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Physiological changes induced by sodium chloride stress in *Aphanizomenon gracile*, *Cylindrospermopsis raciborskii* and *Dolichospermum* sp.

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Abstract :

Due to anthropogenic activities, associated with climate change, many freshwater ecosystems are expected to experience an increase in salinity. This phenomenon is predicted to favor the development and expansion of freshwater cyanobacteria towards brackish waters due to their transfer along the estuarine freshwater-marine continuum. Since freshwater cyanobacteria are known to produce toxins, this represents a serious threat for animal and human health. Saxitoxins (STXs) are classified among the most powerful cyanotoxins. It becomes thus critical to evaluate the capacity of cyanobacteria producing STXs to face variations in salinity and to better understand the physiological consequences of sodium chloride (NaCl) exposure, in particular on their toxicity. Laboratory experiments were conducted on three filamentous cyanobacteria species isolated from brackish (Dolichospermum sp.) and fresh waters (Aphanizomenon gracile and Cylindrospermopsis raciborskii) to determine how salinity variations affect their growth, photosynthetic activity, pigment composition, production of reactive oxygen species (ROS), synthesis of compatible solutes and STXs intracellular quotas. Salinity tolerance was found to be species-specific. Dolichospermum sp. was more resistant to salinity variations than A. gracile and C. raciborskii. NaCl variations reduced growth in all species. In A. gracile, carotenoids content was dose-dependently reduced by NaCl. By contrast, in C. raciborskii and Dolichospermum sp., variations in carotenoids content did not show obvious relationships with NaCl concentration. While in Dolichospermum sp. phycocyanin and phycoerythrin increased within the first 24 h exposure to NaCl, in both A. gracile and C. raciborskii, these pigments decreased proportionally to NaCl concentration. Low changes in salinity did not impact STXs production in A. gracile and C. raciborskii while higher increase in salinity could modify the toxin profile and content of C. raciborskii (intracellular STX decreased while dc-GTX2 increased). In estuaries, A. gracile and C. raciborskii would not be able to survive beyond the oligohaline area (i.e. salinity > 5). Conversely, in part due to its ability to accumulate compatible solutes, Dolichospermum sp. has the potential to face consequent salinity variations and to survive in the polyhaline area (at least up to salinity = 24).

Highlights

► In all species, sodium chloride exposure resulted in reduced growth rates. ► The responses of photosynthetic activity, pigment composition, ROS production as well as the capacity to accumulate compatible solutes were species-specific. ► In part due to its ability to accumulate compatible solutes, *Dolichospermum sp*. was the most tolerant species. ► Low sodium chloride concentrations did not impact STXs production in *Aphanizomenon gracile* and *Cylindrospermopsis raciborskii* while higher concentrations modified the toxin profile and content of *C. raciborskii* (intracellular STX decreased while dc-GTX2 increased).

Keywords : Cyanobacteria, Saxitoxins, Paralytic shellfish toxins, Harmful algal bloom, Sodium chloride, Cyanotoxins

1 Introduction

In the last decades, cyanobacteria blooms have considerably increased in frequency, magnitude and duration in many countries and ecosystems (O'Neil et al., 2012; Paerl et al., 2001; Paerl and Huisman, 2009). This phenomenon is becoming a worldwide public health and environmental concern (Burford et al., 2020). Indeed, some strains of cyanobacteria produce toxic metabolites (cyanotoxins) that are responsible for serious human and animal health problems and can even lead to mortalities in some cases. Mass development of cyanobacteria also strongly impacts ecosystems functioning and human activities. Indeed, it increases turbidity of water and accumulates as thick scums and mats that can lead to anoxia when they decomposed. A variety of odor and taste compounds are also produced making affected waters unsuitable for drinking and recreational purposes (Carmichael, 2001; Dittmann et al., 2012; Paerl et al., 2001). Saxitoxins (STXs) are among the most powerful cyanotoxins with a reported 50% mouse lethal dose of 5-10 µg kg^{-1} (Munday, 2014; Schantz et al., 1975). STXs are a group of 57 alkaloids also known as paralytic shellfish toxins (PSTs) due to their association with paralytic shellfish poisoning (PSP) which occurs after ingestion of shellfish contaminated with PSTs (Wiese et al., 2010). STXs are neurotoxins that selectively block voltage-gated sodium channels present in neuronal cell membranes and prolong the gating of potassium channels in heart muscle cells (Pearson et al., 2010). PSP symptoms includes perspiration, vomiting and cardiovascular failure due to respiratory muscles paralysis and heart malfunctioning (Llewellyn, 2006; Wiese et al., 2010). So far, STXs synthesis has been described in nine genera of filamentous cyanobacteria mainly found in freshwaters: *Dolichospermum* (formerly known as *Anabaena*), *Aphanizomenon*, *Cuspidothrix*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix*, *Raphidiopsis*, *Scytonema* and *Phormidium* (Cirés and Ballot, 2016; Pearson et al., 2016). Water contamination with STXs

produced by these genera is a worldwide phenomenon detected in Asia, North and South America, Europe and Oceania. In addition, the frequency and distribution of STXs-containing cyanobacteria blooms appears to be on the rise (Pearson et al., 2016). In the last 10 years, STXs-producing cyanobacteria (STXs-cyanobacteria) have, for example, been detected for the first time in Arctic (Kleinteich et al., 2013), New Zealand (Smith et al., 2011), Canada (Lajeunesse et al., 2012) and several locations in Europe (Ballot et al., 2010; Jančula et al., 2014; Rapala et al., 2005; Teneva et al., 2010; Wörmer et al., 2011) with some of those blooms reaching high STXs concentrations (such as 25-1000 µg PSTs L^{-1} (Rapala et al., 2005; Wörmer et al., 2011) knowing that in Europe, the acute reference dose for STXs has been fixed at 0.5 µg STX equivalents kg^{-1} of body weight (Paredes et al., 2011)). Recently, climate change has been linked to the extension and intensification of harmful cyanobacterial blooms (Paerl and Huisman, 2009; Paerl and Paul, 2012; Slim et al., 2014). Even though the main influence of climate change is global warming with its direct influence on water temperature, climate change is also likely to induce salinity fluctuations in brackish and fresh waters due to sea level rising (through the penetration of seawater into estuaries over a higher distance and increasing submersions) and modifications in frequency, intensity and duration of precipitations and droughts (Li et al., 2016; Paerl and Paul, 2012). This, associated with the increasing anthropogenic salinization of inland waters, may strongly modify phytoplankton community structure and blooms occurrence (Williams, 2001). It is thought that salinity fluctuations would be favorable for cyanobacteria. Indeed, one of the impacts of salinization is increased vertical density stratification (Paerl and Huisman, 2009 and citations therein). Such conditions would facilitate buoyancy of some cyanobacteria because they have gas vesicles in cells that enable them to float to the water surface and in this way, they outcompete other algae for available light and nutrients (Antunes et al., 2015; Cirés and Ballot, 2016; Li et al., 2016). Additionally, the sparse information on the response of harmful

bloom forming cyanobacteria to salinity seems to indicate that some genera or strains are rather tolerant to salinity changes, sometimes more than their counterpart eukaryotic freshwater phytoplankton species (Brutemark et al., 2015; Moisander et al., 2002; Paerl and Paul, 2012; Preece et al., 2017). The salt tolerance of these cyanobacteria would be one of the causes of the increasing number of harmful cyanobacteria blooms in brackish waters (Paerl and Paul, 2012; Soto-Liebe et al., 2013). This salt tolerance also spotlights estuaries as potential vectors for increasing spread of harmful cyanobacteria through possible transfers along the freshwater-marine continuum (Bormans et al., 2019). Several estuarine localities have already experienced harmful cyanobacteria appearance and sometimes bloom formation in response to salinity fluctuations (John and Kemp, 2006; Preece et al., 2017; Robson and Hamilton, 2013; Ross et al., 2019). Such an increased expansion of harmful cyanobacteria blooms exposes other aquatic organisms and human users of these waters to elevated risks of contamination with cyanotoxins (Paerl and Paul, 2012). Especially since field studies have suggested causal relationships between variations in salinity (or conductivity) and increases in water STXs concentration (Brentano et al., 2016 and references therein). Despite the public health concern and economic importance of such toxic cyanobacteria occurrences, relatively few studies have actually focused on the salt tolerance of STXs-cyanobacteria and the resulting effects on STXs production. This contrasts with the significant amount of publications dealing with STXs production in toxic dinoflagellates (e.g. Aguilera-Belmonte et al., 2013 and references therein) or salt effects on other cyanotoxins such as microcystins (e.g. Georges des Aulnois et al., 2020; Georges des Aulnois et al., 2019; Melero-Jiménez et al., 2020 and references therein). Additionally, some of the STXs-cyanobacteria species remain poorly studied. To our knowledge, this is the case of *Aphanizomenon gracile* that has not been previously used as a model species to study effects of salinity variations on its physiology and toxin production. Some physiological processes potentially involved in salt stress acclimation,

such as photosynthesis, ROS production and accumulation of compatible solutes, have also received little attention in STXs-cyanobacteria. Even in *Cylindrospermopsis raciborskii*, one of the most-studied STXs-cyanobacteria, in which salt stress effects on growth and STX production have been previously described (Carneiro et al., 2013; Ongley et al., 2016; Pomati et al., 2004b). It becomes thus crucial to acquire more knowledge about the physiological response of STXs-cyanobacteria to salt exposure to fully understand their invasive potential. This study investigates impacts of increasing salt concentrations on survival, growth, pigments composition, photosynthetic activity, production of reactive oxygen species and intracellular compatible solutes and STXs contents of STXs-cyanobacteria. The objectives were: 1) to evaluate the capacity of each species to face salinity variations, 2) to identify the physiological processes impacted by salt stress and, as far as possible, their role in salt acclimation, 3) to determine whether the different species use similar strategies to deal with salinity variations and 4) to understand how salinity variations may alter the toxicity (toxin content and profile) of these species. The physiological responses of two freshwater cyanobacteria (*A. gracile* and *C. raciborskii*) grown under controlled conditions were investigated and compared to the responses of a brackish water strain (*Dolichospermum* sp.). The *Dolichospermum* sp. strain was used as a model of cyanobacteria already used to face high salinity exposure in its natural environment.

2 Materials and methods

2.1 Cyanobacteria strains and growth conditions

Two freshwater STX-producing strains, *Aphanizomenon gracile* (PMC627.10, isolated from Champs-sur-Marne in France, Ledreux et al., 2010) and *Cylindrospermopsis raciborskii* (PMC00.01, isolated from Jucazinho in Brazil, Bernard et al., 2003) were obtained from the microalgae and cyanobacteria culture collection of the National Museum of Natural History

(Paris, France). The brackish water non-toxin-producing strain, *Dolichospermum* sp. (CCY9401, isolated from Arcachon in France, salinity = 9) was obtained from the culture collection of the Royal Netherlands Institute of Sea Research (Yerseke, Netherlands). None of these strains has been found to produce detectable amount of anatoxins, homoanatoxins, cylindrospermopsin, desoxycylindrospermopsin, microcystins or nodularins. These three strains were maintained at 25°C under illumination of 40 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR) provided by cool-white fluorescent tubes (Osram lumilux cool daylight L18w/865) with a 12:12 h light/dark cycle. Although they were diazotrophic species, the three strains were maintained in nitrogen replete culture media. *A. gracile* and *C. raciborskii* were maintained in BG11 medium (Allen, 1968; Allen and Stanier, 1968; Rippka et al., 1979). *Dolichospermum* sp. was maintained in BG11 medium in which 9 g L^{-1} NaCl were added because this strain was isolated from brackish waters.

2.2 Salt stress experiment

Cultures in exponential growth phase were used as inoculum for new batch cultures in media containing different amount of NaCl for 4 days. *A. gracile* and *C. raciborskii* were exposed to BG11 (control conditions), BG11 + 2 g L^{-1} NaCL (i.e. 34 mM NaCl), BG11 + 9 g L^{-1} NaCl (i.e. 154 mM NaCl) and BG11 + 15 g L^{-1} NaCl (i.e. 257 mM NaCl). In an estuary, these salinity variations would correspond to a transfer from freshwaters to the oligohaline area (from BG11 to BG11 + 34 mM NaCl) and from freshwaters to the mesohaline area (from BG11 to BG11 + 154 mM NaCl and from BG11 to BG11 + 257 mM NaCl). *Dolichospermum* sp. was exposed to two media in common with *A. gracile* and *C. raciborskii*: BG11 + 154 mM NaCl (which corresponds here to control conditions) and BG11 + 257 mM NaCl. It was also exposed to BG11 + 18 g L^{-1} NaCl (i.e. 308 mM NaCl) and BG11 + 24 g L^{-1} NaCl (i.e. 411 mM NaCl): this corresponds respectively to an increase of 154 and 257 mM NaCl in

comparison to control conditions as it was the case for *A. gracile* and *C. raciborskii*. Each condition was performed in triplicates. Sampling in aseptic conditions was done after different exposure times (0, 1, 4, 6, 24, 48 and 72h). For each treatment, cyanobacteria growth was measured by following chlorophyll a (chl a) content (see below for its measurement) and growth rate (μ) was calculated following Wood et al. (2005).

2.3 Photosynthetic activity and mean chl a-specific absorption coefficient ($\bar{a}^*_{phy}$)

Photosynthetic activity was measured by Pulse Amplitude Modulated (PAM) fluorometry using a PHYTO-PAM fluorometer PHYTO-ED (Heinz Walz GmbH, Effeltrich, Germany). For a detailed description of the PHYTO-PAM, see Kolbowski and Schreiber (1995) and Schreiber (1998). Before measurement, samples were dark acclimated for 15 minutes at sampling temperature. Fluorescence levels F_0 and F_m were respectively measured before and after a saturating pulse (400 ms to 4000 μmol photons quanta m^{-2} s^{-1}) to obtain the maximum quantum yield (F_v/F_m):

$$F_v/F_m = \frac{(F_m - F_0)}{F_m}$$

Rapid Light curves were then constructed by exposing the sample for 10 s to 21 sequential increasing light steps with irradiance levels ranging from 1 to 2064 μmol photons m^{-2} s^{-1}. Actinic light was provided by 16 LEDs peaking at 655 nm. At each light step, the fluorescence levels before and after applying a saturating pulse were measured with four pulse-modulated measuring lights peaking at 470, 520, 645 and 665 nm. At each light step and for each measuring light, the effective quantum yield of photosystem II (ϕPSII also noted $\Delta F/F'_m$) was calculated according to Genty et al. (1989):

$$\phi PSII = \frac{\Delta F}{F'_m} = \frac{(F'_m - F)}{F'_m}$$

where F is the fluorescence level of the light-acclimated sample just prior to the saturating pulse and F'_m is the maximum fluorescence emitted by the light-acclimated sample after the saturating pulse.

ϕPSII measured with the measuring light peaking at 645 nm was then selected to calculate the absolute electron transport rate (ETR) since it targets phycocyanin and was the most appropriate wavelength to our cyanobacteria species. ETR was calculated as:

$$ETR = \phi PSII \times E \times 0.36 \times \bar{a}^*_{phy}$$

where E (µmol photons m^{-2} s^{-1}) is the actinic irradiance, 0.36 is the fraction of absorbed quanta to PSII (fAQ_{PSII}) in cyanobacteria (Johnsen and Sakshaug, 2007) and $\bar{a}^*_{phy}$ (m^2 (mg chl a)$^{-1}$) is the spectrally averaged (400-700 nm) chlorophyll a-specific absorption coefficient (see below for its measurement). The obtained ETR vs. E curves were fitted using the model of Eilers and Peeters (1988) to estimate the maximal light utilization efficiency (α) which corresponds to the initial slope of the curve and, maximum electron transport rate (ETR_m) which is the asymptote of the curve. Curve fitting was carried out using the downhill simplex method of the Nelder-Mead model (1965) and standard deviation of each parameter was estimated by an asymptotic method. All fittings were tested by analyses of variance ($P < 0.001$), residues being tested for normality and homogeneity of variance, and parameters significance by Student's t test ($P < 0.05$). All curve fitting processes and associated statistics were coded under MATLAB R2010b.

At each light step, non-photochemical quenching of fluorescence (NPQ) was calculated from the fluorescence levels measured with the measuring light peaking at 645 nm using the equation proposed by Serôdio et al. (2005):

$$NPQ = \frac{(F'_{mm} - F'_m)}{F'_m}$$

where F'_{mm} is the maximum F'_m value, higher than F_m, that is measured under low actinic irradiance. Then, NPQ_{max} that corresponds to the highest NPQ value measured during ETR vs E curve was extracted.

To measure the mean chl a-specific absorption coefficient ($\bar{a}^*_{phy}$), cells were harvested from 25 mL of culture by centrifugation (4000 g, for 15 min at 25°C) and the pellet obtained was resuspended in 1 mL of Milli-Q water. The optical density (OD) spectrum was then measured between 350 and 850 nm with 1 nm-increment using a spectrophotometer UV-Vis 6705 (Jenway). A cuvette filled with Milli-Q water was used as a reference (blank). The OD between 750 and 800 nm was used to correct for scattering. The absorption coefficient of particles ($ap(\lambda)$, in m^{-1}) was calculated according to the equation of Mitchell et al. (2003):

$$ap(\lambda) = \frac{2.303}{l} [OD_{sample}(\lambda) - OD_{blank}(\lambda) - OD_{null}(\lambda)]$$

where l is the cuvette pathlength, $OD_{sample}(\lambda)$ is the sample absorbance, $OD_{blank}(\lambda)$ is the reference absorbance and $OD_{null}(\lambda)$ is the OD between 750 and 800 nm used to correct for scattering. The depigmented particle absorption coefficient ($ad(\lambda)$, in m^{-1}) was obtained by bleaching the sample with a solution of sodium hypochlorite (0.1% active chlorine). The spectral absorption coefficient for cyanobacteria pigments ($a_{phy}(\lambda)$) was then calculated by subtracting $ad(\lambda)$ to $ap(\lambda)$. $a_{phy}(\lambda)$ was normalized to the chlorophyll a concentration to obtain the chl a-specific absorption ($a^*_{phy}(\lambda)$, in m^2 (mg chl a)$^{-1}$). Finally, the mean absorption coefficient between 400 and 700 nm ($\bar{a}^*_{phy}$, in m^2 (mg chl a)$^{-1}$) was calculated.

2.4 Chlorophyll a, carotenoids, phycoerythrin and phycocyanin content

Chlorophyll a (chl a) and carotenoids concentrations were measured by filtering known volume of sample through 25 mm GF/F glass-fiber filter. The filters were stored at –80°C. Chl a and carotenoids were subsequently dark-extracted in 90% acetone, clarified by

centrifugation and quantified spectrophotometrically. Chl a and carotenoids concentrations were respectively calculated according to the equations of UNESCO (1966) and Strickland and Parsons (1972).

Phycoerythrin and phycocyanin contents were measured using in vivo fluorescence excitation spectra. Prior to the measurements, samples were treated with 50 µM (final concentration) of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, Sigma Aldrich) and illuminated with a strong light to ensure the complete saturation of PSII and avoid variable fluorescence (Duysens, 1972). Spectra were recorded using a microplate reader infinite 200 (Tecan). Excitation light was provided at 5 nm bandwidth from 400 to 700 nm with emission recorded at 730 nm. Phycoerythrin and phycocyanin contents were determined by using respectively the fluorescence intensity measured after excitation at 565-575 and 645-655 nm (Seppälä, 2009). All spectra were normalized at the chl a peak (425 nm) for comparison.

