
RESEARCH PAPERS

Adaptation of Halophytes to a Gradient of Conditions on the Intertidal Zone of the White Sea Coast

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Abstract—A comparative study of structural and functional features of leaves of sea arrowgrass (*tristrennik*, *Triglochin maritima* L.) and saline aster (*Tripolium vulgare* Nees.) growing along a gradient of conditions on the littoral of the intertidal zone of the Pomorsky coast of the White Sea was carried out. The study of leaf morphometric parameters showed that as the gradient of conditions increases from the upper part of the littoral to the conditions of deeper and longer flooding, there is a decrease in the area and leaf structure parameters. The number of stomata decreases, but their area per leaf unit does not change. Higher values of photosynthesis rate, transpiration, stomatal conductance and carboxylation rate were observed in leaves of plants growing near the shoreline and exposed to insignificant level and time of flooding in comparison with plants at the water's edge under prolonged stay under water. Immediately after emergence from prolonged flooding, plants, in addition to utilizing the major carbon dioxide fixation pathway via RBPC/O, turn on the PEP-carboxylase pathway of CO₂ fixation. Functional differences between species are related to the maintenance of gas exchange under flooding conditions: in *tristrennik* due to large funds of storage parenchyma, and in aster due to metabolic switching. The revealed differences explain their different degree of development of seaside territories: *tristrennik*, in comparison with aster, develops a wider range of littoral ecotopes. Additional arguments in support of high plasticity of littoral species in Arctic conditions were obtained.

Keywords: *Triglochin maritima*, *Tripolium vulgare*, halophyte, littoral, photosynthesis, environmental factors, stress tolerance, diurnal rhythmicity, tidal cycle

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INTRODUCTION

Seashores are contact zones between terrestrial and marine ecosystems [1–4] and are characterized by unstable environmental conditions associated with TC. Tidal dynamics cause a periodic twice-daily change in the water and air environments in the littoral and each successive tide in the TC arrives almost 50 min later, ensuring its constant shift in the day [1]. This multidimensional dynamics of vital environmental factors for plants on the coast includes: light intensity and spectral composition, temperature, humidity; atmospheric pressure, salinity, availability of oxygen, carbon dioxide, etc. The change of environments occurs twice a day on the littoral and several times a month on the supralittoral. In addition, all these factors vary along the gradient of flooding from the aboriginal shore to the supralittoral and depend on the level of flooding.

The conditions led to the formation of a unique halophytic complex of higher terrestrial plants on the littoral [2], resistant to daily dynamics of conditions due to the special development of morphological and ana-

tomical structures (aerenchyma, water-storage tissue, mechanical tissue, hydrophobic leaf surface with gas film, powerful root system, reduction of aboveground plant size, increase in leaf thickness, etc.) and physiological and biochemical mechanisms for maintaining water regime, photosynthesis, resistance to lack of oxygen, carbon dioxide, and changes in light conditions. It is assumed that it is the daily flooding (different depth and duration) that is the leading selection factor exerting pressure on the selection of various combinations of functional resistance traits in wetland plants [5].

It has been found that one of the most common mechanisms for maintaining an active functional state in submerged plants is the ability to extract CO₂ from HCO₃⁻ [5, 6]. This mechanism has been described for algae [7, 8], seagrasses [9, 10] and for submerged terrestrial plants [11]. Under water immersion conditions, where the diffusion of gases is dramatically slowed down (almost 100-fold compared to the air environment) and there is a decrease in the availability of inorganic carbon (dissolved bicarbonate) and oxy-

gen [11] for underwater photosynthesis. It has been shown [11] that the general evolutionary direction in the adaptation of salt-tolerant species along an aridity and salinity gradient is associated with a change in photosynthetic types in the C_3 - C_4 -CAM direction.

The utilization of HCO_3^- is often associated with the connection of C_4 - and CAM-metabolism and leads to the hypothesis of a change (or connection) in some aquatic plants of the pathway of carbon metabolism under changes in the external environment. For example, species of *Egeria densa* and *Hydrilla verticillata* (*Hydrocharitaceae*), aquatic annual plants, normally carry out C_3 photosynthesis but can switch to C_4 photosynthesis under carbon limiting conditions [8, 12]. *Isoetes howellii* and *I. sinensis* (*Isoetaceae*), amphibious lycophytes, increase CAM-metabolism activity in leaves that develop under water immersion conditions, but not in leaves developing under terrestrial conditions [13]. In the freshwater plant *Ottelia alismoides* (*Hydrocharitaceae*) (C_4 -plant), all three types of functional rearrangements of the CO_2 -concentrating mechanism have been observed depending on the conditions [14], which, in particular, can increase CAM activity under conditions of low CO_2 content [15].

