Title

- The role of floridoside in osmoadaptation of coral algal endosymbionts to hypersaline stress
- Osmoadaptation of coral algal endosymbionts

Authors

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Summary sentence

In response to salinity stress, algal endosymbionts (Symbiodinium sp.) in corals produce floridoside, an osmolyte with antioxidant potential.

Abstract

The endosymbiosis between Symbiodinium dinoflagellates and stony corals provides the foundation of coral reef ecosystems. The survival of these ecosystems is under threat at a global scale and better knowledge is needed to conceive strategies for mitigating future reef loss. Environmental disturbance imposing temperature, salinity, and nutrient stress can lead to the loss of the Symbiodinium partner, so-called coral bleaching. Some of the most thermotolerant coral-Symbiodinium associations occur in the Persian/Arabian Gulf and the Red Sea, which represent also the most saline coral habitats. We studied whether Symbiodinium alter their metabolite content in response to high salinity environments. We found that Symbiodinium cells exposed to high salinity produced high levels of the osmolyte 2-O-glycerol-α-D-galactopyranoside (floridoside), both in vitro and in their host animals, thereby increasing their capacity and putatively the capacity of the holobiont to cope with the effects of osmotic stress in extreme environments. Given that floridoside has been previously shown to also act as an antioxidant, this osmolyte may serve a dual function. Firstly, as a compatible organic osmolyte accumulated by Symbiodinium in response to elevated salinities, and secondly to counter reactive oxygen species (ROS) produced as a consequence of salinity stress.
MAIN TEXT

Introduction
Coral reefs are biodiversity hotspots of immense biological and economical value (1). The relationship between *Symbiodinium* – an autotroph endosymbiotic dinoflagellate - and scleractinian corals forms the basis of coral reef ecosystems (2). The coral host provides inorganic nutrients and carbon dioxide to the dinoflagellate in exchange for energy in the form of photosynthetically produced carbohydrates (3, 4). This symbiotic relationship is highly sensitive to environmental disturbance. For instance, increases in temperature, salinity, nutrients, and/or high solar irradiance can impair photosynthetic efficiency and enhance the formation of harmful reactive oxygen species (ROS) that must be detoxified by the organism via antioxidants and ROS scavengers (5-8). Otherwise, the environmental stress can ultimately lead to coral bleaching, the visual whitening of corals due to loss of their endosymbionts (5, 8). As a consequence, increasing exposure to environmental stress, in particular to rising seawater temperatures, is threatening the existence of coral reefs at a global scale (9, 10).

In comparison to the detrimental effects of elevated seawater temperatures, much less is known about the effects of increased salinities on corals and their endosymbionts. Yet, reef corals from the Red Sea and the Persian/Arabian Gulf (PAG) are commonly found at salinities of up to 41 in the Red Sea and up to 50 in the PAG at comparably high summer temperatures exceeding 32°C and 35°C in parts of the Red Sea and Persian/Arabian Gulf (PAG), respectively (11-14). In fact, both salinity and temperature in these regions are the globally highest to support reef growth (15). Although the osmotic response of *Symbiodinium* at the molecular level is virtually unknown (16-18), studies on free-living algae suggest that production and accumulation of compatible organic osmolytes (COOs), referred to as osmoadaptation (19), is the most widespread mechanism for adjusting intracellular osmotic pressure in response to elevated salinity; accumulation of inorganic ions and cell volume changes may occur as well. However, since the latter two processes are stressful and can disturb cellular function, they are not considered to represent viable long-term solutions for osmoadaptation (20-23). In contrast, the accumulation of COOs adjust the osmotic pressure and protects proteins from increased ion concentrations (22).

To test whether *Symbiodinium* is capable of synthesizing COOs, we subjected *Symbiodinium* strains from different clades and origins to high salinity stress, both in vitro and in their animal hosts (hereafter referred to as in hospite), and screened for the presence of COOs using gas chromatography-mass spectrometry (GC-MS). We hypothesized that *Symbiodinium* would increase cellular concentrations of COOs in response to elevated salinities and that the response would be similar in vitro as in hospite. Among the utilized COOs, we identified the carbohydrates floridoside, inositol, and mannitol in vitro and in hospite. These compounds can act as both osmolytes and antioxidants, thereby having the potential capacity to convey osmoadaptation to increased salinities and the ability to counter ROS produced as a consequence of salinity stress (24-29).

