: quantitative and qualitative aspects

Jordi Figuerola[1], Andy J. Green[1] and Luis Santamaría[2]

1 Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. Maria Luisa s/n, E-41013 Sevilla, Spain.
2 Netherlands Institute of Ecology, Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands.

Author for correspondence:
Jordi Figuerola, Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. Maria Luisa s/n, E-41013 Sevilla, Spain. E-mail: jordi@ebd.csic.es

Running head: Seed dispersal by a waterfowl community

Summary

1. The effectiveness of a seed disperser depends on the quantity of seed dispersed and the quality of dispersal provided to each seed. How the quantity and quality components of dispersal effectiveness are interrelated and how the characteristics of the dispersers are associated with such components remain largely uninvestigated.

2. The effectiveness of different waterfowl species at dispersing seeds of *Ruppia maritima* was evaluated in a wetland in south-west Spain. Droppings were collected during the autumn and spring waterfowl migration periods and the number of seeds ingested (estimated from seed fragments), undigested and viable in germination trials were determined.

3. Ingestion by waterfowl enhanced the rate of germination and, for several duck species, it also had a positive effect on germinability. Both the presence of seeds in the diet and the effects of gut passage showed high interspecific and temporal variance. Some of the interspecific variation in dispersal quality was related to gut structure: species with heavier gizzards destroyed a higher proportion of seeds and undigested seeds ingested by species with more grit in the gizzard germinated better.

4. In the waterfowl community studied, the quantity and quality components of seed dispersal effectiveness were positively correlated across species.

Key words: seed dispersal, dispersal quality, dispersal quantity, seed germination, germination rate, germination speed, effects of seed digestion by vertebrates, gut structure

Introduction

Many freshwater wetlands are isolated habitats equivalent to ecological islands. This contrasts with the similarities in composition of aquatic plant (Good 1953; Raven 1963) and invertebrate communities (Brown & Gibson 1983) between different catchment areas. Aquatic habitats present relatively low rates of endemicity compared with other ecosystems, suggesting that plants and invertebrates have significant rates of dispersal between wetlands (Santamaría 2002). Since Darwin, the possibility of such dispersal by waterbirds has been recognised (Darwin 1859; Ridley 1930), but little is known about its frequency in the field (see reviews by Figuerola & Green 2002; Green, Figuerola & Sánchez 2002), especially in comparison with the relatively well documented dispersal patterns of many terrestrial plants by frugivorous birds (see review in Janzen 1983; Jordano 2000). Most aquatic plants do not produce fleshy fruits (e.g. *Zannichellia*, *Ruppia*), or produce fruits with a small proportion of edible material (e.g. *Potamogeton*). Hence, internal dispersal often implies the non-intentional ingestion of seeds by herbivores eating vegetative parts and by filter feeding species, or the survival of digestion by granivorous species that destroy an important fraction of the ingested seeds.

Successful internal dispersal of aquatic plants results from the interaction of different stages of seed processing, disperser movements, biotic and abiotic influences on the survivorship of seeds and seedlings, and the growth and reproduction of the resulting individuals. The complete sequence of steps in this process has seldom been considered for any plant species (but see Jordano & Schupp 2000, and references therein). Many of these variables are likely to differ between species of disperser. Disperser effectiveness can be defined as the contribution a disperser makes to the future reproduction of a plant. The ideal measure of dispersal effectiveness is the number of new plants produced as a result of a disperser activity relative to the numbers produced by other dispersers or by seeds that are not dispersed (Schupp 1993). However, due to the difficulties inherent in estimating adult fitness, such a definition has never been applied in the field,

and less complete approaches based on seed viability or seedling survival have been used (Reid 1989). The identification of the attributes of effective dispersers has become a central topic for the progress in the understanding of plant-animal interactions (Izhaki, Walton & Safriel 1991 ; Jordano & Schupp 2000). Many questions remain to be investigated, notably how the different components of seed dispersal effectiveness co-vary between disperser species and how the quantity and quality components of dispersal interact.Seed processing by birds affects the germinability and/or the rate of germination of seeds. A recent review concluded that enhancement of germination occurred twice as often as inhibitions following seed passage through the digestive tract of vertebrates (Traveset 1998; see also Traveset & Verdú 2002). Differences in seed retention time in the disperser digestive tract, or in the type of food ingested with the seeds could partly explain the variation in seed responses among and within plant species, and among disperser species as well (Traveset 1998). The effect on germination is thus likely to be a key factor determining disperser effectiveness, particularly for waterfowl species that ingest seeds mixed with other food (e.g. vegetative plant parts or zooplankton).

Wigeongrass, *Ruppia maritima* (L.), is a submerged angiosperm that inhabits brackish coastal and inland saline waters and presents a worldwide distribution including all the continents and many islands between 69 ^{o} N and 55° S (Verhoeven 1979). Mechanisms of dispersal of *Ruppia* seeds are poorly understood. Agami & Waisel (1988) demonstrated that seeds can resist passage through the gut of fish. *Ruppia* seeds are often consumed by waterbirds (Gaevskaya 1966; Cramp & Simmons 1977 ; Green *et al.* 2002), but no previous information is available for any species of the genus about dispersal by waterfowl.

Here, we evaluate different components of dispersal effectiveness by migratory waterfowl species (10 duck species plus Eurasian Coot *Fulica atra*) in south-west Spain, examining separately the importance of seed ingestion, seed digestion and germinability. Thus, we focus both on the quantity (estimated as the number of seeds per dropping) and two phases (the proportion of seeds that pass the gut intact, and the proportion of intact seeds

that actually germinate) of the quality component of dispersal (Schupp 1983) provided by each species. We expect a decline in seed availability for waterfowl consumption during the winter, due to reduced accessibility after seeds are shed from the plants in autumn and a decrease in density in the seed bank caused by seed predation and germination during winter and early spring. We thus test the hypothesis that the number of seeds dispersed during the autumn will be greater than during the spring, due to these decreases in seed availability and to ongoing consumption by the disperser.

This study represents a first step towards establishing the ecological role of waterfowl in aquatic plant dispersal dynamics, and provides an assessment of the dispersal components of an important fraction of the dispersers in the system studied.

Material and methods

Study area

The study was carried out in 'Veta   la Palma', a modified marsh within the Doñana wetland complex (south-west Spain; 6 ^{o} 14' W, 36 ^{o} 57' N). Veta la Palma is largely managed for fish farming with ca. 40 brackish ponds of rectangular shape and c. 100 ha surface each (total surface 3.125 ha), and also includes 4.442 ha of untransformed, temporary marshs. The area is used by a high number of aquatic birds during the winter (20.000 to 74.000 ducks and coots observed between November 1998 and February 1999, see Table 1). Some islands are present in most of the ponds, and are used by roosting waterfowl during the day. During the study period, most of the ducks wintering in Doñana (total area: 166.000 ha) were concentrated in this locality due to the scarcity of water in other wetlands. *R. maritima* is the most abundant macrophyte in the area. In the sediments, the mean density of seeds in 5 cm deep, 4.4 cm diameter cores was 0.10 seeds/cm ^{3}_(s.e. =0.02, n = 96) in September 1999, and 0.05 (s.e. = 0.01; n = 96) in May 2000, suggesting a reduction in the availability of seeds in the area during the course of the winter (Wilcoxon signed rank tests = 509.5, P = 0.007).

Data collection

Fresh droppings were collected from islands with roosting waterfowl (Table 2). Previous to collecting the droppings, the position of each individual bird was determined by a second observer with the aid of a telescope, making use of the elevated position offered by pond dykes. The observer kept his observation field fixed while the collecter reached the island to collect the droppings. We selected long but narrow (less than one meter wide), roost sites to facilitate the location of individual ducks using natural vegetation or small wooden stakes placed on each island as reference points. In addition to this location method, the differences in specific size of the droppings among the waterfowl species studied (see Bruinzeel *et al.* 1997 for an overview of waterfowl dropping sizes) allowed us to double-check the taxonomic identity of the individual that produced the dropping. Droppings were first collected between 3 November and 3 December 1998, a period overlapping with the end of the autumn southward movements of birds migrating from Central and North Europe (García, Calderón & Castroviejo 1989; Scott & Rose 1996); therefore, they are referred to hereon as 'autumn samples'. A second set of droppings was collected in the period 22-25 February 1999, when many birds were leaving the study area towards their northern breeding grounds, and referred from hereon as 'spring samples' because their collection dates overlap with spring migration of waterfowl.

Faecal samples (one dropping per sample) were stored in individual tubes in the refrigerator until analyses, during the next few days. Given the large number of birds at the study site, all or almost all samples were likely to be from different individuals. We collected samples from more than one individual at the same location. To minimise the chances of collecting droppings from the same individual and of introducing some dependence in the data set (if these birds were more likely to have been feeding in the same area), we specifically aimed at collecting samples from individuals belonging to different waterfowl species when each single location was sampled. Faeces were washed in a 0 .5 mm sieve using tap water. The items remaining

in the sieve were examined under a dissection microscope and plant seeds or invertebrate eggs were identified (see Figuerola, Green & Santamaría 2001a, for a summary of the propagules of other species found in the droppings). In the case of *R. maritima*, we counted separately the number of intact seeds, the number of seeds with a fractured seed coat (hard endocarp) but retaining an intact seed content (embryo plus endosperm), and the number of seed-coat fragments. The first two groups were stored wet (in Eppendorf tubes filled with a few drops of tap water) in the refrigerator and used to test the germination viability of seeds after digestion by waterfowl, and are referred to as "undigested" seeds.

Control seeds (not retrieved from droppings) were collected in November 1998 in two of the ponds used to collect the droppings. They were stored in Eppendorf tubes half filled with tap water and kept in the refrigerator for posterior germination experiments. In addition, a second batch of control seeds was allowed to dry on a sheet of filter paper and were stored dried in the refrigerator until germination trials. Dry and wet seeds were used because *R. maritima* is known to show low germination unless dormancy is broken by drought or, potentially, by gut passage (with limited effects of winter stratification; van Vierssen, van Kessel & van der Zee 1984, Koch & Seeliger 1988). Hence, germination of wet, dormant seeds would have provided a poor indicator of seed viability. Control and duck-ingested seeds were germinated in microtiter trays with each 3 .5 ml cell filled with 2 ml of tap water, placed in germination rooms at 20º C and in the dark. This germination regime was considered optimal for maximising the germination of the experimental seeds (see Koch & Seeliger 1988 ; Koch & Dawes 1991 and Acosta   *et al.* 1998 for the effect of salinity and temperature on germination of *R. maritima*). We used dark conditions because they had been previously found to enhance germination of the local populations of *R. maritima* and *R. drepanensis* (L. Santamaría, unpublished data). Separated samples were randomly distributed over separate cells, receiving a maximum of 10 seeds per cell; samples of more than 10 seeds were thus distributed over a number of adjacent cells. Germination was checked every 2-3 days, until no increase in the germination frequency was detected (42 days in autumn and 58 days in

spring). At the same time, water was replenished and germinated seeds removed. The few seeds that became infected by bacteria and/or fungi (mostly those with a cracked seed coat) were immediately removed and the water of the cell was refreshed. We did not apply antibiotics because we considered that increased infection risk is one of the processes affecting seed viability following gut passage.

We were unable to collect suitable controls in the field at the same time as droppings in spring, because seeds in the field could correspond to uningested seeds or to seeds that have been ingested and defecated during the winter by birds (or fish). For this reason, we used dry seeds as controls, since moderately long dry storage of *R. maritima* seeds resulted in very little variation in their initial germination characteristics. Seeds used as controls in the spring germination trial can not be considered to reliably represent the behaviour of non-ingested seeds in the field, since they have not experienced the same conditions during the winter as seeds collected from spring droppings.

Seed-endocarp fragments recovered from the droppings were dried for two days at 70 ^{o} C and weighed to the nearest 0.1 mg. To estimate the minimum number of seeds represented by the fragments of each dropping, 26 seeds were deliberately broken with the pressure of a finger, the remnants sorted using a 0 .5 mm sieve, dried for two days at 70 ^{o} C, and weighed. After this, the average dry mass of the fragments derived from one seed was estimated to be 4.1 mg (s.e. of measurements of eight different groups of 26 seeds = 0.07). The number of broken seeds in each dropping was estimated as the mass of the fragments extracted from the dropping divided by 4.1, rounded to the upper whole number (e.g. 0.2 rounded up to 1), because the presence of a unique fragment implies the ingestion of at least one seed.

To test the possible role of gut morphology in explaining interspecific differences in quality of dispersal, we studied gut morphology using dead birds. Dead waterfowl were obtained from various sources: illegally shot birds confiscated by the police, birds arriving dead at recovery centres, individuals that died following a toxic spill in the Doñana area (see Aparicio *et al.* 1998; Grimalt, Ferrer & MacPherson 1999) or during an epidemic of avian

bronchitis. The carcasses used to obtain these data had different origins, and this could introduce a source of heterogeneity in the data set. But, since we collected individuals for all species from a diversity of sources, the heterogeneity in the data set would only decrease the changes of detecting differences between species, making our analyses more conservative (instead of causing the patterns of variation reported in the results section). Although ill birds might have stopped feeding for days before dying, a process that could affect gut size, the rather drastic effects of the mortality sources (avian bronchitis, acute toxicity) makes this unlikely – indeed, most of the birds examined presented food in their guts. These corpses were analysed to determine the structure of their guts (see Table 3). Gizzards were opened to extract their contents. Grit was separated from food by decantation, dried at 40° C to constant mass and weighed to the nearest 0.01 g. Empty gizzards were also weighed to the nearest 0.01 g. Intestine length was measured with a ruler to the nearest cm. Since the structure of digestive organs and quantity of grit ingested may change seasonally (Trost 1981; White & Bolen 1985; Mateo 1998), we only considered data from birds collected between October and December (autumn sample) and February-April (spring sample). Unfortunately, the sample size of birds collected in spring was very small (ten individuals of four species) and it was not enough to allow a separate analysis for this season. The repeatability of species means was estimated as the intraclass correlation of the measures obtained from different individuals in the autumn period (see Lessells & Boag 1987; repeatability estimates are generally reduced both by measurement error and intraspecific variability). Estimates of repeatability of species means were 69% for gizzard mass ($F_{7,96}$ = 26.74, $P < 0.0001$), 85% for intestine length ($F_{4,50}$ = 44.78, $P < 0.0001$) and 84% for grit mass ($F_{7,107}$ = 67.06, $P < 0.0001$). Body mass for each species was estimated as the mean for male and female values from Figuerola & Green (2000) for ducks, and Cramp (1980) for coot. We did not obtain morphological data for all the species, so the number of species included in this analysis was lower than the total number of species for which seed survival and germination were measured.

Statistical analyses

To analyse the seasonal or species-specific patterns of variation in number of ingested seeds per dropping, we used General Linear Modelling (GLM). GLM allows a more versatile analysis of correlation than standard regression methods, because the error distribution of the dependent variable and the function linking predictors to it can be adjusted to the characteristics of the data. For these analyses we used a negative binomial error model with a log link function and type III sum of squares, implemented using the GENMOD procedure of the SAS program (v. 8.2, SAS Institute 2000). Poisson errors are widely used for the analyses of count data, but in our analyses they resulted in highly overdispersed models (Crawley 1993), making the negative binomial a more adequate error structure. The differences among species and seasons in the number of seeds surviving gut passage or the number of seeds successfully germinating were analysed with a GLM model with binomial error and a logit link function. This procedure uses the number of seeds ingested or the number used in the germination experiments as the binomial denominator, thus controlling for the effects of sample size on the proportion surviving gut passage or germinating (Crawley 1993). Deviances from the model were scaled with the square root of the ratio deviance/degrees of freedom to correct for the effects of data overdispersion in the statistical test. For germination data, separate models were used for autumn and spring samples because, among other factors, the duration of exposure to germination differed between trials. Unbiased estimates correcting for the effects of number of seeds per sample on the proportion of seeds surviving digestion or germinating were obtained by backtransformation of the estimates obtained from binomial GLM, and the standard error of these estimates was calculated using the Delta method (SAS Institute 1996).

When a factor with more than two levels was significant, the statistical significance of comparisons between levels of the factor were estimated by the Likelihood Ratio statistic (SAS Institute 2000). If the interaction between

factors was significant we used the Wald chi-square test for differences between least-squares means (SAS Institute 2000).

To investigate the relationship between gut morphology and interspecific variation in seed ingestion, seed digestion and seed germinability, the factor 'species' was replaced by mean values of the different morphological characters considered for each species. The proportion of the interspecific variation explained was estimated in separate models for each variable as the ratio of the deviance explained by the morphological variable to that explained by the factor 'species'. The variable explaining more variance was added to the model, and the significance of the remaining variables tested again using type I sum of squares. Log_{10} transformed grit mass values were used in the analyses.

The effects of seed ingestion on seed germination rate were tested in a failure-time analysis by fitting a Cox proportional hazards regression model (e.g. Allison 1995) on data consisting of the number of days between setting for germination and seedling emergence, for each individual seed. Only data for seeds that had germinated by the end of the germination trials were included to separate the effects on germination rate from those on total germination. To account for the effects of digestion by different duck individuals (the 'dropping' effect, i.e. differences between faecal samples) or germination in different random groups (in the case of controls), a replicate effect was added to the model as a random or 'frailty' effect (Mathsoft 1999). Duck species and control treatments were analysed as fixed effects. Ties were estimated using the exact method, in the program S-Plus 2000 (Mathsoft 1999).

The number of seeds ingested per dropping was considered as an estimate of the quantity component of dispersal, whereas the quality component of dispersal was estimated as the proportion of seeds successfully germinating from the total ingested. This quality component includes two phases of the dispersal process: the passage of the seeds through the gut without damage, and then the successful germination of the undigested seeds. The relationship between the quantity and quality components of dispersal was tested in two different ways. First,

interspecifically by analysing the relationship (using Spearman Rank correlation) between the estimates obtained from the model analysing variation in quantity of seeds ingested and those obtained from the model analysing the proportion of these seeds successfully germinating. The second analysis was done intraspecifically, by constructing separate GLM models for each species and season, analysing the relationship between quantity of seeds ingested (as the independent variable) and the proportion of seeds germinating (germinated/ingested as the binomial dependent variable).

To avoid pseudoreplication, in all these analyses the faecal sample (and not the seed) is our unit of analyses, since seeds coming from the same dropping are not likely to constitute independent samples. The number of species differed between analyses because not enough faecal samples with presence of seeds were available for all species.

Results

The quantity component of Ruppia dispersal: Presence of R. maritima in the diet of waterfowl in autumn and spring

The estimated number of ingested seeds per dropping differed between species (GLM χ^2 = 232.59, 8 df, *P* < 0.0001, Table 4), and between seasons (χ^2 = 20.87, 1 df, *P* < 0.0001). A significant interaction between both factors was found (χ^2 = 140.94, 3 df, *P* < 0.0001). Across the two seasons, *Anas acuta* and *A. clypeata* were the species with the highest estimated number of ingested seeds per dropping. *A. platyrhynchos* presented significantly smaller quantities of seeds, and *Fulica atra* was the species with the least seeds per dropping (*P* ≤ 0.05 for all these contrasts). Other species could not be included in these comparisons because droppings were collected in high enough numbers in only one season.

The comparisons within each season indicate that in autumn, *A.clypeata* was the species with the highest number of seeds in the droppings (Table 4). *A. acuta* tended to present less seeds than *A. clypeata* (χ^2 = 3.53, 1 df, *P* = 0.06), but more seeds than the rest of species (χ^2 ≥ 21.13, 1 df, *P* ≤

0.0001 for all the contrasts with other species). *A. strepera, A. platyrhynchos, F. atra* and *Marmaronetta angustirostris* constituted a second group presenting less seeds, and *A. penelope* had the least seeds per dropping. In spring, interspecific differences in seed ingestion were more marked. *A. acuta* was the species with the highest number of seeds per dropping, and tended to have more than *A. platyrhynchos* ($\chi^2 = 3.37$, 1 df, $P = 0.07$). The droppings of both species presented significantly more seeds than those of *A. clypeata* ($\chi^2 \geq 14.45$, 1 df, $P < 0.0001$, for the two independent contrasts). Less seeds were found in droppings of *A. crecca* ($\chi^2 \geq 6.74$, 1 df, $P \leq 0.009$ for all contrasts), and *F. atra* had the least seeds ($\chi^2 \geq 6.74$, 1 df, $P \leq 0.009$, for all contrasts).

Within bird species, the presence of seeds of *R. maritima* changed seasonally. Some species presented more seeds in spring (*A. acuta*, $\chi^2 = 28.67$, 1 df, $P < 0.0001$; *A. platyrhynchos*, $\chi^2 = 143.93$, 1 df, $P < 0.0001$), one species had more in autumn (*F. atra*, $\chi^2 = 32.37$, 1 df, $P < 0.0001$), and *A. clypeata* showed no seasonal change ($\chi^2 = 0.46$, 1 df, $P = 0.50$).

The quality component of Ruppia *dispersal: Seasonal changes in the balance between seed predation and dispersal*

The proportion of undigested seeds in the droppings did not differ between species ($F_{6,292} = 1.57$, $P = 0.16$, Table 4), but a significantly larger proportion of seeds survived digestion in autumn ($F_{1,292} = 5.49$, $P = 0.02$). A significant interaction between the factors species and season was detected ($F_{3,292} = 4.72$, $P = 0.003$). This interaction was related to the larger proportion of undigested seeds in autumn than in spring in the droppings of two species (*A. acuta*, $\chi^2 = 9.07$, 1 df, $P = 0.003$; *A. clypeata*, $\chi^2 = 17.57$, 1 df, $P < 0.0001$), and the lack of seasonal changes in other species (*A. platyrhynchos*, $\chi^2 = 1.40$, 1 df, $P = 0.24$; *F. atra*, $\chi^2 = 0.44$, 1 df, $P = 0.51$). Similarly, a smaller proportion of seeds in the autumn droppings of *A. platyrhynchos* were undigested than in *A. clypeata* ($\chi^2 = 8.21$, 1 df, $P = 0.004$) and *A. acuta* droppings ($\chi^2 = 5.28$, 1 df, $P = 0.02$). However, in spring, a higher proportion of seeds were undigested in *A. platyrhynchos* droppings than in *A. acuta* ($\chi^2 = $

15.87, 1 df, $P < 0.0001$). None of the other comparisons within a given season was significant. In summary, this component of dispersal quality was better for some waterfowl species in autumn than in spring, but no seasonal changes occurred for other species.

The quality component of Ruppia *dispersal: Germination of ingested seeds*

In the autumn samples, total seed germination (after 42 days) differed significantly among species ($F_{6,68} = 5.04$, $P = 0.0003$, Table 5). Post-hoc tests identified two groups of species. A group with a low frequency of germination included *A. acuta* , *A. strepera* and control (non-ingested) seeds stored wet. The second group, with higher germination frequency included *A. clypeata*, *A. platyrhynchos, F. atra* and the group of control seeds stored dry. However, in spring, no significant differences were detected in the germination of undigested and non-ingested (control) seeds ($F_{4,85} = 0.77$, $P = 0.55$).

The comparison of germination patterns for undigested and control seeds indicated that undigested seeds germinated earlier than dry control seeds both in autumn ($\chi^2 = 24.37$, 1 df, $P < 0.0001$) and spring ($\chi^2 = 5.28$, 1 df, $P = 0.02$). In autumn, wet control seeds showed an intermediate germination rate (which did not differ significantly from dry control ($\chi^2 = 0.05$, 1 df, $P = 0.83$) or undigested ($\chi^2 = 1.86$, 1 df, $P = 0.17$) seeds.

Amongst autumn samples, seeds from droppings of *A. platyrhynchos* and *F.atra* germinated at a higher rate than seeds ingested by other species and controls (Fig. 1). Seeds from *A. clypeata* droppings germinated later but still at a higher rate than dry control seeds ($\chi^2 = 15.45$, 1 df, $P < 0.0001$). *A. acuta* seeds showed an intermediate germination rate, not significantly different from any of the other species or the controls ($\chi^2 \leq 3.01$, 1 df, $P \geq 0.08$ for all the contrasts). Amongst spring samples, seeds from *A. platyrhynchos* droppings germinated at a higher rate than control seeds ($\chi^2 = 5.85$, 1 df, $P = 0.02$), and the germination rate of seeds from *A. acuta* droppings was intermediate and not statistically different from those of *A. platyrhynchos* ($\chi^2 = 0.79$, 1 df, $P = 0.37$) or control seeds ($\chi^2 = 2.43$, 1 df, $P = 0.12$).

Intraspecific and interspecific variability in quantity and quality of dispersal

When analysing the proportion of ingested seeds that germinate successfully (i.e. combining the effects due to mechanical seed destruction and the more subtle effects on germination ability), we again detected significant interspecific differences in autumn ($F_{5,153}$ = 3.58, P = 0.004), and a less marked effect in spring ($F_{4,139}$ = 2.55, P = 0.04). In autumn the best dispersal in terms of probability of seed germinating per ingested seed was provided by *F. atra*, *A. clypeata* and *A. platyrhynchos*; the worst by *Ay. ferina*, *A. acuta* and *A. strepera*. In spring *A. platyrhynchos* was a better disperser than *A. clypeata* ($F_{1,139}$ = 5.94, P = 0.02) and *A. acuta* ($F_{1,139}$ = 4.82, P = 0.03), and no seed germinated successfully from *A. crecca* and *F. atra* droppings.

The relationship between the quantity and the quality components of dispersal was analysed by determining how the proportion of seeds that germinated is related to the number of seeds ingested by each waterfowl species. For these analyses we used the parameter estimates obtained from above models. The parameter estimates for the proportion of seeds ingested that germinated presented a marginal positive correlation with the estimates obtained when analysing the number of seeds ingested by each species in each season (Spearman Rho = 0.58, P = 0.06, n = 11 species*season).

The results of analyses of the relationship between quantity and quality of dispersal at the intraspecific level varied among species and seasons. In two cases, the quantity of seeds ingested was not specifically related to the proportion of ingested seeds that germinated successfully (spring samples for *A. acuta* and *A. clypeata*, see Table 6). However, for three other cases, a significant positive relationship between seeds ingested and viability was found (autumn samples of *A. clypeata* and *F. atra*, and spring samples of *A. platyrhynchos*, Table 6).

Seed dispersal effectiveness and its relation to gut morphology

Of the four anatomical traits considered (body mass, gizzard mass, grit mass and intestine length, Table 3), gizzard mass was the variable that best explained the interspecific differences in the proportion of seeds undigested after gut passage (estimate ± s.e. = -0.04 ± 0.01, $F_{1,157}$ = 23.42, P < 0.0001, Fig. 2). Although gizzard mass explained 61% of the interspecific variability, the categorical factor 'species' remained significant when added to the model ($F_{4,153}$ = 3.99, P = 0.004). None of the other variables remained significant after controlling for gizzard mass (P ≥ 0.17 for the other three variables).

