Ecology and life cycle of a *Nitella* sp. from Capel Wetlands, Western Australia

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The morphology, ecology, life cycle pattern and taxonomy of a *Nitella* sp., a charophyte from three sand-mine-void lakes at the Capel Wetlands Centre, were investigated both in the field and in the laboratory. Water depth was positively correlated with the height of the *Nitella* sp. Low numbers of gametangia were associated with high water depth. Life cycle of the charophyte was shown to be adapted to the hydrological regime and water quality of the temporary lakes at Capel Wetlands within the pH range of 5 to 9. Laboratory cultures of the species as well as its occurrence in lakes in the Perth Metropolitan region under various trophic conditions indicate that the species has wide ecological tolerance. The charophyte begins its life cycle with autumn rains filling the temporary wetlands and continues to grow throughout the winter. By spring, reproduction is initiated. Mature oospores are produced by the end of summer, coinciding with the decline in water level followed by the drying of the lakes. Morphology of vegetative and reproductive structures, reproductive state and ultrastructure of oospores were combined to provide a description of the species. The number of accessory branchlets was found to be variable. The *Nitella* species is placed under the subgenus *Decandollea* section *Decandollea* and shares characters of *N. congesta* (R Brown) A Braun and *N. lhotzkyi* A Braun.


INTRODUCTION

Very little published information is available on charophytes of Western Australia. Most of the published works on Australian charophytes are from the Eastern States. Charophytes are pioneers of newly established wetlands (Zanereld 1940) and have been known for their ability to ameliorate water quality and increase productivity of wetlands (John 2003). As water quality of wetlands deteriorates, charophyte diversity tends to decrease (Blindow 1992a; van den Berg et al. 1998; Kufel & Kufel 2002). Identification of charophytes beyond the genus level often requires consideration of not only the morphology of the vegetative structure and arrangement and ultrastructure of reproductive bodies but also the monoecy or dioecy nature of reproduction. A *Nitella* sp. was found to have colonised a number of sand-mine-void wetlands at Capel, Western Australia in the early 1990s. The colonisation started from the bottom end of a chain of 15 lakes when the pH changed from 4 to 5. Colonisation of this charophyte was found to have significantly contributed to the development of the lakes at Capel Wetlands Centre. These wetlands were oligotrophic with high mineral content (John 2003; Annan 2008). The charophyte species in this study has been also found in Lake Leschenaultia and in many urban stormwater wetlands and artificial lakes (e.g. Lake Bungana in Maylands) around Perth. The range of aquatic environments occupied by charophytes, coupled with their response to lake restoration make them relevant in the management of wetlands (Coops 2002).

Charophytes include five distinct genera: *Chara, Nitella, Lamprothamnium, Lychnothamnus* and *Tolypella*. The genus *Nitella* is characterised by forked branchlets (furcation), compressed oospores, terminal antheridia and 10-celled coronula in the oogonium (Wood & Imahori 1965; Casanova 2009). The growth and mortality of charophytes may be influenced by factors such as temperature, water depth, transparency, pH, flow, turbulence, the presence of herbivores, shading by other macrophytes, and nutrient concentrations in the water and sediment (Meiers et al. 1999; Casanova & Brock 1999). Charophyte growth rate differs according to substratum particles (Andrews et al. 1984a) water depth (Andrews et al. 1984b), and to some extent nutrient status (e.g. phosphorus concentration: Blindow 1988; Kufel & Kufel 2002). Charophytes can be classified as annuals or perennials depending on the life cycle pattern. They can be either monoecious or dioecious. In the monoecious species the male and female reproductive bodies are borne on the same individual, can be attached at the same node (conjoined) or at different nodes. Fertilized oogonia develop into oospores.