Results
High levels of floridoside in *Symbiodinium* exposed to high salinities
First, we screened *Symbiodinium* cultures exposed to different salinities (i.e., low salinity: 25, ambient salinity: 38, high salinity: 55) for the presence of carbohydrate COOs. A markedly high abundance of a compound at 31.5 min retention time (RT) in the gas chromatography/mass spectrometry (GC-MS) trace was detected in all four tested *Symbiodinium* cultures under high salinity (i.e. *Symbiodinium microadriaticum* type A1, *Symbiodinium* sp. type A1, *Symbiodinium minutum* type B1, and *Symbiodinium*...
floridoside. This compound was identified as the derivative of floridoside (2-O-glycerol-α-d-galactopyranoside-(hexa-TMS)) by a search against the National Institute of Standards and Technology Mass Spectrometry (NIST MS) library with a reverse match factor of 971/1000 (Table S1). Floridoside concentrations of Symbiodinium strains exposed to high salinity ranged from 50 ± 7 nmol (Symbiodinium minutum) to 490 ± 55 nmol (Symbiodinium psygmophilum) and were consistently represented among the most abundant carbohydrates quantified in this analysis (Table 1). In contrast, floridoside was non-detectable under low salinity conditions for all Symbiodinium strains (Fig. 2a, Table 1). At ambient salinity levels of 38, floridoside was only detected in Symbiodinium psygmophilum at a concentration of 51 ± 14 nmol (Fig. 2a, Table 1). This strain also accounted for the highest measured amounts of floridoside under high salinity levels of 55 (490 ± 55 nmol). By comparison, inositol and mannitol were consistently present at low salinities and showed reduced concentrations at higher salinities for some strains (Table 1).

We identified several other metabolites in the same GC-MS trace and could quantify a total of five additional carbohydrates (i.e., glycerol, glucose, galactose, ribose, and fructose) and four amino acids (i.e., glycine, alanine, valine, and proline) for each of our samples that serve as putative osmolytes. Concentrations of these metabolites were significantly different between different salinities (except proline) and Symbiodinium strains as well as combinations thereof (all P ANOVA < 0.01) (Table S2). This indicates that these metabolites are differentially regulated under changing salinities and in different Symbiodinium strains. Importantly though, only floridoside production showed a substantial increase at high salinities, whereas levels of all other metabolites (including mannitol and inositol) showed inconsistent patterns (Table 1).

Since floridoside can be derived from glycerol and glucose/galactose (30), we investigated changes in the abundance of these molecules in detail (Fig. 1, Fig. 3, Table 1). In all Symbiodinium strains, a decrease in glycerol coincided with the accumulation of floridoside when comparing low to high salinity conditions (Fig. 3, Table 1). Notably, glucose and galactose were enriched under high salinity conditions in Symbiodinium microadriaticum, Symbiodinium sp. type A1, and Symbiodinium psygmophilum: only in Symbiodinium minutum these sugars remained at the same level or showed a slight decrease between low and high salinity conditions (Fig. 3, Table 1).

High levels of floridoside in Symbiodinium from coral holobionts exposed to high salinities

To assess the importance of floridoside in holobionts, we exposed the coral model Exaiptasia associated with Symbiodinium minutum (strain SSB01) (31) and the coral Porites lobata associated with Symbiodinium thermophilum originating from the southern PAG (11) to different salinities and measured floridoside levels. All samples exposed to high salinity displayed a substantial increase of floridoside (Exaiptasia: 94 % increase, P. lobata: 83 % increase) (Fig. 2b, Table S3).

We then compared algal floridoside levels in Symbiodinium from coral holobionts that show different capacities to survive at high salinity. We found that corals that were actively growing and surviving at a salinity of 42 for >24 months, i.e. P. lobata and Hydnophora grandis, had higher floridoside levels compared to those of Porites lichen, which is only capable to survive for a short period of time at this salinity (11) (Fig. 2c, Table S3).

