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Expression of glucose (GLUT) and glycerol (GLP) transporters in symbiotic and bleached *Cassiopea xamachana* (Bigelow, 1892) jellyfish

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Abstract

Cassiopea xamachana is a tropical medusa that lives in symbiosis with dinoflagellate algae, serving as a model organism for symbiotic studies. The symbiosis is necessary for this medusa to complete its life cycle. The symbiotic partners maintain a metabolic exchange of organic molecules that constitute an important source of energy for the animal host, with free organic molecules, like glucose and glycerol, being the primary source. This molecular exchange can be facilitated by cellular internal membrane transport proteins, such as Glucose membrane transporters (GLUTs) and Glycerol transport-like aquaglyceroporins (GLP-like), probably located at the symbiosomal interface. The present study was conducted in October 2021, evaluating the expression of transporter coding genes *GLUT3*, *GLUT8*, and *GLP9* (two genes) by qPCR under conditions of symbiosis and after the loss of symbionts. Symbiotic medusae donated from Xcaret Park, Mexico (20° 34′ 24.59″ N; -87° 07′ 5.40″ W) were sampled and compared to medusae with an experimental decrease of algal symbionts. In agreement with glucose being an important mobile molecule, our results showed higher transcription levels for glucose transporters GLUT3 and GLUT8 in control compared to bleached medusae. By contrast, bleached medusae showed a higher expression of aquaglyceroporin transporters GLP9-1 and GLP9-2, probably associated with glycerol production after lipid catabolism, to compensate for lower organic carbon levels due to the loss of symbionts. Our results highlight the importance of free carbon molecules transported from symbiont to host and agree with glucose being an energy fuel for this symbiotic association.

Keywords Symbiosis · Potential photosynthates · Carbon transport · Integral proteins

Introduction

The symbiosis between cnidarians and dinoflagellate algae in the family Symbiodiniaceae is the basis for coral reef development. Corals can grow in nutrient-poor waters thanks to the support of organic molecules provided by their algal symbionts (Muscatine and Porter 1977). The

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translocation and exchange of organic molecules that are metabolically energetic has been the focus of many studies aimed at understanding the mechanisms that drive and regulate this symbiosis (Hofmann and Kremer 1981; Fitt and Trench 1983; Wakefield and Kempf 2001; Burriesci et al. 2012; Davy et al. 2012; Kopp et al. 2015). The algal symbionts reside in the host gastrodermis surrounded by several membranes of algal origin, plus an outermost host-derived membrane, known as the symbiosome membrane complex (Fitt and Trench 1983; Wakefield and Kempf 2001), and any exchange of organic molecules must be through this selective barrier. Photosynthetic products translocated to the host, or photosynthates, constitute a major source of energy in this symbiosis, with free sugars like glucose and glycerol being the most important (Muscatine 1967; Lewis and Smith 1971; Schmitz and Kremer 1977; Hofmann and Kremer 1981; Gordon and Leggat 2010; Davy et al. 2012; Kopp et al. 2015). The primary source of nitrogenous compounds

to the symbiotic partners is through heterotrophic feeding (Davy et al. 2012).

Transmembrane transporters involved in the distribution and assimilation of nitrogen and carbon have been identified in symbionts of the Symbiodiniaceae family (Aranda et al. 2016). Correspondingly, the animal host must be capable of internalizing photosynthates to cover its basal energetic requirements (Lampert 2016), but these are not thoroughly studied. The molecular exchange of organic compounds could be facilitated by internal membrane proteins located in the cell membranes of each symbiotic partner, and in the symbiosomal interface (Fitt and Trench 1983; Wakefield and Kempf 2001). Some studies have suggested that specialized proteins may be responsible for the transport of organic molecules from symbiont to host, like glucose and glycerol (Sproles et al. 2018). Glucose membrane transporters (GLUT-type) in the Solute Carrier family (SLCA2) are of interest, given their high affinity for glucose and location in internal membranes (Rands et al. 1993; Escher and Rasmuson-Lestander 1999; Wakefield and Kempf 2001; Joost et al. 2002; Wood and Trayhurn 2003; Uldry and Thorens 2004; Wright 2013; Scheepers et al. 2015; Sproles et al. 2018). GLUTs transport glucose down its concentration gradient, hence the name facilitated diffusion (Castrejón et al. 2007). The glucose-sensing function has two components: (1) GLUT-mediated glucose entry into the cell and (2) glucose metabolism through glucokinase phosphorylation (Díaz and Burgos 2002; Castrejón et al. 2007). Glucose selectivity by GLUT transporters is determined by a highly conserved amino acid sequence (QLS) in transmembrane segment seven, the glucose recognition site (Díaz and Burgos 2002). In the symbiotic sea anemone *Exaiptasia diaphana*, GLUT8 exhibited high levels of mRNA expression under symbiotic conditions in contrast to an aposymbiotic condition (Lehnert et al. 2014). This same transporter was later immunologically detected by Mashini and collaborators (2022) in *E. diaphana* infected with homologous and heterologous symbionts in the gastrodermis and epidermis of symbiotic and aposymbiotic anemones; however, these authors did not find differences in the immunodetection of this protein according to symbiotic state.

Glycerol is transported through Intrinsic Channel Proteins of the Superfamily of Major Intrinsic Proteins (MIPs) known as Glycerol Facilitators (GFs), channel-like plasma membrane proteins that can take up or exclude glycerol (Castrejón et al. 2007). Glycerol can also be transported through Aquaglyceroporins (GLPs), which are channels that transport small uncharged molecules, such as glycerol, CO_2, and urea, but exclude water (Rojek et al. 2008). Once glycerol is transported to the symbiosomal space, it must cross the host cell membrane possibly through a GLP, whose transport mechanism may be by diffusion (Castrejón et al. 2007). GLPs are regulated by phosphorylation or glycosylation

(Tamás et al. 1999; Mandal et al. 2012) and by activation of pH-dependent ions or intracellular signals (Von Bülow and Beitz 2015). In aquaglyceroporins (GLPs), conserved motifs ("NPA") form an aromatic/arginine selectivity filter (ar/RSF) that largely determines solute specificity and substrate transport (Deshmukh et al. 2015). In cnidarians, 16 sequences with homology to GLPs have been identified, all with at least six transmembrane domains, characteristic of these proteins, with amino-terminal and carboxy-terminal ends in the cytoplasm (Agre et al. 2002).