The ultrastructure of the oospore wall and sometimes supplemented by chromosome numbers is important in the identification of charophytes (John & Moore 1987; Casanova 1997, 2009; Garcia, 1999, 2001, 2002; Sakayama et al. 2002, 2004, 2005). The oospore of charophytes is protected by a sheath of five spirally disposed cells known as the tube cells and when fertilised, develops a thick multilayered wall. Ridges, which ascend spirally from right to left are formed from the lower lateral walls of these spiral cells and later develop into a prominent flange or wing-like protrusion. The area of the wall lying between each spirally disposed ridge is called the fossa.
Figure 1. (a) Map of Australia showing site. (b) Chain of lakes that make up the Capel Wetlands Centre. The lakes coloured blue are the lakes selected for the project.
The fossa is often ornamented and this is a species-specific feature. Fully mature oospores have characteristic ornamentation, offering more reliable information than non-mature ones since the latter either lack, or have weakly developed, ornamentation (John & Moore 1987).

The objective of this paper is to report investigations on the morphology, life cycle, ecology and identity of a Nitella sp. from the Capel Wetland Centre, Western Australia by field and laboratory culture observations.

MATERIALS AND METHODS

Site description

The charophytes investigated were from Capel (33°S, 115°E), midway between the regional centres Bunbury and Busselton, 200 km south of Perth, Western Australia (Figure 1a). The annual average rainfall for the Capel region is 730.3 mm (Bureau of Meteorology 2008) and the climate is Mediterranean characterised by dry summers and wet winters. The region is originally comprised of mixed woodland interspersed with tuart groves, sandy areas of Banskia woodland and wet swampy areas with peaty edges (Davies 2002).

Since 1956, sand deposits, mainly titaniferous minerals ilmenite and rutile have been mined from Capel (Brooks 1991; Qui et al. 2002). The mining operations were extensive with the removal of large quantities of sand from the orebodies with, significantly, no backfilling. The pits created after the extraction of mineral sands intercepted the water table with the resultant formation of mine voids (pits) which were converted into artificial wetlands by landscaping and creating islands and peninsulas, and planting fringing vegetation between 1975 and 1979. The wetland lakes were originally used as a source for mineral processing. These lakes were acidic with pH ranging from 2 to 4.

A Nitella sp. was later found to have colonised a number of the interconnected linear chain of 15 lakes at the Capel Wetlands Centre. The first record of the Nitella sp. in the lakes was in the early 1990s in two lakes (John 1993). A study conducted in 1987 to assess the water quality of the wetlands indicated that the very low pH posed a problem impeding the development of the lakes. The low pH of the lakes was the result of the acidic effluent water discharged from the processing plant to the top lake in the chain of mine-void lakes. The effluent was neutralised from 1988 to 1993 thus raising the average pH of the lakes to more than 7. In 1996, four more lakes were found to have been colonised by the Nitella species and in 2003, with further increase of pH, 12 lakes were recorded to have the charophyte (John 2003). Its presence was found to have significantly contributed to the development of the lakes at Capel Wetlands Centre. Three lakes, Plover North Lake, Plover South Lake and Nitella Lake, from a chain of mine-void wetlands all of which had abundant charophytes were chosen for this study (Figure 1b).

Morphological studies

Samples of healthy charophyte shoots were collected from the field and washed to remove as much debris as possible and observed under a Leica dissection stereomicroscope. The shoots were excised from the apex downwards and various morphological features such as the main axis, branchlets, primary, secondary and dactyl cells were observed and measured. Both antheridia and oogonia were examined and their dimensions measured under dissection stereomicroscope and compound microscopes and micrographs taken as required.

Scanning electron microscopy (SEM) of oospores

The ornamentation pattern of the oospore can be considered a highly reliable consistent character and therefore of a significant diagnostic feature (Boszke et al. 2008). For the genus Nitella, oospore morphology serves as an indicator in speciation in the absence of breeding studies (Casanova 2009). The oospores were cleaned for scanning electron microscopic observation using the acetolysis method after John & Moore (1987). The cleaned oospores were placed on glass slides and were heated on a hot plate at low heat, until all the water was evaporated. The oospores were then mounted on aluminium stubs with a double-sided transparent adhesive tape, sputter-coated with gold and viewed with a Phillip’s XL 30 Scanning Electron microscope using secondary and backscattered electron detectors with an accelerating voltage of 15kV. Oospore characteristics were outlined following descriptions given by Wood & Imahori (1965), Haas (1994), Garcia (2001, 2002) and Casanova (2009).

