BICARBONATE UTILIZATION POTENTIAL (pH DRIFT) STUDY

Objective

The objective of this study is to determine which of the major submersed aquatic plant species of the Comal River are capable of utilizing bicarbonate (HCO_3^-) as a carbon source for photosynthesis. It is projected that under reduced flows and warmer water temperatures, the pH of the rivers may rise resulting in significantly lower carbon dioxide (CO_2) availability than currently experienced.

Study Location & Background

Plant material will be collected from the Comal River and transported to a controlled growth facility at Baylor University. Plants will be cultured under both CO_2-sufficient and CO_2-stressed conditions.

The potential of several species to utilize HCO_3^- will be evaluated by measuring the rate of photosynthesis as a function of pH. HCO_3^- use by Texas wild-rice (*Zizania texana*) has been previously studied using this method by Power and Doyle (2004). This method relies on the well-known speciation of inorganic carbon in water as a function of pH (figure 1).

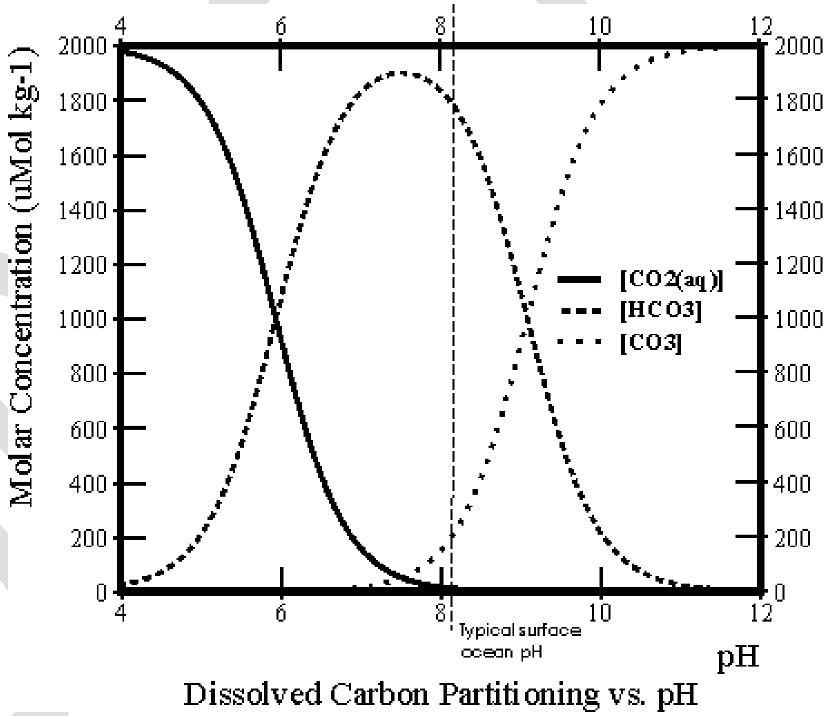

Figure 1: Carbon speciation vs. pH.

All plants can utilize CO_2 for photosynthesis and no plants can directly utilize carbonate (CO_3) as a carbon source. However, some plants can utilize bicarbonate (HCO_3^-) while other plants cannot.

One of the most straightforward ways of determining HCO_3^- utilization is to see how far an aquatic plant can drive (drift) the pH when photosynthesizing in a closed system. As aquatic plants photosynthesize, they consume inorganic carbon and drive the pH up as a result of reduced carbonic acid in equilibrium. Species that are obligate CO_2 users (cannot utilize HCO_3^-)

stop photosynthesizing at a pH where CO_2 disappears from solution (typically pH of 8.8-9.1) depending on temperature and carbonate alkalinity of the water). Species that can utilize HCO_3^- can continue photosynthesis to a much higher pH, typically somewhere between 9.5-10.5 depending on the species affinity for HCO_3^- (some species are better at utilizing HCO_3^- than others).

Figure 2 shows the response observed by Power and Doyle (2004) indicating that Texas wild-rice photosynthesis stops as the pH reaches the point where CO_2 disappears. The zero intercept at the point where CO_2 disappeared is evidence that this species cannot utilize bicarbonate.