By phylogenetic analysis, Sproles and collaborators (2018) identified putative GLUT transporter proteins in cnidarians; these transporters clustered into two classes, regarded as highly conserved human homologs. These same authors identified the GLUT8 transcript in the sea anemone *Aiptasia pallida* (accepted name: *Exaiptasia diaphana*) as being probably located in internal membranes and, for non-symbiotic species, in the plasma membrane, highlighting the importance of these proteins in the exchange of potential photosynthates in the symbiosis. Also, aquaglyceroporins considered by these same authors (Sproles et al. 2018) shared similarities to human GLP3 and GLP9 and suggested the need for experimental evidence to confirm their role in cnidarian transport. Mashini et al. (2022) later reported elevated levels of AQP3 protein in aposymbiotic *Exaiptasia*, suggesting that this was a response to a reduced supply of symbiont-derived organic carbon.

Model organisms, such as the medusa *Cassiopea xamachana*, are an attractive option for studying symbiosis. Unlike corals, the lack of a carbonate skeleton facilitates its handling and cultivation under laboratory conditions (Ohdera et al. 2018). The adult medusa depends on the energy transferred from its symbionts to cover up to 70% of its basal needs (Lampert 2016). About 3 weeks after the symbionts are acquired by asexual polyps, depending upon symbiont species, temperature, and food intake, metamorphosis of the newly symbiotic polyp into a medusa larva occurs (Colley and Trench 1983; Fitt and Costley 1998). In the medusa *Cassiopea andromeda*, glucose and glycerol are the only two free carbon molecules that acquire an isotopic label in short-term studies (under 90 s), suggesting that glycerol may fuel the synthesis of lipids, being rapidly metabolized (Hofmann and Kremer 1981). Further, previous studies have shown that adult medusae lose mass (size and weight) when symbiont density declines or in the absence of light, even when food is supplemented (Lampert 2016). Apparently, when medusae shrink under low light levels, some polyunsaturated fatty acids seem to be transferred from symbiont to host (Mortillaro et al. 2009). We hypothesized that if glucose and glycerol are important carbon molecules for this symbiosis, the expression of coding genes for their transport would differ in medusae with and without symbionts. This work aimed to evaluate the expression levels

of genes coding for glucose transporter proteins GLUT3-like and GLUT8-like, and two genes for glycerol transport through GLP9-like, in the model organism *C. xamachana*, comparing the conditions of symbiosis and bleaching.

Materials and methods

Experimental design

C. xamachana medusae were donated by Xcaret Park, collected at 20° 34′ 24.59″ N; -87° 07′ 5.40″ W from NE Quintana Roo, Mexico in October 2021. They were acclimated in an open system pond (100 L seawater) under natural light and temperature conditions for over a year. Adult medusae with a similar size (6 cm in diameter) were individually transferred to 1 L beakers containing 500 mL of natural seawater (SW) with bubbling air and placed under a roof with indirect natural sunlight. Medusae were not fed during the experimental phase; the SW was replaced daily. Control (n = 3) and bleached (n = 4) medusae were maintained for 21 days at room temperature (28.3 ± 2.27 ℃; SAMMO 2021). Samples from a tentacle of each medusa were taken weekly, starting on day 0. The tentacle fragments were flash frozen in liquid N_2 and stored at -80 °C until further processing. After 14 days, the addition of sugars to the bleached medusae was discontinued (see below) maintaining them for one more week and sampled. At the end of the treatments, medusae were returned to the open system pond. The adults of this medusa associate with *Symbiodinium* type A1 in Florida, *Symbiodinium microadriaticum* (Thornhill et al. 2006). We presume that the same symbiont occurs in the adult medusae we used, as they are from the same geographic area.

Artificial bleaching of medusae

The medusae were artificially bleached by the daily addition of a monosaccharide mix following Pogoreutz et al. (2017), but with a sugar mixture and final concentration of 0.3 mg L^{-1} according to Carabantes et al. (2022). Bleaching of the medusae was evaluated by weekly sampling of a tentacle fragment from each medusa extracted with a Dounce homogenizer. The symbionts were collected by centrifugation (13,000 RPM, 5 min), washed twice with milli-Q water, and resuspended in 500 μL of sterile seawater, adding Lugol (30%) to aid in symbiont counting. Symbiont density was quantified with a hemocytometer on an optical microscope (3 replicate counts per sample), and the data normalized to the wet weight (g). Further, we photographed a portion of a tentacle of each medusa with a fluorescence microscope (Axioskop 40 with aim 20X Tex Red Fs 15) at the beginning and at the end of the 21-day treatment.

RNA extraction

RNA was extracted from a tentacle of each medusa on the initial day, and on days 14 and 21, following Pawlowski et al. (1999) with some modifications. Briefly, frozen tentacle pieces were macerated in liquid N_2 and added as powder to a preheated (95 °C) acidic phenol–buffer mixture and vortexed for two minutes, adding RNase Out. After a 25 min centrifugation at 4 °C, the upper phase was chloroform extracted twice and the RNA precipitated with 8 M LiCl. RNA samples (n = 21) were treated with DNase. RNA integrity was evaluated by electrophoresis on 1% agarose gels. RNA concentration and quality were assessed with a BioSpectrometer (Eppendorf). The extracted RNA (500 ng) was copied to cDNA using the ImProm-II TM Reverse Transcriptase (Promega) with 4 μl of buffer (250 mM Tris–HCl pH 8.3, 375 mM KCl, 50 mM DTT, 25 mM MgCl2), 1 μl dNTPs, 0.5 μl ribonuclease inhibitor, and 1 μl of the RT enzyme. cDNAs were synthesized in a BioRad T100 Thermal Cycler under the following conditions: 25 °C (5 min), 42 °C (1 h 3 min) and 70 °C (5 min). For the corroboration of cDNA synthesis, each gene fragment was RT-PCR amplified using the primers described in Table 1.

