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GAS EXCHANGE AND FLUORESCENCE OF CITRUS ROOTSTOCKS VARIETIES UNDER SALINE STRESS[1]

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ABSTRACT- High salt concentration in water are common in Brazilian semirad region, being important to research alternatives for use this waters on crop, like use of tolerant genotypes to salinity. Thus, in order to evaluate the saline stress perception of citrus rootstocks varieties crop from gas exchange and fluorescence analysis, an experiment was realized in greenhouse at the Center for Science and Technology Agrifood, CCTA, of Federal University of Campina Grande, UFCG, Pombal, PB, Brazil. It was studied in a randomized block design with factorial scheme (2x4), two salinity levels (0.3 and 4.0 dSm-1) and four varieties of citrus rootstocks [1 -common Sunki mandarin (TSKC), 2 - Florida Rough lemon (LRF), 3 -Santa Cruz Rangpur lime (LCRSTC) and 4-Volkamer lemon (LVK)], with three replications. The citrus rootstocks varieties grown on hydroponic system and at 90 days after sowing the plants were evaluated by gas exchange and PSII fluorescence at 0, 24 and 48 hours after application of treatments to determine the times for the physiological establishment of salt stress. The first 48h under saline conditions promoted changes in gas exchange and PSII fluorescence in varieties TSKC, LRF and LCRSTC indicating the begin of physiological stress; the common 'Sunki' mandarin and the 'Florida Rough' lemon are the more sensitive genotypes to saline stress, in order hand the 'Santa Cruz Rangpur' lime and 'Volkamer' lemon are the genotypes more tolerant.
Index terms: *Citrus* spp., salinity, physiology.

TROCAS GASOSAS E FLUORESCÊNCIA DA CLOROFILA EM VARIEDADES DE PORTA-ENXERTOS DE CITROS SOB ESTRESSE SALINO

RESUMO- Águas com maior concentração de sais são comuns em regiões semiáridas, sendo importante viabilizar sua utilização, o que pode ocorrer com o cultivo de genótipos tolerantes à salinidade. Nesse contexto, realizou-se este trabalho, objetivando-se avaliar a percepção do estresse salino em variedades de porta-enxertos de citros, por meio de trocas gasosas e da fluorescência da clorofila a. O experimento foi desenvolvido em ambiente protegido do Centro de Ciências e Tecnologia Agroalimentar, da Universidade Federal de Campina Grande - UFCG, Pombal-PB. Foram estudados, em blocos casualizados e usando o esquema fatorial (2x4), dois níveis de salinidade (0,3 e 4,0 dS m-1) da água usada para o preparo das soluções nutritivas, obtendo-se soluções com 2,6 e 6,3 dS m-1, respectivamente, após acrescentar os fertilizantes, utilizadas na irrigação de quatro variedades de porta-enxertos de citros [1- tangerineira Sunki comum (TSKC); 2- limoeiro Rugoso da Flórida (LRF); 3- limoeiro Cravo Santa Cruz (LCRSTC), e 4-limoeiro Volkameriano (LVK)], com três repetições. As variedades de porta-enxertos de citros foram cultivadas em sistema hidropônico e, aos 90 dias após a semeadura, foram avaliadas quanto aos parâmetros de trocas gasosas e de fluorescência do fotossistema II (PSII), nos tempos de 24 e 48 horas após o início da aplicação dos tratamentos, de modo a determinar o estabelecimento fisiológico do estresse salino. As primeiras 48 horas sob condições de salinidade promoveram alterações nas trocas gasosas e fluorescência do PSII, nas variedades TSKC, LRF e LCRSTC, indicando o início do estresse fisiológico; a tangerineira 'Sunki comum' e o limoeiro 'Rugoso da Flórida' são mais sensíveis à aplicação do estresse, enquanto o limoeiro 'Cravo Santa Cruz' e o limoeiro 'Volkameriano' são genótipos mais tolerantes.
Termos para indexação: *Citrus* spp., solução salina, mudas.

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INTRODUCTION

Brazil is a world reference in the production of fresh fruits, especially the orange-'Doce '[Citrus sinensis (L.) Osbeck] and lime-sour' Tahiti '(C. Latifolia Tanaka), which are responsible for the first and fourth place respectively, in the ranking of fruit exports (AGRIANUAL, 2013).

