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Population Isolation and Stress Tolerance in Rock Pool *Daphnia*

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Abstract

A major concern in conservation biology is the increasing habitat fragmentation causing small and isolated populations, which face the consequences of loss of genetic variability because of genetic drift and inbreeding. The loss of genetic variance and fitness loss due to inbreeding may reduce the adaptive potential of populations to cope with changing environments, especially under stressful environmental conditions. However, previous studies show variable results, and one reason for this may be that most studies were done on laboratory populations and lacking a connection to natural variances. In this study I used field populations of three *Daphnia* species (*D. longispina*, *D. magna*, and *D. pulex*) and high salinity as a stress to investigate how habitat isolation affects mean fitness and population growth. I found that isolation only affected *D. manga* populations but not *D. pulex* and *D. logispina*. Instead, in general, the field salinity that populations just and/or have experienced seems more important for their adaptabilities to tolerate high salinity conditions. The results of this study indicate that the natural environmental conditions that population experienced strongly influence populations' responses and increase their potential to tolerate stress. Thus, besides genetic components, the natural variation in disturbances is also important to be included when considering conservation strategies of species.

Introduction

The increasing human population and its activities have caused vast environmental impacts on wild organisms. Environmental changes from anthropogenic influences, for example, climate changes, water management and pollutions, make environments more stressful to organisms than they were before (World Conservation Monitoring Centre 1992; Rattner 2009). In addition, habitat disappearance and fragmentation have made populations smaller and/or more isolated (Fahrig 2003). Small and isolated populations are not only threatened by demographic and environmental stochasticity, but also genetic deterioration (e.g. inbreeding), which may have negative influence on population persistence (Lande 1988; Keller and Waller 2002; Willi et al. 2006; Laio and Reed 2009).

Populations which are small and isolated for many generations usually face two types of genetic threats: genetic drift and inbreeding (Keller and Waller 2002). Genetic drift, where allele frequencies change randomly in a population may cause alleles to be fixed or lost from the population by chance, which decreases the level of quantitative genetic variation (Lande 1995). This may be deleterious at the population level as potentially beneficial alleles are lost from the population causing longer time for populations to adapt to new circumstances, or populations to be less tolerant towards disturbances (Armbruster & Reed 2005, Willi et al. 2006). Thus, genetic drift may not directly affect mean individual fitness, and hence not threaten populations in the short term, but may be a problem for a population to survive for environmental changes in a longer run. But as genetic diversity is lost through drift, especially rapidly in small populations, it causes restricted opportunities for mating with variant genotypes. Small and isolated populations foster inbreeding via mating among relatives, causing loss of reproductive fitness through increasing homozygosity (Keller and Waller 2002; Laio and Reed 2009). In contrast with genetic drift and other mechanisms which may threaten population persistence, the negative impact from inbreeding occurs most rapidly and poses populations to high extinction risk (Keller and Waller 2002). Such inbreeding which causes a reduction in fitness is called inbreeding depression. Inbreeding depression on small and/or isolated populations is widely demonstrated (Radwan 2003; Reed et al. 2003), but there are genetic mechanisms limiting it (Keller and Waller 2002; Reed 2010). However, those mechanisms which may help with purging the genetic load is only effective enough in particular situations, thus many small populations still cannot avoid inbreeding and inbreeding depression (Keller and Waller 2002).

It is commonly argued that reduced genetic variation generally increases the sensitivity of a population to environmental stress and that this negatively impacts populations persistence (Griffen and Drake 2008; Bijlsma and Loeschcke 2011). Inbred individuals are considered more sensitive to stressful conditions (Armbruster and Reed 2005), supposedly because stress

increases the expression of deleterious alleles (Lynch and Walsh 1998). Studies on laboratory *Drosophila* have shown that inbred/bottlenecked populations have a stronger reduction of fitness or go extinct more quickly than non-inbred/non-bottlenecked populations under stress conditions, for example, high temperature, crowding, saline and ethanol conditions (Frankham et al. 1999; Bijlsma et al. 1999, 2000). Other studies on plants, butterfly and seed-feeding beetle also show similar results (Karlsson and Van Dyck 2005; Briggs and Goldman 2006; Fox et al. 2010). However, several studies do not find fitness costs of low genetic variation on population viability under more stressful environment, indicating that the relationship between inbreeding and environmental stress is complex (Armbruster and Reed 2005; Rogell et al. 2010).