We also checked for homologs of the putative enzyme that converts Glycerol-3-phosphate to floridoside in the available genomes of Exaiptasia and the corals Acropora digitifera and Stylophora pistillata to assess whether cnidarian hosts are in principle able to
synthesize floridoside. Following (30), we found no homologs for the gene that encodes the enzymatically active floridoside phosphate synthase/phosphatase in the red alga *Galdieria sulphuraria* (Gasu_26940) in the corals *Acropora digitifera* or *Stylophora pistillata* and only a partial hit in the genome of *Exaiptasia* CC7 (AIPGENE13675). Conversely, we found putative full-length homologs of the gene in all available *Symbiodinium* genomes, i.e. *Symbiodinium microadriaticum* (Smic14738, Smic32192, Smic6078), *Symbiodinium minutum* (symbB.v1.2.003359, symbB.v1.2.013114, symbB.v1.2.013196), and *Symbiodinium kawagutii* (Skav203497). Hence, the coral genomes investigated do not harbor the enzyme to produce floridoside. Although there is a possibility that *Exaiptasia* is capable of producing floridoside based on a partial hit, this should be clarified in future experiments.

Discussion

In this study, we determined levels of the carbohydrates floridoside, inositol, and mannitol in response to high salinity in *Symbiodinium in vitro* and *in hospite* to assess the capacity of these COOs to fulfill a function in osmoadaptation to high salinities. Notably, other osmolytes that were not measured in our study, e.g. taurine, betaines, or DMSP (32-34), might also contribute to osmoadaptation of *Symbiodinium*. Consequently, our data should not be considered a complete assessment of all osmolytes in *Symbiodinium*. Importantly, however, we identified the osmolyte floridoside consistently and in increased amounts in *Symbiodinium* at high salinities. This shows that cultured *Symbiodinium* cells produce floridoside in response to salinity stress. Elevated floridoside levels were also evident in *Exaiptasia* and *P. lobata* when exposed to high salinity. In particular, we found elevated floridoside levels in *Symbiodinium* of corals capable of long-term survival at high salinity conditions (*P. lobata* and *H. grandis*). As such, our work has uncovered a key COO that we suggest allows *Symbiodinium* to osmoadapt to extreme salinities *in vitro* and *in hospite*. The fact that our results show a consisted accumulation of floridoside in response across a range of *Symbiodinium* strains and experimental conditions provides strong support for the notion that increased floridoside levels constitute part of the osmoadaptive response to high salinity. Our findings also provide insight for our understanding of the role of osmoadaptation in the coral-*Symbiodinium* endosymbiosis with implications for the coral stress response as further discussed below.

**Floridoside as a key osmolyte in *Symbiodinium***

Synthesis of the osmolyte floridoside has been identified as a conserved pathway in evolutionary distinct organisms such as red algae, green algae, and cryptophycae (24, 30, 35). It is produced by uridine diphosphate (UDP) galactosyltransferases via condensation of glycerol-3-phosphate and UDP-galactose (30). UDP-galactose demands can be supplied via starch mobilization resulting in increased glucose/galactose pools, as described for the green algae *Dunaliella* sp. under conditions of high salinity (36). A similar mechanism might explain the increased levels of glucose (in cultures of *Symbiodinium microadriaticum* and *Symbiodinium* sp. *type A1*) and galactose (in all cultured strains) that we measured in *Symbiodinium* at high salinity (Fig. 3, Table 1). The consistent increase of glucose and galactose in concert with the upregulation of floridoside suggests that they fulfill a conserved osmotic adjustment function within the genus *Symbiodinium* (Fig. 3, Table 1). Besides UDP-galactose, the second component required for floridoside synthesis, glycerol-3-phosphate is likely supplied from photosynthesis or via the Calvin cycle (37). Glycerol-3-phosphate can be produced from glycerol, which is considered to be one of the main COOs in marine algae (38), although it has been shown to be released...
under osmotic pressure in *Symbiodinium* (16, 18, 39). Even though our preliminary analysis on available *Symbiodinium* genomes confirmed the presence of homologs for the enzyme that produces floridoside, it will be interesting to check for the presence and identity of the enzymes required for floridoside synthesis in ecologically relevant *Symbiodinium* (e.g., *Symbiodinium thermophilum*), and whether gene expression or duplication can be aligned with strain- or species-specific differences (40).