However, the reaction of plants to daily cyclic environmental changes on the littoral, regulation and mechanisms of organismal responses in plants of the intertidal zone of littoral ecosystems of the northern Holarctic seas are not discussed in the literature known to us. Perennial halophyte species widely represented on the White Sea coast—sea triostrennik (*Triglochin maritima* L.) and salt aster (*Tripolium vulgare* Ness.)—were chosen for the study. Both plants grow along a gradient of conditions on the littoral and are subjected to flooding every day, with the maximum time under water at full tide being up to 3 hours and the minimum time near the shore being about an hour.

MATERIALS AND METHODS

Time and place of investigations. The work was carried out in early July 2019 at the mouth of the Keret River on the western coast of the White Sea (66°17'33" N 33°35'28" E). Keret Bay enters the Karelian shore of the Kandalaksha Bay of the White Sea 22.2 km northwest of Cape Sharapov and is a bucket-type bay that is also an estuary of the Keret River. This is a characteristic water area of the White Sea with a complex bottom topography and an intensified hydrodynamic regime, which is largely determined by tidal dynamics. The average tidal range is from 2 to 3.5 m, the salinity of the bay waters is low—20–22‰, near the estuary 3–12‰ [3]. The climate in the study area is of the temperate type [16]. Summer is cool, the temperature usually stays within 15–20°C. This place of the Subarctic region is characterized by an increase in the length of daylight hours (polar day) during the growing season (June–July).

In the experimental area, a transect was laid out from the bedrock shore to the low tide water cutoff line, where two 0.25 m² trial plots differing in the duration of tidal action were identified. Sample Area 1 (SA1) was located on the upper littoral below the shoreline. The vegetation here forms a closed herbaceous cover dominated by grasses and sedges. The experimental plants are represented by groupings, but the height of the water column at maximum tide does not exceed 20 cm, the studied plants are flooded twice a day by 10–15 cm and the duration of flooding is 30–40 min. Sample area 2 (SA2) was located on a slope in the low part of the littoral. Plants here formed groupings and were flooded with seawater twice a day for 2–3 h. The height of the water column at high tide reached 70 cm. Water salinity at the sampling site was 7‰.

Plant objects. Typical plants of sea coasts dominating on all subzones of the littoral of the White Sea coast—saltwort aster (*Tripolium vulgare* Nees., *Asteraceae*) and sea arrowgrass, triostrennik (*Triglochin maritima* L., *Juncaginaceae*), which were at the flowering stage and represented by well-developed plants, were chosen as objects of the study.

Tripolium vulgare—a biennial herbaceous plant 30–50 cm tall with a short rhizome. Stem is ascending or erect, ribbed in the upper part, branched. Leaves are regular, oblong, glabrous, with reticulate veining, fleshy, upper leaves linear-lanceolate. Inflorescence is a basket 10–15 mm in diameter. It occurs along the banks of rivers flowing into the White Sea in the zone of daily flooding on dried areas with different substrate composition.

Triglochin maritima is a rosette-forming herbaceous perennial up to 70 cm tall. The plant forms turf with a long and branched rhizome and vegetative shoots slightly thickened at the base with fleshy rooting narrowly linear leaves up to 30 cm long. Flower stalks are several or solitary, up to 45–50 cm high, small greenish flowers are bipinnate, collected in dense multiflowered apical clusters. Occurs in estuaries, dominates in the zone of daily flooding, in depressions with clay substrate, where salt water can stagnate [4].

Morphological and anatomical characteristics. For the study, 10 leaves from 5 plants of each species were collected from two habitats. Freshly collected leaves were photographed and their area was determined using ImageJ image analysis program. Leaf thickness was measured on temporary preparations of leaf transverse section in 10-fold repetition. Anatomical structure of leaf epidermis and mesostructure was analyzed in 30-fold repetition. Photographs of anatomical slices and epidermal impressions were obtained using a Primo Star microscope (Zeiss, Germany). Anatomical parameters were measured using the *ADF Image Capture program*.