2.5 Production of reactive oxygen species (ROS)

Intracellular ROS production was assayed by measuring the oxidation of the 2'-7'-dichlorohydrofluorescein diacetate (DCFH-DA, Sigma Aldrich) according to the protocol of Rajneesh et al. (2017) (steps 3-7) with small modifications. Cells were harvested from 5 mL of culture by centrifugation (4000 g for 15 min at 25°C) to obtain cell pellet and supernatant. Supernatant was discarded. Cell pellet was resuspended in 1 mL of phosphate buffer (50 mM) and 2.5 µL of 2 mM DCFH-DA solubilized in ethanol was added. Two blanks were also prepared: 1) a cell pellet harvested from a 5 mL of sample centrifuged and resuspended in 1 mL phosphate buffer to correct for pigments fluorescence in the absence of DCFH-DA and 2) 1 mL of buffer with 2.5 µL of 2 mM DCFH-DA to correct for background fluorescence from potential auto-oxidation of DCFH-DA. Samples and blanks were incubated at room temperature in the dark for 1h on a shaking table (15 rpm). OD_{750} and fluorescence of samples

and blanks were measured using a microplate reader infinite 200 (Tecan). For fluorescence measurements, excitation light was provided at 485 nm and emission was registered between 515 and 600 nm to target fluorescence from 2',7'-dichlorofluorescein (DCF). The relative ROS production was calculated by averaging the fluorescence intensity measured between 525 and 535 nm after blanks correction and was normalized by OD_{750}.

2.6 Extraction and analysis of saxitoxins (STX)

Cyanobacteria were harvested from 25 mL of culture by centrifugation (4000 g for 15 min at 25°C) to obtain cell pellet and supernatant. Cell pellet was resuspended for toxins extraction in 1 mL of 0.1 N acetic acid. Cells were lysed using 0.25 g of glass beads with a ball mills (MM400, Retsch GmbH, Germany) for 30 min at 30 Hz. Samples were centrifuged at 15000 g for 15 min at 4°C. The supernatant was then filtrated through a Nanosep MF centrifugal devices 0.2 µm (4000 g for 15 min at 4°C).

Analysis of STXs was conducted by HPLC-FLD with a protocol adapted from the method of Van de Riet et al. (2009) which uses a reversed-phase LC with post-column oxidation. Samples were analyzed on a Zorbax bonus RP column (4.6 mm x 150 mm, 3.5 µm) using a mobile phase composed of two solvents: one containing 11 mM heptane sulfonate and 5.5 mM phosphoric acid (H_3PO_4) and the second containing 11 mM heptane sulfonate, 16.5 mM H_3PO_4 and 11.5% acetonitrile. The post-column oxidant was a mixture of 100 mM H_3PO_4 and 5 mM periodic acid. 0.75 M of nitric acid was used as a post-column acid. Fluorescence was monitored using an excitation light at 330 nm and emission at 390 nm. Six standard mixtures containing known increasing concentrations of toxins were prepared using solutions of gonyautoxin 1, 2, 3, 4 and 5 (respectively GTX1, GTX2, GTX3, GTX4 and GTX5), decarbamoylogonyautoxin 2 and 3 (dc-GTX2 and dc-GTX3), neosaxitoxin (Neo-STX), decarbamoylsaxitoxin (dc-STX) and saxitoxin (STX) purchased from the Institute for

Marine Biosciences (National Research Council, Halifax, NS, Canada). During HPLC measurements, each run of samples was preceded and followed by these standards mixtures and blanks. Toxins in samples were identified by comparison with the known retention time of each standard toxin (supplementary materials Fig. S1 and S2). Toxins in samples were quantified by comparison of their integrated area to a calibration curve built, for each standard toxin, from integrated areas and known concentrations in mixtures (supplementary materials Fig. S3). Intracellular toxin contents were normalized by chlorophyll a and concentrations were expressed per chlorophyll unit. However to be sure that observed variations did not reflect changes in chl content, toxins were also normalized by OD_{750}.

2.7 Extraction and analysis of DMSP, glycine betaine, methionine and proline

Cyanobacteria were harvested from 15 mL of culture by centrifugation (4000 g for 15 min at 25°C) to obtain cell pellet and supernatant. Cell pellet was resuspended for DMSP, glycine betaine (GB), methionine and proline extraction in 1 mL of 100% methanol (Honeywell). Cells were lysed using 0.25 g of glass beads with a ball mills (MM400, Retsch GmbH, Germany) for 20 min at 30 Hz. Samples were centrifuged at 10000 g for 2 min at 4°C. The supernatant was then filtrated through a Nanosep MF centrifugal devices 0.2 µm (10000 g for 2 min at 4°C).

Intracellular contents in DMSP, GB, methionine and proline were measured by LC-MS/MS on a LC System (UFLC XR, Shimadzu) coupled to a triple quadrupole mass spectrometer (4000 QTrap, ABSciex). A Hypersil GOLD HILIC column (150 x 3.0 mm, 3 µm, ThermoScientific) with a suited guard column was used to perform chromatography using a protocol adapted from Curson et al. (2018). The binary gradient was composed of water/acetonitrile (90:10; V/V) containing 4.5 mM ammonium formate (A) and water/acetonitrile (5:95, V/V) containing 5mM ammonium formate (B). Injection volume was

5 µL and flow rate equaled 0.4 mL min^{-1}. Column temperature was 30°C and that of sample 4°C. Elution gradient was 90% B (0-1 min), 90-45% B (1-8 min), 45% B (8-12 min) and 90% B (12-15 min). A positive ionization mode and MRM with two transitions per compounds was used. Compounds were quantified by comparison with 5-point calibration curves built with standards (Sigma Aldrich) dissolved in 100% methanol at concentrations ranging from 50 nM to 5000 nM. Measurements and data processing were carried out using the Analyst 1.6.3 (ABSciex) software. Intracellular contents in DMSP, GB, methionine and proline were normalized by chlorophyll a and concentrations were expressed per chlorophyll unit. However to be sure that observed variations did not reflect changes in chl content, they were also normalized by OD_{750}.

2.8 Extraction and analysis of mono and disaccharides

Cyanobacteria were harvested from 15 mL of culture by centrifugation (4000 g for 15 min at 25°C) to obtain cell pellets and supernatants. Cell pellets were resuspended in 1 mL of absolute ethanol and incubated for 3h at 65°C. Samples were then filtered through a Nanosep MF centrifugal devices 0.2 µm (8000 g for 1 min at 4°C) to remove salt. Ethanol was evaporated from the salt-free extracts under a stream of nitrogen at 65°C. Dried pellets were suspended in Milli-Q water and sugar contents were analyzed by gas chromatography (GC-FID, Agilent Technologies 6890N) following Adams et al. (1999). Four osidic residues were investigated: sucrose, fructose, glucose and D-trehalose (Sigma Aldrich). Intracellular contents in sucrose, fructose, glucose and D-trehalose were normalized by chlorophyll a and concentrations were expressed per chlorophyll unit. However to be sure that observed variations did not reflect changes in chl content, they were also normalized by OD_{750}.

2.9 Statistical analysis

For each parameter, differences between control and other culture conditions were tested using two-way ANOVA followed by post-hoc multiple comparisons using the Holm-Šidák method (Holm, 1979). Normality and equal variance were tested using respectively the Shapiro-Wilk (Royston, 1982) and Brown-Forsythe (Brown and Forsythe, 1974) tests. All analyses were performed using SigmaPlot 14.0.

3 Results

3.1 Growth and survival

Growth and survival time of *A. gracile* (Fig. 1A) and *C. raciborskii* (Fig. 1B) were inversely proportional to NaCl concentration in culture medium. In BG11, growth of *A. gracile* started after a 48h lag phase (μ=0.12 day^{-1}). In BG11 + 34 mM NaCl, the cell density of *A. gracile* slightly increased during the 72h of experiment and remained close to the BG11 lag phase (μ=0.07 day^{-1}). In BG11 + 9 g. L^{-1} NaCl, the cell density of *A. gracile* moderately decreased after 24h exposure to NaCl and remained stable until the end of the experiment. In BG11 + 257 mM NaCl, the quantity of chl a dropped in the first 6h after the salt treatment and *A. gracile* only survived 24h. *C. raciborskii* only grew in BG11 and BG11 + 34 mM NaCl with a lower growth in BG11 + 34 mM NaCl (μ=0.03 day^{-1}) than in BG11 (μ=0.06 day^{-1}). In BG11 + 154 mM NaCl, *C. raciborskii* survived until the end of the experiment but the cell density declined over time as indicated by the drop in chl a concentration. In BG11 + 257 mM NaCl, *C. raciborskii* only survived 24h. *Dolichospermum* sp. survived until the end of the experiment in all culture media (Fig. 1C). There was no difference in *Dolichospermum* sp. growth in BG11 + 154 mM NaCl (μ=0.17 day^{-1}) and BG11 + 257 mM NaCl (μ=0.17 day^{-1}). Growth in BG11 + 308 mM NaCl and BG11 + 411 mM NaCl was lower than in BG11 + 154

mM NaCl and inversely proportional to NaCl amount (μ=0.10 day^{-1} in BG11 + 308 mM NaCl and 0.08 day^{-1} in BG11 + 411 mM NaCl). In BG11 + 411 mM NaCl, the lag phase was longer than in other media.

3.2 Pigments

In all species, carotenoids concentration changed significantly over time and with NaCl treatments ($p<0.05$). There was a significant interaction between these factors ($p<0.001$). In *A. gracile*, carotenoids concentration differed from control conditions (BG11) only from 4 to 6h of NaCl exposure when carotenoids concentration became lower in NaCl containing media than in BG11 (Fig. 2A). In *C. raciborskii*, carotenoids concentration in BG11 + 34 mM NaCl changed in two steps (Fig. 2B). It increased after 4h exposure and recovered a level not significantly different from control conditions (BG11) after 6h exposure. Then, it increased again after 24h exposure and remained stable until the end of the experiment. In BG11 + 154 mM NaCl, carotenoids concentration was not significantly different from control conditions and remained relatively stable during the 72h of experiment. In BG11 + 257 mM NaCl, carotenoids concentration decreased from 24h exposure. In *Dolichospermum* sp., carotenoids concentration at t=0 was around 2 times lower than in *A. gracile* and *C. raciborskii*. In BG11 + 257 mM NaCl was not significantly different from control conditions (BG11 + 154 mM NaCl) (Fig. 2C). In BG11 + 308 mM NaCl, carotenoids concentration decreased from 48 to 72h exposure. In BG11 + 411 mM NaCl, carotenoids concentration increased after 4h exposure. Then, it recovered a level not significantly different from control conditions and remained relatively stable until the end of the experiment.

Phycocyanin (PC) was the dominant phycobiliprotein in *A. gracile* and *C. raciborskii* while phycoerythrin (PE) dominated in *Dolichospermum* sp. (Fig. 2). In all species, PC and PE content varied significantly over time and with NaCl treatments ($p<0.001$). There was a

significant interaction ($p<0.001$) between these factors. In *A. gracile*, exposure to NaCl decreased PE and PC contents proportionally to NaCl concentration in culture media from 24 to 48h exposure and from 6 to 48h exposure respectively (Fig. 2D, G). After 72h exposure, in media in which *A. gracile* survived PC and PE content did not seem to be further impacted by NaCl. In *C. raciborskii*, variation in PC and PE content in response to NaCl exposure was biphasic (Fig. 2E, H). After 1h exposure, PC and PE content were higher in NaCl containing media than in BG11. Then, from 4 to 24h exposure, there was no significant difference between BG11 and NaCl treatments. After 24h exposure, PC content in BG11 remained stable while it became inversely proportional to NaCl amount in the other media. PE content followed the same pattern as PC but after 48h exposure. In *Dolichospermum* sp., PC and PE content showed similar patterns of variation (Fig. 2F, I). In control conditions (BG11 + 154 mM NaCl), these contents remained stable. By contrast, from 1 to 24h exposure in other media, PC and PE content was proportional to the NaCl amount. After 48h exposure, PC and PE recovered levels not significantly different from control conditions.

3.3 Photosynthetic parameters

The maximum quantum yield (F_v/F_m), measured in the diverse NaCl treatments, ranged from 0.59 to 0.20 in *A. gracile* (Fig. 3A), 0.61 to 0.04 in *C. raciborskii* (Fig. 3B) and 0.52 to 0.23 in *Dolichospermum* sp. (Fig. 3C). In all species, F_v/F_m changed significantly over time and with NaCl treatments ($p<0.001$). There was a significant interaction ($p<0.001$) between these factors. When *A. gracile* was exposed to BG11, BG11 + 34 mM NaCl and BG11 + 154 mM NaCl, F_v/F_m stayed high with few differences between treatments. By contrast BG11 + 257 mM NaCl resulted in cell death after 48h exposure and F_v/F_m decrease drastically (from 0.52 to 0.26) during the last day of life (24h). When *C. raciborskii* was exposed to BG11 + 34 mM NaCl and BG11 + 154 mM NaCl, F_v/F_m increased proportionally to the amount of NaCl

during the first 24h exposure. After 48h in BG11 + 34 mM NaCl, F_v/F_m recovered values not significantly different from control conditions. Inversely, in BG11 + 154 mM NaCl, F_v/F_m dropped from 0.57 to 0.17 and reached values close to zero after 72h exposure. BG11 + 257 mM NaCl resulted in cell death after 48h exposure. F_v/F_m started to decrease after 1h exposure, remained stable during the first 6h and dropped from 0.37 to 0.19 during the last day of life (24h). In *Dolichospermum* sp., F_v/F_m increased over time in all media. During the first 4h exposure, F_v/F_m was not affected by NaCl and only decreased proportionally to the amount of NaCl between 6 and 48h exposure. In BG11 + 257 mM NaCl and BG11 + 308 mM NaCl, F_v/F_m recovered after 72h exposure while, in BG11 + 411 mM NaCl, F_v/F_m stayed lower than in control conditions until the end of the experiment.

In all species, the maximal light utilization efficiency (α) and maximum electron transport rate (ETR_m) varied conjointly. α ranged from 0.0006 to 0.0045 µmol e^- mg chl a^{-1} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$ in *A. gracile* (Fig. 3D), 0.0003 to 0.0038 µmol e^- mg chl a^{-1} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$ in *C. raciborskii* (Fig. 3E) and 0.0003 to 0.0025 µmol e^- mg chl a^{-1} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$ in *Dolichospermum* sp.(Fig. 3F). ETR_m ranged from 0.32 to 3.35 µmol e^- mg chl a^{-1} s^{-1} in *A. gracile* (Fig. 3G), 0.11 to 2.57 µmol e^- mg chl a^{-1} s^{-1} in *C. raciborskii* (Fig. 3H) and 0.22 to 1.84 µmol e^- mg chl a^{-1} s^{-1} in *Dolichospermum* sp. (Fig. 3I). In *A. gracile* and *C. raciborskii*, α and ETR_m changed significantly over time and with NaCl treatments ($p<0.001$). There was a significant interaction ($p<0.001$) between these factors. In *A. gracile*, α and ETR_m increased in response to salt stress after 4-6h exposure. In BG11 + 34 mM NaCl, this increase did not persist as α and ETR_m recovered values not significantly different from the control after 24h exposure. In BG11 + 154 mM NaCl, α recovered after 48h while ETR_m recovered after 72h. In BG11 + 257 mM NaCl, α and ETR_m did not recover and decreased after 24h exposure. In *C. raciborskii* exposed to BG11 + 34 mM NaCl, α and ETR_m were not significantly different from control conditions. While α and ETR_m decreased in BG11 during

437 the first 4-6 hours, in BG11 + 154 mM NaCl, α and ETR_m stayed high and reached their
438 highest values after 24h exposure. Then, they decreased until the end of the experiment. In
439 BG11 + 257 mM NaCl, α did not change over time and was not significantly different from
440 control conditions. ETR_m was significantly lower than in control conditions only after 24h
441 exposure. In *Dolichospermum* sp., α and ETR_m changed significantly over time ($p<0.001$) but
442 there was no significant difference between NaCl treatments.

443 In *A. gracile* and *C. raciborskii*, maximum non-photochemical quenching of
444 fluorescence (NPQ_{max}) changed significantly over time and with NaCl treatments ($p<0.001$).
445 There was a significant interaction ($p<0.001$) between these factors. In *A. gracile*, NPQ_{max} in
446 BG11 + 34 mM NaCl was not significantly different from NPQ_{max} in BG11 (Fig. 3J). In
447 BG11 + 154 mM NaCl, NPQ_{max} increased significantly after 48h exposure and stayed high
448 until the end of the experiment. In BG11 + 257 mM NaCl, NPQ_{max} increased significantly
449 after 24h exposure. In *C. raciborskii*, NPQ_{max} in BG11 + 34 mM NaCl and BG11 + 257 mM
450 NaCl was not significantly different from NPQ_{max} in control conditions (Fig. 3K). In BG11 +
451 154 mM NaCl, NPQ_{max} increased significantly only after 24h exposure. In *Dolichospermum*
452 sp., NPQ_{max} did not change significantly over time and with NaCl treatments (Fig. 3L).
453
454 *3.4 Reactive oxygen species (ROS)*
455
456 In all species, ROS production (Fig. 4) changed significantly over time and with NaCl
457 treatments ($p<0.05$). There was a significant interaction ($p<0.001$) between these factors. In *A.*
458 *gracile*, ROS production in BG11 + 34 mM NaCl was not significantly different from control
459 conditions (Fig. 4A). In BG11 + 154 mM NaCl and BG11 + 257 mM NaCl, ROS production
460 of *A. gracile* was proportional to NaCl amount. In BG11 + 154 mM NaCl, ROS production
461 was biphasic. It started after 6h exposure, remained stable between 6 and 48h exposure and
462 increased after 72h. In BG11 + 257 mM NaCl, ROS production occurred between 4 and 6h

463 exposure. Then, ROS production declined. In *C. raciborskii*, ROS production in BG11 + 34
464 mM NaCl was not significantly different from control conditions except after 24h exposure
465 where it was higher than in BG11 (Fig. 4B). In BG11 + 154 mM NaCl and BG1 1+ 257 mM
466 NaCl, ROS production was biphasic. In BG11 + 154 mM NaCl, a first increase in ROS
467 production occurred after 1h exposure. Then, ROS declined after 4h exposure and a second
468 phase of ROS production took place from 6 to 24h exposure. After, 48h exposure, ROS
469 decreased and became lower than in BG11. In BG11 + 257 mM NaCl, ROS production
470 increased after 1h exposure and remained stable between 1 and 6h exposure. A second peak
471 of ROS production occurred after 24h exposure. In *Dolichospermum* sp., ROS production in
472 BG11 + 257 mM NaCl was not significantly different from control conditions (BG11 + 154
473 mM NaCl) (Fig. 4C). In BG11 + 308 mM NaCl and BG11 + 411 mM NaCl, ROS production
474 was proportional to NaCl amount. In BG11 + 308 mM NaCl, ROS production started after 1h
475 exposure and reached its maximum after 4h exposure. It remained stable between 4 and 24h
476 exposure and recovered its basal level after 48h exposure. In BG11 + 411 mM NaCl, ROS
477 production started after 1h exposure and increased with a maximum reached after 24h
478 exposure. Then, ROS production decreased to a level not significantly different from control
479 conditions and remained stable until the end of the experiment.
480
481 *3.5 Toxins*
482
483 In *A. gracile* samples, two toxin analogs, neosaxitoxin (Neo-STX) and saxitoxin (STX),
484 were detected (supplementary materials Fig. S4). The total amount of paralytic shellfish
485 toxins (PSTs) (Fig. 5A) as well as Neo-STX (Fig. 5B) and STX (Fig. 5C) concentrations
486 varied significantly over time and with NaCl treatments ($p<0.05$). There was a significant
487 interaction between these factors ($p<0.001$). Neo-STX and STX showed similar variations
488 and thus changes in PSTs concentrations were the result of changes in both toxins. PSTs,

Neo-STX and STX concentrations in BG11 + 34 mM NaCl and BG11 + 257 mM NaCl were not significantly different from control conditions in BG11. In BG11 + 154 mM NaCl, PSTs, Neo-STX and STX concentrations were significantly higher than in control conditions and increased with exposure time to NaCl. At t=0, the relative proportions of Neo-STX and STX were respectively 75% and 25% and Neo-STX stayed the dominant analog in all treatments (Fig. 6).

