

Electronic supplementary material (ESM)

Groundwater contamination by sewage causes benthic algal outbreaks in the littoral zone of Lake Baikal (East Siberia)

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General notes on *Spirogyra* biology and diversity in Lake Baikal and on the planet.

Prior to the current ecocrisis, five species of the genus *Spirogyra* were described from bays and shallows of Lake Baikal (for summary, see Iziboldina, 2007). Single vegetative filaments were rarely found in samples collected in open parts of the coastal zone during the 45 years of Iziboldina's macrophytobenthos studies. "Evidently, representatives of the *Spirogyra* genus grow only in the isolated, shallow water bays and gulfs of the Lake with well warmed waters" (Iziboldina, 2007: p. 213). Summarizing all previous data obtained within an 80-year period prior to the current ecological crisis, *Spirogyra* has never created mass blooms and/or giant wash-ups in open parts of the coastal zone.

According to different sources, the genus *Spirogyra* is distributed world-wide and includes about 400–500 species (Guiry and Guiry, 2015; Rundina, 1998). Their identification is complicated and based on the morphology of vegetative, fertile filaments, and the types of conjugation and zygospores. Finding conjugation and zygospores *in situ* is difficult and a rare event. Therefore, scientists often delineate separate morphotypes based on the morphology of vegetative filaments (Hainz et al., 2009). We use this approach here where we focus on two of the most widely distributed and morphotypes in Lake Baikal which together dominate the

biomass of autumn blooms within the lake proper and in some tributaries (ca. $\geq 90\%$) (Timoshkin, 2016). Attached (“morphotype 1”) and free-floating (“morphotype 2”) morphotypes were described previously from the open shallows of Lake Baikal (Timoshkin, 2016). “Morphotype 1” typically occurs on hard substrates and dominates (frequently $> 95\%$ and almost 100% of the projected area) the rocky bottoms near coastal settlements, towns, and areas with high recreational activity (see below) (Fig. S1). Its filaments attach to the rocky bottom and cliffs by rhizoids (Fig. S1 A) with filaments being 40–50 μm in diameter, with a simple cell wall, and three to four visible chloroplasts in young cells at the beginning of their growth (Fig. S1 A–D). The chloroplasts of the “old” cells (predominantly, in autumn) within the filament become solid, so-called “condensed” (Fig. S1 E–F). Actually, at this stage the chloroplast number can be seen in several cells only; those cells are located in either the basal or apical portions of the filament. During the late summer–autumn seasons of 2014–2016 “morphotype 1” dominated at depths 0.5–1.5 m on the rocky substrate of Listvennichnyi, Bol’shie Koty, Aya, Perevoznaya, Kharin-Irgi, Senogda, Frolikha, Ayaya bays, near Baikal’sk, Slyudyanka, Kultuk, Khuzhir, Severobaikal’sk, Zarechnoe settlements (Fig. 1). “Morphotype 2”, the free-floating form, dominates in some tributaries and over sandy substrates in the coastal zone of the lake (Chyornaya, Zhilishche, Bol’shaya Kotinka Streams; near Senogda and Severobaikal’sk). These filaments are 25–40 μm in diameter and usually have one, or more rarely, two chloroplasts (sometimes within the same filament) (Figs. S2 D–E, S3 B–C, S4). In exceptional cases, some filaments may also have rhizoids. Numerous additional morphotypes occur in the coastal zone of the open part of the lake, but as a rule they are rare, comprising a minor component of the samples (usually $< 10\%$ of occurrence)¹. Some of them are shown on the light-microscopic images (Fig. S1 B, E, S4 B, C). “Morphotype 2” dominated at the warm-water shallows and temporary puddles in vicinities of Senogda Bay and Zarechnoe Settlement in June 2016 (Fig. S3 A–C, S4), while the giant amount of rotten *Spirogyra*, covered by sand due to storms and a higher water level in autumn of 2015, mostly consisted of “morphotype 1” (Fig. S3 D). In early summer, in areas of year-round waste water pollution, filamentous algae (dominated by *Spirogyra*) create ball-shaped aggregations, 3–7 mm in diameter, which concentrate on the sandy bottom of the lake (Fig. S4 A).

