

Unsuitable habitat patches lead to severe underestimation of dynamics and gene flow in a zooplankton metapopulation

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Summary

1. Migration and re-colonization enable organisms to persist in metapopulations. Re-colonization success may be limited by the number of arriving migrants or by patch quality. In a well-studied rock pool *Daphnia* metapopulation, it is frequently assumed that re-colonization is limited by the number of arriving migrants, and that all patches are equally suitable for colonization. This assumption strongly influences how observations about dynamics, epidemiology and population genetics for the entire metapopulation are interpreted. Here we test this assumption.

2. In 627 rock pools, we found that high pH, high Ca⁺⁺ and high water conductivity were positively correlated with the presence of *D. magna*. The experimental release of *D. magna* into randomly chosen natural pools revealed the highest colonization success in pools with high pH. Next, we elevated pH and Ca⁺⁺ concentrations in natural pools by adding a system-specific natural source of calcium carbonate (either from crushed oyster shells or from eider duck droppings, which contain blue mussel shells). These treatments led to a rapid increase in pH and Ca⁺⁺ and strongly raised the likelihood that introduced *D. magna* would establish persistent populations. Therefore, we conclude that low pH and Ca⁺⁺ result in unsuitable colonization conditions in two-thirds of the untreated pools.

3. A further experiment revealed that natural colonization rates were about five times higher in calcium-treated pools than in untreated pools. Finally, we observed that eider droppings are more frequently found in the catchment area of occupied pools, than they are in those of unoccupied pools, suggesting that the blue mussel shells contained in the eider droppings play an important role in making pools suitable for colonization and in enabling *D. magna* to persist. Thus, eider ducks are ecosystem engineers in this system.

4. We recalculate typical metapopulation parameters to account for the unsuitable pools, resulting in a much more dynamic picture of this metapopulation than previously believed, with colonization rates and gene flow three to five times higher. These results have strong implications for metapopulation persistence, local and global genetic diversity, genetic rescue, gene flow and local adaptation. Our results emphasize that without verifying patch suitability, estimated rates of metapopulation dynamics can severely underestimate the true rates.

Key-words: colonization, *Daphnia magna*, ecosystem engineering, metapopulation, migration

Introduction

Many species live in spatially delimited local populations, known as metapopulations, connected by migration

(Levins 1970; Hanski & Gaggiotti 2004). Without migration, the metapopulation as a whole would go extinct, as local extinctions would continuously reduce the number of occupied patches (Hanski & Gaggiotti 2004). Classical metapopulation models regard all patches to be of equal quality with the same chance to become colonized (Levins

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1970; Hanski 1999). More complex scenarios consider that colonization is limited by the degree of spatial isolation (Hanski 1999), by local variation in patch size and number of migrants produced (Hanski 1994; Altermatt & Ebert 2010), and by the quality of migrants (Haag *et al.* 2005b). A fundamentally different type of limitation is the suitability of potential patches as habitat (Fleishman *et al.* 2002; Franzen & Nilsson 2010). In the most extreme case, unsuitable patches will not be colonized even if large numbers of migrants arrive. However, local patches may undergo temporary changes in quality (Fleishman *et al.* 2002), making them suitable only at certain times. This profoundly influences metapopulation dynamics, as patch quality becomes a limiting factor for re-colonization. Here, we address the extent to which patch quality limits colonization and how this affects the dynamics of a zooplankton metapopulation.

The successful colonization of empty patches (i.e., establishment) is a convenient way to quantify migration rates among patches, as each colonization event testifies to the arrival of at least one migrant. This method assumes that all patches offer the same quality for incoming migrants to establish themselves successfully. When patches vary in quality – so that some seemingly suitable patches are, in fact, unsuitable for colonization – estimates of metapopulation dynamics are biased: First, all observed dynamics happen in the subset of suitable patches. Thus, the entire metapopulation is smaller but has, at the same time, higher colonization rates per suitable patch. Second, a higher per suitable patch colonization rate means that more migrants arrive at already occupied patches. Increased gene flow has important consequences for the evolutionary ecology of the system (De Meester *et al.* 2002; Fleishman *et al.* 2002). Immigrants can, for example, rescue inbred local populations from extinction (Madsen *et al.* 1999; Ebert *et al.* 2002). In addition, the potential for local adaptation strongly depends on the genetic diversity of a patch (Gandon *et al.* 1996; Kawecki & Ebert 2004), and understanding gene flow is essential for the design of conservation strategies (Hedrick & Kalinowski 2000; Arroyo-Rodríguez *et al.* 2009). To estimate gene flow accurately using data on colonization of empty patches, one must know the proportion of suitable habitat patches among the unoccupied patches.

Patch suitability is linked to the niche concept, which describes the combination of environmental factors under which an organism can persist. Within the boundaries of a species' niche and given no dispersal limitation, colonization should be possible and the population growth rate of immigrants should be positive (Chase & Leibold 2003; Hooper *et al.* 2008). For aquatic organisms such as the freshwater crustaceans in the genus *Daphnia* studied here, niches are often defined by physical and chemical parameters, in particular pH and the calcium concentration. Surveys across lakes and ponds have revealed positive associations between the presence of different cladoceran species and concentrations of pH and calcium in the

water (Potts & Fryer 1979; Tessier & Horwitz 1990; Lehto & Haag 2010). Large cladocerans such as *Daphnia magna* are usually found in water with high pH and calcium levels (Potts & Fryer 1979; Ranta 1979), a link that laboratory experiments have further corroborated (Alstad, Skardal & Hessen 1999; Hessen, Alstad & Skardal 2000; Glover & Wood 2005; Hooper *et al.* 2008). However, these studies were unable to explain why many natural habitats within the range of suitable water parameters do not contain *D. magna* populations (Potts & Fryer 1979; Ranta 1979; Hooper *et al.* 2008). Either these habitats were not colonized because of dispersal limitation, or their suitability, based on a few habitat parameters, was insufficient and they were still quality limited (along with chemical and physical properties, quality includes interactions with other species, such as competition, parasitism and predation). As a third option, it is possible that habitat suitability changes over time, rendering long-term persistence impossible.