In *C. raciborskii*, 3 toxins were detected: decarbamoylogonyautoxin 2 (dc-GTX2), Neo-STX and STX (supplementary materials Fig. S5). An unidentified peak appearing between GTX4 and GTX1 standards was also detected during toxin analyses. The quantity of PSTs produced by *C. raciborskii* was 5-6 times lower than in *A. gracile*. The total amount of PSTs (Fig. 5B) and concentrations of Neo-STX (Fig. 5D), STX (Fig. 5F), and dc-GTX2 (Fig. 5G) changed significantly over time and with NaCl treatments ($p<0.05$). There was a significant interaction between these factors ($p<0.001$). PSTs, Neo-STX, STX and dc-GTX2 concentrations in BG11 + 34 mM NaCl were not significantly different from control conditions in BG11. In BG11 + 154 mM NaCl, PSTs and Neo-STX contents both decreased with exposure time to NaCl. While in control conditions STX concentration increased from 6h, in BG11 + 154 mM NaCl STX content stayed low. By contrast, dc-GTX2 became significantly higher than in control conditions after 6h exposure and then gradually decreased from 24h exposure. From 48h exposure, dc-GTX2 was the only analog detected in this culture medium. In BG11 + 257 mM NaCl, PSTs, Neo-STX, STX and dc-GTX2 concentrations decreased with exposure time to NaCl. At t=0 in BG11, the relative proportions of Neo-STX, STX and dc-GTX2 were respectively 74%, 8% and 18% (Fig. 6). Neo-STX stayed the dominant analog in all treatments during the first 24h exposure. In BG11 + 34 mM NaCl and BG11 + 257 mM NaCl, the relative proportions of Neo-STX, STX and dc-GTX2 followed the same temporal variations than in control conditions in BG11. By contrast, in BG11 + 154 mM

NaCl, dc-GTX2 proportion increased with time exposure to NaCl while those of Neo-STX and STX decreased Fig. 6G). Similar trends of variation were obtained when toxins were normalized by OD_{750} (supplementary materials Fig. S6 and S7). This indicates that observed variations were the results of toxins contents variations and not the results of changes in intracellular chl a concentrations. No toxin was detected in *Dolichospermum* sp. (supplementary materials Fig. S8).

3.6 Methionine

In all species, methionine content varied significantly over time and with NaCl treatments ($p<0.05$). There was a significant interaction ($p<0.001$) between these factors. In *A. gracile* (Fig. 7A) and *C. raciborskii* (Fig. 7B), methionine content in BG11 + 34 mM NaCl was not significantly different from control conditions in BG11. In *A. gracile*, methionine content was proportional to NaCl concentration from 6 to 48h exposure. In BG11 + 154 mM NaCl, methionine concentration increased strongly after 72h exposure. In *C. raciborskii*, methionine content was inversely proportional to NaCl concentration from 6 to 24h exposure. After 48h exposure, methionine content in BG11 + 154 mM NaCl increased and methionine became proportional to NaCl concentration. After 72h exposure, methionine content was again inversely proportional to NaCl concentration. In *Dolichospermum* sp. (Fig. 7C), from 6 to 24h exposure, only the methionine content in BG11 + 411 mM NaCl was significantly different from control conditions in BG11 + 154 mM NaCl. After 48h exposure, only the methionine content in BG11 + 257 mM NaCl was significantly lower than in control conditions. After 72h exposure, methionine contents in BG11 + 257 mM NaCl and BG11 + 411 mM NaCl were both significantly lower than in control conditions while in BG11 + 308 mM NaCl, methionine content increased.

3.7 DMSP, glycine betaine, proline, sucrose, fructose, glucose and D-trehalose

In all species, DMSP and glycine betaine concentrations were below the detection limit (50 nM) in all treatments. In *A. gracile* (Fig. 7D) and *C. raciborskii* (Fig. 7E), proline content changed significantly over time and with NaCl treatments ($p<0.05$). There was a significant interaction ($p<0.001$) between these factors. By contrast, in *Dolichospermum* sp. (Fig. 7F), NaCl treatments did not significantly modify proline content and the maximum level of proline produced was threefold lower than in *A. gracile* and *C. raciborskii*. In *A. gracile*, proline content increased dose-dependently with NaCl after 24h exposure. After 48h exposure, proline content in BG11 + 154 mM NaCl recovered a level not significantly different from control conditions while it increased in BG11 + 34 mM NaCl. After 72h exposure, proline content was again proportional to NaCl concentration. In *C. raciborskii*, proline content decreased dose-dependently with NaCl and was inversely proportional to NaCl concentration after 24h exposure. After 48h exposure, proline content in BG11 + 34 mM NaCl recovered a level not significantly different from control conditions while it increased in BG11 + 154 mM NaCl. After 72h exposure, proline content in BG11 + 154 mM NaCl decreased but it was still significantly higher than in control conditions. In *A. gracile* and *C. raciborskii*, sucrose, fructose, glucose and D-trehalose concentrations were below the detection limit (8 µg L^{-1}) in all treatments. In *Dolichospermum* sp., sucrose concentration was below the detection limit in BG11 + 154 mM NaCl and BG11 + 257 mM NaCl. By contrast, in BG11 + 308 mM NaCl and BG11 + 411 mM NaCl sucrose was detected from 6h exposure (Fig. 8). Sucrose concentration was higher in BG11 + 411 mM NaCl than in BG11 + 308 mM NaCl. In both media, sucrose concentration remained relatively stable until the end of the experiment. The other mono and disaccharides (fructose, glucose and D-trehalose) were below the detection limit in all treatments. Normalization by OD_{750} confirmed that observed variations were the results of changes in intracellular methionine, proline and sucrose in

566 response to NaCl treatments and not related to modifications in intracellular chl a
567 concentrations (supplementary materials Fig. S9 and S10).
568
569 **4 Discussion**
570 *4.1 Growth and salt tolerance*
571
572 *A. gracile*, *C. raciborskii* and *Dolichospermum* sp. presented distinct tolerance and
573 survival capacities to salinity stress. *C. raciborskii* seemed to be the less tolerant as it only
574 survived 24h when it was exposed to the highest salinity and the cell density declined over
575 time after exposure to BG11 + 154 mM NaCl. By opposition, the brackish water species
576 (*Dolichospermum* sp.) was the most tolerant. It survived and grew in all tested salinities. In all
577 species, NaCl dose-dependently slowed down growth. However in *A. gracile* and *C.*
578 *raciborskii*, growth seemed to be inhibited when [NaCl] ≥ 154 mM while in *Dolichospermum*
579 sp., growth at high salinities was reduced but not inhibited. The slow-down or inhibition of
580 growth by NaCl has been frequently observed in several species of cyanobacteria (Carneiro et
581 al., 2013; Chen et al., 2015; Georges des Aulnois et al., 2019; Hagemann, 2011; Pomati et al.,
582 2004b; Swapnil et al., 2017; Tonk et al., 2007). This happens because high NaCl
583 concentrations lower the water potential in the culture medium to an extent that makes it
584 difficult for cyanobacteria to obtain and retain water inside their cells. Turgor, the driving
585 force for growth, is consequently reduced or lost and growth is affected (Hagemann, 2011).
586 Growth reduction may also results from the extra energy expenditure due to Na^+/H^+
587 antiporters activity and implementation of the "salt-out" strategy (Carneiro et al., 2013;
588 Hagemann, 2011). In the present study, intensity of growth slow-down (or inhibition) also
589 depended on cyanobacteria capacity to maintain their photosynthetic activity under salt stress
590 conditions (see section 4.3. for more details). The higher salt tolerance of *Dolichospermum* sp.
591 (*Anabaena* sp.) in comparison to *C. raciborskii* is consistent with the Tonk et al. (2007)'s data

compilation on the salt tolerance of cyanobacteria as well as the previously reported *C. raciborskii* preference for low salinity conditions with optimal growth in freshwaters to oligohaline conditions (Antunes et al., 2015).

4.2 Pigments

Carotenoids content was differently impacted by NaCl treatments in the freshwater (*A. gracile*, *C. raciborskii*) and brackish water (*Dolichospermum* sp.) species. While in *A. gracile*, NaCl reduced carotenoids content from 4 to 6h exposure, in *C. raciborskii* and *Dolichospermum* sp., variations in carotenoids content differed among salinity treatments without any obvious relationships with NaCl concentration. In cyanobacteria, carotenoids are photosynthetic pigments described under salt stress as having a protective role on chlorophyll as well as antioxidant effects on reactive oxygen species (ROS) (Bhargava and Srivastava, 2013). However, in cyanobacteria, effects of NaCl stress on carotenoids content do not seem to be universal. In freshwater cyanobacteria, some authors found a decrease in carotenoids content with increasing NaCl concentration (Chen et al., 2015) while others observed an increase (Kumar et al., 2015; Schubert et al., 1993). A production of carotenoids to protect cells against ROS could not be confirmed in the present study since carotenoids trends did not correspond to ROS variations. For instance, in *Dolichospermum* sp. cultures exposed to 411 mM NaCl, there was an increase in ROS production from 4 to 24h while on the same period carotenoids content decreased from 4 to 6h and then remained relatively stable until 48h. Besides, although ROS variations were biphasic in *A. gracile* and *C. raciborskii*, these species presented completely different trends in their carotenoids content.

While in *Dolichospermum* sp. PC and PE contents increased within the first 24h exposure to NaCl, in both *A. gracile* and *C. raciborskii*, PC and PE contents decreased proportionally to NaCl concentration but with different timing. The decrease of these

phycobiliproteins in response to salinity stress has been described in a freshwater strain of *Microcystis aeruginosa* (Chen et al., 2015) and several species of freshwater filamentous cyanobacteria (Kumar et al., 2015; Lu and Vonshak, 2002). In cyanobacteria, phycobiliproteins constitute phycobilisomes that serve as the primary light-harvesting antenna for photosystems II. Under stress conditions, the constituents of phycobilisomes are often degraded. This degradation could provide the cell with amino acids used for the synthesis of proteins required for the acclimation processes (Grossman et al., 1993). Modifications in PC and PE contents can also have important consequences on cyanobacteria photosynthetic capacities because this can modify energy transfers from phycobilisomes to photosystems II (Schubert et al., 1993; Sudhir and Murthy, 2004; Zhang et al., 2010).

4.3 Photosynthesis

PSII activity was differently impacted by NaCl treatments in *A. gracile*, *C. raciborskii* and *Dolichospermum* sp. In *A. gracile* and *C. raciborskii*, partly due to phycobilisomes modifications, the treatment with the highest NaCl concentration (BG11 + 257 mM NaCl) strongly impacted PSII functioning. The maximum quantum yield (F_v/F_m) and maximum electron transport rate (ETR_m) were reduced and the maximum non-photochemical quenching of fluorescence (NPQ_{max}) increased in *A. gracile*. These results suggest that the stress level generated by 257 mM NaCl was sufficiently high to down regulate the photosynthetic activity and to stimulate photoprotective mechanisms (NPQ). None of these parameters recovered during the experiment leading to cell death. In the other treatments, F_v/F_m was not impacted. The maximal light utilization efficiency (α) and ETR_m increased but recovered with a recovery time inversely proportional to NaCl concentration. NPQ_{max} increased only in BG11 + 154 mM NaCl. Hence, exposure to 34 and 154 mM NaCl reversibly modified photosynthetic activity and slowed down growth. In *C. raciborskii*, F_v/F_m first increased

644 proportionally to the amount of NaCl during the first 24h exposure. Then, F_v/F_m recovered in
645 BG11 + 34 mM NaCl while in BG11 + 154 mM NaCl, it strongly decreased. α and ETR_m
646 were not impacted in BG11 + 34 mM NaCl. In BG11 + 154 mM NaCl, they decreased after
647 24h exposure and did not recover. NPQ_{max} increased simultaneously to α and ETR_m decrease
648 in BG11 + 154 mM NaCl. While exposure to 34 mM NaCl did not severely impacted
649 photosynthetic activity and slightly reduced *C. raciborskii* growth, 154 mM NaCl treatment
650 irreversibly inhibited photosynthesis (both the capacity to absorb light and transport electrons)
651 and led to cell death. In the brackish water species (*Dolichospermum* sp.), NaCl had a weak
652 effect on PSII activity. Growth was thus slowed down but maintained in all salt treatments. α,
653 ETR_m and NPQ_{max} were not affected. Only F_v/F_m decreased proportionally to the amount of
654 NaCl and recovered in BG11 + 257 mM NaCl and BG11 + 308 mM NaCl but not in BG11 +
655 411 mM NaCl.

656 Like in the present study, different effects of salinity stress on cyanobacteria PSII
657 activity have been reported in literature. Although it has been found that PSII of a freshwater
658 strain of *Synechocystis* sp. (PCC6803) were not impacted by a salt stress of 550 mM NaCl
659 (i.e. 32.14 g L^{-1} NaCl) (Jeanjean et al., 1993), the majority of studies on freshwater
660 cyanobacteria observed a decrease of PSII activity under salinity stress (e.g. Chen et al., 2015;
661 Gong et al., 2008; Lu and Vonshak, 2002; Ross et al., 2019). In accordance with the present
662 study, when salinity concentration was sufficiently high to induce a stress, a decreased F_v/F_m
663 was observed in *Spirulina platensis* (Gong et al., 2008; Lu et al., 1999) and *M. aeruginosa*
664 (Georges des Aulnois et al., 2020; Ross et al., 2019). In line with the *A. gracile* and *C.*
665 *raciborskii* results under high salinity concentrations, a decreased ETR_m under salinity stress
666 has also been found in a freshwater strain of *M. aeruginosa* (Chen et al., 2015).

667 The underlying mechanisms of the salt-stress reduction in PSII activity, and
668 photosynthesis in general, are not completely resolved in cyanobacteria. In the freshwater

cyanobacteria: *Synechocystis* sp. and *S. platensis*, it has been suggested that the decreased PSII activity under salt stress is associated with a transition from state 1 to state 2 i.e. a modification in the distribution and transfer of excitation energy from phycobilisomes to PSII and photosystem I (PSI) (Gong et al., 2008; Schubert et al., 1993; Schubert and Hagemann, 1990). In *S. platensis*, Gong et al. (2008) found that salt stress resulted in an increase of state 2 stability at the donor side and this was associated with a dissociation of the PSII extrinsic protein (PsbO) from the thylakoid membrane. The transition to state 2 involves the functional decoupling of phycobilisomes from PSII and their reassociation with PSI. Light energy transfer to PSII is thus reduced in favor of PSI (Joshua and Mullineaux, 2004) and the resulting F_v/F_m decreases (Ogawa et al., 2017). PSII is responsible for the production of NADPH, whereas PSI is involved in ATP generation (Bhargava and Srivastava, 2013). This transition to state 2 and the resulting boost in PSI activity would thus increase the relative amount of ATP produced by photosynthesis. This ATP may be used to fuel P-type ATPases and to respond to the over demand of ATP for Na^+ extrusion and restoration of cellular homeostasis (Bhargava and Srivastava, 2013; Hagemann, 2011). The increase in NPQ_{max} observed in *A. gracile* and *C. raciborskii* is consistent with the occurrence of this state transition because it has been shown in many studies that non photochemical quenching in cyanobacteria largely reflects changes in the PSII fluorescence yield as a result of the state transition mechanism (Lu and Vonshak, 2002 and references therein). In addition to state transition, the decreased F_v/F_m may be due to PSII reaction centers inactivation and inhibition of electron transport at both donor and acceptor sides (Gong et al., 2008; Lu et al., 1999; Lu and Vonshak, 2002). In *S. platensis*, it has been shown that salt stress induced a F_v/F_m decrease associated with a modification of the plastoquinone Q_B niche at the acceptor side (i.e. one of the PSII electron transporters) (Gong et al., 2008).

694 *4.4 Reactive oxygen species (ROS)*

695

696 ROS production was observed in the three tested cyanobacteria species when salinity
697 was sufficiently high to induce a stress. However, the ROS accumulation timing and sequence
698 differed among the three species. In the freshwater species (*A. gracile* and *C. raciborskii)*,
699 ROS accumulation was biphasic. In *A. gracile*, the time at which the first phase started was
700 dependent on NaCl concentration. The higher the NaCl concentration, the sooner ROS
701 accumulated. This trend was not observed in *C. raciborskii* in which ROS accumulation
702 started after 1h exposure at any NaCl concentration. A biphasic ROS accumulation induced
703 by salinity has been previously reported in the freshwater cyanobacteria *Anabaena fertilissima*
704 (Swapnil et al., 2017). According to Swapnil et al. (2017), the first phase would be produced
705 by NADPH oxidases located in the plasma membrane as well as cell wall peroxidases. These
706 ROS would act as signal molecules to regulate ion homeostasis. The second phase would
707 result from altered photosynthetic activity (Swapnil et al., 2017). Altered photosynthetic
708 activity caused by salt stress may generate electrons in excess, which associated with changes
709 at the electron acceptor sides, reduces O_2 and forms ROS (Bhargava and Srivastava, 2013;
710 Swapnil and Rai, 2018). In the same way, a salinity induced increase in respiration may over
711 reduce the ubiquinone pool and lead to electron leakage from complex I and III to O_2 which
712 generates ROS (Swapnil and Rai, 2018; Swapnil et al., 2017). Results from the present study
713 are insufficient to confirm that the first ROS accumulation in *A. gracile* and *C. raciborskii*
714 acted as signal molecules because no measurement of intracellular ionic concentration or
715 NADPH oxidases and cell peroxidases activity has been made. However, the results seem to
716 confirm that ROS production was related to photosynthetic activity. In *A. gracile*, timing of
717 variation in ROS accumulation corresponded to changes in α and ETR_m, while in *C.*
718 *raciborskii*, it corresponded in part to changes in F_v/F_m and ETR_m. In *Dolichospermum* sp.,
719 ROS accumulation was not biphasic and did not seem to be related to PSII activity because it

started before any visible variation in F_v/F_m, α and ETR_m. Additional measurements of PSI activity and respiration would be required to better understand the processes behind this ROS production. The buildup of ROS, such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^·$), may be problematic for prokaryotes such as cyanobacteria because these compounds have the potential to damage cell constituents. It has been shown that ROS production is related to several cellular toxic processes including damages to proteins, membrane lipid peroxidation, enzyme inactivation and DNA breakage (Latifi et al., 2009). ROS accumulation can also lead to the degradation of cellular membranes and consequent toxin release (Ross et al., 2006). This could not be confirmed in the present study because timing of ROS accumulation did not match with the changes observed in toxin content.