Measurement of CO_2 -gas exchange rate. All measurements were made using a LCPro+ portable gas analyzer (ADC BioScientific Ltd, UK) immediately

Table 1. Morphometric indices of leaf structure of *T. vulgare* and *T. maritima* in different littoral ecotopes

Indicators	<i>Tripolium vulgare</i>		<i>Triglochin maritima</i>	
	SA1 upper, shore	SA2 lower, depth	SA1 upper, shore	SA2 lower, depth
Leaf area, mm ²	687.26 ± 49.35*	376.28 ± 35.82	1259.34 ± 168.27	1214.25 ± 158.36
Leaf thickness, µm	892.26 ± 74.29*	658.25 ± 46.28	2301.29 ± 385.26	2214.25 ± 419.32
Palisade mesophyll thickness, µm	348.64 ± 98.60	52286.24 ± 68.95	341.23 ± 68.29	325.36 ± 54.26
Spongy mesophyll thickness, µm	483.25 ± 102.65	311.64 ± 57.46	1627.85 ± 258.23	1624.26 ± 342.58
Thickness of the upper epidermis, µm	21.38 ± 6.38	21.42 ± 6.25	21.34 ± 5.28	21.12 ± 6.45
Thickness of the lower epidermis, µm	21.44 ± 4.28	21.13 ± 3.58	21.52 ± 9.25	20.86 ± 3.95
Number of stomata in 1 mm ² on the upper side of leaf, pcs	48 ± 13*	89 ± 14	74 ± 12	68 ± 9
Number of stomata in 1 mm ² on the lower side of leaf, pcs	79 ± 21*	116 ± 13	78 ± 8	72 ± 6
Stomata area on the upper side of the leaf, µm ²	1062.26 ± 196.35	1126.25 ± 253.21	841.25 ± 69.32	852.36 ± 65.87
Stomata area on the lower side of the leaf, µm ²	1158.96 ± 214.26	1126.52 ± 163.29	847.36 ± 61.29	835.25 ± 74.29

* A difference among corresponding parameters is reliable ($P < 0.05$).

after low tide between 9–16 h on a sunny day for four 4 days. Natural illumination was 1000–1200 µmol photon/m² s and air temperature was 20–25°C. To determine photosynthetic and transpiration rates, 2–3 leaves were taken from each trial area immediately after low tide and placed in a clothespin chamber. After steady-state values were established, CO₂-gas exchange rate, transpiration rate, stomatal conductance, and CO₂ concentration in leaf intercellular spaces were determined. Carbon dioxide dependences of photosynthesis rate were plotted by varying the CO₂ concentration in the air supplied to the assimilation chamber of the gas analyzer within the range of 0–1600 µmol/CO₂ mol at a light intensity of 1200 µmol/m² s. For this purpose, the levels of CO₂ concentration supplied to the leaf chamber were set using the microprocessor of the gas analyzer and changed sequentially: 400, 200, 100, 50, 400, 800, 1200, 1600 µmol/mol.

Determination of carboxylation rate. The maximum carboxylation rate ($V_{c_{max}}$) was determined by analyzing the carbon dioxide curves of CO₂ gas exchange using the model [17] modified [18, 19] and the program Photosyn Assistant Ver. 1.1.2 [20]. According to the authors' equations, the model allows us to determine the maximum rate of carboxylation ($V_{c_{max}}$), the rate of electron transport at light saturation (J_{max}), the rate of triisophosphate utilization, and a number of other parameters.

Determination of FEP carboxylase activity. Enzyme activity was measured in total protein extract from

leaves of plants frozen and stored in liquid nitrogen. A leaf suspension was homogenized in medium containing 50.0 mM HEPES-KOH (pH 8.0), 10.0 mM Mg SO₄·7H₂O, 5.0 mM DDT, 5.0 mM EDTA·Na₄, and 0.5% PVP. The homogenate was centrifuged for 20 min at 10000 g. The supernatant was used to measure the enzyme activity. Measurements were performed spectrophotometrically on a Genesys 10UV instrument (USA) on the oxidation of NAD·H in the conjugated reduction reaction of OAA. 0.1 mL of protein extract (50–100 µg protein) was added to 1.9 mL of reaction mixture containing 50 mM Tris-HCl buffer (pH 8.0), 5 mM MgCl₂, 5 mM NaHCO₃, 1 mM DTT, 0.1 mM EDTA·Na₄, 5 mM PEP-Na, 0.2 mM NAD·H, and 5 EE of malate dehydrogenase. All organic reagents were manufactured by Sigma. Measurements were performed in 3 biological and 3–4-x analytical repetitions.

