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Effects of Salinity on Germination, Seedling Growth and Ecological Properties of *Phragmites australis* Communities in the Estuary of the Chikugogawa River, Southwestern Japan

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Abstract

Salt tolerance of *Phragmites australis* populations was investigated in natural reed habitats in the estuary of the Chikugogawa River, southwestern Japan. *P. australis* populations were selected along the salinity gradient in the estuary, including limnetic (salinity 0.05%), oligohaline (0.4%) and polyhaline sections (2.5%). The ratio of Total-P/Total-N of river water showed high values in the oligohaline section and the above ground biomass, population height and culm diameter of *P. australis* showed maximum values in this section. Sufficient phosphorus supply could sustain the high productivity of the community in the oligohaline section, irrespective of the salinity of inundated water. The seed production of *P. australis* was lowest in the polyhaline section. Thus, the ecological performance of *P. australis* was highest in the oligohaline section and the performance declined with the increasing salinity of the habitat. The effects of salinity on germination and seedling growth were evaluated by means of cultivation in 0.0% - 5.0% (NaCl w/w%) salinity medium. Seeds of *P. australis* collected from every natural population in the estuary failed to germinate at salinity levels above 2.3%. Growth of shoot length and above-ground biomass of seedlings germinated in the fresh water medium were measured for 21 days' exposure to constant salinity solutions ranging from 0.0% - 5.0%. Although mortality was high at salinity levels 3.5% and 5.0%, elongation of shoots of some plants was evident at a salinity level of 5.0%. Seeds from the population in the limnetic section never germinated at a salinity of 3.5%, whereas seedlings obtained from the seeds of the same population germinated in freshwater conditions grew under a salinity of 3.5%. Thus, *P. australis* seedlings have higher salt tolerance compared to that during the germination stage of the seeds. In this study, it is clarified that *P. australis* has low salt tolerance during the germination stage and then it acquires salt tolerance during the stage of seedling growth.

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Keywords

Common Reed; Germination; Salt Marsh; Salt Tolerance

1. Introduction

Phragmites australis (Cav.) Trin. ex Steud. (common reed) is a perennial monocotyledonous Gramineae species growing in fresh and brackish water and forming swamp vegetation along riversides and estuaries. The species is distributed from aquatic to arid communities and has the ability to tolerate high salinity, high acidity, and exposure to sulfides, hydrogen sulfide or ammonia. *P. australis* produces populations with high densities of culms from the river mouth to the fresh water sites in the research area of a river in the northern part of Kyushu Istand, south-western Japan. The main objective of the present study was to clarify eco-physiological properties of the species allowing it to colonize a wide range of salinity gradients and to flourish abundantly in the estuary community.

The salt tolerance of *P. australis* has been widely reported in the literature. *P. australis* distributes at salinity up to 500 mM (NaCl) in an abandoned salt field in Japan [1] (Matoh *et al*., 1988), or up to 22 g/L in the Delaware Estuary in the USA [2] (Hellings and Gallagher, 1992). Matured plants of *P. australis* showed higher salt tolerance, although the growth of plants is usually inhibited in high salinity conditions. Asaeda *et al*. (2003) tested the effects of salinity and cutting of above-ground portions of *P. australis* [3]. Shoot density, shoot height, leaf length, leaf width and panicle formation decreased with both treatment of 3% salinity and shoot cutting. Howard (2010) tested the effect of salinity and soil type on the growth of *P. australis* plants acclimated for 25 months in a greenhouse after filed sampling [4]. Plant height and both above- and below-ground biomass significantly decreased at 1.8% salinity. Thus, *P. australis* is categorized as a halophyte species.

Salt tolerance of *P. australis* has been investigated by ecophysiological analysis. Salt tolerance at the germination stage has been studied by Mauchamp and Mésleard (2001), Gorai *et al*. (2006) and others and they found that the germination of *P. australis* seeds is inhibited at 0.3% - 1.5% salinity [5] [6]. Thus, the salt tolerance of *P. australis* is not high enough at the germination stage to establish populations in a sea water inundated habitat. Lissner and Schierup (1997), Hootsmans and Wiegman (1998), Matoh *et al*. (1988), Gorai *et al*. (2007) and others investigated the salt tolerance of *P. australis* at the seedling stage and found that *P. australis* seedlings survived at 1.8% - 3.5% salinity [1] [7]-[9]. Although the maximum reported salinity for *P. australis* seedling survival differs among various studies, seedlings exhibited a higher salt tolerance than seeds. Lissner and Schierup (1997) clarified that rhizome-grown plants had higher salt tolerance than seedlings and rhizome-grown plants survived up to 3.5% [7], whereas all juveniles died out at 2.25% and higher salinity. It can be concluded that *P. australis* plants acquire higher salt tolerance in the progress of growing stages and adult plants have the highest tolerance to salt. However, the process of acquiring salt tolerance by *P. australis* has not been fully investigated, and ecological analysis has not been adequate concerning salt tolerance of *P. australis* relating the establishment of populations in the natural habitat focusing on the germination and seedling growth. In this paper, we investigated salt tolerance of *P. australis*, including both germination and seedling growth stages, focusing on the ecological properties of natural *P. australis* populations distributed over a wide salinity gradient in the estuary of a river. The objectives of this study are to clarify the ecological properties of *P. australis* with reference to 1) ecological performance along the salinity gradient of riversides of an estuary; 2) salt tolerance at the germination stage of seeds from the different habitats within the salinity gradients; and 3) salt tolerance of seedlings obtained from seeds of different habitats and different salinity conditions at germination (habitat of seed collection x germination condition).

