

PHYSIOLOGICAL RESPONSE OF NATIVE ECOTYPES OF *CLADOPHORA GLOMERATA* (L.) KÜTZING (CHLOROPHYCEAE) IN RELATION TO SALINITY

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Abstract

The effect of different salinities on intracellular minerals, total carbohydrates, soluble sugars, insoluble sugars, ammonia, amides, nitrates, proline, protein and total nitrogen contents of *Cladophora glomerata* was studied. The results revealed that osmoregulation mechanism in this species was only in part via mineral ions concentrations and mainly via proline production. The results suggest *Cladophora glomerata* as a “salinity generalist” species (a species that able to maintain fitness in a wide range of salinities).

Key words: *Cladophora glomerata*, physiological response, ecotypes, salinity.

Introduction

Phenotypic plasticity is the ability of an individual organism to alter its physiology and morphology in relation to changes in environmental conditions (Schlichting, 1986). By the early 1990's, developmental and physiological plasticity had been reported in plants, marine organisms, insects, reptiles, small mammals (Sultan, 1995) and lichens (Pintado *et al.*, 1997). However differences in physiological plasticity have been demonstrated in response to changes in, nutrient concentrations for microalgae (Fabergas *et al.*, 1986), salinity for *Skletonema costatum* (Rijstenbil *et al.*, 1989a), salinity for *Ditylum brightwellii* (Rijstenbil *et al.* 1989b) salinity for *Enteromorpha intestinalis* (Allam, 1994) and salinity and inorganic nitrogen sources for *Dunaliella salina* and *Chlorella salina* (Abdel-Kareem, 1997).

Few originally marine algae have been succeeded in conquering pure fresh water habitats. The diatom *Hyrosera triquetra*, the red alga *Caloglossa agaswgraensis* (Simonsen, 1965), the brown alga *Ectocarpus siliculosus* (Russel and Balton, 1975) and the green alga *Enteromorpha intestinalis* (Allam, 1989; Martins *et al.*, 1999; Kamer and Fong, 2000) live in marine water, but can migrate to fresh water or water fall.

Full realization of algal potentialities to overcome the salinity problem will not be achieved until our understanding of their physiological responses to salt stress is intensively studied. So, this work aims to evaluate the physiological responses of *Cladophora glomerata* in relation to salinity stress and to assess the

metabolites that will be stimulated to carry out the osmoregulatory mechanisms in this species.

Materials and Methods

Algal material and water sampling

Cladophora glomerata was collected from three habitats representing fresh water, estuarine and marine environments at El-Max locality, Alexandria Egypt. The marine sample was collected from the sea, the estuarine sample from the junction between the sea and Lake Mariut under El-Max Bridge while the fresh water sample from the lake itself. Water samples were taken from the same sites; subsurface water samples were collected in polyethylene bottles and conveyed to the laboratory for analysis.

Culturing

The collected algal materials were maintained under laboratory conditions in their natural bathing medium supplied with enriching solution (Boalch, 1961) and two antibiotic mixture (Stein, 1973) in a large aquarium with good aeration for acclimation. The acclimatized axenic progeny of these plants were used as the starting inocula for culture purposes. The samples were cultured in 1 liter capacity flat bottom flasks equipped with inlet and outlet tubes for aeration. Each culture flask contained 500 ml enriched sterilized water of the appropriate salinity and 2 g of axenic fresh algal material. The cultures were vigorously aerated with air containing approximately 0.5% CO₂ passed through sterilized gamma 12 filter units to prevent bacterial contamination. The culture cabinet was provided with cooling unit to adjust the temperature at 25°C and light was supplied by 6 fluorescent lamps giving a total light intensity of 80 μmol m⁻²s⁻¹. The light regime was 16 h light/8 h dark cycles and the flasks were illuminated from below. The algal materials used in biochemical analysis were collected at its exponential growth phase (data of growth curve not shown) after 6 days. Different grades of salinity were prepared by diluting double strength sea water (Starr, 1978).

