Kwansei Gakuin University
Report of Research Outcome

2026/03/17

To President

Department : Science and Technology
Position : Postdoctoral fellow
Name : Matthew Brown

I report the outcome of the research as follows.

Name of the Fund/Program	□Sabbatical leave with grant □Sabbatical leave with no grant □KGU Joint Research □Individual Special Research ☒Postdoctoral fellow ※Please report by designated form as for "International Research Collaboration".
Research Theme	CREST – function of the diatom CO2-concentrating mechanism
Research Site/Venue	Kwansei Gakuin University, Department of Biosciences
Research period	2025/04/01 ～ 2026/03/31 （ 12 month）

◆ **Summary of the research outcome** **(approx. 2,500 words)**

Please write down the outcomes in detail regarding the research theme above.

Our lab's primary research focus has been on the structure and activity of the diatom CO2-concentrating mechanism (CCM). Previous work has identified various molecular components and determined their specific function in terms of importing and transporting carbon through the cell. Carbonic anhydrase (CA) are utilized to convert CO_2 into bicarbonate (HCO_3-) while transporters like bestrophins and SLC pump bicarbonate into the cell and chloroplast. Finally, bicarbonate is converted back into CO_2 by CA to be fixed by the major carbon-fixing enzyme Rubisco. Via a mechanism called liquid-liquid phase separation, Rubisco forms a specialized organelle called a pyrenoid which is essential for high-efficiency carbon fixation. Surrounding the pyrenoid is a specialized protein called PyShell which acts as a proteinaceous barrier, both giving structure to the pyrenoid and preventing the escape of CO_2 before it can be fixed into organic carbon.

My work has focused on two aspects of the CCM. First, while previous research has extensively examined how the different components of the CCM operate under different carbon conditions, none have examined to what degree they are necessary under varying light conditions. In particular, fluctuating light conditions (wherein light intensity varies

over the course of a diel cycle due to movement of cells within the water column, clouds, and the daily course of the sun) is commonly observed in natural systems. Under fluctuating light, cells are constantly moving between conditions of light-limitation and light-saturation. Importantly, this also means that cells are moving between periods of carbon-limitation (when light intensity is high) and carbon-saturation (when light intensity is low). As such, I have studied the importance of various molecular CCM components under fluctuating light conditions. Second, I have tried to contribute to the study of PyShell by complementing knockout mutants lacking the protein with functional versions of it using different molecular approaches. The following is a summary of my research results.

For the first, I have cultivated strains of the diatom *Phaeodactylum tricorntum* lacking different proteins essential for the proper functions of the CCM: specifically the proteins, Theta-Carbonic Anhydrase1, Bestrophin3, and SLC4. Under normal conditions these mutants show notable decreases in photosynthesis due to their impaired ability to transport inorganic carbon into the cell. I have tested both strains under fluctuating light regimes with 1 minute and 5 minute periods, fluctuating between 0 and 600 umol photons m^{-2} s^{-1}. Growth rate was determined via measurements of changes in culture optical density over the course of several days. Data from these experiments showed that the mutant knockout strains for CA1 and BST3 displayed significantly lower growth under fluctuating light, while no difference was observed with SLC4. Subsequent research was focused on BST3 to ascertain any effects on photosynthesis. To determine this, changes in oxygen concentration were measured using a Clark-type oxygen electrode at a constant light level across a range of dissolved inorganic carbon (DIC) concentrations. Cells were suspended in DIC free water and the concentration of DIC was steadily raised using injections of known amounts until O2 production plateaued. From this, it was determined that BST3 mutants showed little to no difference in photosynthesis across DIC compared to wild-type.

Our lab has previously generated knockout strains of *Thalassiosira pseudonana* lacking functional PyShell proteins. To confirm the function of PyShell it is necessary to complement those strains with functional version of the both proteins. To do so, I have been constructing plasmids capable of expressing both proteins under the control of a single promoter by connecting the genes for each using a special 2A peptide. I successfully constructed the plasmids and was able to successfully transform them into our mutant strains. Screening for colonies that contain the plasmid was performed and I isolated a number of potential complement strains. However, it is necessary to determine not just that the plasmids have been taken up by the cells but that the proteins are being properly expressed. To do this, I grew up cultures of both wild-type, mutant, and several of my complement strains and extracted their total protein content. I am currently testing the presence of the proteins using Western Blotting. Confirming the presence of the protein will mean that I have been successful in complementing the strain. Subsequently, I will be testing growth rate and photosynthetic parameters of my complement strains and compare them to wild-type.

Deadline : Within two months after finishing the research period.

Sabbatical leave with grant: Submit this report to President with confirmation by the dean of school you belong to.

※Postdoctoral fellow is required to submit this report with confirmation by the dean of graduate school before the end of employment period.

Where to submit : Organization for Research and Development and Outreach (NUC)

◆We put this report on the web of KGU. If there is any problem about it because of difficulties on your research, please let us know.