In particular the citrus cultivation is based largely on the use of limoeiro- Rangpur (C. limonia Osbeck) as rootstockcitrus (MATTOS JUNIOR et al., 2005). However, with a single rootstock, it is not possible to get the maximum potential of each variety, thus preventing the plant to expresses all its productive capacity, and the vulnerability of the crops in cases of endemic diseases (POMPEU JUNIOR, 2005) . This fact is reflected in the orchards of productivity values in Brazil, where the average is 24.24 t ha^{-1}, a low value when compared to countries like the United States (31.75 t ha^{-1}), South Africa (35.62 t ha^{-1}) and Turkey (40.09 t ha^{-1}) (FAO, 2013).

This difference in productivity is also noted between the regions of the country, like São Paulo and Paraná, for example , where the highest yields are recorded, with 28.43 and 32.48 t $ha^{-1,}$ respectively. Unlike in Bahia and Sergipe, the largest producers in the Northeast, are low yields (15.92 and 14.58 t $ha^{-1,}$ respectively). In this sense, the use of materials with greater yield potential can help increase productivity.

In addition to problems related to lack of genetic diversity in the Brazilian citrus industry, the expansion of its cultivation to other regions of the country depends on numerous factors, such as obtaining tolerant materials to abiotic stresses, like water and salt stress, common in semi-arid areas of the Brazilian Northeast.

Salt stress is one of the main limiting factors to growth and to the productivity of citrus in the Northeast, due to the reduction in water availability to plants, and due to the lower osmotic potential of the soil solution, which implies increased energy expenditure for absorption of water and nutrients (Leonardo et al., 2003). However, these effects vary among species and genotypes of the same species and between plant developmental stages (Ayers; Westcot., 1999; Fernandes et al, 2011), making it possible to identify genetic materials tolerant to this factor, enabling the expansion of new agricultural frontiers.

It should be noted, however, that the identification of citrus genotypes tolerance to salinity may be a long term process, for the need to be analyzed during several stages of growth of the plant, either in the formation of the rootstock , in the initial phase of interaction of the scion and of the rootstock, whether in the field phase, up to the fruit production. One can, however, find indications of tolerant materials, especially rootstocks, by checking the changes in gas exchange and chlorophyll fluorescence, as identified by Silva et al. (2014) who studied physiological mechanisms in trifoliated hybrid citrus and hybrid tangerine 'Sunki', all recommended as rootstock.

The objective was to study the gas exchange and chlorophyll a fluorescence in citrus genotypes, during the initial phase of submission to salt stress in order to identify physiological changes that are indicative of possible tolerance.

MATERIALS AND METHODS

The experiment was conducted in a protected environment (greenhouse) at the Centre for Science and Technology Agrifood - CCTA, the Federal University of Campina Grande - UFCG, located in the municipality of Pombal-PB with the geographic coordinates 6º47'20 "latitude S and 37º48'01 "W longitude, at an average altitude of 184 m.

The two factors that were studied corresponded to two water salinity levels [0.3 (regular drinking water) and 4.0 dS m^{-1} (salinated water supply)] and four genotypes, represented by varieties recommended as rootstock citrus [1 'Sunki' Common tangerine (TSKC) (Citrus sunki (Hayata) hort ex Tanaka.); 2 'Rough Florida' lemon (LRF) (C. jambhiri Lush.); 3 'Rangpur Santa Cruz' (LCRSTC) (Citrus limonia Osbeck), and 4- 'Volkameriano' lemon (LVK) (C. volkameriana V. Ten. & Pasq.)]. Using factorial combinations (2 x 4) as a result of 8 treatments was obtained , which were distributed in randomized blocks and three replications, and the experimental unit consisting of four working plants. It was observed that all genotypes were obtained by the Genetic Improvement Program of Citrus Embrapa Cassava and - PMG Citrus.

The water salinity levels tested were based on the threshold salinity (2 dS m^{-1}), being one level below and one above the threshold salinity of citrus varieties established by Singh et al. (2003).