**Osmoadaptation in the coral-*Symbiodinium* endosymbiosis with implications for the coral stress response**

Our results demonstrate that exposure to high salinities leads to higher endosymbiont floridoside levels *in vitro* and *in hospite*. This may point to that increasing floridoside levels not only increase the capacity of *Symbiodinium* to cope with the effect of osmotic stress in extreme environments, but also the capacity of the holobiont. Further, our results suggest that osmolarity changes within the coral tissue are noticed by endosymbiotic *Symbiodinium*, and thus, both the coral and *Symbiodinium* respond to salinity changes, presumably by adjusting the inner osmolarity to the higher outside salinity. It remains to be determined, however, whether *Symbiodinium* adjust their inner osmolarity the same way *in vitro* as *in hospite* (32). Importantly, potentially any metabolite contributes to the osmolarity, and thus the endosymbiotic environment in coral cells might differ from the seawater environment (38).

Beyond its function as an osmolyte, floridoside has been shown to act as an antioxidant with ROS scavenging properties (25, 26). As such, floridoside has the capability to convey osmoadaptation as well as to counter ROS produced in response to the salinity stress (24, 26-28). In particular, increased ROS detrimentally affects photosystem II in photosynthetic organisms (5, 41-43). Therefore, the production of antioxidants during salinity stress is potentially important for *Symbiodinium*, and floridoside represents an osmolyte that fulfills a ROS scavenging function at the same time (26). Increasing levels of floridoside and oxidative stress in response to increased salinities were shown in the red algae *Gracilaria sordida* (44) and *Gracilaria corticata* (45), respectively. Future work should determine the exact role that floridoside plays in the salinity stress response, either as an antioxidant, as a COO, or both, e.g. by comparing floridoside and ROS levels at ambient and increased salinities.

The notion that ROS producing mechanisms for photosynthetic organisms are similar (if not the same) under salinity and heat stress (42) has also interesting implications for our understanding of the response to heat stress in *Symbiodinium*, and by extension, for the coral hosts. Similar to salt stress, heat stress results in a malfunction of the photosynthetic machinery of *Symbiodinium* and the production of ROS that may damage the algal cells and, in the case of the coral-algal endosymbiosis, may trigger bleaching (46). We find that *Symbiodinium* exposed to high salinity *in vitro* and *in hospite* accumulate high amounts of floridoside. As such, the production of floridoside in high salinity environments may thus increase the ability to sustain heat stress in *Symbiodinium* and, by extension, their coral hosts through scavenging of increased ROS levels by high levels of floridoside. Consequently, the thermal resilience of coral holobionts may potentially increase under conditions of high salinity due to the accumulation and inherent antioxidative capabilities of floridoside. Experimental data connecting increased floridoside levels to decreased ROS and bleaching levels under increased salinities are in demand to support this potential link.

**Materials and Methods**

**Symbiodinium cultures**
Symbiodinium microadriaticum CCMP2467 (type A1, originally isolated from Stylophora pistillata, Red Sea) (40), Symbiodinium sp. type A1 (originally isolated from Astreopora sp., Red Sea) (47), Symbiodinium minutum Mf1.05b (type B1, isolated from Orbicella faveolata, FL, USA) (48, 49), and Symbiodinium psygmophilum Mf10.14b.02 (type B2, isolated from O. faveolata, FL, USA) (50, 51) were cultured in f/2 media without silicium under a photonflux of 108 µmol m⁻² s⁻¹ at 26°C (52). The f/2 media was prepared from sterile filtered Red Sea water (salinity of 38, and complemented with NaNO₃, NaH₂PO₄, vitamins, and trace metals (53). For each strain, we used replicate culture flasks and prepared three salt-adjusted f/2 media (salinities: 25, 38, and 55, following (54)) either by adding appropriate amounts of NaCl or dilution of the media with ddH₂O water. Triplicates of 5 mL of Symbiodinium cultures at exponential growth (10⁻⁵-10⁶ cells mL⁻¹) were transferred to 35 mL salt-adjusted f/2 media for each salinity and incubated for 4 hs under cultivating conditions. Symbiodinium cells were subsequently harvested by centrifugation (4500 x g, 10 min, 4°C). Cells were counted by fluorescence-activated cell sorting (FACS). To do this, 1 ml of each Symbiodinium culture were collected and fixed with formalin. After washing, samples were resuspended in 1 ml of ddH₂O and labeled with SYBR Gold (Thermo Fisher Scientific, USA), of which 150 µl aliquots were supplied for FACS (50 µl counting volume). FACS measurements were conducted in triplicate on a cell analyzer (LSRFortessa, BD Biosciences, USA). Stained DNA/RNA was excited via 488 nm blue laser and emission detected for total nucleotide detection (Alexa Fluor 488 filters, Life Technologies). Detection of valid signals was a combined signal of forward- and side-scattering and both fluorescence signal of SYBR Gold and chlorophyll autofluorescence. FACS data were analyzed by FlowJo 10 flow cytometry analysis software (FlowJo LLC, USA).