Statistical analysis. PAST and MS Excel programs were used for statistical data processing. Comparison of leaf parameters in different experiment variants was performed using ANOVA statistical test at $P \leq 0.05$. The tables show mean values and standard deviation.

RESULTS

Morphological and Anatomical Studies

Analysis of the leaf structure of *T. maritima* and *T. vulgare* plants growing on the upper littoral near the shore and on the lower littoral in deep flooding conditions revealed differences in a number of morphological and anatomical parameters (Table 1). On the lower

Table 2. Gas exchange parameters of *T. maritima* and *T. vulgare* leaves on the transect along the gradient of conditions in the low tide phase

Growing conditions	<i>T. maritima</i>			
	photosynthesis $\mu\text{mol} (\text{CO}_2)/\text{m}^2 \text{ s}$	transpiration $\text{mmol} (\text{H}_2\text{O})/\text{m}^2 \text{ s}$	stomata conductivity $\text{mole} (\text{H}_2\text{O})/\text{m}^2 \text{ s}$	internal concentration of CO_2 , $\mu\text{mol}/\text{mol}$
SA1	$18.1 \pm 2.2^*$	6.4 ± 0.2	$0.26 \pm 0.02^*$	210 ± 15
SA2	$12.1 \pm 2.0^*$	4.7 ± 0.4	$0.18 \pm 0.02^*$	179 ± 13
	<i>T. vulgare</i>			
	photosynthesis $\mu\text{mol} (\text{CO}_2)/\text{m}^2 \text{ s}$	transpiration $\text{mmol} (\text{H}_2\text{O})/\text{m}^2 \text{ s}$	stomata conductivity $\text{mole} (\text{H}_2\text{O})/\text{m}^2 \text{ s}$	internal concentration of CO_2 , $\mu\text{mol}/\text{mol}$
SA1	$11.5 \pm 1.0^*$	4.7 ± 0.4	$0.28 \pm 0.02^*$	304 ± 13
SA2	$5.6 \pm 0.8^*$	1.4 ± 0.2	$0.12 \pm 0.01^*$	271 ± 15

* A difference among corresponding parameters is reliable ($P < 0.05$).

littoral (SA2) under the conditions of prolonged flooding at high tide, *T. vulgare* plants formed smaller leaves, the area decreased by 46% and the thickness decreased by 26% compared to plants on SA2. The area and thickness of *T. maritima* leaves did not differ on the upper and middle littoral (Table 1).

The epidermis of leaves of *T. maritima* and *T. vulgare* is single-layered with stomata located on the adaxial and abaxial sides of the leaf. The thickness of the upper and lower leaf epidermis did not vary depending on the growing conditions. Calculation showed that the number of stomata in aster plants growing in deep flooding conditions (SA2) increased on the upper side of the leaf by 47% and on the lower side by 29% compared to plants on the upper littoral (SA1). Whereas in triostrennik plants the number of stomata does not change depending on the ecotope (Table 1).

Physiological and Biochemical Studies

Comparison of the two species showed that for various indices of CO_2 -gas exchange (photosynthesis rate, transpiration and stomatal conductance) higher values were obtained in plants near the shore on the upper littoral (SA1), and at depth (SA2) to a different extent, but all values decreased (Table 2). Thus, photosynthesis intensity in plants at SA2 was decreased by 34% in triostrennik and 52% in aster; transpiration value in triostrennik by 27% and in aster by 70%, stomatal conductance in triostrennik by 31% and in aster by 58% and internal CO_2 concentration value in triostrennik by 15% and in aster by 11% were lower than in plants at SA1. The comparison showed that the studied parameters decreased more significantly in aster plants—by 52–70%, and in triostrennik only—by 27–30% compared to plants on SA2. A smaller decrease was observed only for CO_2 concentration in leaf intercellular spaces.

Comparative analysis of carbon dioxide curves of gas exchange of littoral plants according to the model [18–20] showed that the rates of individual reactions

of light and dark stages of photosynthesis differ significantly (Table 3).

