

Study on the phenology of *Chara vulgaris* in Xin'an Spring, north China

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Abstract The phenology of *Chara vulgaris* was studied. The specimens were collected four times from April 2004 to January 2005 (one sampling per season). The water temperature, pH, dissolved oxygen, specific conductance, maximum width and maximum depth were monitored at every sampling time. Eighteen main morphological characteristics of the *Chara vulgaris* were also observed and measured under the microscope. The results showed that all the environmental factors had different seasonal patterns and some of the morphological characteristics had significant fluctuations, indicating differences in their seasonality. At the same time, some morphological characteristics were affected by the environmental parameters to some extent and the effect was primarily exhibited in its vegetative proportions; there was little effect on the sexual reproductive characteristics. Therefore, the relatively stable sexual reproductive characteristics can be used to identify the species.

Keywords *Chara vulgaris*, China, environmental factors, seasonal dynamics, Xin'an Spring

1 Introduction

The charophyte is a freshwater algae with the largest and most complex structure (Moore, 1986). *Chara vulgaris* L., the most representative one, was named earliest in the division (Linnaeus, 1753).

Chara vulgaris occurs in all continents (Groves and Bullock-Webster, 1924; Fritsch, 1935; Zaneveld, 1940; Olsen, 1944; Wood and Imahori, 1965), and has been found in 24 provinces in China (Han and Li, 1994; Ling et al., 2000).

There has been much research about the flora of Charophytes, including a few reports about their relationships to environmental parameters (Vaidya, 1966; Luo and Yu, 1999; Fu et al., 2001a, 2001b; Zhang et al., 2002; Lan et al., 2003). However, regarding their seasonal dynamics, reports are rare (Hussain et al. 1996).

Charophyta have great economic importance. The water in which the Charophyta grow is always extremely clear. This may be partly ascribed to the fact that Charophyta are able to purify the water by retaining mud particles between the whorls of their branchlets (Zeneveld, 1940). A great number of insects, crustaceans, snails and other organisms take shelter in the dense masses of Charophyta and feed on them. In addition, some fishes make nests among Charophyta (Zeneveld, 1940). Importantly, decayed Charophyta can be used for manure, especially for correcting the acidity of soils (Zeneveld, 1940; Wasmund 1933). Besides this economic importance, Charophyta have other uses. For example, it can also be used for fish-culture, polishing-paste, mud-bathing, therapy, clarification of sugar, and luring noxious insects (Zeneveld, 1940).

With the increasingly global environmental pollution, many water bodies, including streams, are being polluted to varying extents. As a result, charophytes have become a highly endangered group of algae (Kotta et al., 2004). Studies of the effects of environmental factors on the seasonal dynamics of Charophyta are of great importance.

2 Materials and methods

2.1 Site description

Located at 36°20'–36°27' N and 113°26'–113°27' E in a river valley at the borders between Lucheng, Licheng and Pingshun counties of Shanxi Province, Xin'an Spring is the second largest cold spring in north China. It lies to the south of the Taihang Mountain. The area has a mid-low

mountainous landscape with a continental climate. The annual average rainfall is 586 mm. The spring basin comprises two spring groups, Shihui and Wangqu. The spring is almost 16 km long (Fig. 1).