Growth studies in the field and laboratory

Charophyte samples were harvested monthly from May to November in 2004 from Nitella Lake, Plover North and Plover South Lakes at the Capel Wetland Centre. Measurement of height, number of nodes and number of branches were recorded for 25 individuals during each sampling. Height was measured from the apical meristem to the point of attachment of rhizoids. Nodes and branches were also counted along the axes for each individual. The growth measurements continued until the first sighting of reproductive bodies. Data on length were used to calculate mean growth rates taking elongation of the shoot as a proxy for growth in the field using formula 1 (below). One-way ANOVA was used to compare means of the three different groups (i.e. individuals from Nitella Lake, Plover North Lake and Plover South Lake) to find out whether there was any significant growth difference among the three lakes. Statistical package SPSS Grad Pack 15.0 was used for all the statistical analyses.

Sediment samples containing oospores of charophytes were collected from Nitella Lake, Plover North and Plover South Lakes (at a depth of ~30 cm and a distance of 1 m from the western banks) in 2004. The sediment samples were dehydrated by evaporation and equal amounts of sediment were transferred into three aquarium tanks (30 L glass tanks) in the greenhouse at the Department of Environmental and Agriculture, Curtin University of Technology. Twenty litres of lake water was added slowly to each tank. The aquaria were aerated with pumps and air-stones and left in natural day length conditions from 30 April to 30 November 2004 (244 days). Water levels were maintained by adding lake water.
The germination and growth of germinants were monitored. Measurement of height, number of nodes and branch lengths were recorded for 20 individuals (selected at random and tagged loosely by carefully tied red-coloured thread around the thallus) from each tank. This was done from the onset of emergence of the germinants up to the first sighting of reproductive bodies. Height was measured from the apex of the thallus to the upper surface of sediment where charophyte rhizoids were located. The number of nodes and branches were counted along the axes of tagged individuals. One-way ANOVA was used to compare means of the three different groups to see whether there were any statistically significant differences. The growth rates $G_t$ (mm/day) were calculated from the shoot length data using the formula:

$$G_t = \frac{H_t - H_0}{t}$$

where $H_0$ is the length of the shoot at time zero and $H_t$ is the length of the same shoot after a period of $t$ days.

### Water depth vs height of Nitella

Two sites (one shallow and the other deeper) were selected from Plover South Lake (shallow, 33°36.19’S, 115°30.11’E and deep, 33°36.17’S, 115°30.25’E) in 2005 and the mean heights of the Nitella sp. shoots were measured against the water depth monthly from June to November. Plover South Lake was selected because it was the deepest among the three lakes for the study. Data obtained were subjected to regression analysis to determine any statistically significant relationship between water depth and height of Nitella sp. shoots.

### Life cycle studies of the Nitella sp. in the field and laboratory

The morphological changes of the Nitella sp. cultured were observed fortnightly in the field and on daily basis in the laboratory culture. Timing of production of gametangia (antheridia and oogonia) and the duration of their maturity were recorded. The reproductive bodies were collected, measurements taken and features viewed under a Leica stereomicroscope. Where possible, photographs of the stages were taken. Male and female shoots from the tagged samples in the culture tanks and from field samples were counted to determine the sex ratio.

Frequent monitoring of the water quality of the lakes were done during the duration of this study with additional sampling in 2008.