Transcripts encoding GLUT and GLP transporter proteins in *C. xamachana*

Nucleotide sequences reported in the *C. xamachana* genome (https://cassiopeabase.org/resources/c-xamachana-genome/) indicated genes that potentially code for glucose and glycerol transporters. We identified ten transcripts with homologies for *GLUT1, GLUT10*, four transcripts for *GLUT3* and

Table 1 Specific primer sequences for fragment amplification of genes coding for *GLUT3*-like, *GLUT8*-like, *GLP9*-like (genes 1 and 2), and *FE1α*

Target gene	Sequence
GLUT3-like	Amplification product length: 166 bp
CxGLUT6F	5′-GTC CCT TTT ACG GCA CTG GT -3′
CxGLUT6R	5′-AGA GTT GGA CGT CCG ATG AC -3′
GLUT8-like	Amplification product length: 189 bp
CxGLUT8F	5′- GTC ACC AAG CTG TTT CCA CA-3′
CxGLUT8R	5′- ATC TCG CAC AGT CTC AGT AGG A-3′
GLP9-1-like	Amplification product length: 177 bp
CxGLP91F	5′- GAT CAG CTT CTT GCA ACT -3'
CxGLP92R	5′- TGG AAT CAA GTC TCT TGC-3'
GLP9-2-like	Amplification product length: 180 bp
CxGLP91F	5′- GAT CAG GTT TTT GCC ACT -3'
CxGLP92R	5′- TGG GAT AAG ATC ACG TGC -3'
FE1α	Amplification product length: 300 bp
PF-Feα1F	5′- CCT CCA TAC TCT GAA CCA AGG TTT AAT G -3'
PF-Feα1R	5′- CCA ATT CCA CCA ATC TTG TAT ACA TCC TG -3'

GLUT8, and three transcripts from *GLP9* in the genome. From these sequences, a BLAST alignment (https://blast. ncbi.nlm.nih. gov/) with sequences for *GLUT* and *GLP* transporters from other cnidarians, with identities > 50%, was identified. We selected one sequence for *GLUT3* (> TRINITY_DN109051), one sequence for *GLUT8* [Transcript (3204 bp)/CDS Sequence (1545 bp)], and two sequences for the *GLP9* gene (Transcript (807 bp)/CDS Sequence (807 bp; Transcript (945 bp) /CDS Sequence (945 bp)). Conserved motifs were identified in these translated sequences after an alignment made in the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). The presence of motifs that fulfill the function of sugar transport in GLUT (GR, GK, GRR, and PETK) (Joost and Thorens 2001), and glycerol transport in GLP (two NPA motifs and a D residue) (Agre et al. 2002) were corroborated (Supplementary Fig. S1).

The identity of the amplified fragments corresponding to the *GLUT3, GLUT8*, and two *GLP9* genes was confirmed by endpoint PCR amplification using the primers designed (Table 1). Further, the amplification products were purified using the Wizard® SV Gel and PCR Clean-Up System and sequenced at the Institute of Cellular Physiology at UNAM. Through a BLAST search (https://blast.ncbi.nlm.nih.gov/), we corroborated the identity of all the genes (Supplementary Fig S2).

qRT-PCR assays

Quantitative RT-PCR assays were performed in the StepOnePlus thermocycler (Applied Biosystems). Samples of 20 ng of cDNA from tentacles of *C. xamachana* were used in each amplification reaction, and the expression of fragments corresponding to genes *GLUT3*, *GLUT8*, *GLP9*-1, and *GLP9*-2 were evaluated in control (n = 3) and bleached (n = 4) medusae at times 0, 14, and 21 days. A fragment of the gene encoding Elongation Factor 1 alpha (EF1α) from *C. xamachana* was used as reference (Nicot et al. 2005; Cabrales-Arellano et al. 2017). The amplification reactions contained 2X the reaction buffer with intercalating agent (QuantiTect® SYBR®-Green, BioRad), 5 μM forward primer, 5 μM reverse primer, water, and 20 ng of cDNA as template. The amplification conditions were: one cycle at 95 °C for 10 min, 40 cycles: 95 °C for 15 s, and 55 °C for 1 min, and 60 °C for 1 min; and for the melting curve 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. To determine cycle quantification (CT) values for each gene fragment amplified, the dissociation curve analysis method was used, setting the fluorescence umbral to 0.05. CT values for each gene were averaged and normalized to the *FE1α* reference gene. The 2-ΔΔCT relative quantification method (Livak and Schmittgen 2001) was performed to determine the fold change in expression. The stability for the expression of the reference gene was calculated using free access programs (GenNorm, Δ-CT, and BestKeeper). Means and standard deviations were determined for each sample.

Statistical analysis

A T-Student test was used for comparing the density of symbionts and umbrella size for the sampling times on each experimental treatment. One-way Analysis of Variance was used to assess differences in the expression levels of the selected genes, between the conditions of symbiotic and bleached medusae, with an alpha of 0.05. Pairwise multiple comparisons were run with a Tukey test (Supplementary Material SM1).

Results

Cassiopea xamachana experimental bleaching

Symbiont density in the bleached treated medusae diminished significantly after 7 days of treatment, going from 1.53×10^7 cells g^{-1} at day 0 to 0.033×10^7 cells g^{-1} at day 14 (t-value $_{0vs7d} = 4.73$). After 14 and 21 days, symbiont density remained low, showing 84–99% fewer symbionts from the initial time; symbiont density was also significantly different (t-value $_{7vs14d} = 7.90$; t-value $_{14vs21d} = 8.80$). Control medusae conserved their symbionts after 21 days (Fig. 1a); the data collected at each time point were not different from the initial symbiont density (t-value $_{0vs7d} = -1.95$, t-value $_{7vs14d} = -2.08$, t-value $_{14vs21d} = -2.01$). We observed a decrease in umbrella size in bleached medusae from 6.5 cm on day 0 to 4.5 cm on day 21; however, control medusae also reduced their size from 6.5 cm at time 0 to 5.7 cm on day 21 (Fig. 1b). Paired t-test comparisons, however, indicated no significant differences in umbrella size between control and bleached medusae on any of the sampling days (t-value $_{0vs7d} = 0.00$, t-value $_{7vs14d} = 4.68$, t-value $_{14vs21d} = 0.52$) The phenotypic characteristics showed the absence of brownish color due to the diminishing of symbionts in bleached medusae, corroborated by fluorescence microscopy (Supplementary Fig. S3).