4.5 Toxins

Changes in cyanobacteria toxin content in response to environmental conditions are an important consideration when estimating the risk of animal and human exposure. In the present study, toxin content of *A. gracile* did not seem to be affected in a dose dependent-manner by salinity variations, since no significant changes in PSTs, Neo-STX and STX concentrations and proportions has been observed at the lowest as well as the highest NaCl concentrations tested. Only exposure to BG11+ 154 mM NaCl induced a significant increase in toxin content associated with an increase in ROS, methionine and proline contents after 72h exposure. There was also no apparent relationship between growth rate and toxin content. To our knowledge, *A. gracile* has not been previously used as a model species to study salinity effect on the production of STX and its analogs. Light, temperature and nutrients are the main abiotic factors that have been investigated in *Aphanizomenon spp.* (see Cirés and Ballot, 2016 for a review). The absence of an evident relationship with growth rate is consistent with

746 previous studies, since to date, no clear relationship between toxin content and growth rate
747 has been found in *Aphanizomenon spp*. (Casero et al., 2014; Cirés and Ballot, 2016; Preußel et
748 al., 2009). Toxin production by *C. raciborskii* seemed to be more sensitive to salinity
749 variations. A decreased PSTs concentration was observed rapidly after exposure to the two
750 highest NaCl levels tested. The sensitivity of STX content to NaCl stress in *C. raciborskii* is
751 in agreement with previous studies on the Brazilian freshwater *C. raciborskii* strain T3
752 (Carneiro et al., 2013; Ongley et al., 2016; Pomati et al., 2004b). However, results from the
753 different studies are not completely consistent, since some authors found that NaCl stress
754 resulted in intracellular STX accumulation in a dose-dependent manner (Carneiro et al., 2013;
755 Pomati et al., 2004b) while, others observed a decrease (Ongley et al., 2016).
756 Although it is well known that STX, and its analogs, perturb voltage-gated Na+
757 channels in vertebrate cells, the stimuli inducing (or repressing) their production within
758 cyanobacteria cells as well as their metabolic role for the producing organisms remain unclear
759 (D'Agostino et al., 2016; Ongley et al., 2016; Soto-Liebe et al., 2012). The fact that STX
760 seems to respond to salinity variations, more precisely intracellular sodium levels, led to think
761 that STX biosynthesis might be influenced by the protective mechanisms that cyanobacteria
762 use to maintain homeostasis or that PSTs themselves could interact with ion transport (Pomati
763 et al., 2004b; Soto-Liebe et al., 2012). In the Brazilian freshwater *Raphidiopsis brookii* strain
764 D9, exposure to NaCl has been shown to stimulate the transport of STX outside of the cell
765 likely through an active transport involving the multidrug and toxic compound extrusion
766 (MATE) family of transporters (Soto-Liebe et al., 2012). This toxin transport would act as a
767 protective mechanism to face salinity variations. However, by examining expression of genes
768 involved in STX biosynthesis (*sxtA*) along with that of MATE transporters in *R. brookii* D9
769 and *C. raciborskii* T3, Ongley et al. (2016) found that both species showed opposing
770 transcriptional responses to the same NaCl stress. In the present study, extracellular level of

771 STX has not been monitored and it could not be confirmed whether any extracellular transport
772 of STX occurred in response to NaCl exposure. *Dolichospermum* sp. did not produce any
773 toxins under the studied conditions, and yet was the most salt tolerant strain. This suggests the
774 involvement of other metabolites to ensure the protection against salinity stress.

775 In *C. raciborskii*, results from the present study showed shifts in STX and dc-GTX2
776 proportions after exposure to 154 mM NaCl. Few studies have investigated how the different
777 PSTs analogs produced by a single cyanobacteria species may vary in response to NaCl stress
778 exposure. In *C. raciborskii* T3, Carneiro et al (2013) observed that NaCl modified the ratio of
779 STX to Neo-STX. The relative proportion of STX increased but Neo-STX remained the major
780 analog. The physiological reason of this shift remained unexplained. However, in *R. brookii*
781 D9, Soto-Liebe et al. (2012) indicated that in the presence of the ionophore monensin that
782 removes the Na^+ gradient between the cytoplasm of cyanobacteria cells and culture medium,
783 GTX2/3 levels were not impacted while, after 1h exposure, intracellular levels of STX
784 decreased. These authors suggested that STX synthesis is sensitive to the Na^+ gradient or that
785 high Na^+ concentrations may have affected enzymes involved in STX biosynthesis (Soto-
786 Liebe et al., 2012). This could explain why STX, which is the precursor of both Neo-STX and
787 GTX2/3 (D'Agostino et al., 2014; Soto-Liebe et al., 2010), can be differently affected by
788 NaCl exposure than Neo-STX and GTX2/3. In the case of STX and dc-GTX2, the situation is
789 different because STX is not the direct precursor of dc-GTX2 (D'Agostino et al., 2014). The
790 suggestions of Soto-Liebe et al. (2012) remain, nonetheless, plausible. Distinct degradation
791 rates have also been suggested to explain imbalance among PSTs analogs (Pomati et al.,
792 2004a). Further research is, however, needed to better understand the underlying processes
793 responsible for these shifts in STX and dc-GTX2 as well as why similar modifications have
794 not been observed after exposure to 257 mM NaCl. Since toxicity of PSTs analogs is different
795 (Wiese et al., 2010), the shifts in the relative proportions of PSTs analogs observed in *C.*

796 *raciborskii* in response to NaCl exposure means that salinity has the potential to modify the
797 toxicity of this species. This is an important consideration when evaluating the risk of toxicity
798 for animals and human beings.

799 Ongley et al. (2016)'s results, associated with those from the present study, showed
800 that cyanobacteria with PSTs analogs in common can differently respond to the same salinity
801 stress. This means that the physiological response involved in PSTs biosynthesis under NaCl
802 stress is species-specific.

803

804 *4.6 Methionine and compatible solutes*

805

806 Methionine is essential in all organisms as it is both a proteinogenic amino acid and a
807 component of the cofactor S-adenosyl methionine (SAM) (Ferla and Patrick, 2014). In
808 cyanobacteria, PSTs would be biosynthesized via a pathway requiring 3 precursors: arginine,
809 acetate (via acetyl-coenzyme A) and methionine methyl (via SAM) (Kellmann et al., 2008).
810 In the present study, variations in methionine content in response to NaCl exposure were
811 species-specific. The smallest variations were observed in *Dolichospermum* sp. Methionine
812 content in *A. gracile* and *C. raciborskii* showed opposite variations during the first 24h of
813 NaCl exposure. In *A. gracile*, methionine content increased in a dose dependent-manner with
814 NaCl concentration. By contrast, it decreased in *C. raciborskii*. A potential link between the
815 available methionine pool and PSTs content was difficult to establish with the present results.
816 The hypothesis that, in comparison to *C. raciborskii*, toxin content in *A. gracile* was less
817 sensitive to NaCl because methionine content increased in response to NaCl exposure
818 whereas it decreased in *C. raciborskii* is plausible. However, this does not explain why, in *C.*
819 *raciborskii*, STX level remained high in the cells presented the lowest methionine content (i.e.
820 in BG11 + 257 mM NaCl) while in BG11 + 154 mM NaCl, STX concentration was low with
821 more methionine available. Beside their role in toxin biosynthesis, methionine enters in

proteins composition. Variations in methionine content under salt stress, in toxic as well as non-toxic cyanobacteria, may thus result from protein synthesis alteration and anabolic processes. In stressed cyanobacteria cells, it has been shown that protein synthesis is usually modified leading to reduced synthesis of most normal cell proteins while synthesis of a set of special stress proteins is induced or enhanced (Fulda et al., 1999). Production of free methionine takes place in two possible ways: synthesis via homocysteine and release from protein degradation by proteases (Gröne and Kirst, 1992).

Under salt exposure, it has been shown that cyanobacteria may accumulate compatible solutes to compensate for differences in water potential between their intracellular medium and the surrounding environment (Hagemann, 2011; Klahn and Hagemann, 2011). These compatible solutes are of different chemical nature including carbohydrates, polyols, organosulfur compounds, amino acids and their derivatives (Oren, 2007). In the present study, the presence and potential accumulation of 7 compounds (DMSP, glycine betaine, proline, sucrose, fructose, glucose and D-trehalose) previously described as compatible solutes in microalgae and cyanobacteria was investigated. None of the three cyanobacteria species presented a detectable accumulation of DMSP, glycine betaine, fructose, glucose or D-trehalose in response to NaCl exposure. DMSP has been described as a constitutive compatible solute (Stefels, 2000). It has been found to increase with salinity in several microalgae species and has been detected in several cyanobacteria genera including *Anabaena* species and *Aphanizomenon flos-aquae* (Oren, 2007; Steinke et al., 2018). However, the measured concentrations in cyanobacteria were low and it is still unclear to what extent cyanobacteria rely on DMSP for osmotic balance (Oren, 2007). Considering the environment from which the three cyanobacteria species were isolated (fresh- and brackish waters), it is not surprising that they did not accumulate glycine betaine. Indeed, among cyanobacteria, a correlation has been found between their final salt tolerance and major compatible solute they

accumulate. Glycine betaine is usually synthesized by halophytic strains able to grow in saturated salt concentrations (Hagemann, 2011 and references therein). Moderately halotolerant (generally marine) strains accumulate glucosylglycerol as major compatible solute and sometimes glucosylglycerate as secondary solute. Freshwater strains with low halotolerance mainly synthesize sucrose and/or trehalose. Among the three species investigated, only *Dolichospermum* sp. isolated from brackish waters was found to accumulate sucrose in response to the two highest salinity levels tested. This agrees with the list of compatible solutes found in *Dolichospermum* (*Anabaena*) species (supplementary table in Hagemann, 2011). This is also in line with the sucrose accumulation observed in a particularly salt-tolerant *M. aeruginosa* strain, isolated from brackish waters (Georges des Aulnois et al., 2019). The protective role of sucrose during salt stress was further confirmed in the same *M. aeruginosa* strain by the over-expression of genes involved in its synthesis in response to salinity increase (Georges des Aulnois et al., 2020).

The presence of proline was found in all three cyanobacteria. In *Dolichospermum* sp., proline did not seem to be used as a compatible solute because NaCl exposure did not significantly modify its intracellular concentration. *A. gracile* and *C. raciborskii* showed different patterns in proline variations. In *A. gracile*, proline accumulated proportionally to NaCl concentration at two different times: 24h and 72h. In *C. raciborskii*, after a first phase during which proline content was inversely proportional to NaCl concentration, proline accumulated in a dose-dependent manner after 48h of NaCl exposure. However, with the present results, it is difficult to confirm with certainty the role of proline as a compatible solute in *A. gracile* and *C. raciborskii* because unlike most other compatible solutes, proline is used in primary metabolism (Hagemann, 2011). In addition, in plants, proline has been recognized as a multifunctional amino acid (Szabados and Savouré, 2010). It has been proposed to act as a compatible solute, to be a ROS scavenger, to function as a molecular

chaperone stabilizing the structure of proteins and to act as a signaling molecule to modulate mitochondrial functions (Ben Rejeb et al., 2014; Szabados and Savouré, 2010; Verbruggen and Hermans, 2008). In *A. gracile* and *C. raciborskii*, ROS decreased when proline accumulated in cells respectively after 24 and 48h exposure to NaCl. However, it remains difficult to interpret this as a cause and effect relationships confirming that proline acted as a ROS scavenger. In *A. gracile* and *C. raciborskii*, proline trends were similar to variations in methionine content. This suggests that proline content may also have been influenced by protein synthesis alteration and anabolic processes. There are extensive reports on salt stress induced proline accumulation in higher plants (see Mansour and Ali, 2017 for a review). By contrast, in cyanobacteria, not much work has been done to understand the role of proline in salinity stress tolerance. The action of proline as a potential compatible solute has been shown in *Nostoc muscorum* (Singh et al., 1996). A mutant strain, over accumulating proline due to their deficiency in proline oxidase activity, has been found to be protected against the lethal effects of 150 mol m^{-3} NaCl. The culture medium supplementation with proline, and its subsequent uptake, offered a similar protection to the parent strain. Intracellular proline content has also been shown to naturally accumulate in response to NaCl exposure in an halophilic *Synechococcus* sp. strain (Fulda et al., 1999), cyanobacteria isolated from rice fields (*Cylindrospermum* sp. (Chris et al., 2006) and *Anabaena variabilis* (Syiem and Nongrum, 2011)) and two strains of *M. aeruginosa* (one isolated from a freshwater lake and the other from a brackish water reservoir) (Georges des Aulnois et al., 2019). In *Synechococcus* sp., proline accumulation preceded the synthesis of glycine betaine (Fulda et al., 1999).

5 Conclusions

The brackish (*Dolichospermum* sp.) and freshwater (*A. gracile*, *C. raciborskii*) species presented different tolerance to salinity stress. In estuaries, *A. gracile* and *C. raciborskii* would not be able to survive beyond the oligohaline area while *Dolichospermum* sp. seems to be able to colonize a larger zone and to potentially survive up to the polyhaline area. In all species, NaCl exposure resulted in reduced growth rates. By contrast, the responses of photosynthetic activity, pigment composition, ROS production as well as the capacity to accumulate compatible solutes were species-specific. *A. gracile* and *C. raciborskii* responded differently to salinity stress even though they were isolated from similar systems (freshwaters). This illustrates the diversity of mechanisms operated by cyanobacteria to face salinity variations. Low sodium chloride concentrations did not impact STXs production in *A. gracile* and *C. raciborskii* while, higher concentrations modified the toxin profile and content of *C. raciborskii* (intracellular STX decreased while dc-GTX2 increased). Since toxicity of PSTs analogs is different, these variations in toxin profile imply that salinity variations have the potential to modify the toxicity of this species. This is an important fact to consider when assessing the risk of animal and human exposure to cyanobacterial PSTs.

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Figures captions

Fig. 1: Growth curves of A) *Aphanizomenon gracile* and B) *Cylindrospermopsis raciborskii* in BG11, BG11 + 34 mM NaCl, BG11 + 154 mM NaCl and BG11 + 257 mM NaCl. C) Growth curves of *Dolichospermum* sp. in BG11 + 154 mM NaCl, BG11 + 257 mM NaCl, BG11 + 308 mM NaCl, and BG11 + 411 mM NaCl. Data are average of triplicate cultures and error bars represent standard deviation

Fig. 2: Carotenoids content (in µg (µg chl a)$^{-1}$) (A, B, C), phycoerythrine content (in relative units) (D, E, F) and phycocyanin content (in relative units) (G, H, I) over time and along a salinity gradient in *Aphanizomenon gracile* (A, D, G, J), *Cylindrospermopsis raciborskii* (B, E, H, K) and *Dolichospermum* sp. (C, F, I, L). Data are average of triplicate cultures and error bars represent standard deviation

Fig. 3: Maximum quantum yield (F_v/F_m) (A, B, C), maximal light utilization efficiency (α in µmol e^- mg chl a^{-1} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$) (D, E, F), maximum electron transport rate (ETR_m in µmol e^- mg chl a^{-1} s^{-1}) (G, H, I) and maximum non-photochemical quenching (NPQ_{max}) (J, K, L) over time and along a salinity gradient in *Aphanizomenon gracile* (A, D, G), *Cylindrospermopsis raciborskii* (B, E, H) and *Dolichospermum* sp. (C, F, I). Data are average of triplicate cultures and error bars represent standard deviation

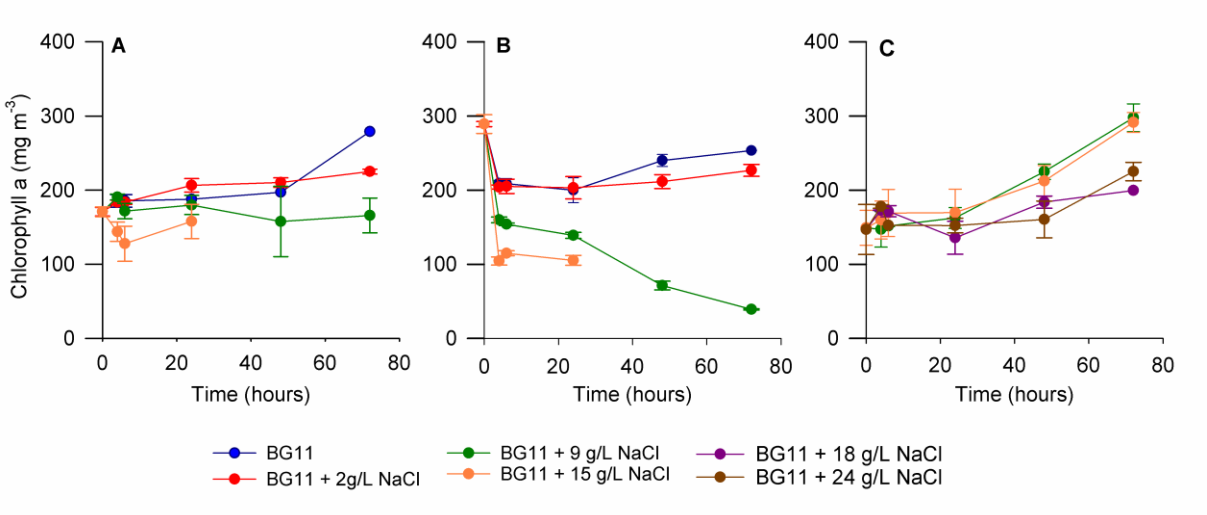
Fig. 4: ROS production measured as 2',7'-dichlorofluorescein (DCF) fluorescence level (in relative units) over time and along a salinity gradient in *Aphanizomenon gracile* (A), *Cylindrospermopsis raciborskii* (B) and *Dolichospermum* sp. (C). Results are represented as relative changes from the respective control conditions. Data are average of triplicate cultures and error bars represent standard deviation

Fig. 5: Intracellular quota of paralytic shellfish toxins (PSTs) (A, B), neo-saxitoxin (C, D), saxitoxin (E, F) and decarbamoylogonyautoxin 2 (G) over time and along a salinity gradient in *Aphanizomenon gracile* (A, C, E) and *Cylindrospermopsis raciborskii* (B, D, F, G). Data are average of triplicate cultures and error bars represent standard deviation. Please note the different y-axis limits

Fig. 6: Intracellular proportions of paralytic shellfish toxins analogs in *Aphanizomenon gracile* (A, B, C, D) and *Cylindrospermopsis raciborskii* (E, F, G, H) exposed to BG11 (A, E), BG11 + 34 mM NaCl (B, F), BG11 + 154 mM NaCl (C, G) and BG11 + 257 mM NaCl (D, H)

Fig. 7: Intracellular quota of methionine (A, B, C) and proline (D, E, F) over time and along a salinity gradient in *Aphanizomenon gracile* (A, D) and *Cylindrospermopsis raciborskii* (B, E) and *Dolichospermum* sp. (C, F). Data are average of triplicate cultures and error bars represent standard deviation

Fig. 8: Intracellular sucrose accumulation in *Dolichospermum* sp. after a salt shock