Higher values of almost all indicators of carbon dioxide gas exchange of plants under conditions of elevated CO_2 content were found in plants growing in the upper littoral, near the shore (SA1), and in plants of the middle littoral at SA2 to a different extent, but almost all values decreased. Thus, the maximum rate of CO_2 uptake in triostrennik plants at SA2 was reduced by 40% and in aster by 24%, the maximum rate of carboxylation in triostrennik was reduced by 40% and in aster only by 10%, the rate of electron transport in both species was reduced, but in triostrennik by 41% and in aster by 22%. The rate of triose phosphate utilization was reduced by 43% in SA2 triostrennik and by only 10% in aster. However, the carboxylation efficiency was 30% higher in triostrennik and 82% higher in aster under deep flooding conditions at SA2. Differences between species were revealed by the rate of dark respiration, which decreased by 30% in triostrennik plants at SA1 and increased by 82% in aster. The carbon dioxide compensation point in both species decreases, but in triostrennik by 9%, and in aster by 22%. It should be noted that there are important differences between the responses of the species: triostrennik decreases by 10–40%, while aster decreases only by 10–20%. However, aster plants showed an increase in carboxylation efficiency under conditions of deep flooding at SA2 by 82%, while that of triostrennik only by 30%, as well as differences in the value of dark respiration, which increased at SA2 by 82%, while that of triostrennik decreased by 30% compared to plants at the coastal ecotope SA1.

Carboxylation Enzyme Activities

The activities of PEP-carboxylase as well as RBPC/O (Table 4) showed that the studied enzymes were active in both ecotopes. Thus, the activity of PEP-carboxylase was almost 2 times higher in triostrennik on the middle littoral under conditions of deep

Table 3. Analysis of photosynthetic apparatus activity of *T. maritima* and *T. vulgare* under conditions of elevated CO₂ concentration [17]

Parameters/Variant	<i>Triglochin</i>		<i>Tripolium</i>	
	SA1 shore	SA2 depth	SA1 shore	SA2 depth
Maximum CO ₂ uptake rate, $\mu\text{mol}/\text{m}^2 \text{ s}$	143.2 $\pm$ 7.7*	7.9 $\pm$ 8.1	87.8 $\pm$ 3.6*	67.1 $\pm$ 8.1
Maximum carboxylation rate, $\mu\text{mol}/\text{m}^2 \text{ s}$	60.1 $\pm$ 4.3*	37.3 $\pm$ 1.8	61.3 $\pm$ 3.3	55.6 $\pm$ 1.8
Carboxylation efficiency, $\mu\text{mol CO}_2/\text{Pa}$	0.45 $\pm$ 0.05	0.59 $\pm$ 0.13	0.47 $\pm$ 0.05*	0.86 $\pm$ 0.13
Electron transport rate at light saturation, $\mu\text{mol}/\text{m}^2 \text{ s}$	170 $\pm$ 16*	101 $\pm$ 14	199 $\pm$ 14*	155 $\pm$ 12
Triose phosphate utilization rate, $\mu\text{mol}/\text{m}^2 \text{ s}$	17.2 $\pm$ 1.2*	7.4 $\pm$ 0.8	11.1 $\pm$ 1.2	10.0 $\pm$ 0.8
Rate of dark CO ₂ release, $\mu\text{mol}/\text{m}^2 \text{ s}$	10.9 $\pm$ 0.8*	7.7 $\pm$ 1.0	2.9 $\pm$ 0.8*	5.3 $\pm$ 1.0
CO ₂ compensation point, $\mu\text{mol CO}_2/\text{mol}$	223.6 $\pm$ 8.4*	204.4 $\pm$ 6.3	85.6 $\pm$ 5.4*	66.4 $\pm$ 6.3

* A difference among corresponding parameters is reliable ($P < 0.05$).