Grit mass explained 73% of the interspecific differences in the proportion of undigested seeds that germinated successfully (1.22 ± 0.38, $F_{1,58}$ = 10.26, P = 0.002, Fig. 3). After controlling for grit mass, interspecific differences were no longer significant ($F_{3,55}$ = 1.25, P = 0.30), nor were any of the other variables considered (P ≥ 0.50). Grit mass also explained 36% of the interspecific differences in the proportion of ingested seeds that germinated (0.65 ± 0.26, $F_{1,157}$ = 6.23, P = 0.01). After controlling for grit mass, gizzard mass still explained a significant portion of interspecific variability (-0.03 ± 0.02, $F_{1,156}$ = 4.50, P = 0.03). In the model controlling for grit and gizzard mass, neither the factor 'species' ($F_{3,153}$ = 2.26, P = 0.08) nor any of the other variables considered were significant (P ≥ 0.07).

Discussion

Our results demonstrate the importance of waterfowl as dispersers of seeds of *R. maritima* at a local scale. Given the high number of wintering waterfowl in the area, the elevated number of seeds surviving ingestion and the germinability of defecated seeds, the number of seeds travelling within or through our study area transported by ducks must be very high on a daily basis. This waterfowl mediated transport is likely to influence   *R. maritima* population structure, given that one immigrant per generation seems enough to counteract population differentiation due to genetic drift (Wright 1951). However, the real impact of waterfowl on *R. maritima* population dynamics is

difficult to evaluate without information on how successful dispersal translates into plant fitness. In any case, our results illustrate the potential importance of waterfowl-mediated transport for the colonisation of new areas, or the re-colonisation of temporary wetlands after periods of drought. Fish constitute another potential disperser of *Ruppia maritima*, given that a fraction of seeds can resist ingestion by some fish species and germinate in a higher proportion than control uningested seeds (Agami & Waisel 1988). However, unlike fish, waterfowl can potentially disperse the ingested seeds between hydrologically unconnected wetlands and even across isolated river basins. How fish and waterfowl differ in the quantity and quality components of *Ruppia maritima* dispersal is currently unknown.

Another important result of our study is the high potential for dispersal not only in early winter (when the seeds have recently been released from the plants), but also in early spring. This is a major difference with many previous studies on the dispersal of terrestrial fruiting plants, where dispersal is concentrated in the fruiting season (Izhaki & Safriel 1985). This difference is probably related to the filter feeding mechanism used by most waterfowl, that can facilitate the ingestion of seeds already deposited in sediments even when the birds are not feeding primarily on them (e.g. when searching for benthic invertebrates). Orth, Luckenbach & Moore (1994) considered that waterfowl were not good candidates for the dispersal of *Zostera marina* because waterfowl arrive at their study area after the period of seed release. However, our results indicate that this does not preclude dispersal by waterfowl, because seeds can be consumed several months after their production, making the examination of droppings necessary to exclude the presence of viable seeds.

We have found a marked interspecific and seasonal variation in the ingestion of seeds of *R. maritima* by waterfowl. The pattern of interspecific variation varied among seasons, but despite changes in their relative ranks, two species showed high seed consumption over both seasons (*A. clypeata* and *A. acuta*). A high variability in the proportion of seeds destroyed during gut passage was also found. The proportion of seeds surviving gut passage was larger in autumn than in spring. However, this difference was only

significant in the two species with high seed consumption (*A. acuta* and *A. clypeata*). At least two non-exclusive factors can explain the higher survival of seeds of ingestion in autumn. Firstly, some species may have more developed gizzards in autumn than in spring (see below), thus destroying a larger fraction of the seeds ingested. Secondly, a larger fraction of seeds may have survived ingestion in autumn due to the large quantities ingested by some species in this season, and the positive relationship found by us between the quantity of seeds ingested and the proportion of viable seeds in the droppings (see results). The effects of gut passage on seed viability also differed between species and seasons. As a consequence we found a high seasonal and interspecific variability in the potential for dispersal, a feature that characterises bird-mediated seed dispersal dynamics in some terrestrial systems, leading to non-equilibrium states of bird-plant interactions, that generally operate against mutual adaptations of interacting organisms (see Jordano 1994; Herrera 1998). Such instability makes the inference of selection pressures from field data difficult, because the conditions of the system change both seasonally and among years. In our study system, only two species behaved consistently as a good (*A. clypeata*) or as a poor (*A. crecca*) disperser of *R. maritima. A. clypeata* preys mainly on invertebrates and is characterised by a highly specialised bill (Cramp & Simmons 1977). Many seeds can be accidentally ingested by shovelers when filtering food and they may pass undamaged through their small gizzard holding little grit, as characteristic of carnivorous species (see Kehoe & Ankney 1985; Barnes & Thomas 1987). In addition to interspecific differences and seasonal changes in the rates of seed consumption, size of faecal droppings is an uncontrolled factor affecting the estimate of dispersal quantity used in this study. But, given the limited variation in dropping size and faecal output rate that has been observed in four of the duck species used here (or which can be expected from the other given their similar size and structure; e.g. dropping length ranged from 24.9 to 46.3 mm, see Apendix 1 in Bruinzeel et al. 1997) and the large magnitude of interspecific variation in the number of seeds/dropping (mean seed abundance ranged from 0.4 to 12.1 in autumn samples and from

0.2 to 50.3 in spring), we consider it very unlikely that this variability was only explained by differences in faecal output rate or dropping size.

In general, our study indicates a positive relationship between the quantity and quality components of dispersal in the waterbird guild studied. The species (and in many cases the individuals) consuming more seeds produced droppings with a higher proportion of viable seeds. Such a relationship has also been reported at the intraspecific level in *A. crecca* (Tamisier 1971, p. 284). In terrestrial systems, an analysis with three species of birds also supported the existence of a positive relationship between quantity and quality components of dispersal (Larson 1991). Schupp (1993) also concluded that the quantity and quality components of dispersal were positively correlated, based on the reanalysis of data on dispersal of *Virola surinamensis* by six species of birds (see also Howe & van de Kerckhove 1981). However, this may not be the general case, since the two species of birds studied by Reid (1989) differed in the relative quality and quantity of dispersal provided to the plant. Clearly, more studies including a significant number of disperser species are necessary.

To our knowledge, this is the first study that documents differences in the viability of seeds after gut passage by most sympatric species of a given community of birds, largely composed by species with close phylogenetic and ecological affinities (all Anatinae except for one ralid). Previous studies have reported differences in viability after digestion by disperser species pertaining to different taxonomic classes (a deer and an iguana, Mandujano, Gallina & Bullock 1994; a lizard and a bird, Nogales, Delgado & Medina 1998). Recently, Traveset, Riera & Mas (2001) reported differences in the effect of ingestion of seeds by two species of passerine birds. Our study expands these results showing that even closely related species (most of the species in our study are members of a single genus) can have very different effects on seed germination. These differences occurred both in autumn and spring; however, they varied over time, making it difficult to generalise them. What are the reasons for these interspecific differences? As already mentioned, birds differ in their gut structure and this necessarily affects the treatment suffered by ingested food. Interestingly, we have found strong

correlations between the gut structure of different species and digestive efficiency of ingested seeds. Species with heavier gizzards destroyed a higher fraction of the seeds (supporting the assumptions of previous work, e.g. Proctor, Malone & De Vlaming 1967) while the abundance of grit was positively associated with the proportion of seeds germinating after gut passage. The effects of gut ingestion on seed viability can be derived from the mechanical treatment in the gizzard and/or the chemical treatment in the gut (Lohammar, 1954; Telstscherova & Hejny, 1973, Sylber 1988). Our results indicate that the grinding effect in the gut is central to the effects of ingestion by birds on germination. A recent study in *Potamogeton pectinatus* concluded that the effects of ingestion by ducks upon germination are similar to the behaviour of seeds after scarification with sand, but completely different from those obtained after various periods of acid incubation (Santamaría *et al.* in press, see also Spence *et al.* 1971). Additionally, the apparent improvement in seed viability can also be explained by a selective destruction of seeds during gut passage, if for example larger seeds are more likely to resist ingestion (since larger seeds generally have higher germinability and shorter germination times; Greipsson & Davy 1995; Bond, Honig & Maze 1999). However, smaller seeds seem more likely to survive digestion by birds in both interspecific (De Vlaming & Proctor 1968) and intraspecific analyses (Figuerola *et al.* 2001b). In trials with captive ducks, Charalambidou, Santamaría & Langevoort (2001) found no evidence for an effect of bird passage on *Ruppia maritima* seed viability due to size differential destruction of seeds. In summary, our study shows that gizzard size has an important effect on the survival of seeds following ingestion by birds, and that grit has an important role in explaining the effects of gut passage on the viability of seeds.

Differences in the retention time of seeds in the gut are another possible reason for the observed differences in seed viability after ingestion by different waterfowl species. In captivity, we have found important differences between duck species in retention time and a significant effect of retention time on the viability of *Scirpus maritimus* seeds (Figuerola *et al.* 2001b). Together, these two effects may translate into interspecific

differences between waterfowl in the viability of ingested seeds (Figuerola *et al.* 2001b, see also Traveset 1998). However, the effect of retention time upon viability cannot be generalised over species, since e.g. it was not significant in another plant species tested (*Scirpus litoralis*, Figuerola *et al.* 2001b) and in *Solanum americanum* (Wahaj *et al.* 1998). Although experiments in captivity showed that the viability of undigested *R. maritima* seeds varied with retention time, neither the retention time of seeds not the patterns of variation in viability over retention time varied among duck species (Charalambidou *et al.* 2001). In our study, the relationship between intestine length and seed viability was weak and disappeared after controlling for the quantity of grit in the gizzard, suggesting that interspecific differences in retention time may be of minor importance. Body mass (a important correlate of intestine length, Herrera 1986; and of intensity of frugivory in terrestrial systems, Jordano 2000) was also a poor predictor of interspecific differences in seed viability.

Ingestion by waterfowl also accelerated germination rate, a frequent phenomenon in studies with birds (Traveset 1998). In autumn, the germination rate of control seeds stored wet and dry did not differ, indicating that wet storage caused higher germination rates only when seeds undertook gut passage. The comparable germination rate of dry and wet controls thus suggests low seed coat permeability as one of the mechanisms responsible for seed dormancy in this species, and increased permeability following gut passages as one of the mechanisms that overcomes it. Although such an effect has often been considered beneficial for the plant, this remains unclear. Research on non-ingested seeds indicate that early germination can result in a competitive advantage over other seedlings (Zimmerman & Weis 1984; Waller 1985), but it can also increase seedling mortality due to exposure to harsh weather or pathogens (e.g. Traveset 1990). Clearly, the costs and benefits of early germination due to internal dispersal remain unevaluated, and experimental comparisons of the fate of ingested and control seeds would provide interesting results. In any case, ingestion by birds will diversify the germination response of seeds, a favourable process in environments

with unpredictable climatic conditions (Harper 1977; Izhaki & Safriel 1990 ; Traveset *et al.* 2001).

The effects of seed passage through vertebrate guts on germination are not consistent among plant species (Traveset 1998). Given that temporal factors (changes in seed maturity or in disperser gut) can affect germination after passage, it is possible that this inconsistency is related to experimental conditions (see Figuerola & Green 2002, for a review of some problems associated with such experiments). As examples of seasonal changes in gut characteristics, Mateo (1998) reported an increase in quantity of grit ingested by *A. platyrhynchos* from October to March and no seasonal changes in *A. acuta*. However, gizzard mass decreased from autumn to early spring in *A. platyrhynchos* in Texas (Whyte & Bolen 1985), and increased in *A. strepera* in Louisiana (Paulus 1982). All this variability is likely to affect both the proportion of seeds surviving gut passage and their posterior capacity to germinate.

In summary, waterfowl constitute an important dispersal agent for *Ruppia maritima*. Our results show that dispersal is not only concentrated during the seed production phase, but is also prolonged at least up to the end of the winter and early spring. Interestingly, a high seasonal and interspecific variation for potential for dispersal was found. Some of the interspecific variation was related to gut structure. The proportion of seeds destroyed during gut passage was related to gizzard mass and the quantity of grit was related to the germinability of seeds. Some of the seasonal variation could also be related to seasonal changes in gut morphology and/or grit consumption. Ingestion by waterfowl enhanced the rate of germination and, for several duck species, it also had a positive effect on the germinability. The quantity and quality components of seed dispersal effectiveness were positively correlated across waterfowl species, and species consuming more seeds also provided better dispersal for the plant (for the phases of dispersal quality considered in this study: seed survival to digestion and seed germinability). Other phases of dispersal quality can modify our estimates of effectiveness in the field. The spatial redistribution of the seeds due to waterfowl transport can be an important factor affecting the seed rain

shadows of *Ruppia maritima*, and possible differences in the conditions for germination and seedling survival among the sites where seeds are defecated by waterfowl would be an interesting topic for further research in the system studied.

Acknowledgements

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Table 1. Numbers of ducks and coot counted in the study area in aerial surveys done on 6 November 1998 and 23 February 1999 and mean numbers (and range) counted during four monthly surveys over the period November 1998-February 1999. Source: Equipo de Seguimiento de Procesos Naturales, Estación Biológica de Doñana, CSIC, unpublished data.

Species	6/11/98	23/02/99	Nov. 1998 - Feb. 1999
Anas acuta	2000	1900	4153 (1900-9280)
Anas clypeata	19270	6480	15495 (6480-23700)
Anas crecca	2800	650	6030 (650-15870)
Anas penelope	14380	0	5148 (0-14380)
Anas platyrhynchos	2900	2000	3815 (2000-5340)
Anas strepera	700	30	1018 (0-3340)
Aythya ferina	900	6270	3466 (900-6270)
Fulica atra	13360	2260	5565 (2260-13360)
Marmaronetta angustirostris	1	0	51 (0-165)
Netta rufina	400	0	308 (0-800)
Tadorna tadorna	0	292	226 (0-310)
Unidentified anatidae	16000	400	8350 (400-16000)
Total	72711	20282	53625 (20282-74345)

Table 2. Number of faecal samples examined for each waterfowl species during autumn and spring 1998-99. The total number of droppings examined, the number of droppings with   *Ruppia* remains (1), with undigested *Ruppia* (2) and the total number of undigested seeds counted (3) are given for each species and season.

Species	Autumn				Spring			
	Total	(1)	(2)	(3)	Total	(1)	(2)	(3)
Anas acuta	17	13	5	79	34	31	26	558
Anas clypeata	40	35	22	382	31	28	12	108
Anas crecca	8	4	2	4	22	15	0	0
Anas penelope	23	5	1	4	5	3	0	0
Anas platyrhynchos	62	32	7	25	63	58	39	957
Anas strepera	21	14	6	24	-	-	-	-
Aythya ferina	17	13	2	2	1	1	1	12
Fulica atra	92	54	20	87	70	13	4	6
Marmaronetta angustirostris	10	5	1	3	-	-	-	-
Netta rufina	3	1	1	1	3	2	1	36
Tadorna tadorna	-	-	-	-	4	4	2	13
Total	293	176	67	611	233	155	85	1690

Table 3. Gut structure in waterfowl collected from Doñana (south-west Spain). Mean±se and sample size (in parentheses) are given.

	Gizzard mass (g)	Grit mass (g)	Intestine length (cm)
Anas acuta	18.1±3.7 (2)	0.47±0.24 (2)	-
Anas clypeata	8.8±0.4 (34)	1.05±0.10 (41)	213±5 (34)
Anas platyrhynchos	36.4±2.0 (11)	2.45±0.44 (11)	150±18 (3)
Anas strepera	25.8±3.3 (5)	2.84±0.56 (5)	102 (1)
Aythya ferina	41.9±6.6 (3)	2.17±0.56 (3)	-
Fulica atra	44.7±4.3 (27)	8.78±0.57 (28)	115±6 (12)

Table 4. Mean number ± s.e. and range of ingested seeds per dropping ('Ingested'), undigested seeds per dropping (i.e. seeds that retained intact the embryo and endosperm; 'Undigested'), and estimated proportion of undigested seeds that they represent ('%undigested' = undigested/ingested). 'Estimated%' refers to the percentage of undigested seeds as estimated from the GLM models, which accounts for the differences in the number of seeds contained in each dropping (see Crawley (1993). '*n*'= number of droppings examined. The number of ingested seeds is based on the total number of droppings examined, whereas both the number and the percentage of undigested seeds are based on the number of droppings with any seed remaining (thus the different *n* values). Only species in which at least five droppings contained *Ruppia maritima* seed remains were included in these analyses.

	Autumn samples							
Species	Ingested ± s.e.	Range	*n*	Undigested ± s.e.	Range	*n*	%undigested ± s.e.	Estimated% ± s.e.
Anas acuta	5.7 ± 3.2	0 - 54	17	6.1 ± 4.8	0 - 53	13	36.2 ± 12.8	81.4 ± 16.4
Anas clypeata	12.1 ± 3.3	0 - 119	40	10.9 ± 3.4	0 - 107	35	48.9 ± 7.0	79.1 ± 7.4
Anas penelope	0.4 ± 0.2	0 - 5	23	0.8 ± 0.8	0 - 4	5	16.0 ± 16.0	44.4 ± 54.0
Anas platyrhynchos	1.3 ± 0.4	0 - 19	62	0.8 ± 0.5	0 - 15	32	12.8 ± 4.7	31.7 ± 18.2
Anas strepera	2.0 ± 0.7	0 - 10	21	1.7 ± 0.8	0 - 9	14	27.1 ± 9.3	57.1 ± 25.0
Aythya ferina	0.9 ± 0.2	0 - 3	16	0.2 ± 0.1	0 - 1	13	6.4 ± 4.8	12.5 ± 40.5
Fulica atra	1.6 ± 0.3	0 - 16	92	1.6 ± 0.5	0 - 14	54	24.7 ± 4.8	59.2 ± 13.3
Marmaronetta angustirostris	0.8 ± 0.4	0 - 4	10	0.6 ± 0.6	0 - 3	5	15.0 ± 15.0	37.5 ± 57.2
	Spring samples							
Species	Ingested ± s.e.	Range	*n*	Undigested ± s.e.	Range	*n*	%undigested ± s.e.	Estimated% ± s.e.
Anas acuta	50.3 ± 4.9	0 - 122	34	18.0 ± 4.2	0 - 85	31	24.7 ± 3.8	32.6 ± 8.7
Anas clypeata	9.7 ± 3.8	0 - 102	31	3.9 ± 2.3	0 - 65	28	24.4 ± 5.9	36.0 ± 20.7
Anas crecca	0.8 ± 0.1	0 - 2	22	0 ± 0	0	15	0	0
Anas platyrhynchos	29.9 ± 4.4	0 - 136	63	16.5 ± 4.0	0 - 132	58	31.6 ± 4.5	50.8 ± 8.3
Fulica atra	0.2 ± 0.1	0 - 4	70	0.5 ± 0.2	0 - 3	13	25.0 ± 11.3	35.3 ± 8.7

Table 5. Percentage and estimated percentage (± s.e.) of apparently viable seeds recovered from waterfowl faeces that germinated in autumn samples (after 42 days) and in spring samples (after 58 days). Percentage germinating was estimated from the ratio number of seeds germinating/number of undigested seeds in the dropping. Estimated percentage was estimated from the GLM models and accounts for the differences in the number of seeds contained in each dropping. Note that when the number of seeds is the same in all the samples (the case of controls) the germinability estimated directly from the sample and from the GLM model is the same. ' *n* samples' refers to the number of droppings (undigested seeds) or the number of random germination batches (control seeds).

Autumn	% germinating	Estimated %.	*n* samples	*n* seeds
Anas acuta	17.6 ± 9.1	8.9 ± 16.3	5	77
Anas clypeata	50.8 ± 8.1	19.9 ± 7.4	22	217
Anas platyrhynchos	46.7 ± 21.1	44.0 ± 28.9	6	16
Anas strepera	25 ± 17.1	12.5 ± 29.5	6	25
Fulica atra	29.2 ± 8.6	40.2 ± 15.5	20	87
Dry control[*]	50.0 ± 5.2	50.0 ± 14.4	10	100
Wet control[*]	8 ± 3.7	8.0 ± 20.4	5	50

Spring	% germinating	Estimated %	*n* droppings	*n* seeds
Anas acuta	30.8 ± 7.0	30.6 ± 14.9	26	558
Anas clypeata	19.0 ± 9.2	21.8 ± 31.5	12	124
Anas platyrhynchos	25.5 ± 5.9	32.1 ± 11.4	38	955
Fulica atra	0	0	4	6
Dry control[*]	58.4 ± 6.8	58.4 ± 35.1	10	100

[*] Seeds from the plant, stored in dry or wet conditions (see methods).

Table 6. Relationship between the estimated quantity of *Ruppia maritima* seeds ingested and the proportion of the ingested seeds that germinated, given separately for each waterfowl species (i.e. comparing faecal samples within species). Estimates correspond to the slopes obtained from the GLM models, with binomial error and a logit link.

Species	Season	estimate ± s.e	*F*	df	*P*
Anas acuta	Spring	0.015 ± 0.009	2.36	1,29	0.14
Anas clypeata	Autumn	0.011 ± 0.003	11.35	1,33	0.002
	Spring	-0.020 ± 0.012	3.65	1,25	0.07
Anas platyrhynchos	Spring	0.012 ± 0.005	5.26	1,56	0.03
Fulica atra	Autumn	0.157 ± 0.045	14.06	1,52	0.0004

Figure 1. Germination rate, expressed as the parameter estimates obtained from separate Cox regressions for autumn and spring *Ruppia maritima* germination data. Bars correspond to model coefficients (+ 1 s.e.). A larger coefficient indicates faster seed germination. The coefficients for dry control seeds were set to zero. Since data comes from different germination trials and regression curves, coefficients for autumn and spring are not directly comparable. No data were available for *A. clypeata*, *F. atra* and wet controls in spring.
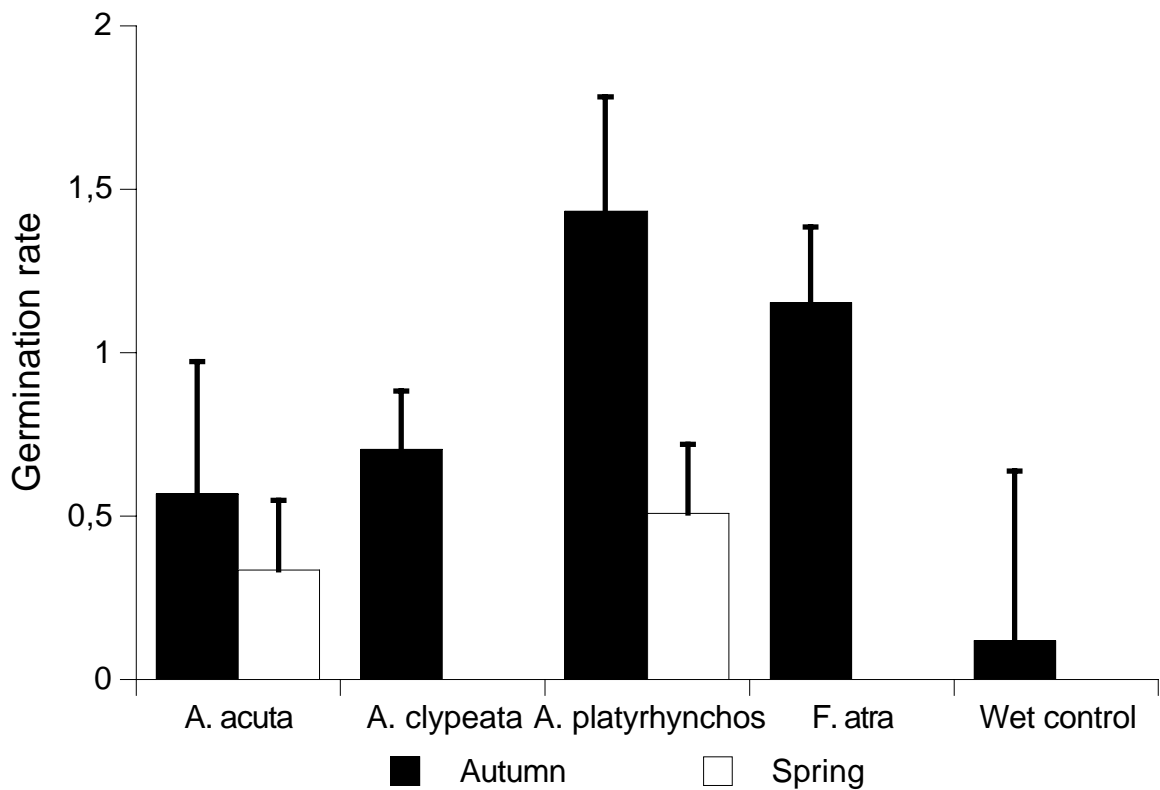
Figure 2. Relationship between gizzard mass and estimated proportion of *Ruppia maritima* seeds surviving digestion. Filled triangles show the values predicted for each species according to a GLM model and open circles the values observed for each species. Codes correspond to the first three letters of the specific latin name.
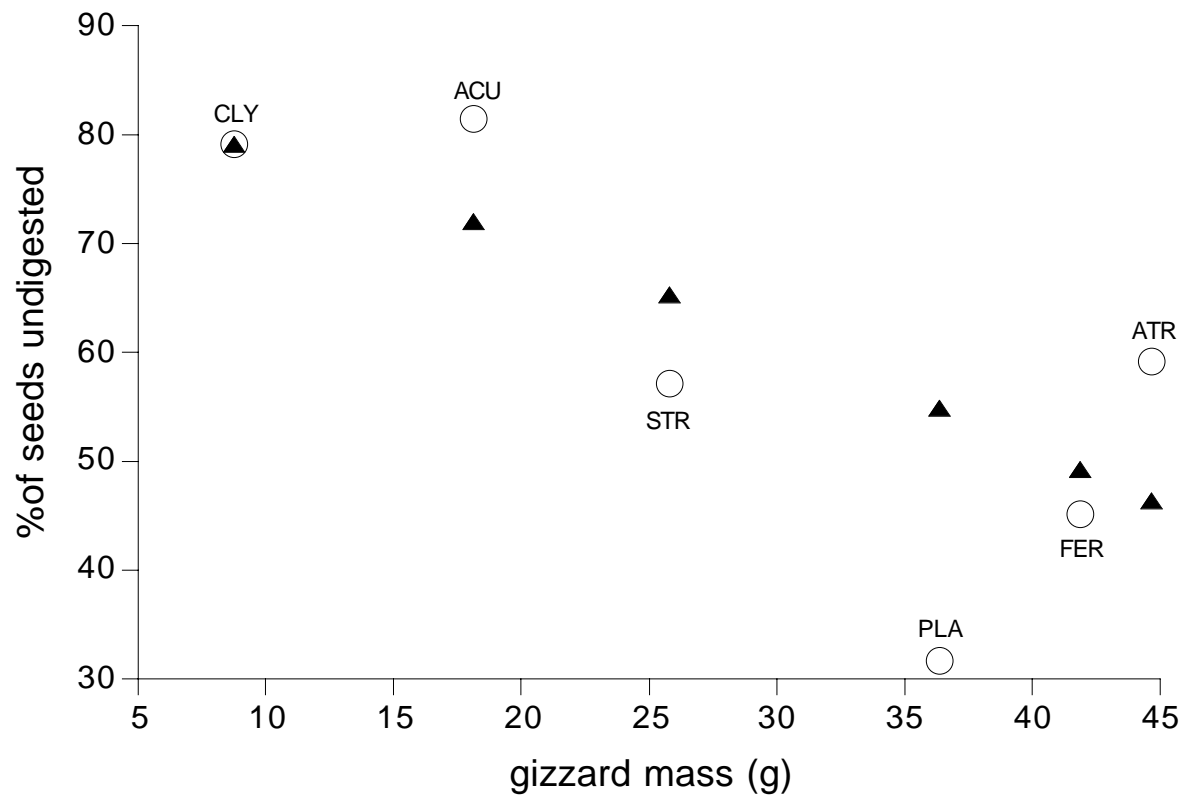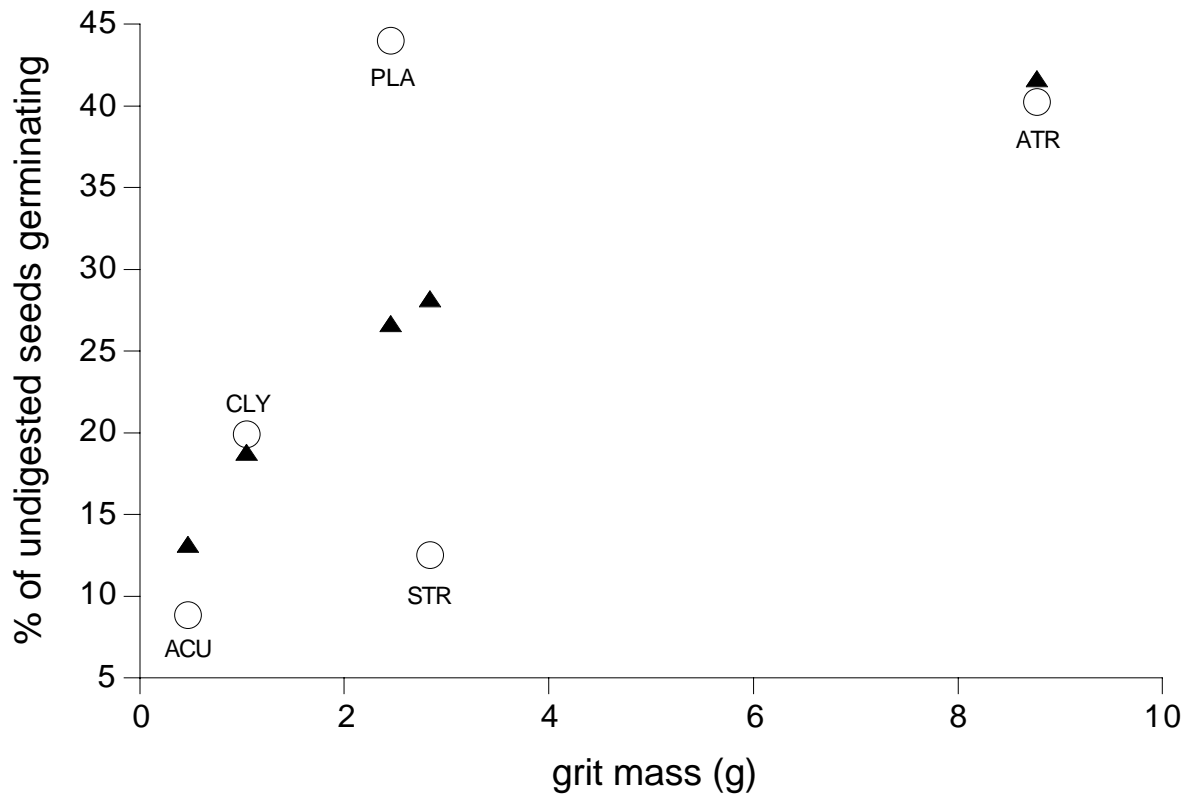
Figure 3.   Relationship between grit mass and the proportion of undigested seeds of *Ruppia maritima* germinating. Filled triangles show the values predicted for each species according to a GLM model and open circles the values observed for each species.