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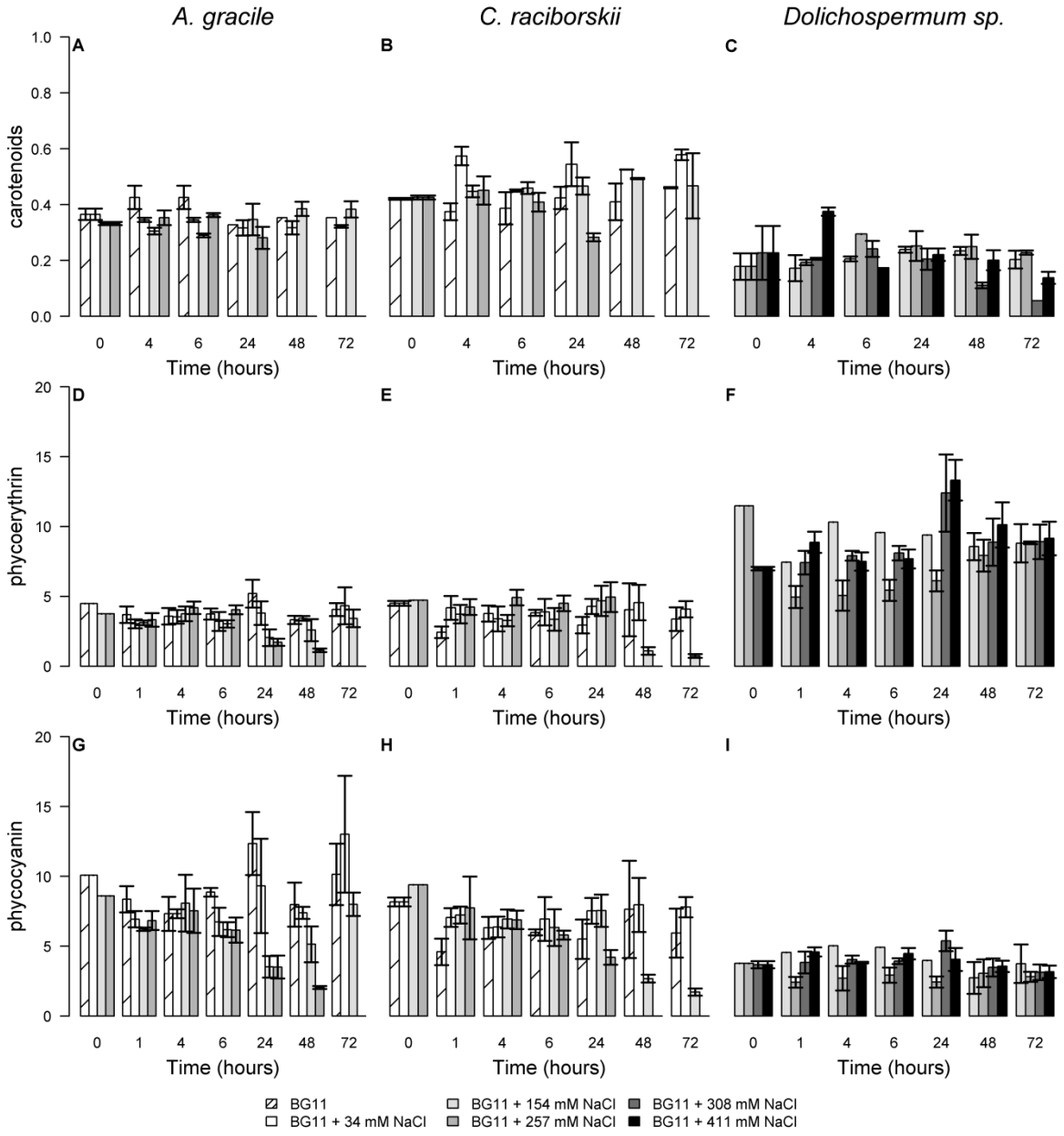

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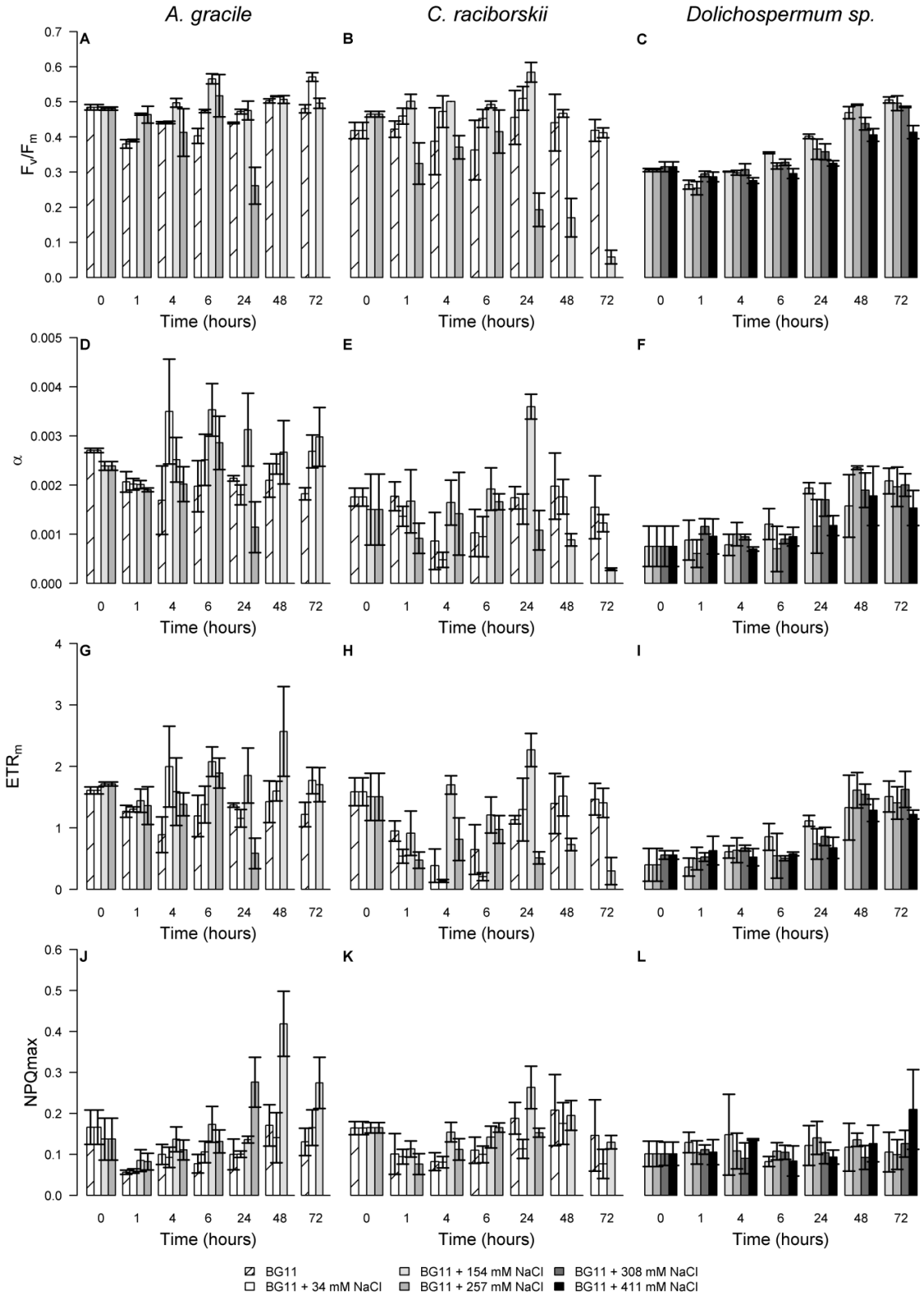

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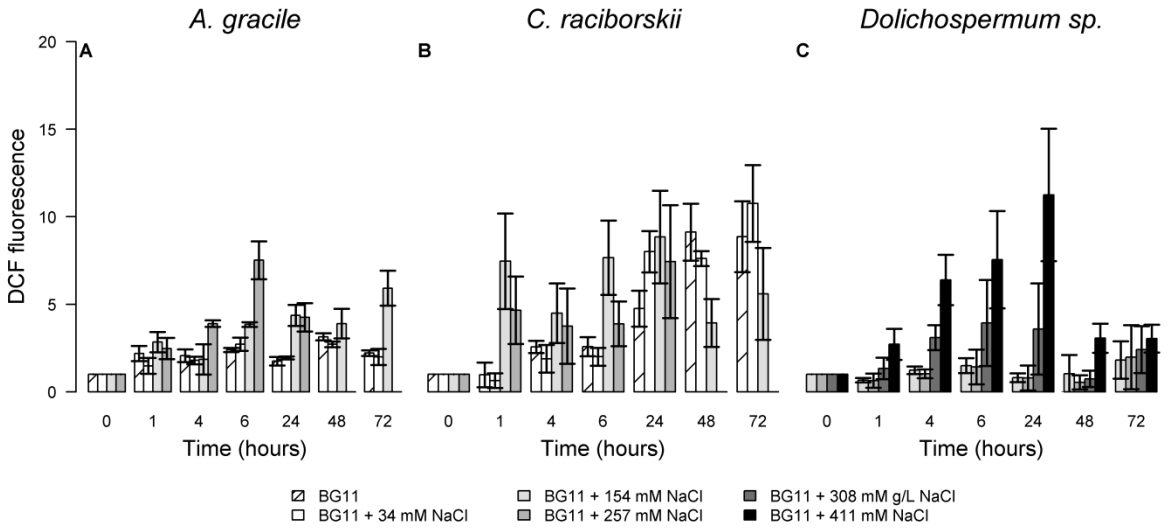

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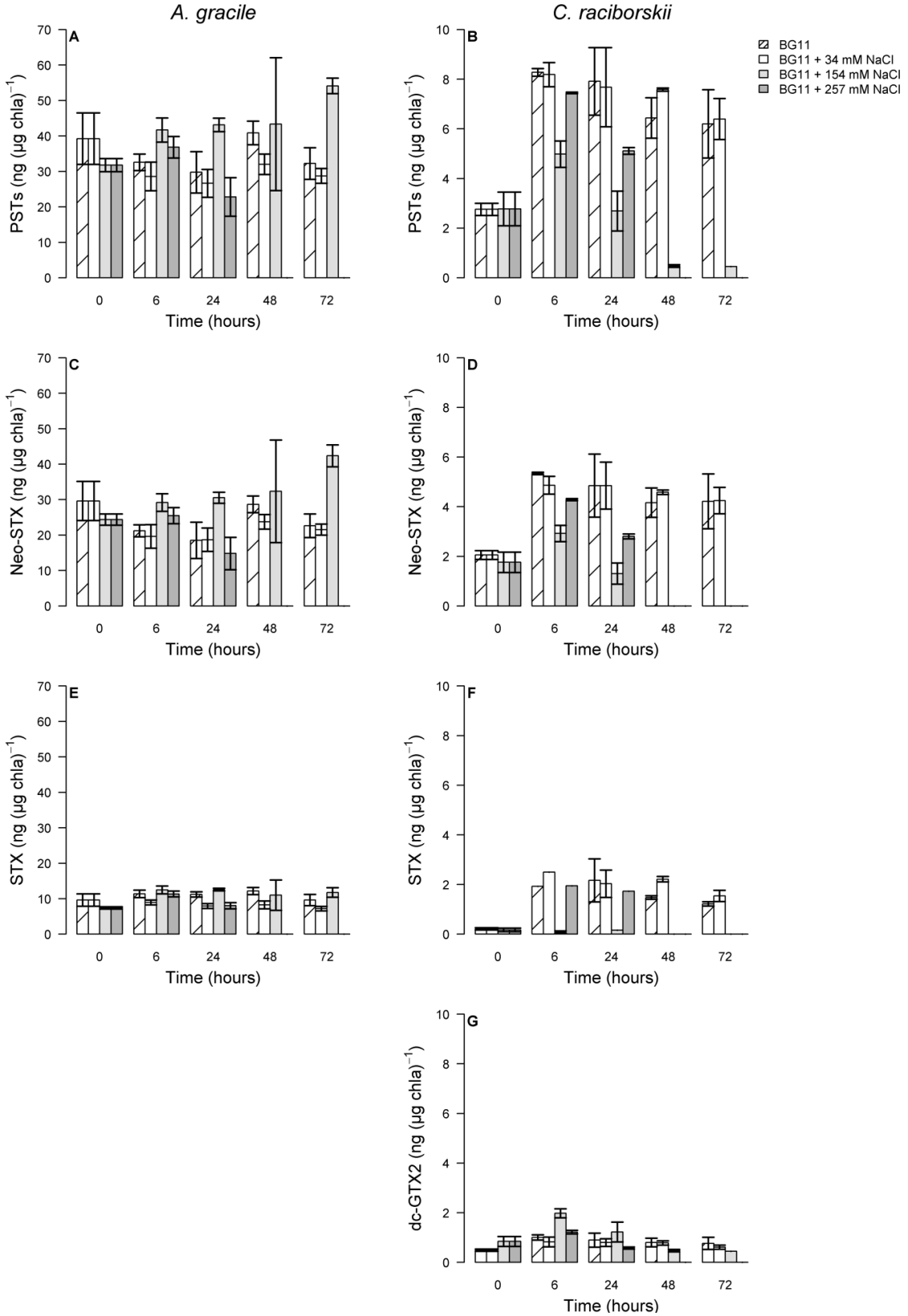

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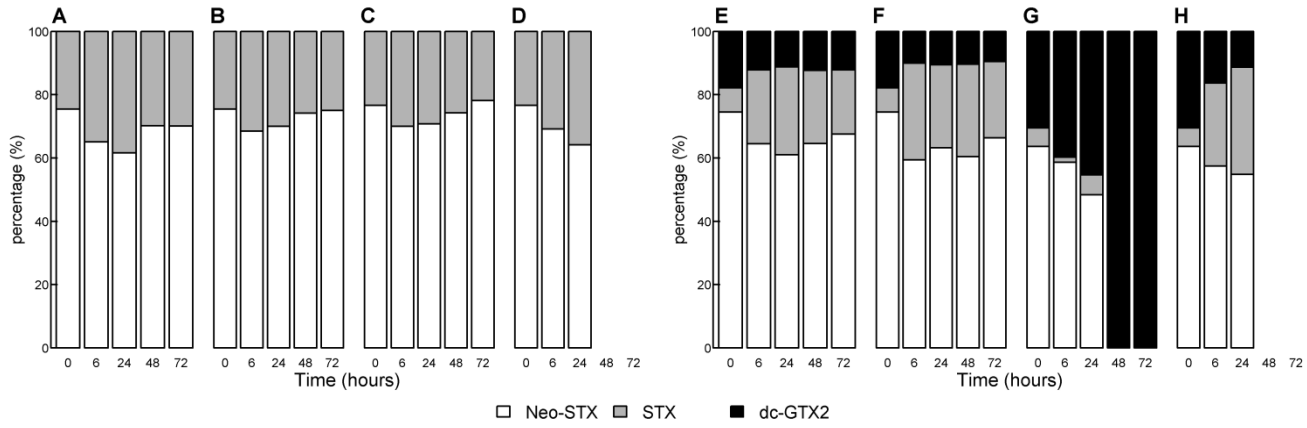

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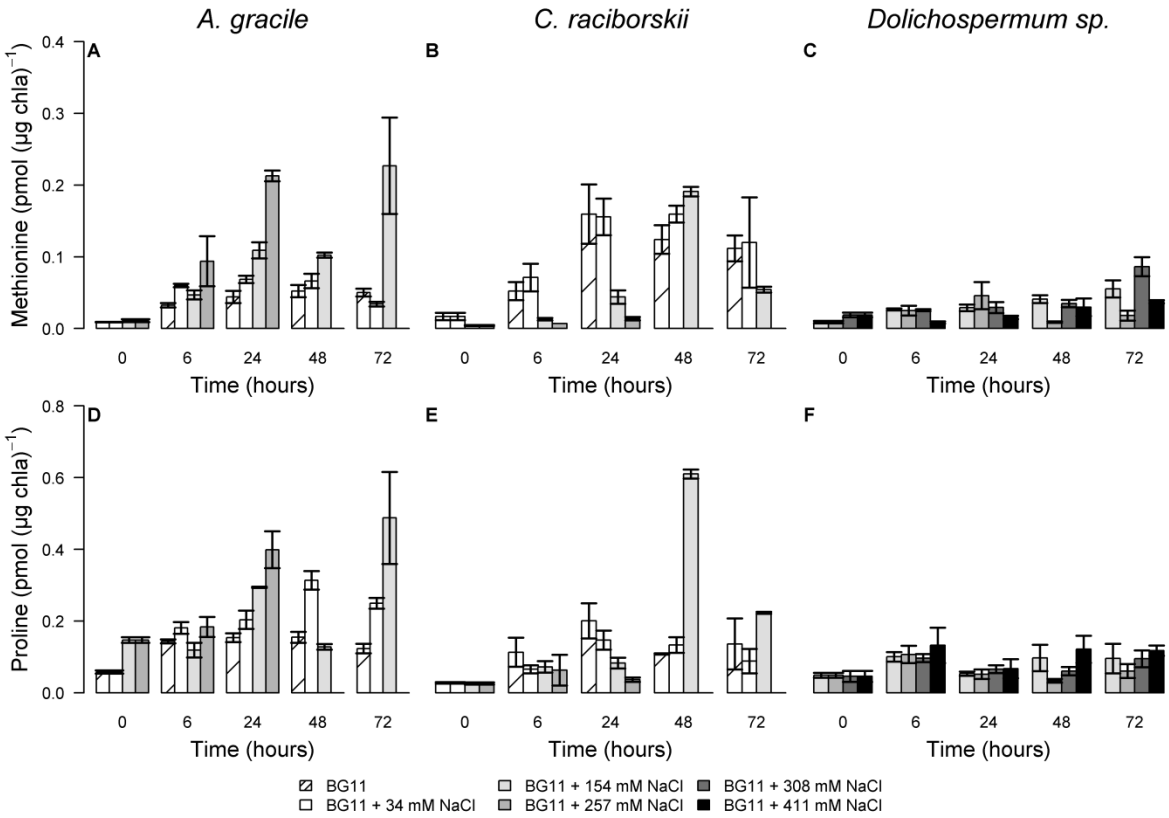

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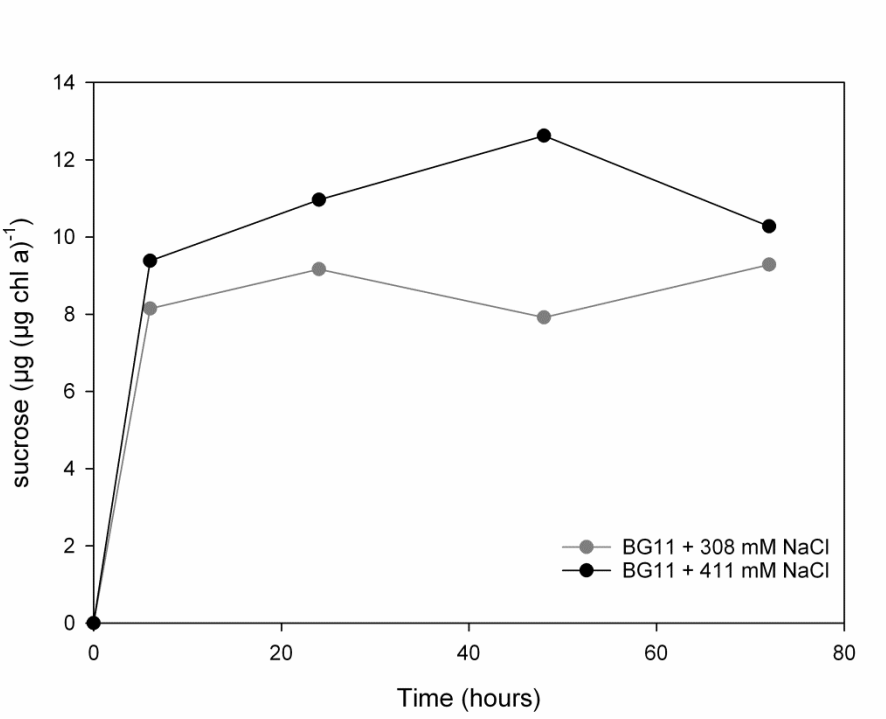


Fig. 8: Intracellular sucrose accumulation in *Dolichospermum* sp. after a salt shock

Supplementary materials

Physiological changes induced by sodium chloride stress in *Aphanizomenon gracile, Cylindrospermopsis raciborskii* and *Dolichospermum* sp.