The seeding and the initial growth of the seedling , and the transplanting of different genotypes occurred in containers of 1,500 ml capacity, and washed coconut fiber substrate, to avoid interference of salts present in the material in the availability of nutrients. It is noteworthy that the nutrient solutions used followed the recommendations of Hoagland and Arnon (1950), which, however, added 25% EDTA iron in order to meet the nutritional needs of

plants in relation to this micronutrient in accordance with observations in the preliminary test. In Table 1, one can check the result of chemical analysis of this nutrient solution methodology determined from EMBRAPA (2009).

The seeds, properly selected and treated with thiram disulfide fungicide (4g, 1 kg of seeds) were sown at a rate of three per container, at an approximate depth of 2 cm. Containers used for cultivation of citrus trees were "Leonard vessels", fitted with Pet bottles (Santos et al., 2009). The upper vessel was filled with 1.5 L of the substrate and, at the bottom, remained the Hoagland nutrient solution under continuous flow.

After preparation, the Leonard vessels were wrapped with double-sided plastic, in order to reduce evaporation of the solution; Moreover, each vessel was connected via hose to a tank containing the nutrient solution which, in turn, enabled the continuous supply of solution to the containers, and the level of this container was checked daily for volume and electrical conductivity of the solutions which were indicators for solution increase or for the changing of the containers , this way ensuring that the substrate always remains with moisture next to the container capacity.

After germination, when the seedlings were with three or more pairs of true leaves, the thinning was performed , leaving only one individual per container, keeping only those that represented the plant pattern of each genotype, to select seedlings of nucellar origin (originating from their parent plants). They adopted all controls for weed herbs and prevention and control of pests, recommended in the production of nursery trees (MATTOS JUNIOR et al., 2005).

The saline water up to 4.0 dS m^{-1}, used in mixture with the nutrient solution was prepared so as to have an equivalent ratio of 7: 2: 1, among Na: Ca: Mg, and is considered, therefore, ECw, and the relation between salt concentration (10 mmol L^{-1} * 1 = dS m^{-1} CE_A), extracted from Rhoades et al. (1992) CE_A valid from 0.1 to 5.0 dS m^{-1} that fits the level tested, based on the existing water supply in place. This relationship was chosen because it reflects the predominant ions in water sources used for irrigation in small Brazilian Northeast properties (Medeiros et al., 2003). The preparation of the solution with different electrical conductivity (EC) was given by the addition of salt water until it reached the desired level of EC, giving values using a portable conductivity meter set to the temperature 25 ° C.

In the water supply (0.3 dS m^{-1}) and water salinity (4.0 dS m^{-1}) nutrient solutions were added which gave an increase of 2.3 dS m^{-1} in the electrical conductivity of water , which was measured daily with use of a portable conductivity measuring unit adjusted to 25 ° C; thus, the solutions available to the plants had 2.6 dS m-^{1} into S_1e6.3 dS m^{-1} in S_2.

90 days after sowing, the citrus genotypes were evaluated for physiological establishment in relation to salt stress, measuring up the CO_2 assimilation rate (A) (mmol m-^{2} s-^{1}) transpiration (E) (mmol H_2O m-^{2} s-^{1}), stomatal conductance (gs) (mol of H_2Om-^{2} s-^{1}) and internal CO_2 concentration (Ci) of the first mature leaf, measured from the apex, using the portable photosynthesis measuring "LCPro +" of ADC BioScientific Ltda. With this data, the efficiency of water usage (USA) (A / T) [(ol m-^{2} s-^{1}) (H_2O mmol m-^{2} s-^{1}) -^{1}], and the instantaneous efficiency of carboxylation Φc (A / C) (KONRAD et al., 2005) could be estimated. In the same leaves, which were analyzed for gas exchange , foliar tweezers were placed and, after a period of 30 minutes of dark adaptation (KONRAD et al., 2005) the fluorescence parameters such as : initial fluorescence (Fo) , maximum fluorescence (Fm), variable fluorescence (Fo-Fm) and quantum efficiency of photosystem II (Fv / Fm), were determined using the PEA equipment - Hansatech. Both gas exchange and fluorescence were evaluated in the 0; 24 and 48 hours after beginning the application of nutrient solutions added with saline water.

The results were evaluated by analysis of variance, the 'F' test. In cases of significance a mean clustering test was performed (Scott and Knott to 5% probability) for the factor genotype was studied for each level of salinity (Ferreira, 2011).