Biochemical analysis methods

The amount of sodium, potassium and calcium in the algal material was determined according to the method described by Chapman (1976). The carbohydrate contents were assayed by the procedure described by Muratl et al. (1968). Ammonia was determined colorimetrically using the phenol method as reported by Booth and Lobring (1973). Amides and nitrates were determined according to Naguib (1964) and Johnson and Ulrich (1950) respectively. Free proline in water extract of the algal material was estimated as recommended by Bates *et al.* (1973), protein content was assayed by the method described by Hartee (1972) while the total nitrogen content assayed by the method described by Barakat *et al.* (1986). All experiments described were conducted

independently twice with three replicates each, the mean values given in results representing averages of six assays.

Results and Discussion

Organisms are not just slaves to the physical environments, they adapt themselves and modify according to changes in these environments so as to reduce the limiting effects of environmental factors. Species with wide geographical ranges almost always develop locally adapted populations called ecological races or ecotypes that have optima and limits of tolerance adjusted to local conditions (Fretwell, 1972). Accordingly, we have three ecotypes of *Cladophora glomerata* grown naturally in three different salinity habitats: marine, estuarine and fresh water.

The analysis of water collected from these three habitats (Table 1) revealed that the total alkalinity, total hardness and calcium content are nearly the same in the three habitats. However, sea water was found to be the richest in the content of chloride, nitrate and magnesium followed by estuarine water then fresh water. Meanwhile, the estuarine water was the richest in silicate, while the fresh water was the richest in sulphate content. These variations, mainly in salinity, brought about a multitude of structural response mainly in body size, where there was a gradual decrease in body size from marine to estuarine and then to fresh water samples.

Table (1): Analysis of water in the three habitats of *Cladophora glomerata*.

Content of water sample	Habitat		
	Sea water	Estuarine water	Fresh water
Total alkalinity (g/L)	0.940	1.000	1.000
Chloride (mg/L)	8.800	3.600	2.750
Nitrate (mg/L)	0.120	0.050	0.020
Total hardness as carbonate (g/L)	2.980	1.600	1.320
Hardness as Ca carbonate (g/L)	1.000	1.040	1.140
Hardness as Mg carbonate (g/L)	1.980	0.560	0.180
Calcium (g/L)	0.400	0.417	0.456
Magnesium (g/L)	0.481	0.136	0.437
Sulphate (g/L)	0.113	0.125	0.520
Silicate (g/L)	3.000	4.000	2.000
Salinity ‰	37.500	17.000	5.000

When marine macroalgae subjected to increase or decrease in salinity, they are able to regulate their intracellular osmotic pressure by adjusting cellular ion concentrations (K^+ , Na^+ , Ca^{++} and Cl^-) and/or concentrations of organic solutes (Allam, 1989; Thomas *et al.*, 1990; Kirst, 1990 and others). Evidently such variation plays a major role in enabling particular species to inhabit a variety

of saline habitats. This is not absolutely true regarding ions contents in the three ecotypes in their natural habitats (Table 2). Although Ca^{++} and Cl^- contents were higher in the marine sample, it is not so for both K^+ and Na^+ contents. Meanwhile, highest K^+/Na^+ ratio was recorded in estuarine sample, which suggest a special osmoregulatory mechanism to overcome salinity fluctuation characterizing its natural habitat, since plants with high K^+/Na^+ ratio had wider salt tolerance (Thomas *et al.*, 1990).

Table (2): Analysis of some carbohydrate, nitrogen and minerals fractions of the three ecotypes of *Cladophora glomerata*.