### RESULTS

#### Morphological studies

The vegetative features of the Nitella sp. are illustrated in Figure 2(a). The apical meristem and the main axis are ensheathed by mucilage. From the apex (topmost whorl) downwards, the number of branchlets at the nodes varied. The number of branchlets from the 1st to 3rd nodes from the apex were consistently 8 (Figure 2b). However, from the 4th node, the number of branchlets changed. At the 4th node, in addition to the primary branchlets, accessory branchlets were seen either at developing or developed stages. The total number of branchlets at the 4th node ranged from 15 to 20. From the 4th node downwards to the rhizoids, the accessory branchlets were consistent and well developed. However, at the lowest part of the thallus, the accessory branchlets were absent. Additionally, the accessory branchlets were irregularly arranged in between the primary branchlets, some above and some below the node. The primary rays (labelled P in Figure 2i) were observed to furcate into 5 or 6 secondary rays. The secondary rays (labelled S in Figure 2k) terminated into 5–6 dactyls (labelled D in Figure 2k) which were 2-celled. The antheridia were solitary, orange coloured and had 8 shield cells, 375–450 μm in diameter and located terminally at the point of furcation (Figure 2f, g). Oogonia were gold coloured and 1–3 at points of furcation on female individuals (Figure 2h–m). They were 650–750 μm long and 400–500 μm wide with 7–9 convolutions. Coronula in 2 rows of 5 and were 30–45μm high and 50–60μm wide at the base.

#### Oospore structure by SEM

Oospores were identified using the following parameters and descriptions. Results were compared with those of Wood & Imahori (1965), Garcia (1999, 2001, 2002) and Casanova (2009). The length was 415–500 μm; width 300–400 μm and length:width ratio 1.0–1.6. The shape of the oospore is described from different views: apical view, flattened (Figure 3a); lateral view, compressed (Figure 3b); ridges are prominent, 7–9 in number (Figure 3b); basal view (Figure 3c); colour is light-brown but turns dark-brown when mature. The oospore membrane ornamentation is imperfect reticulate (Figure 3d).

#### Growth studies in the field and laboratory

The highest growth rate in the field was observed in Plover South Lake in winter corresponding with the lowest mean temperature 18°C (Table 1). The mean

<table>
<thead>
<tr>
<th></th>
<th>Plover South Lake (mm/day)</th>
<th>Mean Temperature (°C)</th>
<th>Plover North Lake (mm/day)</th>
<th>Mean Temperature (°C)</th>
<th>Nitella Lake (mm/day)</th>
<th>Mean Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>0.52±0.30</td>
<td>23.0</td>
<td>0.61±0.30</td>
<td>22.0</td>
<td>0.50±0.13</td>
<td>23.4</td>
</tr>
<tr>
<td>Winter</td>
<td>1.92±0.89</td>
<td>18.0</td>
<td>1.17±0.62</td>
<td>18.7</td>
<td>0.79±0.33</td>
<td>19.2</td>
</tr>
<tr>
<td>Spring</td>
<td>0.95±0.29</td>
<td>24.5</td>
<td>0.74±0.65</td>
<td>23.8</td>
<td>0.36±0.36</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Table 1 Field growth rate (mm/day) of Nitella sp. in Plover South, Plover North and Nitella Lakes with corresponding mean temperature (°C) in 2004. n = 25.
Figure 2. Morphology of *Nitella* sp. from Capel Wetlands. (a) Habit showing whorls of branchlets and internodes; towards the apex the internodes are compacted; whorls of branchlets spread out as mucilage was shed off. Scale bar, 5 mm. (b) Whorls of branchlets at third node without accessory branchlets. Scale bar, 500 μm. (c) Whorls of primary branchlets with developing accessory branchlets (three arrowed) at fourth node from the apex of thallus. Scale bar, 500 μm. (d) Whorls of primary branchlets and developed accessory branchlets (two arrowed) at fourth node from the apex of thallus. Scale bar, 500 μm. (e) Whorl of primary branchlets from the mid-portion of thallus with accessory branchlets. Scale bar, 1 mm. (f, g) antheridia (globules), male sex bodies. Scale bar, 400 μm. (h) Developing oogonia (nucules) showing five tube cells. Top view of the coronula cells (arrowed). (i) Developing oogonium at point of furcation of a primary branchlet/ray. P, primary branchlet/ray. (j) Fertilised oogonia at a point of furcation of a primary branchlet. C, coronula of 10 cells in two tiers. (k) Developing oospores at a point of furcation of a primary ray. P, primary ray; S, secondary ray; D, dactyl; E, end cell. (l) Mature oospores at points of furcation of primary rays. (m) Whorl of oogonia with outer covering mucilage. Mucilage was stained with toluidine blue. Scale bar, 450 μm.
height, mean number of nodes and mean number of branches of shoots in the field were also highest in the Plover South Lake (Table 2). In the laboratory, the highest growth rate was in the Plover North Tank in winter corresponding with the lowest temperature of 12°C (Table 3). The mean height, mean number of nodes and mean number of branches of the *Nitella* sp. in the laboratory was also highest in the Plover North Tank (Table 4). Overall, growth rates were higher in the field than in the laboratory. *Nitella* sp. showed tolerance for high pH, conductivity and salinity in the field (Table 5) and laboratory (Table 6). However, when the lakes became acidic (pH 3.47–4.28) in 2008, no charophytes were observed.