Exaiptasia and coral cultures
Anemones of the single, clonal genotype Exaiptasia (55) strain CC7 in association with Symbiodinium strain SSB01 (Symbiodinium minutum) were generated and reared as described previously (31). Anemones were cultured under a 12 hs/12 hs light/dark cycle under a photonflux of 20-40 µmol m⁻² s⁻¹ at 25°C and fed twice a week with Artemia. To avoid Artemia contamination, food supply was ceased five days prior to experiments. For experimental treatments, a six-well cell culture plate was prepared with 6 mL autoclaved Red Sea water (salinity: 38) per well. Seawater in three wells was adjusted to salinity of 35 and to 42 in the other three wells by addition of ddH₂O and NaCl, respectively. One Exaiptasia polyp was transferred into each well (i.e., triplicate samples for both salinities and one polyp for each sample and extraction were used) and kept at a 12 hs/12 hs light/dark cycle at 25°C for 15 hs. Subsequently, each Exaiptasia colony was transferred into a 1.5 mL cryotube, rinsed thrice with filtered seawater, and frozen and stored in liquid nitrogen.

Corals were kept in long-term culture (>24 months) in different compartments of the experimental coral mesocosm facility at the University of Southampton at salinity levels matching those of their habitats of origin (salinity 42, P. lobata, Persian/Arabian Gulf (11), salinity ~36.5, P. lichen, H. granidis, Indopacific (11, 56, 57). P. lobata were additionally cultured under reduced salinity conditions of 34 for >24 months. Corals were kept at a temperature of 26°C with a 10 hs/14hs light/dark cycle under a photonflux of 150 µmol m⁻² s⁻¹ (11). Light and temperature levels suitable for long-term culture of the corals were established during previous work (56, 57). These three species were studied owing to their different capacity for survival at elevated salinities: Porites lichen associated with Symbiodinium sp. type C96 (short-term survival, (11)), P. lobata with S. thermophilum (long-term survival (11, 14)), and Hydnophora grandis with Symbiodinium sp. type C40.
(long-term survival). In the latter case, survival capacity was determined by incubating ten replicate colonies >24 months at a salinity of 42. In contrast to other Indopacific species (11), these corals have not suffered any mortality and have been actively growing during this time (57). In the present experiments, replicate colonies of *P. lichen* and *H. grandis* previously cultured at lower salinities (34 and 36.5) were gradually adjusted to the high salinity of 42 over two days before being moved to the high salinity compartment for 12 days prior to sampling. All replicate colonies were produced by earlier fragmentation. Using an airbrush, coral tissue was blasted off the skeleton with ice-cold sterile-filtered freshly prepared artificial seawater with the same salinity as the culture rearing water. Three coral colonies were used per species to extract zooxanthellae. Algal cells were precipitated from the homogenate by centrifugation at 2,500 x g for 5 min at 4°C. Cells were washed twice (to remove the host tissue fraction and to prevent residual salt to interfere with the downstream GC-MS analyses), first in precooled seawater, then in ddH₂O, each step followed by centrifugation and re-suspension. The exposure to ddH₂O was limited to ~30 s to minimize potential effects of the hypoosmotic environment. After the final centrifugation step, all liquid was removed and the cell pellet was lyophilized for 14 hs.