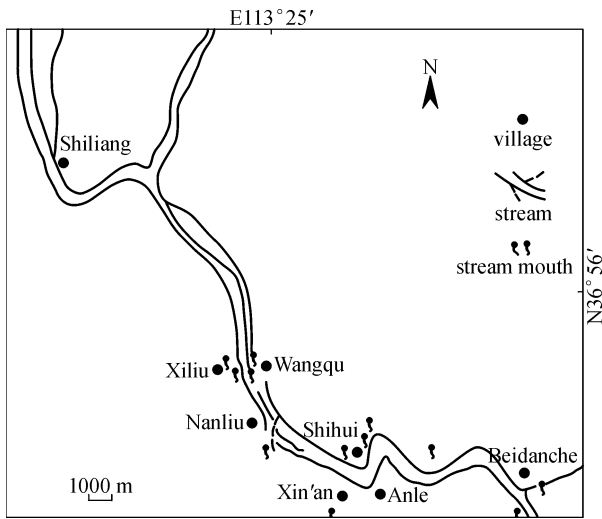


Fig. 1 Distribution of the sampling sites in Xin'an Spring

2.2 Specimen collection and treatment

We collected the specimens four times (one sampling per season) from April 2004 to January 2005 in Xin'an Spring. At the same time, we investigated the environmental factors such as water temperature (JPB-607 Equipment, Shanghai), pH (Model pH-4, Shanghai), specific conductance (Model DDB-303A Conductivimeter, Shanghai), dissolved oxygen (JPB-607 Equipment, Shanghai), and maximum depth and width (tapeline). The specimens were collected by hand where possible. The collected plants were stored in labeled polythene bottles, and then preserved in 4% formaldehyde. Finally, they were identified under the microscope (OLYMPUS BX51, Japan).

A total of 18 morphological characteristics were observed and measured under the microscope: height of the frond, diameter of the stem, length of internode, length of stipulode, diameter of stipulode, diameter of cortex, length of spine-cell, length of branchlet, diameter of branchlet, length of bracteoles, length of bract-cell, diameter of antheridium, diameter of oogonium, length of oogonium, number of convolutions, diameter of oospore, length of oospore, and number of stria. Before microscopic observation, we dandified the plant with hydrochloric acid and then mounted the fragments on glass

Table 1 Data of phenology of *Chara vulgaris* and environmental parameters in Xin'an Spring

| | April 2004 | July 2004 | October 2004 | January 2005 |
|--|---------------|---------------|---------------|---------------|
| water temperature/°C | 10.5 | 24.1 | 16.7 | 9 |
| pH | 7.73 | 7.58 | 7.65 | 7.76 |
| maximum depth/cm | 45 | 45 | 47 | 43 |
| maximum width/m | 12 | 12 | 15 | 13 |
| dissolved oxygen/mg·L ⁻¹ | 10.1 | 10.7 | 9.7 | 9.3 |
| specific conductance/μS·cm ⁻¹ | 966 | 915 | 916 | 955 |
| height of frond/cm, (n = 5 – 10) | 24 ± 1.7 | 26 ± 2.7 | 28 ± 3.2 | 17 ± 2.4 |
| diameter of stem/μm, (n = 15) | 602 ± 87.2 | 600 ± 100 | 673 ± 104.5 | 593 ± 172.3 |
| length of internode/mm, (n = 15) | 11 ± 2.1 | 18.9 ± 7.5 | 16.1 ± 4.2 | 25.1 ± 4.9 |
| length of stipulode/μm, (n = 15) | 131 ± 23.3 | 119 ± 25.6 | 147 ± 44.5 | 170 ± 68.3 |
| diameter of stipulode/μm, (n = 15) | 76 ± 19.6 | 73 ± 14.2 | 70 ± 8.2 | 82 ± 19.3 |
| diameter of cortex/μm, (n = 15) | 40 ± 14.9 | 39 ± 14.5 | 58 ± 6.3 | 47 ± 10.3 |
| length of spine-cell/μm, (n = 15) | 63.5 ± 27.5 | 52.7 ± 18.6 | 89.3 ± 34.8 | 78.6 ± 29.4 |
| length of branchlet/mm, (n = 15) | 15.6 ± 5.5 | 17.7 ± 4.1 | 12.1 ± 2.6 | 15.8 ± 3.9 |
| diameter of branchlet/μm, (n = 15) | 414 ± 129.3 | 384 ± 81.0 | 239 ± 38.7 | 303 ± 11.6 |
| length of bracteoles/μm, (n = 15) | 350 ± 45.2 | 456 ± 58.6 | 532 ± 67.9 | 467 ± 36.3 |
| length of bract-cell/μm, (n = 15) | 1721 ± 1056.7 | 4120 ± 1206.3 | 1480 ± 260.4 | 1360 ± 345.2 |
| diameter of antheridium/μm, (n = 50) | 290 ± 45.3 | 323.6 ± 71.0 | 255.6 ± 116.2 | 302.1 ± 59.4 |
| diameter of oogonium/μm, (n = 50) | 342.5 ± 42.2 | 380.0 ± 84.7 | 329.3 ± 53.4 | 360.0 ± 66.0 |
| length of oogonium/μm, (n = 50) | 610 ± 116.3 | 535 ± 81.8 | 642.3 ± 121.5 | 639.2 ± 114.8 |
| number of convolutions, (n = 50) | 11.9 ± 3.0 | 9.4 ± 1.7 | 12.3 ± 3.2 | 11.6 ± 2.4 |
| diameter of oospore/μm, (n = 50) | 263.8 ± 34.2 | 294.3 ± 52.6 | 254.2 ± 29.8 | 276.5 ± 49.3 |
| length of oospore/μm, (n = 50) | 532.1 ± 47.8 | 493.6 ± 32.4 | 552.0 ± 63.2 | 548.5 ± 54.8 |
| number of stria, (n = 50) | 10.3 ± 2.8 | 8.4 ± 0.7 | 11.8 ± 3.5 | 10.6 ± 2.4 |

