Rikunomizu（*Limnology in Tokai Region of Japan*）**60** : 35 - 39（2013）

Regional Report

Formation process of conjugation and zygospores of a filamentous green alga, *Spirogyra* species collected from a lowland marsh, Naka-ikemi, Tsuruga, Fukui, Japan

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Abstract

The formation process of conjugation and zygospores of a *Spirogyra* species collected from a Japanese lowland marsh on 12 March 2012 is described using microscopic photographs of the *Spirogyra* filaments, and physical and chemical conditions of their sampling site were shown in order to consider the factor inducing conjugation. Single cell of the *Spirogyra* was 40-50 μm wide and 60-100 μm long with plane end cell walls. One chloroplast was making 2-5 turns in each cell. Conjugation of the *Spirogyra* was scalariform-type, and tubes elongated both sides of the filaments. Fertile and sterile cells were mostly cylindrical, sometimes enlarged or inflated. Characteristics of fully-ripened zygospores were ellipsoidal, with a smooth medium spore wall and brownish color. The *Spirogyra* in this report was identified as *S. variformis* TRANSEAU. Changes in NO_3^--N concentration and water temperature seem to be factors inducing conjugation.

Key words: conjugation, Naka-ikemi, *Spirogyra variformis* TRANSEAU, zygospore

（Received: 12 October 2012, Accepted: 6 December 2012）

Introduction

Genus *Spirogyra*（Zygnemataceae）is a common group of filamentous green algae observed in many freshwater environments. Their formation process of conjugation and zygospores are generally introduced in sexual reproduction in biology textbook. Although conjugation of *Spirogyra* is a well-known phenomenon, little is understood about its detailed processes and mechanism, because of difficulty in reproducible induction of conjugation in the laboratory（Ikegaya et al., 2012）. Because the *Spirogyra* species has been classified by the shape and color of conjugation and fully-ripened zygospores formed in sexual reproduction（Yamagishi, 1966; Yamagishi, 1977）, determination of the exact species is very difficult. Many studies of *Spirogyra* classification have been carried out using samples collected from natural environments. However, only a few studies have showed the water environmental condition of the sampling site（*e.g.* Nozaki, 2010）. In the present report, the formation process of conjugation and zygospores of a *Spirogyra* species collected from a Japanese lowland marsh in early spring of 2012 is described using microscopic photographs of the *Spirogyra* filaments, and physical and chemical conditions of their sampling site were shown in order to consider the factor inducing conjugation.

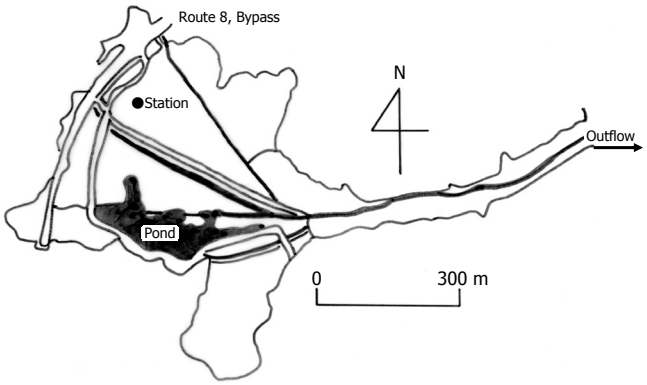

Fig. 1. Map of the sampling site, Naka-ikemi marsh.

Methods

This study was carried out in a Japanese lowland marsh, Naka-

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Fig. 2. Sampling station.

ikemi, Tsuruga, Fukui Prefecture, located at latitude 35° 39' N and longitude 136° 06' E near the center of Tsuruga City. The marsh is in a hilly district with an elevation of 50-60 m and extends 1.3 km from east to west and 0.5 km from north to south, with a total area of approximately 0.25 km^2 (Fig. 1). More information about the location of the study site was provided in Nozaki et al. (2009). Samples of *Spirogyra* filaments and water were collected from a small pool within a reed zone on 12 March 2012 (Fig. 2). Water temperature, pH (WAK-pH, Kyoritsurika Co.) and electric conductivity (CM21P, TOA-DDK Co.) were measured at the sampling station. Samples were stored in a box with ice and were returned to the laboratory within 8 hours after sampling.