Effect of passage through duck gut on germination of fennel pondweed seeds [†]

Luis Santamaría[1,3], Iris Charalambidou[1], Jordi Figuerola[2] and **Andy J. Green[2]**

With 1 figure and 3 tables

[1] Authors' address: Netherlands Institute of Ecology, Centre for Limnology, PO Box 1299, 3600 BG Maarssen, The Netherlands.

[2] Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. de María Luisa s/n, E-41013 Sevilla, Spain.

[3] Corresponding author: santamaria@cl.nioo.knaw.nl

Abstract: Vertebrates are important seed dispersers for many plants. In addition to transport of seeds, ingestion often affects the proportion or rate of seed germination. We present one of the first studies comparing the effects of different waterbird species on the seeds of a subcosmopolitan pondweed, Potamogeton pectinatus. We also present the first comparison of the effects of digestion by ducks (mallard *Anas platyrhynchos*, shoveler *A. clypeata* and wigeon *A. penelope*) and physical-chemical "simulation of digestion" on pondweed seed germination. In two experiments differing in the length of the preceding stratification period, two to three individuals per duck species were force-fed 150 seeds each. Average retrieval did not differ significantly between species. Germination rate was higher for duck ingested seeds, intermediate for scarified seeds and lowest for the controls and acid treated seeds, independently of the length of the stratification period. Total germination, however, did not differ significantly among duck-ingested, scarified, control and acid treated seeds. Consequently the changes in germination rate after ingestion by ducks seem related to the grinding treatment in the gut and unrelated to exposure to acidic conditions. Migratory ducks constitute a legitimate disperser of *P. pectinatus*.

Introduction

Many species of freshwater organisms have wide geographic ranges and inhabit waterbodies that are separated by extensive terrestrial or oceanic areas (DE VLAMING & PROCTOR 1968). This has often been interpreted as evidence that these organisms have an effective and readily available means of dispersal (MALONE 1965), and birds have been considered the main candidate for dispersal (DARWIN 1859, OKAMURA 1997).

Transport of resting stages by birds can either be external (ectozoochory) with the disseminules adhering to feathers, feet and bill or internal (endozoochory) via the digestive tract (THIENEMANN 1950). A number of studies have concentrated on internal transport in waterbirds and a wide variety of aquatic organisms such as algae and freshwater invertebrates (reviewed in FIGUEROLA & GREEN 2002, BILTON et al. 2001) have been observed to survive passage through the digestive system of ducks.

Furthermore, it has been suggested that gut passage can trigger the germination of seeds with a thick coat and/or prolonged dormancy, such as the fruits of *Potamogeton* pondweed species (GUPPY 1897, LOHAMMAR 1954, SMITS et al. 1989). SMITS et al. (1989) found that passage through the mallard *Anas platyrhynchos* gut increased the proportion of seeds germinating in fennel pondweed *Potamogeton pectinatus*, but not in *P. obtusifolius* and *P. natans*. However, germination rate (i.e. the speed of germination from the moment at which seeds are set to germinate) was not monitored and no statistical comparison was attempted. Similarly, AGAMI & WAISEL (1986) reported a significant increase in the germination of *Najas marina* seeds following mallard gut passage: control seeds showed lower total germination than duck-ingested and mechanically cracked seeds. Germination rates of cracked and digested seeds seemed comparable, but no statistical comparison was made.

The effects of ingestion by birds can be due to the grinding effect in the gizzard or the acidic treatment in the gut. In most studies simulating the effects of these processes, seed germination was strongly enhanced by seed scarification (mechanical removal of the soft epicarp+mesocarp), (CROCKER

1907, LOHAMMAR 1954, YEO 1965, SPENCE et al. 1971) and its combination with high temperature (LOHAMMAR 1954), while the effect of chemical treatments was weaker (LOHAMMAR 1954). However, TELSTSCHEROVA & HEJNY (1973) reported strong effects of sulphuric-acid incubation but weak effects of scarification. However, no study has compared the effect of gut passage on pondweed germination to the effect of the physical-chemical 'simulation of digestion' treatments.

Most of the studies mentioned above used relatively short germination runs (up to 30 days). In one of the few studies that used long germination runs (> 4 weeks), seed germination continued well beyond, and often peaked after week four (LOHAMMAR 1954, with *P. pectinatus* and *P. lucens*). Hence, detected effects on total germination in previous studies may have been confounded with a short-term effect on germination rate (see TRAVESET 1998 for review).

Furthermore, most previous studies have used no or rather short periods of seed stratification previous to gut passage (or its simulation) and no stratification following it. However, LOHAMMAR's (1954) data strongly suggest that the effect of gut-passage simulation might only be evident following stratification of the treated seeds. *Potamogeton* seeds are often consumed by ducks in autumn/winter (CRAMP & SIMMONS 1977, TAMISIER & DEHORTER 1999) and seeds do not germinate until the next spring. Hence, in the field seed consumption is often both preceded and followed by several months of stratification. We thus decided to test the effect of duck digestion under prolonged stratification conditions. In this study we investigated how germination rates and viability of *P. pectinatus* seeds are affected by ingestion by ducks, and how these effects are related to the mechanical and chemical components of gut passage. The effects of duck species and seed stratification are also investigated in two separate experiments.

Materials and methods

Three mallard (*A. platyrhynchos*), three wigeon (*A. penelope*) and two shoveler (*A. clypeata*) were used in our experiments. These are migratory

duck species potentially capable of moving pondweed seeds over long distances (ROSE & SCOTT 1997). The mallard had been captured from the wild as adults while the widgeon and shoveler were one year-old   birds born in captivity. Prior to the experiments, they were housed in outdoor facilities at Heteren, The Netherlands, and fed on a stable diet of commercial pellets (Anseres 3 ® Kasper Faunafood) and mixed grains (HAVENS Voeders) for over a year. During the experiment, they were kept individually in wooden cages (0.60 m x 0.50 m x 0.50 m) with a mesh floor (mesh size 12 mm) and removable plastic trays under each cage. The birds were caged overnight with water and pellets to familiarise them with experimental conditions. Pellets and water were available ad libitum throughout the experiments.

P. pectinatus seeds were collected in September 1998 from a population growing in an artificial lake originally created for sand-gravel extraction and situated in Engelbert (Groningen, The Netherlands). All seeds were stored in a large plastic container filled with tap water in a refrigerator (darkness, 5±1 °C: stratification pre-treatment) for 7 (experiment 1) or 12 months (experiment 2). Following the experimental treatments, treated and control seeds were stored for 3 months under the same conditions (stratification post-treatment: experiments 1 and 2). We aimed at providing stratification conditions equivalent to those of Central-Northern European winters. For this purpose, and because we used mild stratification temperatures (5 °C) instead of chilling, in experiment 1 we extended the stratification for three months longer than the typical 6-7 month winter period (pre+post stratification: 10 months). Experiment 2 (pre+post stratification: 15 months) tested whether our treatment was enough to maximise total germination or whether longer stratification periods would still result in increased germination.

For the experiments, seeds were randomly assigned to the following treatments: control (kept at room temperature while the other treatments took place), duck gut passage (with 2 mallard and 2 shoveler for experiment 1, and 3 mallard and 3 wigeon for experiment 2), scarification (for experiments 1 and 2) and chemical treatment with sulphuric acid (experiment 2 only).

Experiment 1 was carried out on 19-20 April 1999 and experiment 2 on 22-23 September 1999. At the beginning of each experiment, each duck was force-fed with 150 seeds (except for one individual fed with only 80 seeds, due to the accidental loss of part of the seeds during the feeding procedure). To facilitate force-feeding, groups of 20-25 seeds were placed in soft pellets made from Anseres soaked in water. The pellets were placed on the posterior part of the tongue and pushed down into the pharynx. The duck faeces were collected in the removable trays 6 and 22 hours after ingestion. Both experiments were terminated after 22 h. Immediately following collection, the faeces were sieved (sieve size 1 mm) and intact seeds were retrieved and counted. We considered seeds to be intact when there showed no visible damage to the endocarp (such as cracks in the seed wall or the opening of the seed's dorsal trap door) that resulted in exposure of the embryo. In this and other experiments (Charalambidou, unpublished data), the low proportion of 'damaged' seeds (i.e. those with broken endocarp and exposed embryo) collected always failed to germinate.

Scarification consisted of filling up triplicate 250ml plastic flasks with seeds and wet gravel (2-4 mm grain size) and shaking them for 12 h using electric test-tube shakers. Seeds were then separated from the gravel and rinsed.

Chemical treatment consisted of the immersion of triplicate batches of 50 seeds in separate test tubes containing 1 M H_2SO_4 for 5, 10, 15, 30, 60 or 120 minutes. Seeds were then thoroughly rinsed. The acid solution utilised (pH $\approx$ 0.3) was chosen to mimic conditions of the avian gut, which show hydrogen-acid concentrations of 0.2-1.2 for the proventriculus and 0.7-2.8 for the gizzard (VISPO & KARASOV 1997). We used a relatively high acidity to facilitate comparison with TELTSCHEROVA & HEJNY (1973) who treated seeds with 'concentrated sulphuric acid for 50, 30 and 10 minutes'.

Retrieved, scarified, chemically-treated and control seeds were then stored in tap water at 4 ºC in a refrigerator for three months (stratification post-treatment, identical for experiments 1 and 2), then set to germinate in microtiter plastic trays. Each cell (3.5 ml in volume) was half-filled with tap water (approx. 2 ml) and individual samples were placed in separate cells

with a maximum of 10 seeds per cell. The trays were placed in a climate room at 15 hours light / 9 hours dark at 20 ºC. Germination was checked weekly and the experiments were terminated after 9 or 10 weeks (experiments 1 and 2, respectively).

The effect of seed treatment on total germination (cumulative germination at the end of the germination run) was tested by means of Generalised Mixed-model ANOVAs using the GLIMMIX models of SAS (SAS INSTITUTE INC. 1996). The model included 'treatment' (gut passage vs. scarification vs. control) as a fixed effect and 'block' (i.e. each individual duck for the retrieved seeds and each replicate batch for the scarified and control seeds) as a random effect. We used a logit link for binomial data on the response variable 'proportion of germinated seeds' (number of germinated seeds / total number of seeds). When the model estimation algorithms failed to converge with this response variable, we re-expressed it as binomial data (a 0-1 variable, with one case per individual seed) to obtain a more consistent convergence (SAS INSTITUTE INC., 1996).

We analysed experiments 1 and 2 separately. Due to the low number of individual ducks used, we split the ANOVA to increase the chance of detecting duck vs. control differences. Thus we first performed an ANOVA in which the seeds digested by the two different duck species used were lumped in a single category ('duck digested seeds') and compared it with the control and scarified seeds. Then in a separate ANOVA we compared the germination of duck-ingested seeds between duck species. Since these two ANOVAs were orthogonal, no Bonferroni correction was needed.

The effect of seed treatment on seed germination rate was tested by fitting a Cox proportional hazards regression model (e.g. ALLISON 1995) to the number of days between setting for germination and seedling emergence, for each individual seed. Cox's proportional hazards model is one of the regression techniques belonging to the broad category of 'survival analyses', used to determine the existence of significant correlations with certain independent variables when the dependent variable of interest (survival or failure time) corresponds to time until the occurrence of a particular event (in our case, germination) and it is most likely not normally distributed (since

survival times usually follow an exponential or Weibull distribution). Note that each germination event is equivalent, for our purpose, to the loss of an individual in the population of non-germinated seeds. The proportional hazard model is not based on any assumptions concerning the nature or shape of the underlying survival distribution, since it models the underlying hazard rate (rather than survival time) as a function of the independent variables (HARRELL 2001).

Only data from seeds that had germinated by the end of the experiment were included, to separate the effects on germination rate from those on total germination (see above). Fixed effects and specific fixed-effects contrasts were similar to those described above (see also Table 2). To account for the effects of digestion by different individual ducks (within the 'duck' treatment), immersion in acid or scarification in different batches, or germination of control seeds on different random groups, a replicate effect was added to the model as a random, or 'failtry' effect. Ties were estimated using the exact method. Survival analyses were computed using S-Plus 2000 (MATHSOFT 1999).

Results

Average retrieval of intact seeds was 11 % for mallard and 29 % for shoveler (experiment 1) and 5 % for mallard and 11 % for wigeon (experiment 2), with large variation among individual ducks (Table 1).

1. Experiment 1 (7 months pre-treatment stratification)

Seed treatment significantly affected the germination rate, but the seeds had achieved a comparable total germination at the end of the germination run (Tables 2 and 3, Fig. 1a). Contrasts among treatments (following Bonferroni correction; experimentwise error rate, $P_{EER}=0.05$, comparisonwise error rate, $P_{CER}=0.017$) showed that, following gut-passage, seeds had higher germination rates than the scarified and control seeds (Table 3, Fig.1a). Scarified seeds had higher germination rates than control seeds (Table 3, Fig.1a).

Germination rate and total germination did no t differ significantly between duck species (Tables 2 & 3, Fig.1a).

2. Experiment 2 (12 months pre-treatment stratification)

Seed treatment significantly affected the germination rate, but the seeds had achieved a comparable total germination at the end of the germination run (Tables 2 & 3 ; Figs. 1b,c). Contrasts among treatments (following Bonferroni correction, as above) showed that, following gut-passage, seeds had significantly higher germination rates than the scarified, acid-incubated and control seeds (as indicated by the higher parameter estimate obtained for duck-ingested seeds in the Cox regression model, Table 3; Figs. 1b,c). Scarified seeds had significantly higher germination rates than the acid-incubated and control seeds, which did not differ significantly (Table 3, Figs. 1b,c).

We were not able to test for differences among acid-incubation times or for specific contrasts between each acid-incubation time and the control, because the Cox regression model failed to converge. Independently of the length of the incubation period, immersion in 1 M H_2SO_4 never stimulated seed germination. It seemed rather to have a negative effect on total germination (Fig. 1c), although the differences among acid-incubation (all treatments pooled) and control were not significant (Tables 2 & 3).

Germination rate and total germination did not differ significantly between duck species (Tables 2 & 3, Fig. 1b).

Discussion

Our results show that *P. pectinatus* seeds can withstand duck gut passage and germinate afterwards. Our retrieval of intact seeds is comparable to the findings of SMITS et al. (1989; 20 % for mallard and 23 % for coot) and AGAMI & WAISEL (1986; 26 to 34 % retrieval of *N. marina* seeds ingested by mallard). We found large variation in seed retrieval among individual ducks (Table 1).

In both our experiments, total cumulative germination (after 9 and 10 weeks) did not differ significantly between treatments (duck ingestion,

scarification and control). Germination following gut passage or scarification was 37 to 61 %, comparable to previous work using duck-ingested seeds or simulating gut passage (CROCKER 1907, LOHAMMAR 1954, YEO 1965, SPENCE et al. 1971, TELSTSCHEROVA & HEJNY 1973, SMITS et al. 1989). Total germination of the untreated seeds was 33 % in experiment 1 and 55 % in experiment 2, much larger than found in previous work, a difference that we attribute to the longer stratification period we used.

Germination rate, on the other hand, was significantly higher in seeds treated with duck gut passage and, to a lesser degree, in those with surface scarification. Even after applying large stratification periods to break seed dormancy, untreated seeds took five weeks longer to germinate (cumulative germination was comparable at day 35, Fig. 1). Thus, in climates with cold winters (temperate to sub-arctic region), gut passage will not enhance germination as opposed to seeds remaining dormant in the seed bank, but will result in an earlier germination within the same growth season. Consequently, our results support the hypothesis that duck digestion affects the rate of germination but not the viability of the digested seeds (see also TRAVESET 1998).

Whether an earlier germination represents fitness advantage is not self-evident: it will depend on a number of factors affecting seedling mortality and growth (advantage of an extended growth season, early mortality due to late frost or spring storms, seedling competition, etc.). It is possible, however, that the short growth seasons that characterise temperate and sub-arctic climates are the features for which early germination is most likely to represent a fitness advantage, at least in the years with mild springs. More generally, the coexistence of digested and undigested seeds within a given seed cohort will result in increased diversification of seed germination patterns, which can favour the colonisation of habitats characterised by unpredictable climatic conditions.

Incubation in concentrated sulphuric acid did not result in increased total germination or higher germination rates (Fig. 1). Our results contradict the findings of TELSTSCHEROVA & HEJNY (1973) and indicate that, until more specific tests are carried out, incubation in digestive chemicals cannot be

assumed to mimic duck gut passage, but suggest that the abrading of seed coats can be partially responsible for changes in germination patterns.

Passage through the gut of different duck species did not result in significant differences in seed germination. However, this result may be explained by the small number of individuals used, the large variation in seed retrieval found among individuals and the fact that we did not control for retention time (time spent by each individual seed in the gut, which may have varied between species). In any case, our data are in agreement with a recent review by TRAVESET (1998), who concluded that interspecific differences among animals that ingest seeds have a limited effect on seed germination.

The flyways of tens of millions of migratory ducks overlap with the global distribution of *P. pectinatus* (ROSE & SCOTT 1997), and our study shows they have the capacity to disperse viable seeds during local movements or long-distance migrations. In this experiment, viable *P. pectinatus* seeds were retained in the duck gut for longer than 6 hours. The flying speed for *Anas* ducks ranges from 60 to 78 km/h (WELHAM 1994), hence waterfowl within distances of approximately 400 km may regularly disperse fennel pondweed seeds. More detailed work on the relationship between retention time in the gut and seed viability is however needed before advancing hypotheses on the spatial scale at which dispersal is likely to take place.

Acknowledgements

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Table 1. *Potamogeton pectinatus* seed retrieval following passage through the gut of different duck species. 'Experiment 1' and 'Experiment 2' were preceded by periods of seed stratification (storage at 5 °C) of 7 and 12 months respectively.

|	Duck species	Duck individual	Number of seeds ingested	Number of seeds retrieved	% seeds retrieved
Experiment 1	Mallard	1	150	12	8
		2	80	10	12
	Shoveler	1	150	31	21
		2	150	56	37
Experiment 2	Mallard	1	150	8	5
		2	150	14	9
		3	150	0	0
	Wigeon	1	150	2	1
		2	150	44	29
		3	150	5	3

Table 2. Effect of *Potamogeton pectinatus* seed treatment on total seed germination (i.e. cumulative germination at the end of the germination run, i.e. day 63 for experiment 1 and day 70 for experiment 2). Results of Generalised Mixed Models on seed germination over time. ϕ = extra-dispersion parameter. The scaled chi-square can be interpreted as a lack-of-fit statistic for the fixed component of the effect (SAS INSTITUTE INC. 1996); hence, degrees of freedom and P-value are also provided.

	Model Statistics				**Type 3 tests of fixed effects**			
	ϕ	**Scaled chi-square**	**df**	**P**	**df$_N$**	**df$_D$**	**F**	**P**
Experiment 1:								
Ducks/scarified/control	0.98	157.5	161	0.56	2	5	3.07	0.13
Mallard/Shoveler	1.01	71.3	72	0.50	1	1	1.89	0.40
Experiment 2:								
Ducks/scarified/acid/control	0.94	1236	1252	0.49	8	16	1.46	0.25
Mallard/Wigeon	1.03	68.0	68	0.48	1	1	0.19	0.74

Table 3. Effect of treatment on *Potamogeton pectinatus* seed germination rate. The days to germination of each individual seed were   fitted to a Cox proportional hazards regression model (MATHSOFT 1999). Parameter estimates indicate differences in the hazard rates under different treatments, relative to an arbitrarily-chosen category of reference (indicated in the table with zero values, and assigned to the 'control' treatment whenever available). Increasing parameter estimates thus indicate increasing germination rates, with negative values indicating lower germination rates than the category of reference.