Table 2 One-way ANOVA comparing mean height, mean number of nodes and mean number of branches per shoot of *Nitella* sp. in the field in 2004. α = 0.05, n = 25.

<table>
<thead>
<tr>
<th></th>
<th>Plover South Lake</th>
<th>Plover North Lake</th>
<th>Nitella Lake</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean height (mm)</td>
<td>159.20±110.47</td>
<td>124.38±77.18</td>
<td>83.86±47.02</td>
<td>1.69</td>
<td>0.21</td>
</tr>
<tr>
<td>Mean number of nodes</td>
<td>10.59±4.80</td>
<td>9.47±4.09</td>
<td>4.61±2.14</td>
<td>4.78</td>
<td>0.02*</td>
</tr>
<tr>
<td>Mean number of branches</td>
<td>4.21±3.00</td>
<td>1.53±1.39</td>
<td>1.40±0.70</td>
<td>4.60</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Table 3 Laboratory growth rate (mm/day) of *Nitella* sp. in aquaria with corresponding mean temperature (°C) in 2004. n = 20.

<table>
<thead>
<tr>
<th></th>
<th>Plover South Lake (mm/day)</th>
<th>Mean Temperature (°C)</th>
<th>Plover North Lake (mm/day)</th>
<th>Mean Temperature (°C)</th>
<th>Nitella Lake (mm/day)</th>
<th>Mean Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>0.47±0.30</td>
<td>20.3</td>
<td>0.50±0.37</td>
<td>21.2</td>
<td>0.26±0.16</td>
<td>22.2</td>
</tr>
<tr>
<td>Winter</td>
<td>0.67±0.12</td>
<td>14.1</td>
<td>1.01±0.11</td>
<td>12.0</td>
<td>0.59±0.26</td>
<td>13.7</td>
</tr>
<tr>
<td>Spring</td>
<td>0.22±0.14</td>
<td>23.2</td>
<td>0.07±0.10</td>
<td>27.7</td>
<td>0.43±0.34</td>
<td>25.8</td>
</tr>
</tbody>
</table>
Water depth vs height

Regression analysis of water depth and mean height per shoot of *Nitella* sp. in Plover South Lake Site 1 was significant at the 5% level, \( F = 148.13, \) \( R \text{ Square} = 97.4\% \), \( P = 0.00 \) (Figure 4). Regression analysis of water depth and mean height per shoot of *Nitella* sp. in Plover South Lake Site 2 was significant at the 5% level, \( F = 35.86 \), \( R \text{ Square} = 90.0\% \), \( P = 0.00 \) (Figure 5). The high R-square values showed that the height of *Nitella* sp. was associated with water depth. The deeper the water, the higher the height of shoots observed.

Life cycle studies of the *Nitella* sp. in the field and laboratory

This species was found to be dioecious, producing separate male and female reproductive structures on separate individuals. The male individuals produced orange-coloured octoscutate antheridia (with eight shield cells) and were borne terminally. This occurred within a period of 2–4 weeks after which no more antheridia were seen. The oogonia which were initially somewhat gold coloured, were observed to have become dominant two weeks after the development of the antheridia. After 3–5 weeks of production of the oogonia, oospores were observed. The oospores reached maturity with a colour change to dark-brown during summer.