Additional temperature data supports the viewpoint on the *Spirogyra* tolerance to significant temperature changes.

¹ Study of Baikal *Spirogyra* taxonomy, based on comparative-morphological and molecular-biological approaches, is currently being performed (Volkova et al., 2013, 2015).

Figure S10 presents the typical diel variation in temperature near the substrate at 3 m depth along the west coast of the south basin in summer. These measurements were obtained using TidBit Stow Away Loggers. In August–September 2015, when the *Spirogyra* populations were already in full bloom, the temperature ranged from 8–17.3°C during a 24 h period (i.e., a gradient of almost 10°C). Numerous similar examples of significant daily changes of surface and bottom water temperature within the period of open waters can be found in the electronic data base (Timoshkin et al., 2017). This means that the *Spirogyra* populations and other benthic inhabitants of the Lake Baikal coastal zone (0.5–10 m depths) experience large diel changes in temperature on a daily basis.

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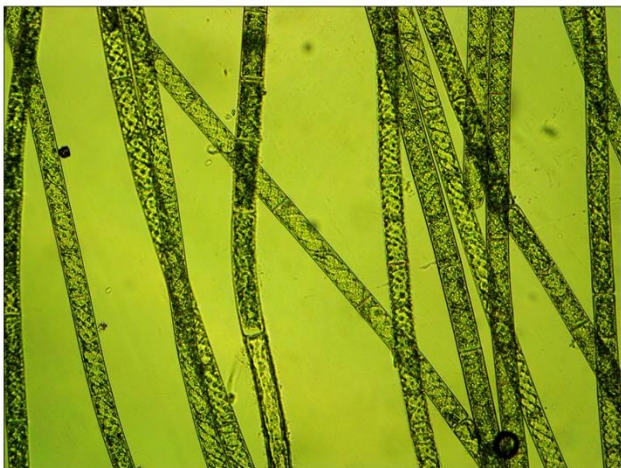
ESM figure captions



A _____



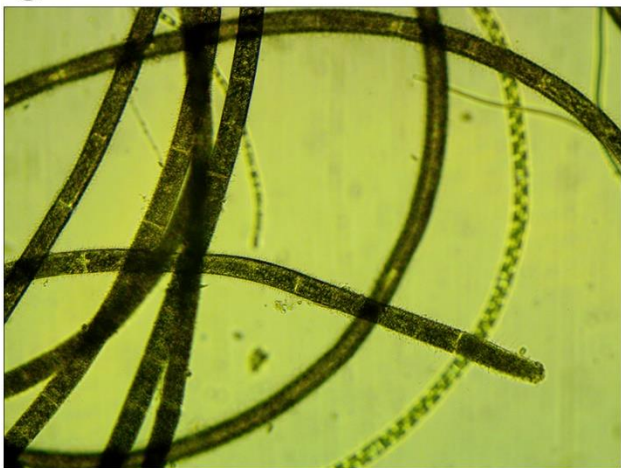
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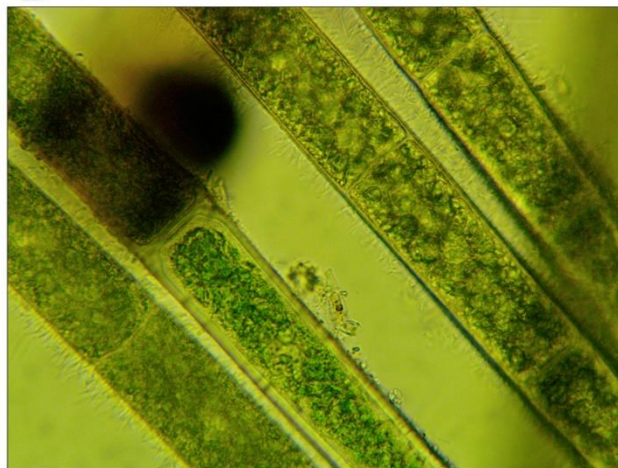
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Figure S1. Light-microscopic images of *Spirogyra* sp. “morphotype 1”: young (A–D) and mature (E–F, condensed chloroplasts) filaments, with rhizoid (A), conjugating cells (B, D) and zygospores (D). Bol’shie Koty Bay, near dock B, 1.5-m depth, August 31, 2015 (A–D) and October 29, 2015 (E–F). Scale bars: A, C, E = 0.5 mm, B, D = 0.2 mm, F = 0.1 mm.

