$\delta^{13}C$ and N Contents of Two Aquatic Plants, *Spaganium fallax* and *Schenoplectus mucronatus*, in a Subtropic Mountainous Lake

Wen-Yuan Kao[1,2*]

1. Institute of Ecology and Evolutionary Biology, 1 Roosevelt Rd., Sec. 4, Taipei 106, Taiwan.
2. Department of Life Science, 1 Roosevelt Rd., Sec. 4, Taipei 106, Taiwan.
** Coresponding author. Email: wykao@ntu.edu.tw*

(Manuscript received 3 September 2009; accepted 2 November 2009)

ABSTRACT: *Spaganium fallax* and *Schoenoplectus mucronatus* subsp. *robustus* are emergent monocot plants dominating a subtropical mountainous lake, the Yuanyang Lake (YYL), in Taiwan. The photosynthetic pathways and the ecophysiology of these two species were studied in this study. I first analyzed $\delta^{13}C$ of fractions of *S. fallax* (submerged leaves and roots of seedlings, and submerged and emergent parts of leaves, and roots of mature plants) and *S. mucronatus* (emergent culms and roots of mature plants) to identify their photosynthetic pathway. I then investigated monthly variation in $\delta^{13}C$ and nitrogen content of emergent parts of the two species during 2003 to evaluate the integrated photosynthetic response. The leaf carbon isotope ratio of both emergent plants was within the range of most C_3 plants indicating that they used C_3 photosynthetic pathway. Similar pattern of seasonal variation in leaf nitrogen content and $\delta^{13}C$ values was found. Spring leaves (or culms in *S. mucronatus*) had significantly higher leaf nitrogen content and more positive $\delta^{13}C$ values than summer leaves (or culms). Consequently, there are significantly positive correlations between $\delta^{13}C$ and leaf nitrogen content in both species. The result suggests that changes in photosynthetic capacity might contribute to the seasonal variation in $\delta^{13}C$ in both species. In comparison between two species, *S. fallax* had significantly more positive $\delta^{13}C$ and higher leaf nitrogen content than *S. mucronatus* during most of the sampling months. The higher leaf nitrogen content found in *S. fallax* might also contribute to its more positive $\delta^{13}C$ values than *S. mucronatus*.

KEY WORDS: Yuanyang Lake Natural Preserve, *Spaganium fallax*, *Schoenoplectus mucronatus* subsp. *robustus,* nitrogen content, stable carbon isotope ratio.

INTRODUCTION

Because of loss of suitable habitats, the number of aquatic plant species worldwide has greatly reduced in the past decades. Improving our understanding of aquatic plants and their habitats is a critical first step in helping to combat the losses. Unfortunately, compared to ecological studies on terrestrial plants, those of aquatic plants are relatively rare. Research on aquatic plants distributed in mountain lake is even less.

Sparganium, a genus of monocot plant containing about 20 species, is distributed mainly in temperate regions. There is only one species of *Sparganium*, i.e. *S. fallax*, in Taiwan and it is distributed in mountain lakes at mid elevation (Yang et al., 2001). The Yuanyang Lake (YYL) Nature Preserve is one of the six Taiwan Long-Term Ecological Research sites. One of the reason for declaration of the area as a preserve is to protect the emergent plant *S. fallax* Graebn. In the lake, *S. fallax* is neighbored by *Schoenoplectus mucronatus* subsp. *robustus* (Chou et al., 2000), also a monocot plant. *Schenoplectus* is a genus of about 80 species with a cosmopolitan distribution. Seven species of *Schenoplectus* are reported in Taiwan and are mainly distributed in wetlands from low to mid elevation (Yang et al., 2001). To my knowledge, the photosynthetic performance of these two species has not been studied. The objectives of this study were to understand their photosynthetic pathway and to compare the ecophysiology of these two emergent plants in the subtropical mountainous lake, YYL.