**Table 4** One-way ANOVA comparing mean height, mean number of nodes and mean number of branches per shoot of *Nitella* sp. in the laboratory in 2004. \( \alpha = 0.05 \), \( n = 20 \)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plover South Lake</th>
<th>Plover North Lake</th>
<th>Nitella Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean height (mm)</td>
<td>Mean±sd</td>
<td>Mean±sd</td>
<td>Mean±sd</td>
</tr>
<tr>
<td>2004</td>
<td>54.53±17.33</td>
<td>98.56±40.85</td>
<td>41.76±24.82</td>
</tr>
<tr>
<td>2008</td>
<td>98.56±40.85</td>
<td>12.69±4.84</td>
<td>7.84±2.50</td>
</tr>
<tr>
<td>Mean number of nodes</td>
<td>Mean±sd</td>
<td>Mean±sd</td>
<td>Mean±sd</td>
</tr>
<tr>
<td>2004</td>
<td>8.57±2.09</td>
<td>12.69±4.84</td>
<td>7.84±2.50</td>
</tr>
<tr>
<td>2008</td>
<td>12.69±4.84</td>
<td>7.84±2.50</td>
<td>0.75±0.56</td>
</tr>
<tr>
<td>Mean number of branches</td>
<td>Mean±sd</td>
<td>Mean±sd</td>
<td>Mean±sd</td>
</tr>
<tr>
<td>2004</td>
<td>0.84±0.69</td>
<td>1.37±1.12</td>
<td>0.75±0.56</td>
</tr>
<tr>
<td>2008</td>
<td>1.37±1.12</td>
<td>0.75±0.56</td>
<td>0.75±0.56</td>
</tr>
</tbody>
</table>

**Table 5** Water-quality parameters measured in the field during growth of *Nitella* sp. in 2004 and 2008.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plover South Lake</th>
<th>Plover North Lake</th>
<th>Nitella Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.94–9.15</td>
<td>3.47</td>
<td>7.88–8.25</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>1232–2290</td>
<td>1174–1462</td>
<td>1268–782</td>
</tr>
<tr>
<td>Salinity (ppm)</td>
<td>604–1069</td>
<td>408–699</td>
<td>125–378</td>
</tr>
</tbody>
</table>

**Table 6** Water-quality parameters measured in the laboratory during growth of *Nitella* sp. in 2004.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plover South Lake</th>
<th>Plover North Lake</th>
<th>Nitella Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>9.05</td>
<td>8.85</td>
<td>9.13</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>2640</td>
<td>2460</td>
<td>1440</td>
</tr>
<tr>
<td>Salinity (ppm)</td>
<td>1338</td>
<td>980</td>
<td>781</td>
</tr>
</tbody>
</table>

**Figure 4.** Mean height of shoot of *Nitella* sp. in Plover South Lake Site 1 (deep).
In the field study, the charophyte again showed vegetative growth for approximately 25 weeks before undergoing reproduction. The production of reproductive bodies was observed from late October to early November (late spring). Ratio of male to female individuals in all three lakes was ~1:1 with no statistical difference among lakes or between field-grown and cultured populations. A schematic presentation of the life cycle of the *Nitella* sp. summarising the observations is shown in Figure 6.