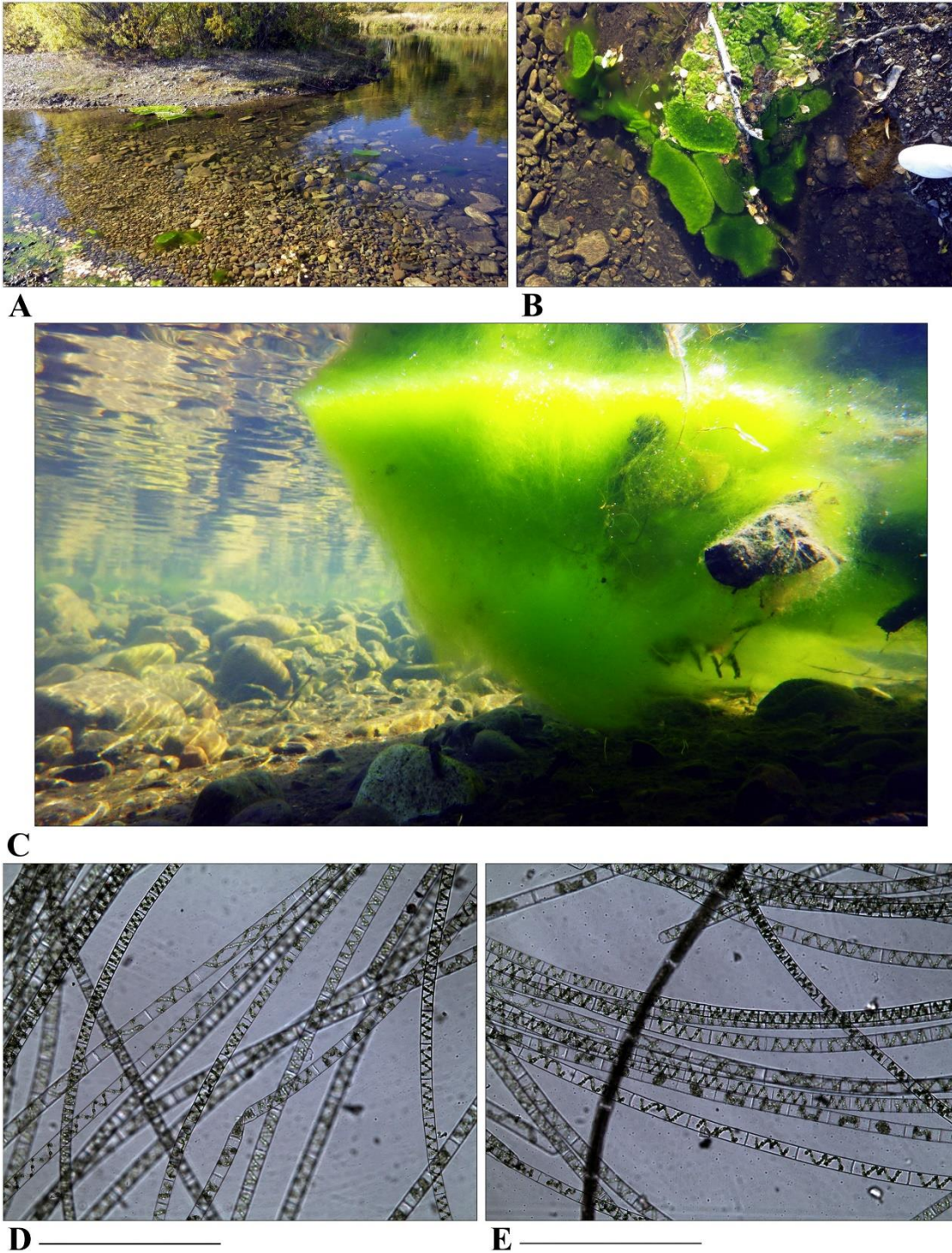