Fraction	Algal ecotype		
	Sea water	Estuarine water	Fresh water
Carbohydrate fractions			
Total carbohydrates ($\mu\text{g}/100$ g fresh wt.)	356.000	310.000	98.400
Soluble sugars ($\mu\text{g}/100$ g fresh wt.)	22.080	19.480	10.920
Insoluble sugars ($\mu\text{g}/100$ g fresh wt.)	333.920	290.520	87.500
Nitrogen fractions			
Ammonia ($\mu\text{g}/100$ g fresh wt.)	0.900	2.800	2.600
Amides ($\mu\text{g}/100$ g fresh wt.)	8.400	7.860	8.220
Nitrates ($\mu\text{g}/100$ g fresh wt.)	2016.000	2284.000	3072.000
Soluble nitrogen (mg/g dry wt.)	4.200	4.500	4.000
Insoluble nitrogen (mg/g dry wt.)	5.800	3.000	48.000
Total nitrogen (mg/g dry wt.)	10.000	7.500	52.000
Protein ($\mu\text{g}/100$ g fresh wt.)	252.000	567.000	445.000
Proline ($\mu\text{g}/100$ g fresh wt.)	2.400	1.800	0.600
Minerals fractions			
Chloride (Cl^- mg/100g fresh wt.)	30.700	27.300	24.800
Calcium (Ca^{++} $\mu\text{g}/\text{g}$ dry wt.)	126.780	73.940	77.460
Potassium (K^+ $\mu\text{g}/\text{g}$ dry wt.)	29.720	35.480	33.270
Sodium (Na^+ $\mu\text{g}/\text{g}$ dry wt.)	106.960	107.960	105.860
K^+/Na^+ ratio	0.278	0.329	0.314

Therefore, the osmoregulation mechanism in *Cladophora glomerata* was suggested to be only in part via mineral ions concentrations. This suggestion was supported by the results obtained when the alga cultivated in different salinities. Although there is a proportional relationship between salinity and Cl^- content, the K^+ , Na^+ and Ca^{++} concentrations increase gradually by increasing salinity up to 45‰ or 55‰ in most cases (Figure 1). However, the increase of salinity more