**DISCUSSION**

**Morphological studies**

The charophyte resembles closely *Nitella hyalina*, *Nitella lhotzkyi* and *Nitella congesta* (placed in the subgenus *Decandollea* by Casanova (2009)) in vegetative morphological features and share characteristic mucilage ensheathment over the meristem and axis. This species in Capel Wetlands was initially identified as *N. hyalina* in previous studies before the lifecycle was investigated.
(John & Gayton 1994; Ward et al. 1997; John 2003). N. lhotzkyi produces mucilage ensheathment over the apical meristem and most of the vegetative body. However, N. hyalina is monoecious, while N. congesta and N. lhotzkyi (Wood & Imahori 1965) are dioecious. In comparison with N. lhotzkyi, the branchlets with the mucilaginous ensheathment at nodes on the axis of N. congesta are spheroid and compact which has also been observed in the current species. However, in N. lhotzkyi, the branchlets are found to be of two kinds in each whorl (Wood & Imahori 1965) and consistently spread out: normal branchlets are 7–11 in a whorl with only 1 or 2 furcation and the accessory branchlets are consistently present at each node on the entire thallus (Wood & Imahori 1965). The current Nitella sp. was found to have consistently 8 primary branchlets at each node and the branchlets are not spread out in two rows as in N. lhotzkyi, although the accessory branchlets are irregular and shorter than the primary branchlets.

In the population of Nitella studied, the accessory branchlets could not be observed as a consistent feature at all nodes. This made identification of species very difficult. However, careful observations showed that the accessory branchlets develop later at some nodes away from the tip (apical meristem) of the thallus. It was observed that many new branchlets were being produced in between the primary branchlets. From our observations, it is clear that as the growth proceeds, more accessory branchlets would be produced from the top downwards. However, there is no mention about the sequential development of accessory branchlets from the top downwards in the literature. Whether the variability in the accessory branchlets is a plastic character is unknown. Examination of specimens collected from an artificial eutrophic wetland from Maylands, in the Perth region, clearly show the accessory branchlets developing from the 3rd to 4th node from the apex up to a maximum number of 20 accessory branchlets.

Another characteristic feature was the shape of the oogonium. The shape of the oogonium in the current species is similar to that of N. congesta as described by Wood & Imahori (1965). Wood & Imahori (1965) also described the oospore membrane ornamentation of N. lhotzkyi as finely granulate and that of N. congesta as ‘slightly spongy’. The current species of Nitella has a reticulate oospore ornamentation. The oospore-based determination of some species of charophytes still remains very difficult (Boszke et al. 2008). According to Casanova (1997), morphological variations of the charophyte oosporcs can be associated with the type of meristem and most of the vegetative body. N. lhotzkyi produces oospores that are spheroid and compact which has also been observed in the current species. However, in N. congesta the branchlets with the mucilaginous ensheathment at nodes on the axis of N. congesta are spheroid and compact which has also been observed in the current species. However, in N. lhotzkyi, the branchlets are found to be of two kinds in each whorl (Wood & Imahori 1965) and consistently spread out: normal branchlets are 7–11 in a whorl with only 1 or 2 furcation and the accessory branchlets are consistently present at each node on the entire thallus (Wood & Imahori 1965). The current Nitella sp. was found to have consistently 8 primary branchlets at each node and the branchlets are not spread out in two rows as in N. lhotzkyi, although the accessory branchlets are irregular and shorter than the primary branchlets.

Ecology and life cycle of the Nitella sp.
Charophytes are sensitive to water-quality parameters, especially pH (García 2002). From this study the lower limit of pH of this species was found to be between 3.5 and 5. This species of Nitella showed tolerance to high conductivity.

After fertilization, the zygotes underwent maturity as temperature rose during late spring and early summer. At that stage, the oospores became darker and shrunk in size (mature oospore). Oospores were then released into the sediment as the standing water dried out. Thus, the life cycle of the Nitella sp. was well adapted to the hydrological regime of the lakes. The production and release of oospores was triggered to coincide with the summer drawdown, depositing the oospores in the sediment to ensure species success. On the onset of rains during late autumn/early winter, mass germination of oospores was observed. That was followed by a slow period of growth and then accelerated vegetative growth and finally reproductive phase.