Expression levels of glucose and glycerol putative transporters in symbiotic and bleached medusae

The expression levels for the gene fragments *GLUT3*, *GLUT8*, *GLP9-1*, and *GLP9*-2 in bleached medusae and the controls, at day 0, were set at 1 ± 0, normalized to the reference gene (Supplementary Table S2). Results showed that the expression of GLUT transporters was not significantly different after 14 days of treatment in control and bleached medusae; however, control medusae showed a tendency to diminish their expression, while
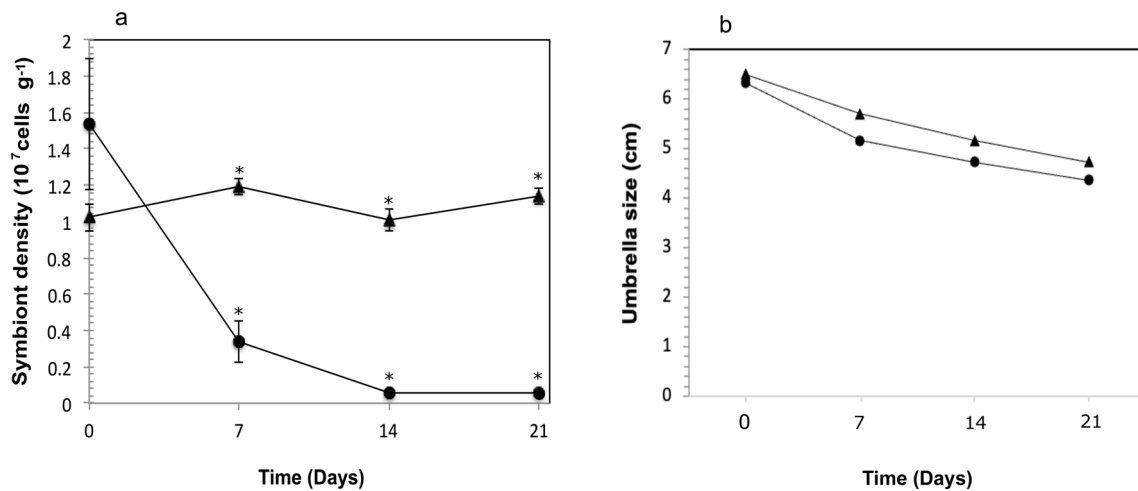
Fig. 1 *Cassiopea xamachana* condition after experimental treatments. (**a**) Symbiont density (1×10^7 cells g.$^{-1}$) and (**b**) umbrella size (diameter in cm) in control (filled triangles) and experimentally bleached (filled circles) medusae, during 21 days of treatment (mean values $\pm$ sem; n = 3 for control, n = 4 for bleached medusae)

bleached medusae increased it (Fig. 2a). The comparative CT values (2-$\Delta\Delta$Ct) after 21 days indicated a decrease in the expression levels of *GLUT3* (X $\pm$ SE = 0.519 $\pm$ 0.395, n = 4) and *GLUT8* (X $\pm$ SE = 0.225 $\pm$ 0.210, n = 4), with significantly different values compared to the symbiotic control (ANOVA: $F_{(1,17)}$ = 5.263, p = 0.009, for *GLUT3*, and $F_{(1,17)}$ = 5.382, p = 0.008, for *GLUT8*) (Supplementary Fig S3, Supplementary Material SM1).

Gene expression values for the *GLP9*-1 gene were significantly lower in the controls at day 14 (ANOVA: $F_{(1,17)}$ = 142.570, p < 0.001) (Fig. 2b) with a lower symbiont density in bleached medusae (0.056 $\times$ 107 cells g^{-1}) (Fig. 1a); however, both *GLP9* genes changed their expression in bleached medusae (X $\pm$ SE = 1.332 $\pm$ 0.0816, n = 4 for *GLP9*-1, and X $\pm$ SE = 1.517 $\pm$ 0.668, n = 4 for *GLP9*-2) (Fig. 2b, Supplementary Tables S1 and S2). The sugar treatment was discontinued after 14 days, maintaining the medusae for seven further days in a bleached condition without food. At this time (21 days of treatment), symbiont density remained low with 0.004 $\times$ 10^7 cells g^{-1} in contrast to 1.269 $\times$ 10^7 cells g^{-1} in the control (Fig. 1a). An increase in the expression levels of *GLP9*-1 (X $\pm$ SE = 3.045 $\pm$ 0.287, n = 4) and *GLP9*-2 (X $\pm$ SE = 2.718 $\pm$ 0.654, n = 4) was observed, with expression levels significantly different from the symbiotic control on day 21 (ANOVA: $F_{(1,17)}$ = 15.170, p < 0.001) (Fig. 2b, Supplementary Tables S1 and S2, Supplementary Material SM1).

Discussion

Our knowledge of the symbiosis between algae and cnidarians is still incomplete. We have a limited understanding of the cellular and molecular mechanisms for the biosynthesis and translocation of carbon from symbiont to host. In the present study, we measured the expression of selected genes that code for glucose and glycerol transporters in *Cassiopea xamachana*, identified as main carbon compounds potentially translocated to the medusa host (Hoffman and Kremer 1981). The addition of sugars successfully diminished the symbiont density in the experimental bleaching, as was demonstrated by Carabantes et al. (2022). GLUT transporters only showed a significant variation in their expression after 21 days of treatment in both conditions, although control medusae increased them, while bleached medusae decreased them. These results suggest that control medusae, being at low light, were probably increasing their chance of acquiring any organic carbon that the symbionts might transfer. Also, in *Exaiptasia diaphana*, it was documented that anemones with symbionts showed significantly higher levels of GLUT8 than aposymbiotic ones (Mashini et al. 2022). According to these same authors, the GLUT8 transporter localized to the symbiosome membrane complex of *E. diaphana*, although not exclusively. This observation, along with our results, suggests that this GLUT8 protein could be involved in the transport of symbiont-derived glucose in the medusa as well.