2. Materials and Methods

2.1. Study Sites

The surveyed *Phragmites australis* community was in the estuary of the Chikugogawa River, northern Kyushu, south-western Japan (**Figure 1**).

Three stands with different salinity of river water were selected for the ecological survey and seed collection

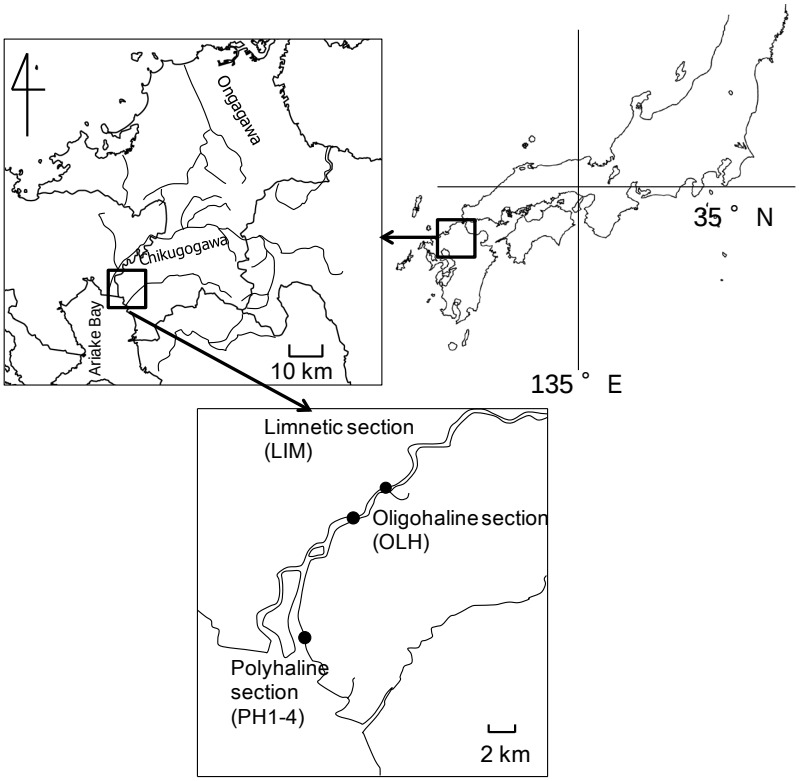

Figure 1. Map showing the research area and data sampling stands in the estuary of the Chikugogawa River, northern Kyushu, south-western Japan. Three stands with different salinities of river water were selected from the river mouth to the 25 km point upstream from the river mouth: limnetic section (LIM; maximum salinity = 0.05%, oligohaline section (OLH; maximum salinity = 0.4%, and polyhaline section (PH; maximum salinity = 2.5%). Four vegetation zones of pure *P. australis* stand with differing community heights and culm densities were recognized in the oligohaline section, and the four stands (PH1 - PH4) were located 0.0 m, 8.0 m, 17.7 m and 21.0 m from the shore line.

of *P. australis*, based on a preliminary survey of river water salinity between river mouth to the 25 km point upstream from the river mouth (**Table 1**): limnetic section (LIM; maximum salinity = 0.05%), oligohaline section (OLH; maximum salinity = 0.4%), and polyhaline section (PH; maximum salinity = 2.5%). Four vegetation zones of pure *P. australis* stands with different community heights and culm densities were recognized in the oligohaline section, and subsequently four stands (PH1 - PH4) were located 0.0 m, 8.0 m, 17.7 m and 21.0 m from the shore line at low tide. At the PH site, PVC pipes (130 cm length, 3.0 cm diameter) with pores at 10 cm intervals were inserted vertically into the sediment at 2.0 m intervals from the river to the river bank and the water table depth was measured. Water table depth was transformed to water level by using the elevation at each PVC pipe. Inundation period at each point was estimated by comparing the reference water level (Rokugo-ro-bashi; data from the Chikugogawa River Office).

2.2. Physical and Chemical Environments of the Study Sites

Total nitrogen (TN) and total phosphorus (TP) of the surface river water were determined so as to estimate the nutrition conditions at the three study sites. Water sampling was done at high tide 3 times during the spring tide (2 June, 15 June, 28 June 2007) and 4 times during the neap tide (8 June, 22 June, 6 July, 23 July 2007) at 10 points from the river mouth to the point 23.4 km from the river mouth of the Chikugogawa River. TN was determined by measuring the UV absorption (220 nm) after potassium peroxio disulfate—NaOH digestion. TP was determined by measuring molybdenum blue absorption (885 nm) after digestion by potassium peroxo disulfate. Standard solutions for the TN and TP measurements were prepared using Milli-Q water including 3.0% NaCl.