Charophyte life history is characterised by monocarpy or polycarpy based on the reproductive patterns of the alga. Polycarpic charophytes reproduce several times in a lifetime whereas monocarpic ones reproduce once and die. Monocarps are divided into those that reproduce at an early stage and grow for a long time and those that grow vegetatively for some time before undergoing reproduction and then death (Harper 1977; Casanova 1993). In this study, the Nitella sp. exhibited the latter type of monocarpy in the laboratory cultures and in two of the Capel lakes which are typically temporary. Thus the Nitella sp. acted as an annual in the temporary wetlands. However, in the deepest lake, Plover South Lake (with a depth of about 2.5 m) had sparse reproductive bodies and continued to grow vegetatively. This can be interpreted as an indication of the
adaptability of the *Nitella* sp. in deep waters to survive as a facultative monocarpic species.

Sex allocation in charophytes is complicated and is influenced by geographical and environmental factors (Cox 1981; Lloyd 1982). The male shoots became the most abundant followed by the female shoots becoming the most abundant later. This protandrous phenomenon has been explained as a temporal niche separating sexes (Cox 1981). The production of antheridia before that of oogonia presupposes that it may be less expensive to produce antheridia than oogonia (Casanova 1994). Therefore assuming that the production of antheridia required fewer resources than that for oogonia, antheridia were produced earlier than oogonia. Charophytes can reproduce by asexual means, for example, by the generation of secondary protonema from the bulbils in permanent water bodies. In this case, the plant energy is allocated toward vegetative growth instead of sexual reproduction. In ephemeral water bodies, however, charophytes reproduce by sexual means whereby drought resistant oospores are produced. Thus more resources are allocated towards oospore production rather than vegetative growth (Casanova 1994).

Description of *Nitella* sp. from Capel Wetlands Centre, Western Australia

Wood (1972) mentioned the occurrence of the species in Victoria and Western Australia. An illustration of the vegetative and reproductive structures of the species is given in Wood & Imahori (1965). A description of the *Nitella* sp. from Western Australia based upon populations at Capel is presented below.

The alga is dioecious, mostly up to 33 cm high but more than 1 m in deeper lakes and dark green in colour with slender axes, 0.7–1.0 mm in diameter. There were 8 primary branchlets (3.0–7.0 mm long) at each node but up to 20 branchlets where accessory branchlets were present. The accessory branchlets are irregularly arranged. Apical part of shoot was covered with mucilage making it compact (Figure 2a). The internode lengths varied according to water depth; shorter towards the apical meristems and longer downwards along the axis. The primary rays furcate into secondary rays (length 1.0–2.3 mm which are 5–6 in a whorl (reduced when replaced by an oogonium), one being percurrent. The secondary rays furcate into 5–6 dactyls which are 2-celled; end cell transparent in the population studied. They were elongated, 100–200 μm long (including acuminate end cell). Both the internode and whorls of branchlets are ensheathed by mucilage. As mucilage is shed off in the lower portion of shoot, branchlets appear to spread out (Figure 2c). Accessory branchlets were found at some 3 nodes below the terminal meristem in between normal branchlets. Oogonia are 1–3 aggregated at nodes, 650–750 μm long, 400–500 μm wide and with 7–9 convolutions. Coronula are 30–45μ high and 50–60μ wide at the base. Antheridia are solitary, terminal on males (Figure 2f), 375–450 μm in diameter. Oospores are light-brown or dark-brown (when fully mature), 415–500 μm long, 300–400 μm wide; striae of 7–9 broad, prominent ridges with an imperfect reticulate ornamentation.

In conclusion, based upon mucilaginous ensheathment, vegetative morphology, structure of gametangia, oospore structure and sexual state, the population of *Nitella* sp. in Capel Wetlands and some Perth wetlands treated in this paper could not be placed either in *N. congesta* or *N. lhotzkyi*. Casanova (2009) provided the most up-to-date overview of *Nitella* in Australia and summarised the characters of the subgenera of *Nitella*. Accordingly, this species can be placed in the subgenus *Decandollea* and section *Decandollea* to which the above two species of *Nitella* have been allotted. However, of all the subgenera of *Nitella*, *Decandollea* remains problematic (Casanova 2009). Further work is required to delineate species boundaries within the section.

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