Here we test if patch quality limits *D. magna* colonization and persistence and if pH and calcium concentration are adequate indicators of patch suitability. We address three main questions: First, does patch quality play a role in the colonization success of empty patches? To answer this question, we surveyed natural rock pool populations and carried out release experiments in the field. Second, are there biotic or abiotic factors that can influence patch suitability? We looked particularly at factors that influence water pH and calcium concentration, such as the locally common droppings from eider ducks (*Somateria mollissima*), which are rich in calcium carbonate from crushed bivalves, the main food of eiders (Öst & Kilpi 1998). We hypothesized that eider droppings influence water chemistry in a way beneficial for *D. magna* colonization, making the eider ducks ecosystem engineers. Third, can natural colonization rates be elevated by manipulating patch quality? We address this question by monitoring natural colonization in ponds where we manipulated the water quality experimentally. Using these results, we update previous estimates of metapopulation parameters.

THE STUDY SYSTEM

Daphnia magna is a planktonic cyclical parthenogen. With an adult size up to 5 mm and a generation time of 7–15 days at 20 °C, it is often found in freshwater rock pools on the Skerry Islands of the Baltic Sea. *Daphnia* reproduce mostly asexually but may reproduce sexually, resulting in resting stages (=ephippia) for dispersal and to endure harsh winter conditions or summer droughts. In the Tvärminne archipelago of south-western Finland where our study was conducted, an average of 17% of pools are occupied by *D. magna*; 16% of the occupied pools go extinct per year, and re-colonization approximately balances this loss in the long run (Pajunen 1986; Pajunen & Pajunen 2003).

Factors that influence *Daphnia* habitat characteristics include interspecific competition, the presence of parasites and predators, isolation in space, salinity, pH, organic matter and pool size (Ranta 1979, 1982; Pajunen 1986; Weider & Hebert 1987; Wilson & Hebert 1992; Bengtsson 1993; Bengtsson & Ebert 1998; Ebert, Hottinger & Pajunen 2001; Pajunen & Pajunen 2007). Many of these factors are correlated with each other (Ebert, Hottinger & Pajunen 2001; Pajunen & Pajunen 2007), making it difficult to determine cause and effect relationships. Here we focus on calcium concentration and pH, which are positively correlated in natural lakes and ponds (Hooper *et al.* 2008), as in our metapopulation (Pajunen & Pajunen 2007). Ca^{++} and pH of Baltic rock pools are influenced by different factors. The granite bedrock of the islands in our study area usually contains little calcium, so rainwater collected from the catchment area is low in calcium and slightly acidic (Lehto & Haag 2010). One source of calcium is the brackish water of the Baltic Sea ($85 \text{ Ca}^{++} \text{ mg L}^{-1}$), which waves and spray bring into the rock pools. Droppings of eider ducks are the second main source of calcium. The ducks feed predominantly on blue mussels (*Mytilus edulis*), and their droppings, which contain large amounts of crushed mussel shells, are found in the catchment area of the pools. The islands serve as breeding and resting habitat for the birds. Both calcium sources (sea water and duck droppings) influence pools closer to the shoreline more than pools further inland. The pH of pool water may be as low as 4, resulting from run-off water from peat bogs in forests further away from the sea. Calcium carbonate from eider duck droppings may buffer low pH.

Materials and methods

SPATIAL PATTERN OF WATER CHEMISTRY AND *D. MAGNA* DISTRIBUTION

In summer 2000, we visited 627 rock pools on 20 islands of the Tvärminne archipelago ($59^{\circ}50'35 \text{ N}$, $23^{\circ}15 \text{ E}$) and recorded the presence and absence of *D. magna*, water pH (using a KCl electrode) and conductivity (using a Consort C531, Turnhout, Belgium). We quantified Ca^{++} concentration using a colorimetric titration kit (Merck, Darmstadt, Germany; detection limit: 1 mg L^{-1}). Sixteen of these islands belong to a long-term study area, and some of these data have been used in an earlier publication (Pajunen & Pajunen 2007).

RELEASE EXPERIMENT 1: COLONIZATION INTO UNTREATED ROCK POOLS

The aim of this experiment was to test whether natural rock pools without *D. magna* populations are suitable for colonization with *D. magna*. Absence of *D. magna* was checked using small hand nets (0.2-mm mesh size) that we passed several times through the water, reaching into every part of the pool. If five to eight trials did not reveal any *Daphnia*, we scored the rock pool as being free of *D. magna*. The study included 29 rock pools on two islands (9 on Branskär, 20 on Furuskär). We excluded pools

that showed factors known to make them unsuitable for long-term persistence of *D. magna*, i.e. pools with fish, pools with intensive plant growth (which dry up within 2 weeks without rain), pools very close to the shoreline, pools with very dark water (bog type; typically at the edge of the forest) and pools less than 10-cm deep (high desiccation risk). In the 29 study pools, we measured water conductivity and pH. We quantified water colour, which can range from clear to dark brown depending on the inflow of water from surrounding bogs, as in Ebert, Hottinger & Pajunen (2001). We estimated pool volume by assuming the pool to have the shape of an inverted pyramid (longest length \times longest width \times greatest depth/3).

Four days after taking these measurements, we released 200 parasite-free *D. magna* into each pool. These animals had been propagated asexually in 100-L mesocosms filled with natural rock pool water and placed outside close to natural rock pools. The pH of the mesocosms was 7.6. Each rock pool received animals from all mesocosms, constituting the same mix of ages and genotypes. Ten days after the release, we checked all pools for the presence or absence of *D. magna* as described above.

RELEASE EXPERIMENT 2: ESTABLISHMENT SUCCESS AFTER OYSTER SHELL ADDITION

To test if water pH determines the success of *Daphnia* colonization, we manipulated 28 of 55 rock pools by adding calcium carbonate as crushed oyster shells to increase the pH and calcium concentrations in the water. We used bivalve shells because they are a natural component of this ecosystem. Commercially available oyster shells (Oytaco, Frederikssund, Denmark) constitute nearly 100% crushed shells (mainly calcium carbonate), with 36% calcium and 5% ash, insoluble in HCl. This experiment took place on the same islands as release experiment 1, but 2 years later. Pools were chosen based on the same criteria as before. None of the pools contained *D. magna* in the year this experiment started, or in the previous year. Twelve rock pools that failed to become colonized in release experiment 1 were also used in release experiment 2, but were equally distributed across islands and treatments.