RESULTS AND DISCUSSION

Studying gas exchange through the unfolding of the factors in all variables measured at 24 and 48 h (Table 2), it was found, regardless of the concentration of salts in solution, higher average values in the 'Rough Florida' lemon tree especially with respect to the stomatal conductance (gs), transpiration (E) and net photosynthesis (A) did not differ statistically, however, from the 'Rangpur Santa Cruz' and the 'Volkameriano' lemon, implying greater physiological potential observed in these lemons compared with the common 'Sunki' tangerine . This result was similar to that reported by Silva et al. (2014) studying hybrid trifoliated and other citrus hybrids under salt stress, highlighting the effect of salinity on gas exchange of some genotypes, which the authors consider to be sensitive to salinity.

When analyzing the effect of salinity solutions in gas exchange variables , an increase in conductance of stomata, Photosynthesis and transpiration was noted in general when plants were exposed to treatment with the higher salt concentration (Table 2) except for what happened at 24h with LVK, verifying reduction of gas values and A. The increase in gas exchange, observed in most genotypes and saline conditions may be related to the fact, in an attempt to lower the effect of stress, an increase in net CO_2 assimilation has occurred. This effect may be associated with secondary routes for the production of organic compound protectors, such as proline, so that they are compartmentalized in the vacuole, as explained by Taiz and Zaiger (2009) and is related to increased synthesis and absorption solutes, as observed in the physiological mechanisms of lemon 'Rough Florida'.

On the other hand, the increase in gas exchange can lead to higher consumption of water and of salts, causing excessive accumulation of specific ions in the plant (FLOWERS, 2004; FLOWERS; FLOWERS, 2005), further aggravating the effects of salt stress.

In detailing the interaction between the factors, it might be noted that the largest increase caused by exposure to saline solution occurred in the lemon tree 'Rough Florida' on the stomatal conductance, an increase of 57.1% and 78.6% at 24 and 48 hours after the start of the application, respectively (Table 2), in fact net photosynthesis also occurred, where 33.8% and 51.2% increase was found in the values with increasing salt concentration, respectively. This behavior was also observed in common 'Sunki' mandarin with 107.1% increase in CO2 assimilation rate. Thus, there is indication that these genotypes are more sensitive to increased salt concentrations.

Minor changes in gas exchange were observed in Rangpur lime Santa Cruz lemon , and 'Volkameriano ' lemon . In the case of CO_2 assimilation rate, at 24 hours, an increase of 24.4% of net LCRSTC photosynthesis and a reduction of 9.6% in LVK was noticed , already at 48 hours, and in both cases there was an increase in photosynthesis , with values of 26.4% and 31.4% in value, respectively, lower than those observed in other genotypes, indicating greater stability in gas exchange, thus indicating tolerance. Corroborating this statement, Brito (2010), studying the tolerance of citrus genotypes, the rootstock formation stage and after grafting, that salt stress was also found, in these genotypes, the highest survival rates, even applying water 4.0 dS m^{-1}, beginning at 60 days and ending at 330 days after sowing.

It is interesting to note that both the 'Rangpur Santa Cruz' and the 'Volkameriano' are seen as tolerant to salinity by Brito et al. (2008) and Fernandes et al. (2011), confirming that the low change in gas exchange in early stress is indicative of promising materials.

In contrast, genotypes which exhibit large changes in gas exchange process during the first hours of submission to salinity conditions may be sensitive or have moderate restriction salinity, triggering the physiological mechanisms that allow its adaptation to stress conditions, as occurred with common 'Sunki' mandarin and the 'Rough Florida' lemon tree. Similarly, two hybrids of 'Sunki' tangerine were studied by Rochdi et al. (2005) for 60 days and tissue culture conditions, observing that they were sensitive to the nutritive solution with a concentration of 70 mM, which is equivalent to 7 dS m^{-1}, indicating that the observed increment can mean increased sensitivity to stress. Similarly, Sant'Anna (2009) studied the gas exchange in plant ne 'Sunki Florida' tangerine under progressive water stress, and also noticed increased sensitivity of this genotype, reinforcing the claim that this material is sensitive and that the variables of gas exchange are important tools in identifying stress and tolerant genotypes.