Turbidity of water samples was measured with a water analyzer (WA1, Nippon Denshoku Co.) using pre-filtered water. Water sample was transferred to a glass fiber filter (GF-75, Advantec Co.) in preparation for the analysis of water color and nutrient concentrations. Water color was also measured with a water analyzer (WA1, Nippon Denshoku Co.). Nutrients analyses were as follows: NH_4^+-N (Solorzano, 1969), NO_2^--N (Bendschneider and Robinson, 1952), NO_3^--N (Kalff and Bentzen, 1984) and PO_4^{3-}-P (Murphy and Riley, 1962).

Spirogyra filaments were placed in a 100-mL glass beaker filled with tap water in our laboratory was kept on a shelf in front of a window facing south. *Spirogyra* filaments were observed under an optical microscope (BX 51, Olympus Co.), and microscopic photographs of conjugation and zygospores were taken by digital camera (Camedia C-5060, Olympus Co.).

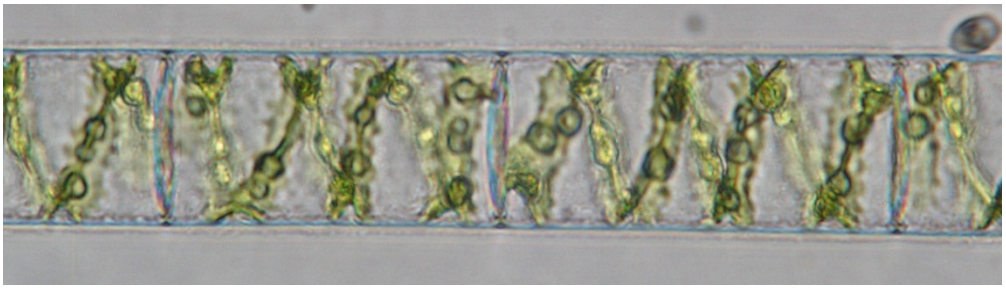
Results and Discussion

The formation process of conjugation and zygospores of the *Spirogyra* filaments is illustrated in Figure 3a-f. A single *Spirogyra* cell measured 40-50 μm wide and 60-100 μm long with plane end cell walls. One chloroplast was making 2-5 turns in each cell (Fig. 3a, b). Sexual reproduction of the *Spirogyra* was started by formation of papilla on cells opposite each other (Fig. 3b). After elongation of the papilla, 2 filaments aligned and conjugation tubes were formed between cells. Conjugation of the *Spirogyra* was scalariform-type and tubes elongated both sides of the filaments (Fig. 3d). Fertile and sterile cells were mostly cylindrical, sometimes enlarged or inflated. Zygospores matured by approximately 40-50 days after sampling. Characteristics of fully-ripened zygospores were ellipsoidal, smooth medium spore wall and brownish color (Fig. 3e, f). The *Spirogyra* in this report is identified to be *S. variformis* TRANSEAU according to monographs (Transeau, 1938; Yamagishi, 1966; Yamagishi, 1977).