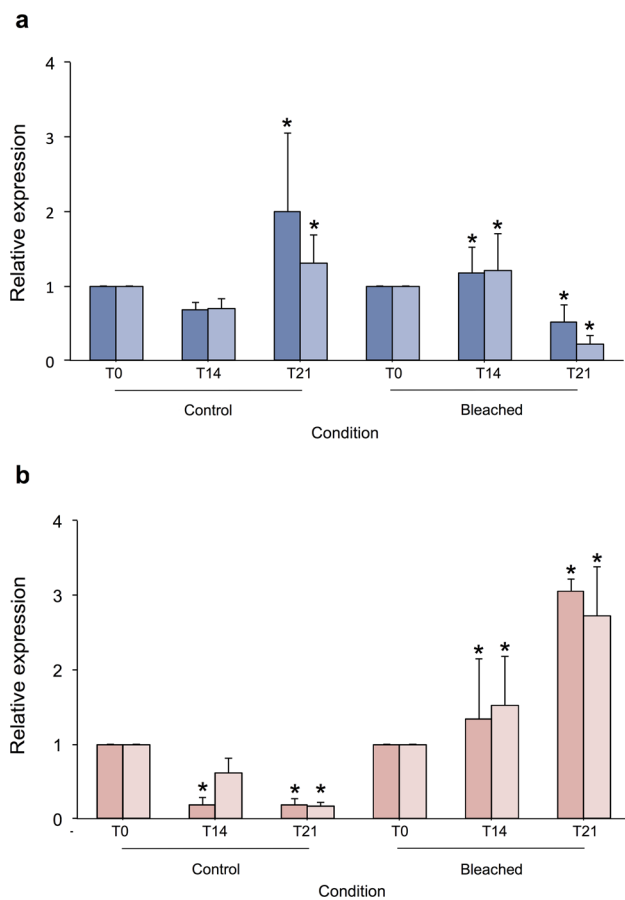In symbiotic medusae, the expression of glycerol transporters diminished with time, perhaps from being under

Fig. 2 Gene expression levels for glucose and glycerol transport genes in symbiotic and bleached medusae. Gene expression levels for (**a**) glucose transport genes *GLUT3* (dark blue) and *GLUT8* (clear blue) and (**b**) glycerol transport genes *GLP9*-1 (dark peach) and *GLP9*-2 (clear peach) measured in symbiotic (Control, n = 3) and bleached medusae (n = 4), sampled at day 0 and on days 14 and 21 of treatment. Sugar addition in bleached medusae was discontinued after 14 days. *Denotes significant differences ($p < 0.001$) in expression levels for each gene from its control

stressful conditions in the vessels (e.g., low circulation and reduced water volume). However, it is also possible that the aquaglyceroporins we assayed are not responsible for transporting glycerol from symbiont to host. In bleached medusae, the diminishing density of symbionts affected the levels of mRNA transcripts for the selected glycerol genes. After 14 days, bleached medusae significantly increased the number of transcripts for the two aquaglyceroporins compared to the control. Previous studies have reported an increase in the levels of glycerol of approximately three-fold in bleached anemones (Molina et al. 2017), suggesting that host animals may degrade lipids to compensate for the loss of symbionts, as has also been documented to occur in the face of nutrient deficiency (Fitt and Pardy 1981). Moreover, after discontinuing the addition of sugars, these same transporters showed further increases in expression

at 21 days, consistent with our interpretation. The catabolism of carbohydrate and lipid reserves, when compared between symbiotic and aposymbiotic states, increased in *E. diaphana* anemones in symbiosis with a heterologous symbiont, *Symbiodinium trenchii*, that conferred metabolic and transcriptomic profiles in between the symbiotic and aposymbiotic states (Matthews et al. 2017). It seems that with less appropriate symbionts, or in their absence, the animal host may utilize reserves to compensate for the loss of symbiont-derived organic carbon; this has also been observed in medusae, even when food is supplemented (Lampert 2016).

In our results, the high expression levels of the two genes encoding glycerol transporters suggest that bleached medusae were degrading lipids and transporting glycerol through a GLP-type protein to compensate for the absence of symbionts, given the lack of glucose on which they depend as an essential metabolite. Since glycerol is produced by lipid degradation and is used for gluconeogenesis, its transport within the cnidarian host via a GLP9 transporter could also be modulating fat metabolism (Hibuse et al. 2006; Madeira et al. 2015). In the study by Molina et al. (2017), the reduction of the glucose reserve in the anemone *Exaiptasia pallida* (*E. diaphana*) due to a diminished symbiont density led to an increase in glycerol-3 phosphate (G3P), as well as a decrease in the specific activity of glycerol-3 phosphate dehydrogenase, suggesting that no lipids were being synthesized. These results were consistent with a steady specific activity of the enzyme Glucose-6-phosphate dehydrogenase (G6PDH), which participates in the pentose pathway where NADPH is regenerated for anabolic metabolism and in fatty acid biosynthesis (Park et al. 2015). Further, in the coral *Acropora aspera* after ^{13}C labeling experiments, fatty acids and lipogenesis intermediates acquired the label in symbiotic specimens (Hillyer et al. 2017); however, after 9 days under high-temperature conditions, the breakdown of energy stores was detected, diminishing such compounds. It would be worthwhile coupling studies of the expression of transporters with metabolic profiles in this medusa, to achieve a better understanding of the dynamics involved.