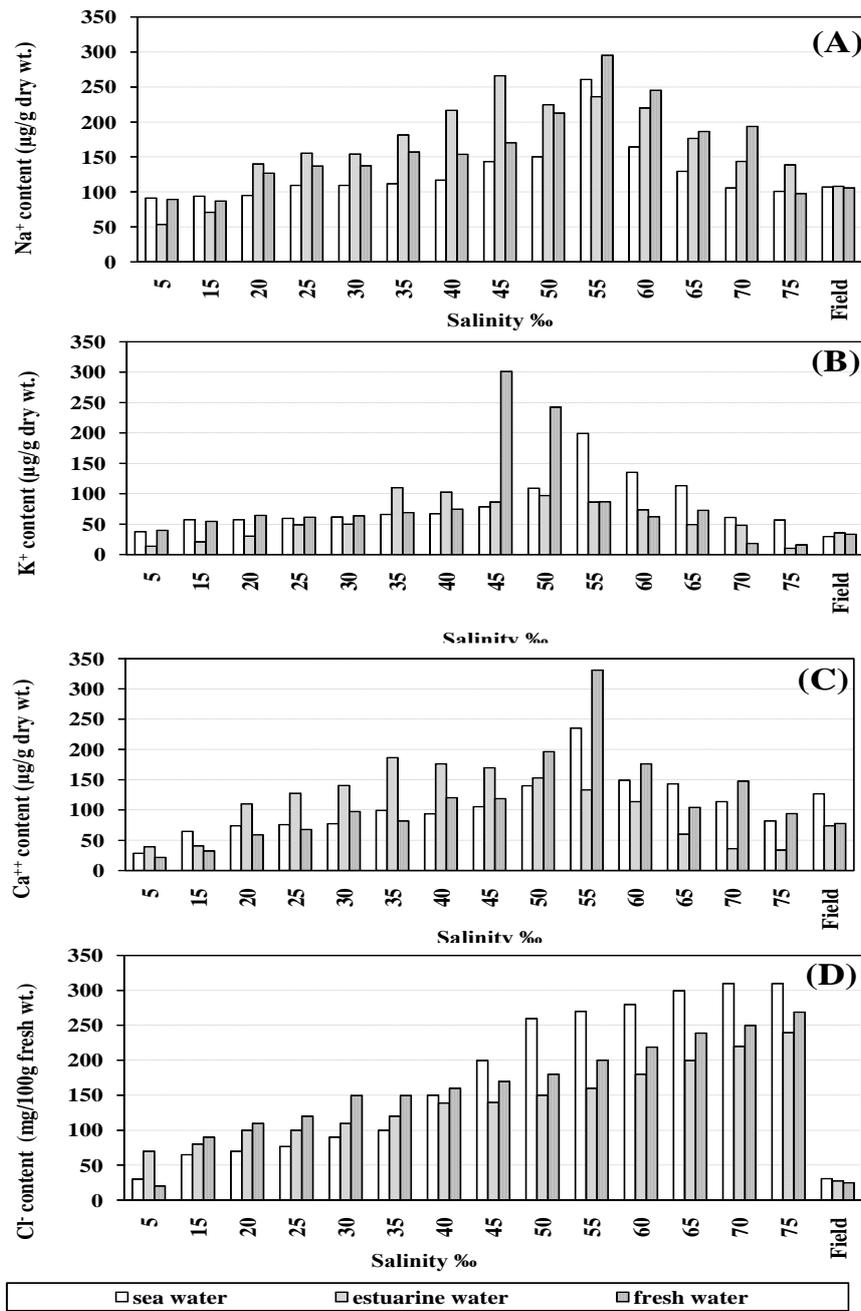


Figure 1. Effect of different salinities on the intracellular minerals contents of *Cladophora glomerata* collected from different habitats, (A) Na⁺, (B) K⁺, (C) Ca⁺⁺ and (D) Cl⁻.

than 55‰ led to a decrease of K^+ , Na^+ and Ca^{++} concentrations and led also to the interaction of another osmoregulator, proline (Figure 4).

Marine sample in its natural habitat showed higher proline content than both estuarine and fresh water ones, which provide physiological adaptation to local environmental salinity conditions. Meanwhile, cultivation in different salinities showed a proportional relationship between salinity and proline content in the three ecotypes. Proline accumulation may be considered either as an indicator of salt-induced water deficit or as a temporary deviation of the metabolic pathway from glutamic acid to proline (Imamul Hug and Larher, 1985). However, formation and degradation of proline could be regarded as one of the main osmoregulatory mechanisms necessary to maintain a suitable osmotic pressure within the cells of many algae (Allam, 1989; Abdel-kareem, 1997; Abde-Rahman *et al.*, 2005) and act as a compatible solute in the important role of balancing cytoplasmic and vacuolar water potential (Gorham, 1995; Gul and Khan, 2008).

On the contrary, protein content dropped with the elevation of salinity above 35‰ (Figure 4). This may be due to increased synthesis of proline accompanied the breakdown of protein due to salinity stress. These results have been documented by many previous studies (e. g. Abdel-Basset, 1986; Allam, 1989; Abdel-Kareem, 1997). Total carbohydrate contents in plants cultured at all salinity variations (Figure 2) were always less than the results obtained from native field plants. However, the content of total carbohydrates in cultivation was found to be higher in marine than both estuarine and fresh water samples. Samples of all habitats showed a marked decrease in the content of total carbohydrates, soluble and insoluble sugars when cultured at higher salinity levels (40-50‰). The decrease in the content of insoluble sugars due to salinity stress was recorded in many algae belonging to different groups (e. g. Rijstenbil *et al.*, 1989 a & b; Karestn and Kirst, 1989 & 1990). This may interpreted to the inhibitory effect of high salinity upon photosynthetic process. Meanwhile, the decrease of soluble sugars because of salinity stress could be due to photosynthesis and/or respiration inhibition (Ziska and Teramura, 1992; Larkum and Wood, 1993; Ekelund, 2000).