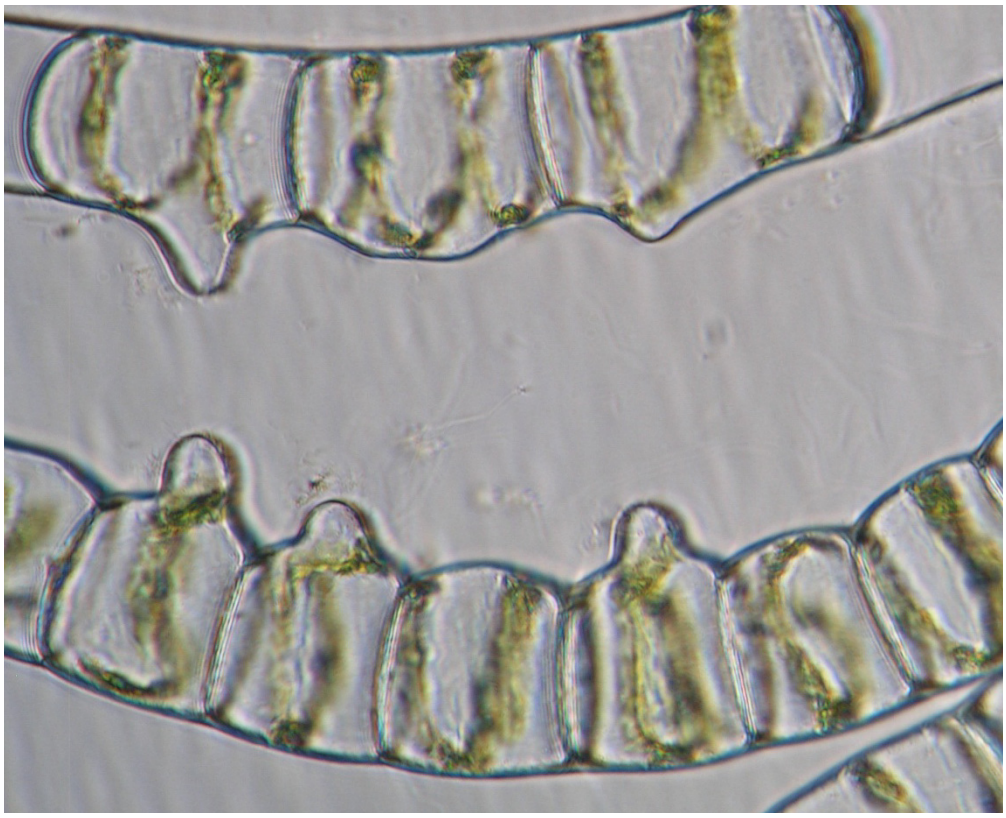
Physical and chemical conditions of the water sample collected from sampling station are shown in Table 1. Electric conductivity (25.9 mS m^{-1}) and NO_3^--N concentration (1383 μg L^{-1}) in the station were relatively higher than those of other areas in this marsh (Nozaki and Tuji, 1999; Tuji et al., 1999). In this study, the *Spirogyra* filaments were incubated using tap water of our laboratory (Nagoya City, Aichi Prefecture), and its NO_3^--N concentration was only about 500 μg L^{-1} (Nozaki, 2009). The role of nitrogen depletion and light intensity were suggested as key factors for inducing conjugation (Yamashita and Sasaki, 1979). Thus, the decline of dissolved nitrogen concentration seems to be a factor inducing conjugation in this study. Although the water temperature at the sampling station was 9.3 ℃ , the temperature condition of our laboratory where the *Spirogyra* filaments were incubated was clearly higher than that of the station. Thus, the rise in water temperature is regarded as another factor controlling conjugation of the *Spirogyra*. To understand in more detail the factors affecting the conjugation and zygospore formation of the *Spirogyra*, further field and laboratory studies are warranted.

Table 1. Characteristics of water quality in sampling station on 12 March 2012.

Sampling time	13:17
Water temperature (℃)	9.3
pH	6.5
Turbidity (degree)	1.6
Water color (degree)	4.0
Electric conductivity (mS m^{-1})	25.9
NH_4^+-N (μg L^{-1})	5.6
NO_2^--N (μg L^{-1})	0.8
NO_3^--N (μg L^{-1})	1383.0
PO_4^{3-}-P (μg L^{-1})	4.5