Daphnia is a filter-feeding planktonic crustacean. It reproduces by cyclical parthenogenesis. Before winter in northern Europe, sexual reproduction produces resting eggs (ephippia) for surviving over winter and also acts as dispersal stage transporting by wind, water currents, and birds; in the rest of seasons. *Daphnia* mainly reproduces asexually for up to 12 generations, but under harsh conditions sexual reproduction can also occur under the summer season (Ebert et al. 2002; Haag et al. 2002). *Daphnia* occur in many different aquatic habitats of which rock pools is one. Rock pools are semi-permanent structures with fresh to saline water and often found in rocky outcrops along the Baltic Sea coast. They are patchily distributed and not physically connected to each other (Haag et al. 2002; Östman 2011a). Rock pool *Daphnia* populations are subdivided in discrete habitat patches, thus the population in each rock pool is considered as a metapopulation of whole population in an area (Ebert et al. 2002; Haag et al. 2002; Östman 2011a). Extinctions and colonizations of rock pool *Daphnia* are frequent. Colonizations may happen only by one or few individuals and increase in population size is in a short time entirely by asexual preproduction (founder effect). In addition, the population dynamic of *Daphnia* may be unstable causing periods of low population densities. Thus, the population of a rock pool may go through genetic bottlenecks very often that may affect their genetic diversity (Haag et al. 2002; Östman 2011b). Moreover, inbreeding is common in rock pool *Daphnia* system. A rock pool metapopulation obtain outbreed opportunities when new individual disperse from other rock pools, but it is restricted by the limitation of *Daphnia* dispersal ability (isolation distances). If there are no new individuals joining the population, inbreeding is unavoidable during sexual reproduction (Haag et al. 2002; Östman 2011a). Founder effects, frequent bottlenecks and inbreedingmay thus affect the genetic diversity and population growth rates (Ebert et al. 2002; Östman 2011b).

These characters make the rock pool *Daphnia* system suitable for researching fragmentation effects on metapopulations. Previous studies have already investigated the fitness difference between inbred and outbred *Daphnia*, but most of these studies were done on laboratory

populations and focused on only one species. The response to disturbances which relate to natural variation in isolation/population size of field populations is largely missing. There are three species of *Daphnia* in the rock pools along the Baltic Sea coast, *D. magna*, *D. pulex* and *D. logispina*. The three species have some differences in life histories and habitat requirements but as they occur in the same habitats they share the same environmental stresses, for example, high salinity, although the tolerance differs between species (Östman 2011a), giving an opportunity to compare the effects of fragmentation between closely related species.

This study aims to investigate how isolation of habitats affects rock pool *Daphnia* populations' mean fitness and population growth under a stressful condition, high salinity. Although the genetic analysis has not finished it is reasonable to assume that the more isolated populations also generally have a lower genetic diversity. The hypothesis I test is that populations that are more isolated have lower survival and fitness under environmental stress. To be specific, I focus on the following questions: (1) What is the effect of isolation on stress tolerance and adaptability of populations of the three species? (2) Do the responses to saline manipulation differ between species? (3) Besides isolation, is there any other variable affecting population survival and fitness under salinity stress?

To answer these questions, four natural populations with different isolation level of each *D. magna*, *D. pulex* and *D. logispina* were studied under laboratory conditions during experiments with manipulating salinity as an environmental stress. In order to understand the populations' response from natural conditions, I used field collected *Daphnia* and not laboratory lineages reproduced in the lab.

Method

Field data

Field data was collected from 112 rock pools around the Island of Gräsö (N 60° 30', E 18° 25) between 2007 to 2010, in total 1216 records. A detailed description of the area and sampling procedure is available in Östman (2011a). In short, each rock pool was visited three to nine times per year. At each sampling occasion density of *Daphnia* was estimated from 3-6 liter of pool water, depending on densities, filtered through an aquarium net. If densities were low (<1 ind/liter), densities were estimated visually, and individuals sampled by sweeping the pool water. For each rock pool and sampling occasion, water conductivity (salinity) was measured with a conductivity meter.