The content of nitrogen fractions (ammonia, amides, nitrates, protein and total nitrogen) of the cultured species showed the highest values mostly at 35‰ salinity and in part at 40‰ salinity (Figures 3 and 4). Increasing salinity above 40‰ led to markedly drop in these fractions, results parallel to those of carbohydrate contents (Figure 2). This drop of fitness contents due to salinity stress could suggest being the cost of plasticity.

Costs of plasticity were detected in low percentage of cases (Van Kleunen and Fischer, 2005 and 2007), this may indicate that costs are rare or difficult to detect. The fact that plasticity observed in nature is often lower than expected

Physiological response of native ecotypes of *Cladophora glomerata* (L.) Kützing (Chlorophyceae) in relation to..

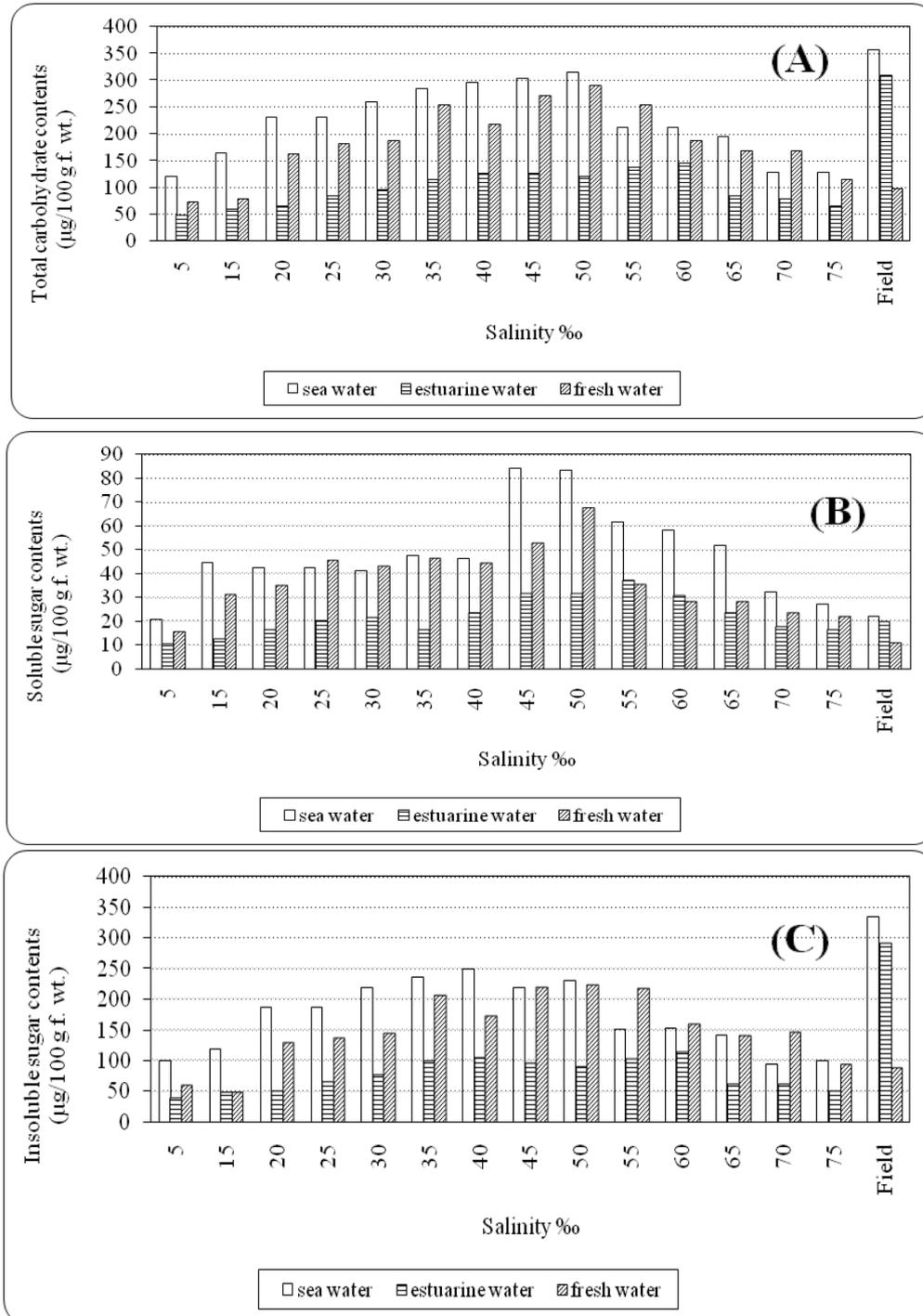


Figure 2. Effect of different salinities on total carbohydrates (A), soluble sugars (B) and insoluble sugars (C) contents of *Cladophora glomerata* collected from different habitats.