Emilie Houliez, Enora Briand, Florent Malo, Georges-Augustin Rovillon, Fabienne Hervé, Elise Robert, Laetitia Marchand, Agata Zykwinska, Amandine Caruana

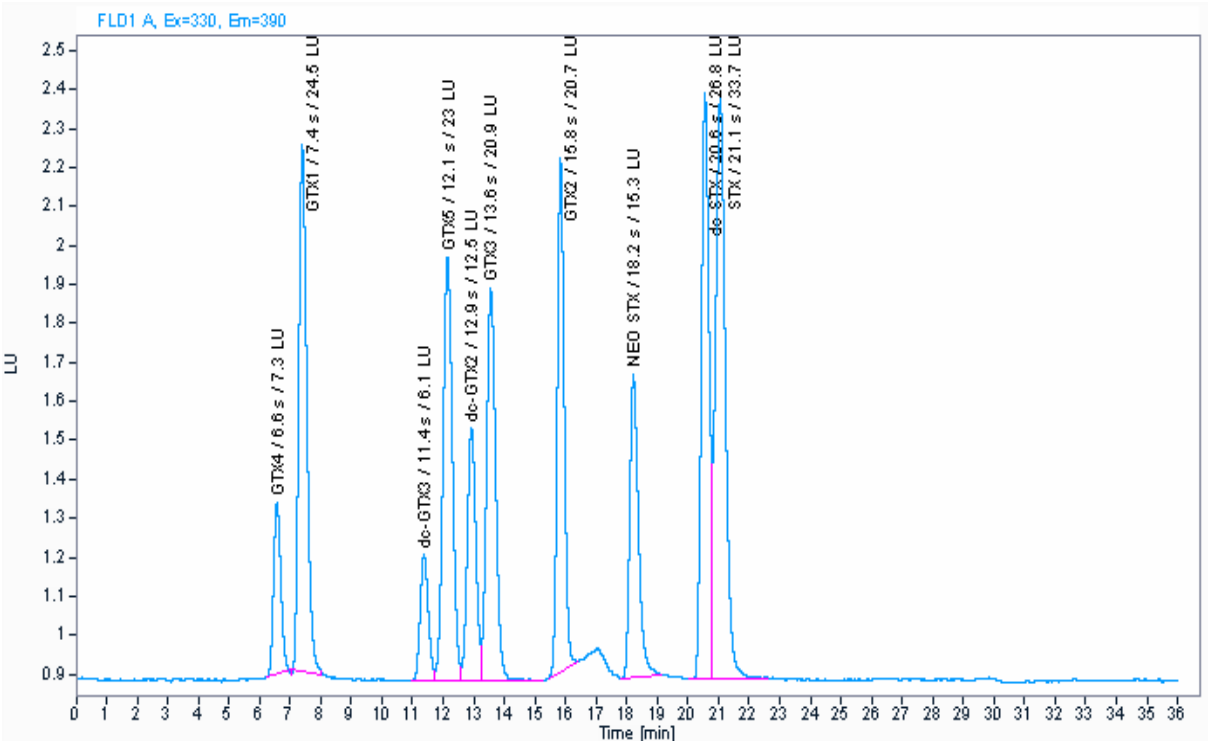


Fig. S1: High performance liquid chromatogram showing the retention time of paralytic shellfish toxins (PSTs) standards: gonyautoxin (GTX4), gonyautoxin (GTX1), decarbamoylogonyautoxin (dc-GTX3), gonyautoxin 5 (GTX5), decarbamoylogonyautoxin 2 (dc-GTX2), gonyautoxin 3 (GTX3), gonyautoxin 2 (GTX2), neosaxitoxin (NEO STX), decarbamoylsaxitoxin (dc-STX) and saxitoxin (STX)

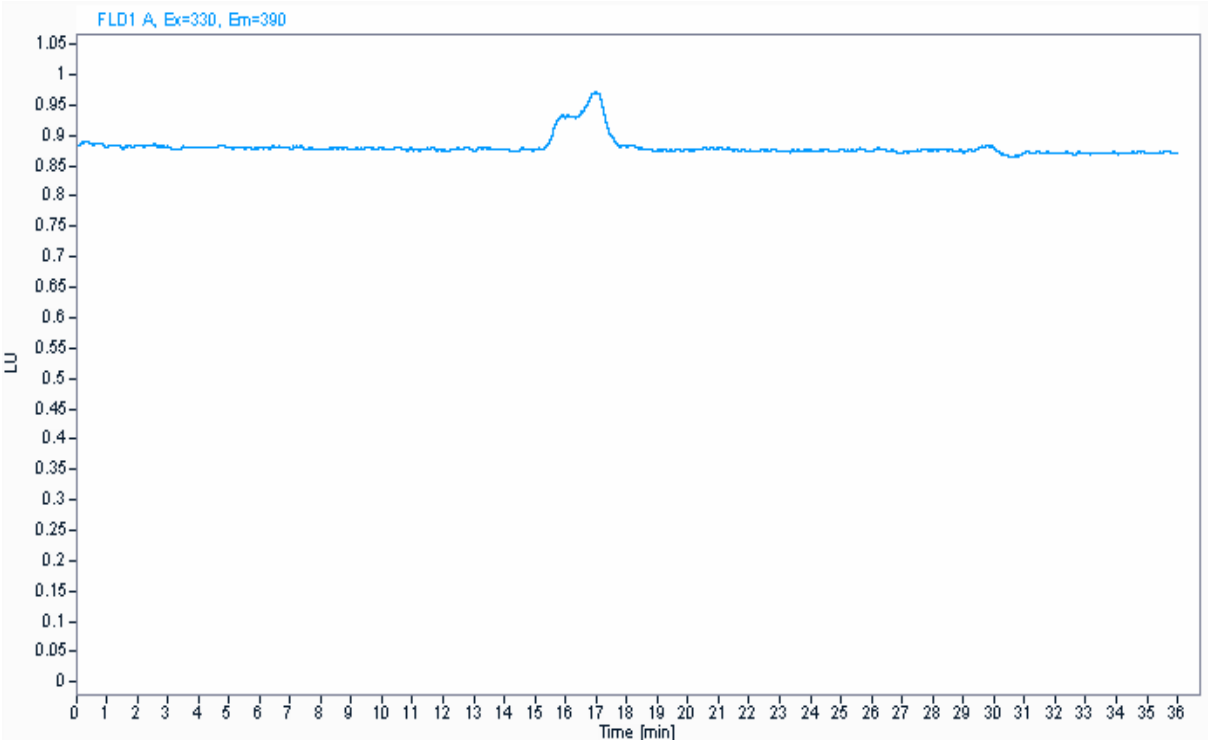


Fig. S2: High performance liquid chromatogram of a blank

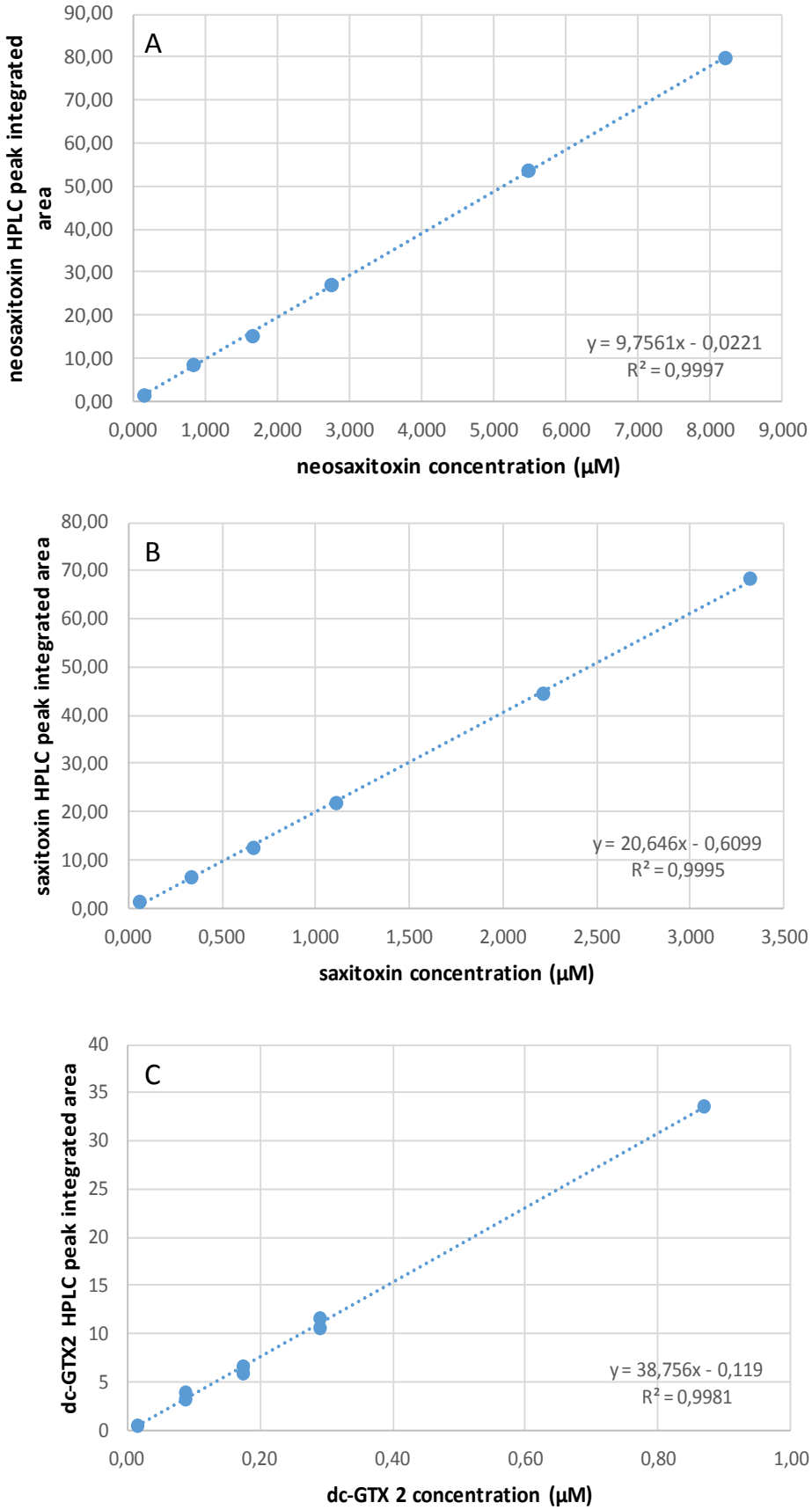


Fig. S3: A) Neosaxitoxin calibration curve. B) Saxitoxin calibration curve. C) Decarbamoylogonyautoxin 2 (dc-GTX2) calibration curve

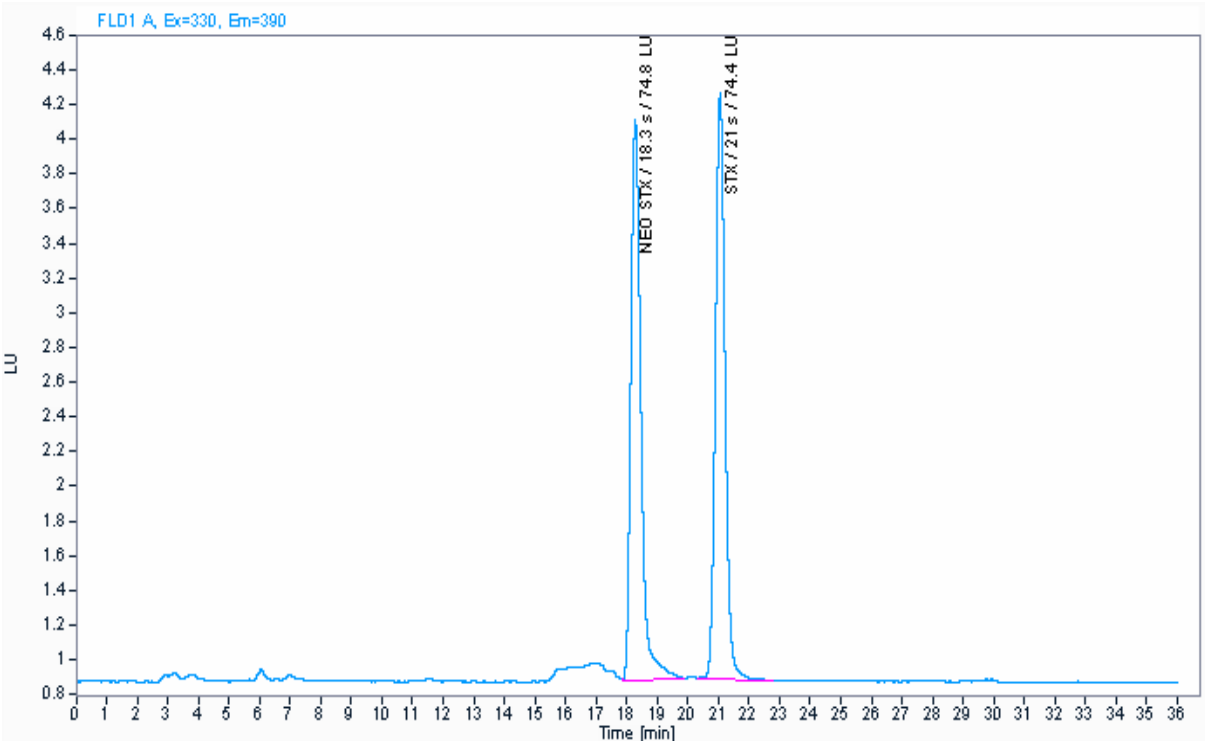


Fig. S4: High performance liquid chromatogram of a sample of *Aphanizomenon gracile* (PMC627.10) culture

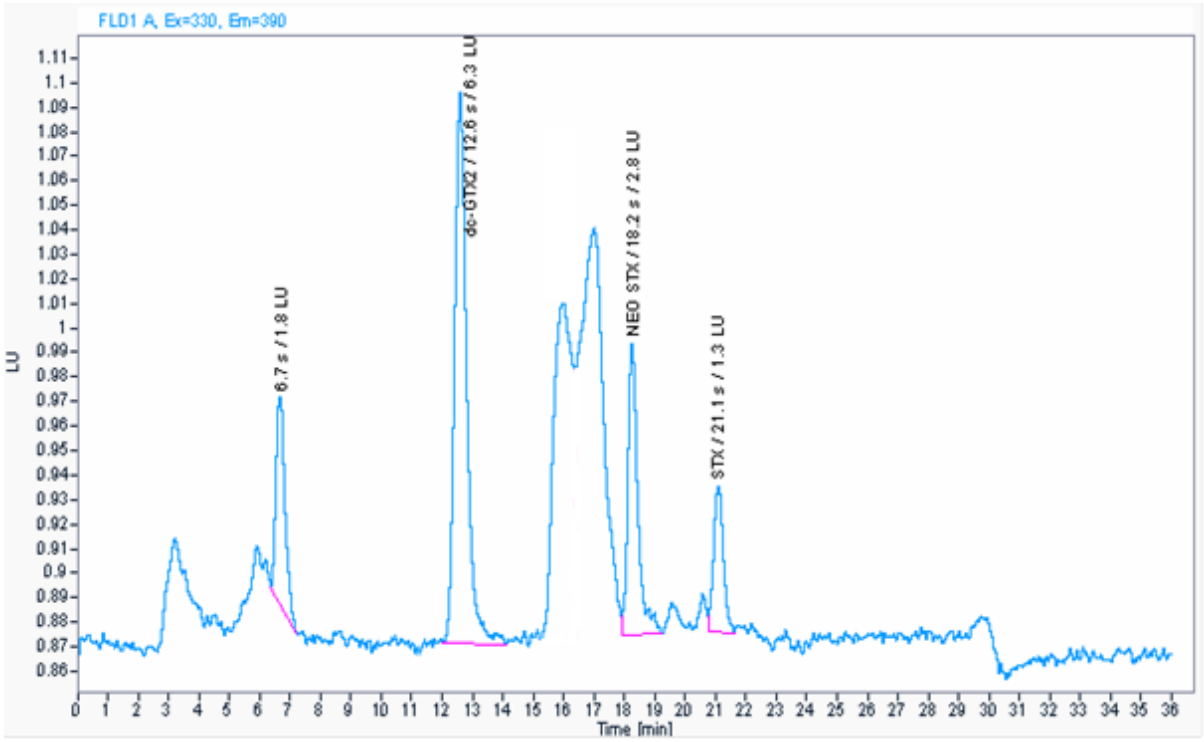


Fig. S5: High performance liquid chromatogram of a sample of *Cylindrospermopsis raciborskii* (PMC00.01) culture