	χ^2	df	P	Parameter estimates ± standard error
Experiment 1							
Duck spp. pooled				*Control*	*Duck*	*Scarified*	
Model fit	48.9	2	<0.0001	0	3.54±0.64	1.37±0.47	
Contrasts:							
Duck vs. control	30.9	1	<0.0001				
Duck vs. scarified	14.5	1	0.0001				
Scarified vs. control	8.55	1	0.003				
Between duck spp.				*Mallard*	*Shoveler*		
Mallard vs. shoveler	0.01	1	0.93	0	0.03±0.35		
Experiment 2							
Duck spp. pooled				*Control*	*Duck*	*Scarified*	*Acid*
Model fit	190.0	14.9	<0.0001	0	3.01±0.48	1.47±0.37	0.44±0.33
Contrasts:							
Duck vs. control	39.2	1	<0.0001				
Scarified vs. control	15.3	1	<0.0001				
Acid vs. control	1.8	1	0.18				
Duck vs scarified	10.6	1	0.001				
Duck vs. acid	40.1	1	<0.0001				
Scarified vs. acid	12.7	1	0.0004				
Between duck spp.				*Mallard*	*Wigeon*		
Mallard vs. wigeon	0.62	1	0.43	0	-0.34±0.44		

Figure 1. Effect of experimental treatments (scarification, passage through duck gut and acid incubation) on the germination of *Potamogeton pectinatus* seeds. (a) Seeds stratified 7 months before and 3 months after applying the experimental treatments. (b) and (c) Seeds stratified 12 months and 3 months after applying the experimental treatments. 'Shoveler', 'mallard' and 'wigeon' are the duck species that ingested the seeds in the gut passage treatment. '5' acid', '10' acid', etc. are the durations of seed incubation in sulphuric acid (in minutes).
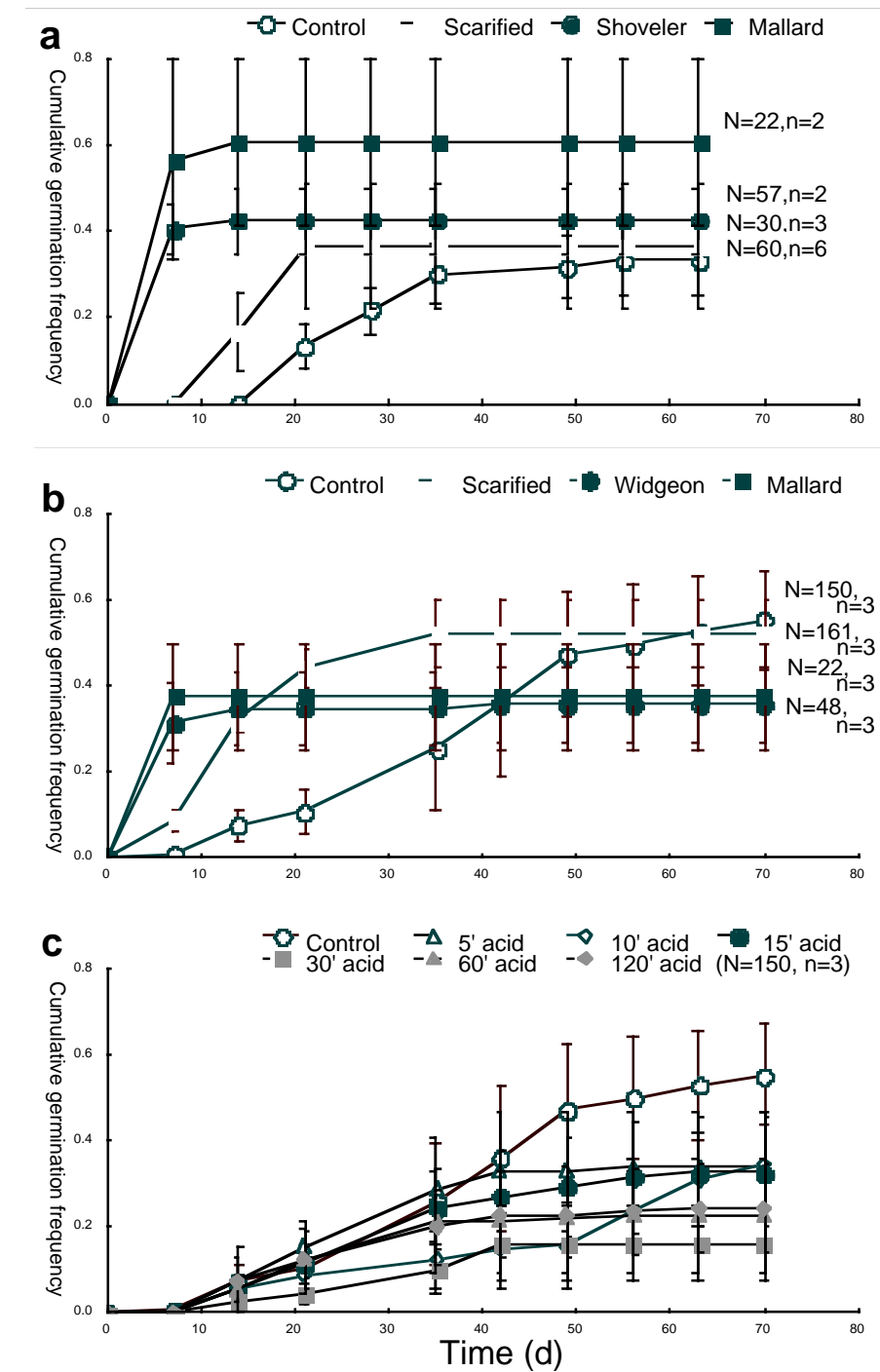
Effects of seed ingestion by ducks on the fennel pondweed *Potamogeton pectinatus* fitness

Jordi Figuerola[1], Luis Santamaría[2], Andy Green[1], Isabel Luque[1], Raquel Alvarez[3] and Iris Charalambidou[2]

1 Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. María Luisa s/n. E-41013 Sevilla, Spain. E-mail: jordi@ebd.csic.es

2 Netherlands Institute of Ecology, Centre for Limnology, PO Box 1299, 3600 BG Maarssen, The Netherlands.

3 Biology and Ethology Unit, Facultad de Veterinaria, University of Extremadura, 10071 Cáceres, Spain

Author for correspondence:

Jordi Figuerola
Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. María Luisa s/n. E-41013 Sevilla, Spain. E-mail: jordi@ebd.csic.es

Fax: 34 95 4621125
e-mail: jordi@ebd.csic.es

Submitted

Abstract

The ingestion of seeds by vertebrates can affect the germinability or germination rate of seeds. It is however unclear whether an earlier germination necessarily results in enhanced plant fitness. In the case of fennel pondweed *Potamogeton pectinatus*, effects of seed ingestion by ducks on both germinability and germination rate have been reported from laboratory experiments. We performed an experiment under controlled conditions in the field, to determine the effects of seed ingestion by ducks on seed germination, seedling survival, and plant growth and asexual multiplication. Seeds were fed to three captive shovelers *Anas clypeata*, and planted in pots placed outdoors in water-filled containers. Experimental ingestion by ducks took place both at the start and end of the winter. Germination and survival were measured monthly. Standing crop and investment in vegetative parts (shoot and roots), asexual propagules (tubers) and sexual reproduction (seeds) were determined in autumn. More duck-ingested than control (undigested) seeds germinated in early winter, but this difference disappeared for seeds planted in late winter, when the duck-ingestion and control treatments were preceded by three-months of stratification. None of the variables measuring seedling survival and plant performance varied between duck ingested and control seeds. Since winter temperatures do not provide for seed stratification in southern Spain, we conclude that under our experimental conditions (no herbivory or intra-specific competition), ingestion by ducks in early winter resulted in increasing pondweed fitness due to enhanced seed germinability, without other costs or benefits for the plant.

Introduction

The effects of ingestion by vertebrates on the capacity of seeds to germinate have been studied in many systems, and effects on the germinability of seeds or the rate of germination have often been reported (Traveset 1998). Although changes in these parameters have generally been assumed to have an adaptative value for the plants, this may not be necessarily true. Increases or decreases in germination rate can both be interpreted as adaptive responses of plants to adjust their germination patterns to habitat characteristics (e.g. Barnea et al. 1991). Under field conditions, seedlings are exposed to different selective pressures. Although an early germination can reduce the levels of intra- and interspecific competition (Garwood 1983, Loiselle 1990), early-growing seedlings can also be exposed to harsher climatic conditions (as suggested by Janzen 1981), to increased damage by pathogens (e.g. fungus infection, Traveset 1990) or to predation by herbivores (Figuerola and Green, in press). Consequently, the final outcome of seed ingestion by vertebrates is not clear, and is most likely to depend on both the characteristics of each specific plant-disperser system and the local conditions. Unfortunately, information on the fitness of vertebrate ingested and control (non-ingested) seeds in the field is scarce.

The direct or indirect effects of ingestion by vertebrates may last long after the period of seed germination and have long term impacts on plant fitness. Firstly, effects of gut passage on germination phenology may affect the timing available for growth and reproduction. Secondly, seed ingestion may select for certain seed characteristics that enhance survival of gut passage (for example, smaller seeds or those with harder seed coats, Agami and Waisel 1988). For example, larger seeds may be more likely to be damaged during gut passage (Figuerola and Green 2002) and represent a larger reward for vertebrate and invertebrate seed predators (Hulme 1993, Van der Wall 1994, Moegenburg 1996, Alexander et al. 2001). However, seedlings originating from larger seeds tend to have increased survival and establishment (Schaal 1980, Foster 1986, Susko and Lovett-Doust 2000),

and a higher competitive ability (Houssard and Escarré 1991, Turnbull et al. 1999).

In this paper, we experimentally tested the short term (germinability) and long term (growth and reproduction) effects of seed ingestion by ducks under controlled conditions that excluded the effects of herbivory and intra-specific competition. Ingestion by vertebrates was simulated at two different moments (both at the beginning and end of winter) to separate the direct effects of gut passage on seedling growth and survival, from the indirect effects of changes in seed germination phenology on plant fitness. For this purpose, we monitored the fate of the resulting seedlings until the end of the growing season.

Methods

Seeds of *Potamogeton pectinatus* were collected in Veta la Palma ,Doñana Natural Park (6^0 14' W, 36^0 57' N), on October 2000, separated from debris and kept moist, at 5 ±1 °C and in darkness until further use. All the seeds collected were haphardadly grouped into 24 batches of 50 seeds, which where then randomly assigned to the different treatments (two treatments, control vs. ingested, and two experimental runs, early vs. late winter). Seeds were feed to 3 shovelers *Anas clypeata* kept in captivity (150 seeds each), although undamaged seeds were only recovered from two of them in the second experimental run (late winter). Droppings produced during the 24 hours after feeding were collected and sieved to separate undamaged seeds. Control and ingested seeds were stored wet in the refrigerator until the day before planting (within one week after retrieval). A random subsample of the 200 control seeds originally selected for each experimental run was planted, in order to have comparable numbers of duck-ingested and control seeds.

Each seed was planted in a separate plastic pot (1 L volume, 11.5 cm upper diameter), filled with a mixture of potting clay and sand (1:3 by dry weight) covered with 1-2 cm of washed aquarium sand.   Pots were randomly interspersed inside five (three for the early winter run, and two for the late winter run) separate containers (of 550 L volume and 110 cm length x 95 cm

width x 65 cm height, with 60 pots per container) filled with groundwater from a local sandy aquifer that discharges into the Doñana marshes. Each container was partially shaded by roofing and covered with 35% neutral density shading net (® Agralin-N35) to reproduce shading conditions typical of the shallow lakes inhabited by *P. pectinatus* and to limit the amount of debris and insects falling into the water.

Seeds were set to germinate on 10 November 2000 (early winter run) and 29 march 2001 (late winter run) and the resulting plants were cultivated until 1 October 2001. At harvest, sediment was washed off using pressurised water and plants and tubers collected on a 2 mm sieve. We first measured the length of the longest shoot and number of ramets per plant. Above-ground (shoots) and below-ground (roots+rhizomes) parts were then separated and their biomass measured (dry weight, after drying at 60 °C for at least 48 hours). Tubers were weighted individually, and the number of tubers, flowers and seeds counted. Plant biomass was determined as the sum of the dry mass of shoots, roots+rhizomes, flowers, seeds and tubers.

Statistical analyses

Differences in germinability between duck-ingested and control seeds were tested with a GLM mixed model. Models were fitted using Restricted Maximum Likelihood with binomial errors for presence/absence response variables and normal errors for continuous response variables. A two-level, fixed-effect factor (duck ingested versus control seeds) and one random factor (water tank) were included as independent variables in the model. To produce normal distributions, the square root of the number of shoots and the logarithm of shoot length were used in the analyses. The relative investment in above- and below-ground dry mass was estimated as the residuals of a Reduced Major Axis (RMA) regression between shoot mass and roots+rhizomes mass. RMA instead of OLS regression was used because both the dependent and independent variables were measured with error (see Green 2001 for a discussion of the use of Model II residuals in ecology). Differences between treatments in the number of sexual and asexual

structures were assessed using Wilcoxon signed-rank tests, because data were not normally distributed and the reduced sample size did not allow control of the effects of replicate tanks. Differences in survival between both experiments were tested with Chi-square with the Yates correction for continuity.

Results

Effects of ingestion on germinability

Germinability was significantly greater for duck digested seeds than for controls in the early winter run (χ^2=15.21, 1 df, p<0.0001), but no significant differences were detected in the late winter run (χ^2=0.84, 1 df, p=0.36, Figure 1). In each run, the great majority of seeds germinated within the month following planting (95% and 100% for early and late winter). Mortality was very low, but significantly higher in early winter (8%) than late winter (0%, χ^2=5.80, 1 df, p=0.02). The five plants that died were equally distributed among treatments (3 controls and 2 duck digested seeds, χ^2=0.71, 1 df, p=0.40).

Effects of ingestion on development

No significant differences between duck-ingestion treatments were found in the number of ramets per plant (early winter, $F_{1,49}$=0.67, p=0.42; late winter, $F_{1,93}$=0.09, p=0.76; Table 1), in maximum shoot length (early winter, $F_{1,49}$=3.77, p=0.06; late winter, $F_{1,93}$=0.91, p=0.34), in the length of the first internode (early winter, $F_{1,49}$=0.34, p=0.56; late winter, $F_{1,93}$=0.97, p=0.33), in shoot mass (early winter, $F_{1,49}$=0.10, p=0.75; late winter, $F_{1,93}$=0.05, p=0.83), in the mass of the roots+rhizomes (early winter, $F_{1,49}$=0.16, p=0.69; late winter, $F_{1,93}$=0.46, p=0.50), in total biomass (early winter, $F_{1,49}$=0.11, p=0.74; late winter, $F_{1,93}$=0.38, p=0.54) or in shoot-to-root allocation (early winter, $F_{1,49}$=0.02, p=0.89; late winter, $F_{1,93}$=0.35, p=0.56).

Effects of ingestion on sexual and asexual reproduction

No significant differences between duck-ingestion and control treatments were found in the number of tubers produced per plant (early winter, $Z=1.03$, $p=0.30$; late winter, $Z=0.18$, $p=0.86$; Table 1), in the proportion of plants producing tubers (early winter, $\chi^2=0.01$, 1 df, $p=0.96$; late winter, $\chi^2=1.39$, 1 df, $p=0.24$), in flower and seed production per plant (early winter, $Z=0.00$, $p=1.00$; late winter, $Z=1.31$, $p=0.19$), in the proportion of plants producing flowers (early winter, $\chi^2=0.48$, 1 df, $p=0.49$; late winter, $\chi^2=0.16$, 1 df, $p=0.69$) or in the proportion of plants producing reproductive organs (sexual + asexual; early winter, $\chi^2=0.38$, 1 df, $p=0.54$; late winter, $\chi^2=0.54$, 1 df, $p=0.46$).

Discussion

The ingestion of seeds by vertebrates is an important process affecting the distribution, structure and composition of plant communities (see Fenner 2000). In our experiment, ingestion by ducks had a positive, short-term effect on the germinability of seeds. However, such effect was apparent only if seeds were ingested at early winter and disappeared for seeds ingested at late winter. Apparently, gut passage can break the dormancy of pondweed seeds (see also Lohammar 1954, Smits et al. 1989), and is thus equivalent to the effects of winter stratification (simulated here through a three-months storage at 5 °C). Such possible interaction between gut passage and other dormancy-breaking processes (such as stratification or drought, Probert 2000, Murdoch and Ellis 2000) can explain the great diversity of results obtained when analysing the effects of gut passage on seed germination patterns (see Traveset 1998).

It is important to note, however, that the population chosen for our study does not naturally undergo periods of winter stratification as severe as those imposed by our seed storage conditions previous to the second (late-winter) experimental run. This is clearly indicated by the lower germinability of the control seeds from early winter (which were exposed to natural conditions in the field) as compared with the late winter controls, and by the high

germination rate of control seeds from early winter (95% germinated during the first month, namely November). If winter stratification would contribute to faster or enhanced germination, it would have resulted in either a slower germination rate (i.e. in progressive germination through the whole winter) or in a second peak of germination in early spring or early winter planted seeds. Seed storage at low temperature was necessary to prevent seed germination before the late-winter experiment was carried out, but its use means that the germination rates in late winter are not representative of actual field conditions (mean field air temperature was superior to 11 ^{0}C during all the experiment). Our interest in using the late-winter ingestion run was to evaluate whether there were fitness costs or benefits that followed from germinating in early (as opposed to late) winter (i.e. posterior to the germination events), and separating them experimentally from the potential costs of undergoing duck gut passage.

If winter temperatures in the field are not low enough to ensure dormancy breaking via seed stratification, enhanced seed germination following gut passage represents a genuine enhancement of fitness. Even if dormant seeds enter the seed bank and suffer insignificant mortality there, a delay in establishment of at least one growth season is most likely to represent a serious handicap for such dormant seeds, particularly in populations likely to be exposed to high predation of seeds by waterfowl (e.g. Van Eerden, 1990; Green et al. 2002). Perennation of plants through tubers, which have a higher competitive ability than seeds owning to their higher germinability and much larger reserve storage (Van Wijk, 1989; Spencer and Ksander, 1995), could enhance the fitness advantage of early germinating seeds. However, seed banks represent an insurance against habitat instability (Thompson 2000) and consequently all aspects of habitat stability and seed bank dynamics should be considered before concluding that germination after duck digestion is a better option than dormancy.

Despite the important effect of duck ingestion on early-winter germinability, we failed to found any subsequent effect of pondweed fitness. Plants grown from ingested and non-ingested seeds showed comparable seedling survival, dry-mass accumulation, shoot-to-root allocation, clonal

multiplication and sexual reproduction. We must thus conclude that, under the controlled conditions of our experiment (i.e. in the absence of herbivory pressure and intra- or inter- specific competition), enhancement of germinability represents the only significant effect of gut passage on the long term fitness of seedlings.

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Table 1. Characteristics of the plants originating from duck ingested and control seeds. Mean±s.e. and sample size (in brackets) are reported for original data.

	Early winter		Late winter	
	Control	Duck ingested	Control	Duck ingested
Shoot number	18.5±1.1 (18)	17.2±0.9 (36)	19.6±1.0 (50)	20.5±1.0 (46)
Maximum shoot length	57.35±2.52 (18)	54.30±1.76 (36)	56.05±1.04 (50)	54.68±1.07 (46)
Internode length	40.1±2.9 (18)	43.4±2.3 (36)	41.8±1.6 (50)	43.7±1.5 (46)
Shoot mass	0.920±0.079 (18)	0.848±0.052 (35)	0.930±0.049 (50)	1.002±0.055 (46)
Roots+rizome mass	0.480±0.049 (18)	0.436±0.034 (36)	0.408±0.021 (50)	0.443±0.023 (46)
Plant mass	1.403±0.123 (18)	1.284±0.081 (35)	1.340±0.065 (50)	1.455±0.075 (46)
Number flowers+seeds	2.0±1.0 (3)	1.7±0.7 (3)	3.2±1.0 (5)	4.1±3.0 (7)
Number of tubers	2 (1)	1.3±0.2 (7)	1.3±0.3 (3)	1.3±0.3 (6)
% germinating	21.8 (101)	50.7 (75)	83.3 (60)	76.7 (60)
% flowering	16.7 (18)	8.3 (36)	10.0 (50)	15.2 (46)
% tuberizing	5.6 (18)	19.4 (36)	6.0 (50)	13.0 (46)

Figure 1. Proportion of seeds germinating (+s.e.) for fennel pondweed seeds planted in early and late winter.
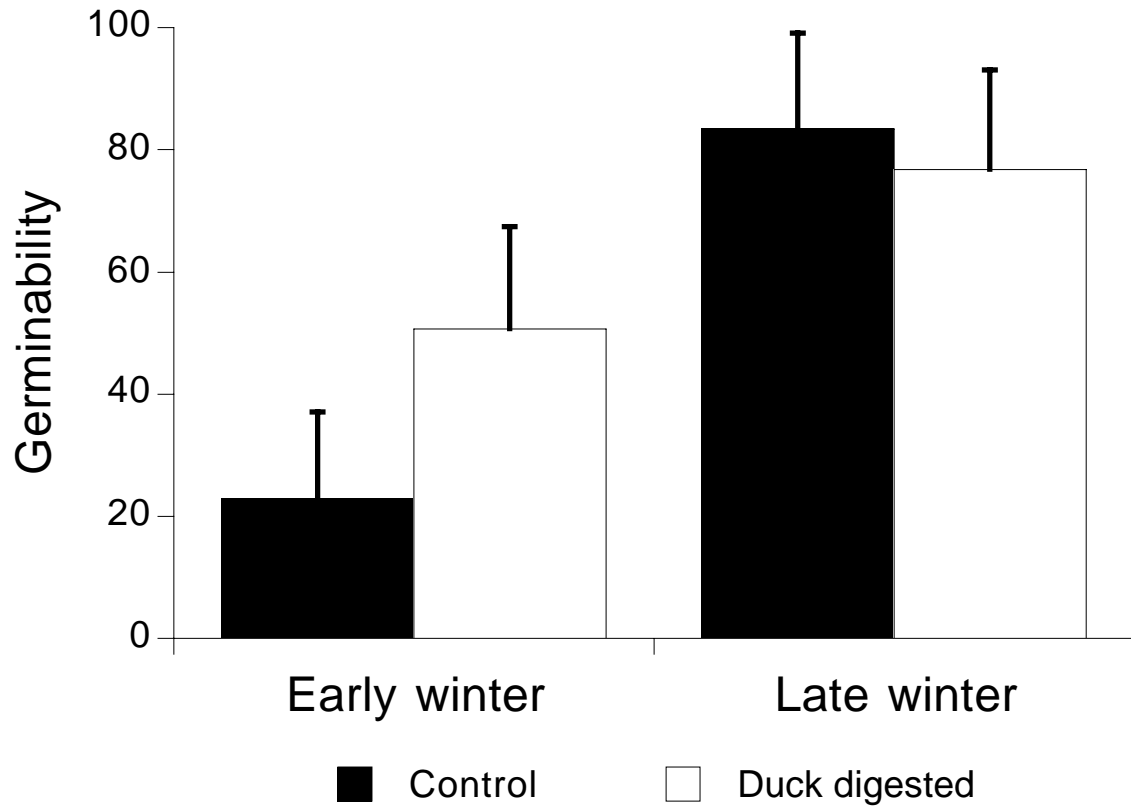
Effects of seed ingestion and herbivory by waterfowl on seedling establishment: a field experiment with wigeongrass *Ruppia maritima* in Doñana, south-west Spain

JORDI FIGUEROLA AND ANDY GREEN

Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. María Luísa s/n, 41013 Sevilla, Spain. E-mail: jordi@ebd.csic.es

Abstract

The ingestion of seeds by vertebrates usually affects the viability and/or germination rate of seeds. Increases in germination rate following passage through the vertebrate gut have often been assumed to be favourable for seedling survival and plant fitness, but this assumption has never been tested experimentally. Given that numbers of herbivorous waterfowl are higher in winter in Mediterranean wetlands, herbivory pressure there will be higher for early growing plants. In a factorial experiment we investigated the effects of seed ingestion by ducks (shoveler, *Anas clypeata*) on the survival of wigeongrass *Ruppia maritima* seedlings in the field in Doñana (south-west Spain), under differing exposures to herbivory by waterfowl and fish. We planted ingested and non-ingested seeds in December, using exclosures to protect half of the them from herbivores. The number of plants established from ingested and non ingested seeds was similar inside exclosures, but fewer plants survived until June-July from ingested than from non-ingested seeds when exposed to natural levels of herbivory. In conclusion, increases in germination rate after ingestion are not necessarily beneficial for the plant, and the final outcome depends on complex interactions with other factors such as herbivore abundance.

Introduction

The dispersal of seeds is an important ecological process and the effects of ingestion by vertebrates on the capacity of seeds to germinate have been studied in a large number of systems. Ingestion by vertebrates usually affects the germinability of seeds (i.e. proportion of seeds that germinate), or the rate of germination (i.e. inverse of the time between seeding and start of germination, see review by Traveset 1998). Traditionally, faster germination after ingestion has been considered as intrinsically beneficial for the plant (but see discussion in Traveset 1998). This assumption is partly supported by the high growth rate and higher survival of seedlings germinating earlier (Zimmerman & Weis 1984, Waller 1985, Bush & Van Auken 1991, Seiwa 1998). However, these relationships between early germination and fitness have been deduced from natural variation in germination times of non-ingested seeds. Early germination can also result in different risks due to adverse climatic conditions or a higher probability of predation or pathogen attack as reported for some plant species (Jones & Sharitz 1989, Traveset 1990). However, to the best of our knowledge, no field data are available on the success of ingested seeds in comparison to non ingested seeds in any system.

The final results of such opposing processes on germination phenology is likely to depend on the characteristics of each species and the conditions available for establishment in a given area. Field experiments are necessary to determine the ultimate consequences for plant fitness of seed ingestion by vertebrates in terms e.g. of seedling survival or reproductive success. One potential source of variability is the abundance of herbivores. For example, herbivory pressure has been reported to affect the interactions between plants and their pollinators (Herrera 2000), change the optimal flowering phenology (Pilson 2000), and determine the structure and diversity of plant communities (Huntly 1991). In the case of submerged plants in temperate or Mediterranean wetlands, the advancement of germination time may expose the seedlings to extensive feeding by concentrations of wintering

waterfowl, which feed extensively on macrophytes (Lodge et al. 1992, Idestam-Almquist 1998).

Wigeongrass *Ruppia maritima* (L.) is an aquatic angiosperm that inhabits brackish coastal and inland saline waters with a nearly cosmopolitan distribution (Verhoeven 1979). Mechanisms of dispersal of *Ruppia* spp. are not well understood, but their seeds and green parts are important waterfowl foods (Gaevskaya 1966, Cramp & Simmons 1977). Agami & Waisel (1988) demonstrated that seeds can resist passage through the gut of fish. In the same Mediterranean wetland used in the current study, Figuerola et al. (2001) reported the presence of undamaged seeds of *Ruppia maritima* in 22.9% and 35.8% of the waterfowl droppings examined in early and late winter respectively. As for many other plant species, the rate at which seeds of *Ruppia maritima* germinated over time increased for duck ingested seeds as compared to control (non-ingested) seeds (Figuerola et al. submitted). The proportion of seeds germinating after ingestion by shoveler *Anas clypeata* was the same as for control seeds (Figuerola et al. submitted).

In this paper we study the consequences of *Ruppia maritima* seed ingestion by ducks in a field experiment with two different levels of herbivore pressure. We test two hypotheses, firstly, that ingestion by vertebrates enhances the establishment of seedlings, and secondly that the presence of herbivores early in the growing season can reduce (or reverse) the benefits of ingestion by vertebrates, by decreasing seedling survival.

Methods

The study was performed in 'Veta la Palma' (36^0 57' N, 6^0 14' W), a brackish marsh in Doñana, south-west Spain. The area is largely managed for fish farming with 37 ponds of rectangular shape and ca. 100 ha surface area (total surface 3,125 ha), and also includes 4,442 ha of untransformed temporary marshes. Salinity ranged from 10 to 17 g/l at the time of study. The ponds contain estuarine fish such as *Dicentrarchus labrax*, *Mugil cephalus* and *Sparus auratus* at unknown densities. The area is very important for waterfowl (*sensu* Rose & Scott 1997: ducks, coots, waders, gulls, herons,

flamingos, spoonbills, etc.) and in many winters most of the ducks in Doñana concentrate in these ponds due to the scarcity of natural, seasonal habitats. Waterfowl in Veta la Palma were counted monthly via ground surveys as part of Doñana Biological Station's monitoring program (see Fig. 1 for details).
In September 1999, *Ruppia maritima* seeds were collected from the shores of several ponds in Veta la Palma. The seeds were separated from debris and stored dry in plastic vials in the refrigerator. In December 1999, seeds were force-fed to three captive shovelers (*Anas clypeata*) and the droppings were collected the next day. Immediately after collection, droppings were sieved (mesh-size: 0.5 mm) and groups of 10 whole seeds were extracted and stored dry in separate Eppendorf tubes in the refrigerator for less than a week until planting in the field. Three such feeding sessions were conducted on successive days to obtain the 240 duck ingested seeds necessary for the experiment.

The effects of gut passage and herbivores on seed establishment were studied simultaneously by means of a factorial experiment. On 12 December 1999, seeds were planted in plastic pots with ten in each pot, and four pots in each replicate block. The pots were filled up to five cm from the top with sediment from one pond in the study area and passed through an autoclave for 30 minutes to kill any seeds present in the sediment.   *Ruppia* seeds were then randomly distributed on the pot surface and pushed one cm below the sediment surface. The pots were partially buried in the sediments on the bottom of the ponds, but the upper five cm were left rising above the sediment surface to protect the content of the pots from water currents. The pots within a replicate block were distributed on the four corners of an imaginary square of 50 cm side. Two of the pots contained duck ingested seeds and the other two non-ingested control seeds. The no herbivore treatment consisted of protecting one control and one experimental pot within each block with a wire mesh cage (1x0.5 m, mesh size 1 cm). These exclosures were high enough to reach the water surface. The herbivore treatment consisted of the two pots outside the exclosure. Thus each replicate block had four pots with two inside an exclosure. Six replicate blocks (i.e. six exclosures and 24 pots) were used

in each of two ponds, randomly changing the spatial distribution of the treatments in each block.

The two levels of factors led to the following four treatments: 1) control seeds exposed to herbivores, 2) duck ingested seeds exposed to herbivores, 3) control seeds protected from herbivores and 4) ingested seeds protected from herbivores.