For each year the density of each species in each rock pool was calculated as the estimated cumulative density between 1 May and 30 September from the observed densities at samplings (see Östman 2011a for a detailed description). Yearly conductivity was estimated as averages from sampling occasions. Population isolation (I) for rock pool *i* and species *s* was calculated on a yearly basis as a modified version of the incidence function (Hanski 1994):

$$I_{si} = \log_e\left(\sum_{i=1, i\neq j}^{n} a_s d_{ij}^{-z_s} Occ_{js}\right) \qquad \text{(equation 1)}$$

A higher value of I_{si} means more populations of species *s* closer to rock pool *i*. d_{ij} is the distance (meters) between rock pool *i* and rock pool *j*. Occ_{js} is 1 if rock pool *j* is occupied by species *s* (that year) and otherwise 0. a_s and z_s are the species specific scaling coefficients between the colonization rate and distance between rock pools, see Östman (2011a). .

Sampling area

The sampling area for the experiments, Ugglan, is a peninsula of the island Gräsö situated at the Baltic Sea coast off Sweden (60°29.85'N, 18°25.77'E), and one of the area included for the field data analyses. All rock pools are situated less than 20 m from the coast line in a 15000 m^2 area. The average elevation above sea level is around one meter (range 0.5-4 m). The environment is characterized by bare rock with some sparse low vegetation and shrubs. Rock pools are semi-permanent water bodies between 1 to 20 m^2 (average 2.2 m^2), 20-50 cm deep (average 35 cm) in rock crevices. In the area there is almost a hundred rock pools but only a bit more than 30 have been observed to be inhabited by *Daphnia* populations, many others are too small or dry out too quickly to suit *Daphnia* or appear to be too saline for

Daphnia. The average pH of the rock pools inhabited by *Daphnia* is 8.4 (range 6.5 – 10), and average measured conductivity is 100 µS, around 0.02 psu (range 20 - 2000 µS, around 0 – 2.44 psu). All three *Daphnia* species occur in the area, *D. longispina* is usually the most frequently occurring species with around 15 populations per year. There are usually around 5-10 populations per year of *D. magna* and *D. pulex*.

More than 300 individuals from each of four populations per species were sampled from rock pools at Ugglan on September 6th, 2011. All chosen rock pools only contained a single *Daphnia* species. Each population was chosen so that they differed in isolation levels and average rock pool salinity. Salinity, pH, and temperature of rock pools were measured at collection. All collected *Daphnia* were stored in 17°C at Uppsala University and fed by algae (*Scendesmus* sp.) every second day. 48 individuals from each population were picked out for genetic analysis on 9th September 2011. For the second experiment (Salt addition experiment) there was not enough *Daphnia* from some populations. *Daphnia* was collected again from these rock pools on September 27th, 2011. However, for one *D. magna* population (M3), new *Daphnia* wasn't found in the rock pool. New collected *Daphnia* was stored together with the old one in the same box.

Salinity treatment experiment

The salinity treatment experiment was conducted between September 12th to October 6th, 2011, in a 19°C room at the Evolutionary Biology Centre, Uppsala University. Three treatments were used for *D. magna* (Ambient, 1.5 psu salinity, 3.0 psu salinity) and four treatments for *D. pulex* and *D. logispina* (Ambient, 0.75 psu salinity, 1.5 psu salinity, 3.0 psu salinity).The salinity levels were decided according to the field salinity condition. For example, the highest salinity used in the experiment (3 psu) was chosen base on the maximum field salinity of populations. Because of the fact that *D. pulex* and *D. logispina* were not as abundant as *D. magna* in saline rock pools (Östman 2011b), one more lower salinity treatment (0.75 psu) was added on these two species in order to see populations' changes clearly. All populations were set up in three-liter transparent plastic boxes with 2.5 liter of their natural rock pool water. The ambient treatment was the natural salinity in the rock pool water. The other salinity levels were created by a mix of field water with MQ water and sea salt to manipulate different salinity levels (0.75 psu salinity, 1.5 psu salinity, and 3.0 psu). Each treatment was replicated three times, thus in total nine subpopulations of each *D. magna* population and twelve subpopulations for each *D. pulex* or *D. longispina* population. 25 *Daphnia* individuals were put in to each box, from all life stages if possible. The *Daphnia* was fed with an algae suspension every second day, 25ml/20ml/15ml for *D. magna*/*D. pulex*/*D.*

longispina each time, depending on the different body size of each species. The experiment was continued for two weeks. After seven days, population size of each box (subpopulation) was counted (Mid-term sampling) visually after the water had been filtered through an aquarium net. Population size was counted again at day 14 (final collection) with the same method above. At the same time fecundity was estimated under a dissecting scope. Fecundity was considered as a proxy of the individuals quality in each subpopulation.