slides. Finally, the data were analyzed statistically (SPSS, version 11.5).

3 Results

3.1 Seasonal dynamics of morphological characteristics

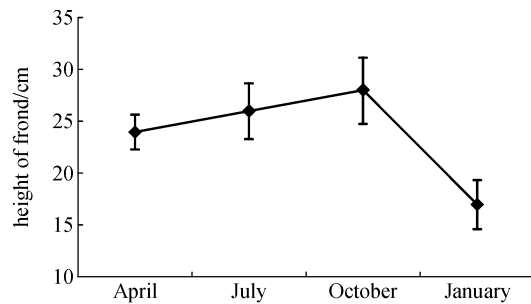
The results of One-Way ANOVA revealed that only three (16.67%) morphological characteristics had differences during the four seasons: length of internode ($F = 13.868$, $P < 0.05$, Table 1, Fig. 2), diameter of cortex ($F = 5.298$, $P < 0.05$) and diameter of branchlet ($F = 6.294$, $P < 0.05$). Although there were also some fluctuations among the other morphological characteristics, they did not vary significantly during the whole period. At the same time, nine (50.00%) maximum values of morphological characteristics were observed in the fall (October 2004), among which there were four (22.22%) sexual reproductive characteristics.

3.2 Seasonal dynamics of environmental factors

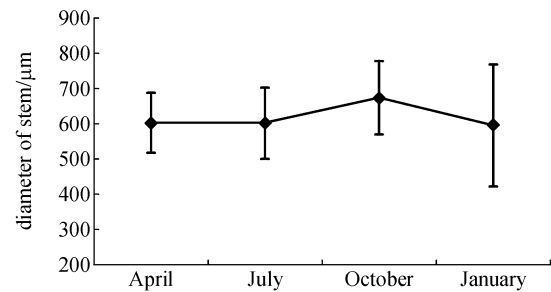
The extent of fluctuations in environmental factors during the whole sampling period was different (Table 1 and Fig. 3). The dissolved oxygen was obviously higher in July than in the other three seasons, and the water temperature had the same trend. The maximum depth was observed in October, while the water width had few fluctuations. The specific conductance was higher in April and January. The pH varied during the four sampling seasons, but only with small fluctuations.

3.3 Relationships of morphological characteristics

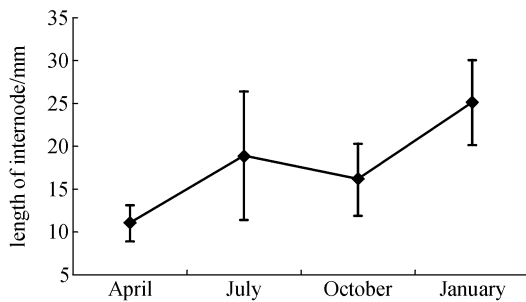
There were positive correlations between the length of the branchlet and diameter of the antheridium ($r = 0.988$, $P < 0.05$), the length of the bract-cell and number of stria ($r = 0.987$, $P < 0.05$), the diameter of the antheridium and that of the oogonium ($r = 0.964$, $P < 0.05$), the diameter of the antheridium and that of the oospore ($r = 0.956$,