The samples from the first pond were collected on 3 June 2000. Samples in the second pond were collected on 17 July. The plants were well grown, and had reached the water surface, but had not completed seed production (which spans from June to September in La Camargue at 46^0N, Verhoeven 1979). The presence and number of plants in the pots was scored. Germination could not be monitored during the experiment since high water turbidity made impossible to check the pots without disturbing the experiment. Our intention was to measure biomass of each sample, but most of the samples were lost during a fire in the laboratory.

Statistical analyses

The significance of the effects of herbivores and duck digestion was assessed by fitting a General Linear Model with repeated measures to the data. Since the count data were highly right skewed due to the abundance of zeros (pots with no plants growing), a negative binomial error structure and a log link function were used in the model (Crawley 1993). A repeated SUBJECT effect was included in the model to clump the data from the four treatments within each independent replicate (see Stokes et al. 1995, SAS Institute 1996). Replicates were nested within a pond effect to control for the different collection date and conditions in the two ponds. Main effects (duck ingestion and herbivory treatments) and the two-way interaction were fitted using type 3 sum of squares, and tested using chi-square distribution (SAS Institute 2000). All calculations were done with SAS version 8.2 (SAS Institute 2000). Overall the design used was similar to an ANOVA design with randomised blocks, but the use of GLM was necessary because the count data did not fit the normality assumption of traditional ANOVA methods (Crawley 1993).

Results

In June-July, a significant effect of duck digestion was detected on the number of plants growing in the pots, with less plants growing in pots with ingested seeds (χ^2=4.05, p=0.04). Although the herbivore exclusion treatment did not have a significant effect on the number of plants growing (χ^2=0.16, p=0.69), the herbivore and digestion treatment showed a significant interaction (χ^2=4.22, p=0.04). This interaction was due to a lower number of plants growing in the duck ingested pots, but only when exposed to herbivores (χ^2=12.30, p=0.0005, see Fig. 2). Control and duck ingested seeds had the same survival rate when protected from herbivory (χ^2=0.00, p=1.00). The number of plants growing in control pots was not significantly affected by the herbivore treatment (χ^2=1.42, p=0.23), but for duck ingested seeds there was a tendency for more plants to occur when protected from herbivores (χ^2=3.75, p=0.05).

Discussion

When *Ruppia maritima* plants were exposed to natural levels of herbivore presence (albeit in an artificial marsh), duck ingested seeds survived worse and produced fewer recruits in the population than non ingested seeds. However, under reduced herbivore grazing, both ingested and non-ingested seeds produced the same number of plants. We suggest that this difference was due to the increase in germination speed of seeds of *Ruppia maritima* ingested by ducks, already demonstrated by us (Figuerola et al. submitted). This early germination of ingested seeds exposes seedlings for more days to herbivory during the winter period of higher waterfowl presence (see Fig. 1), when water temperatures and *Ruppia* growth rates are relatively low (Verhoeven 1979). Seeds germinating later in the season suffer a lower risk of predation because of the shorter time overlap of plant growth with high waterfowl presence. By the end of January and beginning of February, most herbivorous waterfowl in Doñana start to migrate to breeding areas in

northern Europe (Figure 1, Scott & Rose 1996). Ingested seeds protected from herbivores in exclosures were not exposed to such predation risk due to early germination and showed a similar survival to control seeds. This suggests that, in this study, the influence of early germination on plant establishment was unrelated to climatic effects, and that the differences in the survival of plants from ingested and non ingested seeds were not just an artefact of differences in the number of seeds germinating for each treatment.

Herbivory commonly affects the hierarchies in plant communities (Huntly 1991), and our study suggests that this effect can extend to an intraspecific level by reducing the possibilities of establishment of seeds ingested by waterfowl. Thus, benefits of seed ingestion by waterfowl could be derived from escape (deposition of the seeds away from the mother plant), directional (transport to favourable areas for germination) and colonisation (transport of the seeds to new localities) effects of seed dispersal (Howe & Smallwood 1982, Figuerola & Green 2002). However, these benefits will be countered by costs derived from exposure to higher herbivore pressure under early germination.

Asynchronous germination can be favoured in environments with unpredictable climatic conditions (Harper 1977), as is the case in seasonal Mediterranean wetlands that tend to have unpredictable flooding cycles (Pearce & Crivelli 1994). The changes in the patterns of germination associated with seed ingestion by vertebrates result in a diversification of the germination patterns of seeds. In dry years it is possible that plants germinating from ingested seeds have a higher fitness than   non ingested seeds because only early growing plants are able to complete the seed production cycle. Furthermore, spatial variation in the density of herbivores is likely to translate into differing effects of the observed interaction between germination rate and herbivory. In our study site, another exclosure experiment in natural *Ruppia* beds has shown that grazing by waterfowl has a strong effect on *Ruppia* biomass and survival (authors, unpublished data). However, similar experiments on coastal marshes in Louisiana found that the effects of waterfowl on *Ruppia* were undetectable (Hunter 2000). In such low herbivory conditions, early germination might provide benefits via competition

for light and space, as has been demonstrated in a number of different plant species (e.g. Garwood 1983, Jones & Sharitz 1989). In addition, other factors such as salinity, temperature and turbidity can influence *Ruppia* growth (Verhoeven 1979, Santamaría et al. 1996), and may in turn influence the consequences of the herbivory-duck ingestion interaction.

In conclusion, increases in germination rate after seed ingestion by waterfowl are not necessarily beneficial. Among other potential sources of variation, herbivore abundance is a clear factor affecting the consequences of seed ingestion for plant fitness.

Acknowledgements

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Figure 1. Results of ground waterfowl counts in 'Veta la Palma' from December 1999 to August 2000. Filled circles show the total number of waterfowl (*sensu* Rose & Scott 1997: ducks, coots, waders, gulls, herons, flamingos, spoonbills, etc.) and open circles to the number of ducks and coot. Source: Equipo de Seguimiento de Procesos Naturales, Estación Biológica de Doñana.
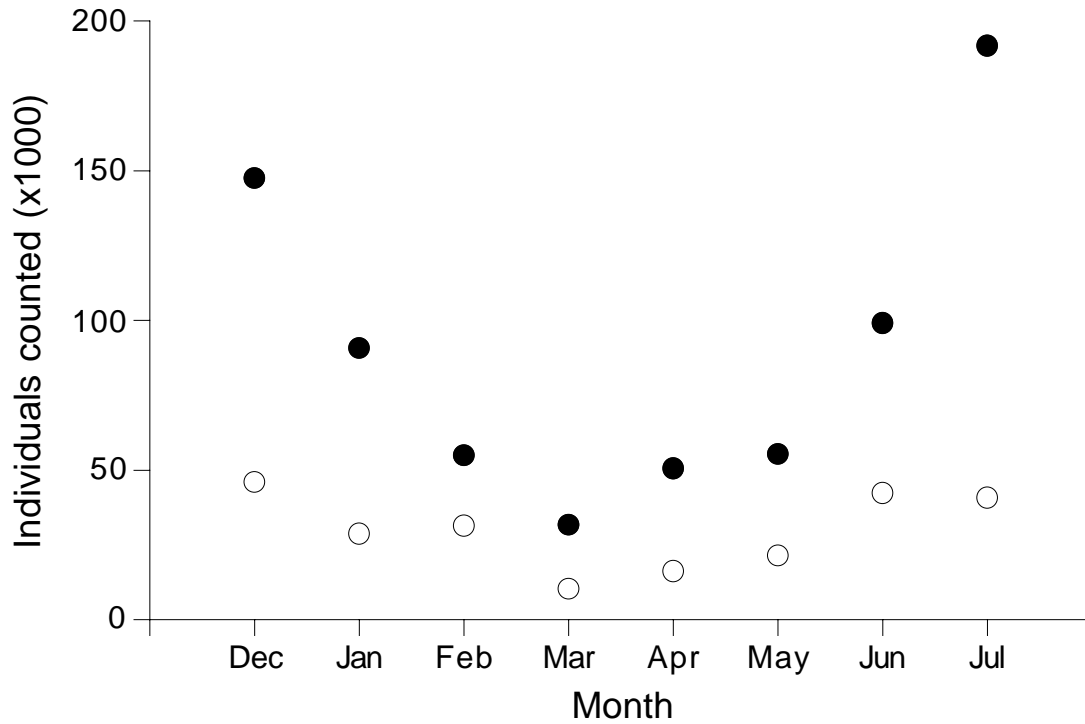
Figure 2. a) Mean number of *Ruppia* plants growing for each treatment (12 replicates per treatment were used). Error bars correspond to the standard errors. Treatments correspond to seeds that have been ingested and defecated by *Anas clypeata*, or to control non-ingested seeds. These seeds were planted inside exclosures protecting the plants from herbivory or exposed to natural levels of herbivory by waterfowl and fish. b) Mean number of plants growing for each treatment (and standard errors), after controlling for block differences in a GLM with a negative binomial error distribution and a log link (see Crawley 1993).

a)

b)
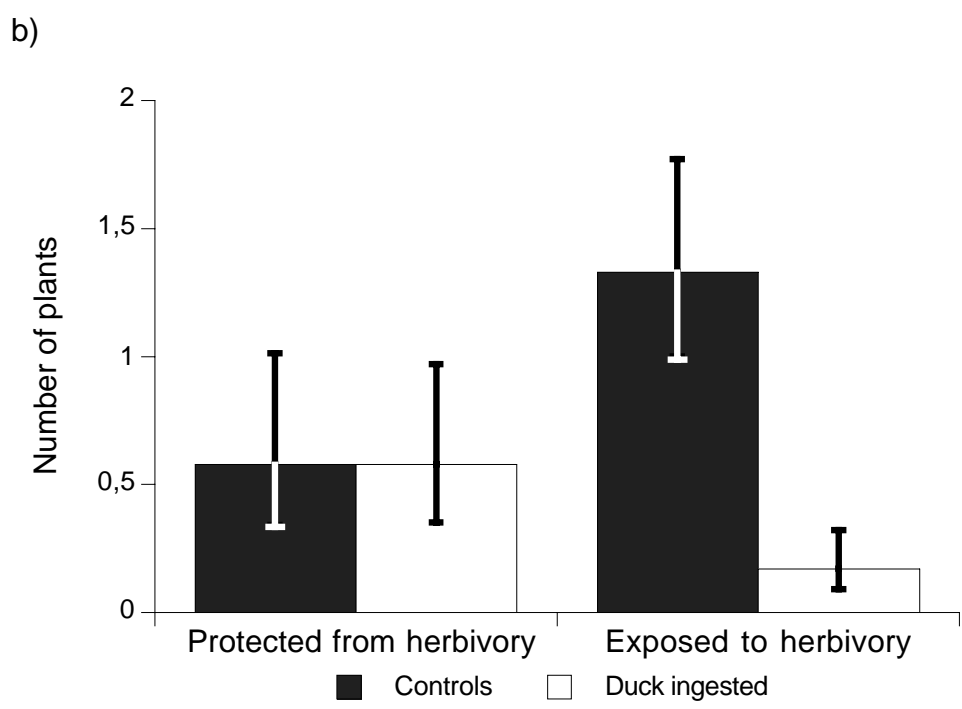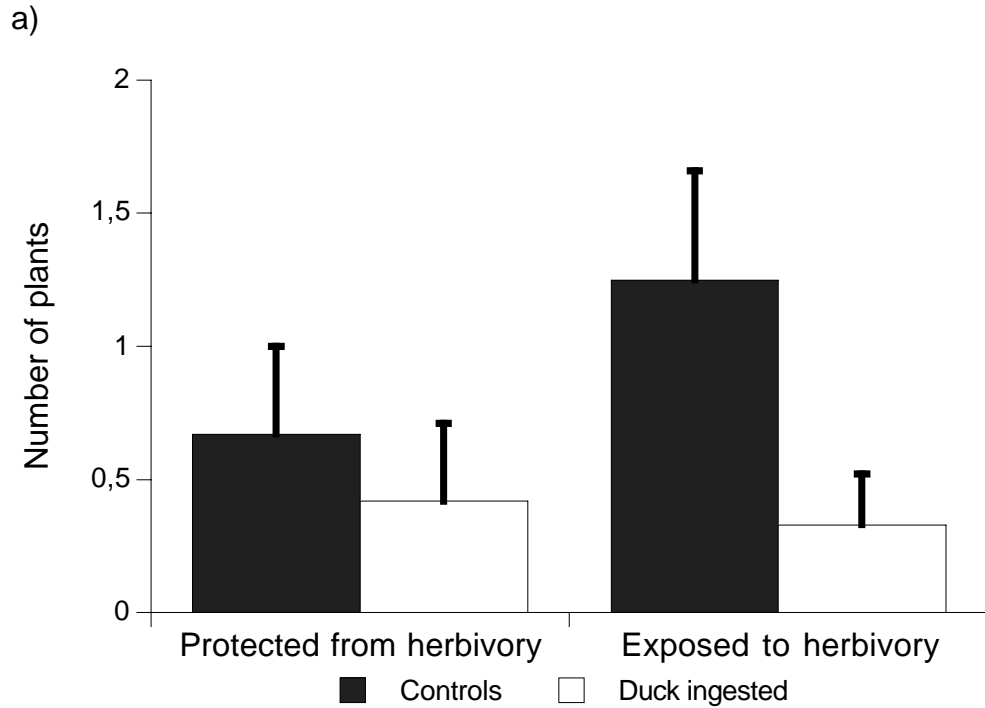
How far can the freshwater bryozoan *Cristatella mucedo* disperse in duck guts?

Iris C. Charalambidou[1], Luis Santamaria[1] and **Jordi Figuerola[2]**

With 1 figure and 1 table

[1] **Authors' addresses:** Netherlands Institute of Ecology, Centre for Limnology, PO Box 1299, 3600 BG Maarssen, The Netherlands. E-mail: chara@cl.nioo.knaw.nl.

[2] Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. de María Luisa s/n, E 41013 Sevilla, Spain.

Submitted

Abstract:

Statoblasts of *Cristatella mucedo* were fed to two duck species, shoveler (*Anas clypeata*) and pintail (*A. acuta*), to assess whether endozoochorous dispersal may contribute to the metapopulation structure revealed by population genetic studies of this bryozoan. Eight individuals (four per duck species) were force-fed 500 statoblasts each. The number of intact statoblasts retrieved from the duck droppings up to 48 hours after ingestion and their retention times (i.e. the amount of time from ingestion to excretion) were recorded. Retrieval of intact statoblasts did not differ significantly between duck species (37 ± 29 % for pintail and 13 ± 21 % for shoveler, average ± SE) and the pattern of retrieval was identical. Most statoblasts (79-96% and 51-96% of those retrieved, from pintail and shoveler respectively) were recovered during the first four hours after ingestion. Maximum retention times were 32 hours for shoveler and 44 hours for pintail. A few statoblasts retrieved two hours following gut passage germinated, but none in the control group did. We provide evidence that the potential for waterfowl dispersal of *C. mucedo* statoblasts is much higher for short distances of up to 300 kilometres, but still possible over longer distances.

Introduction

The dormant stages of aquatic organisms have been considered as a means to disperse long distances in the digestive tract (endozoochory) or stuck to the plumage and feet of birds (epizoochory) (FIGUEROLA & GREEN 2002). *Cristatella mucedo* Cuvier 1798 (Phylum Bryozoa, Class Phylactolaemata) is a freshwater bryozoan distributed throughout the Holarctic. Overwintering occurs as statoblasts which are small (<1 mm), multicellular, amictic dormant stages (OKAMURA 1997) with highly resistant chitinous valves (FREELAND et al. 2000). Microsatellite studies of populations of *C. mucedo* collected along a major waterfowl migratory route in North West Europe provide evidence for a large scale metapopulation structure (FREELAND et al. 2000). Transport of statoblasts by waterfowl has been proposed to determine the metapopulation dynamics within this region.

Bryozoan statoblasts have been identified as food items in the gizzards of a number of duck species including pintail (*Anas acuta*) and shoveler (*Anas clypeata*) (ANDERSON 1959), while BROWN (1933) demonstrated that statoblasts of some bryozoa can germinate after ingestion by mallard (*Anas platyrhynchos*). Although survival to gut passage indicates that endozoochorous dispersal is possible, detailed information on the rates of excretion over time is necessary to conclude that long distance dispersal is likely to occur. Nevertheless, ecological studies addressing and quantifying animal-mediated dispersal of freshwater invertebrates are rare (BOHONAK & WHITEMAN 1999). Moreover, experimental work with captive waterbirds has usually provided basic and non-conclusive data. For example, only two studies cite the numbers of invertebrate dormant stages ingested and excreted by the birds (BROWN 1933, MELLORS 1975). In these, a low number was administered (BROWN 1933, MELLORS 1975), the methodology of retrieval from droppings was not accurate (BROWN 1933) and the number of experimental animals was extremely low ($N = 1$ and 2, BROWN 1933, $N = 1$, MELLORS 1975). Detailed information on the rates of excretion over time is also lacking although some basic data have been collected for the brine shrimp (*Artemia salina*) (PROCTOR et al. 1967, MACDONALD 1980). Furthermore, few water bird species have been utilized

in such studies (MELLORS 1975, MACDONALD 1980) of which only one duck species, the mallard (e.g. BROWN 1933).

In this study we fed statoblasts of C. *mucedo* to pintail and shoveler, two species specialized in different diets: shoveler feed mainly on zooplankton, while pintail consume a wide variety of plant and animal materials. In autumn and winter plant materials dominate pintail diets (CRAMP & SIMMONS 1977). We then retrieved the intact statoblasts excreted in the ducks' droppings. Our objectives were: 1. to estimate the proportion of intact statoblasts retrieved from the droppings and their retention time (from ingestion to excretion), and establish whether they are consistent with long-distance dispersal and, 2. to compare the retrieval and retention time for two waterfowl species, pintail and shoveler, that greatly differ in their feeding habits.

Materials and methods

C. mucedo statoblasts were collected from lakes in Southern England (Hinksey Lake, Oxfordshire; Tufty's Corner and White Swan Lake, Berkshire) in autumn 1998. Aliquots of 50 statoblasts were placed in 1 .5 ml Eppendorf tubes filled with distilled water and stored frozen at –20 °C throughout winter. One day before the experiment they were left to thaw in a refrigerator (temperature: 4 °C) and on the day of the experiment were kept at room temperature (20 °C) for 3 to 4 hours.

Four pintail and four shoveler, all female one year-old birds born in captivity, were used in this study. Prior to the experiment they were housed in outdoor facilities and fed on a stable diet of commercial pellets (Anseres 3 @ Kasper Faunafood) and mixed grain (HAVENS Voeders) for one year. Although they had access to the same diet the shoveler preferentially consumed more pellets, which are animal based, while the pintail tended to favor the seed based mixed grain diet. During the experiment (18 to 20 April 1999), they were kept individually in wooden cages (0.60 m x 0.50 m x 0.50 m) with a mesh floor (mesh size 12 mm) and removable plastic trays placed

under each cage. Birds were placed in the cages the night before the experiment. Food pellets and water were available   ad libitum .

Each duck was force-fed with 500 statoblasts. Force-feeding was necessary to ensure that an exact amount and time of ingestion were known. To facilitate force-feeding the statoblasts were placed inside small pellets made with Anseres soaked in water. Each pellet contained 100 statoblasts. Duck faeces were collected in the removable trays every four hours up to 48 hours after ingestion. They were immediately sieved (sieve size 150 µm) and intact statoblasts were retrieved, counted, placed in tap water in closed plastic containers and stored at 4 °C in a refrigerator. The experiment was terminated when no intact statoblasts were retrieved from any of the experimental animals after one collection period of four hours. The efficiency of the retrieval of statoblasts from the droppings was initially tested with 0   .5 µm plastic beads that were fed and retrieved from mallards, which resulted in recoveries of 90-95% of the plastic beads.

We assessed germination of the retrieved (N = 736 and 265, for pintail and shoveler, respectively) and of non-ingested (controls, N = 100) statoblasts. All statoblasts were positioned in microtitre plastic trays. Each cell was filled half way with distilled water and individual samples were placed in separate cells with a maximum number of 5 statoblasts per cell. On 6 May 1999 the trays were positioned in a climate room set at 15 hours light / 9 hours dark and 15 °C (simulating the light/temperature conditions of mid spring in Southern England and Central Europe). Germination was checked every two days and was scored when an ancestrula was observed. The germination trial was terminated on 31 August 1999.

The number of statoblasts retrieved from the droppings was analysed with Generalized Linear Models, using binomial error distribution and a logit link function. 'Duck species' was introduced as a fixed factor and 'duck individual' as a random factor. The analysis was performed using the GLIMMIX procedure in SAS (SAS INSTITUTE INC. 1996). Differences in the retention time of statoblasts between the two duck species were tested by fitting a Cox proportional hazards regression model (e.g. ALLISON 1995) to data consisting of the number of hours between ingestion of the statoblasts

and their recovery in droppings. As in the previous analysis, species was introduced as a fixed effect, and to account for the effects of digestion by different duck individuals a replicate effect was added to the model as a random, or 'failtry' effect. Ties were managed using the exact method, and survival analysis was computed using S-Plus 2000 (MATHSOFT 1999).

Results

The model used to analyse the number of statoblasts retrieved from droppings fitted the data well (Deviance = 3587, df = 3998, P > 0.9) and no evidence of over- or under-dispersion was found (ϕ=0.99). The proportion of retrieved statoblasts did not differ significantly between species ($F_{1,3}$ = 2.55, P = 0.21, Table 1). Average retrieval estimated by the GLIMMIX procedure was 37 ± 29 % and 13 ± 21 % for pintail and shoveler, respectively (average   ± standard error, following back-transformation from the logit link and application of the delta method, respectively ; SAS INSTITUTE INC. 1996, 1997).

Most statoblasts were retrieved within the first 4 hours after ingestion (79-96% and 51-96% of retrieved statoblasts, for pintail and shoveler, respectively), less between 4 and 8 hours (pintail = 3-16 % and shoveler = 4-49 %) and very few after 8 hours (pintail = 0.3-10% and shoveler = 0-12%) (Fig. 1). The probability of retrieval of intact statoblasts over retention time did not significantly differ between pintail and shoveler (Cox's Regression Model, R^2 = 0.001, χ^2 = 1.42, df = 1, P = 0.23). Maximum retention times recorded were 44 hours for pintail and 32 hours for shoveler (Table 1).

Only three statoblasts germinated (two retrieved from pintail and one from shoveler faeces). They were all obtained from faeces collected two hours after ingestion. None of the controls germinated (Table 1).

Discussion

In this study we provide evidence that the pintail and shoveler, and probably other duck species, are likely dispersal agents of *C. mucedo.* However, our results indicate a sharp distinction between the possibilities for short and long distance dispersal. In order to obtain reliable estimates of the

recovery rate of intact statoblasts from the droppings and their viability after gut passage, we fed a high number of statoblasts (500) to each duck. Although we recovered a fairly high proportion of statoblasts (13 to 37% on average), most were recovered by 4 hours and only a very small fraction (<12%) was recovered after 8 hours following ingestion (Fig. 1). Maximum records of 44 and 32 hours of retention time for pintail and shoveler, respectively imply that few statoblasts also remain intact for longer retention times. These results would indicate that since the majority of statoblasts are retrieved at retention times up to 4 hours and only small numbers at later stages, they would stand much higher chances of being dispersed over short rather than longer distances. Since the flying speed of *Anas* ducks ranges from 60 to 78 km/h (WELHAM 1994), our results would suggest that dispersal of statoblasts after ingestion by ducks is most likely to occur at distances of less than 250 to 300 kilometers, and extremely unlikely (but possible) at distances more than 500 to 600 kilometers.

Similar experiments with the brine shrimp (*Artemia salina*) support this observation. Although the data presented were not complete, PROCTOR et al. (1967) found that the first intact eggs fed to killdeer (*Charadrius vociferous*) were usually retrieved in 5-15 minutes following their ingestion, the peak number was reached at 1 $^1/_2$ hours, a small number was retained as long as 8 hours and a very small proportion were retrieved afterwards (up to 24-26 hours following their ingestion). Similarly, MACDONALD (1980) found that faeces of one shelduck (*Tadorna tadorna*) and one flamingo (*Phoenicopterus ruber roseus*) contained large numbers of cysts until 2 and 3 hours after ingestion respectively, while very low numbers were recovered afterwards (until at least 38 hours later).

Furthermore, we provide the first experimental study on freshwater invertebrates to utilize pintail and shoveler instead of mallard. The average proportion of ingested *C. mucedo* statoblasts retrieved from the droppings of the pintail and the shoveler did not differ significantly between species owing to high individual variation among replicate ducks (measured ranges of statoblast retrieval were 4-70% for pintail and 7-17% for shoveler). On the other hand, the pattern of retrieval of statoblasts over time was nearly

identical in both duck species. This indicates that interspecific differences seem to have limited impact on this aspect of dispersal, similarly to reports for terrestrial plant-disperser systems (TRAVESET 1998).

Only three (0.3%) of the statoblasts recovered after ingestion germinated, while none of the controls did. Few data are accessible for *C. mucedo* that is known to have a low germination success (SMYTH & REYNOLDS 1995), and probably our inability to successfully germinate controls reflect the use of inadequate germination conditions. However, our results show that *C. mucedo* statoblasts are able to germinate following duck gut passage, and although most of them will be dispersed over short to medium distances (<300 km) waterfowl provide a vehicle for less frequent phenomena of transport over long distances (>1000 km).

Acknowledgements

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Table 1. Results of force-feeding and germination experiments. N = number of individual ducks (= replicates) per species. Maximum retention time = maximum time after ingestion at which at least one intact statoblast was recovered from the droppings.

Treatment	Survival to gut passage				Germination	
	N	Ingested	Retrieved (average ± SE)	Maximum retention time (h)	Set to hatch	Hatched
Pintail	4	500	183 ± 70	44	736	2
Shoveler	4	500	66 ± 11	32	265	1
Control					100	0

Figure 1. Percentage and standard error values (± SE) of intact statoblasts of *Cristatella mucedo* retrieved from the droppings of the pintail and shoveler over retention time (i.e. time retained in the gut, from ingestion to excretion). The black bars represent night period collections (from 22:00 to 06:00 hours).
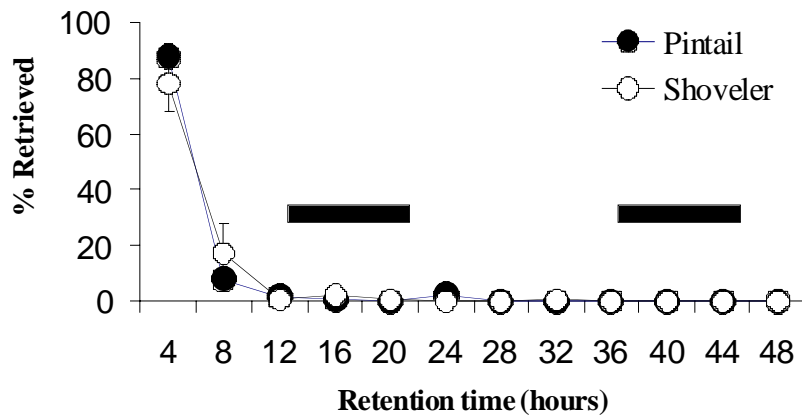
Part 4

Long distance dispersal of aquatic organisms:

The role of waterfowl migration

Effects of premigratory fasting on the potential for long distance dispersal of seeds by waterfowl: an experiment with Marbled Teal

JORDI FIGUEROLA[1] and ANDY J. GREEN [2]

[1]Department of Applied Biology, Estación Biológica de Doñana, Avda. María Luisa s/n. E-41013 Sevilla. E-mail: jordi@ebd.csic.es

[2]Department of Applied Biology, Estación Biológica de Doñana, Avda. María Luisa s/n. E-41013 Sevilla. E-mail: andy@ebd.csic.es

Author for correspondence: Jordi Figuerola, Department of Applied Biology, Estación Biológica de Doñana, Avda. María Luisa s/n. E-41013 Sevilla. E-mail: jordi@ebd.csic.es

Submitted

Abstract

The possible effect of premigratory fasting on the potential for long distance dispersal of seeds by migratory birds was tested experimentally using nine captive Marbled Teal. A mixture of two different species of *Scirpus* seeds (one with larger seeds) and plastic markers of two different sizes (1.0 and 0.5 mm Ø) was force-fed to the birds at the start of the experiment. Five individuals were exposed to 8 hours of food deprivation (fasting phase), followed by a period of 8 hours of food and water deprivation (simulated flight phase). Four control individuals were exposed to the simulated flight phase but not to the previous fasting phase. Whilst the larger seeds and markers were almost absent from the droppings collected after more than 16 hours, a significant number of the smaller seeds and markers were collected. When resuming feeding after the period of simulated flight, birds exposed to periods of premigratory fasting defaecated more seeds of the smaller species and more small markers than control individuals. These results suggest that premigratory fasting can favour the long-distance dispersal of seeds by birds via the retention of propagules in the gut.