Prior to the experiment, we measured water conductivity, pH, water colour, Ca^{++} concentration, pool dimensions and recorded the pool's distance to both the sea and to the next tree following Ebert, Hottinger & Pajunen (2001). Pools were assigned to two treatment groups by balancing locations (within and between islands), distance to the sea and trees, pool volume, pH, calcium concentration, conductivity and water colour, such that none of these variables differed among the two treatment groups (all *T*-tests $P > 0.25$). One of the two groups of pools was assigned to be a control by coin toss; the other was assigned to receive crushed oyster shells. Pools in the oyster shell group received 1 kg material evenly spread over the water surface.

To verify that the oyster shell material was free of *Daphnia* resting eggs, we placed 200 g of the shells in each of fifteen 10-L buckets, added 6 L rock pool water and placed them near natural rock pools. Five of these buckets (positive controls) were also treated with about 200 g of sediment from natural rock pools containing *D. magna* resting eggs. No *Daphnia* hatched in buckets treated with only oyster shells, whereas all five positive controls had hatchlings.

A week after we applied the treatment (June 2000), we again measured water chemistry and released *c.* 200 *D. magna* (from

mesocosms, as before) into each pool. We then left the pools alone until we checked for *D. magna* in August 2000 and again in May 2001 and May 2002. On each sampling date, but the last, we also measured water chemistry.

RELEASE EXPERIMENT 3: ESTABLISHMENT SUCCESS AFTER ADDITION OF EIDER DUCK DROPPINGS

To test if the calcium-rich droppings of the eider duck (*Somateria mollissima*) influence water quality, we collected about 15 kg of eider droppings on islands outside our study area. The average dry weight of an eider dropping is 13.9 g (range 10–18 g, $n = 80$). Droppings were dried in the sun, and a homogenous mixture was produced. We then tested this material for the absence of *Daphnia* resting eggs using the experimental procedure described above for the oyster shells. No *Daphnia* were hatched in buckets containing only eider droppings, whereas all positive controls had hatchlings.

We chose 30 pools on the islands of Branskär, Sundholmen and Furuskär that fulfilled the criteria outlined above and assigned them to two treatment groups as described for experiment 2. We added 700 g of eider dropping material to half of the pools and measured pH, calcium concentration and conductivity both before (July 21, 2002) and after the application of the treatment (July 26, 2002, August 12, 2002, May 15, 2003 and July 14, 2003). On July 26, 2002, we released 100 *D. magna* into each pool and checked for their presence whenever we took water samples. In July 2003, one pool had fallen dry and one had *Daphnia* but not enough water for chemical analysis. The strong results of release experiment 2 encouraged us to decrease the amount of material added to the treated pools and to half the number of released *Daphnia*.

QUANTIFICATION OF NATURAL COLONIZATION IN TREATED POOLS

Seventy-one unoccupied pools on nine islands were chosen as for the previous experiments. Pools were assigned randomly to two treatment groups. Twenty-five were left untreated, whereas we added 1 kg of oyster shells to each of the 46 remaining pools. One year later, all pools were visited and checked for the presence of *D. magna*.

SURVEY FOR D. MAGNA AND EIDER DUCK DROPPINGS

To further investigate the possible link between eider ducks and rock pool suitability, all rock pools in our long-term *Daphnia* metapopulation study area (Pajunen & Pajunen 2003) were checked for the presence or absence of *D. magna* in early August 2010. In mid-August 2010 (10–12 days after the last rain), we visited all islands with at least one population of *D. magna* (10 islands) and recorded the presence of eider droppings in the catchment area of the pool. This survey needed to be done during a period without rain, as rain washes the droppings into the rock pools.

STATISTICAL ANALYSIS

If not specified otherwise, statistics were done with the software package R (version 2.15.1)(R Development Core Team 2008).

Continuous variables were examined for their distribution and, in the case of Ca concentration and conductivity, were \log_{10} transformed.

We used the generalized linear model (GLM) in R to test for associations between *D. magna* and quantitative pool variables [e.g. model <- glm(daphnia ~ ph, family = binomial, data = dat-new), summary(model)]. Models were examined for possible overdispersion, which was negligible, so uncorrected standard errors were used. Spearman rank correlations were used to correlate abiotic parameters from the field survey on water chemistry. Data in 2×2 contingency tables were analysed with Fisher exact tests and odds ratios and 95% confidence intervals were calculated.

The treatment effects on water chemistry in release experiments 2 and 3 were analysed using mixed models. pH and $\log_{10}(\text{Ca}^{++} + 1)$ were normally distributed. Treatment and sample date were included as fixed effects (nominal data structure). Individual pools were nested in treatment and assigned random effects. The interaction of treatment and sampling date was included. This mixed model analysis was performed using the statistics software JMP (version 9.0.3). The presence/absence data of *D. magna* in these rock pools were analysed for each of the three samples separately using Fisher exact tests. We used the Holm- (sequential) Bonferroni method to correct for the family-wise error rate.

Results

SPATIAL PATTERN OF WATER CHEMISTRY AND D. MAGNA DISTRIBUTION

Water chemistry across the 627 rock pools varied strongly: pH varied from 4.38 to 11.03 (mean = 8.33), calcium ranged from below 1 (i.e., undetectable) to 332 mg L⁻¹ (mean = 26.1, median = 16.0) and water conductivity ranged from 12 to 34 200 $\mu\text{S cm}^{-1}$ (mean = 1780, median = 470). All three parameters were correlated with the presence of *D. magna*, such that pools with higher pH (GLM: Slope \pm SE = 0.159 \pm 0.073, z -value = 2.17, $P = 0.03$), higher $\log_{10}(\text{Ca}^{++} + 1)$ (0.680 \pm 0.197, z -value = 3.46, $P = 0.0005$) and higher $\log_{10}(\text{conductivity} + 1)$ (0.399 \pm 0.137, z -value = 2.93, $P = 0.0035$) were more likely to harbour a *D. magna* population (Fig. 1). All three parameters were positively correlated with each other (Spearman correlations: pH – Ca^{++} : $\rho = 0.50$, $P < 0.0001$; pH – conductivity: 0.34, $P < 0.0001$; conductivity – Ca^{++} : 0.78, $P < 0.0001$).