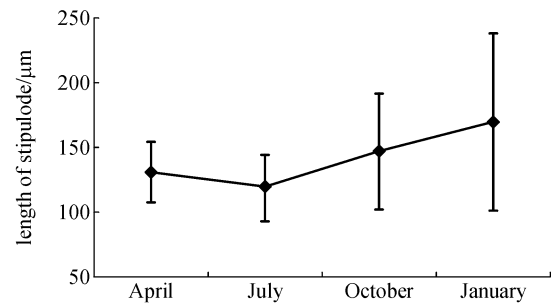
(a)



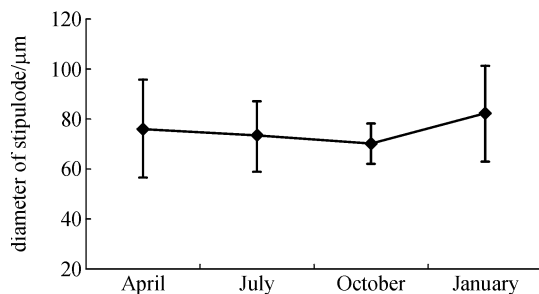
(b)



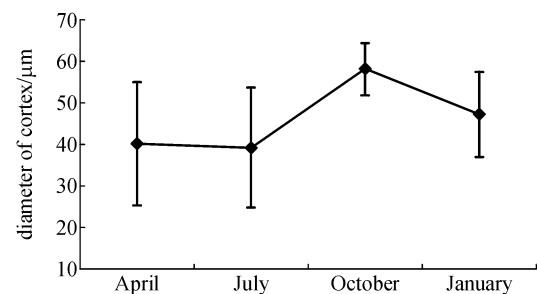
(c)



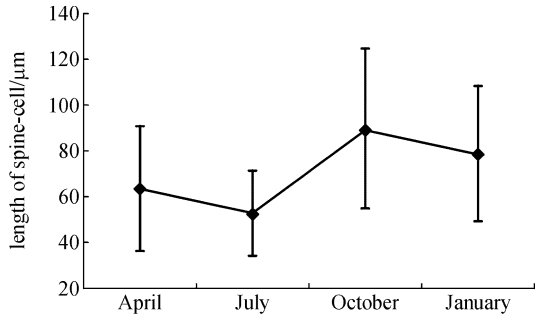
(d)



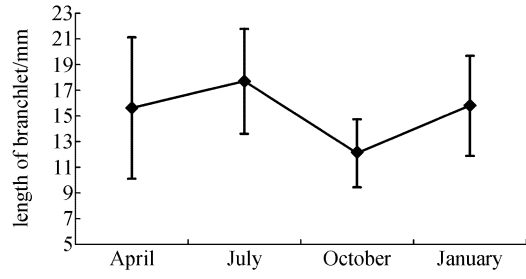
(e)



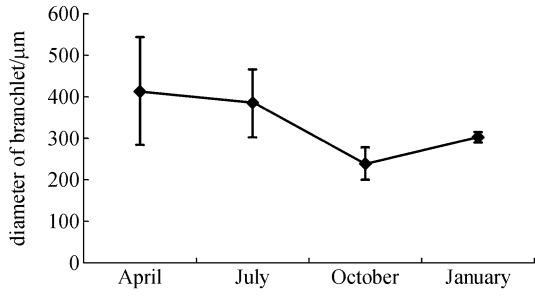
(f)



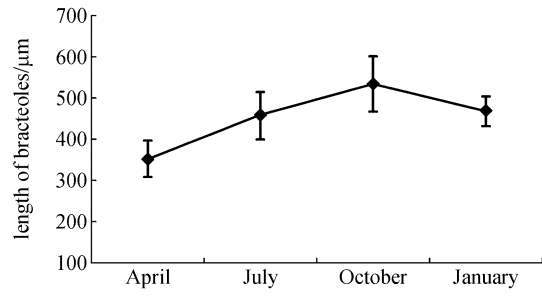
(g)