Two stable carbon isotopes, ^{12}C and ^{13}C, are found in nature. The relative content of ^{12}C and ^{13}C in a matter is generally expressed as $\delta^{13}C$ values. During the process of photosynthetic carboxylation, the carboxylation enzyme ribulose bisphosphate carboxylase (rubisco) discriminates against ^{13}C more than phosphoenol pyruvate carboxylase (O'Leary, 1981; Roeske and O'Leary, 1984). Consequently, C_3 plants contain less ^{13}C than C_4 plants. Among plants measured, $\delta^{13}C$ values of C_3 plants vary from -34 to -23 ‰, and those of C_4 plants approximately -15 to -7 ‰ (O'Leary, 1988). CAM plants use the same two carboxylating enzymes as do C_4 plants. Hence, obligate-CAM species tend to have $\delta^{13}C$ values similar to C_4 plants, while facultative-CAM plants intermediate between C_4 and C_3 plants (-22 to -10 ‰). Compared to those of terrestrial plants, $\delta^{13}C$ values of submerged plants are more complicated by the possible different forms of C utilized and the variation in $\delta^{13}C$ of these sources C (Keeley, 1988). Analyses of $\delta^{13}C$ of submerged plants might provide information about their carbon source in addition to the photosynthetic pathways.

In addition for providing a powerful method for determining the pathway of CO_2 fixation of terrestrial

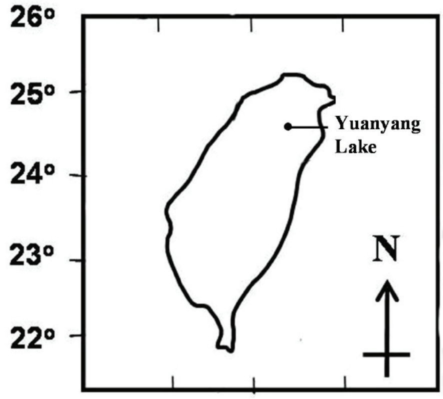

Fig. 1. The location of Yuanyang Lake Natural Preserve in Taiwan.

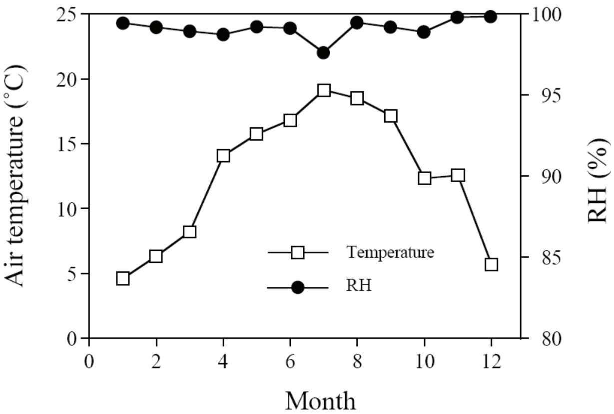

Fig. 2. The monthly average of air temperature and relative humidity at Yuanyang Lake, 2003.

plants, stable carbon isotopes analysis has also become a widely used tool in plant physiological ecology after the demonstration that $\delta^{13}C$ values in C_3 plants are a reliable long-term measure of the ratio of intercellular (C_i) to ambient (C_a) atmospheric concentrations of CO_2 (Farquhar et al., 1982). The ratio of Ci/Ca, estimated from gas exchange measurement, is often used as an indicator of instantaneous photosynthetic water use efficiency (Jones, 1992).

To obtain an integrated picture of physiological and year-round photosynthetic responses of *S. fallax* and *S. mucronatus* in the YYL, in addition to analyze $\delta^{13}C$ values of fractions of the two emergent plants, I also investigated monthly variation in the ratio and nitrogen contents in emergent part of the two plants. Specifically, I asked following questions. (1) What are the $\delta^{13}C$ values of the two aquatic plants? Are there variation in $\delta^{13}C$ in plant parts and compartments of these two aquatic plants? (2) Is there seasonal variation in the $\delta^{13}C$ in the emergent part of the two aquatic plants? If so, what factors might contribute to the seasonal variation?

MATERIALS AND METHODS

Schoenoplectus mucronatus subsp. *robustus* and *Sparganium fallax* were sampled from the Yuanyang Lake, Taiwan (Fig. 1). The Yuanyang Lake Natural Preserve (24º 35' N, 121º 24' E) is located in the northern part of Hsinchu County in Taiwan. The monthly mean air temperature ranged from 5 to 17.5 ºC, and the mean annual rainfall was ca. 3000 mm (Hwang et al., 1996). The climate of the area is classified as temperate heavy moist (Liu and Hsu, 1973). The Yuanyang Lake (YYL), an oligotropic lake at elevation of 1670m, is at the edge of the Nature Reserve. *S. fallax* and *S. mucronatus* dominated the lake habitat with the former covering ca. 1.6 ha and the later 0.6 ha of the area (Hwang et al., 1996). In YYL, *S. fallax* grew in water less than 2 m deep, seedlings were submerged in the water, while mature plants had emergent leaves with some lower part remaining submerged. *S. mucronatus* populations occupied marsh area and at the borders of the lake (Huang et al., 1996). In contrast to *S. fallax*, most of populations of *S. mucronatus* with only roots immersed in the water, while shoot were above water surface. The leaves of *S. mucronatus* are much reduced and culms are the dominant part (Yang et al., 2001).

