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# A tribute to George Bowes: linking terrestrial and aquatic botany

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George E Bowes was brought up in England and finished his PhD in 1967 at the University of London. He then 'crossed the pond' to take a postdoctoral position at the University of Illinois where he worked with Bill Ogren, followed by a year as a Carnegie Fellow at the Carnegie Institute at Stanford University. In 1972 he moved to the Botany Department at the University of Florida where he spent the rest of his career.

It was at Illinois that he and Bill Ogren undertook the defining work on Ribulose-bis-phosphate carboxylase-oxygenase (rubisco), discovering the oxygenase reaction and its role in photorespiration, and thus explaining the oxygen effect on photosynthesis in $C_3$ plants (Bowes et al., 1971; Ogren and Bowes, 1971). They also found that, while rubisco is inhibited by oxygen, phospho*enol*carboxylase (PEPC) is not, explaining the differential effect of oxygen on $C_3$ and $C_4$ plant photosynthesis (Bowes and Ogren, 1972). Bill Ogren (Ogren, 2003) provides some fascinating information about the difficulties he and George faced in publishing this work and persuading the photosynthesis community that it was correct. It is hard, now, to overestimate the importance of this work. The name of the enzyme (then ribulose-diphosphate carboxylase) was changed to recognize its oxygenase activity but more importantly this fundamental discovery altered the course of photosynthesis research and influenced many fields including plant and algal ecology and physiology, biochemistry and biogeochemistry, climate change and food security.

Shortly after joining the University of Florida, George began to study aquatic plant photosynthesis. His research trajectory was initiated by the discovery of 1) differing $CO_2$ compensation points among several aquatic species, and 2) the observation with Scott Holaday of environmental impacts on intraspecific variation in $CO_2$ compensation points (Van et al., 1976). His graduate students, Jocelyne Ascencio and Mike Salvucci, found that increased temperature and photoperiod reduced the $CO_2$ compensation point  and photorespiration in the submerged macrophyte *Hydrilla verticillata* (Salvucci and Bowes, 1981; Ascencio and Bowes, 1983). The reduced compensation point was associated with increased activity of PEPC, and several enzymes associated with $C_4$ photosynthesis, and decreased activity of photorespiratory enzymes (Holaday and Bowes, 1980; Salvucci and Bowes, 1981). Finally, pulse-chase results suggested that *Hydrilla* had a $C_4$-type photosynthetic system (Holaday and Bowes, 1980; Salvucci and Bowes, 1983). An interesting area of complexity opened up when they found that another submerged macrophyte, *Myriophyllum spicatum*, lacked the requisite enzyme activities, despite showing the physiological characteristics of $C_4$ photosynthesis. Experiments with an inhibitor of carbonic anhydrase suggested that this enzyme facilitates bicarbonate use as part of a $CO_2$ concentrating mechanism (Salvucci and Bowes, 1982, 1983) underlining the diversity in the way that freshwater macrophytes acquire inorganic carbon for photosynthesis. Subsequent studies on *Hydrilla* indicated that Kranz anatomy, typical of many terrestrial $C_4$ plants, was lacking. Immunogold labeling showed that there was an intracellular separation of Rubisco and PEPC in leaves of $C_4$-type plants: rubisco was confined to the chloroplasts and PEPC to the cytosol, i.e., there was an intracellular separation of the $C_4$ and Calvin cycles (Reiskind et al., 1989). Later studies demonstrated that the chloroplast was the site where $CO_2$ was concentrated, with $CO_2$ concentrations estimated to be 400 μM (Reiskind et al., 1997). *Hydrilla* was the first known single-cell $C_4$ system, although this has been found subsequently in certain terrestrial $C_4$ plants (Voznesenskaya et al., 2001; Edwards et al., 2004).

In the mid-1990s George made a leap into studying the molecular details of the *Hydrilla* $C_4$ system. Three key $C_4$-enzymes: PEPC, pyruvate Pi dikinase (PPdK) and NADP-malic enzyme (NADP-ME) are up-regulated during induction of the $C_4$-photosynthetic state. Three

PEPC isoforms were identified in *Hydrilla* (Magnin et al., 1997; Rao et al., 2002). One of these forms was up-regulated during induction, only expressed in $C_4$-type leaves and had the kinetic characteristics of the $C_4$-isoform (Rao et al., 2008). Terrestrial $C_4$-PEPC isoforms possess a serine moiety at the amino acid position 774 near the carboxy terminus, based on the *Flaveria* sequence (Blasing et al., 2000). The *Hydrilla* $C_4$-PEPC isoform contains alanine at this position like all $C_3$-isoforms, despite being similar kinetically to the $C_4$-isoform (Rao et al., 2008). Two isoforms of PPdK and three isoforms of NADP-ME were also detected (Rao et al., 2006a; Rao et al., 2006b; Estavillo et al., 2007). One NADP-ME isoform was from the chloroplast, up-regulated in the light with kinetic characteristics intermediate between $C_3$ and $C_4$ NADP-ME isoforms of terrestrial plants (Estavillo et al., 2007). Over many years, work by George Bowes and his co-workers have established *Hydrilla* as a $C_4$-NADP-ME plant and it is now one of the most completely studied $C_4$ plants on the planet.