KEY WORDS: habitat colonisation, migration, Scirpus litoralis, Scirpus maritimus, seed dispersal

Many aquatic plants and invertebrates lack the capacity to colonise new areas by themselves, but it is striking how they often occupy wide geographic ranges (Good 1953, Raven 1963). Waterfowl (ducks, geese and swans) have been proposed as important agents for the dispersal of aquatic organisms by transporting the propagules adhered to their body, or internally in the digestive tube (Darwin 1859, Ridley 1930). This idea appeared a long time ago in the literature, yet little information is available on the real role of waterfowl as dispersors of aquatic organisms (but see De Vlaming & Proctor 1968). For successful long-distance internal dispersal by waterfowl we can identify three primary stages that must be accomplished. 1) birds should ingest the propagules, 2) the propagules should be retained enough time inside the duck to allow the arrival of the bird to a new area and 3) the propagules must survive digestion and find the conditions necessary for development in the new areas they are dispersed to.

Evidence in support of the accomplishment of these three stages has been provided in laboratory and field studies (De Vlaming & Proctor 1968, Proctor 1968). However, one question remains uninvestigated. Some authors have suggested that birds may empty their guts prior to the start of migration (Morton 1967). The period of migratory fasting reported in several different bird species gives support to this hypothesis (Fransson 1998). Although experiments in captivity have shown that seeds can be retained for periods of more than 24 hours, it is unclear if any seeds will remain in the gut to be dispersed if birds initiate a period of fasting prior to the migratory flight.

To assess the impact of premigratory fasting on the potential for long distance seed dispersal by waterfowl, we performed an experiment under controlled conditions in captivity.

Material and Methods

The experiment was performed in the installations of the Wildlife Recovery Centre in Doñana National Park on Marbled Teal (*Marmaronetta angustirostris*), a duck species that makes extensive migratory and dispersive movements within its range (Green 1993, Navarro & Robledano 1995). Nine

captive bred Marbled Teal were individually housed in cages (three meters long by three meters wide), with wire mesh covering the sides and roof. The floor of the cages was occupied by a rectangular concrete pond which was surrounded by a band of soil (approximately 0.5 meter wide) covered with a layer of fine sand. Prior to the start of the experiment, the ponds were emptied, allowed to dry and the drainage pipe was sealed with plastic bags and adhesive tape. Two of the cages lacked a concrete pond, and all the soil was covered with fine sand. The birds, previously housed together in a different cage, were redistributed individually in each of the cages the night before the start of the experiment (18 October 1999). Water and food (commercial duck-food pellets) were provided *ad libitum* on separate dishes.

The next morning a mixture of 100 seeds of *Scirpus maritimus*, 100 seeds of *Scirpus litoralis*, 60 plastic markers of 1.0 mm diameter and 100 plastic markers of 0.5 mm diameter were force-fed to each individual. Both these seeds were collected by us from Doñana Natural Park, and are major food items of the Marbled Teal in the Mediterranean region (Green & Selva 2000). Seeds of both species can retain their viability after digestion by Marbled Teal (J. Figuerola, A.J. Green and L. Santamaría, unpublished data). The provision of food and water was constant for four individuals (control). Food was removed from the cages of the other five individuals (experimental) to simulate a period of fasting. After eight hours, the water supply was removed for both control and experimental birds to simulate a period of no availability of food and water during a nocturnal migratory flight. Both controls and experimental birds were then exposed to a second period of fasting because fasting is obligatory during migratory flight. After another eight hours (i.e. 16 hours following the ingestion of the seeds), both food and water supply were returned. Droppings were collected after two hours, four hours and at four hour intervals up to 16 hours after the start of the experiment. Unfortunately, a storm started aprox. 19 hours after ingestion of the seeds, washing away part of the droppings produced in the 16-25 hour interval. A final collection of droppings was conducted 32 hours after the start of the experiment. This last collection included part of the droppings produced in the 16-25 hour interval (those not washed away by the rain) and all the droppings

produced from 25 to 32 hours. Since part of the droppings produced (and consequently, part of the defaecated seeds and markers) were washed away by the rain, we did not analyse the total proportions of seeds and markers recovered throughout the experiment. Instead, we compared the absolute number of the different seeds and markers recovered in each phase of the experiment.

Results

The experiment can be divided into three clear phases: the treatment phase (with access to water in the first eight hours for experimental birds, and access to food and water for control birds), the flight simulation phase (without access to food or water during the 8-16 hour period) and a final refuelling phase (16-32 hours). The seeds of one species (*S. maritimus*) and one size of the markers (1 mm Ø) were hardly represented in faeces in the last period of the experiment, since only one individual duck presented seeds/markers in its droppings (Fig. 1). Both cases were due to the same experimental bird with eight seeds and one marker in its droppings collected during the 16-32 hour period. Given their anecdotical presence in the droppings in the last phase of the experiment, no statistical analyses were performed for these two seed/marker types.

No significant differences in the number of the other seeds (*S. litoralis*) or markers (0.5 mm Ø) were observed between experimental and control birds during the treatment period (i.e. first eight hours) of the experiment (markers, z=0.86, P=0.39, *S. litoralis*, z=0.61, P=0.54, Fig. 2). During the flight simulation phase, controls defaecated significantly more *S. litoralis* seeds than experimental birds (z=2.05, P=0.04), but no differences occurred in the number of markers in the droppings (z=0.13, P=0.89). After termination of the fasting period, the droppings collected from experimental birds contained significantly more 0.5mm Ø markers (z=2.02, P=0.04) and *S. litoralis* seeds (z=2.05, P=0.04) than droppings from control individuals (Fig. 2).

Discussion

An increasing number of studies are rep orting short-term morphological changes in birds during migration (also known as phenotypic flexibility, Piersma & Lindström 1997, Piersma 1998). Field data indicate that, during migration, individuals seem to reduce the size of their digestive organs (gizzards, livers, kidneys and guts) and increase their fat deposits (i.e Piersma & Gill 1998, see also Gauthier et al. 1984). Experiments in captivity have concluded that changes in digestive organs in birds during migration are similar to those experienced by individuals exposed to short periods of fasting (Biebach 1998). Such a reduction in the activity of digestive organs can have opposing consequences for the probability of dispersal of propagules by waterfowl. On the one hand, the number of propagules retained in the digestive tube may be reduced, since most of the tube is probably emptied before the start of migration. On the other hand, this reduction in the digestive activity may allow very long retention times for the propagules remaining inside the birds.

In our experiment, both control and experimental birds defaecated fewer seeds and markers of the larger size during the last phase of the experiment (when long distance dispersal would be likely to occur). We suggest this size effect can be explained by the negative relationship between seed size and retention time in the digestive tube already reported in cedar waxwings (Levey & Grajal 1991). Larger seeds could also be less resistant to digestion by ducks (De Vlaming & Proctor 1968), leaving fewer viable seeds to be defaecated. However, large markers (1.0 mm Ø) were also retained for shorter periods than smaller ones (0.5 mm Ø), suggesting that seed size is an important variable affecting the potential for endozoochorous dispersal.

In our experiment, we explored the potential effects of premigratory fasting on seed dispersal. Our results indicate that, although fasting individuals defaecated a large number of seeds in the first few hours after feeding, they retained *S. litoralis* seeds for a longer period that control individuals. Thus, premigratory fasting may increase the potential for long distance dispersal of propagules by increasing the number of seeds retained

for a long time (more than a day) at the potential cost of a reduction in the number of seeds retained for intermediate periods of time (8-16 hours). Clearly, a period of fasting prior to the start of migration should not be considered an unassailable barrier for the occurrence of bird mediated long distance dispersal. More research is needed to study the effects of fasting over longer periods of time (>16 hours), and to investigate the effects of fasting on the future viability of the retained seeds.

Our experiment provides further evidence that migratory waterfowl play a major role in the long-distance dispersal of aquatic plants. Marbled Teal make regular movements between wetlands such as Doñana and El Hondo in Spain, and Sidi Moussa in Morocco (Green 1993, Navarro & Robledano 1995), all sites holding *S. litoralis* and *S. maritimus* and separated by more than 500 km from each other. We expect the teal to regularly move viable *Scirpus* seeds between such wetlands.

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Figure 1. Number (mean±SE) of (a)   *Scirpus maritimus* seeds and (b) 1.0 mm Ø markers found in the droppings collected from five experimental and four control Marbled Teal during the three phases of the experiment. ◨ experimental ducks ☐ control ducks (not subjected to the first 8 h fasting period).
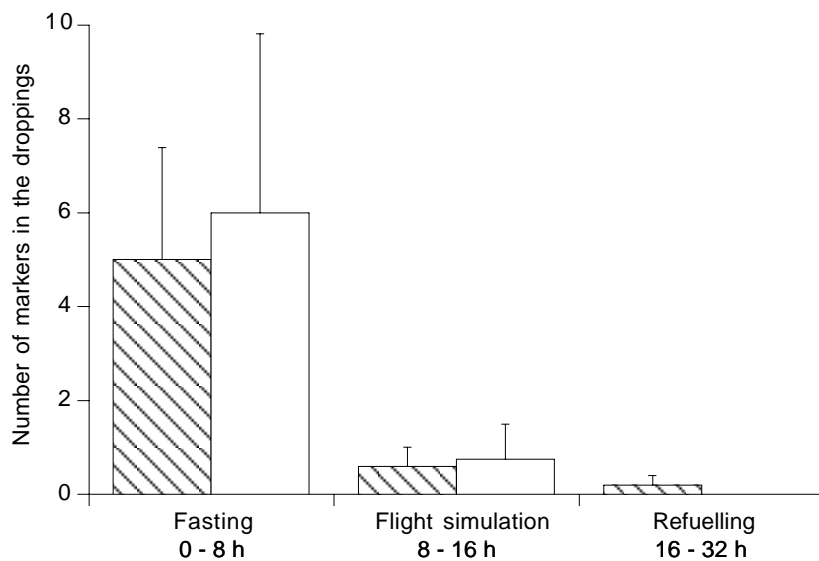
a)
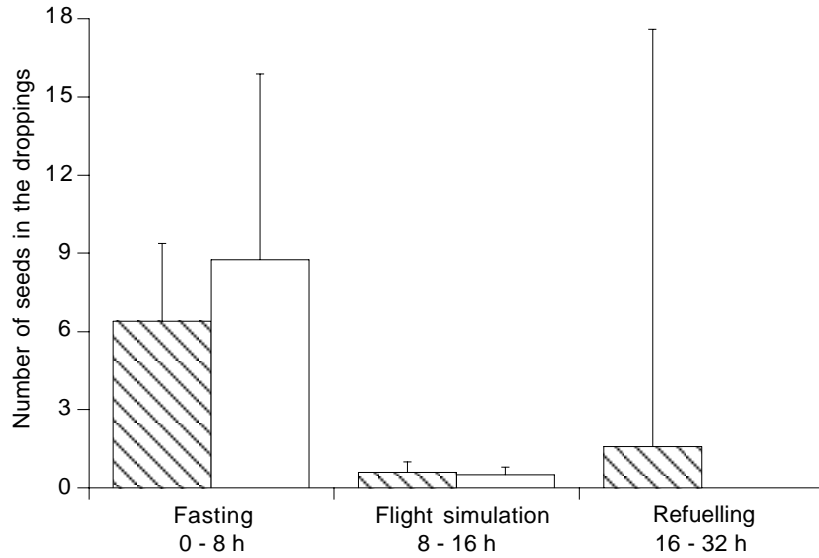
b)

Figure 2. Number (mean±SE) of (a) *Scirpus litoralis* seeds and (b) 0.5 mm Ø markers (b) found in the droppings collected from five experimental and four control Marbled Teal during the three phases of the experiment. * Indicates a significant difference ($P<0.05$) between control (not subjected to the first 8 h fasting period) and experimental birds. ▨ experimental ducks ☐ control ducks.

a)
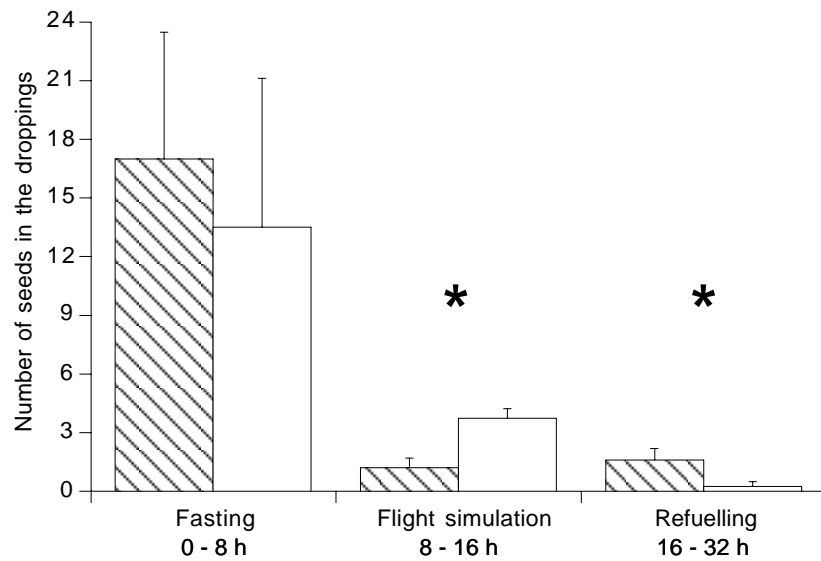
b)
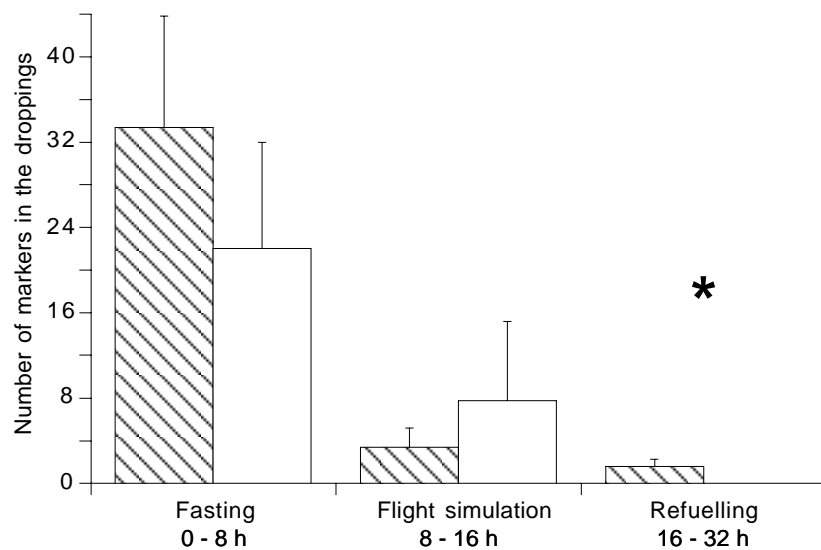
First evidence of waterfowl mediated gene-flow in aquatic invertebrates

Jordi Figuerola [*], Andy J. Green [*], Paul D. N. Hebert [†] & Thomas C. Michot [‡]

[*] Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. Maria Luísa s/n, 41013 Sevilla, Spain

[†] Department of Zoology, University of Guelph, Ontario, Canada N1G 2W1

[‡] USGS, National Wetland Research Center, 700 Cajundome Boulevard, Lafayette, Louisiana 70506, USA

'The wide distribution of fresh-water plants and of the lower animals, ..., apparently depends in main part on the wide dispersal of their seeds and eggs by animals, more especially by fresh-water birds, which have great powers of flight, and naturally travel from one piece of water to another', Charles R. Darwin 1859.

The capacity of aquatic plants and invertebrates to colonise new habitats and distribute themselves over large geographic ranges has long fascinated naturalists[1]. Although, recent genetic analyses have determined that some morphospecies correspond in fact to cryptic species complex[2], it remains true that many taxa are widely distributed. Such ubiquity has been ascribed to the potential for waterfowl to transport propagules of such organisms[3,4]. Waterbirds carry viable plant and animal propagules both externally (attached to plumage or feet) and internally (after surviving digestion)[5,6], but none has yet found a way to quantify the direction and distance in which they are moved (i.e. the propagule shadows) and the capacity to establish in new populations of dispersed propagules. Although gene flow between different catchments has often been demonstrated, as has the rapid expansion of introduced aquatic species, this could potentially be due to other dispersal mechanisms such as wind, rain or human activities, and no direct evidence exists of waterfowl mediated long distance dispersal[7]. In thisstudy we use molecular estimates of gene flow for four aquatic

invertebrates and ringing data for waterbirds in North America to present the first evidence of a direct link between waterfowl migration and aquatic invertebrates dispersal.

North America is traditionally divided into four flyways for Anatidae (ducks, geese and swans) movements (Fig. 1). However, this is a major simplification, and ducks (usually the most important dispersers of aquatic invertebrates owing to their abundance and habitat use)[6] do not have discrete flyways but rather probability matrices of movements between one area and another. Thus, in order establish a clear hypothesis for the consequences of waterfowl dispersal, it is essential to quantify their movements. We used ringing data to produce a matrix of distances based on long distance movements of waterfowl, and tested its effectiveness in explaining the genetic structure of 3 cladoceran crustaceans and 1 bryozoan over North America.

The correlation between genetic and geographic distance between invertebrate populations ranged from r=0.27 to 0.44 (see Table 1), whereas the correlation with waterfowl movements ranged from r=-0.24 to -0.50. Waterfowl movements explained a greater amount of variance in genetic distances than geographic distances for three of the species analysed (*Daphnia ambigua*, *Daphnia laevis* and *Cristatella mucedo*), and the opposite occurred for *Sida crystallina*. After controlling for geographic distances, waterfowl movements still explained a significant fraction of variation in genetic distances for three of the species studied, *D. ambigua* (r=-0.30, p=0.01), *D. laevis* (r=-0.31, p=0.03) and *C. mucedo* (r=-0.29, p=0.05). For *S. crystallina* waterfowl movements were unrelated to genetic distances after controlling for geographic distances (r=-0.08, p=0.21).

Our results suggest that bird movements influence the genetic structure of aquatic invertebrate populations. For three of the four species analysed waterfowl migratory routes explained a significant amount of genetic variation. There are a number of biases associated with ringing data mainly due to the heterogeneity of the ringing effort and recovery probabilities in space [8].

Furthermore, birds may not move directly between the ringing and recovery areas but rather stop by other wetlands with little hunting activity. Furthermore, we combined ringing data for a list of species that vary both in their movement patterns and probably also in their relative importance as dispersers of different invertebrates[6]. All these factors make our tests conservative since there is no *a priori* reason to expect an association between genetic distances of aquatic organisms and such biases in the ringing data.

The poor relationship between waterfowl movements and genetic distance in *S. crystallina* is consistent with unusually high levels of genetic divergence between lineages in this species. This was considered due to a reduced capacity to disperse through waterfowl[9], as supported by our results. Interestingly, *S. crystallina* propagules have a completely different structure than *Daphnia* eggs, and appear less suitable for dispersal by waterfowl. They show a reduced tolerance to desiccation (less than 24 hours[10]) in comparison with *Daphnia* eggs[11] and visually appear less resistant to digestion. Resistance to ingestion by waterfowl has been demonstrated for several *Daphnia* species and for statoblasts of *C. mucedo*[12].

Dispersal of aquatic organisms across Canada has been thought to be greatly facilitated by the formation of great postglacial lakes during deglaciation[13], and this could be an important factor shaping *S. crystallina* genetic structure given the more northerly distribution in comparison to the other three species analysed. However, waterfowl movements remained unrelated to *S. crystallina* genetic structure even when excluding data from Canadian localities (results not presented).

In order to understand how aquatic organisms can react to environmental change[14], or to assess the possibilities of controlling invasive aliens[15], we need to improve our understanding of their mechanisms of dispersal. Our results suggest that birds disperse organisms over large geographic ranges, and provide a novel way to analyse interspecific differences in the success of bird mediated dispersal. To assess the role of bird mediated dispersal in generating and maintaining aquatic communities structure and diversity we need to clarify how much can be generalised the

relation between bird movements and genetic structure we have demonstrated for the first time. These should be complemented by comparative analyses of the resistance of different propagules to ingestion by waterfowl (important for internal transport), and their capacity of adhesion and resistance to desiccation (important for external transport).

Methods

mtDNA sequences and genetic distances

MtDNA sequences from four published studies were obtained from GENBANK. The *Daphnia laevis* dataset consists of 20 haplotypes of the mitochondrial 12S rRNA (569 bp). Data were obtained for 20 localities from 10 states[16]. For *Daphnia ambigua*, data consisted of 43 sequences (31 haplotypes) of a 640 bp fragment of the cytochrome c oxidase subunit I (COI). Overall, 33 localities from 15 states were sampled [17]. *Sida crystallina* data consisted of 40 haplotypes of a 614 bp fragment of COI, from 44 localities sampled in 16 states [9]. The last dataset consisted of 53 haplotypes of 363 bp of cytocrome b from *Cristatella muceda* Data was available for 20 localities of 9 states[18].

To calculate genetic distances we used the MODELTEST program [19] to select the evolutionary model best fitting to the characteristics of the data using the AIC criterion (Table 1). Since the model selected for *D. laevis* resulted in non-estimable distances, the next model with the lowest AIC was used. Interstate differences were calculated as the mean distance between populations in different states less the average of genetic distances between populations in the same state [20].

Waterfowl movements

Recoveries from waterfowl in North America (USA and Canada) were obtained from the Bird Banding Laboratory (US Geological Survey). The genera included in the analyses were *Aythya* (5 species), *Anas* (9 sp.), and *Fulica* (1 sp.). Overall, 1,517,976 recoveries were suitable for constructing the waterfowl movements matrix, although the number of recoveries used for each dataset was smaller because genetic data were available for a different number of states in each study (see Table 1). A matrix was constructed with

the number of individuals ringed and recovered in states where genetic information for each species of invertebrate studied. This information was transformed into a matrix of similarities based on Steinhaus index [21] using the program R-Package.

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Table 1. Relationships between genetic, geographic and bird movements matrices

Description of the test	C. mucedo		D. ambigua		D. laevis		S. crystallina	
	r	p	r	p	r	p	r	p
Genetic vs km	0.40	0.05	0.29	0.05	0.27	0.06	0.34	0.007
Genetic vs bird	-0.48	0.04	-0.39	0.01	-0.40	0.007	-0.25	0.03
Genetic vs bird controlling km	-0.28	0.05	-0.29	0.02	-0.31	0.03	-0.07	0.26
Number of states	9		15		10		16	
Number of bird recoveries used	310,668		359,586		301,320		251,123	
Evolutionary model	K81uf+I+G		TrN+I		GTR+I		HKY+I+G	

Pair-wise correlations between genetic distances and geographical distances (km) or similarities based on waterfowl migration (bird) were calculated using Mantel Test[21]. The relative contributions of bird distances to explaining genetic distances while controlling for geographic distances were estimated using partial Mantel tests. The number of states and bird recoveries, as well as the evolutionary model used to calculate genetic distances are reported.

Figure 1. Similarities based on waterfowl ring recoveries between Louisiana and the other states with genetic material sampled for *Daphnia ambigua*. White arrows indicate a similarity index < 0.05, black arrows a index > 0.10 and grey arrows a intermediate index. For the 14 states at least some recoveries exist involving individuals ringed or recovered at Louisiana.
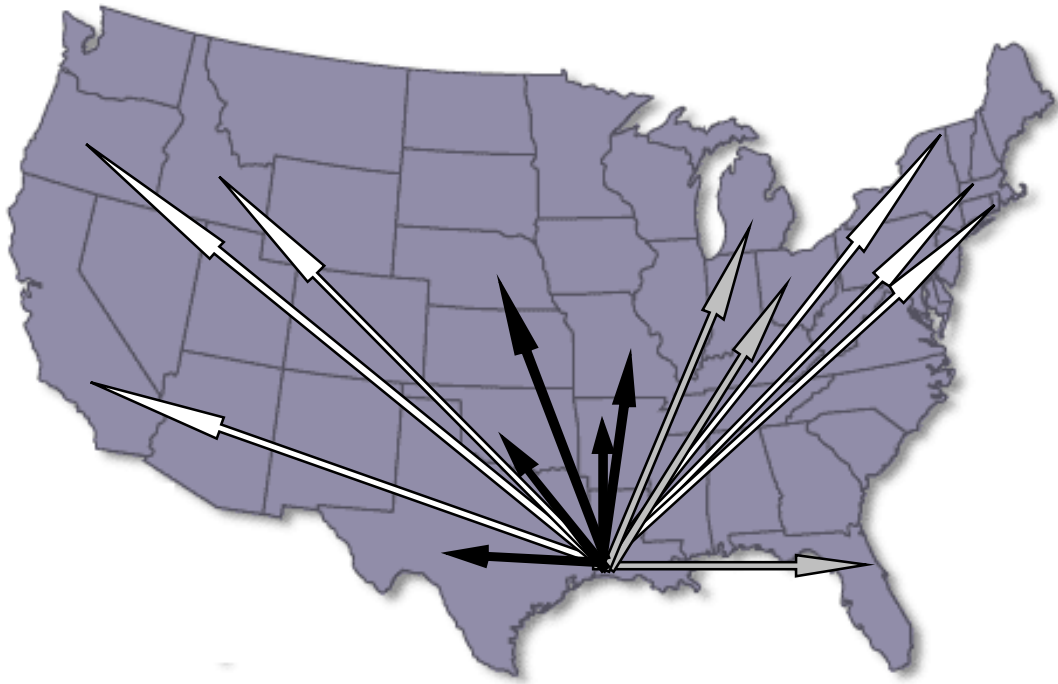
Part 5

Local adaptation as a limitation for long distance dispersal

Running head: Plant performance across latitude

Plant performance across latitude: the role of plasticity and local adaptation

L. Santamaría[1], J. Figuerola[2], J.J. Pilon[1], M. Mjelde[3], A.J. Green[2], T. de Boer[1], R.A. King[4], R.J. Gornall[4]*

[1] Netherlands Institute of Ecology (NIOO-KNAW), Center for Limnology, PO Box 1299, 3600 BG Maarssen, the Netherlands.