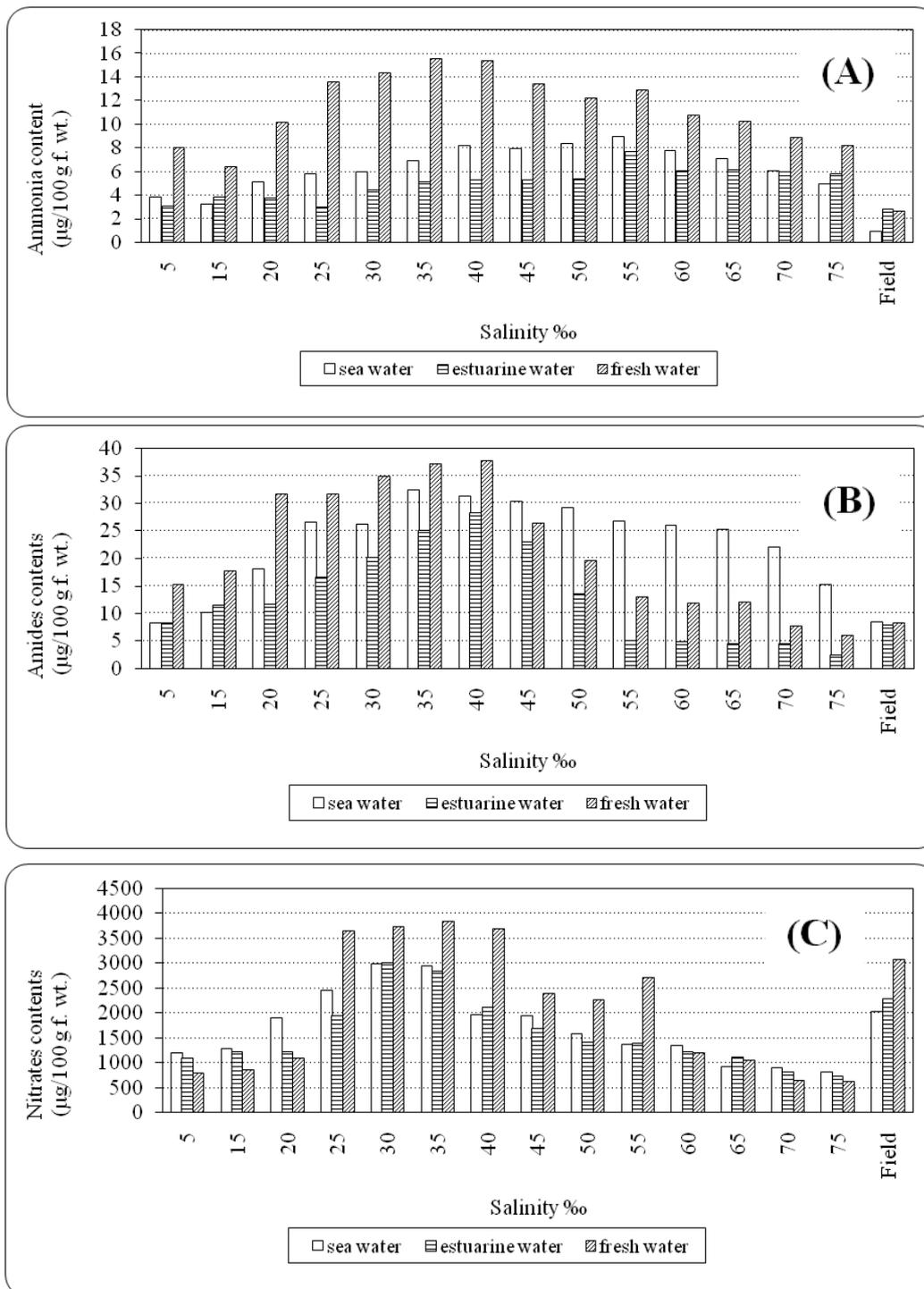


Figure 3. Effect of different salinities on ammonia (A), amides (B) and nitrates (C) contents of *Cladophora glomerata* collected from different habitats.