By studying the fluorescence of chlorophyll *a* , at 24 and 48 hours after the start of applying the solutions, the only effect for treatments for maximum and variable fluorescence (Table 3), highlighting, as well as gas exchange, major changes in tangerine common 'Sunki' and 'Rough Florida' lemon , noting the increase in the maximum fluorescence of 9.75% and 25.6%, and 13.6 and 34.5% in the variable fluorescence, respectively. These results imply greater loss of energy by chlorophyll complex, which can be related to the greater difficulty of plants to absorb water and optimize the process of light energy usage . These findings may be related to increased stomatal conductance and CO_2 assimilation rate observed in these genotypes at 48 hours of application of saline treatment referencing, therefore, the sensitivity of these individuals to salinity (Table 3). These results corroborate those proposed by Baker and Rosenqvst (2004), in which plants exposed to abiotic stress suffer alterations in the functional state of the thylakoid membranes of chloroplasts, occurring frequent changes in the characteristics of fluorescence signals, these can be perceived by fluorescence study the leaf.

The 'Cravo Santa Cruz' lemon and 'Volkameriano' lemon did not change significantly in their physiology, due to the application of saline

treatment during the first 48 hours of exposure to treatment, which can be related to the greater degree of tolerance of these varieties to salinity conditions than the others studied (Table 3).

In general, one can say that the plants that were evaluated for up to 48 hours under salt stress were in the first stage of the stress, corresponding to osmotic stress where there is a restriction on the absorption of water and nutrients by reducing the osmotic potential, which led to restrictions in the use of light by chlorophyll, triggering an increase in gas exchange, especially in genotypes that are more sensitive.

TABLE 1- Concentration of nutrients in the nutrient solution for hydroponics proposed by Hoagland and Arnon (1950), set to citrus. Pombal-PB, 2014.

Nutrients	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Mo
							(mmol L^{-1})					
Concentration	15	1	6	5	2	2	0,0625	0,01	0,05	0,003	0,0008	0,001

TABLE 2- Internal concentration of CO_2 , transpiration, stomatic conductance , CO_2 assimilation rate , water efficiency and instantaneous carbolixation efficiency of the citrus varieties, 24 and 48 hours after the begining of stress in hydroponic culture. Pombal - PB, 2014.

	Salinity Exposure				Salinity Exposure			
	24 hours		48 hours		24 hours		48 hours	
Var.*	0,3 dSm^{-1}	4,0 dSm^{-1}	0,3 dSm^{-1}	4,0 dSm^{-1}	0,3 dSm^{-1}	4,0 dSm^{-1}	0,3 dSm^{-1}	4,0 dSm^{-1}
	Internal CO_2 Concentration				CO_2 assimilation rate (*A*) (µmol m^{-2} s^{-1})			
TSKC	264 bA	225 bA	309 aA	262 aA	3,15 bA	3,39 bA	1,67 bA	3,46 bA
LRF	245 aA	251 aA	259 bA	256 bA	8,40 aA	11,24 aA	7,78 aB	11,77 aA
LCRSTC	262 aA	253 aA	255 bA	251 bA	6,87 aA	8,55 aA	6,95 aA	8,79 aA
LVK	233 aA	223 bA	234 bA	237 bA	9,04 aA	8,17 aA	8,08 aA	10,62 aA
	Transpiration (*E*) (mol of H_2O m^{-2} s^{-1})				Efficiency of water usage (USA) (*A*/*E*) [(µmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$]			
TSKC	1,10 cA	0,93 cA	1,08 bA	1,16 cA	3,02 aA	3,59 aA	1,65 bA	2,99 bA
LRF	2,39 aA	3,11 aA	2,19 aB	3,09 aA	3,62 aA	3,62 aA	3,61 aA	3,81 aA
LCRSTC	2,18 bA	2,52 bA	1,89 aA	2,34 bA	3,13 aA	3,38 aA	3,71 aA	3,73 aA
LVK	2,30 bA	2,06 bA	1,88 aA	2,51 bA	3,99 aA	4,06 aA	4,30 aA	4,25 aA
	Stomatic conductance (mol of H_2Om^{-2} s^{-1})				Instantaneous corboxilation efficiency Φc (*A*/Ci)			
TSKC	0,05 bA	0,04 dA	0,05 bA	0,06 bA	0,012 bA	0,015 bA	0,005 bA	0,013 bA
LRF	0,14 aB	0,22 aA	0,14 aB	0,25 aA	0,034 aA	0,045 aA	0,030 aB	0,046 aA
LCRSTC	0,12 aA	0,16 bA	0,12 aA	0,16 aA	0,027 aA	0,034 aA	0,028 aA	0,035 aA
LVK	0,14 aA	0,12 cA	0,11 aA	0,18 aA	0,039 aA	0,037 aA	0,035 aA	0,045 aA