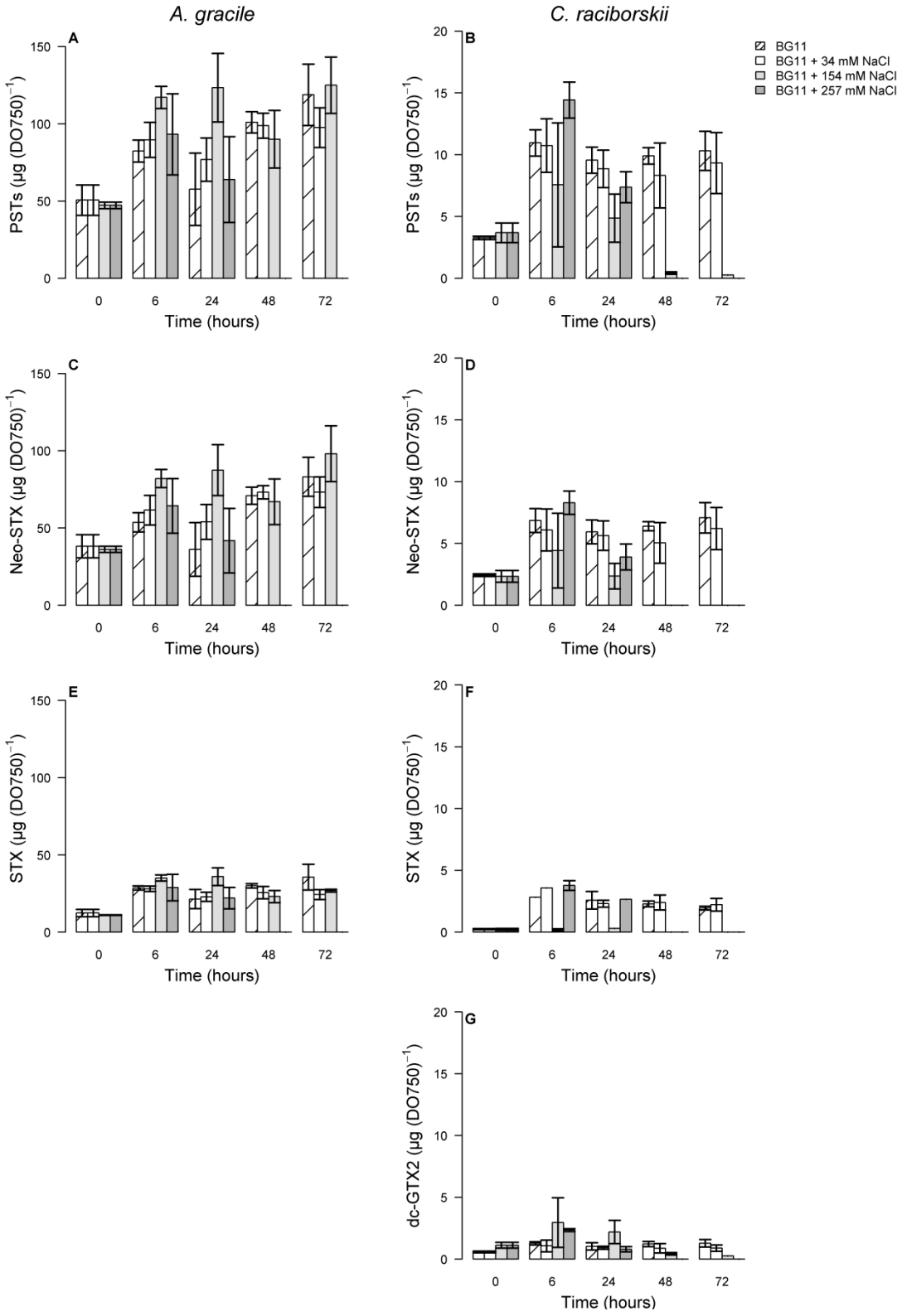
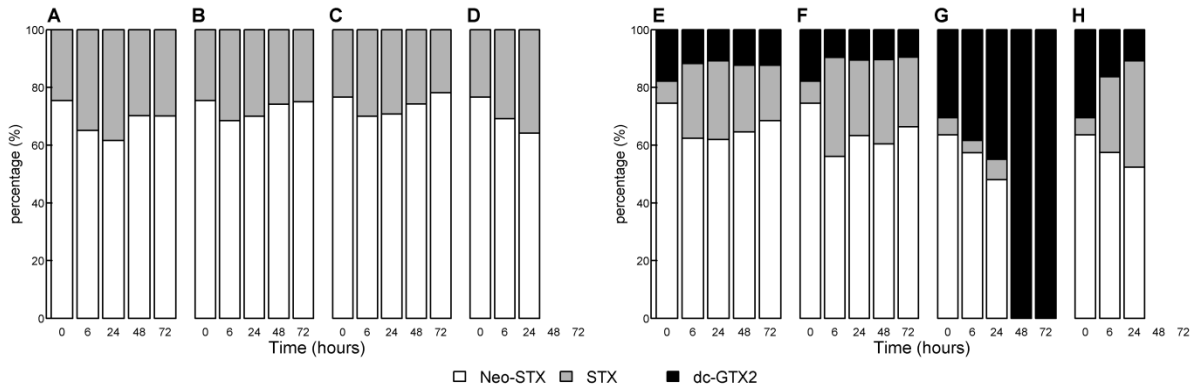


Fig. S6: Intracellular quota of paralytic shellfish toxins (PSTs) (A, B), neo-saxitoxin (C, D), saxitoxin (E, F) and decarbamoylogonyautoxin 2 (G) over time and along a salinity gradient in *Aphanizomenon gracile* (A, C, E) and *Cylindrospermopsis raciborskii* (B, D, F, G). Data are average of triplicate cultures and error bars represent standard deviation. All toxins values were normalized by OD_{750}. Please note the different y-axis limits



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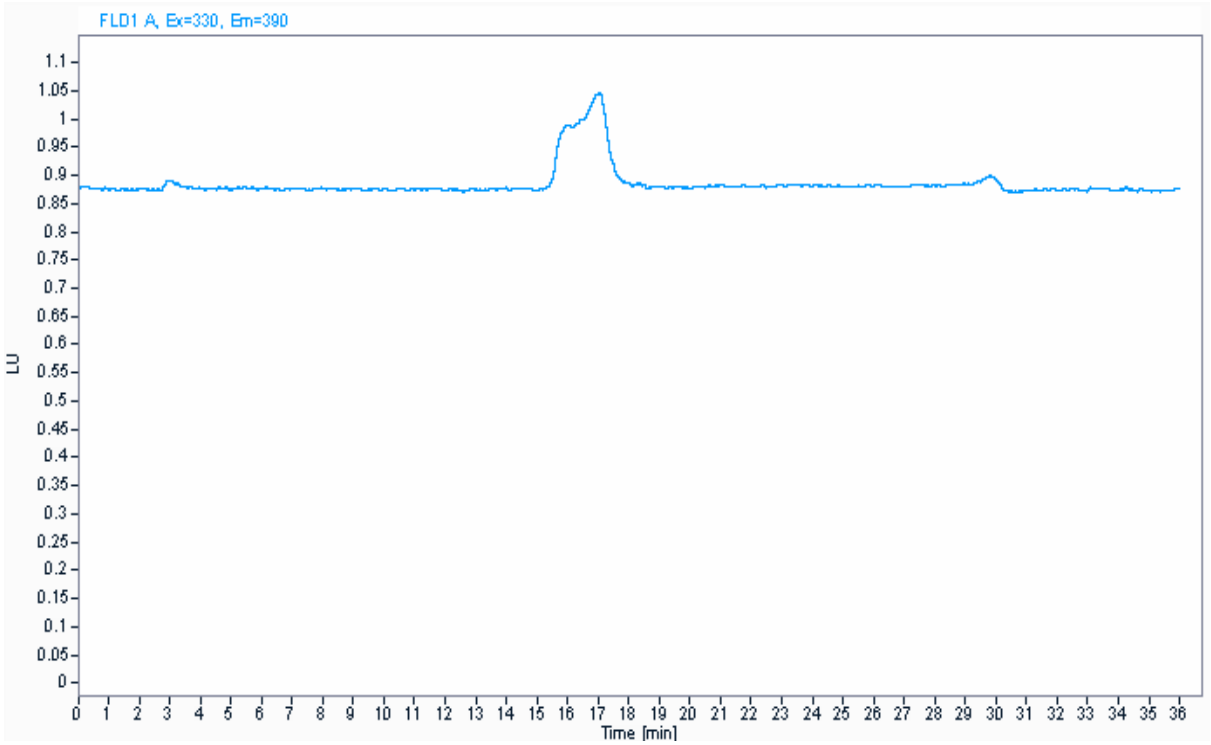

Fig. S8: High performance liquid chromatogram of a sample of *Dolichospermum* sp. (CCY9401) culture

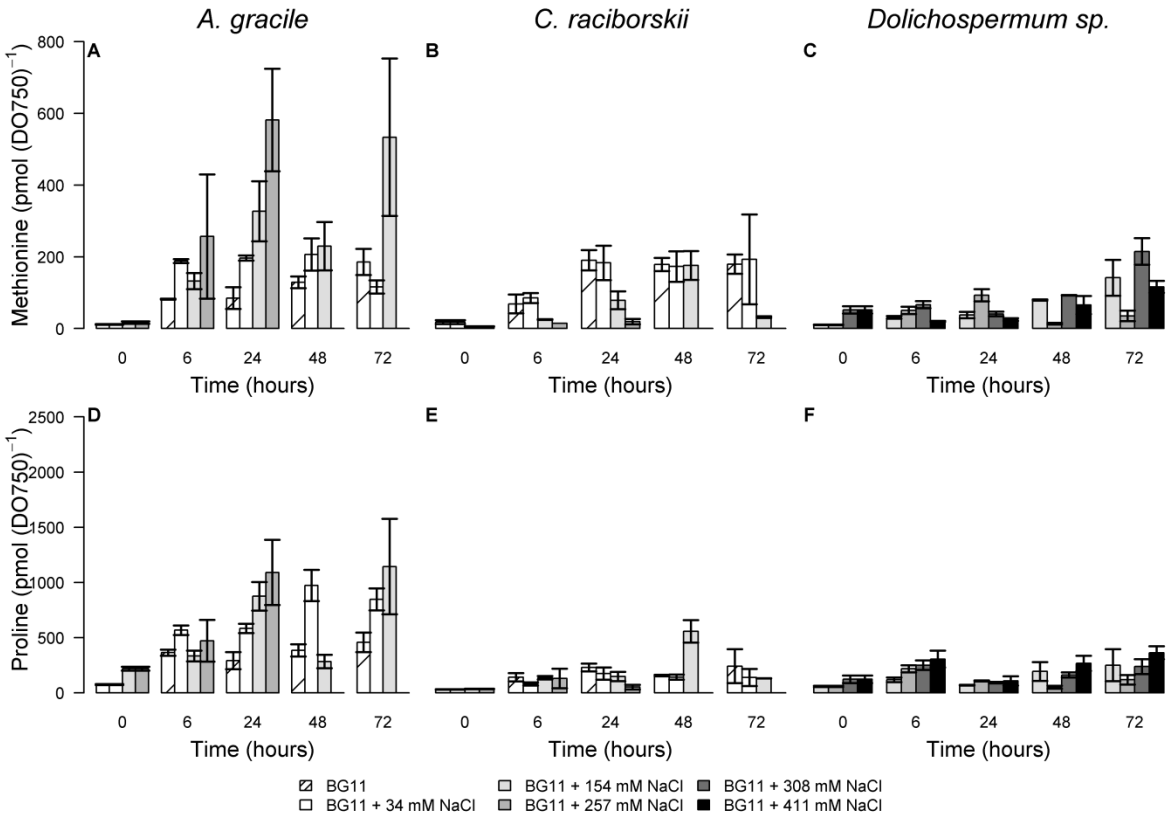

Fig. S9: Intracellular quota of methionine (A, B, C) and proline (D, E, F) over time and along a salinity gradient in *Aphanizomenon gracile* (A, D) and *Cylindrospermopsis raciborskii* (B, E) and *Dolichospermum* sp. (C, F). Data are average of triplicate cultures and error bars represent standard deviation. All concentrations were normalized by OD_{750}

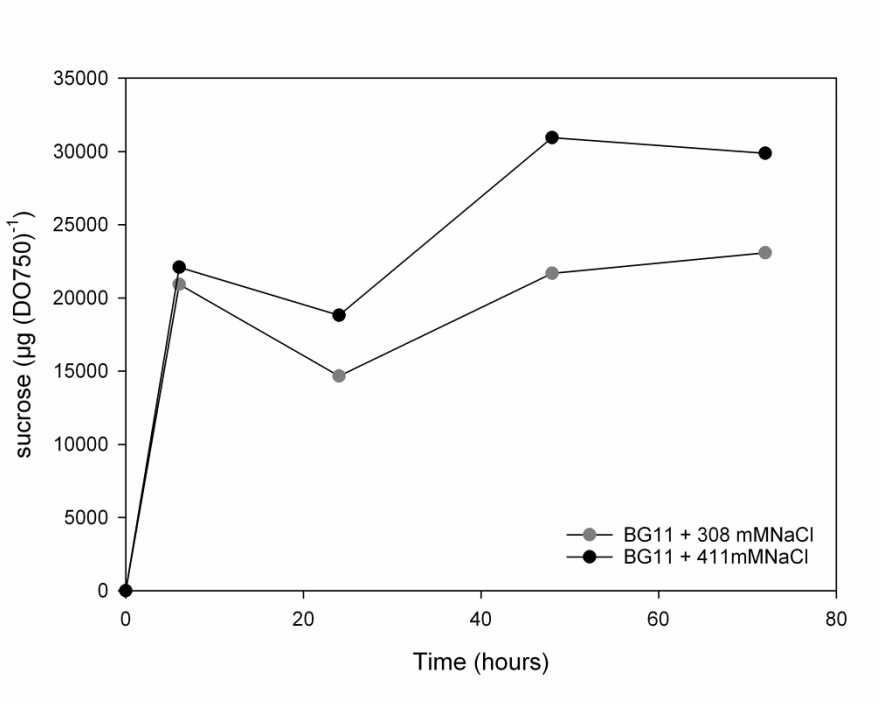

Fig. S10: Intracellular sucrose accumulation in *Dolichospermum* sp. after a salt shock when concentrations were normalized by OD_{750}

Figure1

Click here to access/download;Figure;figure1.TIF

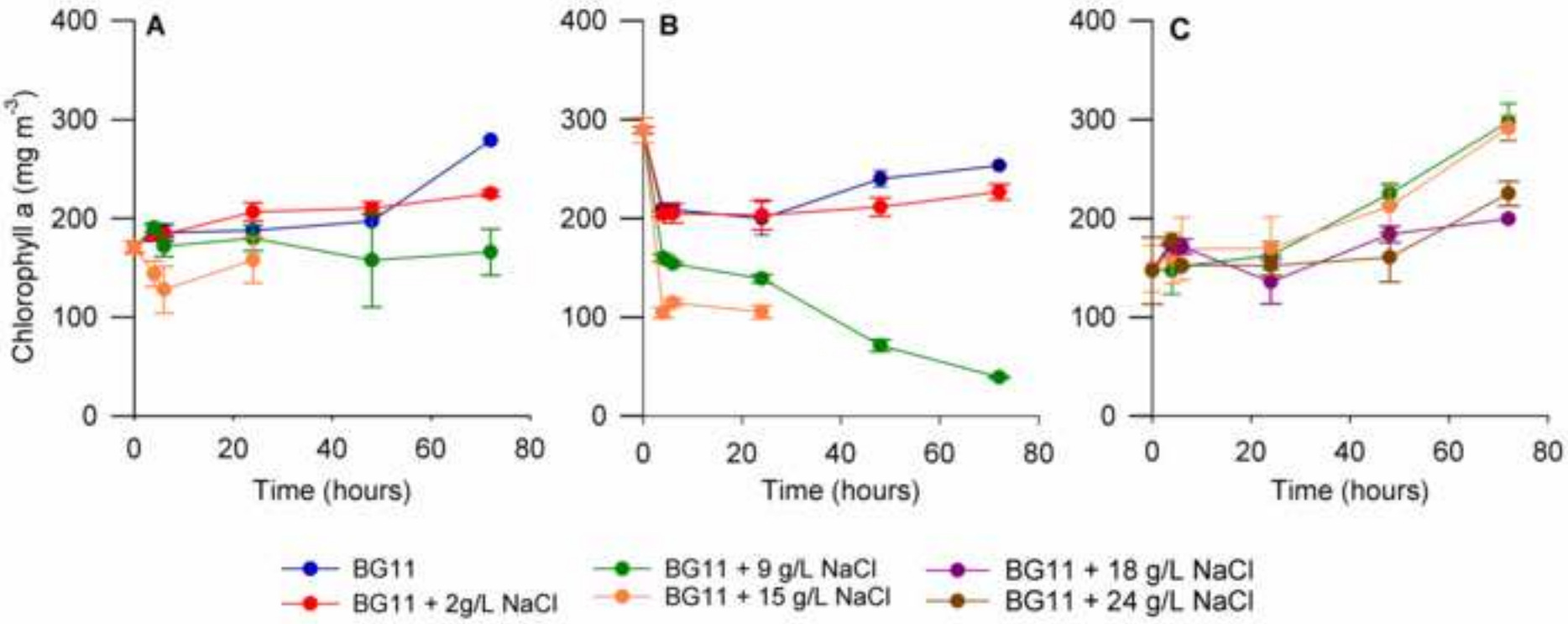

Figure2

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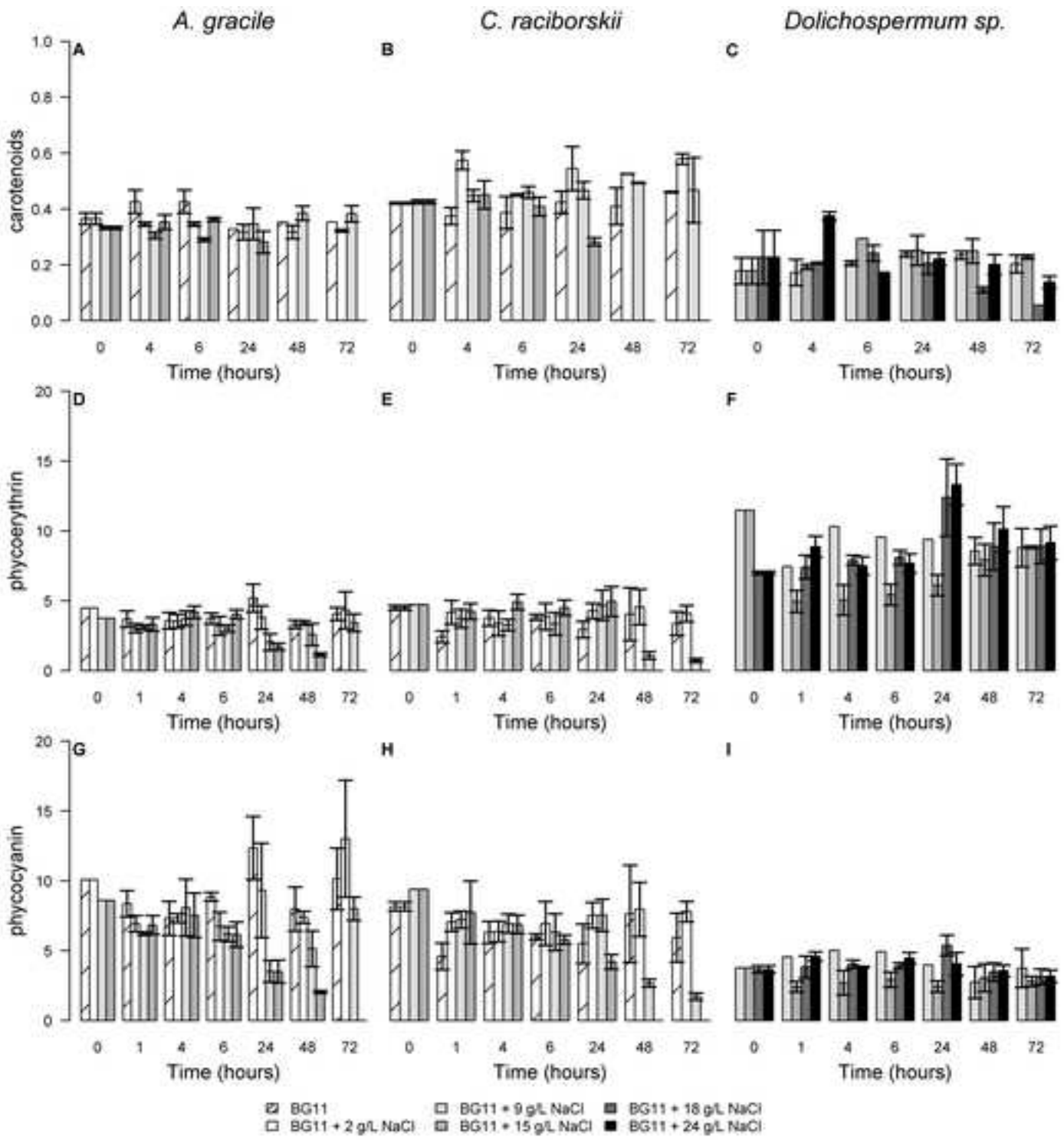