Table 4. Carboxylase activity in *T. maritima* and *T. vulgare* on the transect along the gradient of conditions on the littoral during the low tide phase

<i>T. maritima</i>		
Parameters	PEP carboxylase, $\mu\text{mol}/\text{mg (Pr) min}$	RBPC/O, $\mu\text{mol}/\text{m}^2 \text{ s}$
Upper littoral SA1 shore	0.0254 $\pm$ 0.0012*	60.1 $\pm$ 4.3*
Lower littoral SA2 depth	0.0443 $\pm$ 0.0006	37.3 $\pm$ 1.8
<i>T. vulgare</i>		
Upper littoral SA1 shore	0.0202 $\pm$ 0.0009*	61.3 $\pm$ 3.3*
Lower littoral SA2 depth	0.0392 $\pm$ 0.0008	55.6 $\pm$ 1.8

* A difference among corresponding parameters is reliable ($P < 0.05$).

flooding of SA2 than on the upper littoral (SA1), and the activity of RBPC/O—on the contrary—was higher on SA1 and decreased almost 2 times in plants on the middle littoral (SA2). In aster, PEP-carboxylase activity was 2-fold higher in SA2 plants under high flooding conditions, and RBPC/O activity was similar in both ecotopes. Species comparison showed that both species had higher similar values of PEP-carboxylase activity on the lower littoral under deep flooding (SA2) conditions. Higher and similar RBPC/O activity values were obtained on the upper littoral (SA1) in both species, but in aster the same values were observed in both ecotopes. In triostrennik, RBPC/O activity in the lower littoral (SA2) decreased by almost 40%.

DISCUSSION

Comparison of the results of leaf anatomical indices and functional activity of photosynthetic apparatus shows that the studied plant species differ significantly in structural and functional indices under conditions of the TC tidal phase. The effect of long-term flooding of plants at SA2 has a great influence on growth and functional activity of both halophyte species, but the response of these species is different. In terms of the

complex of anatomo-morphological structures, more significant effects were observed in plants growing under deep flooding conditions in the middle littoral at SA2: aster forms smaller (78% less) but thicker (30%) leaves, while triostrennik shows the same trend, but the changes are much smaller. In plants of both species, a similar response of changes at the level of the stomatal apparatus is observed at SA2: their number increases significantly (almost twice), while the area of stomata slightly decreases. These structural unidirectional changes show that the role of stomata in moving from the coastal ecotope of SA1 to the middle littoral of SA2 changes, and their quantitative increase on both sides of the leaf in plants at (SA2) indicate their participation in the adaptation of plants to flooding conditions in the littoral.

According to the parameters of CO₂-gas exchange in plants under natural growing conditions, it turned out that in both species high values of activity of all functional indices are observed at SA1 in comparison with plants at SA2, where all indices decrease. This effect is more significant in aster (about 50%) compared to triostrennik (about 30%). Comparative analysis of assimilative activity of two plant species according to the model of Farquhar et al. [17] also showed

differences in plant response depending on the species and conditions of their growth on the littoral. Aster plants on SA1 growing under conditions of short-term daily flooding showed an increase in the maximum rate of CO₂ uptake, carboxylation rate, electron transport, triose phosphate utilization, and carboxylation efficiency in response to an increase in CO₂ content compared to triostrennik growing under the same conditions. The rate of dark respiration was also higher in aster on SA2 compared to SA1 in contrast to triostrennik, which showed a decrease in respiration of plants growing on SA2 compared to SA1.

Of particular note is the fact that plants of both species remain highly active at SA2, where plants can stay in flooding conditions for more than 6 hours per day due to two tidal cycles. This phenomenon may be related to the mechanism of maintaining their functional activity through internal reserves and/or supplementation of CO₂ and O₂ from the aquatic environment where these gases are present [6]. This hypothesis is supported by the data on the existence of mechanisms of CO₂ entry from water into leaf mesophyll in seagrasses (*Zostera*, *Rupia*) [10] and in rice plants [21]. The fact of opening of stomata in halophytes of the intertidal zone during the tide also supports this hypothesis [22].