Table 1. Environmental parameters and vegetation structure of *Phragmites australis* community in the estuary of the Chikugogawa river, south-western Japan.

Section	Polyhaline				Oligohaline	Limnetic
Sites	PH1	PH2	PH3	PH4	OLH	LIM
Distance from the river mouth (km)	0.00[1]				12.92	16.40
Averaged salinity of river water (-) [n = 7]	17.5[1]				1.1	0.3
Maximum salinity of river water (-)	25.0[1]				4.0	0.5
Averaged salinity of river water at the spring tide (-) [n = 3]	21.9[1]				1.3	0.5
Distance from the shore line (m)	0	8	18	21	0	0
Flooding period at the spring tide (min/d)	452.0 ± 97.9	257.4 ± 54.0	0	0	-	-
NaCl concentration in soil (%)						
0 - 10 cm depth horizon	0.440	0.099	0.045	0.033	0.027	0.007
10 - 20 cm depth horizon	0.395	0.267	0.073	0.041	0.025	0.011
20 - 30 cm depth horizon	0.411	0.223	0.071	0.070	0.029	0.015
30 - 40 cm depth horizon	0.404	0.300	0.078	0.078	0.045	0.014
Culm density (number/m^2)	51.3	91.3	18	9.3	60	34
Shoot biomass (kg/ m^2)	0.410	1.096	0.288	0.316	12.012	4.624
Seed production (number/ m^2)	0	8141	1307	7136	20905	57186
Seed weight (mg/100 seeds)	19.3 ± 1.2[2]				25.6 ± 6.6	11.0 ± 3.7

Salt contents in sediments at each site were determined for a sediment core of 60 cm collected using a Tomas type peat sampler (Nose Tekkosho, Okayama, Japan) on 26 October 2007. The sediment core was divided into 10 cm segments and dried at 105˚C for more than 72 hours. Milli-Q water (50 ml) was added to the dried sediment (10 g) and was shaken vertically at 200 rpm in a 100 ml polyethylene bottle for 2 hours. The supernatant solution was filtered with filter paper (5C, Advantec Co. Ltd., Tokyo, Japan) and 0.2 mm cellulose acetate membrane filter. Chloride ion concentration was determined by ion chromatography (Dionex Model DX-120, Japan Dionex Co. Ltd., Tokyo, Japan) and then the salt content of each sediment was calculated.

In order to determine the soil salinity change caused by tidal change, EC of the sediment pore water was measured twice during a 24 h period at the three sites. At the PH site, PH 2 was selected for the measurement of sediment pore EC. An electrode for EC measurement (ES-12, Horiba Co. Ltd., Kyoto, Japan) was placed at a 15 cm depth from the sediment surface and EC was recorded at 10 min intervals.

2.3. Ecological Properties of *Phragmites australis* Stands

Culm density was determined in August 2008 at each site. Stand height (height of culms), diameter of culms at base and dry weight of above-ground portions of culms were measured for randomly selected 10 ramets at each site. Shoot biomass was calculated by multiplying culm density and averaged culm dry weight at each site.

All inflorescences within 1 m^2 were harvested in December 2008 and the number of inflorescences was counted. Inflorescences were divided into three size classes: large, medium and small. Spikelets were eliminated and the whole spikelets within each size class were weighed. 100 randomly selected spikelets were weighed and the number of seeds was counted for each size class. Seed setting ratios were calculated for each of the size classes and then the numbers were summed to obtain the seed setting rate in 1 m^2.

2.4. Effects of Salinity on Germination and Seedling Growth

Response of *Phragmites australis* germination and seedling growth to salinity was investigated through laboratory cultivation by controlling medium salinity from 0.0% to 5.0% (W/W) NaCl. Seeds of *P. australis* were ob-

tained from each population in December 2007. Seeds were removed from the spikelets and stored in a refrigerator at 5˚C before sowing. Seeds (100 individuals) were sown on absorbent cotton in Petri dishes and soaked with water of various salinities: 0.0, 0.2, 0.75, 2.3, 3.5 and 5.0% NaCl solution. Maximum salinity of the cultivation medium was determined at ca. twice the maximum salinity in the field populations of *P. australis* at surveying sites. Here the experimental design was 6 populations × 6 salinities. Cultivation was done in a growth chamber at 20˚C with illumination of ca. 150 μmol/m^2·s PPFD for 12 days. The number of germinated seeds was counted 12 days after sowing.

Effects of salinity on seedling growth were investigated by using the seedlings germinated on freshwater-soaked absorbent cotton in Petri dishes. Germinated seedlings (n = 2) were transplanted to salt-free sand in plastic pots. Pots with seedlings were placed in a plastic vat and filled with solutions of differing salinities up to the soil surface. Salinities of the medium were 0.0, 0.2, 0.75, 2.3, 3.5 and 5.0% NaCl solution, and the experimental design covered 6 populations × 6 salinities. Pure water was supplied every day to maintain a constant water level. Plants were transplanted 14 days after germination. Cultivation medium was replaced every 7 days and adjusted to maintain a constant salinity. Seedlings were situated in 14L(25˚C - 29˚C)/10D(19˚C - 24˚C) conditions with illumination of ca. 150 μmol/m^2·s PPFD for the lighting condition. Shoot length was measured every day for all living seedlings, and seedlings were harvested 21 days after transplantation. Harvested plants were dried at 80˚C for more than 48 h and then individual dry weights were determined.