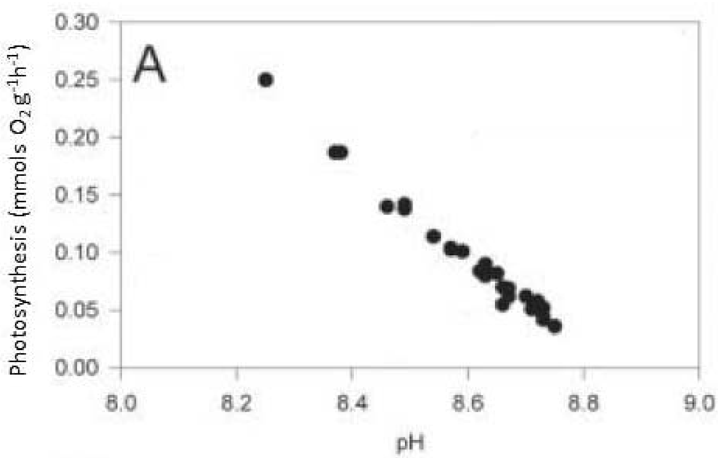

Figure 2: TX wild-rice photosynthesis vs. pH (from Power & Doyle, 2004)

Unfortunately, comparable data for other native and non-native submersed species on the Comal and San Marcos Rivers does not exist, hindering projections of which species are likely to survive and dominate under altered flow and temperature regimes.

Materials and Methods

Plants collected from the Comal River will be returned to the labs at Baylor University and analyzed for bicarbonate utilization potential within 72 hours of collection. Plants will be maintained at river temperatures and under light-sufficient for growth. We will utilize a method similar to that of Power and Doyle (2004). Oxygen and pH will be measured repeatedly in a closed system to allow estimation of apparent photosynthesis as a function of pH. The alkalinity of the solution used will be determined by titration with dilute hydrochloric acid according to standard methods. The total inorganic carbon (C_T) as well as the speciation of inorganic carbon as CO_2, HCO_3 or CO_3 will be made by standard methods based on known alkalinity, pH and temperature. The pH endpoint for each species will vary depending on the ability of that species to utilize bicarbonate as well as the overall affinity for bicarbonate (if any). The ratio of total inorganic carbon (C_T) of the solution is only modestly impacted by removal of CO_2 (typically <

5%) while removal of HCO_3 has a much stronger impact on C_T. Neither impact the overall alkalinity of the waters since the buffering capacity lost by inorganic carbon consumed is balanced by production of OH^- ion. Consequently, the C_T:Alk ratio is an excellent and sensitive indicator of bicarbonate utilization. The ratio for obligate CO_2 users is very close to 1.0, while that of bicarbonate users is well below 1.0.

The target species for this study include *Hygrophila*, *Ludwigia*, *Cabomba*, *Vallisneria*, *Sagittaria* and one or more of the bryophytes in the river. Additional species will be considered as time and resources allow. The assays will be replicated at least five times for each species.

In order to determine if growth under CO_2-stressed conditions induces the physiological ability to utilize bicarbonate, each of the species tested will also be grown under CO_2 stressed conditions. These conditions will either be simulated at the Baylor Aquatic Research labs or in conjunction with the Low-Flow Threshold study being conducted at the San Marcos Aquatic Resource Center (ARC) in San Marcos, TX. Following at least two weeks of exposure to CO_2 stressed conditions, the plants will be assayed for bicarbonate utilization potential as described above. At least five replicate analyses will be made for each species tested.

Data analyses

The affinity of each species for bicarbonate under current river flow conditions will be evaluated from the results of the assays conducted immediately upon collection from the river. The key response variable will be the C_T:Alk ratio at the pH at which photosynthesis ceases as an indicator of bicarbonate utilization potential. To evaluate if bicarbonate utilization potential was induced due to growth under CO_2 stressed conditions, we will compare the bicarbonate utilization affinity before and after two weeks of growth under CO_2 stressed conditions.

Comparison among species will be made by one way analysis of variance (ANOVA) following data normalization (if necessary). The response variable will be the C_T:Alk ratio at which each species ceases photosynthesis. Significant ANOVA's will be followed by a Tukey's test to evaluate which species have significantly different bicarbonate affinities.