Our results suggest that lipolysis might be involved in the cellular metabolism of energy reserves in bleached medusae. In this process, triglycerides (TAG) and diglycerides (DAG), which are chief energy reserves in symbiotic cnidarians (Imbs et al. 2010; Garret et al. 2013; Hillyer et al. 2017; Imbs et al. 2021), are hydrolyzed into fatty acids and glycerol by the action of enzymes known as lipases, found in the adipose tissue of animals (Li et al. 2012). The acyl-CoA produced in the fatty acid degrading process is transported into the mitochondria as acyl-carnitine, where β-oxidation occurs. In this oxidative process, the resulting acetyl-CoA is a substrate for the citric acid cycle that will later generate energy in the form of ATP. Our results regarding the

increased expression in GLP9 transporters suggest that bleached medusae could be shifting their metabolism toward the catabolism of lipid stores, explaining the higher expression of glycerol transporters.

Other studies suggest that the loss of symbionts can alter the nutrient cycle in cnidarian symbioses: the cessation in the transfer of photosynthates "gradually reduces the translocation of carbon by the algal symbionts", leading the symbiotic system to a carbon-limited state (Rädecker et al. 2021), but this would depend on lipid stores as suggested by our results. Further, glucose is an important energy molecule for this medusa, evidenced by the decrease in GLUT3 and GLUT8 transcript levels after a significant reduction of symbionts. By contrast, *E. diaphana* anemones were found to have the same intensity in the immunodetection of GLUT8 in symbiotic and aposymbiotic organisms (Mashini et al. 2020), suggesting a tighter relationship of the medusa with its symbionts. Interestingly, increased levels of GLUT8 transporter were found in symbiotic anemones harboring heterologous symbionts like *Symbiodinium microadriaticum*, with higher photosynthetic rates, suggesting an increased effort by the host to acquire photosynthetic products (Mashini et al. 2020). Alternatively, the synthesis of glucose from the glycerol produced by lipid degradation through gluconeogenesis would be unfavorable, since it is an energy-consuming pathway (Molina et al. 2017). Finally, the present work provided experimental evidence for glucose transport in *C. xamachana* jellyfish involving proteins GLUT3 and GLUT8, as previously proposed by phylogenetic analysis (Sproles et al. 2018). However, evidence for their location in inner membranes and other transporter proteins, such as SGLTs, also needs experimental support for their role in carbon exchange in the cnidarian–dinoflagellate symbiosis. Finally, even though the bleaching of medusae appears to have been homogeneous, we worked with tentacle fragments which could have introduced variations in the quantification of transcripts unrelated to gene expression. Therefore, our results should be taken with caution.

In conclusion, the loss of symbiont cells in *C. xamachana* decreased the levels of transcripts for the glucose transporters. Our results showed higher expression of glucose transporters GLUT3 and GLUT8 in control compared to bleached medusae, in agreement with glucose being a mobile molecule. There is no significant glucose transport in the absence of symbionts, as the decreased expression of *GLUT3* and *GLUT8* in bleached medusae suggests. Previous studies suggested the function of GLUT8 as a hexose transporter in intracellular membranes and in the symbiosomal membrane; our results consistently showed transcript levels for GLUT8 decreasing significantly on day 21 in bleached medusae. The results also suggest that symbiont-derived glucose was probably replaced in bleached medusae by lipid catabolism, which increased *GLP9* expression, associated with glycerol transport probably within the host tissues. We also observed that sugar addition alone was insufficient to cover the host's energetic needs after 14 days of treatment, leading to a steady loss of umbrella size in bleached medusae. The greatest size reduction was for the bleached medusae at day 21, coincident with the decrease in the expression of glucose transporters GLUT3 and GLUT8.

Declarations

Conflict of interest The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval The jellyfish used are not held to Official Mexican Standard 059 (NOM-059) as native fauna at risk. The jellyfish were kindly donated by Xcaret Park, Quintana Roo.

References

Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S (2002) Aquaporin water channels-from atomic structure to clinical medicine. Physiol J 542(1):3–16. https://doi.org/10.1113/jphysiol.2002.020818

Aranda M, Li Y, Liew YJ, Baumgarten S, Simakov O, Wilson M, Voolstra CR (2016) Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. Sci Rep 6(1):1–15. https://doi.org/10.1038/srep39734

Burriesci MS, Raab TK, Pringle JR (2012) Evidence that glucose is the major transferred metabolite in dinoflagellate-cnidarian symbiosis. J Exp Biol 215(pt19):3467–3477. https://doi.org/10.1242/jeb.070946

Cabrales Arellano P, Islas Flores T, Thomé PE, Villanueva MA (2017) Indomethacin reproducibly induces metamorphosis in *Cassiopea xamachana* scyphistomae. PeerJ 5:e2979. https://doi.org/10.7717/peerj.2979

Carabantes N, Cerqueda-García D, García-Maldonado JQ, Thomé PE (2022) Changes in the bacterial community associated with experimental symbiont loss in the mucus layer of *Cassiopea xamachana* jellyfish. Front Mar Sci 9:1–13. https://doi.org/10.3389/fmars.2022.879184

Castrejón V, Carbó R, Martínez M (2007). Mecanismos moleculares que intervienen en el transporte de la Glucosa. Revista de Educación Bioquímica 26(2):49–57. https://www.medigraphic.com/pdfs/revedubio/reb-2007/reb072b.pdf

Colley NJ, Trench RK (1983) Selectivity in phagocytosis and persistence of symbiotic algae by the scyphistoma stage of the jellyfish *Cassiopea xamachana*. Proc R Soc 219(1214):61–82. https://doi.org/10.1098/rspb.1983.0059

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinofagellate symbiosis. Mcirobiol Mol Biol Rev 76(2):229–261. https://doi.org/10.1128/MMBR.05014-11

Deshmukh RK, Vivancos J, Ramakrishnan G, Guérin V, Carpentier G, Sonah H, Labbé C, Isenring P, Belzile FJ, Bélanger RR (2015) A precise spacing between the NPA domains of aquaporins is essential for silicon permeability in plants. Plant J 83(3):489–500. https://doi.org/10.1111/tpj.12904

Díaz HDP, Burgos HLC (2002) ¿Cómo se transporta la glucosa a través de la membrana celular? *IATREIA* 15(3):11. Encyclopaedia de la Vida. 2014. *Cassiopea xamachana* upsidedown jellyfish. (http://eol.org/pages/203396/overview, consulted June 23rd, 2019).