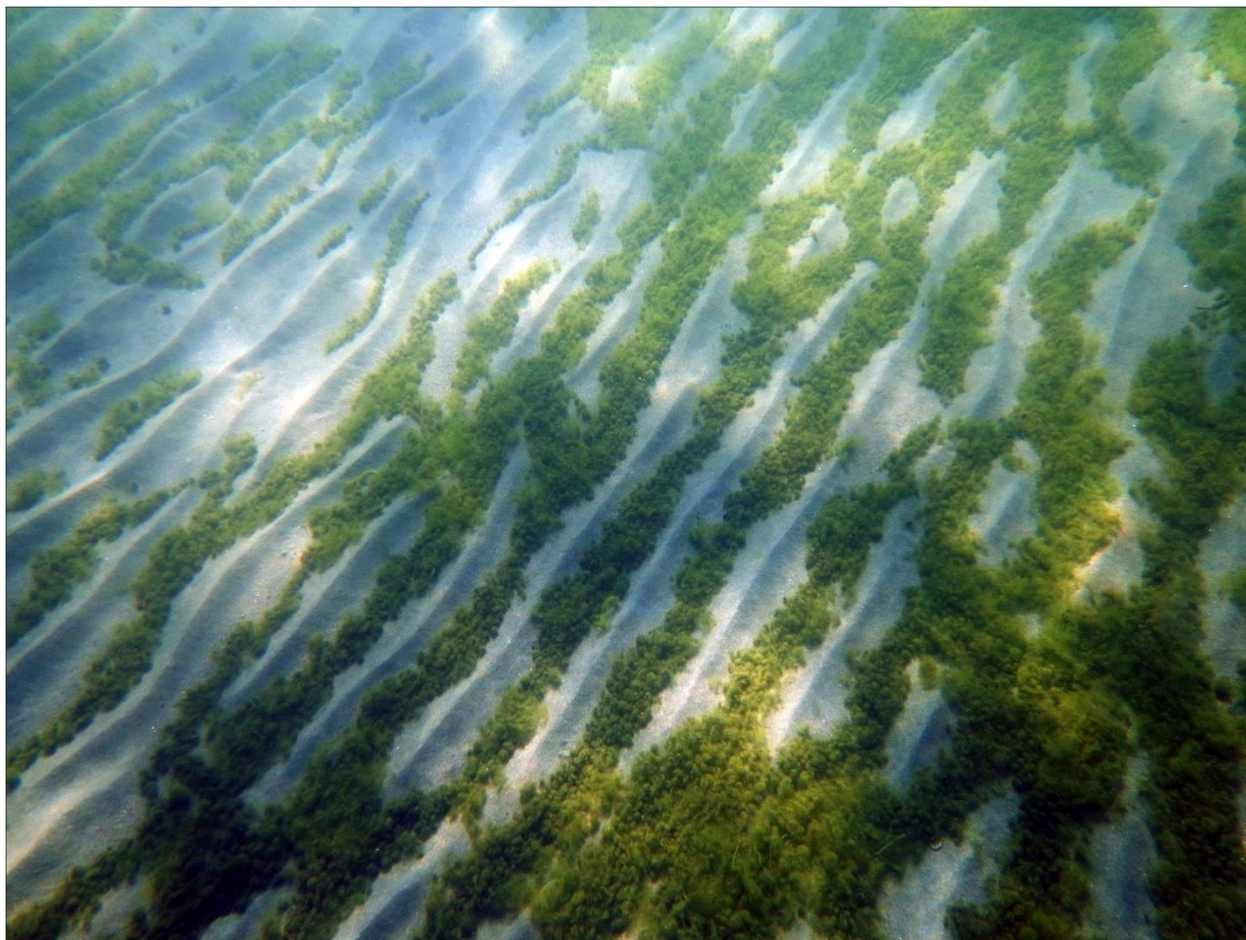