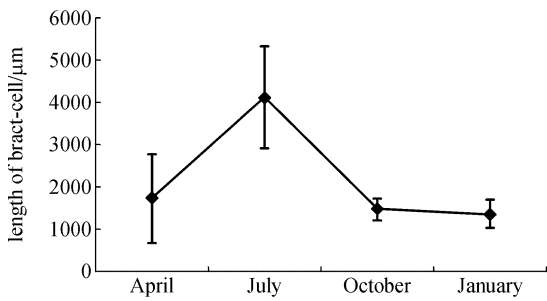
(h)



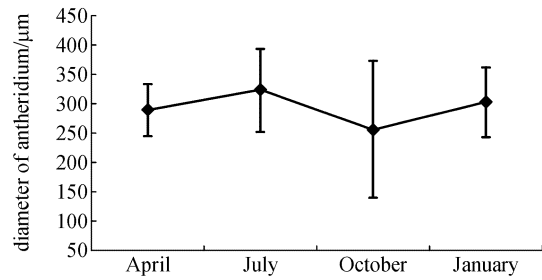
(i)



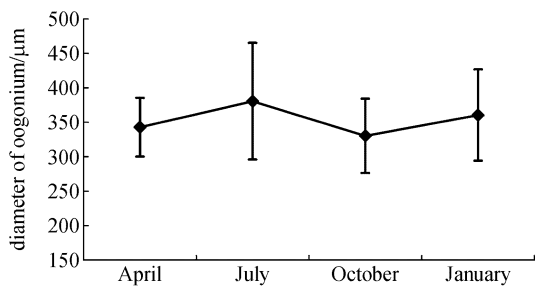
(j)



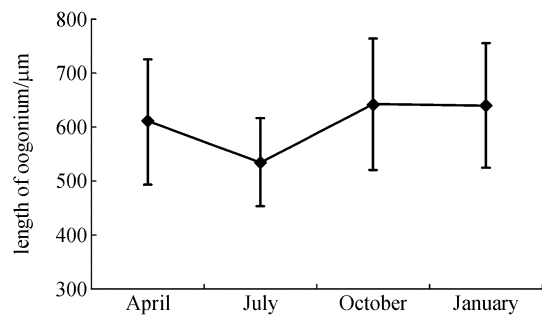
(k)



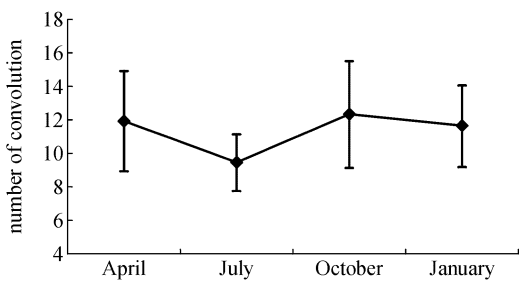
(l)



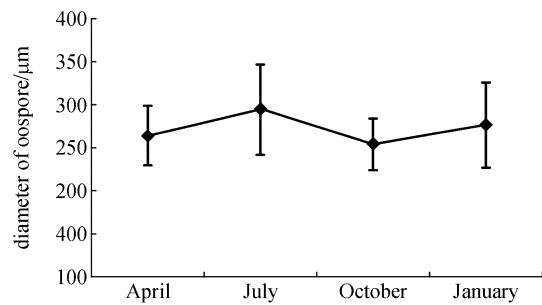
(m)



(n)



(o)



(p)

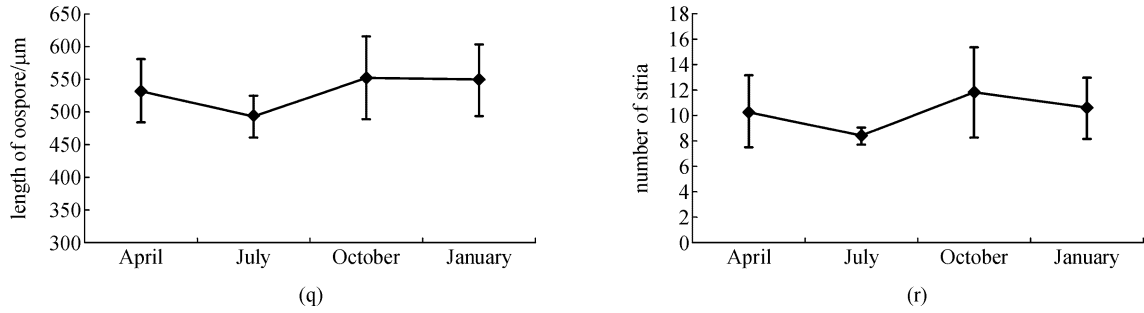


Fig. 2 Seasonal dynamics of morphological characteristics of *Chara vulgaris* in Xin'an Spring