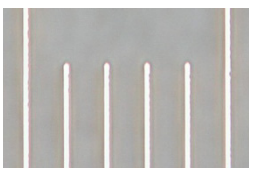


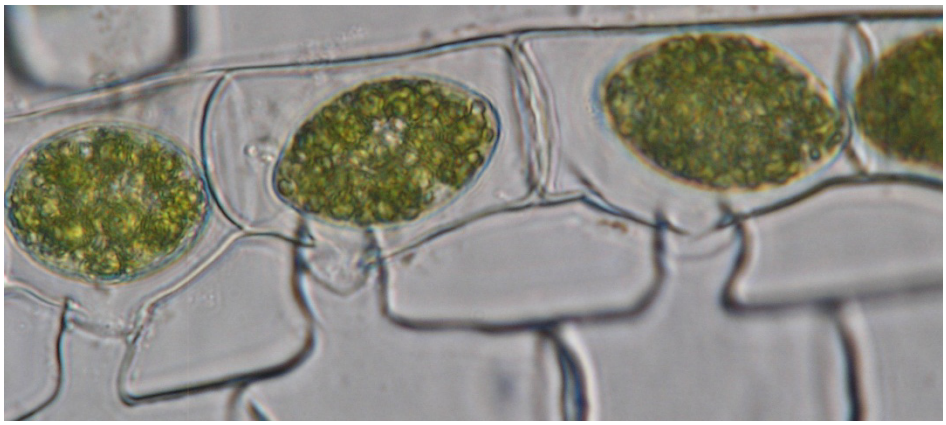
a) × 400
March,12

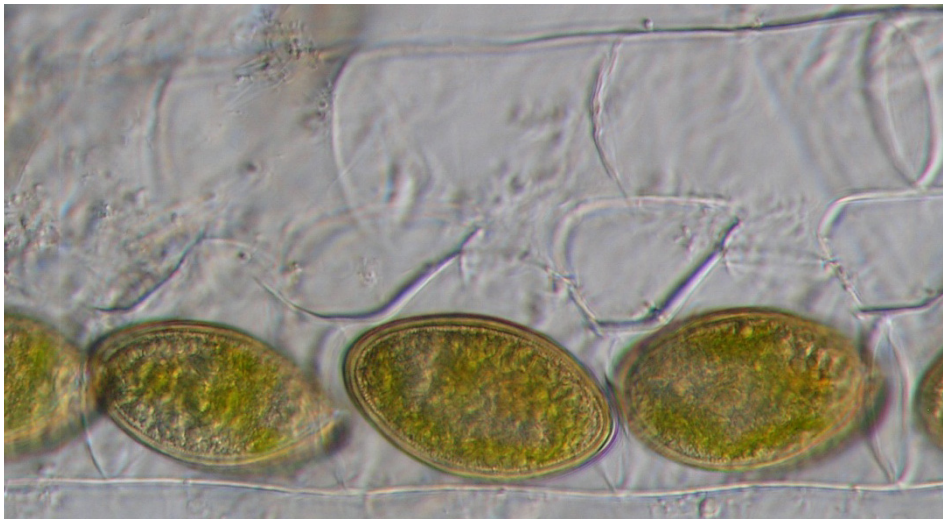


b) × 400
March,26

c) × 400
March,26







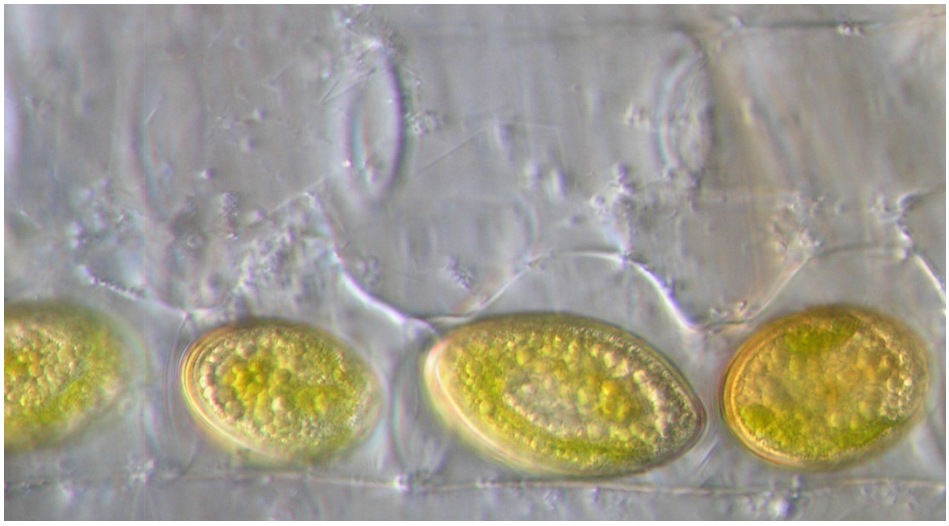


Fig. 3a-f. Microscopic photographs of formation process of conjugation and zygospores of the *Spirogyra* collected from Naka-ikemi marsh on 12 March 2012.

Acknowledgements

The author is grateful to Yuki MORI of the School of Education, Sugiyama Jogakuen University, for her generous assistance in the field sampling. The author wishes to thank Dr. Takashi TASHIRO and Tomohiro YOSHIIKE of the Nagoya University for their help in this study. This research was supported by a Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science (No. 24501114) to Kentaro NOZAKI.

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(Editor: Professor Dr. Noriko ISHIDA,
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摘　要

中池見湿地（福井県敦賀市）で採集された糸状緑藻
アオミドロ（*Spirogyra* 属）の接合体と接合胞子の形成過程

野崎健太郎

2012 年 3 月 12 日に中池見湿地から採集されたアオミドロの接合体と接合胞子の形成過程を顕微鏡写真と採集地点の水環境と共に記述した。細胞は，幅 40 ～ 50 μm，長さ 60 ～ 100 μm で，細胞間の隔膜は平板状であった。葉緑体は 1 本で，2 ～ 5 回転していた。接合体は，並び合った 2 本の糸状体の両方から接合管が伸び，梯子状に形成された。接合体は大部分が円筒状であったが，時には拡張していた。熟した接合胞子は，楕円形で，胞子中層膜は黄褐色で平滑であった。これらの特徴から，*Spirogyra variformis* TRANSEAU と同定した。硝酸態窒素濃度と水温の変化が接合体の誘導に影響していると思われた。

キーワード：接合体，中池見湿地，
Spirogyra variformis TRANSEAU，接合胞子

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