3. Results

3.1. Nitrogen and Phosphorus Conditions in the Estuary of the Chikugogawa River

In order to evaluate the nutritional conditions of river water in the *Phragmites australis* stands, we determined the Total-P (TP) and Total-N (TN) within the range including the investigation sites as well as tidal change.

Although TP and TN fluctuated largely due to tidal change, TN decreased from the river mouth moving upstream, whereas TP showed a maximum in the OLH section (ca. 13 km from the river mouth; **Figure 2**). Thus, the TP/TN ratio showed a maximum at the OLH site (TP/TN = 0.11), implying phosphorus supply was sufficient there.

3.2. Salinity in the *P. australis* Communities

Salt contents in sediment in the PH section showed gradients from the river to the river bank (**Table 1**). Sediment at PH1 showed the highest salinity in soil, and salinity decreased toward PH4. Salinity of sediments was nearly constant from the surface to 40 cm depth, except for the surface at PH2. Salinity in soil below the 10 - 20 cm horizon showed higher values than the surface soil at PH2. Salinity of sediments in the OLH section was lower than in the PH section and that at LIM was the lowest among the investigated sites. Clay deposited from the surface to at least 60 cm depth at PH1, whereas a sand layer of 15 cm thickness overlays the clay sediment at PH2. Sediment at PH3 and PH4 was sand from the surface to at least 40 cm depth. Clay was deposited from the surface to at least 60 cm depth in the PLH and LIM sections.

3.3. Ecological Properties of *P. australis* Communities

Stand height of the *P. australis* community in the PH section tended to increase from the river side (PH1) to the river bank site (PH4; **Figure 3**).

The community in the OLH section showed the highest stand height among the study sites. Above-ground biomass showed the same tendency as stand height, and the OLH stand exhibited an extremely high biomass among the study sites.

Seed setting rate per 1 m^2 in the LIM section community showed the highest value among the study sites, whereas the community in the PH section, especially in the PH1 community, exhibited an extremely small seed setting rate (**Table 1**).

3.4. Effects of Salinity on Germination and Seedling Growth of *P. australis*

The germination rate of *P. australis* tended to decrease with increasing salinity of cultivation medium except for seeds obtained from the PH1 site (**Figure 4**).

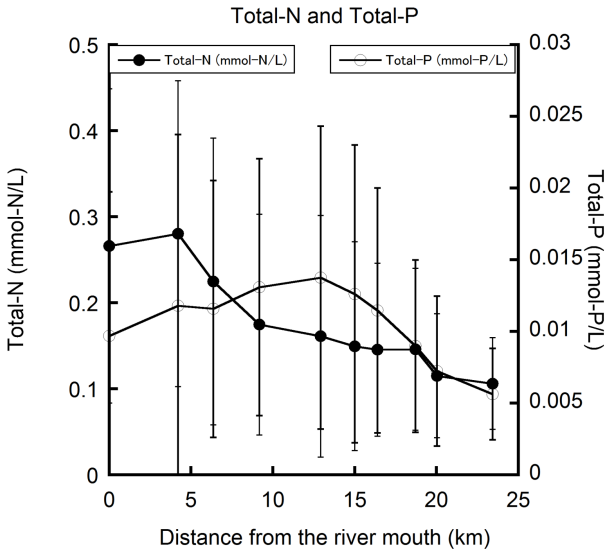

Figure 2. Total nitrogen (TN; ●) and total phosphorus (TP; ○) of the surface water of the Chikugogawa River from the river mouth to the 23.4 km point upstream from the river mouth. Water samplings were made 3 times during the spring tide (2 June, 15 June, 28 June 2007) and 4 times during the neap tide (8 June, 22 June, 6 July, 23 July 2007) at 10 points. Means and SD are presented.