Salt addition experiment

The salt addition experiment lasted between September 30th to October 28th 2011, under the same condition as the former experiment. The salt addition experiment used identical three-liter plastic boxes with 2.5 liter water. Instead of natural water, a mix between *Daphnia* medium (Ebert 2006) and MQ water (approximate 1:1) which had a salinity of 0.25 psu was used. 25 individuals of each *Daphnia* population were put in each box, of all life stages if possible. The source populations for this experiment were sampled on two different occasions, Sep. 6th and 27th, 2011. The *Daphnia* was fed with an algae suspension every second day, with the same volume as the former experiment. Each population had three replicates. To begin with, the salinity of the water was increased with 0.25 psu per day until salinity was 3.0 psu. Then 0.5 psu/day of salinity was added from the 11th to 20th day until salinity was 7.5 psu. After the 21st day, 0.75 psu/day of salinity was added until salinity was 11.25 psu. The population size of each box was observed and recorded visually in the boxes everyday. When observing that the population went extinct, it was sampled by aquarium net to confirm the extinction. The experiment ended when the Daphnia in all boxes had gone extinct (6.0 psu in *D. pulex* and *D. longispina*, 11.25 psu in *D. magna*).

Data analysis

In the field data yearly population densities of respective species was related to mean salinity (represented by conductivity) and isolation levels by generalized linear model (GLM) in order to understand the association between population density and environmental factors among field populations. Because the interaction between salinity and isolation is of particular interest it was included in the model. Densities and salinity levels were $\log_e$-transformed prior to analysis.

For each species separately the population size of the mid-term and final collection, and

fecundity in the salinity treatment experiment was first analyzed in relation to treatment and population and their interaction with a two-way ANOVA. If the interaction between treatments and populations was significant a second analyze was conducted to study what population features that best explained this interaction. Instead of using population as a category variable population features was used as continuous variables in a generalized linear mixed model (GLMM) with treatment as a category variable and population as random effect. The population features investigated was isolation level, current salinity (measured when collecting Daphnia from rock pools), mean salinity, and maximum salinity measured in the field between 2007-2011. Which population variables that best fitted the results from the salinity treatment experiment was evaluated by the Akaike's Information Criteria (AIC, Burnham & Anderson 1998).

Extinction rank recorded from salt addition experiment between populations was analyzed with Kruskal-Wallis rank sum test (K-W test). If the extinction rank differed between populations, a GLMM was applied with extinction rank as dependent variable and population isolation levels and rock pool salinity levels as fixed effects and population as a random effect. Because the rock pool salinity wasn't measured on Sep. 27th, only isolated level, mean salinity and maximum salinity were used for analysis of salt addition experiment. All statistical analyses were done with the software "R".

Results

Field data

The relation between population density, isolated level and salinity among natural rock pools differed between the three *Daphnia* species. The population density of *D. magna* had a significant relation to isolation level (GLM, $t = -2.1$, $df = 105$, $P < 0.05$), and salinity (GLM, $t = 2.5$, $df = 105$, $P < 0.05$). Populations that were more isolated or occurred under more saline conditions had lower population densities. There were no significant associations between population density of *D. pulex* and isolation level (GLM, $t = -0.55$, $df = 64$, $P = 0.6$) or mean rock pool salinity (GLM, $t = -0.33$, $df = 64$, $P = 0.7$). Neither for *D. longispina* were there any significant associations between population density and isolation level, nor mean rock pool salinity (GLM, isolation level $t = 0.074$, $df = 67$, $P = 0.9$; salinity $t = -0.751$, $df = 67$, $P = 0.5$). None of the species had any significant interaction between isolation level and mean rock pool salinity (GLM, *D. magna* $t = -0.37$, $df = 105$, $P = 0.7$; *D. pulex* $t = 0.18$, $df = 64$, $P = 0.9$; *D. longispina* $t = 1.1$, $df = 67$, $P = 0.3$)

Salinity treatment experiment

The average population size at the end of experiment for different treatments and populations of the three species is shown in Figure 1 and the statistical results are presented in Table 1. Of the *D. magna* populations, population M1 and M3 survived in the highest salinity treatment whereas the other two went extinct. None of the *D. pulex* and *D. longispina* populations survived in the highest salinity treatment. Only one of the four populations of respective species survived in the 0.75 psu salinity treatments.