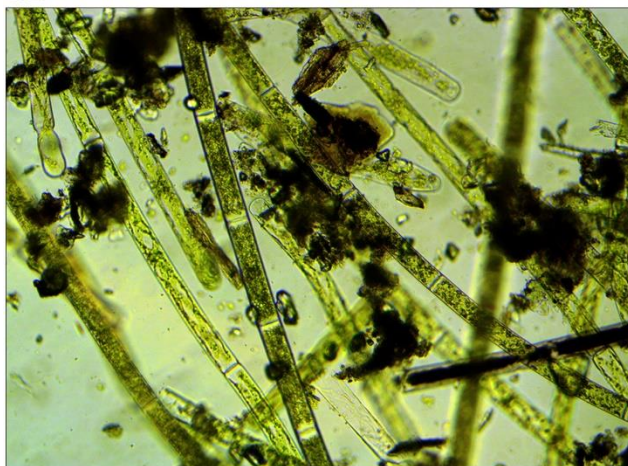
Figure S2. *Spirogyra* sp. “morphotype 2”: A–B — free-floating algal “clouds”; C — underwater clouds, near the river mouth; D, E — light microscopic images of the filaments. Chyornaya Stream (Fig. 1), September 20, 2015. Scale bars: D, E = 0.5 mm.