**Metabolite extraction and recovery**

Cell pellets from *Symbiodinium* cultures, *Symbiodinium* extracted from coral tissues, and whole-animal symbiotic *Exaiptasia* (due to low biomass) were resuspended and washed with 30 mL sterile seawater on ice, pelleted, and washed for ~30 s with further 5 mL ddH₂O to remove residual salt. After a further centrifugation step, pellets were re-suspended in 5 mL ddH₂O and cells disrupted by tip-ultrasonication for 4 min at 3 s pulsing, 6 s pause. Cell debris was removed by centrifugation at 20,000 g for 20 min at 4°C. Proteins/DNA/RNA were removed by ethanol precipitation by adding 9 parts of -20°C ethanol to 1 part supernatant. The precipitate was pelleted and removed by centrifugation, while the supernatant was frozen in liquid nitrogen and lyophilized. Dry samples were dissolved in 240 µL ddH₂O water, spiked with 10 µL of internal standard (1 µg/µL hydroxy benzylic acid (HBA) in ddH₂O), transferred into GC vials, and dried under vacuum. For derivatization, 50 µL of MOX reagent (2 % methoxamine HCL in pyridine) was added to each sample and the solution was heated to 75°C for 1 h. Afterwards, 100 µL of MSTFA solution (MSTFA, 1 % TMCS, ThermoScientific) was added and samples were heated for 1 h at 75°C. Each sample vial was centrifuged at 2,000 x g for 10 min, and 100 µL of the supernatant was transferred to glass inserts placed inside GC vials.

**GC-MS analysis, quantification, and analysis**

Derivatized carbohydrates, amino acids, and further intracellular compounds were characterized and quantified by GC-MS. For separation a HP-5ms column (Agilent Technologies, USA) and a temperature profile starting at 70°C was chosen. Temperature was increased by 6°C min⁻¹ up to 230°C, followed by 60°C min⁻¹ at a maximum of 280°C and held for 4 min. Metabolites were quantified by standard curves produced with pure glucose (99.5 %, Sigma, GER) and glycerol (ACS reagent ≥99.5 %, Sigma) with 60, 30, 10, 1 and 0.1 µg of both compounds. The calibration standards were spiked with 1 µg of HBA and derivatized for GC-MS and analyzed as described above. All samples were prepared and measured in triplicates. GC-MS data were processed (i.e., background subtraction, peak picking, and integration; OpenChrom v. 0.901, Lablicate UG, GER) and MS-ionisation spectra identified (NIST MS Software 2.0, Agilent Technologies). Statistical testing was conducted on normalized quantities of metabolites (nmol) using ANOVAs and Tukey’s HSD post-hoc tests to assess differences between pairwise comparisons (for *Exaiptasia* at a salinity of 35, we only obtained 1 measurement, and
hence, no statistical testing could be conducted). In the case of cultured *Symbiodinium*, we normalized to 100,000 cells mL\(^{-1}\). For *Exaiptasia*, we used one animal of equal size and age per replicate and extraction. Correspondingly, we measured total holobiont osmolyte and floridoside levels in *Exaiptasia*. For corals, *Symbiodinium* extracts were normalized over dry weights.

**Floridoside homologs in cnidarian and *Symbiodinium* genomes**
We searched for homologs of the putative enzyme that converts Glycerol-3-phosphate to floridoside Gasu\_26940 (30) in the available symbiotic cnidarian and *Symbiodinium* genomes via BLASTp on reefgenomics.org (58) using an e-value cutoff of <10\(^{-5}\). Briefly, the amino acid sequence for Gasu\_26940 was queried against the genomes of *Exaiptasia* (31), *Acropora digitifera* (59), and *Stylophora pistillata* (60), as well as against the genomes of *S. microadriaticum* (40), *S. minutum* (49), and *S. kawagutii* (61).

### H2: Supplementary Materials

Table S1. Metabolites in GC-MS traces and Identification information for NIST MS 2.0 Library Search.
Table S2. Statistical evaluation of metabolite concentration changes of carbohydrates and amino acids across four *Symbiodinium* strains at three salinities.
Table S3. Overview over floridoside amounts in cnidarian holobionts at different salinities.

**References and Notes**


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**Author contributions:** MAO, TR, CRV designed and conceived the experiments. MAO, TR, CRV generated, analyzed, and interpreted data. CRV, CDA and JW contributed cultures/reagents/materials. CRV, TR, CDA, JW, and MAO wrote the manuscript.