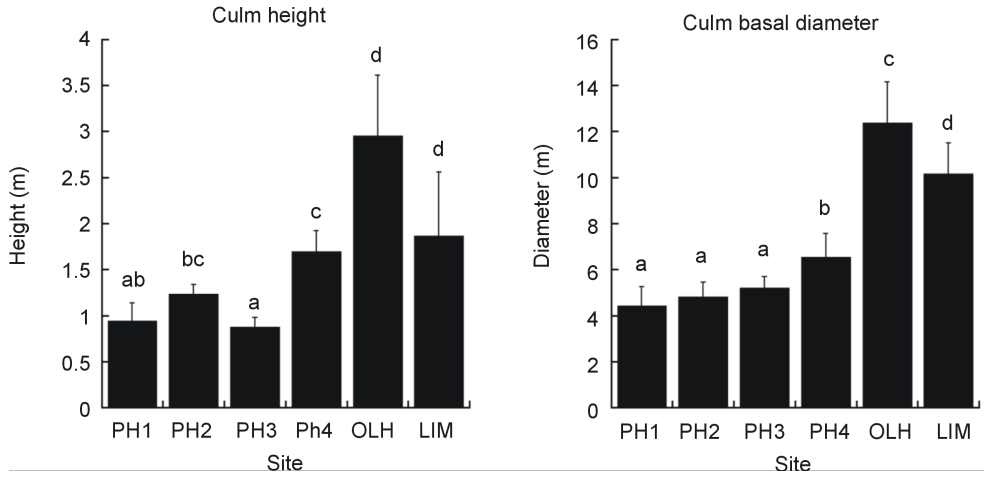

Figure 3. Heights and diameters at the base of culms of *Phragmites australis* in the polyhaline section (PH1-PH4), oligohaline section (OLH), and limnetic section (LIM) collected in August 2008. Stand heights (height of culms) and diameters of culms at base were measured for 10 randomly selected ramets at each site. Means sharing the same letter are not significantly different by Tukey HSD test.

Seeds from the PH1 site showed a maximum at 0.75% NaCl solution. Germination was <20% when the salinity of the medium exceeded 2.3%, but seeds from populations other than that in LIM germinated (although the germination rate was <10%) at a salinity of 3.5%. Germination was not observed at a salinity of 5.0%.

Elongation of seedlings of *P. australis* when salinity was >2.3% was significantly lower than elongation under freshwater conditions (data not presented). Dry weight after 21 days' cultivation showed smaller values in salt-containing medium than in freshwater conditions for seeds obtained from the PH2 and PH4 sites, whereas maximum dry weight growth was observed at 0.7% for PH1 and LIM populations, and 0.2% for PH3 and OLH populations (**Figure 5**).