Figure3

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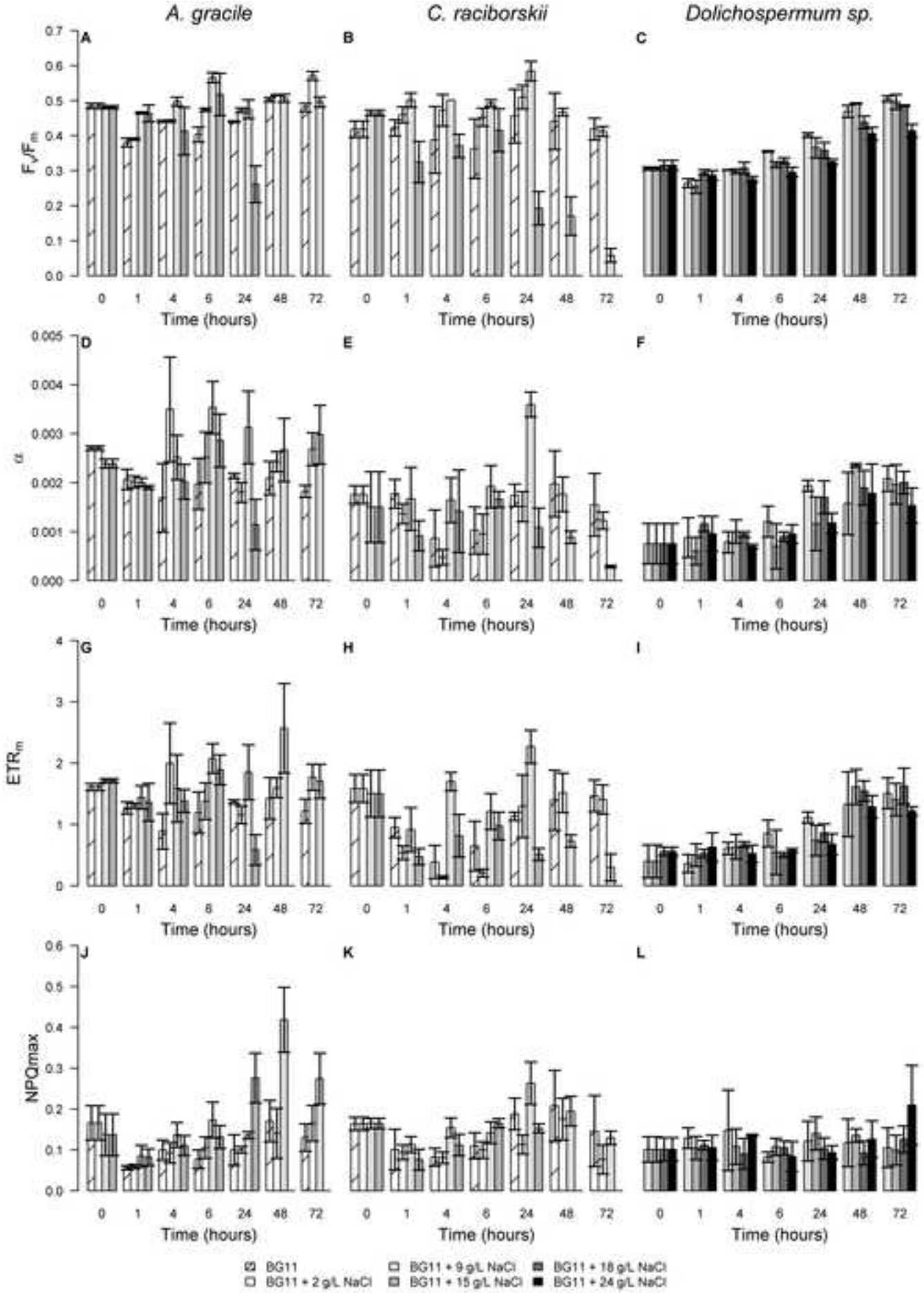

Figure4

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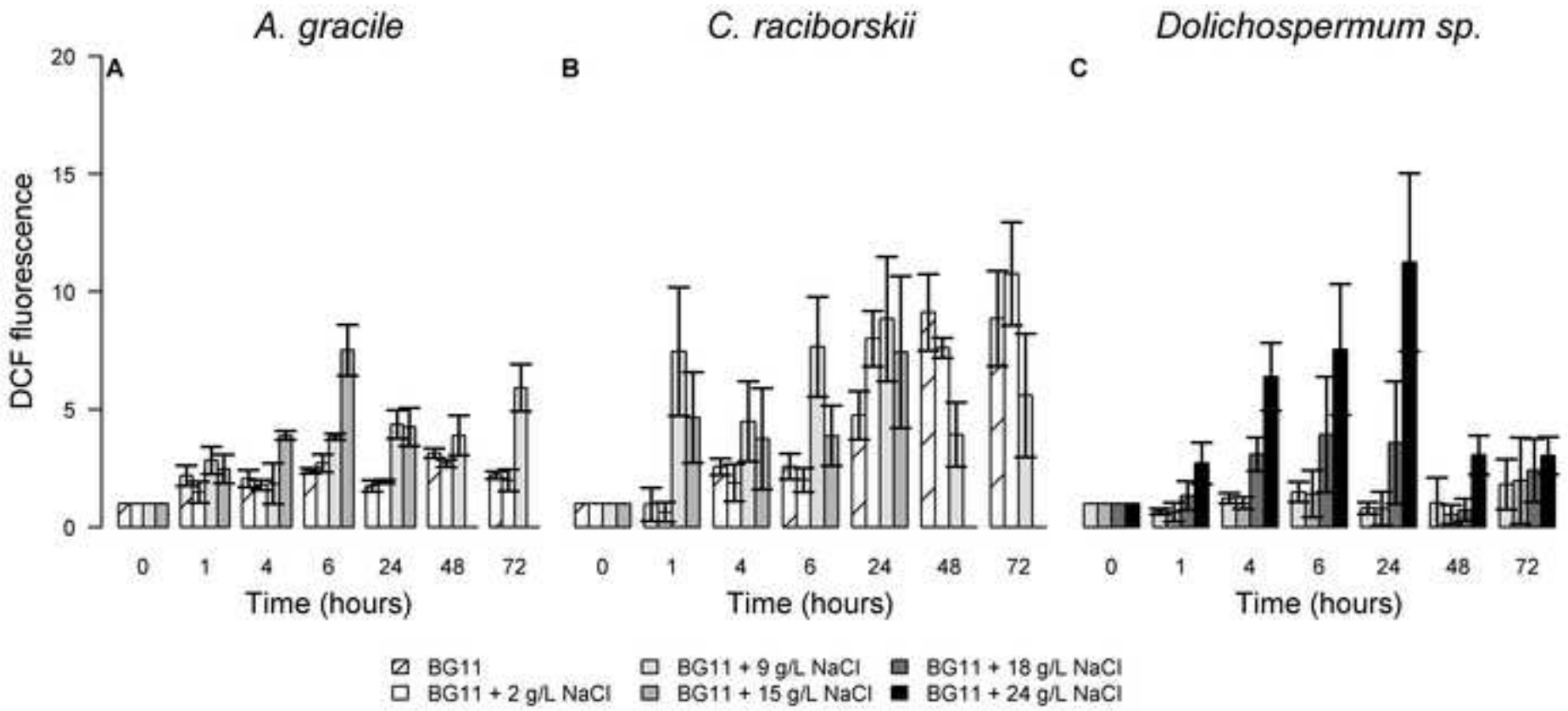

Figure5

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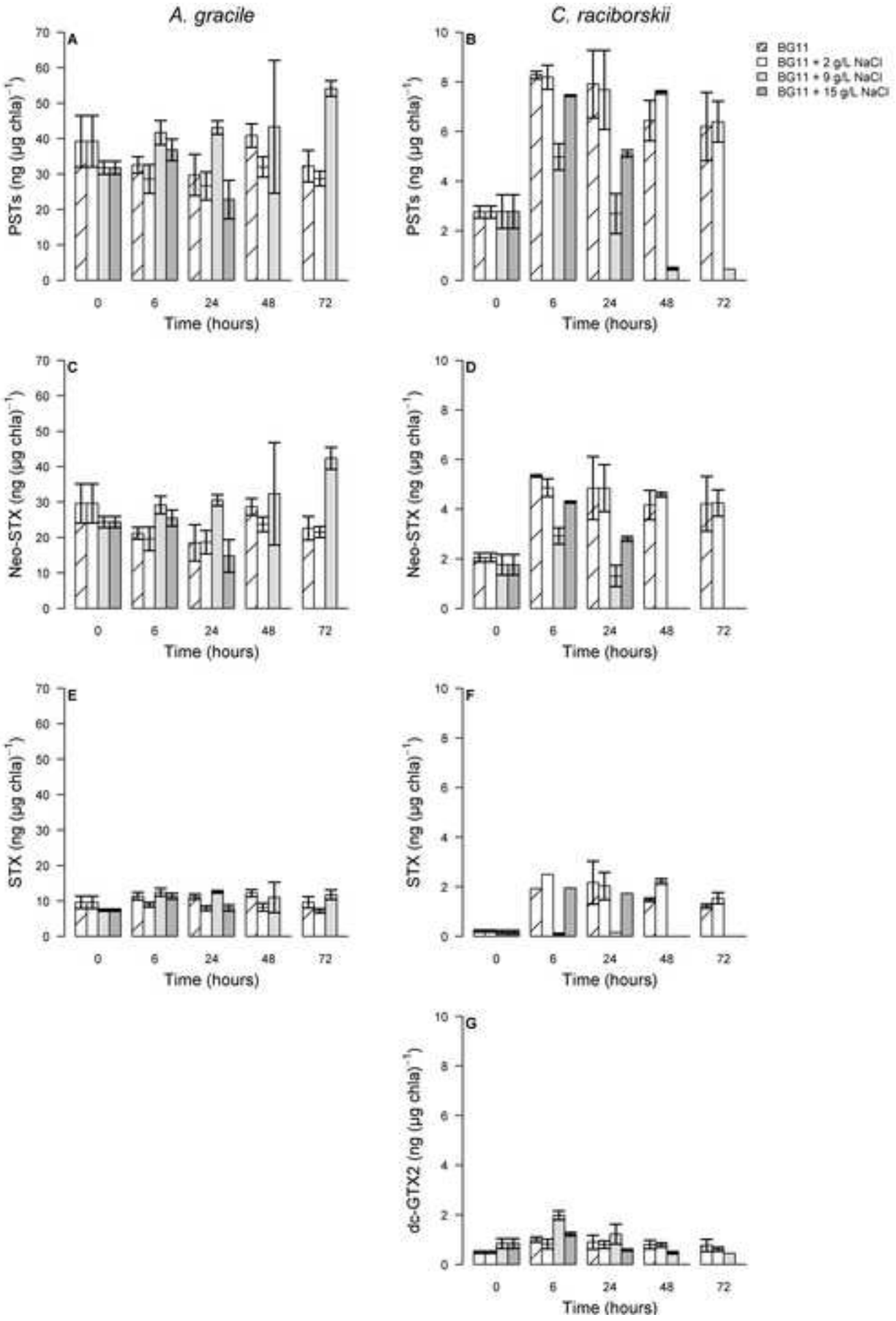

Figure6

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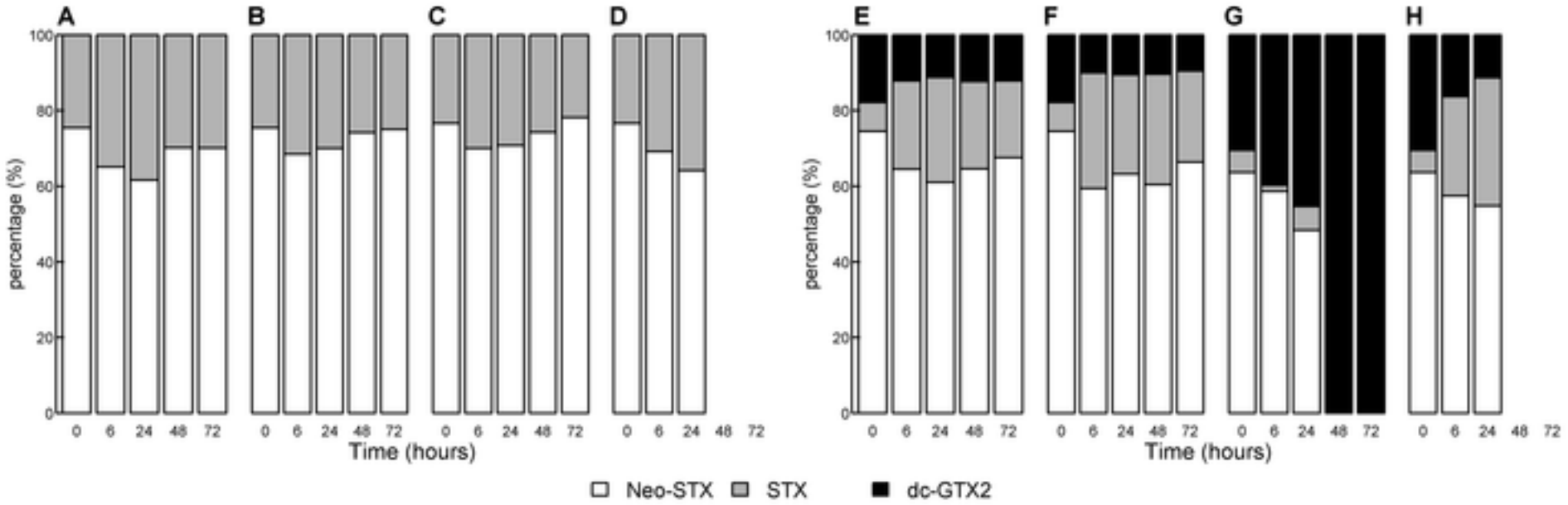

Figure7

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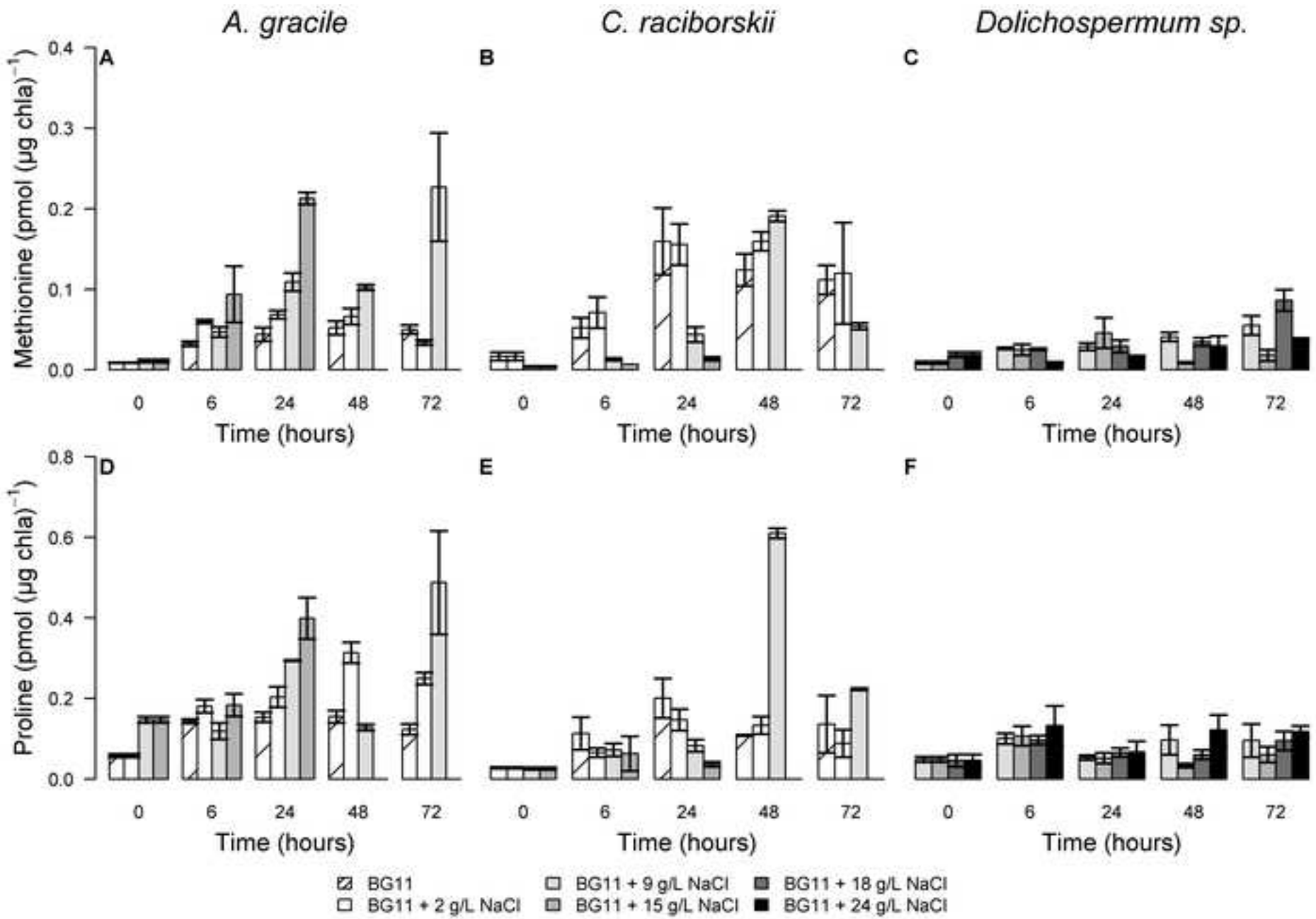
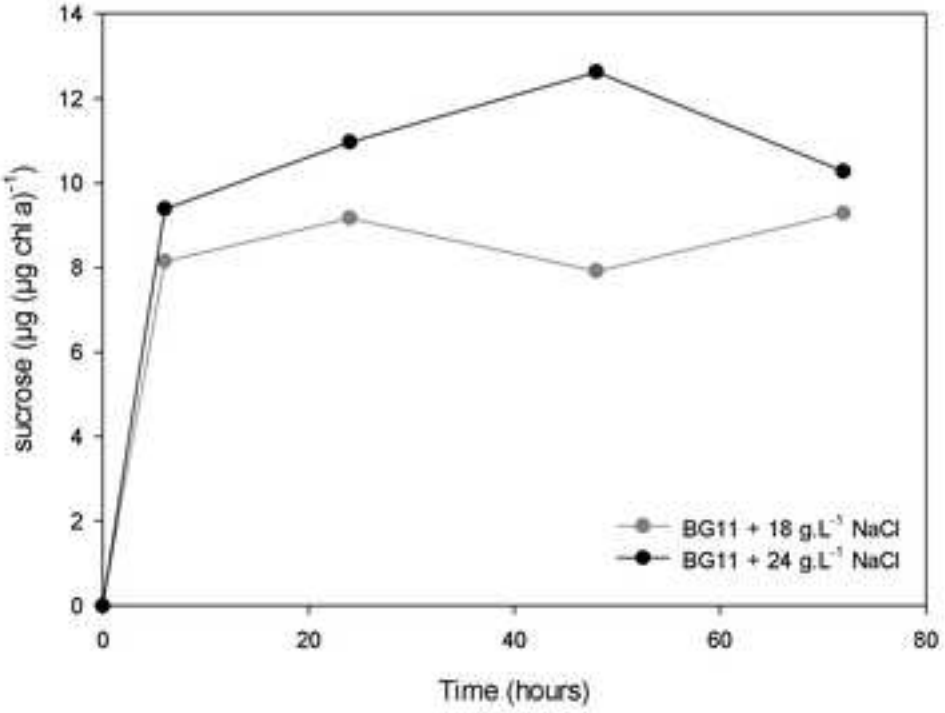


Figure8

Click here to access/download;Figure;figure8.TIF



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