$P < 0.05$), the diameter of the oogonium and the length of the oospore ($r = 0.999$, $P < 0.01$), the length of the oogonium and that of the oospore ($r = 0.999$, $P < 0.01$), and the length of the oospore and the number of stria ($r = 0.955$, $P < 0.05$).

There were negative correlations between the height of

the frond and the diameter of the stipulode ($r = -0.989$, $P < 0.05$), the diameter of the cortex and that of the branchlet ($r = -0.960$, $P < 0.05$), the length of the bract-cell and that of the oogonium ($r = -0.963$, $P < 0.05$), and the length of the bract-cell and the diameter of the oospore ($r = -0.972$, $P < 0.05$).

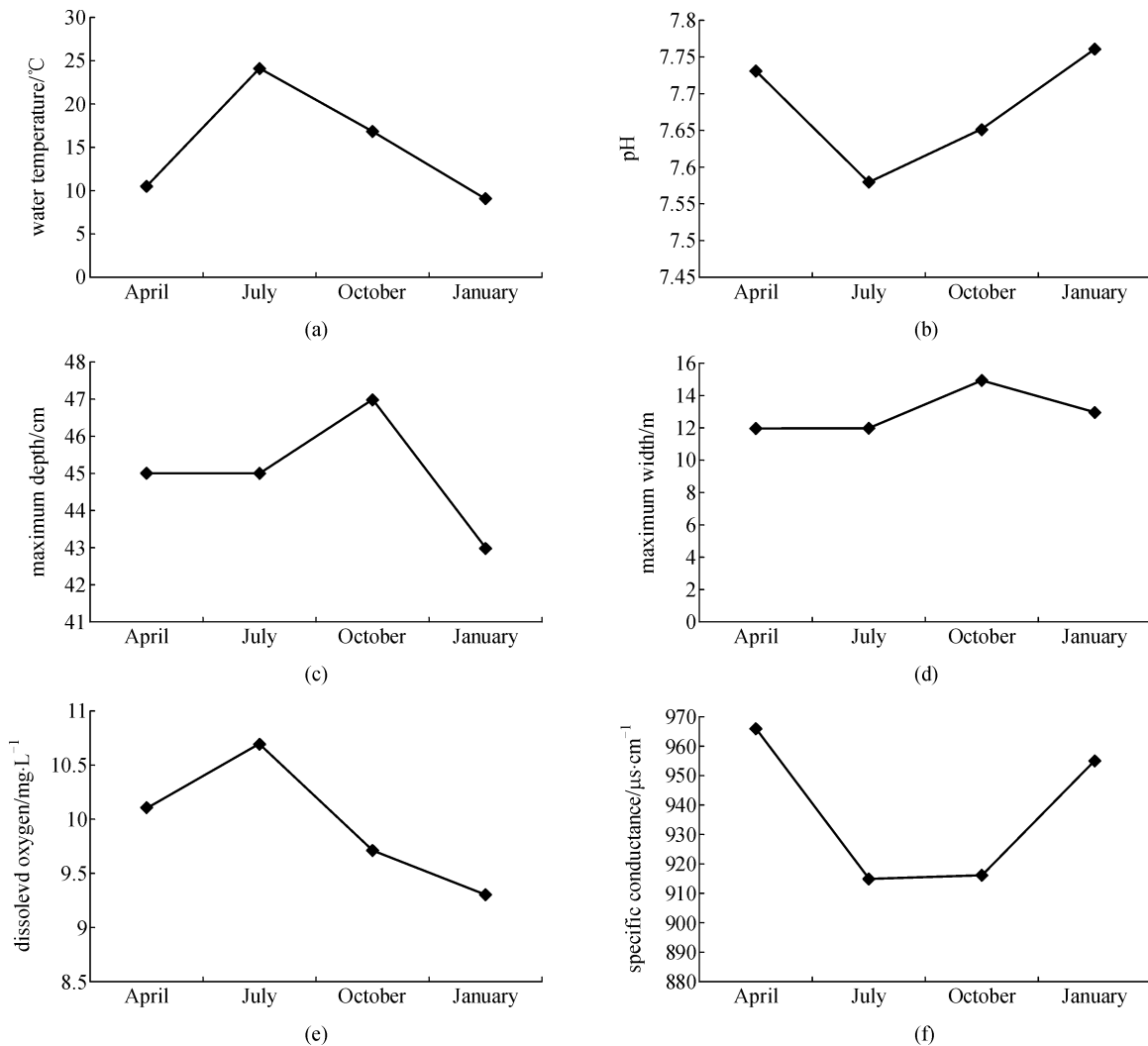


Fig. 3 Environmental variables during the sampling period in Xin'an Spring

3.4 Relationships between environmental factors and morphological characteristics

There were significantly positive correlations between the dissolved oxygen and the diameter of the stem ($r = 0.994$, $P < 0.01$), the maximum width and the diameter of the cortex ($r = 0.996$, $P < 0.01$).

On the other hand, there were negative correlations between the dissolved oxygen and the length of the stipulode ($r = -0.971$, $P < 0.05$), the maximum depth and diameter of the stipulode ($r = -0.956$, $P < 0.05$), and the maximum width and diameter of the branchlet ($r = -0.951$, $P < 0.05$).

4 Discussion

In this study, we measured the morphological characteristics of *Chara vulgaris* in four seasons. The results showed that there were different extents of variations in the investigated morphological characteristics during the whole sampling period, and some differences were significant, indicating differences in their seasonality. Furthermore, all of those morphological characteristics with significant differences were vegetative proportions. This was expected because the reproductive characteristics are relatively steady. Therefore, they are always used as important characteristics to identify the species. This is accordant with the previous report that the production of gametangia was not strictly controlled by seasonality (Hutchinson, 1975).