Figure S3. Free-floating *Spirogyra* “morphotype 2” in Senogda Bay, June 18, 2016. A (left) — aerial view of coastal clouds of *Spirogyra*, summer, 2016. B, C — light microscopic images of filaments. D — decayed algae being cleaned up by volunteers from Severobaikal’sk City. Scale bars: B, C = 0.5 mm.



A



B



C

Figure S4. Underwater balls of algae on sandy substrate, 0.5-m depth, opposite Zarechnoe Settlement, ca. 3 km west of Tyya River, September 25, 2016. A — general view. B — light microscopic images of the balls, consisting mostly of several *Spirogyra* morphotypes, *Oedogonium* sp., detritus and other filamentous green algae. Scale bars: B, C = 0.5 mm.

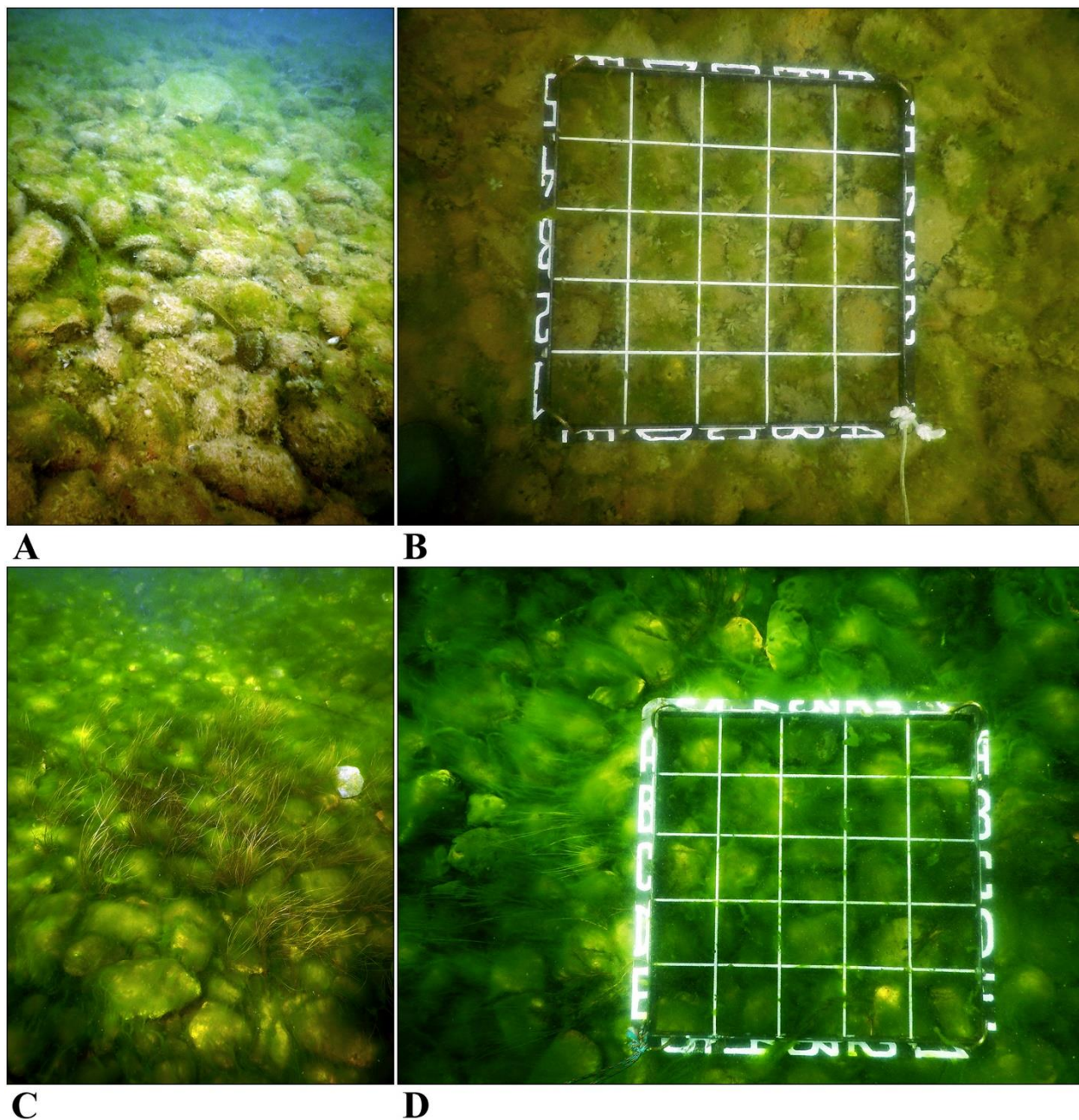


Figure S5. Underwater photos of benthic algal blooms in the coastal zone of Bol'shie Koty Bay, 1.5-m depth, near dock B (see Figure 4). July 23, 2016 (A–B) when the typical Baikalian pattern of benthic algae was present (mostly *Didymosphenia*, *Ulothrix*, Chrysophyceae spp) and August 31, 2016 (C–D) when *Spirogyra* morphotype 1 covered 80–100% of the substrate. Frame size: 50 X 50 cm. See text for additional detail.