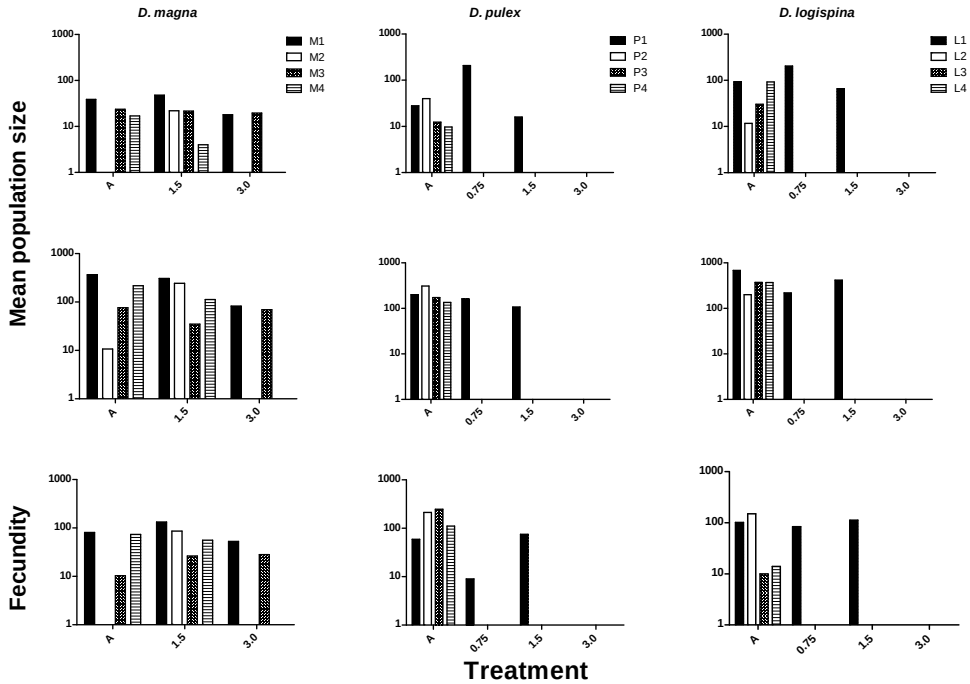

Fig 1. The average density and fecundity of the three species for the different salinity treatments. The first row shows the mean population size collected in the mid-term of the experiment; the second row shows the mean population size collected in the end of the experiment; and the bottom row shows fecundity collected in the end of the experiment. The y axis are mean population size for the top two rows and fecundity for the bottom row; the x axis is salinity manipulated in the experiment (A= Ambient, 0.75 psu, 1.5 psu, and 3.0 psu).

Table 1. F value of the salinity treatment of the three species with ANOVA

	D. magna			D. pulex			D. logispina		
	M	F	Fec.	M	F	Fec.	M	F	Fec.
Population	58.53	14.01	17.97	249.96	485.64	18.66	1158.93	950.82	115.04
Treatment	25.48	11.72	10.59	360.39	1728.08	266.09	1630.06	2707.71	136.11
Pop.×Treat.	15.09	7.78	10.84	84.79	165.07	20.47	283.41	243.55	26.56

n = 36 in *D. magna*; n = 48 in *D. pulex* and *D. logispina*

M = mid-term collection; F = final collection; Fec. = fecundity collected in the end of experiment

All values in the table are statistical significant ($P < 0.01$)

The results of how different rock pool and population variable could explain the differences between *D. magna* populations in the salinity treatment experiment are shown in Table 2. The average difference (across all treatments) between *D. magna* populations in density at the end and the middle of the experiment, as well as fecundity at the end of the experiment were all best explained by isolation level. But the *D. magna* populations' response to salinity treatments, i.e. the interaction between population and treatment, was best explained by the rock pool salinity at sampling (Current salinity, Table 2).