A survey was conducted in June of 2002 to determine the $\delta^{13}C$ values and to investigate if there was variation in $\delta^{13}C$ among organs and different growth stage of both plants. Submerged seedlings (n = 3) of *S. fallax* were sampled and fractionated into submerged leaves and roots while mature plants (n = 3) into emergent part and submersed part of leaves, and roots. In contrast, due to most of populations of *S. mucronatus* with only roots immersed in the water, while shoot (mainly culm) above water surface, mature plants (n =3) were fractionated into emergent culms and roots. Fraction of samples were rinsed with distilled water, oven dried (60 ºC) for at least 48 hr and then ground to a fine powder with an electric grinder.

To investigate if there was seasonal variation in leaf $\delta^{13}C$, five fully developed, healthy and emergent part from five populations of each species were collected monthly in 2003. Leaves were processed as described above. The air temperature and relative humidity were recorded (Fig. 2) in a meteorological station established in 1992.

Stable carbon isotope and leaf nitrogen content analyses were conducted by a continuous flow system in which an elemental analyzer (NA 1500, Fison, Italy) was connected to an isotope ratio mass spectrometer (Delta S, Finnigan Mat, Germany). $\delta^{13}C$ were calculated as: $\delta^{13}C$ (‰) = [(R_{sample} / $R_{standard}$) – 1] * 1000, where R is the ratio of heavy-to-light isotope. The universally agreed standard for ^{13}C is Pee Dee Belemnite (R = 0.011237) (Hayes, 1983).

Table 1. Mean ¹³C (‰) values of fractions of sympatric *S. fallax* and *S. mucronatus* plants in Yuanyang Lake, Taiwan.

Plant	Mean ± s.e.
S. fallx	
Seedling	
Submerged leaf	-27.2 ± 0.3[a]
Root	-26.5 ± 0.3[a]
Mature plant	
Emergent leaf	-28.6 ± 0.4[a]
Submerged leaf	-28.2 ± 0.7[ac]
Root	-28.0 ± 0.6[ac]
S. mucronatus	
Emergent culm	-30.8 ± 0.3[b]
Roots	-29.3 ± 0.1[bc]

Means followed by different superscripts are significant at P = 0.05 (Tukey's test)

RESULTS

δ^{13}C of plant fractions: Table 1 shows δ^{13}C of fractions of *S. fallax* and *S. mucronatus*. In *S. fallax*, no significant difference was found in δ^{13}C values among submerged leaves of seedlings, emergent and submerged leaves of mature plants.

In both species roots had slightly more positive δ^{13}C values than emergent parts, the enrichment was 1-2 ‰ (average 1.5 ‰) in *S. mucronatus*, while 0.1-1.0 ‰ (average 0.6 ‰) in *S. fallax*.

In comparison between roots or emergent parts of two species, *S. fallax* had significantly more positive δ^{13}C values than *S.mucronatus*.

Monthly variation in leaf N content and δ^{13}C: Seasonal variation in nitrogen content and δ^{13}C values of emergent parts was found and the pattern was similar between the two species (Fig. 3). In general, leaves (or culms in *S. mucronatus*) had higher N content in Spring (Feb., March and April) than in Summer months (June, July and August) (Fig. 3A). The difference in N contents between the two seasons was greater in *S. mucronatus* than that in *S. fallax*. In comparison of emergent parts between two species, *S. fallax* had significantly higher N content than *S .mucronatus* during most of the sampling months, except in January, April and May both species had similar N contents, while in December *S. fallax* showed significantly lower N contents than *S. mucronatus*.