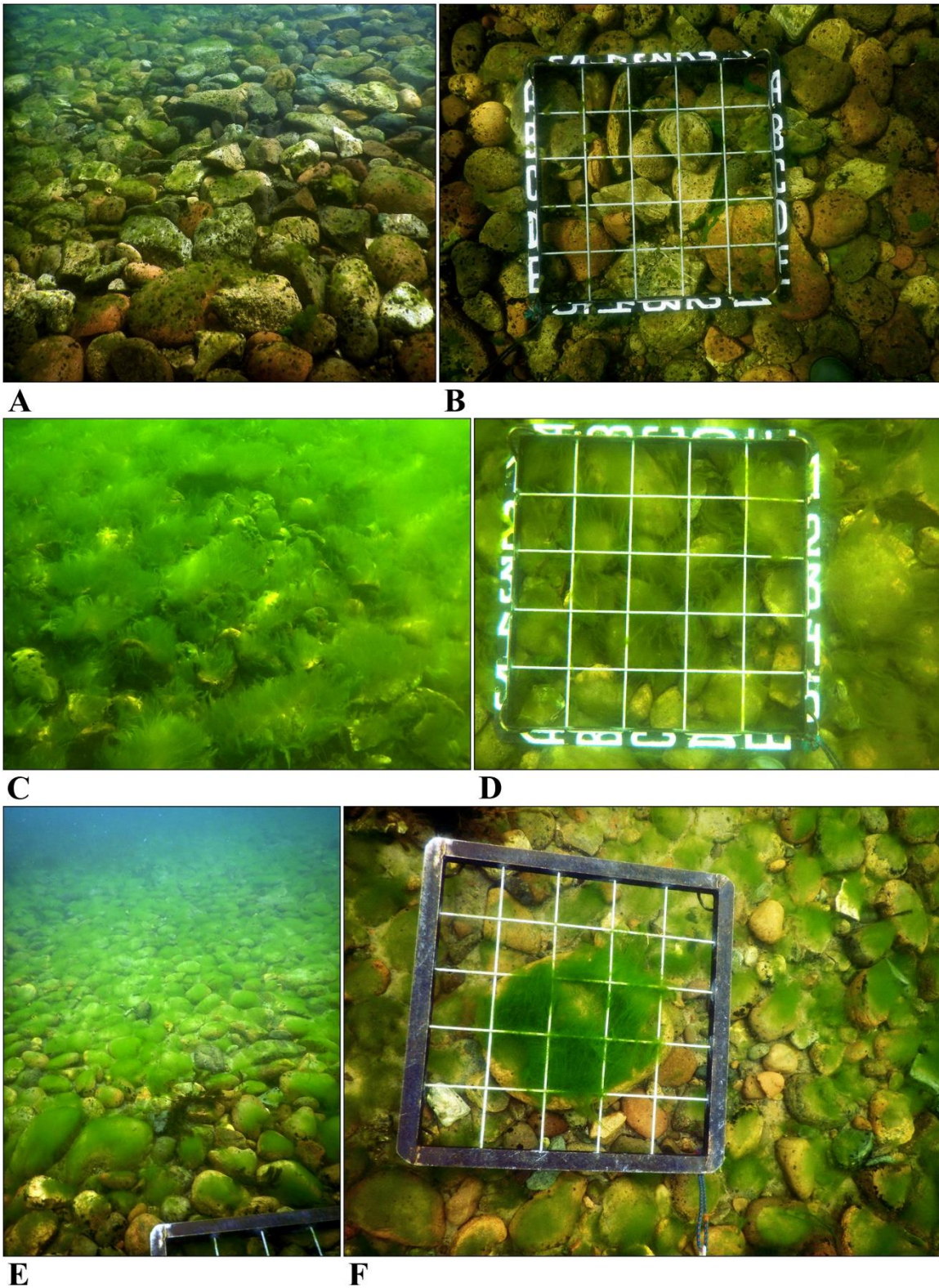


Figure S6. Underwater photos of the benthic algal blooms (predominantly, of *Spirogyra* “morphotype 1”) in the coastal zone of Bol’shie Koty Bay, 1.5-m depth, about 200 m south of dock A (A–B) where there are no buildings near the shoreline and near dock B (C–F) in front of

the field station of Irkutsk State University. September 20, 2016 (A–D) and October 21, 2016 (E–F). See Figure 4 caption and text for additional detail.