Table 2. Summary of relations between density and rock pool variables in *D. magna* by F value

	Df	Mid-term collection	Final collection	Fecundity
Isolation	1	37.06**	18.86**	22.26**
Current salinity	1	16.06**	2.29	0.87
Treatment	2	7.71**	5.90**	4.55*
Iso.×Treat.	2	1.24	1.31	1.69
Current sal.×Treat.	2	2.59	3.00	3.93*

n = 36, * P < 0.05, ** P < 0.01

The *D. pulex* population that survived in the salinity treatments (P1) was the population that originated from the rock pool with highest current salinity (AIC = 71.71; Table 3). Isolation level did not seem to be associated with the density of *D. pulex* at the end of the experiment (GLMM, F = 0.1, df = 1, P > 0.05), whereas mean field salinity was significant (GLMM, F = 160.58, df=1, P < 0.01) but had a higher AIC-value (AIC = 83.12).

The results of fecundity of *D. pulex* was also explained by the current salinity (AIC = 120.6, Table 3). Mean field salinity also showed a significant association with fecundity of *D. pulex* but with higher AIC-value than current salinity (GLMM, F = 59, df=1, P < 0.01, AIC = 137.15). Isolation level did not show any association with population densities from the salinity experiment (Final collection: GLMM, F = 0.3, df = 1, P > 0.05).

Table 3. Summary of relations between density and rock pool variables in *D. pulex* by F value

	Df	Mid-term collection	Final collection	Fecundity	Extinct rank
Current salinity	1	114.35**	357.97**	57.62**	13.53**
Treatment	3	141.97**	1088.68**	276.96**	63.12**
Current sal.×Treat.	3	91.74**	306.03**	61.90**	14.38**

n = 48, * P < 0.05, ** P < 0.01

The difference in density at end of the salinity experiment between *D. longispina* populations was also best explained by current salinity in the rock pool at sampling (Table 4). The population that survived in saline treatments was the one living in the highest current salinity

rock pool (Table 5). Neither isolation level nor mean field salinity showed any evident association with the densities at the end to the experiment (GLMM: Isolation F = 0, df= 1, P >>0.05; Mean salinity F = 0.1, df= 1, P >>0.05).

Table 4. Summary of relations between density and salinity at sampling (Current salinity) in *D. longispina* by F value

	Df	Mid-term collection	Final collection	Fecundity	Extinct rank
Current salinity	1	40.77**	118.43**	16.02**	5.16*
Treatment	3	201.97**	928.61**	60.51**	51.49**
Current sal.×Treat.	3	94.00**	241.57**	27.49**	3.53**

n = 48, * P < 0.05, ** P < 0.01

Table 5. Summary of all environmental factors' values used for analysis

	Isolation level	Current field salinity (psu)	Mean field salinity (psu)	Maximum field salinity (psu)
D. magna				
M1	-1.222	0.44	1.43	2.03
M2	-2.186	0.10	1.32	2.63
M3	-1.848	0.68	4.18	5.70
M4	-1.974	～0	0.43	4.30
D. pulex				
P1	0.203	0.17	1.25	2.55
P2	0.240	～0	～0	0.3
P3	0.157	～0	～0	0.27
P4	0.293	～0	～0	2.18
D. logispina				
L1	-0.353	0.51	0.47	1.13
L2	-0.580	～0	～0	0.18
L3	-0.710	～0	0.89	2.47
L4	0.275	～0	0.05	0.42

Salt addition experiment

Fig. 2 shows the average extinction rank of populations in the salt addition experiment. The difference between *D. magna* populations was close to significant (Kruskal-Wallis rank sum test, df = 3, P = 0.055). However, in contrast to the salinity treatment experiment, the two populations that survived longest (M2, M4) were the populations that went extinct in the high salinity treatment. For *D. pulex*, the difference in extinction rank between populations was significant (Kruskal-Wallis rank sum test, df =3, P = 0.039), but not for *D. longispina* (Kruskal-Wallis rank sum test, df =3, P = 0.5). The best variable explaining populations' differences for *D. pulex* was the maximum of field salinity (GLMM, F = 11, df = 1, P < 0.01). However, for *D. magna* no variable investigated (isolation level, average field salinity, and maximum of field salinity) was associated with average population extinction rank. The best variable for *D. magna* was the maximum of field salinity (Salinity: GLMM, F = 3, df = 1, P > 0.05).