[2] Doñana Biological Station (CSIC), Department of Applied Biology, Avda Maria Luisa S-N, E-41013 Seville, Spain.

[3] Norwegian Institute for Water Research (NIVA), PO Box 173 Kjelsas, N-0411 Oslo, Norway.

[4.] Department of Biology, University of Leicester, University Road, Leicester LE1 7RH UK

*Corresponding Author. Phone: +31-(0)294-239358, Fax: +31-(0)294-232224. Email: santamaria@cl.nioo.knaw.nl

Submitted

Abstract: Geographic variation can lead to the evolution of different local varieties within a given species, therefore influencing its distribution and genetic structure. We investigated the contribution of plasticity and local adaptation to the performance of a common aquatic plant (*Potamogeton pectinatus*) in contrasting climates, using reciprocal transplants at three experimental sites across a latitudinal cline in Europe. Plants from 54 genets, originally collected from 14 populations situated within four climatic regions (sub-arctic, cold-temperate, mild-temperate and Mediterranean) were grown in three different localities within three of these regions (cold-temperate: Norway; mild-temperate: the Netherlands; Mediterranean: Spain). Tuber production was highest for the mild-temperate genets, irrespective of locality where the genets were grown. Selection coefficients indicated that populations at the European center of the species distribution perform better than all other populations, at all sites. However, marginal populations showed changes in life history traits, such as compressed life cycles in the north and true perenniality in the   south, that may allow them to perform better locally, at the limits of their distribution range. Our results thus suggest that local adaptation may overlap spatially with center-periphery gradients in performance caused by genetic factors (such as genetic drift and inbreeding in range-marginal populations).

INTRODUCTION

Common plant species with wide distributions may perform well in a wide range of environmental conditions (Joshi et al. 2001). However, the capacity of individual genotypes to perform well across the full range of conditions is often limited (De Witt et al. 1998). Widespread terrestrial species are often characterized by both phenotypic plasticity and high levels of genetic variation (Bradshaw 1984) and by local specialization to particular environmental conditions (Joshi et al. 2001, Van Tienderen 1990). In contrast, aquatic plant species, many of which are also widespread, have often been shown to have limited genetic variation, suggesting the common occurrence of general-purpose genotypes among them (Barrett et al. 1993, Santamaría 2002). General-purpose genotypes are able to maintain a high fitness over broad ranges of environmental conditions, through compensatory plastic responses in morphology, physiology and/or phenology (adaptive plasticity ; Schlichting 1986, Dudley & Schmitt 1995).

One of the main sources of environmental variation, particularly at continental scales, is climate. This is reflected in world-wide vegetation zones, which follow the latitudinal pattern of prevailing environmental conditions (e.g. Walter 1973); and in global patterns of plant distribution, which are largely explained by species-specific responses to temperature and precipitation (e.g. Woodward & Williams 1987, Woodward 1990). Solar irradiance and temperature decrease with increasing latitude, while summer photoperiod increases. Since these variables are known to influence many aspects of plant life (Berry & Raison 1981, Björkman 1981, Salisbury 1981), widely distributed plant species may be expected to show phenotypic variability across latitude. Genetically based latitudinal variation in phenology (Potvin 1986), growth (Chapin III & Chapin 1981, Sawada et al. 1994, Li et al. 1998) and sexual reproduction (Aizen & Woodcock 1992, Winn & Gross 1993) has been reported among terrestrial species. In general, ecotypes originating from higher latitudes show relatively small statures, early flowering and decreased fecundity due to lower growth rates or short developmental cycles (Chapin III & Chapin 1981, Potvin 1986, Winn & Gross 1993, Li et al. 1998). In the context of aquatic plants, while some

information is available for seagrasses (Philipps et al. 1983) and marine macro-algae (Orfanidis & Breeman 1999), latitudinal adaptations in freshwater angiosperms have received little attention. Because the latter are among the most widely distributed plants (Santamaría 2002) and represent a key component of shallow and littoral aquatic ecosystems (Carpenter & Lodge 1986), we wanted to determine the extent to which their broad distributions are related to local specialization of populations from different climatic regions.

The contribution of environmental and genetic variation to phenotypic variability and performance can be assessed using reciprocal-transplant experiments, where significant home versus away differentials are assumed to reflect local adaptation (e.g. Joshi et al. 2001). Reciprocal transplants have typically been performed over small scales and with terrestrial species (Bradshaw 1984, Schmid 1985, Linhart & Grant 1996), often revealing the existence of local adaptation (e.g. McGraw & Antonovics 1983, Nagy & Rice 1997). Population differentiation in morphological and physiological traits has also been reported for a few aquatic species, typically involving laboratory experiments rather than outdoor transplants (e.g. Koch & Dawes 1991, Barrett et al. 1993). Recently, Joshi et al. (2001) extended the replant-transplant approach to a European scale, aiming to correlate the difference in home versus away phenotypic performance with climatic distances between sites. They found the overall performance of three grassland species to decline with increasing transplanting distance, being highest for home replants (Joshi et al. 2001). However, climatic distance was not correlated with selection indices and only accounted for 18% of the variation related to geographic distance, suggesting that the observed pattern of variation could be caused by factors other than climate (such as biotic influences). Since Joshi et al. (2001) performed their experiment directly in local field sites, they were not able to evaluate the contribution of local (e.g., edaphic or biotic) conditions *versus* climatic environmental variation to the observed patterns of local specialization. In this study, we attempted to minimize local variation by standardizing most of the environmental variables, e.g. soil characteristics and water chemistry, that are not related to climatic conditions, i.e. temperature,

irradiance and photoperiod. Furthermore, we attempted to evaluate the relative contribution of plasticity to the observed patterns of phenotypic variation and to remove the influence of environmentally-induced maternal effects (Rossiter 1998) by multiplying all sampled genotypes under standardized conditions during a complete growing season prior to transplanting their clonal propagules to all our experimental sites.

MATERIALS AND METHODS

Study species

Potamogeton pectinatus L. (fennel pondweed) is a submerged angiosperm with a pseudo-annual life cycle and a cosmopolitan distribution (Hultén & Fries 1986). Plants grow during spring-summer and survive the winter by means of subterranean tubers (specialized asexual propagules). Under mild climatic conditions, *P. pectinatus* may grow as a perennial (Yeo 1965). Annual seed production varies greatly and its contribution to yearly population recruitment is generally considered to be minimal in temperate regions (e.g. Van Wijk, 1989a). Instead, seeds might be important for dispersal and establishment after disturbances (Van Wijk, 1989a). Given the limited importance and reliability of short-term sexual fecundity estimates, we considered that asexual population growth rate (r), a surrogate of genet survivorship, represents a better fitness correlate than fecundity (see also Crone 2001).

Reciprocal transplant experiment

In summer-autumn 1998 and spring 1999, tubers and/or rhizome fragments were collected from at least four spaced (>10 m) individuals at each of 14 localities in Europe and North Africa, grouped within five discrete latitudinal ranges taken to represent different climatic regions (Fig.1, Appendix A). In summer 1999, we grew a single tuber or rhizome fragment from each sampled individual, i.e. one putative genet, under outdoor, common-garden conditions in Heteren (the Netherlands), in order to obtain clonal propagules (tubers). The Mediterranean populations failed to produce tubers under such circumstances, since they are

probably truly perennial; hence we kept the plants growing during the following winter in indoor aquaria and utilized rhizome fragments as propagules. Growing the plants indoors during the winter was also necessary for a few Swedish genets, due to their low tuber production. For such genets, both tubers (from the previous autumn) and rhizome fragments (from living plants) were used as propagules in the transplant experiment. Out of five clonal fragments collected from Loch Gelly (Scotland), only three clonal lines became established during the propagation phase; hence, we were only able to use three genets for this population.

Potamogeton pectinatus hybridizes with closely related species of the subgenus *Coleogeton*. In northern regions (UK, Denmark, Norway, Sweden and Russia), at least two of the hybrids, viz. *P.* x *suecicus* (*P. pectinatus* x *P. filiformis*) and *P.* x *bottnicus* (*P. pectinatus* x *P. vaginatus*), are very difficult to discriminate from *P. pectinatus* on the basis of morphological traits (Preston *et al.* 1998; Preston *et al.* 1999). For this reason, the identity of the collected genets was confirmed by means of RFLP analysis of nuclear and chloroplast DNA extracted from freshly grown material, following the methods described by King *et al.* (2001). In addition, ISSR 'fingerprint' data were used to discriminate between different *P. pectinatus* genets; i.e. to ensure that the putative genets selected for the experiment did indeed represent different genotypes (see Appendix B for details). A high proportion of the clones sampled from one of the Russian populations (Srednii Shar) were identified as hybrids; hence, we were only able to use three *P. pectinatus* genets for this population.

For the transplant experiment (summer 2000), we grew plants from the 54 clonal lines at three distant localities, situated at different latitudes and having contrasting climates (Doñana, SW Spain; Heteren, the Netherlands; and Oslo, SE Norway; Fig. 1, Appendix A). The provenance of the genets corresponded to the three experimental localities, together with Scotland (situated between two of the experimental sites; Fig. 1) and northern Russia (situated in the northern range limit of the species). The design was not fully balanced, owing to shortage of tubers from a few genets: although we aimed to use five replicates per genet

and growing site, a total of 613 plants was finally grown (204 in Norway, 207 in the Netherlands and 202 in Spain), corresponding to a factorial combination of 3 sites x 54 genets using an average of 3.8 replicates. At each experimental locality, plants were cultivated under common-garden conditions (Appendix C).

Plant propagules were planted in mid-May 2000, by inserting them carefully through the first 2 cm of washed aquarium sand into the clay-sand sediment mixture. Plants were cultivated until the end of September, when they were harvested by washing off the sediment using pressurized water and a 2 mm sieve. In the laboratory, we measured the length of the longest shoot and the number of ramets per plant, and separated the above-ground (shoots) and below-ground (roots + rhizomes) fractions to measure their dry weight (after 24h at 70°C). Tubers were weighed individually (fresh weight) and a random subsample was used for dry weight determinations (as above, N=240). Based on this subsample, the dry weight of the remaining tubers was estimated from linear regressions relating dry weight to fresh weight (separately for each experimental locality and region of genet origin; R^2 always above 0.95).

In pseudo-annual plants, asexual rates of population growth (r) are best estimated as the product of asexual fecundity (af), calculated here as the average number of tubers per genet and recorded separately at each experimental site, and survival probability (p), calculated here as the proportion of plants that produced at least one ramet or one viable tuber, for each genet and experimental site. Thus $r = p$ x af. We then used r as a measure of plant performance or fitness, and calculated selection coefficients for each genet i relative to the best-performing genet at each particular experimental site as $s_i =$ 1- (r_i / r_{max}). s_i thus ranges from 0 (for the most successful genet at each site) to 1 (indicating complete selection against a given genet). This procedure may underestimate the performance of perennial plants growing in mild climates, since genets that produced no tubers have a $s_i =$1. For this reason, fitness estimates of plants that produced no tubers but did not senesce by the end of the experiments, i.e. the Mediterranean genets growing in Spain, were also calculated using asexual rates of population growth based on the average

number of ramets per plant, i.e. the number of potentially independent clonal individuals, instead of the number of tubers. Fitness estimates using tubers or tubers+ramets were almost identical; hence, we only report on the latter.

Statistical analysis

All variables measured (total biomass, vegetative biomass and tuber production; number of tubers and tuber size; shoot length, shoot-root ratio and number of ramets; and clonal survival) and fitness estimates were analyzed by means of hierarchical, mixed-models ANOVAs (Appendix D), performed in the General Linear Models module of Statistica 5.5 (Statsoft, 1999). The main effects attributable to environment ('site') and genetics (decomposed in 'region', 'population' and 'genet') were interpreted to represent the general superiority of plants growing at, or originating from a particular site; while interaction effects indicate that different strains respond differently at different sites (similar to Joshi et al. 2001). In the site x region matrix (Table 2), a home effect is represented by values found on the diagonal in bold and an increasing distance effect is reflected by values further away from the diagonal in bold.

RESULTS

During the experimental period (May-September 2000), pronounced climatic differences existed between the experimental sites in Norway, the Netherlands and Spain. *P. pectinatus* genets that were grown at higher latitudes experienced lower air and water temperatures, lower levels of daily irradiance and longer photoperiods than those grown in the south (Fig. 1).

Environmental ('site') and genetic ('region', 'population' and 'genet') effects, as well as their interactions on life-history characters were statistically significant in most cases (Appendix E). Environmentally induced variation occurred in all variables measured, reflecting an increase in performance with decreasing latitude (Fig. 2). Genetic effects occurred mostly at regional level, with almost no significant differences among populations and few among genets. Most genetic x environmental variation was explained by region x site

interactions, with few significant interactions at population and genet levels. Propagule size and type had limited effects on plant growth, affecting significantly only a few variables.

Biomass yield (total, vegetative and tubers) increased significantly when the plants were grown at sites of decreasing latitude (ANOVA: $P<10^{-10}$ for all three variables; Fig. 2A and D). Total biomass yield and tuber production were higher for mid-latitude genets (Netherlands/England and Scotland) and lower for northern (Swedish/Russian) and southern (Spanish/Moroccan) genets (ANOVA, factor 'region': $P<0.001$ for both variables). Vegetative biomass followed the same trend, except for the Mediterranean genets grown in Spain, which had the highest biomass yield observed in the experiment (Fig. 2A) (ANOVA: $P<10^{-8}$ for region, $P=0.023$ for site*region). Even in their 'native' experimental site, however, most clonal replicates of the Mediterranean genets failed to produce tubers (Fig. 2D).

Swedish genets, and those Mediterranean genets that tuberized, produced fewer but larger tubers (Fig. 2E and F; ANOVA, factor 'region': $P<0.0006$)) and showed also the largest plasticity in tuber size, which was significantly reduced when the plants grew in 'less favorable' experimental sites (further north than Spain for the Mediterranean genets and in Norway for the Swedish genets) (ANOVA, site*region: $P<10^{-9}$; Fig. 2F).

Shoot length increased with decreasing latitude of origin, being maximal for the Mediterranean genets (ANOVA, factor 'region': $P=0.0048$). It also tended to decrease from southern to northern experimental sites (ANOVA: $P<10^{-5}$; Fig. 2C). The relative differences in phenotype among experimental sites varied according to the region of origin. For the Mediterranean genets, there were large differences in shoot length between the experimental site in Norway and those in the Netherlands and Spain, which did not differ significantly from each other (Scheffé post-hoc tests: $P<10^{-5}$, $P<10^{-8}$ and $P=0.99$, respectively). For the Russian genets, the pattern was reversed: large differences occurred between the experimental site in Spain and those in the Netherlands and Norway, which were not significantly different (Scheffé post-hoc tests: $P=0.037$, $P=0.0001$ and

P=0.99, respectively). For the Dutch/English and Swedish genets, differences between sites were more homogeneous; no significant variation in shoot length was detected for the Scottish genets.

Ramet production increased at experimental sites from north to south (ANOVA: $P<10^{-12}$; Fig. 2B), while shoot-to-root ratio tended to be lower at the Norwegian site (ANOVA: $P<10^{-4}$; data not shown). Variation among regions of origin was correlated for both variables. Scottish and Dutch/English genets showed the largest plasticity in ramet production (Fig. 2B) and lowest plasticity in shoot-to-root ratio. Russian, Swedish and Mediterranean genets showed larger plasticity in shoot-to-root ratio and a narrower plastic range in ramet production (Fig. 2B).

Seed production was very low. Only Mediterranean genets growing in Spain set fruit (2 out of 16 genets). On average, 14% of all Mediterranean plants growing in Spain produced seeds.

Plant survival varied significantly among sites, and among the regions and populations where the genets originated (ANOVA: P=0.0019, P=0.0015 and P= 0.023, respectively). However, none of the interactions with experimental site was significant, i.e. genetic and environmental effects on survival were additive. Survival was significantly lower in Norway than in the Netherlands and Spain (P<0.003, Scheffé post-hoc tests), which did not differ significantly (P=0.44). The Mediterranean genets showed a significantly lower survival than all other genets ($P<10^{-7}$ for all Scheffé post-hoc comparisons involving the Mediterranean genets) which did not differ significantly among themselves (P>0.18 ; data not shown). The type of propagule from which the plants grew had no significant effect on survival. Irrespective of type, propagule size was, on the other hand, positively correlated with survival (ANOVA: P=0.007 for tubers, $P<10^{-5}$ for rhizomes).

Selection coefficients varied significantly among sites and among the populations where the genets originated (ANOVA: P=0.0007 and P=0.008, respectively) and were significantly affected by the site x region interaction (ANOVA: P=0.0012). At all experimental sites, mid-latitude genets (i.e. those from Scotland and Netherlands/England) had the highest fitness, while those

from the Mediterranean and Swedish regions had the lowest (Table 2). Russian genets had intermediate fitness in Spain and the Netherlands and higher fitness, though not significantly different from the mid-latitude genets, in the Norwegian site (Table 2).

DISCUSSION

Our results show that most *P. pectinatus* genets can grow and reproduce asexually at distant latitudes. Sub-arctic and temperate genets grew and produced tubers at the three experimental sites (Norway, the Netherlands and Spain). For all genets, independent of the region and population of origin, biomass yield and tuber production increased when grown at decreasing latitude. This might suggest that, in Europe, optimal conditions for growth of *P. pectinatus* are encountered in the Mediterranean region. It should be noted, however, that habitat availability in that region is limited by the scarcity of permanent waterbodies (since most shallow lakes dry up during the summer) or by major fluctuations in water depth (Gafny & Gasith 1999). Hence, *P. pectinatus* is most abundant at middle latitudes in Europe (Hultén & Fries 1986), although the potential for clonal growth and multiplication is higher in the south (see also Yeo, 1965).

Tuber production was highest for the mid-latitude genets, i.e. those from the Netherlands and the UK (52-56°N), irrespective of the experimental site where they were grown. The second component of survivorship, plant survival during the growth season, did not vary significantly among non-Mediterranean genets. Selection coefficients thus indicated that populations from middle latitudes perform better at all sites. Local adaptation seems to play a minor role in the phenotypic performance of these plants. Our results are more in agreement with the observation that range-marginal populations show decreased fitness due to genetic processes, such as genetic drift and inbreeding (Jonas & Geber, 1999). It is noteworthy that a different component of asexual fitness, ramet production, shows a comparable pattern of variation among genets.

However, in our high-latitude experimental site (Norway), Russian genets showed their maximum fitness, although not significantly higher than the   Dutch and Scottish genets. For the Russian genets, marginal changes in survival seem to compensate for the decreased tuber production when growing under the limiting conditions typical of cold-temperate and sub-arctic sites. Our results are consistent with common-garden experiments demonstrating that high-latitude populations of *P. pectinatus* complete their life-cycle and produce tubers earlier (Pilon et al. 2002). Compressed life-cycles are generally predicted to increase survival in the north, where summers are short and the conditions in late spring and early autumn are unpredictable, but at the cost of reduced plant growth and propagule size when growing in more favorable environments.

Mediterranean genets failed to produce tubers by mid-September at the three experimental sites. Although they had a biomass yield comparable to all other genets, except the Russian, they had a higher proportion of non-senescent biomass and much thicker rhizomes at harvest. In the temperate and sub-arctic region, surviving without tubers (i.e. as a perennial) is rarely possible owing to the severe winters with their drastically reduced daylengths, low water temperatures and frequent frost damage in shallow areas; hence, Mediterranean genets would not be able to establish. In Mediterranean climates, on the other hand, with their extended spring-autumn growth seasons and mild winters, perenniality and/or delayed tuberization are likely to result in increased competitive ability.

Hence, Mediterranean genets seem to be   locally-adapted: owing to their perennial life-cycle, they would be unable to become established in temperate to sub-arctic populations, but they may have a competitive advantage in climates with mild winters. Correlated changes in shoot length, shoot-to-root ratio and tuber size are consistent with this hypothesis. Shoot length increased with decreasing latitude of origin of the genets. Early elongation of shoot internodes is a well-known response to shading, and high plasticity in this trait confers an advantage in environments when competition for light is a significant selection factor (e.g. Dudley & Schmitt, 1995; Schmitt et al., 1995). In southern sites, characterized by high population densities and high biomass standing crop (due

to the mild winters and better growth conditions during spring-summer ; Santamaría, personal observation), this trait will probably confer a competitive advantage to the local genets. Mediterranean genets showed greater plasticity in shoot-to-root ratio (a trait also involved in light-competition responses) than the mid-latitude genets, and they produced larger tubers, which are known to confer a competitive advantage in dense populations (Spencer & Ksander, 1995). It is however remarkable that the Swedish genets showed a similar pattern of variation. Until more information becomes available on the relative contribution of tuber size and shoot-to-root allocation to competition and plant survival under different climatic regimes, it is difficult to interpret these traits more accurately.

Our data thus seem to point to two contrasting situations. On the one hand, variation in life-history traits and fitness estimates suggests that genets originating from temperate, mid-latitude regions have general purpose genotypes that are able to grow optimally at very distant latitudes. On the other hand, range limit populations show changes in their life-history (compressed life cycles in the north, true perenniality in the south) that may allow them to perform better locally. This discrepancy may be caused by limitations in the duration of our experiment, which does not allow for long-term estimates of fitness (over several clonal generations) that would take into account variable   life-histories among different genets. Alternatively, we may be looking at the spatial overlap of two different processes. Local adaptation may be constrained, but still arise among populations showing center-periphery gradients in plant performance due to genetic effects (as described above). Under such circumstances, the likelihood of finding observable effects reflecting local adaptation, i.e. home versus away advantages, will be highest in experimental manipulations over large geographic areas and strong environmental gradients, particularly those covering the full gradient between both distributional range limits in broadly distributed species (as we did here).

Finally, it is worth noting that tuber size is likely to interact with the results reported in this experiment. We standardized tuber and rhizome size, to minimize their influence on the outcome. It may be, however, that tuber size is one of the

traits influencing survival in a given region. Indeed, we have observed significant variation in the size of the newly produced tubers among genets (genetic effects), among experimental sites (plastic effects) and among their combinations (genetic variation in the reaction norm). Unfortunately, it is very difficult to predict and interpret the effect of propagule size for two reasons. Firstly, there is a trade-off between the increasing construction costs of larger propagules and the increased plant fitness they confer through increased sprouting success, competitive ability and resistance to partial damage (Spencer & Ksander, 1995; Santamaría & Rodriguez-Gironés, 2002; Westoby et al., 1992). Hence, information on both aspects of the trade-off is needed for an interpretation of tuber size variation. For example, Swedish genets that produce larger tubers might benefit from the higher initial growth rates they confer, which is of particular importance in cold regions with short summers. However, the high construction costs of large tubers might constrain their production in the colder and shorter summers of sub-arctic Russia. Secondly, tuber size shows broad plastic variation, e.g. larger plants tend to produce larger tubers. Plants might thus optimize the fitness of their asexual descendants through plastic changes in tuber size (adaptive plasticity). In this respect, progress in the understanding of the regulation of propagule size and its effects on plant fitness will contribute significantly to the understanding of plant local adaptation as a response to spatial heterogeneity.