Both species also had seasonal variation in δ^{13}C values of emergent parts (Fig. 3B), the pattern was similar to that of nitrogen content. Emergent part had more positive δ^{13}C in spring than in summer. In comparison between two species, *S. fallax* had significantly more positive. δ^{13}C than *S. mucronatus* during most of the sampling months, except in December during the period *S. fallax* showed significantly more negative δ^{13}C than *S. mucronatus*.

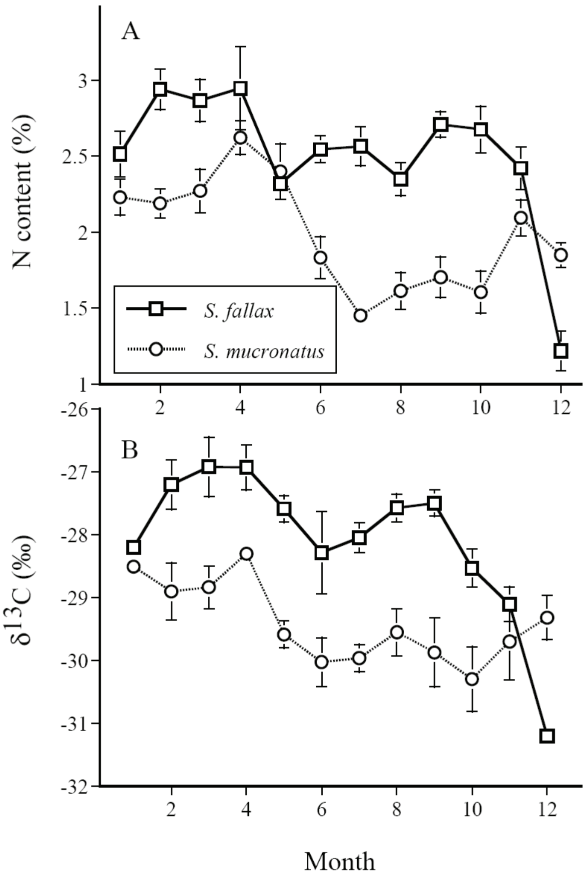

Fig. 3. The monthly average of leaf (or culm) nitrogen contents (A) and δ^{13}C value (B) of *S. fallax* and *S. mucronatus* in Yuanyang Lake, 2003.

The relationship between N contents and δ^{13}C: When δ^{13}C of emergent parts was plotted against corresponding nitrogen content, significantly positive, linear relationships were found between monthly average N content and δ^{13}C for both species (Fig. 4).

DISCUSSION

Though some aquatic plants have been shown to utilized CAM or C_4 photosynthetic pathways when in submerged condition (Robe and Griffiths, 2000, Ueno, 1996), the carbon isotope ratio measured for fractions of both aquatic species (Table 1) or for emergent parts year round (Fig. 3) were all within the range of most C_3 plants (O'Leary, 1988) indicating that they used C_3 photosynthetic pathway. In addition, the result that submerged leaves and emergent leaves of *S. fallax* had similar δ^{13}C values (Table 1) indicated that the δ^{13}C value of carbon source utilized by submerged leaves was not significantly different from that uptake by emergent leaves.

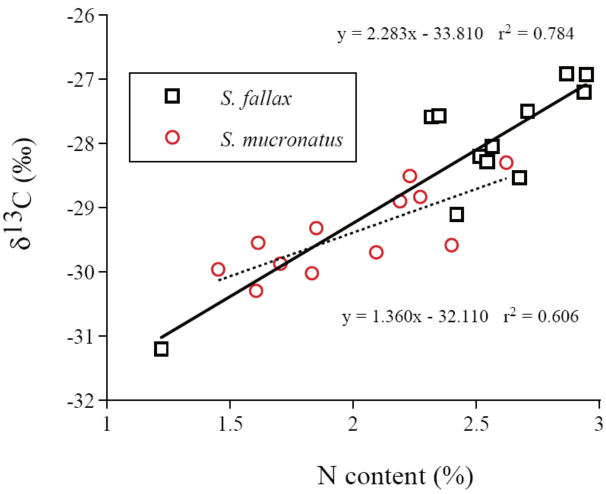

Fig. 4. Relationships between monthly average of leaf (or culm) nitrogen contents and δ^{13}C value of *S. fallax* and *S. mucronatus* in Yuanyang Lake, 2003.