RELEASE EXPERIMENT 1: COLONIZATION INTO UNTREATED ROCK POOLS

Ten days after releasing *D. magna* into 29 rock pools, we found living animals in 13 pools (45%). The presence of *D. magna* was positively correlated with pH (GLM: Slope \pm SE = 2.244 \pm 1.02, z -value = 2.20, $P = 0.028$; Fig. 2). The presence of *D. magna* was negatively correlated with water colour (GLM: Slope = -5.97 \pm 3.02, z -value = -1.98, $P = 0.048$; Fig. 2), with dark water pools hosting fewer *D. magna* populations and having lower pH (Spearman rank correlation: $\rho = -0.64$, $n = 29$,

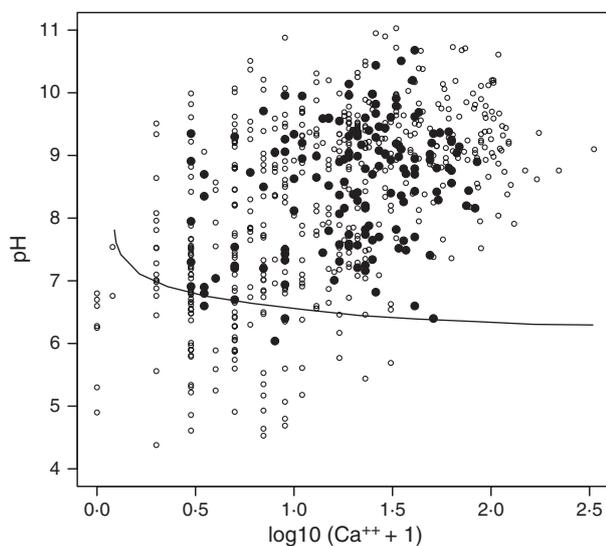


Fig. 1. Scatter plot of pH and Ca^{++} concentration (mg L^{-1}) in pools occupied by *Daphnia magna* (filled circles) and pools without *D. magna* (open circles). The solid line is the zero growth line for *D. magna* from Hooper et al. (2008), below which population growth was reported to be negative.

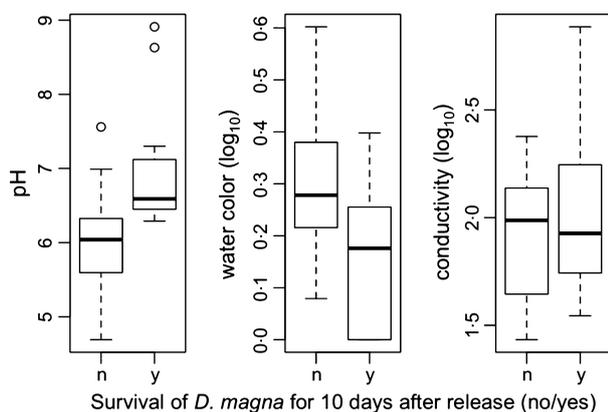


Fig. 2. Box-whisker plots for pH, water colour and conductivity for pools in which released *Daphnia magna* survived for 10 days or disappeared (release experiment 1). pH and water colour significantly explained the survival of *Daphnia* but not conductivity (see text for details). The boxes show the median (thick line) and the 25th and 75th percentiles, respectively. The whiskers show the maximum and minimum values, or, in case of outliers (shown as open circles), 1.5 times the interquartile range of the data.

$P = 0.0002$). Water conductivity did not explain *Daphnia* establishment success (GLM: Slope = 1.187 ± 1.12 , z -value = 1.06, $P = 0.29$; Fig. 2) and did not correlate significantly with either water colour (Spearman $\rho = -0.29$, $P = 0.12$) or pH ($\rho = 0.16$, $P = 0.41$). Ca^{++} was not assessed in this experiment.

RELEASE EXPERIMENT 2: ESTABLISHMENT SUCCESS AFTER OYSTER SHELL ADDITION

The treatment with crushed oyster shells resulted in a highly significant increase in pH (1–1.4 units) and calcium

concentration (5–20 mg L^{-1} ; Table 1, Fig. 3). In spring, water quality is influenced by snowmelt, which led to a drastic Ca^{++} decline (often below the detection limit of 1 mg L^{-1}) in control pools in 2001, but had hardly any impact on treated pools (Fig. 3). Strong temporal dynamics of pH were visible, with changes in pH over up to two units (Fig. 3). Over 2 years, *Daphnia* persisted much better in pools treated with oyster shells than in untreated pools (Table 2A, Fig. 4), indicating a long-lasting effect on patch suitability. All pools with persisting *Daphnia* populations had pH values above 6.5.

RELEASE EXPERIMENT 3: ESTABLISHMENT SUCCESS AFTER ADDITION OF EIDER DUCK DROPPINGS

Eider droppings had the same effect on the rock pool water as the addition of oyster shells in the previous experiment. pH and Ca^{++} increased as soon as a week after treatment (Table 1, Fig. 3). Rock pools treated with eider droppings showed much higher *Daphnia* establishment rates than untreated pools (Table 2B, Fig. 4). The relatively low value for treated pools on May 2003 may be due to the fact that our assessment took place relatively early in the season. Spring emergence of *Daphnia* from resting eggs can be delayed by 2–3 weeks in cold springs. The lowest pH value recorded in a pool with a *D. magna* population was 6.5 (4.4 without *Daphnia*).