Different very small Letters indicate significant difference between genotypes in the respective level of electric condutividade (CEa), for the test of Skott-knott, 5% of probability; different capital letters indicate significant difference enter the levels of salinity (CEa) applied in the respective genotype, for the test of Tukey, 5% of probability. Var.: Varieties; *: LCRSTC: lemon tree `Cravo Santa Cruz' (limonia Citrus Osbeck); TSKC: tangerineira `Sunki' [C. sunki (Hayata) hort. former Tanaka] common election; LVK: lemon tree `Volkameriano' (C. *volkameriana* V. Ten. &Pasq.); LRF: lemon tree `Rugoso' election `of Flowery' (the C. *jambhir*i Lusch.).

TABLE 3 - Inicial Flourescence , Maximum Flourescence , Variable Flourescence , and quantum fotosystem II efficiency of the citrus varieties 24 and 48 hours after the begining of stress in the hydroponic culture . Pombal - PB, 2014.

Initial Flourescence (Fo)				
Var.*	Exposed to salinity			
	24 hours		48 hours	
	0,3 dSm^{-1}	4,0 dSm^{-1}	0,3 dSm^{-1}	4,0 dSm^{-1}
TSKC	488 aA	414 aA	454 aA	441 aA
LRF	423 aA	395 aA	440 aA	424 aA
LCRSTC	467 aA	456 aA	514 aA	502 aA
LVK	470 aA	463 aA	472 aA	526 aA
Maximum Flourescence (Fm)				
TSKC	2079 bA	2151 bA	1968 aA	2160 bA
LRF	2376 bA	2069 aB	1882 aB	2365 bA
LCRSTC	2154 aB	2373 bA	2259 aA	2393 aA
LVK	2293 aA	2267 aA	2271 aA	2286 aA
Variable Flourescence (Fv)				
TSKC	1591 aA	1736 bA	1513 aB	1719 bA
LRF	1953 aA	1674 aB	1442 aB	1940 bA
LCRSTC	1687 aB	1917 aA	1745 aA	1890 aA
LVK	1823 aA	1803 bA	1798 aA	1760 aA
Quantum fotosystem II efficiency (Fv/Fm)				
TSKC	0,76 aA	0,81 aA	0,77 aA	0,80 aA
LRF	0,82 aA	0,81 aA	0,76 aA	0,82 aA
LCRSTC	0,78 aA	0,81 aA	0,77 aA	0,79 aA
LVK	0,80 aA	0,80 aA	0,79 aA	0,77 aA

Different very small Letters indicate significant difference between genotypes in the respective level of electric condutividade (CEa), for the test of Skott-knott, 5% of probability; different capital letters indicate significant difference enter the levels of salinity (CEa) applied in the respective genotype, for the test of Tukey, 5% of probability. Var.: Varieties; *: LCRSTC: lemon tree `Cravo Santa Cruz' (limonia Citrus Osbeck); TSKC: tangerineira `Sunki' [C. sunki (Hayata) hort. former Tanaka] common election; LVK: lemon tree `Volkameriano' (C. *volkameriana* V. Ten. &Pasq.); LRF: lemon tree `Rugoso' election `of Flowery' (the C. *jambhiri Lusch.*).

CONCLUSIONS

There are alterations in gas exchange and chlorophyll fluorescence in citrus genotypes, especially to those who are sensitive, with up to 48 hours of submission to salt stress.

The common 'Sunki' tangerine and the 'Rough Florida' lemon tree proved to be more sensitive to the application of salt stress.

The 'Cravo Santa Cruz' lemon and 'Volkameriano' behaved as less sensitive to salinity.

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