Both aquatic plants had significantly higher N content in spring than in summer (Fig. 3A). The result is consistent with a previous study by Hwang et al. (1996). The authors found that an increase in nitrogen content of above ground tissue corresponded to the onset of growth of both species in spring. In their study, they also found a similar trend in below ground tissue of the aquatic plants. Accordingly, it is suggested that the increase in nitrogen content of emergent parts in spring was not due to the redistribution of the nutrient between above- and below-ground tissues, however, an increasing in nitrogen uptake might contribute to the result.

Significant differences in leaf δ^{13}C between seasons and between plant species were found in the aquatic plants (Fig. 3B). Farquhar et al. (1982) developed an equation to explain the variation in leaf δ^{13}C value in C_3 plants:

$$\delta^{13}C_{leaf} = \delta^{13}C_{atm} - a - (b-a)^* C_i/C_a ,$$

where $\delta^{13}C_{atm}$ is the isotopic ratio of CO_2 available to leaves, a is the fractionation caused by diffusion (4.4 ‰), b is the fractionation associated with carbon fixation (27 ‰), C_i is the intercellular CO_2 concentration and C_a ambient CO_2 concentration. According to the equation, δ^{13}C of leaves is enriched when photosynthetic gas exchange is more limited by leaf conductance relative to carboxylation (or photosynthetic demand), i.e. a lower ratio of C_i/C_a. In contrast, if diffusion limitation is reduced, the relative influence of enzymatic discrimination increases favoring ^{13}C depletion during fixation, would result in leaves of more negative δ^{13}C values. Accordingly, leaf δ^{13}C can be used as an indictor reflecting the long-term, integrated effect of factors affecting inward CO_2 diffusion (leaf conductance) and CO_2 consumption (carboxylation) (Farquhar et al., 1989; Ehleringer et al., 1993). According to the mechanisms of ^{13}C discrimination during photosynthesis, the result that spring leaves (or culms) had more positive δ^{13}C values than summer leaves (or culms) implied that leaves (or culms) in spring time operated at a lower C_i/C_a ratio than those in summer season. A decrease in water availability or a large vapor pressure deficit (VPD) would decrease stomatal conductance and reduce C_i/C_a (Schulze et al., 1987). However, these two conditions do not seem to exist in the study site. Because both plants were rooted in sediment containing saturation water year round, they were unlikely to be limited by soil moisture. A large VPD was not expected in the YYL area where the air humidity approached saturation for most of time during the year (Fig. 2). Hence, the more positive leaf (or culm) δ^{13}C in spring was unlikely resulting from drought or VPD effect. An increase in photosynthetic capacity (carboxylation efficiency) would also result in a reduction in C_i/C_a ratio (Korner et al., 1988). The result that there were significantly positive correlation between monthly mean δ^{13}C and leaf (or culm) nitrogen content in both species (Fig. 4) suggests that seasonal variation in δ^{13}C might due to changes in photosynthetic capacity. Accordingly, nutrients may have dictating the effect on leaf (or culm) δ^{13}C of both species. Similarly, Schulze et al. (1998) found that variation in leaf N determined community-averaged leaf δ^{13}C in northern Australia. Compared to low-light grown leaves, high-light grown leaves have high nitrogen content and more positive δ^{13}C values (Ehleringer et al., 1986). Thus, it is possible that seasonal variation in leaf δ^{13}C might be caused by seasonal changes in light intensity. In this study, I did not measure this environmental factor. However, Huang et al. (1996) reported that the area received more daily solar radiation in summer than in spring. Accordingly, the seasonal variation in leaf (or culm) nitrogen content and δ^{13}C was unlikely caused by changes in light intensity. Water temperature is another possible factor would affect the nitrogen metabolism and photosynthetic activity of aquatic plants. In the present study, the water temperature was not monitored. This factor should be included in the future study.

Because majority of leaf nitrogen content is bound in photosynthetic enzyme, leaf photosynthetic capacity is generally linear related to leaf nitrogen content in wild plants (Field and Mooney, 1986). The result that *S. falalx* had significantly higher leaf N content than *S. mucronatus* during most of the sampling period (Fig. 3A) suggests that the former might have a higher photosynthetic capacity than the later. This might also explain why *S. fallax* had higher annual production than *S. mucronatus* found by Huang et al. (1996).