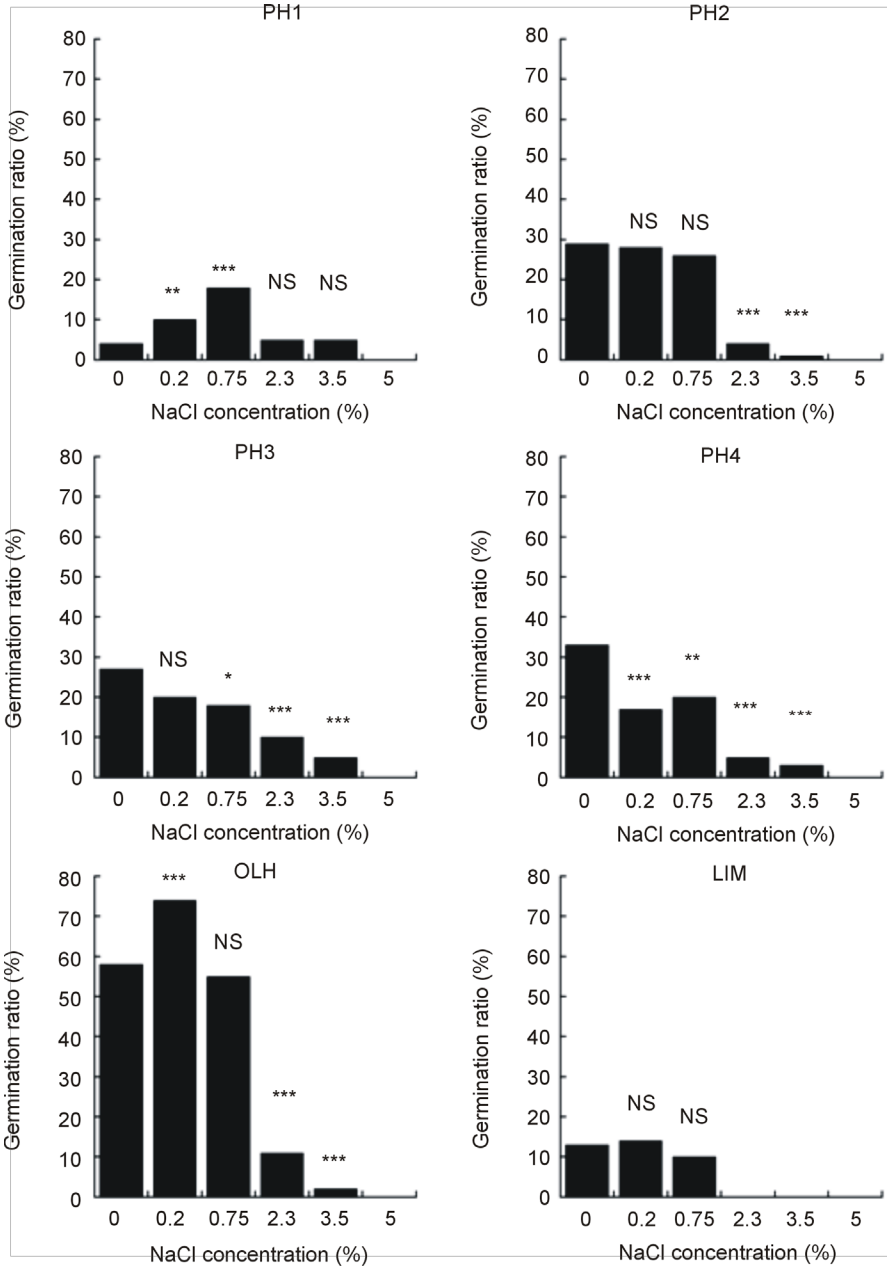

Figure 4. Germination rates of seeds of *Phragmites australis* obtained from polyhaline section (PH1-PH4), oligohaline section (OLH), and limnetic section (LIM) in December 2007. Percentages of germinated individuals among 100 seeds are shown for these salinity levels: 0.0, 0.2, 0.75, 2.3, 3.5 and 5.0% NaCl solution. Germination rates for treatments of 0.2, 0.75, 2.3, 3.5 and 5.0% NaCl solution were compared to the freshwater treatment and evaluated by the χ^2 test. Significance level ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: not significant.

Seeds from the LIM section germinated under freshwater conditions showed elongation and dry weight growth at a salinity of 3.5%, although seeds from the LIM site never germinated with the same salinity medium. Seedling growth was observed at a salinity of 5.0% for seeds from PH2, PH4 and OLH populations, although seeds from every site failed to germinate at 5.0% salinity. Differences in dry weight growth for varying treatments were evaluated using the Kruskal-Wallis test, and it was found that only locality of population of seed source was significantly different (**Table 2**), whereas two-way ANOVA showed significant differences between locality and salinity treatments.

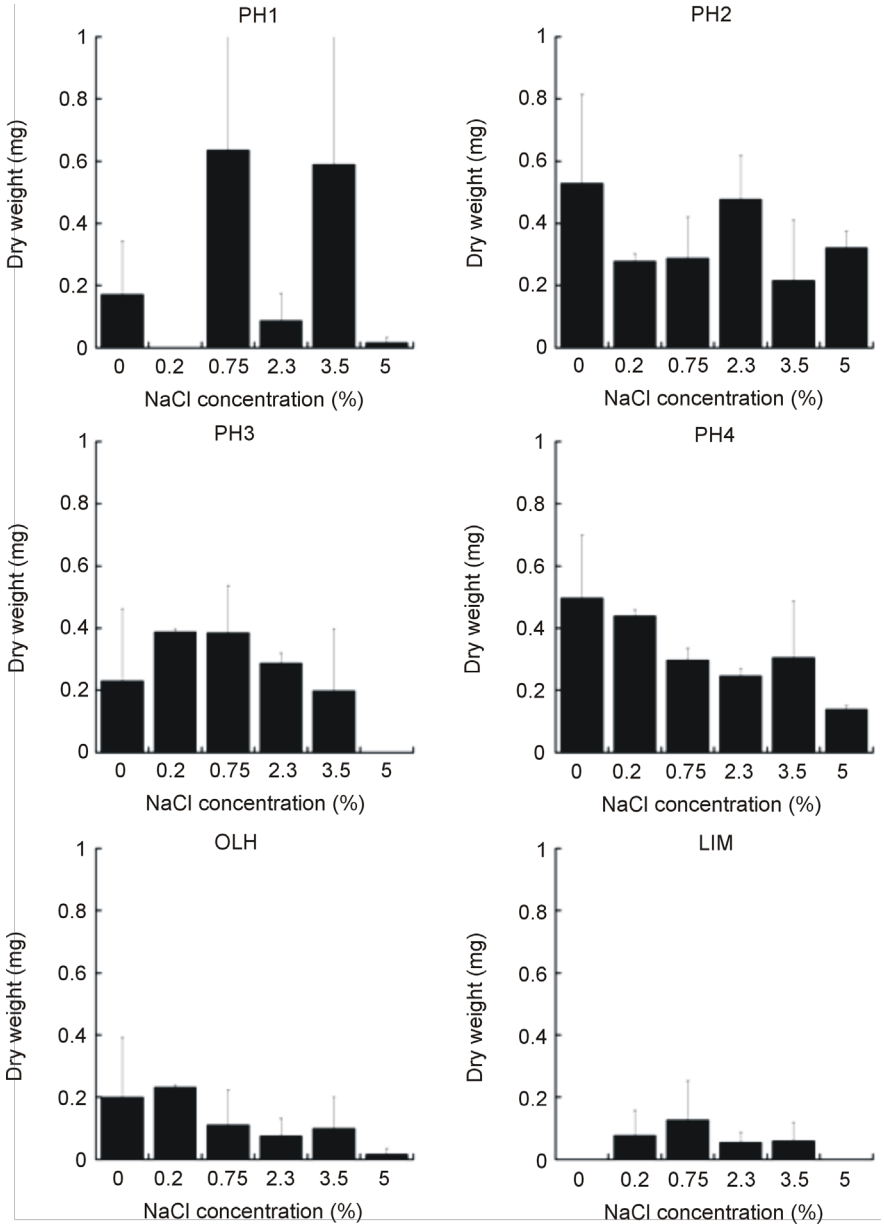

Figure 5. Dry weights of individual seedlings of *Phragmites australis* grown on mediums with salinities of 0.0, 0.2, 0.75, 2.3, 3.5 and 5.0% NaCl solution. Seeds obtained from the polyhaline section (PH1-PH4), oligohaline section (OLH), and limnetic section (LIM) in December 2007 were sown on freshwater medium and transplanted 14 days after sowing (n = 2). Pots with seedlings were placed in a plastic vat and filled with water of differing salinity up to the soil surface; dry weight was measured 21 days after transplanting. Varying salinity treatments were not significantly different among each of the habitats of seed collection, based on the Kruskal-Wallis test ($p > 0.05$).