QUANTIFICATION OF NATURAL COLONIZATION IN TREATED POOLS

Of the 25 untreated pools, 12% were colonized by *D. magna* 1 year later. This is significantly less than pools

Table 1. Fixed-effect tests for treatment and sample effects in the oyster shell (A) and eider dropping (B) experiments. Only samples after application of the treatment are included. Sample and treatment are fixed effects; individual pools are nested in treatment (random effect). See Fig. 3 for a graphical display

Response variable	Source	DF	DF _{Den}	F Ratio	$P > F$
(A) Release experiment 2: oyster shells					
pH	Treatment	1	53.09	35.24	<0.001
	Sample	2	104.3	14.67	<0.001
	Treatment* sample	2	104.3	1.85	0.161
$\log_{10}(\text{Ca}^{++} + 1)$	Treatment	1	53.22	159.49	<0.001
	Sample	2	104.9	52.27	<0.001
	Treatment* sample	2	104.9	24.87	<0.001
(B) Release experiment 3: eider droppings					
pH	Treatment	1	28.24	12.50	0.0014
	Sample	3	82.39	18.02	<0.001
	Treatment* sample	3	82.39	2.77	0.0466
$\log_{10}(\text{Ca}^{++} + 1)$	Treatment	1	28.29	109.54	<0.001
	Sample	3	82.56	59.11	<0.001
	Treatment* sample	3	82.56	19.11	<0.001

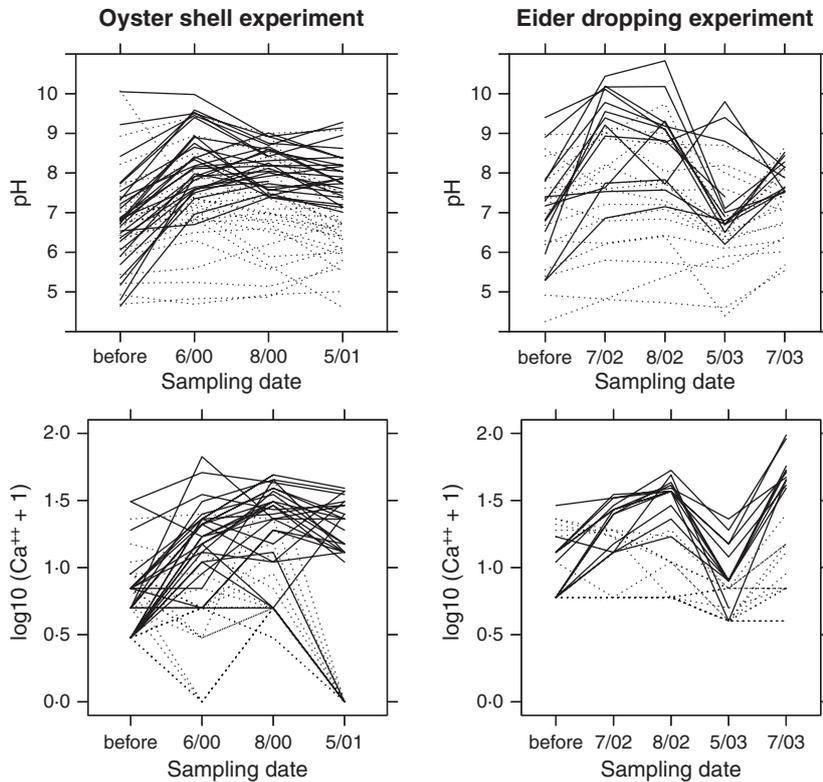


Fig. 3. Changes in pH and Ca^{++} (mg L^{-1}) over time in pools treated with crushed oyster shells (solid lines, left panels) or untreated controls (dashed line, left panels) and in pools treated with eider duck droppings (solid lines, right panels) or untreated controls (dashed lines, right panels). 'Before' indicates pH and Ca^{++} before the treatment was applied (about a week before the second data assessment); these samples are not included in the statistical analysis in Table 1.

Table 2. Records and Fisher exact tests for *Daphnia magna* presence and absence in release experiments 2 (A) and 3 (B). Uncorrected *P* values are given for each date. All tests remain significant at $P < 0.05$, after Holm-(sequential) Bonferroni correction for the family-wise error rate. We lost two replicates due to tectonic activity in winter 2001, and several pools during dry spells in May 2002 and July 2003.

Treatment		Sampling date		
(A) Release experiment 2: oyster shells				
		August 2000	May 2001	May 2002
Oyster shells	<i>Daphnia</i> present	22	19	17
	<i>Daphnia</i> absent	6	7	7
Control	<i>Daphnia</i> present	10	6	5
	<i>Daphnia</i> absent	17	21	20
Total number:		55	53	49
	<i>P</i> -value (Fisher test)	0.0026	0.0003	< 0.0001
(B) Release experiment 3: eider droppings				
		August 2002	May 2003	July 2003
Eider dropping	<i>Daphnia</i> present	11	6	9
	<i>Daphnia</i> absent	2	7	3
Control	<i>Daphnia</i> present	1	1	1
	<i>Daphnia</i> absent	16	16	16
Total number:		30	30	29
	<i>P</i> -value (Fisher test)	< 0.001	0.025	< 0.001

treated with oyster shells (41%; Fig. 5; Fisher exact test, $P = 0.015$), yielding an odds ratio of 5.05 (95% confidence interval: 1.24–30.1).

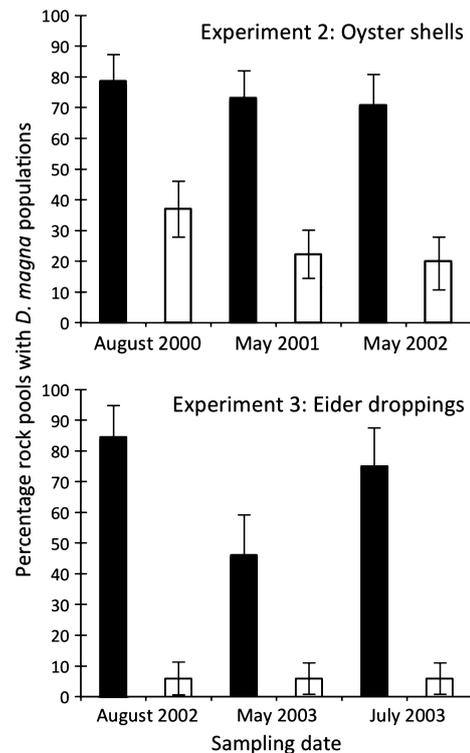


Fig. 4. Percentage of rock pools in which *Daphnia magna* established populations following treatments with oyster shells (upper graph, release experiment 2) or eider droppings (lower graph, release experiment 3). Treatment studies (\pm binomial errors) are shown in black bars; controls (without treatment) with open bars.