Although continuing to work on freshwater aquatics, George also dived into the sea by making annual collecting 'cruises' to the Bahamas and the Florida Keys. Two coenocytic green macroalgae became the focus of this work: *Codium decorticatum* and *Udotea flabellum*. Physiological studies showed that photorespiration in *Udotea* was low, as was PEPC activity (Reiskind et al., 1988). However, the activities of phospho*enol*pyruvate carboxykinase (PEPCK) in both carboxylating and decarboxylating modes and the activities of the other requisite $C_4$-cycle enzymes were sufficiently high to allow a $C_4$-like photosynthetic system to operate (Reiskind et al., 1988). Treatment with a PEPCK inhibitor resulted in reduced photosynthetic rates, increased $O_2$ sensitivity and reduced labeling of $C_4$-acids as initial products of photosynthesis, suggesting that *Udotea* operated a $C_4$ system based on PEPCK with a spatial separation of carboxylase (cytosol) and decarboxylase (chloroplast) activities (Reiskind and

Bowes, 1991). In an evolutionary sense, the $C_4$ system in *Udotea* appears to be the oldest known of any photoautotroph. George continues to be active in marine research and has produced a recent review with colleagues from around Florida on the impact of global climate change on ocean acidification, and its effect on seagrasses and macroalgae (Koch et al., 2013). Meanwhile, George's interest in rubisco continued in the terrestrial environment in collaboration with Mike Salvucci and Gabriel Holbrook, and several agronomists at the University of Florida. Working on *Nicotiana rustica*, Mike, Gabe and George reported a chloroplastic phosphatase that in the light, and particularly in the presence of NADPH, degrades a naturally occurring inhibitor of rubisco activity, Carboxy-arabintol-1 phosphate (Holbrook et al., 1989; Salvucci and Holbrook, 1989). This was another major discovery on the fundamental mechanisms of photosynthesis.

One key concern today that requires a knowledge of plant ecophysiology and biochemistry, is the adequate production of food to support the growing human population against the background of a changing climate. The current concentration of 400 ppm $CO_2$, up 75 ppm from the late 1960s, will tend to increase rates of photosynthesis as the oxygenase function of rubisco will be suppressed, decreasing photorespiratory $CO_2$ loss and increasing ATP/NADPH redirection to photosynthetic assimilation (Bowes, 1991; Long et al., 2004). Rising temperature, however, may have a negative effect in some areas. A comparison of two rice cultivars exposed to 350 and 700 ppm $CO_2$ under varying day/night temperature regimes showed that, while photosystem II efficiency was largely unaffected, rubisco gene expression, protein content and activity were adversely affected by elevated temperature (Gesch et al., 2003). Leaf photosynthetic rates were negatively impacted at the higher $CO_2$ concentration and highest temperature regime with some cultivar differences (Gesch et al., 2003). Agricultural productivity could be increased further if the low photorespiratory rates and potentially higher productivity of

$C_4$ plants could be transferred into current important $C_3$ crops, such as rice. George has worked actively in this area because *Hydrilla*, as a single-cell $C_4$ plant that lacks the structural complexity of Kranz anatomy, is an excellent model for engineering a $C_3$ plant with $C_4$ characteristics (Bowes et al., 2002; Bowes, 2011).

In addition to George's scientific achievements, he was an excellent teacher of undergraduate courses teaching not only the subject but also how to think scientifically and how to ask and answer scientific questions. George's research lab was a mini United Nations with representatives from around the world. This led to many collaborations, which are reflected in the contributions in this issue. It was also a fertile ground for many jokes, especially between the Yanks and the Brits, and a war fought some 235 years ago. Despite all of the above, George found time to serve as Chair of the Botany Department (1998 – 2006). Faculty members describe him as a fair and effective leader with the ability to maintain a balance among the different disciplines.

Of course, this particular issue is also celebrating another aspect of George's academic life, the editing of the scientific journal *Aquatic Botany*. George served on the editorial board from 1982 before taking over as Editor in Chief in 1995 when J.M.A. Brown retired. He stood down in 2013 after 19 years as editor. One feature of George's career is his contribution to terrestrial, freshwater and marine science, which is proof that excellent science can cross scientific disciplines. We have tried to represent some of this diversity in this Special Issue.

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