Seedling growth from seeds at PH sites showed higher figures than was the case with seeds from OLH and LIM sites, based on comparisons using pooled data including all salinity treatments (**Figure 6**).

4. Discussion

Maximum salinity of the water at the *Phragmites australis* habitat in the estuary of the Chikugogawa River was 2.5%, a value comparable to the distribution limit of the *P. australis* in the Mediterranean population [5]

Table 2. Two way ANOVA of the effect of locality of seeds and salinity on dry weight growth of *Phragmites australis* seedlings. significance level; **: $p < 0.01$, *:$p < 0.05$, NS: not significant.

Sources	Sum of square	d.f.	Averaged square	F	P	Kruskal-Wallis test
Locality	0.825	5	0.165	5.194	**	**
NaCl	0.609	5	0.122	3.831	*	NS
Locality × NaCl	1.376	21	0.066	2.061	NS	
Error	0.604	19	0.032			
Sum	3.210	50				

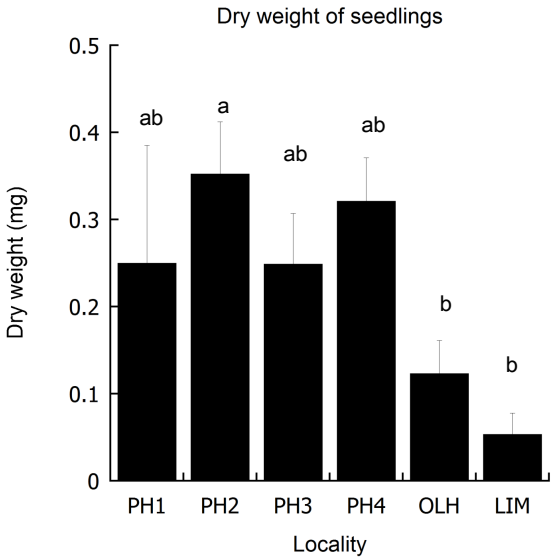

Figure 6. Dry weights of individual seedlings of *Phragmites australis* obtained from seeds in the polyhaline section (PH1-PH4), oligohaline section (OLH), and limnetic section (LIM) in December 2007. All the treatments of different salinity in **Figure 5** were pooled. Means sharing the same letter are not significantly different by Tukey HSD test.

(Mauchamp and Mésleard 2001). EC of the sediment pore water at the PH2 site was constant during the tidal fluctuation and remained ca. 2.3 - 2.7 S/m, whereas EC of the water at the surface showed clear response to tidal change (<0.1 S/m at low tide to 3.5 S/m at high tide). Thus, the rhizosphere of the *P. australis* population at PH2 was constantly in high salinity conditions. Site PH1 was located at a lower elevation site than PH2. Inundation of high salinity water during the high tide period as well as constant high salinity of rhizospheres at PH1 and PH2 would limit the population height, culm density, shoot biomass and seed setting rate at these sites. Population height and shoot biomass showed a maximum at the OLH site, whereas seed setting rate showed a maximum at the LIM site. Thus, seed production of *P. australis* is strongly affected by the salinity of the inundated water and sediment.

Seeds from the LIM site never germinated in a cultivation medium containing 3.5% NaCl, whereas seeds from other sites all germinated at the same salinity, although the germination rate was lower than with the conditions for lower salinity cultivation. No seeds germinated when the NaCl concentration was 5.0%, so no *P. australis* population in the estuary of the Chikugogawa River would reproduce when the salinity reaches 5.0%.

As for salt tolerance at the germination stage, Gorai *et al.* (2006) showed that the germination ratio of seeds from *P. australis* population in saline locations was 98% at 0 mM salinity, decreased to 16% at 50 mM and was 0% at 500 mM [6]. Thus, *P. australis* does not have high salt tolerance during the germination stage. Mauchamp and Mésleard (2001) tested the germination of *P. australis* from three habitats with different soil salinities and

germinated seeds at 0% - 2.5% salinity levels. Germination ratios decreased at 1.5% and higher salinity [5]. Salt tolerance levels between populations were significantly different, but tolerance capabilities were independent of the salinity of original populations. Zehra and Khan (2007) compared the effects of NaCl and sea salt solutions with the same electric conductivity values on germination of *Phragmites karka* [10]. Sea salt inhibited germination more than NaCl at lower temperatures (10˚C - 20˚C), whereas NaCl inhibited germination more at higher temperatures (25˚C - 35˚C). Our results showed almost the same or slightly higher salt tolerance of *P. australis* at the germination stage compared to that presented in these studies, and seeds from high salinity habitats have higher salt tolerance at the germination stage, although germination rates of seeds from saline habitats were lower.