Escher SA, Rasmuson-Lestander A (1999) The *Drosophila* glucose transporter gene: cDNA sequence, phylogenetic comparisons, analysis of functional sites and secondary structures. Hereditas 130(2):95–103. https://doi.org/10.1111/j.1601-5223.1999.00095.x

Fitt WK, Costley K (1998) The role of temperature in survival of the polyp stage of the tropical Rhizostome jellyfish *Cassiopea xamachana*. J Exp Mar Biol Ecol 222(1–2):79–91. https://doi.org/10.1016/S0022-0981(97)00139-1

Fitt WK, Pardy RL (1981) Effects of starvation, and light and dark on the energy metabolism of symbiotic and aposymbiotic sea anemones, *Anthopleura elegantissima*. Mar Biol 61(2–3):199–205. https://doi.org/10.1007/BF00386660

Fitt WK, Trench RK (1983) Endocytosis of the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal by endodermal cells of the scyphistoma of *Cassiopea xamachana*. J Cell Sci 64:195–212. https://doi.org/10.1242/jcs.64.1.195

Garrett TA, Schmeitzel JL, Klein JA, Hwang JJ, Schwarz JA (2013) Comparative lipid profiling of the cnidarian *Aiptasia pallida* and its dinoflagellate symbiont. PLoS ONE 8(3):e57975. https://doi.org/10.1371/journal.pone.0057975

Gordon BR, Leggat W (2010) *Symbiodinium*-invertebrate symbioses and the role of metabolomics. Mar Drugs 8(10):2546–2568. https://doi.org/10.3390/md8102546

Hibuse T, Maeda N, Nagasawa A, Funahashi T (2006) Aquaporins and glycerol metabolism. BBA 1758(8):1004–1011. https://doi.org/10.1016/j.bbamem.2006.01.008

Hillyer KE, Dias D, Lutz A, Roessner U, Davy SK (2017) ^{13}C metabolomics reveals widespread change in carbon fate during coral bleaching. Metabolomics 14(1):12. https://doi.org/10.1007/s11306-017-1306-8

Hofmann DK, Kremer BP (1981) Carbon metabolism and strobilation in *Cassiopea andromeda* (Cnidaria: Schyphozoa): Significance of endosymbiotic dinoflagellates. Mar Biol 65(1):25–33. https://doi.org/10.1007/BF00397064

Imbs AB, Latyshev NA, Dautova TN, Latypov YY (2010) Distribution of lipids and fatty acids in corals by their taxonomic position and presence of zooxanthellae. Mar Ecol Prog Ser 409:65–75. https://doi.org/10.3354/meps08622

Imbs AB, Ermolenko EV, Grigorchuk VP, Sikorskaya TV, Velansky PV (2021) Current progress in lipidomics of marine invertebrates. Mar Drugs 19(12):660. https://doi.org/10.3390/md19120660

Joost HG, Thorens B (2001) The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. Mol Membr Biol 18:247–256. https://doi.org/10.1080/09687680110090456

Joost H, Bell GI, Best JD, Birnbaum MJ, Charron MJ, Chen YT, Doege H, James DE, Lodish HF, Moley KH, Moley JF, Mueckler M, Rogers S, Schu A, Seino S, Thorens B (2002) Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol Endoc M 282(4):E974-976. https://doi.org/10.1152/ajpendo.00407.2001

Kopp C, Domart-Coulon I, Escrig S, Humbel BM, Hignette M, Meibom A (2015) Subcellular investigation of photosynthesis-driven carbon and nitrogen assimilation and utilization in the symbiotic reef coral *Pocillopora damicornis*. Mar Biol 6(1):e02299-e2314. https://doi.org/10.1128/mBio.02299-14

Lampert KP (2016) *Cassiopea* and its zooxanthellae. In: The Cnidaria, Past, Present and Future: The World of Jellyfish and Her Sisters. Eds. S. Goffredo and Z. Dubinsky *Springer Sci Rev* Pp. 415–423. https://link.springer.com/chapter/https://doi.org/10.1007/978-3-319-31305-4_26

Lehnert EM, Mouchka ME, Burriesci MS, Gallo ND, Schwarz JA, Pringle JR (2014) Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. G3: Genes Genomes Genet 4(2):277–295. https://doi.org/10.1534/g3.113.009084.

Lewis DH, Smith DC (1971) The autotrophic nutrition of symbiotic marine coelenterates with special reference to hermatypic corals. I. Movement of photosynthetic products between the symbionts. Proc Royal Soc B 178(1050):111–129. https://doi.org/10.1098/rspb.1971.0055

Li X, Benning C, Kuo MH (2012) Rapid triacylglycerol turnover in *Chlamydomonas reinhardtii* requires a lipase with broad substrate specificity. Eukaryot Cell 11(12):1451–1462. https://doi.org/10.1128/EC.00268-12

Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2-\Delta\Delta CT$ Method. Methods 25(4):402–408. https://doi.org/10.1006/meth.2001.1262

Madeira A, Moura TF, Soveral G (2015) Aquaglyceroporins: implications in adipose biology and obesity. CMLS 72(4):759–771. https://doi.org/10.1007/s00018-014-1773-2

Mandal G, Sharma M, Kruse M, Sander-Juelch C, Munro LA, Wang Y, Vilg JV, Tamás MJ, Bhattacharjee H, Wiese M, Mukhopadhyay R (2012) Modulation of *Leishmania major* aquaglyceroporin activity by a mitogen-activated protein kinase. Mol Microbiol 85(6):1204–1218. https://doi.org/10.1111/j.1365-2958.2012.08169.x

Mashini AG, Oakley CA, Grossman AR, Weis VM, Davy SK (2022) Immunolocalization of metabolite transporter proteins in a model Cnidarian-Dinoflagellate symbiosis. Appl Environ Microbiol 88(12):e00412–22. https://doi.org/10.1128/aem.00412-22

Matthews JL, Crowder CM, Oakley CA, Davy SK (2017) Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. PNAS 114:13194–13199. https://doi.org/10.1073/pnas.17107331