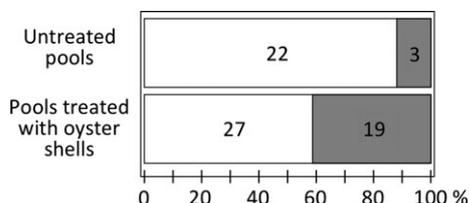


Fig. 5. Mosaic plot showing natural colonization (in grey) in treated vs. untreated rock pools. Untreated pools were colonized less often than treated pools. Numbers inside each field are the number of rock pools in each combination.

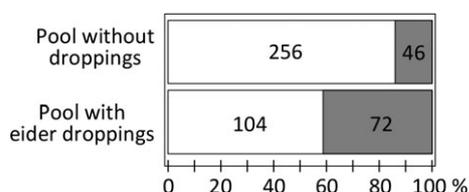


Fig. 6. Mosaic plot showing results of a rock pool survey on 10 islands to determine the presence of *Daphnia magna* in rock pools (in grey pools with *Daphnia*) and eider duck droppings in the catchment area of the pool. Pools without eider droppings in the catchment area are less likely to have *D. magna* populations. Numbers inside each field are the number of rock pools studied.

SURVEY FOR *D. MAGNA* AND EIDER DUCK DROPPINGS

Our surveys of rock pools that had both *D. magna* populations and eider duck droppings in the catchment area confirmed our hypothesis of a positive association [Fig. 6; Fisher exact test, $P < 0.00001$; odds ratio 3.84 (95% confidence interval: 2.44–6.09)].

ESTIMATING METAPOPULATION PARAMETERS

The results of our experiments allow us to adjust previous estimates of metapopulation parameters (Pajunen 1986; Pajunen & Pajunen 2003). To do this, we considered the set of pools included in these earlier studies to represent the entire set (i.e. 100%) and adjusted the estimates for the revised metapopulation relative to this. The revised metapopulation is the subset of pools suitable for *D. magna* (summarized in Table 3).

Of a total of 73 untreated pools into which we released *D. magna* in three different *D. magna* release experiments, 49 (67%) failed to establish populations (binomial confidence interval (b.c.i.) 56.2–77.8%). Excluding six pools (four of which had failed) from the second release experiment that had already been used in the first experiment did not change this result: 45 of 67 failed (67%, b.c.i.: 54.6–78.2). Thus, only 33% of the unoccupied pools were suitable for *D. magna*. To calculate the proportion of all pools suitable for *D. magna*, we accounted for the proportion of occupied pools. The long-term average for this proportion is 17% (Pajunen & Pajunen 2003). Of the

Table 3. Parameter estimates of the previous (old) and revised *Daphnia magna* metapopulation model. The revised model is based on a reduced number of pools after unsuitable pools are excluded. bci: binomial confidence interval

	Estimate
Old Model (all pools considered suitable)	
Proportion of all unoccupied pools considered suitable	100%
Average proportion of pools occupied (Pajunen & Pajunen 2003)	17%
Colonization (per year) of unoccupied pools	
Experimental data (this study; colonization of untreated pools; 3 of 25)	12% (bci: 2.6–31%)
Observational data (Table 2, Pajunen & Pajunen 2003). Mean and range of yearly estimates	4.47% (1.7–8.5)
Extinctions of populations (per year; Pajunen & Pajunen 2003)	16%
Revised model (only a portion of pools are suitable)	
Proportion of all unoccupied pools which are suitable (bci: 21.8–45.4)	33%
Proportion of all pools which are suitable	44.5%
Proportion of suitable pools which are occupied	38.2%
Colonization (per year) of suitable and unoccupied pools	
Experimental data (this study; colonization of pools made suitable with oyster shells; 19 of 46)	41% (bci: 27–56%)
Observational data [from Table 2 of Pajunen & Pajunen (2003) and corrected by accounting that only 33% of unoccupied pools are suitable; $4.47 \times (100/33) = 13.5$]	13.5%
Extinctions of populations (per year; Pajunen & Pajunen 2003)	16%

remaining 83% of unoccupied pools, one-third (33%) is suitable. Thus, we can add to the 17% suitable pools a further 27.5% ($33/100 \times 83 = 27.5$), resulting in an estimated 44.5% ($=27.5 + 17$) of all pools suitable for *D. magna* colonization. With these calculations, we were able to estimate the proportion of occupied pools among all suitable pools to be 38.2% ($17/44.5 \times 100$).

Our quantification of natural colonization in manipulated pools resulted in an estimate of 41% (19 of 46) colonization in treated (putatively suitable) pools and 12% (3 of 25) for untreated pools. Thus, whereas 12% is an estimate for the colonization rate in Pajunen's (1986) model, we obtain 41% as an estimate for the colonization rate of the revised model (assuming that all pools treated with oyster shell are suitable). Twelve per cent (b.c.i.: 2.6–31%) is considerably, but not significantly, higher than the 4.47% average for the years 1983–1998 (Pajunen & Pajunen 2003). The year in which we performed our experiment (2003–2004) had the highest recorded annual colonization rate in our study population (Altermatt, Pajunen & Ebert 2008), possibly explaining the high colonization rates in our experiment. Another way to express colonization rates

in our revised model is by considering them in relative terms. This can be done in two ways: First, the number of natural colonizations in pools treated with oyster shells suggests a fivefold increase (ODDs ratio 5.05, confidence interval: 1.2–30.1). Second, across all three release experiments, *D. magna* failed to establish populations in 67% of releases into untreated pools. Not counting these 67% unsuitable pools, thus, the colonization rate is threefold higher in the revised model. Together, we arrive at an estimated colonization rate for the revised model that is roughly three- to fivefold higher than the previous estimate. Finally, the number of extinctions remains the same in the revised model, with an average of 16% extinctions per year.