Dry weight growth and elongation of seedlings after 21 days' cultivation in different salinity media did not show significant differences among the salinity regimes of the cultivation media. Elongation of *P. australis* seedlings did not show significant differences among the locality regimes of seeds, whereas dry weight did show significant differences among locality regimes ($p < 0.05$).

Mauchamp and Mésleard (2001) showed that dry weight growth of *P. australis* showed significant negative correlation with the salinity of the cultivation medium [5]. They transplanted the plants with developed rhizomes, whereas we used seedlings at 14 days after germination under freshwater conditions. Seedlings are highly vulnerable to salt stress, and so observations using the seedlings will provide information on the population establishment in habitats with salt stress. Because of the low germination rate (<10%) in the fresh water medium together with the high mortality of seedlings after transplantation, the available seedlings for experiments on seedling growth under salt stress were quite limited in number.

Mauchamp and Mésleard (2001) showed that seedling growth of *P. australis* was inhibited at levels over 0.75% salinity and almost stopped at 2.0% [5]. Three-month juveniles raised in freshwater medium were exposed to 2.5% salinity for 25 days, and the plants recovered growth after being transplanted to freshwater medium. Gorai *et al*. (2007) tested the seedling growth of *P. australis* raised from seeds obtained from saline locations [9]. Seeds were germinated on organic soil, transplanted at 2 weeks of age to freshwater medium for 9 weeks, and then transplanted to 0 - 600 mM NaCl medium for 5 weeks. Biomass and RGR of plants at 0 mM medium showed a maximum, whereas plants survived at 600 mM. Matoh *et al*. (1988) investigated the seedling growth of *P. australis* by using seeds from an abandoned salt field germinated in freshwater medium and transplanted at 5 weeks after germination to 0 - 500 mM NaCl medium [1]. Dry weights of seedlings 6 weeks after transplantation did not show significant differences at levels of 0 and 300 mM of NaCl in the cultivation medium. Hootsmans and Wiegman (1998) showed that seedlings exposed to 1.8% salinity for 3 weeks recover growth after beingtransplanted to freshwater medium [8]. Lissner and Schierup (1997) investigated the salt tolerance of seedlings of *P. australis* obtained from brackish marshes [7]. Seedlings from seeds germinated in freshwater medium for 10 weeks were transplanted to 0% - 5% salinity and all the juveniles died out at 2.25% and higher salinity.

Seeds from the LIM site in our study never germinated in cultivation medium containing 3.5% NaCl, but seedlings from the seeds obtained from the LIM site showed shoot growth at 3.5% NaCl cultivation medium. This implies that salt tolerance of *P. australis* at the seedling stage is higher than at the germination stage, and the seedlings acquire higher salt tolerance after germination in a low salinity environment. Lissner and Schierup (1997) showed that matured *P. australis* plants have higher salt tolerance than juvenile plants [7]. Haslam (1971) also showed the higher salt tolerance [11] of matured *P. australis* plants, and these studies imply the acquisition of higher salt-tolerating ability during the development of plants. We compared the salinity tolerance of seeds and seedlings of *P. australis* obtained from the same population and showed that seedlings of *P. australis* had higher salt-tolerating ability than seeds, largely in agreement with these earlier studies. According to Lissner and Schierup (1997), all juveniles of *P. australis* died out at 2.25% and higher salinity, whereas rhizome-grown plants die out at 3.5% and higher salinity. Thus, rhizome-grown plants obtained much higher salt tolerance than seedlings [7].

Some recent studies investigating invasion of *P. australis* in salt or brackish marshes showed there are differences in the introduced populations, especially haplotypes, compared to the native populations in the ecological performance of *P. australis* (Lissner *et al*. 1999; Burdick and Konisky 2003; Philipp and Field 2005; Vasquee *et al*. 2005; Vasquee *et al*. 2006) [12]-[17]. Thus, the genetic properties of the *P. australis* populations could be important factors determining the salt tolerance of the species. Our investigation showed the local populations of *P. australis* within the limited habitat of an estuary had ecological varieties, ignoring genetic variance between

populations. Genetic variations of populations in relation to salt tolerance may be important aspects for the analysis of salt tolerance of *P. australis* populations.

5. Conclusion

Phragmites australis in the fresh water habitat of the estuary of the Chikugogawa River showed lower salt tolerance at the germination stage compared to that of the seeds from the habitats with higher salinity. Seeds from the freshwater habitat showed vegetative growth in the same salinity conditions in which other seeds never germinated. Salt tolerance of the populations was partly determined by the habitat conditions of salinity, but seedlings from freshwater habitats acquire higher salt tolerance at the seedling growth stage after being germinated under freshwater conditions. Thus, a population of *P. australis* in a high salinity habitat of the estuary community can only be established by vegetative reproduction from a population established in a low salinity habitat if the population is regenerated from seeds.

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