**Competing interests:** The authors declare that they have no competing interests.

**Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data are available from authors upon request. Contact corresponding author for animal specimens.
Fig. 1. Gas chromatography analysis of carbohydrate osmolyte diversity in *Symbiodinium psygmophilum* at three salinity levels. Low salinity level of 25 (green), ambient salinity level of 38 (black), and high salinity level of 55 (red). The trace from 26 to 32 min retention time (RT) shows a floridoside peak at RT 31.5 min. The abundances are normalized to an internal standard (ISTD). The chemical reaction depicts synthesis of floridoside (3) as a product of uridine diphosphate (UDP)-galactose (1) and glycerol-3-phosphate (2). Of note, nearly identical traces were obtained for the other three *Symbiodinium* strains.

Fig. 2. Floridoside levels of *Symbiodinium in vitro and in hospite* at different salinities. Floridoside levels represent measured concentrations (nmol) per 10⁵ cells ml⁻¹ of algal cells (for cultured *Symbiodinium*), per dry weight (mg) of whole tissue homogenate (for *Exaiptasia*), and per dry weight (mg) of isolated *Symbiodinium* (for coral samples). Data obtained for each experiment were normalized to the highest value (set to 100%). (a) Floridoside levels in four cultured *Symbiodinium* strains *in vitro* at low (25), ambient (38), and high salinities (55). Smic: *Symbiodinium microadriaticum* (type A1), Sym A1: *Symbiodinium* sp. type A1, Smin: *Symbiodinium minutum* (type B1), Spsy: *Symbiodinium psygmophilum* (type B2). (b) Floridoside levels for the holobiont *Exaiptasia* (strain CC7) symbiotic with *Symbiodinium minutum* (strain SSB01) at salinities of 35 (1 replicate) and 42 (3 replicates) and for *Symbiodinium thermophilum* isolated from *Porites lobata* cultured at salinities of 34 and 42. (c) Floridoside levels of *Symbiodinium* from corals with a different long-term survival capacity at high salinities after incubation at a salinity of 42. Floridoside concentrations were determined for *Symbiodinium* sp. type C96 (*Porites lichen*; short-term survival), *S. thermophilum* (*P. lobata*, long-term survival), and *Symbiodinium* sp. type C40 (*Hydnophora grandis*, long-term survival). Error bars denote standard error (SE). Letters indicate Tukey's HSD post-hoc differences based on pairwise comparisons of ANOVA results (groups with different letters are significantly different at *P*<0.01 for (a) and *P*<0.05 for (c)).

Fig. 3. Osmolyte levels of floridoside and intermediates (glycerol, glucose, and galactose) at three salinities across four *Symbiodinium* strains. Glucose and galactose can be metabolized to glycerol (via the Calvin cycle) and cover UDP-galactose and glycerol-3-phosphate demands for floridoside synthesis. Floridoside, glycerol, glucose, and galactose levels for (a) *Symbiodinium microadriaticum* (type A1), (b) *Symbiodinium* sp. type A1, (c) *Symbiodinium minutum* (type B1), (d) *Symbiodinium psygmophilum* (type B2) cultures at low salinity (25, light gray), ambient salinity (38, gray), and high salinity (55, black) after 4 hs at 108 μmol photons m⁻² s⁻¹. Letters indicate Tukey's HSD post-hoc differences based on pairwise comparisons of ANOVA results (groups with different letters are significantly different at *P*<0.05). Tukey's post-hoc tests were not performed if ANOVAs yielded a non-significant F-ratio, designated as *n.s.* (not significant).
Table 1. Compatible organic osmolyte (COO) concentrations of four *Symbiodinium* strains at three salinities. Carbohydrates (floridoside, inositol, mannitol, glycerol, glucose, galactose, ribose, and fructose) and amino acids (glycine, alanine, valine, and proline) were quantified by GC-MS. COO levels are provided in nmol and normalized to GC-MS internal standard HBA and 1 x 10^5 cells ml⁻¹ (i.e., concentration is nmol/10^5 cells or nmol/ml culture). *Symbiodinium microadriaticum* (type A1); *Symbiodinium* sp. type A1; *Symbiodinium minutum* (type B1); *Symbiodinium psygmophilum* (type B2); SE = standard error.

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Figure 2

(a) In vitro

(b) In hospite

(c) In hospite

- Aiptasia
- P. lobata

- short-term survival
- long-term survival

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