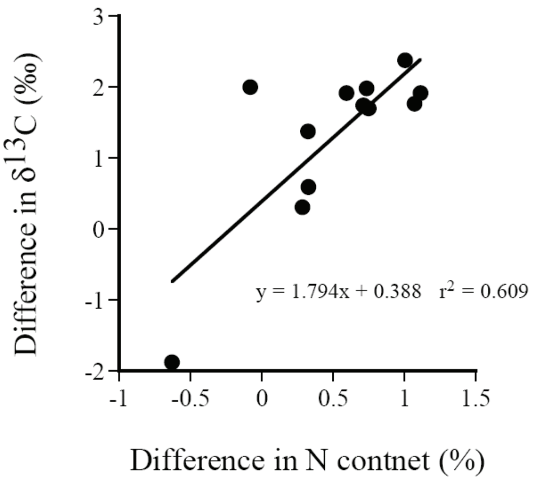

Fig. 5. The relationship between the difference in δ^{13}C and that in nitrogen content between *S. fallax* and *S. mucronatus* for each sampling month in 2003.

In addition to have a higher N content, *S fallax* also had significantly more positive δ^{13}C value than *S. mucronatus* during most of the sampling period (Fig. 3B). To explore the possible reason for the difference, I analyzed the difference in δ^{13}C (Δδ^{13}C) and that in nitrogen content (ΔN) between the two species for each sampling month. A significantly positive, liner relationship between Δδ^{13}C and ΔN was found (Fig. 4). Thus, higher nitrogen content in S. *fallax* might contribute to its more positive δ^{13}C values than *S. mucronatus*. With higher leaf nitrogen content, *S. fallax* might have a higher photosynthetic capacity resulting in a more positive δ^{13}C than *S. mucronatus*.

In conclusion, stable carbon isotope analysis indicated that the two dominant aquatic plants, *S. fallax* and *S. mucronatus*, in YYL used C_3 photosynthetic pathway. Though soil moisture availability and atmospheric moisture were constant in the study site, seasonal variations in leaf δ^{13}C and foliar nitrogen content were found in both aquatic plants. There is positive correlation between leaf (or culm) δ^{13}C and leaf (or culm) nitrogen contents implying that changes in photosynthetic capacity contributed to the seasonal variation in δ^{13}C in both species. The higher leaf nitrogen content found in *S. fallax* might also contribute to its more positive δ^{13}C values than *S. mucronatus*.

LITERATURE CITED

Berry, J. A. 1989. Studies of mechanisms affecting the fractionation of carbon isotopes in photosynthesis. In: Rundel, P. W. J. R. Ehleringer and K. A. Nagy (eds), Stable Isotopes in Ecological Research. Springer-Verlag, New York, USA. pp. 82-94.

Chou, C.-H., T.-Y. Chen, C.-C. Liao and C.-I Peng. 2000. Long-term ecological research in the Yuanyang Lake forest ecosystem. I. Vegetation composition and analysis. Bot. Bull. Acad. Sin. **41**: 61-72.

Ehleringer, J. R., C. B. Field, Z. F. Lin and C.-Y. Kuo. 1986. Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. Oecologia **70**: 520-526.

Farquhar, G. D., M. H. O'Leary and J. A. Berry. 1982. On the relaltionship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. Aust. J. Plant Physiol. **9**: 121-137.

Field, C. and H. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnish, T. J. (ed.), On the Economy of Plant Form and Function. Cambridge University Press, Cambridge. pp. 25-55.

Hays, J. M. 1983. Practice and principles of isotopic measurements in organic geochemistry. In: Meinschein, W.G. (ed.), Organic Geochemistry of Contemporaneous and Ancient Sediments. Society of Economic Paleontologists and Mineralogists, Bloomington, Indiana. pp. 5-31.

Huang, Y.-H., C.-W. Fan and M.-H. Yin. 1996. Primary production and chemical composition of emergent aquatic macrophytes, *Schoenoplectus mucronatus* spp. *robustus* and *Sparganium fallax*, in Lake Yuan-Yang, Taiwan. Bot. Bull. Acad. Sin. **37**: 265-273.

Kao, W.-Y, C.-S. Lu and Y.-C. Chang. 2004. Foliar nutrient dynamics of five dominant plant species in Yuanyang Lake Nature Preserve, Taiwan. Taiwania **49**: 49-56.

Keeley, J. E. 1989. Stable carbon isotopes in vernal pool aquatics of differing photosynthetic pathways. In: Rundel, P.W., J.R. Ehleringer and K.A. Nagy (eds.), Stable Isotopes in Ecological Research. Springer-Verlag, New York. pp. 82-94.