The question arises about the mechanisms of inorganic CO₂ entering the plant stomata, which is in very low concentrations in the aquatic environment. It is known that plants that are under water for a long time (aquatic plants) can use another carbon source—bicarbonate (HCO₃³⁻). It has been found that in seaweeds and vascular plants under submerged conditions, CO₂ concentrating mechanism (CCM) operates with the participation of carboanhydrase, which accelerates the dehydration of bicarbonate to CO₂ and optimizes the operation of RBP carboxylase [23]. Studies have shown (Table 4) that the stomata in aster and triostrennik plants are open under water under slightly alkaline pH conditions during the daylight period of flooding. Gas films, through which gas exchange can be successfully carried out, are considered as a mechanism that allows CO₂ to enter the open stomata [24]. However, it is believed that these films can only be formed in plants with a hydrophobic leaf surface structure, which is not seen in the species studied. However, in the present work, evidence was obtained that the halophyte species under study have a connection with the water space during the tide. One of the evidences of the possibility of CCM functioning in littoral plants is the lower carbon dioxide compensation point at SA1 compared to plants at SA2: in triostrennik by 10%, and in aster by 30%. The differences in the two species may indicate a different contribution of CCM to daily carbon metabolism: it is more significant for aster plants compared to triostrennik. This is also confirmed by the data on the greater stimulation of all processes in aster under CO₂ addition. However,

triostrennik is characterized by large volumes of air-bearing parenchyma in both aboveground and underground organs, which can also provide maintenance of gas exchange under water [4].

Under conditions of relatively low concentrations of CO₂ in leaf intercellular space, an alternative pathway of carbon dioxide fixation may be activated in plants by increasing the activity of PEP-carboxylase via phosphorylation [25, 26]. The activity of this enzyme per fresh leaf weight may increase not only due to de novo synthesis, but also due to the “reserve” pool of the inactive form of the enzyme, which under flooding conditions is phosphorylated and transformed into the active state, and when plants leave the flooding is dephosphorylated and inactivated. This pathway of enzyme activation may be more significant under littoral conditions than de novo synthesis. It is known that under conditions of decreased carbon dioxide concentration there is an increase in the reaction rate of the glycolate pathway, a decrease in the reaction rate of RBPC/O carboxylation and PGA synthesis. The ratio of the two carboxylation pathways under conditions of reduced carbon dioxide concentrations will be shifted towards an increase in the activity of PEP-carboxylase, since the enzyme has lower Km values, uses HCO₃³⁻ as a substrate, and thus gains an advantage in carbon dioxide assimilation. This is not hindered by a possible decrease in temperature during the tide, since the temperature optimum of cytoplasmic PEPC of C₃-plants is lower than that of C₄-plants [27]. This mechanism also explains its high values in both plant species on SA2 in the aftermath of flooding compared to the SA1 data. RBPC/O activity in both species is high and similar in the high littoral—SA1, and in the middle littoral—SA2 it decreased almost 2 times in triostrennik compared to SA1, while in aster it remained high.

It is known from the literature [28] that, along with classical CAM-plants, many plant species are capable of assimilating significant amounts of carbon by “CAM metabolism” not only in the dark but also in the light. “CAM-metabolism in the light” is related more to a deficiency of reducing agent rather than CO₂ under these conditions. As suggested by the authors of this hypothesis, the CO₂-storing mechanism could be either a carboanhydrase or a PEP-carboxylase mechanism, both of which can operate under low-light conditions. Assuming that the inducible CO₂-storing mechanism is switched on during the flooding stage in both species, new more optimal conditions for plant viability are created. This hypothesis is supported by our data on higher activity (2 times more) of PEP-carboxylase in both species after prolonged flooding on SA1 compared to the data on SA2.

When plants are flooded, respiration processes are activated. aster has lower values of dark respiration compared to triostrennik by 2-fold and lower values are observed in aster at SA1 and in triostrennik at SA2.

These results may indicate different mechanisms of maintaining gas exchange under flooding conditions. In aster, this may be due to metabolic switches, while in triostrennik it may be due to oxygen consumption from large funds of storage parenchyma.

The study showed that two dominant species, well adapted to littoral conditions, have different adaptation mechanisms. If the plants of triostrennik, which occupies a wider range of ecotopes on the intertidal zone, the structural features play a major role, while for aster - the lability of metabolic processes. For the conditions of northern Holarctic coastal territories under conditions of short vegetation period structural adaptations are apparently more promising, which provides triostrennik with a wider range of ecotopes on the intertidal zone.

ABBREVIATIONS AND NOTATION

CCM	CO ₂ -concentrating mechanism
CCP	carbon dioxide compensation point
[CO ₂]	carbon dioxide concentration
TC	tidal cycle
PEPC	phosphoenolpyruvate carboxylase
RBP	ribulose biphosphate
RBPC/O	ribulose-1,5-bisphosphate carboxylase/oxygenase
SA	sample area
TPU	triosophosphate utilization rate

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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