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Table 1: Selection coefficients. Values between brackets indicate 95 % confidence intervals. Different letters indicate significant differences between regions of origin for a given experimental site, with lettering order indicating increasing selection coefficients (Scheffé post-hoc tests, P<0.05). N = number of genets.

| Region of origin:	Norway				Netherlands				Spain				N
Russia	**0.523**	[0.313,	0.732]	a	0.666	[0.550,	0.782]	ab	0.612	[0.486,	0.739]	ab	12
Sweden	**0.987**	[0.960,	1.015]	b	0.889	[0.812,	0.966]	bc	0.908	[0.864,	0.952]	b	12
Scotland	0.848	[0.685,	1.011]	ab	**0.360**	[0.154,	0.567]	a	0.161	[-0.354,	0.675]	a	3
Netherlands/UK	0.732	[0.570,	0.895]	ab	**0.464**	[0.317,	0.610]	a	0.455	[0.319,	0.591]	a	12
Spain/Morocco	1			b	0.997	[0.994,	1.001]	c	**0.902**	[0.851,	0.952]	c	16
Mean	0.826	[0.753,	0.899]		0.746	[0.673,	0.819]		0.698	[0.623,	0.773]		55
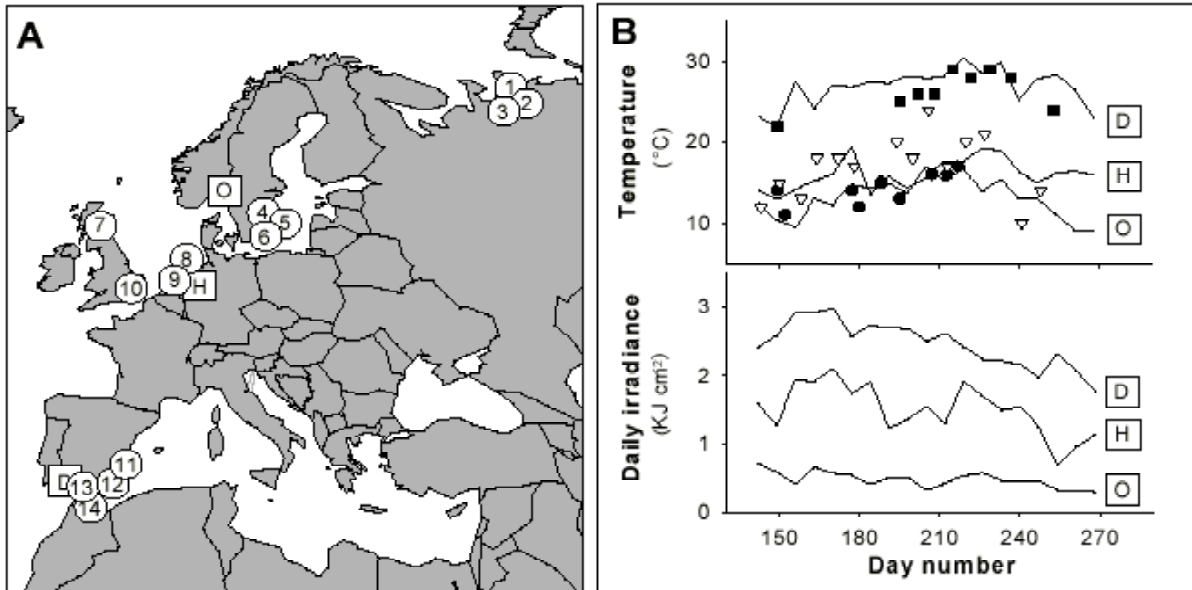
Figure 1. (**A**) Geographic origin of the *P. pectinatus* populations used in the transplant experiment (circles with population numbers) and location of the three experimental sites where they were grown (squares). In the squares 'O' refers to Oslo (Norway), 'H' to Heteren (The Netherlands) and 'D' to Doñana (Spain). Population numbers are use to give entry to geographic characteristics of populations in Appendix A. (**B**) Weekly averages of mean air temperature (lines), mean water temperatures (symbols) and daily irradiance at the three experimental sites for the period between May 15th and September 24th 2000.
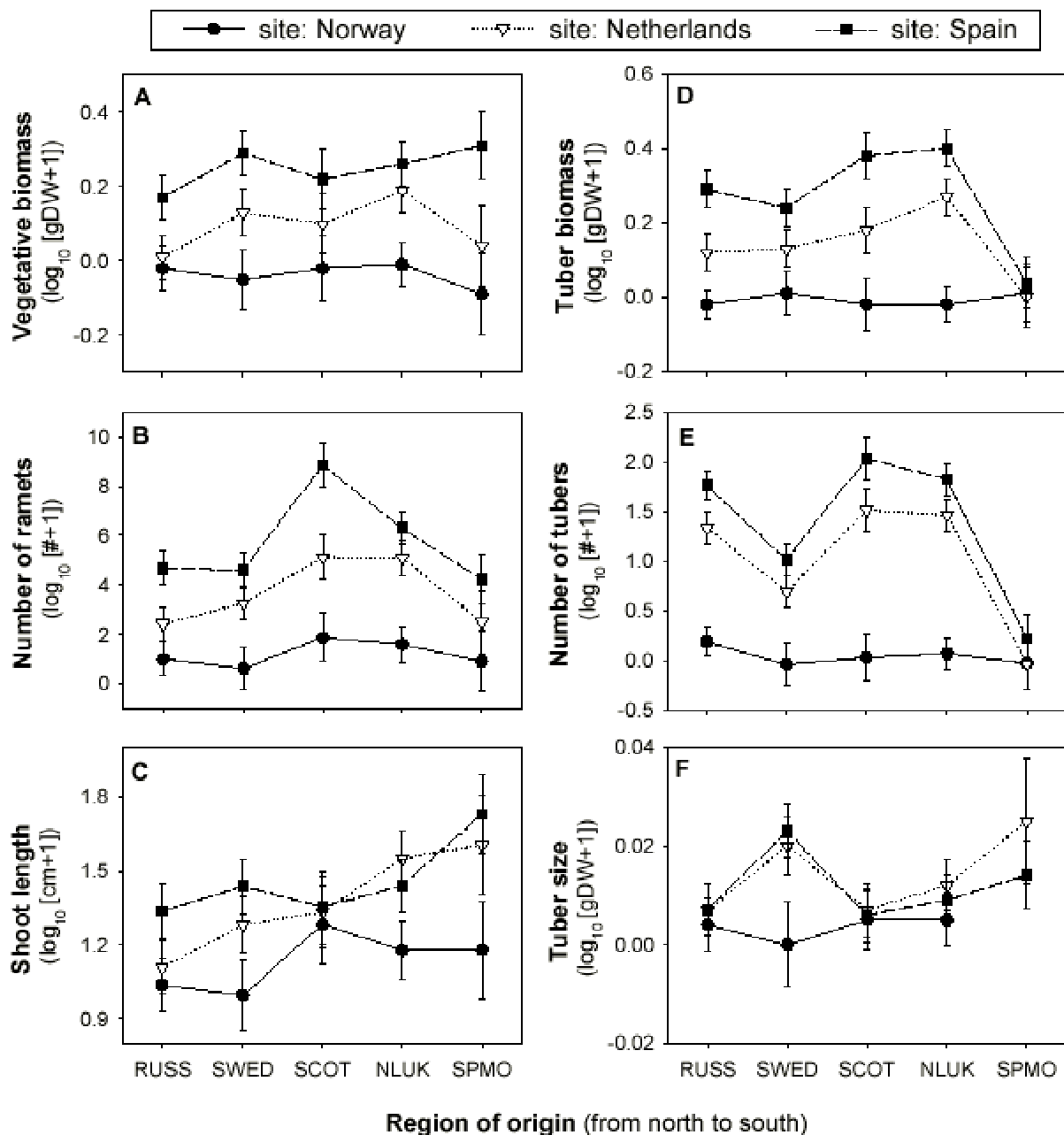
Figure 2. Vegetative biomass (**A**), number of ramets (**B**), shoot length (**C**), tuber biomass (**D**), number of tubers (**E**) and tuber size (**F**) for *P. pectinatus* genets originally collected at five different regions across Europe (RUSS: Northern Russia, SWED: Sweden, SCOT: Scotland, NLUK: The Netherlands and United Kingdom, SPMO: Spain and Morocco) and grown under common garden conditions at three experimental sites (Norway, The Netherlands and Spain). Shown are log-transformed means ± standard error. Means were computed at the covariate's mean mean ($\log_{10}$ tuber mass: 0.017; $\log_{10}$ rhizome length: 0.28).

DIGITAL APPENDICES:

L. Santamaría, J. Figuerola, J.J. Pilon, M. Mjelde, A.J. Green, T. de Boer, R.A. King, R.J. Gornall. Year. Plant performance across latitude: the role of plasticity and local adaptation. *Ecology* VOL: pp-pp.

Appendices

Appendix A: Geographic characteristics of the populations and experimental sites.
Ecological Archives **A/E/M000-000-A#.**

Appendix B: ISSR 'fingerprinting' method.
Ecological Archives **A/E/M000-000-A#.**

Appendix C: Plant cultivation methods.
Ecological Archives **A/E/M000-000-A#.**

Appendix D: Statistical methods.
Ecological Archives **A/E/M000-000-A#.**

Appendix E: Results of mixed-model General Linear Modelling.
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Appendix A. Geographic characteristics of the populations and experimental sites.

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Region	No.	Population	Latitude	Longitude	Climatic region	No. clones
Northern Russia	1	Zelonii Island	68.28°N	54.15°E	Sub-arctic	4
	2	Gusinec Shar	68.25°N	53.72°E		4
	3	Srednii Shar	68.10°N	53.63°E		3
Sweden	4	Sandviken	58.80°N	17.68°E	Temperate-cold	4
	5	Vassviken	58.82°N	17.63°E		4
	6	Sillensvik	58.82°N	17.65°E		4
Scotland	7	Loch Gelly	56.15°N	3.30°W	Temperate	3
Netherlands & UK	8	Lauwersmeer	53.33°N	6.23°E	Temperate-mild	4
	9	IJsselmeer	52.85°N	5.68°E		4
	10	Ouse Washes	52.79°N	0.37°E		4
Spain & Marocco	11	Santa Fé	38.22°N	0.58°W	Mediterranean	4
	12	El Hondo	38.12°N	0.42°W		4
	13	Veta la Palma	36.93°N	6.22°W		4
	14	Sidi Moussa	35.24°N	2.94°W		4

Experimental site	Country	Latitude	Longitude	Climatic region
Oslo	Norway	59.92°N	10.77°E	Temperate-cold
Heteren	The Netherlands	51.92°N	5.85°E	Temperate-mild
Donaña	Spain	37.00°N	6.45°W	Mediterranean

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Appendix B. ISSR 'fingerprinting' method.

To obtain ISSR phenotypes for each of the accessions, nuclear DNA was PCR amplified using inter-simple sequence repeat (ISSR) primers from the University of British Columbia primer set nine and two double-anchored primers supplied by Dr M.J. Wilkinson (University of Reading). Following an initial screen of 35 primers, four were chosen for further use. They were primer 840 ({GA }8YT), primer 841 ({GA}8YC), primer 887 (DVD{TC}8) and CAC-1 (TG{CAC}5GT). Reactions were carried out in a total volume of 25µl consisting of 16.86µl double distilled water, 2.5µl PCR buffer (Bioline), 2.5µl dNTPs (2mM), 1.25µl magnesium chloride (50mM), 0.33µl primer (15µM), 0.3125 units of BIOTAQ DNA polymerase (Bioline) and 1.5µl genomic DNA (5ng/µl). After an initial 3 min denaturing step at 93 ⁰C, reactions were amplified for 40 cycles of 93 ⁰C for 20s, 30s annealing at either 52 ⁰C or 55 ⁰C and extension at 72 ⁰C for 45s. Amplifications were run on 1.6% agarose gels and visualized by staining with ethidium bromide (0.5µg/µl). Bands were scored manually and a table of presence/absence of ISSR fragments was compiled. For nearly all samples, reproducibility of primer-phenotypes was checked by means of duplicate DNA extractions and PCR amplifications.

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Appendix C. Plant cultivation methods.

Each replicate consisted of an individual plant, grown from a tuber or a rhizome fragment, planted in 4 L pots (upper diameter: 21 cm) filled with a mixture of sand and potting clay (in a dry weight ratio of 3:1) covered with 2 cm of washed aquarium sand. At each experimental site, the pots were randomly interspersed among 9 containers (110 cm long x 95 cm wide x 65 cm high) filled with water. To minimize variability in water chemistry, given the higher nutrient load and the higher mineral content that characterize the available surface water in Spain and the Netherlands respectively, water was obtained from a nearby oligotrophic lake in Norway and pumped from nearby sandy aquifers in the Netherlands and Spain. Total-P and total-N concentrations in the sediment mixture were 290 mg P kg^{-1} and 650 mg N kg^{-1}, representing a supply of 1396 mg P and 5109 mg N per pot, enough to prevent nutrient-limitation of plant growth (Van Wijk, 1989b). Since the water supplied a minimal quantity of nutrients in comparison with the sediment, we assumed that the variability in nutrient levels in the different water sources was negligible and could be discounted. We placed the containers in open sunlight, although in Norway and Spain they received some shading in the afternoon, caused by nearby trees and buildings respectively. Each container was covered with neutral-density shading net (Agronet$^{®}$-N35; reducing light availability by 35%) to reproduce the conditions typical of the shallow lakes inhabited by *P. pectinatus* and limit the amount of debris and insects falling into the water.

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Appendix D. Statistical methods.

Dependent variables were $\log_{10}$ or square root transformed as necessary to assure normality and remove heteroscedasticity. In the ANOVAs, the fixed factor 'experimental site' was factorially crossed with the fixed factor 'region of origin', with the random factor 'population' (which was nested within 'region of origin') and with the random factor 'genet' (which was nested within 'population'). We regarded 'experimental site' and 'region of origin' as fixed factors because we were interested in their interaction with each other. In addition, we added a factor coding the type of propagule from which the individual plants grew (tubers vs. rhizome fragments) and two continuous variables to account for the size of the propagule ('initial tuber fresh weight' and 'length of the initial rhizome fragment'). For this analysis, we only used data from those plants that became established, i.e. that produced at least one ramet or one viable tuber.   Survival and fitness estimates were obtained as a single value per genet and experimental site; hence, mixed-model ANOVAs left out the factor 'genet' and its interactions, and the covariables 'type of propagule', 'average initial tuber fresh weight' and 'average length of the initial rhizome fragment' were entered as the average values per genet per site.

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Appendix E. Results of mixed-model General Linear Modelling.

	Main effects				Interactions			propagule type	propagule size	
	site (S)	region (R)	population (P)	clone (G)	S x R	S x P	S x G		tuber	rhizome
df(factor)	2	4	8	36	8	16	64	1	1	1
df(error)	19-21	9-10	19-23	73-76	17	77-80	284-296	284-296	284-296	284-296
Total biomass	163.4 ***	16.7 ***	0.3 NS	1.7 *	2.3 $	1.7 $	1.0 NS	2.2 NS	0.2 NS	2.9 $
Vegetative biomass	103.0 ***	21.1 ***	0.2 NS	1.6 *	3.1 *	1.6 $	1.0 NS	2.6 NS	0.2 NS	3.3 $
Tuber biomass	121.5 ***	11.7 ***	0.6 NS	1.9 *	7.7 ***	1.8 *	1.2 NS	0.6 NS	0.8 NS	0.5 NS
Number of tubers	272.6 ***	11.5 ***	0.8 NS	1.7 *	13 $	1.6 $	1.2 NS	2.1 NS	0.1 NS	2.0 $
Tuber size	59.0 ***	13.3 ***	2.2 $	2.3 **	21 ***	0.3 NS	1.1 NS	0.3 NS	13.2 ***	0.3 NS
Shoot-to-root ratio	16.3 ***	3.3 $	1.8 NS	1.6 *	6.9 ***	0.9 NS	1.2 NS	3.9 *	1.0 NS	1.8 NS
Shoot length	25.1 ***	8.2 **	1.2 NS	0.9 NS	2.5 $	1.6 $	2.1 ***	1.3 NS	5.7 *	2.0 NS
Number of ramets	184.7 ***	14.4 ***	1.7 NS	1.3 NS	4.8 **	1.4 NS	1.2 NS	0.7 NS	0.1 NS	2.1 NS
df(factor)	2	4	9		8	18		1	1	1
df(error)	20-21	11-13	21-24		19-21	115-118		115-118	115-118	115-118
Survival	8.5 **	8.4 **	2.7 *		0.7 NS	0.9 NS		1.0 NS	7.5 **	24.6 ***
Selection coefficients	10.7 ***	1.4 NS	3.5 **		5.3 **	1.4 NS		0.1 NS	20.1 ***	8.3 **

NOTE: Table reports F-values and significance levels. Error degrees of freedom vary within the reported ranges due to missing values in some of the dependent variables. *** P<0.001, ** P<0.01, * P<0.05, $ P<0.10.

Conclusions

1. Transport of propagules of aquatic organisms by waterbirds is a frequent process at a local scale. This transport occur both by the ingestion and defaecation of propagules and by the transport of propagules adhered to the feathers and body of the birds.

2. Bill morphology explained a significant portion of the interspecific differences in the abundance of propagules in duck faeces. Duck species with a coarse filtering structure in the bill had a higer abundance of undigested propagules in their faeces.

3. There is a high interspecific and seasonal variablity in the quantity and quality components of dispersal of *Ruppia maritima* seeds provided by waterfowl. Gizzard and grit mass were related to interspecific differences in the proportion of seeds destroyed during digestion and with the viability of seeds after ingestion.

4. *Potamogeton pectinatus* seeds germinate at a higher rate after ingestion by ducks. This effect on germination appears more related to the scaritication process in the gut than to the acidic treatment during gut passage.

5. No effects of ingestion by ducks were detected on the developent of *Potamogeton pectinatus* seedlings in experiments done in tanks exposed to climatic conditions similar to those in the field. A higher germinability of ingested seeds planted at the start of winter was detected in comparison with non ingested seeds. After a period of winter stratification in the freezer, the germinability of seeds was similar for ingested and uningested seeds.

6. The herbivory by waterfowl can have an effect on the cost-benefits trade-off resulting from *Ruppia maritima* seed ingestion by birds. Ingested and

uningested seeds produced a similar number of plants when protected from natural levels of herbivory in the field. However, a reduced number of plants originated from ingested seeds when exposed to herbivore presence.

7. The statoblasts of *Cristatella mucedo* can survive ingestion by ducks, supporting the possible role of waterfowl migration in explaining the high gene flow between populations of this bryozoan. Most propagules remained inside birds gut for a short time period (less than 4 hours), but a small number of statoblasts were retained for much longer times (up to 44 hours). Although most of the propagules will be transported over short distances, the potential exists for long-distance dispersal between localities in the migratory flyway of birds.

8. If birds start a period of fasting immediately before starting the migratory flight, this may have an effect on the potential for long distance dispersal. Experiments in captivity simulating a fasting period before migration indicate that fasting increased the number of propagules retained for long time periods (16 hours), and that were defecated after the end of fasting. Consequently, migratory fasting can increase the potential for long distance dispersal.

9. Waterfowl movements estimated from ring recoveries explained a significant amount of variance in the patterns of gene flow for three invertebrate species over North America. For another species, bird migration appeared unrelated to bird movements, probably due to a reduced resistance of propagules of this species to ingestion and dessication.

10. The capacity of tubers of *Potamogeton pectinatus* to grow over a gradient of latitudes in Europe was assimetric. Genotypes from North Europe grow without problems in southern Europe, however genotypes from southern Europe have a lower survival and poorer development when growing

under the climatic conditions of North Europe. These suggest that, whilst north-south dispersal will not be limited by the components of local adaptation studied, the dispersal from south to north will be limited by a reduced fitness compared to local genotypes.

Conclusiones

1. La dispersión por aves acuáticas de propágulos de organismos acuáticos es un hecho frecuente a una escala local. Este transporte se produce tanto a través de la ingestión y defecación de propágulos, como mediante el transporte de propágulos adheridos al plumaje o las patas.

2. La morfología del pico explicó una parte importante de la variación en el número de propágulos dispersados por vía interna. Las especies con una estructura de filtrado mas fino en el pico presentaron una mayor abundancia de propágulos no digeridos en las heces.

3. Se dio una importante variación interespecífica y estacional en la cantidad y calidad en la dispersión de semillas de *Ruppia maritima* que proporcionó cada una de las especies de aves estudiadas. El tamaño de la molleja y la cantidad de grit aparecieron como las variables morfológicas con un mayor efecto sobre la proporción de semillas digeridas y sobre la viabilidad de las semillas que sobreviven la ingestión.

4. Las semillas de *Potamogeton pectinatus* germinaron antes al ser ingeridas por patos. Estos efectos sobre la germinación parecen mayoritariamente debidos a la escarificación producida por el grit en la molleja, y no al tratamiento ácido sufrido en el tubo digestivo.

5. Los experimentos en tanques expuestos a las condiciones climáticas del campo no permitieron detectar ningún efecto de la ingestión por pato sobre el posterior desarrollo de las plántulas de *Potamogeton pectinatus*. Sólo se detectó una mayor germinabilidad de las semillas ingeridas y plantadas a principio del invierno. Después de un período de estratificación en la nevera durante el invierno, la germinabilidad de las semillas fue similar en ingeridas y no ingeridas.

6. La herbivoría por parte de la abundante población de aves acuáticas invernantes en Doñana parece afectar la balanza de costes y beneficios de la ingestión de semillas de *Ruppia maritima* por las aves. Mientras las semillas ingeridas y las control originaron un número similar de plantas en condiciones de ausencia de herbívoros, las semillas ingeridas dieron lugar a un menor número de plantas que las control al estar expuestas a los niveles naturales de herbivoría en la zona.

7. Los estatoblastos del briozoo *Cristatella mucedo* son capaces de sobrevivir la ingestión por anátidas, apoyando la hipótesis de que las aves acuáticas pueden mantener el flujo génico entre las distintas poblaciones de este organismo. Los tiempos de retención en cautividad fueron cortos (inferiores a 4 horas), pero un pequeño número de estatoblastos fueron retenidos por períodos mucho más largos de tiempo (hasta 44 horas). Esto sugiere que, aunque la mayoría de propágulos serán transportados sobre distancias cortas, parte de los propágulos podrían potencialmente ser dispersados entre localidades origen y destino del vuelo migratorio.

8. En el caso de que las aves realicen un período de ayuno antes de iniciar la migración, esto podría afectar la posibilidad de dispersión de propágulos a larga distancia. La simulación de períodos de ayuno en cautividad indicó un aumento en el número de propágulos retenidos durante períodos largos de tiempo (16 horas), que eran defecados una vez se reinició la ingestión del alimento.

9. El análisis de las recuperaciones de anillamientos de anátidas y fochas en Norteamérica indicó que los patrones de migración de las aves explicaban una fracción importante de la estructura genética en 3 de las 4 especies de invertebrados acuáticos analizados. Para estas mismas especies, la variabilidad genética explicada por los movimientos de las aves es mucho mayor que la explicada por la distancia geográfica entre las poblaciones estudiadas.

10. La capacidad de los tubérculos de *Potamogeton pectinatus* para desarrollarse en un gradiente de latitud en Europa fue asimétrica. Mientras los genotipos originarios del norte de Europa no tuvieron ningún problema para establecerse en el Sur de Europa, los genotipos del sur de Europa presentaron una menor supervivencia y desarrollo al experimentar las condiciones climáticas del Norte de Europa. Esto sugiere que, mientras la dispersión norte-sur no estará limitada por los componentes de adaptación local estudiados, la dispersión sur-norte estará limitada por una menor eficacia biológica respecto a los genotipos locales.

Crónica de una tesis

Mucha gente ha hecho posible la realización de esta tesis. Para ajustarnos a la realidad empezaré por toda la gente que participó en las maratonianas jornadas de anillamiento en el Delta de l'Ebre y Delta del Llobregat entre el 1991 y el 1998. Mucho tiempo ha pasado, pero la experiencia e información recogida en ese momento fue lo que me abrió las puertas de la EBD y lo que ha hecho posible que haya podido escribir una tesis. Por este motivo la ayuda de Albert Bertolero, Albert Manero, Dani González, Jordi Martí, Juan Diego González, Lluis Gustamante, Luís Gómez, Luís Miguel Copete, María Jesús García, Michael Lockwood, Montse Panyella, Quim Bach, Paco Cerda, Ricard Gutiérrez, Roger Jovani, Teresa Rivas, Tomás Montalvo y Xavier Riera (y muchos otros), aunque lejos de Doñana, ha sido imprescindible para la realización de esta tesis.

Durante los años de peregrinación en pos de una beca me beneficié de la ayuda y amistad de un buen número de personas.

Juan Aguilar Amat fue el primero que confió en mi en Sevilla, y gracias a él pude realizar mi tesina y dar los primeros pasos en el mundo de la investigación. Jacint Nadal me acogió en el Departament de Biologia Animal de la Universitat de Barcelona, aún a costa de alguna que otra refriega con miembros del departamento reacios a admitir que un estudiante de Barcelona tuviera un director en Doñana. El Dr. Nadal mantuvo su apoyo y le estoy agradecido por toda su ayuda y la sinceridad de todos sus consejos. También en Barcelona pude disfrutar de los consejos y ayuda de Juan Carlos Senar, al que conocí cuando él andaba liado con su tesis y que me acogió durante la Prestación Social Sustitutoria en condición de 'técnico en colorimetría en aves' y con quien pude estrechar una amistad, ya iniciada varios años atrás, e iniciar una colaboración científica que espero dure muchos años más.

Una vez terminada la tesina, se estaban acabando las excusas para seguir investigando y las esperanzas de poder iniciar una tesis. Y ahí apareció el curso del Ventorrillo; allí pude disfrutar de un espectacular ambiente científico que recomendaría a cualquiera dispuesto a iniciar una

tesis. Allí conocí a unas maravillosas personas (Pilar López, José Martín, Xim Cerdá, Iván Gómez, y muchos otros), que me brindaron toda la ayuda y el apoyo que por desgracia nunca tuve en la UB. De allí salí animado a seguir con la peregrinación en pos de una beca y gracias a un 'scholarship' de un mes de duración de la Anglo-Catalan Society, me fui con el petate a la University of Bristol con Tamas Székely, dispuesto a emigrar a tierras inhóspitas a ver si ahí había más suerte. De nuevo la experiencia fue muy positiva científicamente, pero de pelas pocas.

Y he aquí que por fin apareció Andy Green, que estaba buscando becario. A Andy le debo la oportunidad de haber realizado esta tesis. La colaboración con Andy ha sido muy buena y espero dure muchos años más. Andy se ha volcado en ayudarme y ha soportado pacientemente al pedazo animal de su becario. Estos cuatro años no han estado libres de quebraderos de cabeza para Andy: mi promiscuidad filogenética, lo variado de mis locuras científicas y la atracción de mi furgoneta hacia áreas no circulables, han sido una constante en estos años (y de lo primero creo que no te vas a librar nunca, espero!).

La llegada a Doñana fue perfecta (bueno, si obviamos el episodio d e la furgoneta, el freno de mano y el barranco). Xim e Iván me acogieron en seguida y presentaron en los círculos de sociedad de la EBD. Durante estos años he disfrutado de la amistad de todos (bueno, de la mayoría) de la gente que ha corrido por ahí. Begoña Martínez, Carlos Alonso, Claudia Keller, David Serrano, Elena Angulo, Esperanza Ursua, Eva Casado, Gema García, Isabel Luque, Iván Gómez, Javier Juste, José María Fedriani, Juan Manuel Grande, Julio Blas, Pepe Tella, Sonia Cabezas y Xim Cerdá han sido unos maravillosos compañeros de correrías por Sevilla y han estado allí tanto en los momentos buenos, como durante la plaga de mariposas. Gracias a Stefani pude instalarme rápidamente en Sevilla, ahorrándome la tediosa faena de buscar piso. El zulo se popularizó en la EBD y por él pasaron varias docenas de amigos durante sus estancias en Sevilla. Por su aguante y resistencia hay que señalar a Javier Marchamalo, pero sin ninguna duda, el campeón de resistencia fue Pedro Corbacho que subsistió en el zulo durante su estancia en Sevilla, tiempo en que me ayudó a filtrar y analizar multitud de

muestras de lodo. En esos menesteres también me beneficié de la ayuda de Marta Sánchez, Jane Orr y varios estudiantes de la Universidad de Sevilla. Rocío Fernández fue de gran ayuda en la siempre agradable tarea de abrir mollejas de pato. Iván Gómez fue tan incauto de dejarme su traje de buzo para que no pasara frío recogiendo las muestras en pleno invierno, todavía lo tengo al lado de la mesa.

En la Reserva, Rafa Laffite y Miguel Ángel Bravo facilitaron la instalación de los experimentos en el Bolín y solventaron todos los problemas que surgieron durante esta fase de mi tesis. Manolo Máñez suministró eficazmente todos los datos de censo que necesité, y que para variar siempre le pedía a última hora y con prisas. Luís García, Héctor Garrido y Fernando Ibáñez llevaron a cabo todos los censos de aves que he podido usar en esta tesis. El 'equipo lodos' (José Luís Arroyo, Antonio Sánchez, Hugo, Mari Medina) colaboraron en la recogida de muestras. Gracias a todo el personal del Coto y del Palacio, cuyo trabajo permitió que nos pudiéramos olvidar del comer y del dormir y centrarnos en batir lodo para las plantas o en contar 'semillitas'.

El Parque Nacional de Doñana permitió el uso de sus instalaciones en el Acebuche para la realización de una serie de experimentos. Celia Sánchez, Pablo Pereira y la guardería del Parque Nacional facilitaron el uso de estas instalaciones. Otros experimentos se realizaron en la Cañada de los Pájaros, gracias a la colaboración de Plácido Rodríguez y Maribel Adrían.

A Marta Sánchez, Elena Solís, Mercedes, Elvira y Raquel Alejandre les tocó pelearse con muestras de lodo y menudos de pato y demás aves acuáticas. Cristina-Belén Sánchez, Gema García, Eva Casado, Judith Hidalgo y Natividad Pérez (Matati), cuidaron de las macetas en el Bolín. Begoña Gutiérrez, Cristina-Belén Sanchez, Isabel Luque, Pedro Corbacho, Raquel Álvarez y Susana Basilio me ayudaron en los experimentos del Acebuche y Begoña tambien cuido del pato de la 11.

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ayudado en distintas fases de la realización de estos trabajos. Héctor, Julio y Cristina han realizado los dibujos incluidos en la tesis.

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