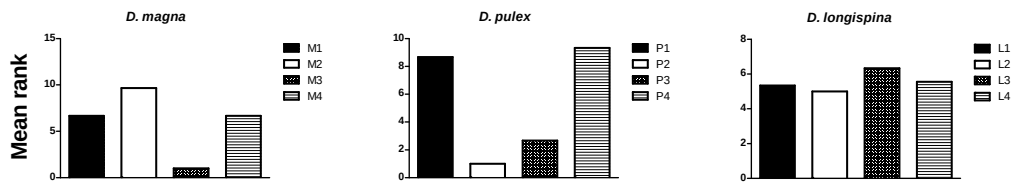

Fig 2. The extinction rank of different populations from the three species. The y axis is the average extinction rank of three repeats in populations, and x axis locates different populations of the species.

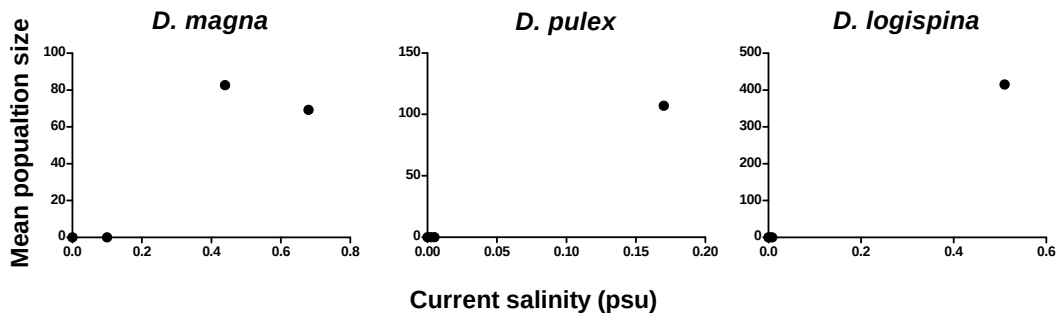

Fig 3. Association between current salinity and mean population size from final collection of the three species.

Discussion

From the combined results (Table 1, Figure 1), it is clear that treatments affected population densities, and that the tolerance or adaptability of different populations to salinity stress differed between populations in all three species. However, only for *D. magna* population isolation level could explain any variance between populations. For *D. pulex* and *D. longispina*, there is no support for the hypothesis that more isolated populations have lower population density (lower fitness or population growth rate) under neither ambient environment nor stressful environment. Instead, the salinity in the rock pool at sampling (current salinity) seemed to be the most important factor affecting populations' tolerance or adaptability to increased salinity, which is out of the original prediction.

The explanation for the non-significant associations between isolation level and population density for *D. pulex* and *D. logispina* may relate to a more specific requirement of rock pool quality for these two species. Östman (2011a) investigated the abundance-occupancy relationships of rock pool *Daphnia*. He found that both probability of occupation and abundance of *D. magna* increased with decreasing isolation distance, but abundance was also influenced by salinity. This fits the results of field data analysis in which population densities of *D. magna* had a significant relation to isolation level and salinity. Also the results from the salinity treatment experiment showed that isolation level and a salinity variable (current salinity) both influenced population densities. For *D. pulex*, abundance and occupation were both associated with rock pool depth and salinity. There was a positive association between rock pool depth for both abundance and occupation for *D. longispina*, and occupation probabilities of *D. longispina* may have a negative association with isolation distance but not for abundance. This suggests that factors of habitat quality (e.g rock pool depth and salinity) are more important for the population density and persistence of *D. pulex* and *D. longispina* than isolation distances. Reflecting to the salinity treatment experiment, because isolation level is not the factor that affects the population distribution of *D. pulex* and *D. logispina*, the assumption that more isolated populations have lower genetic inflow may not be true.

Although the effect of current salinity is not as strong as isolation level in the statistical result for *D. magna*, there is still a clear positive relationship (Table 5 and Fig 3). The populations of all three species that lived in the more saline rook pools displayed better tolerance to the higher salinity treatments, although the highest current salinity of each species were lower than any of the salinity treatments. This result indicates that the current environmental condition that *Daphnia* face is more important for populations tolerating rapid stressful conditions. Bijlsma and Loeschecke (2005) have pointed out that phenotypic plasticity and/or the presence of (partially) resistant genotype are two factors which determine the ability of an individual/population to cope with changing and stressful conditions. In their recent study (Bijlsma and Loeschecke 2011), they mentioned that plastic responses often give a more short-term and emergent solutions to deal with rapid stress conditions, whereas long-term responses may more depend on the genetic variation of the population for evolutionary adaptation, although plastic responses and genetic response may not be completely independent. These explanations suggest that phenotypic plasticity (e.g physiological plasticity) of *Daphnia* might play an important role on the salinity treatments experiment.