It is of significance that nine (50.00%) maximum values of morphological characteristics were observed in the fall (October 2004), and among them there were four (22.22%) sexual reproductive characteristics. Undoubtedly, this suggests that there is a hearty reproductive ability of *Chara vulgaris* in the fall. This finding is consistent with the report that most Charophyta with ripe oospores were found from August to October, several months after the hottest season (Imahori, 1954). However, it is inconsistent with that of *Batrachospermum gelatinosum* (Rhodophyta), which was observed to develop well from late fall to early summer the next year, peaking in May (Xie, 2004). This is because *Chara vulgaris* has its own developmental rules.

Through the relationship analyses of the morphological characteristics, we found some inevitable correlations, for example, between the length of the oogonium and length of the oospore, and the length of the oospore and the number of stria. This indicates that the development of *Chara vulgaris* is regulated by its own biological rhythm to some extent.

Moreover, from the analysis of the relationship between environmental factors and morphological characteristics, we found some correlations between environmental factors and the vegetative proportion of the frond, for example, the positive correlation between the dissolved oxygen and the

diameter of the stem. This finding confirms the results stated by Imahori (1954) that the Charophyta were occasionally destroyed when the elimination of oxygen in water exceeded a limit. Meanwhile, we also found a negative correlation between the maximum water depth and the diameter of the stipulode. This is also similar to the report by Imahori (1954), indicating that a large number of Charophyta grew and flourished in rather clear and shallow waters.

However, we failed to find any correlation between environmental factors and the sexual reproductive characteristics. This differs from the previous report that the production of gametangia was essentially a reaction to local environmental conditions and an increase in photoperiod (Hutchinson, 1975).

In summary, the growth and development of *Chara vulgaris* are affected by environmental factors to some extent, and the influence is primarily exhibited in its vegetative proportions. However, it also has its own biological rhythm.

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