Discussion

We asked three questions in the introduction and can now answer all three questions affirmatively. Our field survey and the release experiments showed that pools with high pH and Ca^{++} are more likely to have *D. magna* populations, and that the artificial increase of these factors increased colonization, establishment success and persistence drastically. The experimental addition of eider duck droppings, which are often found in the catchment area of natural pools, was shown to make pool water suitable for colonization by *D. magna*. Finally, natural colonization rates were higher in pools treated with crushed mussel shells than in untreated pools, suggesting that water quality limits the colonization of unoccupied pools. Together, these data suggest that the metapopulation is only about half as big as previously assumed (Table 3) and, as a consequence, much more dynamic. Gene flow within the metapopulation is higher than previously assumed, which has important consequences for the evolution of the system. Our study highlights that if one estimates key parameters of metapopulation dynamics by counting colonization events without verifying the suitability of the potential patches, parameter estimates will potentially underestimate the true rates substantially.

POOL QUALITY, COLONIZATION AND GENE FLOW

Previous studies of the *Daphnia* rock pool metapopulation assumed that the colonization of empty pools was limited by the number of migrants (Hanski & Ranta 1983; Huguény, Cornell & Harrison 2007). This assumption is consistent with the finding that isolated pools have lower colonization rates than pools near occupied patches (Pajunen 1986; Pajunen & Pajunen 2003). Differences in patch quality had been discussed before, but were considered to play a minor role (Ranta 1979, 1982; Hanski & Ranta 1983; Pajunen & Pajunen 2007). However, our current study shows that patch quality, in contrast, plays a rather important role in determining the distribution and patch occupancy of *D. magna*.

Pools stocked with oyster shells or eider droppings became colonized by natural immigrants much more

frequently than untreated pools, confirming that natural colonization is at least partially limited by habitat quality. Thus, more immigrants arrive at pools than previously estimated by gross colonization rates, which included about 55% unsuitable pools (Hanski & Ranta 1983; Pajunen & Pajunen 2003). Release experiments and the field survey revealed that populations only persisted in pools with $\text{pH} \geq 6.5$ and Ca^{++} concentrations of $\geq 2 \text{ mg L}^{-1}$. Surprisingly, pools with calcium and pH levels that were too low represented only ~17% of all pools in our study area (Fig. 1), although the total proportion of unsuitable pools was about 55% (Table 3). Thus, although *D. magna* requires pH and calcium levels to be within the right chemical range to establish a population, these niche dimensions are not sufficient to explain patch suitability. The same may be true for the large proportion of unoccupied ponds reported in Great Britain with pH and Ca^{++} levels in the right range (Potts & Fryer 1979). While we found that pools are indeed limited by patch quality, our data are also consistent with some degree of limitation by other factors, including number of immigrants. About 60% of pools treated with oyster shells in experiment 2 were not colonized within a year, possibly because no migrants arrived at these pools. It is likely that most of these pools were of appropriate quality, as only about 20% of treated pools in our release experiment failed to be colonized. Thus, our data do not invalidate the assumption that the number of migrants limits colonization, but places it side-by-side with water quality as a limiting factor. A plausible scenario is that pool quality is limiting when inter-pool distances are small and migrants are more abundant, whereas migration may limit colonization of isolated pools.

Our revised model suggests a colonization rate of empty pools roughly three- to fivefold higher than previously believed. This is also a more accurate estimate of the arrival rate of migrants at extant pools, suggesting that gene flow is also three- to fivefold higher than previously assumed. Understanding the magnitude of gene flow in metapopulations is important for studying the drivers and consequences of local genetic diversity (Haag *et al.* 2005a; Altermatt & Ebert 2008), genetic rescue of inbred populations (Ebert *et al.* 2002) and local adaptation (Kawecki & Ebert 2004; Altermatt, Hottinger & Ebert 2007). But this finding is also of general interest for understanding the evolutionary genetics of metapopulations in which gene flow among patches is a central issue (De Meester *et al.* 2002; Hanski & Gaggiotti 2004).

ROCK POOLS ARE CONSTANTLY CHANGING

Half of the pools previously considered to be suitable habitat for *D. magna* are, in fact, suitable. A key question for understanding the overall dynamics of the system is now whether suitability is a permanent feature of a habitat patch or whether it changes over time. If pools change in quality to become uninhabitable, it may explain in part

the high local extinction rate [16% per year (Pajunen & Pajunen 2003)] observed in this metapopulation. Thus, pool quality would not only be a predictor for colonization success but also for persistence and extinction.

Freshwater rock pools are inherently unstable. On the Skerry islands of the Baltic Sea, pools can only exist in a rocky belt between the shore line and the plant-dominated interior of the islands. The width of this belt ranges from about 0.5–10 m above sea level (but may be up to 80-m wide, depending on the slope of the shore). A pool protected from the sea would quickly be invaded by the roots of perennial plants, drastically increasing evaporation (Altermatt, Pajunen & Ebert 2009), or would accumulate organic matter eventually turning the pool into a bog. The geology of the rock pool belt is also influenced by post-glacial uplifting (Kakkuri 1987) and long-term sea-level changes (Leppäranta & Myrberg 2009), but these processes are slow relative to the strong dynamics of the metapopulation itself.

The interplay of the sea and terrestrial plants determines the pH and calcium of the pools. Humic acids from plants and bogs cause a drop in pH after strong rainfalls or melting snow. On the other hand, calcium arrives into the pools with seawater waves and spray. Both factors are strongly affected by weather conditions (e.g. rain, wind, evaporation), are highly dynamic and cause changes in water quality across the seasons (Fig. 2). Eider droppings seem to have a buffering effect on these dynamics. Individual pools can accumulate sediments of up to 20 cm of mussel shells from eider droppings (D. Ebert, V.I. Pajunen, pers. observation). Pools without eider droppings are much less suitable for *D. magna*, making eider ducks an ecosystem engineer for this metapopulation. Consistent with this is our finding of a positive correlation between eider droppings in the catchment area of pools and the presence of *D. magna* in the pool. This correlation might be stronger than reported here, as our assessment is based on a single time point, whereas mussel shells are long lasting, which ensures that they remain present even when pools dry up (Altermatt, Pajunen & Ebert 2009). Thus, eider droppings can be a long-lasting buffer against low water pH and low calcium levels, even across summer drought and harsh winters, and they widen the belt around the islands in which suitable rock pools are found. Without this effect, the number of suitable pools would presumably be much lower, maybe even below the minimal number and density necessary to maintain the metapopulation on a given island.