Compared to the salt addition experiment, populations of all three *Daphnia* species could survive in much higher salinity conditions if the salinity increased gradually. This shows that *Daphnia* species have high adaptive potential to high salinity but the short term tolerance was not that wide. The plasticity of *Daphnia* decides how wide it can adapt form the original environment to a new one in short time. In my result that populations from rock pools with high current salinity showed better adaptability to higher salinity maybe because of the response of plasticity. Moreover, because the experiment only continued for two weeks, the results may just reflect the short-term physiological adjustment of individuals and couldn't prove that populations' evolutionary adaptation will have the same trend.

The results of salt addition experiment showed different extinction times in *D. magna* and *D. pulex* populations but the reason is not very clear. Two reasons may make the results of the salt addition experiment indistinct. The first is that current salinity wasn't included in the analysis. If current salinity affected the population response for different salinity treatment a lot, then it's possible it also played an important role in this experiment. In addition, the current salinity of populations may explain why *D. magna* population M2 and M4 survived in higher salinity treatment but went extinct earlier in salt addition experiment. Unfortunately, the current salinity was not measured for this experiment. Secondly, the time span the populations of each of the three species died off was rather short, almost within two days. This short interval of extinction may cause demographic stochasticity to be important for the order of extinction. However, the maximum field salinity seems at least to be one reason affecting extinction time of populations in *D. pulex* (and is the best chosen variable for *D. magna*, too) in the salt addition experiment. It indicates the environmental condition that

populations have experienced in the history is more important for the populations' resistance towards stress, maybe indicating a genetic effect. Reed (2010) pointed out the genetic factors do not act in a vacuum, whereas they interact with the environment and the power of selection. In this case, populations of *D. pulex* (and maybe also *D. magna*) may already went through selection of high salinity tolerance, and the populations that went extinct later were the once with high salinity tolerance genotype. Thus, although the genetic variance of the population is less, the population still displayed better tolerance of salinity.

Other factors may also cause the results do differentiate from the original prediction. First, the persistence time of populations may vary. Haag et al. (2002) mentioned that allelic diversity of *Daphnia* increases about 10% in number of alleles per year. If this is the case, then the long persisting populations may have higher genetic variance even though they are more isolated. The colonization and extinction rate are around 10-20% in the study area (Östman 2011a), meaning populations in the field may have various persistence times, which gives a probability that the populations used in experiments may have had different genetic diversity caused by how long they had persisted. Second, although previous study established the existence of strong inbreeding depression in Daphnia (Haag et al. 2002), it is normally considered that selfing species shows little loss in fitness upon inbreeding comparing to species morally outcross (Bijlsma and Loeschcke 2011), suggesting that only very inbred *Daphnia* population may get significant negative effects from inbreeding. The different isolation level in this study may have caused different genetic variance, but these differences may have not been large enough to cause variation in inbreeding depression.

Some studies have pointed out the population expression of inbreeding under stress may be different if combining with field environmental conditions (e.g. predators, food resource), and that is one of the reasons causing laboratory experiments to not match field studies (Griffen and Drake 2008, Bijlsma and Loeschcke 2011). The samples in this study were from field directly, thus some field environmental factors (e.g. field salinity) were still included even if the study was done in laboratory condition. In general, the field environmental conditions that population experienced (especially the current condition) strongly influence populations' responses when they face the same stress element (salinity, in this study) for all three *Daphnia* species. Although isolation may reduce the ability of populations to cope with stress, the experience from original environmental condition increase the potential to tolerate stress. The mechanisms behind this adaptation is either because of phenotypic plasticity or also includes genetic changes needs further investigation. For conservation implications, although the *Daphnia* species may partially differ in their biology to other species threaten by fragmentation, the result suggest that in not extremely inbreed populations, a "natural" variation in disturbances can be as important as increasing gene flow between populations for the persistence of species under stressful conditions.

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