Korner, C, G. D. Farquhar and Z. Roksandic. 1988. A global survey of carbon isotope discrimination in plants from high altitude. Oecologia **74**: 623-632.

Liu, T. and K.-S. Hsu. 1973. Ecological study on Yuen-yang Lake Natural Area Reserve. Bull. Taiwan For. Res. Inst. **237**: 1-32.

Jones, H. G. 1992. Plants and Microclimate. A quantitative approach to environmental plant physiology, Cambridge University Press, Cambridge.

O'Leary, M. H. 1981. Carbon isotope fractionation in plants. Phytochemistry **20**: 553-567.

O'Leary, M. H. 1988. Carbon isotopes in photosynthesis. BioScience **38**: 328-336.

Robe, W. E. and H. Griffiths. 2000. Physiological and photosynthetic plasticity in the amphibious, freshwater plant, *Littorella uniflora*, during the transition form aquatic to dry terrestrial environments. Plnat, Cell and Environemnt **23**: 1041-1054.

Roeske, C. A. and M. H. O'Leary. 1984. Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose biphosphate. Biochemstry **23**: 6275-6285.

Schulze, E. D., N. C. Turner, T. Gollen and K. A. Shackel. 1987. Stomatal responses to air humidity and to soil drought. In: Zeiger, E., G.D. Farquhar and I.R. Cowan (eds), Stomatal Funciton. Stanford University Press, Standard, California. pp. 311-321.

Schulze, E. D., R. J. Williams, G. D. Farquhar, W. Schulze, J. Langridge, J. M. Miller and B. Wakler. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Aust. J. Plant Physiol. **25**: 413-425.

Ueno, O. 1996. Immunocytochemcial localization of enzymes involved in the C_3 and C_4 pathways in the photosynthetic cells of an amphibious sedge, *Elocharis vivipara*. Planta **199**: 394-403.

Yang, Y-P., S-H. Yen and C.-K. Lin. 2001. Illustrated Guide to Aquatic Plants of Taiwan. Council of Agriculture, The Executive Yuan of Taiwan.

臺灣亞熱帶高山湖泊兩種水生植物 (東亞黑三稜和水毛花) 穩定性碳同位素比值和氮含量變化

高文媛[1,2*]

1. 國立臺灣大學生態學與演化生物學研究所，106 台北市羅斯福路四段 1 號，臺灣。
2. 國立臺灣大學生命科學系，106 台北市羅斯福路四段 1 號，臺灣。
* 通信作者。*Email: wykao@ntu.edu.tw*

(收稿日期：2009 年 9 月 3 日；接受日期：2009 年 11 月 2 日)

摘要：東亞黑三稜和水毛花是鴛鴦湖生態保護區內兩種優勢水生單子葉植物，本文探討其所使用的光合作用途徑和其生理生態學。本文首先分析東亞黑三稜(沉水植株、成熟植株葉挺水部份和沉水部份、根)植體和水毛花挺水部份(稈)及根部穩定性碳同位素比值 (δ^{13}C)，比較同種水生植物其植體不同部位是否有不同的 δ^{13}C；其後比較這兩種水生植物成熟植株挺水部份其 δ^{13}C 和氮含量的月變化。主要目的在確定這兩種水生植物其所使用的光合作用途徑；同時探討限制這兩種水生植物光合作用速率的因子。分析結果發現這兩種水生植物其植體各部位 δ^{13}C 值均落在 C_3 型植物範圍內，顯示其使用 C_3 光合作用途徑。兩種植物其挺水部份 δ^{13}C 和氮含量有季節性變化，春季高於夏季，且兩者 δ^{13}C 值和氮含量呈線性正相關，顯示兩者 δ^{13}C 之季節性變化和二氧化碳固定作用(carboxylation)效率有關。在年內同一月份，東亞黑三稜挺水葉之 δ^{13}C 值和氮含量都高於水毛花挺水稈。東亞黑三稜有較高的氮含量，因此可能有較高的二氧化碳固定作用效率，以致其挺水葉的 δ^{13}C 值高於水毛花挺水稈。

關鍵詞：鴛鴦湖生態保護區、水毛花、東亞黑三稜、氮含量、穩定性碳同位素比值。