The impact of eider ducks is, not only variable across islands but also across time. During the nesting season, eiders seek cover on forested islands, whereas later in the season they prefer more exposed islands. Terrestrial predators such as fox and mink threaten ducks in and close to the forest, whereas avian predators such as the sea eagle and eagle owl, threaten them more on the open islands (Kilpi & Öst 2002; Jaatinen, Öst & Lehtonen 2011). Eider ducks may change their roosting sites in

response to the dominant predators. Mink invaded the region only during the last century, and bird predators went through population crashes in the last 100 years. For example, the sea eagle (*Haliaeetus albicilla*) population recovered dramatically from near extinction in our study area, which caused a strong increase in predation on eiders on open islands (Jaatinen, Öst & Lehtonen 2011). A speculative chain of events is that humans influence the sea eagle population, which influences the eider duck distribution, which influences rock pool water chemistry, which ultimately influences *D. magna* distribution.

Our discussion highlights the dynamic nature of the rock pool habitat and how it is influenced by biotic and abiotic factors. Although we identified some of the factors that make patches suitable for colonization, our study does not explain the high local extinction rates observed in this metapopulation (Table 3). It is currently unclear whether *D. magna* populations go locally extinct because pools become unsuitable, or if intrinsic factors, such as parasitism, competition and inbreeding, lead to extinction. Distinguishing among these alternatives is of fundamental interest for our understanding of metapopulation biology (Fleishman *et al.* 2002; Hanski & Gaggiotti 2004; Franzen & Nilsson 2010).

PH AND CALCIUM CONCENTRATION DO NOT EXPLAIN EVERYTHING

Because eider droppings are a system-specific feature of this ecosystem and influence calcium concentration and pH simultaneously, we therefore did not attempt to separate the effects of pH and calcium. Considering the distribution of both parameters (Fig. 1), low pH seems to play a stronger role than low calcium in explaining the distribution of *D. magna* in the metapopulation. Plotting the calcium and pH data on the same plot with the zero population growth trajectory reported by Hooper *et al.* (2008) shows that pH has a strong influence on *D. magna* population growth over the relevant parameter space in our study area, whereas calcium's impact is weak. In contrast to pools with a pH below 6.5 (the tolerance level for *D. magna*), pools with very low calcium concentrations are not common except for short periods such as after snow melt (Fig. 1). The zero growth trajectory captures our data rather well, with only a few occupied pools falling below, but close to, the line. Our data also correspond well to those from Potts & Fryer (1979) in Great Britain, who found *D. magna* only in ponds with pH ranging from pH 6.9 to 10.2 and with Ca^{++} per L from 5 to 164 mg.

During our experiments, about 20% of pools treated with oyster shell and eider dropping failed to establish populations from artificially introduced *D. magna* (Table 2). As pH and calcium in treated pools responded quickly to treatment (Fig. 2), this suggests that other factors may also prevent *Daphnia* from establishing populations. Pajunen & Pajunen (2007) and Ranta (1979) reported weak correlations between the presence of

D. magna and indicators of primary production, which is mostly influenced by nutrients from bird droppings. This may relate to our experiment, as we observed initially a slightly higher (but not significant) establishment rate in the experiment using nutrient-rich eider droppings than in treatments using nutrient-poor oyster shells (Fig. 4). Stochastic events may also explain the failure of *D. magna* establishment in some treated rock pools. However, although we cannot exclude this possibility, we consider it unlikely, as we never observed failed colonizations under similar conditions in several outdoor mesocosm experiments at our field site (for example Haag & Ebert 2004; Altermatt, Hottinger & Ebert 2007; Altermatt & Ebert 2008). Natural antagonists may also prevent successful colonization, but again, this is unlikely because we excluded pools with fish, only released parasite-free *Daphnia* and most of the pools used in the experiments had no other *Daphnia* species. A final factor may be that our release experiments were carried out with *Daphnia* in its planktonic phase, and colonization of empty patches is most likely in the majority of cases by dispersing resting eggs. However, although the establishment of new populations from resting eggs may follow different rules than establishment from planktonic animals, the consistency of the results from the release experiments and the field survey suggests that this difference would not be large. In summary, about 20% of pools with pH and calcium levels in the right parameter range were not suitable for *D. magna* colonization, indicating that the right pH and calcium level are in and of themselves not sufficient to guarantee the persistence of *D. magna*. These additional factors remain to be elucidated.

Conclusions

Through this study, a picture emerges of a highly dynamic *D. magna* metapopulation with high migration and colonization rates among suitable pools but also high extinction rates that approximately balance the rate of new colonizations. Whereas previous studies on *D. magna* metapopulation dynamics focused on abiotic interactions, we show here that a key player in these dynamics is the eider duck, whose droppings, rich in calcium carbonate, influence pool suitability. To understand the long-term dynamics of this system, and metapopulations in general, we now need to answer two further questions: Are local extinctions driven by changes in patch quality that make the pools temporarily unsuitable or do populations go extinct due to intrinsic factors? To answer this question, it is necessary to monitor patch suitability across time and link it to colonization and extinction. The second question addresses the role of eider ducks as rock pool system engineers, or more generally, any factor that plays a role in making metapopulation patches suitable. Can the *D. magna* metapopulation persist in the absence of eider ducks? While our experiments suggest that eider ducks play a strong role in making patches suitable, they

also suggest that eider droppings do not in and of themselves explain all variation. Given that metapopulation persistence probably depends on the proportion of suitable patches, a metapopulation on an island without eider ducks may only be able to persist if enough pools are suitable even without the impact of eider droppings. This condition may only occur on islands with many pools, likely big and ecologically diverse islands. In other words, by making a larger fraction of patches suitable, eider ducks reduce the minimal number of rock pools on an island required for local metapopulation persistence. Eider ducks may, thus, not be the crucial factor for the overall existence of the *D. magna* metapopulation on the Skerry islands of the Baltic Sea, but they are likely to be a key player in maintaining it over larger regions and with higher proportions of occupied pools than would otherwise be possible.

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