Molina VH, Castillo-Medina RE, Thomé PE (2017) Experimentally induced bleaching in the sea anemone *Exaiptasia*, supports glucose as the main metabolite associated with its symbiosis. J Mar Biol 3:1–7. https://doi.org/10.1155/2017/3130723

Mortillaro JM, Pitt KA, Lee SY, Meziane T (2009) Light intensity influences the production and translocation of fatty acids by

zooxanthellae in the jellyfish Cassiopea sp. J Exp Mar Biol Ecol 378:22–30. https://doi.org/10.1016/j.jembe.2009.07.003

Muscatine L (1967) Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host. Science 156(3774):516–519. https://doi.org/10.1126/science.156.3774.516

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Biosci Res 27:454–460. https://doi.org/10.2307/1297526

Nicot N, Hausman JF, Hoffman L, Evers D (2005) Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. J Exp Botany 56(421):2907–2914. https://doi.org/10.1093/jxb/eri285

Ohdera AH, Abrams MJ, Ames CL, Baker DM, Suescún-Bolívar LP, Collins AG, Freeman CJ, Gamero- Mora E, Goulet TL, Hofmann DK, Jaimes-Becerra A, Long PF, Marques AC, Miller LA, Mydlarz LD, Morandini AC, Newkirk CR, Putri SP, Samson JE, Stampar SN, Steinworth B, Templeman M, Thomé PE, Vlok M., Woodley CM, Wong JCY, Martindale MQ, Fitt WK, Medina M, (2018) Upside-down but headed in the right direction: review of the highly versatile *Cassiopea xamachana* system. Front Ecol Evol 6:35. https://doi.org/10.3389/FEVO.2018.00035

Pawlowski J, Bolivar I, Fahrni JF, DeVargas C, Bowser S, (1999) Molecular evidence that *Reticulomyxa filosa* is a freshwater naked Foraminifer. J Eukaryot Microbiol 46(6):612–617. https://doi.org/10.1111/j.1550-7408.1999.tb05137.x

Park JJ, Wang H, Gargouri M, Deshpande RR, Skepper JN, Holguin FO, Juergens MT, Shachar-Hill Y, Hicks LM, Gang DR (2015) The response of *Chlamydomonas reinhardtii* to nitrogen deprivation: A systems biology analysis. Plant J 81(4):611–624. https://doi.org/10.1111/tpj.12747

Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Voolstra CR, Wild C (2017) Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching. Glob Chang Biol 23:3838–3848. https://doi.org/10.1111/gcb.13695

Rädecker N, Pogoreutz C, Gegner HM, Cárdenas A, Roth F, Bougoure JJ, Guagliardo P, Wild C, Pernice M, Raina J, Meibom A, Voolstra CR (2021) Heat stress destabilizes symbiotic nutrient cycling in corals. PNAS 118(5):e2022653118. https://doi.org/10.1073/pnas.2022653118

Rands ML, Loughman BC, Douglas AE (1993) The symbiotic interface in an alga-invertebrate symbiosis. Proc Royal Soc B 253(1):161–165. https://doi.org/10.1098/rspb.1993.0097

Rojek A, Praetorius J, Frøkiaer J, Nielsen S, Fenton RA (2008) A current view of the mammalian aquaglyceroporins. Annu Rev Physiol 70:301–327. https://doi.org/10.1146/annurev.physiol.70.113006.100452

SAMMO (2021) Universidad Nacional Autónoma de México, Instituto de Ciencias del Mar y Limnología, Servicio Académico de Monitoreo Meteorológico y Oceanográfico, Puerto Morelos Q. Roo México. www.sammo.icmyl.unam.mx. Accessed Oct 2021

Schmitz K, Kremer BP (1977) Carbon fixation and analysis of assimilates in a coral-dinoflagellate symbiosis. Mar Biol 42(4):305–313. https://doi.org/10.1007/BF00402192

Scheepers A, Joost HG, Schürmann (2015) The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. JPEN 28(5):364–371. https://doi.org/10.1177/0148607104028005364

Sproles AE, Kirk NL, Kitchen SA, Oakley CA, Grossman AR, Weis VM, Davy SK (2018) Phylogenetic characterization of transporter proteins in the cnidarian-dinoflagellate symbiosis. Mol Phylogenet Evol 120:307–320. https://doi.org/10.1016/j.ympev.2017.12.007

Tamás MJ, Luyten K, Sutherland FC, Hernandez A, Albertyn J, Valadi H, Li H, Prior BA, Kilian SG, Ramos J, Gustafsson L, Thevelein JM, Hohmann S (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. Mol Microbiol 31(4):1087–1104. https://doi.org/10.1046/j.1365-2958.1999.01248.x

Thornhill DJ, Daniel MW, LaJeunesse TC, Schmidt GW, Fitt WK (2006) Natural infections of aposymbiotic *Cassiopea xamachana* scyphistomae from environmental pools of *Symbiodinium*. J Exp Mar Biol Ecol 338:50–56. https://doi.org/10.1016/j.jembe.2006.06.032

Uldry M, Thorens B (2004) The SLC2 family of facilitated hexose and polyol transporters. Pflug Arch Eur J Physiol 447(5):480–489. https://doi.org/10.1007/s00424-003-1085-0

Von Bülow J, Beitz E (2015) Number and regulation of protozoan aquaporins reflect environmental complexity. Biol Bull 229(1):38–46. https://doi.org/10.1086/BBLv229n1p38

Wakefield TS, Kempf SC (2001) Development of host- and symbiont-specific monoclonal antibodies and confirmation of the origin of the symbiosome membrane in a cnidarian-dinoflagellate symbiosis. Biol Bull Rev 200(2):127–143. https://doi.org/10.2307/1543306

Wood IS, Trayhurn P (2003) Glucose transporters (GLUT and SGLT): Expanded families of sugar transport proteins. BJN 89(1):3–9. https://doi.org/10.1079/BJN2002763

Wright EM (2013) Glucose transport families SLC5 and SLC50. Mol Aspects Med 34(2–3):183–196. https://doi.org/10.1016/j.mam.2012.11.002