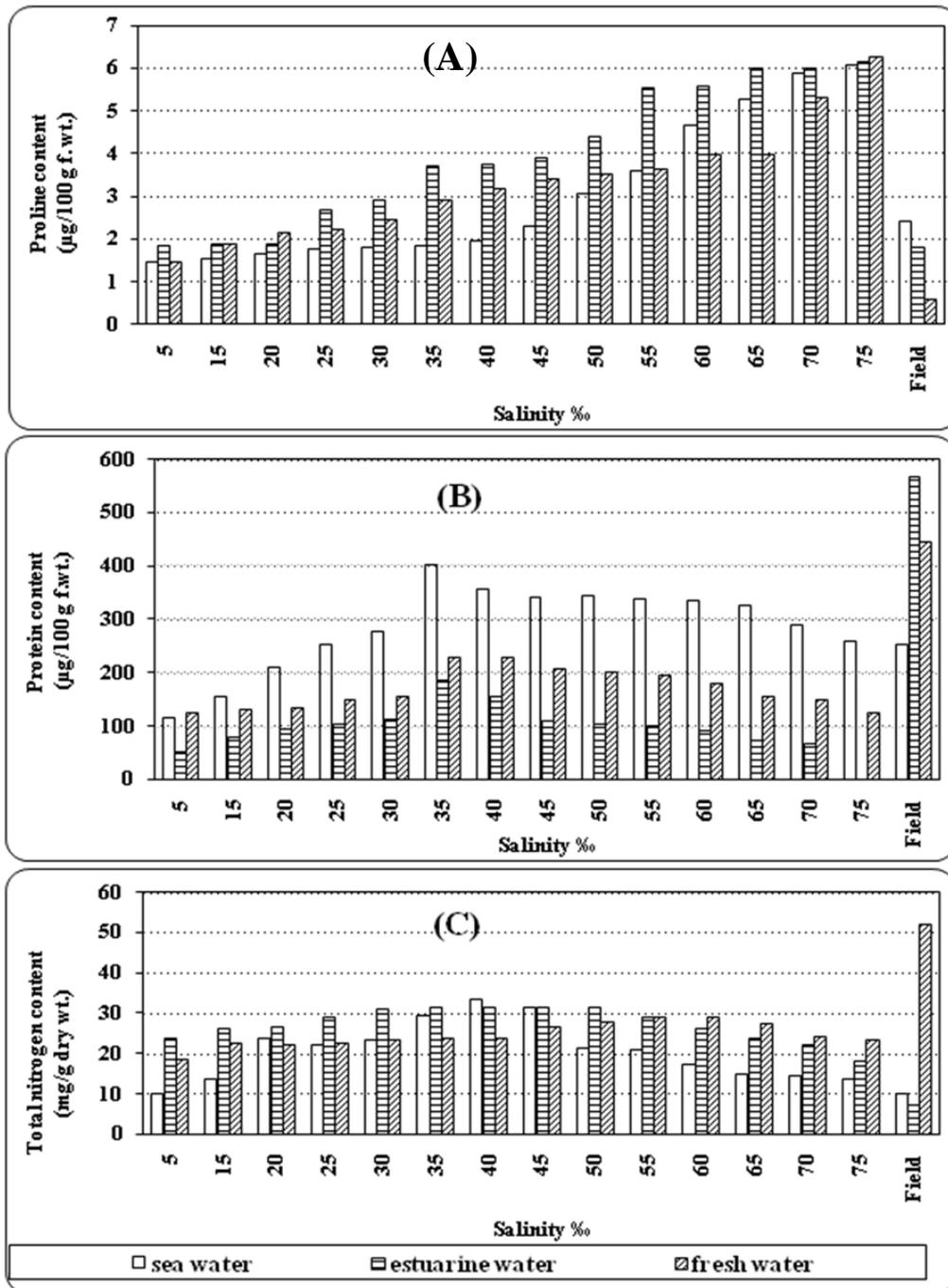


Figure 4. Effect of different salinities on proline (A), protein (B) and total nitrogen contents (C) of *Cladophora glomerata* collected from different habitats.

suggests the existence of costs and limits of plasticity (Valladares *et al.*, 2007). However, costs may vary in magnitude depending on environmental conditions and they seem to be significant in stressful environments (Steinger *et al.*, 2003). As Charles R. Darwin in 1859 said "it is not the strongest of the species that survive or the most intelligent but the ones most responsive to change", the ecological breadth of species may partly explained by their capacity to show plastic response to environment (Sultan, 2001; Gonzalez and Gianoli, 2004; Saldana *et al.*, 2005).

Not only *Cladophora glomerata* could live over a wide range of salinities- as it was collected from fresh, estuarine and sea water, but also it showed promising quantities of fitness component in the three habitats (Table 2). At the same time, it confirmed a good example for adaptation to the micro-component of the surrounding water. Although there are great differences in most mineral fractions of the three types of water (Table 1), *Cladophora glomerata* showed promising contents of most fitness components. Moreover, when it exposed to salinity stress, it still sustained a hopeful contents of fitness component (Figures 2, 3 and 4).

From the above discussion, it is noteworthy to suggest *Cladophora glomerata* a "salinity generalist" (a species that able to maintain fitness over a wide range of salinities), equable to ecological generalist that was suggested by many authors for a species that able to maintain fitness in over wide range of environments (e. g. Lortie and Aarssen, 1996; Sultan, 2001; El-Kenany, 2006).

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الإستجابة الفسيولوجية لطحلب كلادوفورا جلوميراتا (طحالب خضراء) نتيجة للملوحة

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يهدف هذا البحث إلى تقييم الإستجابة الفسيولوجية نتيجة للإجهاد الملحي في طحلب كلادوفورا جلوميراتا، ومعرفة النواتج الأيضية التي تعمل على التنظيم الأسموزي للطحلب. وللتوصل إلى ذلك تم دراسة أثر التركيزات المختلفة للملوحة على محتوى الطحلب من المعادن والكربوهيدرات والسكريات الذائبة والغير ذائبة والأمونيا والأميدات والنيترات والبروتينات والنيتروجين الكلي. وقد أوضحت النتائج أن التنظيم الأسموزي لطحلب كلادوفورا جلوميراتا يتم جزئياً من خلال التحكم في تركيز الذائبات المعدنية، ويتم بشكل أساسي من خلال الإنتاج المتزايد للحمض الأميني برولين كلما زادت الملوحة. هذا وقد أدت النتائج أيضاً إلى إقتراح مصطلح "واسع الإنتشار الملحي" والذي يمكن أن يطلق على طحلب كلادوفورا جلوميراتا نظراً لمقدرته على التكيف مع مدى واسع من الملوحة.