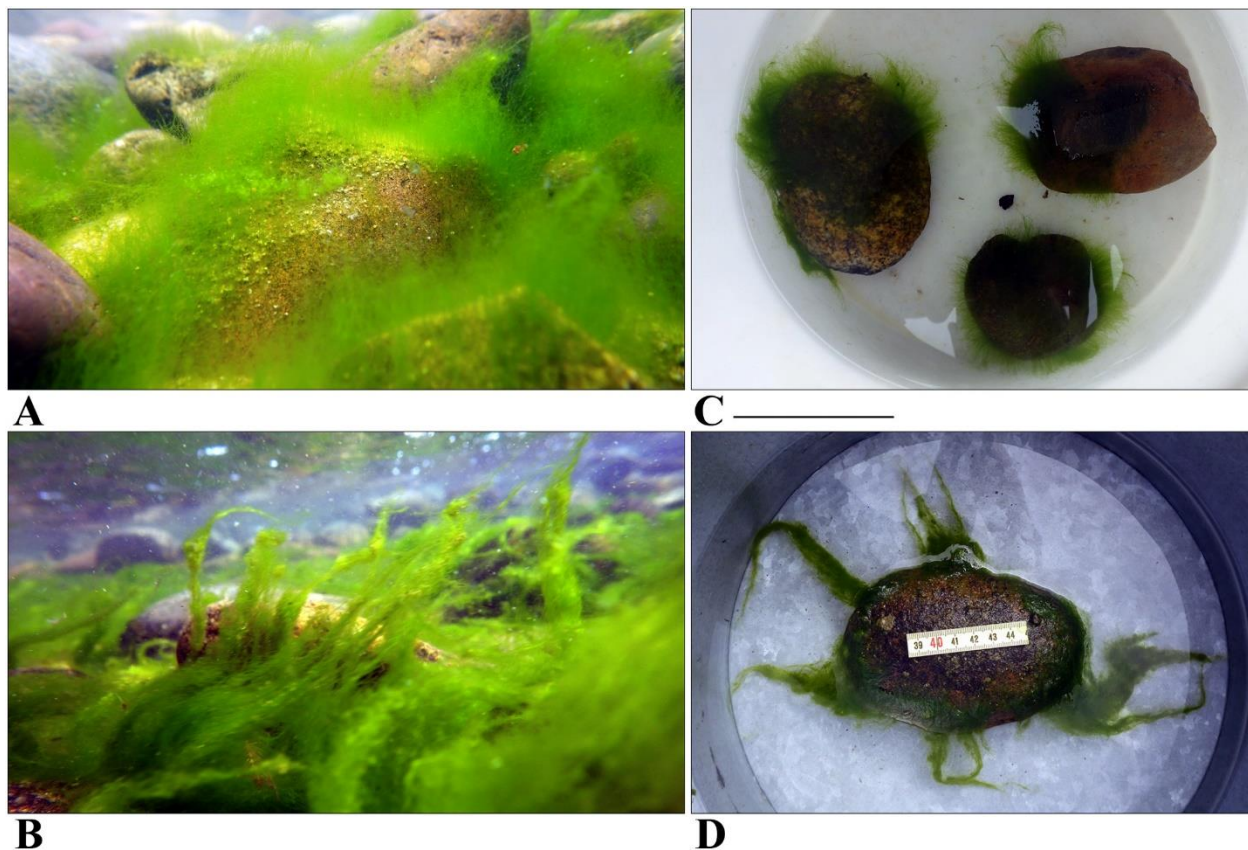


Figure S7. Underwater and aerial images of stones with young (A, C) and mature (B, D) *Spirogyra* “morphotype 1” from the “LFS patch”. Photos taken in the end of July–August (A, C) and September, 2015 (B, D), respectively. Scale bar: C = 10 cm.

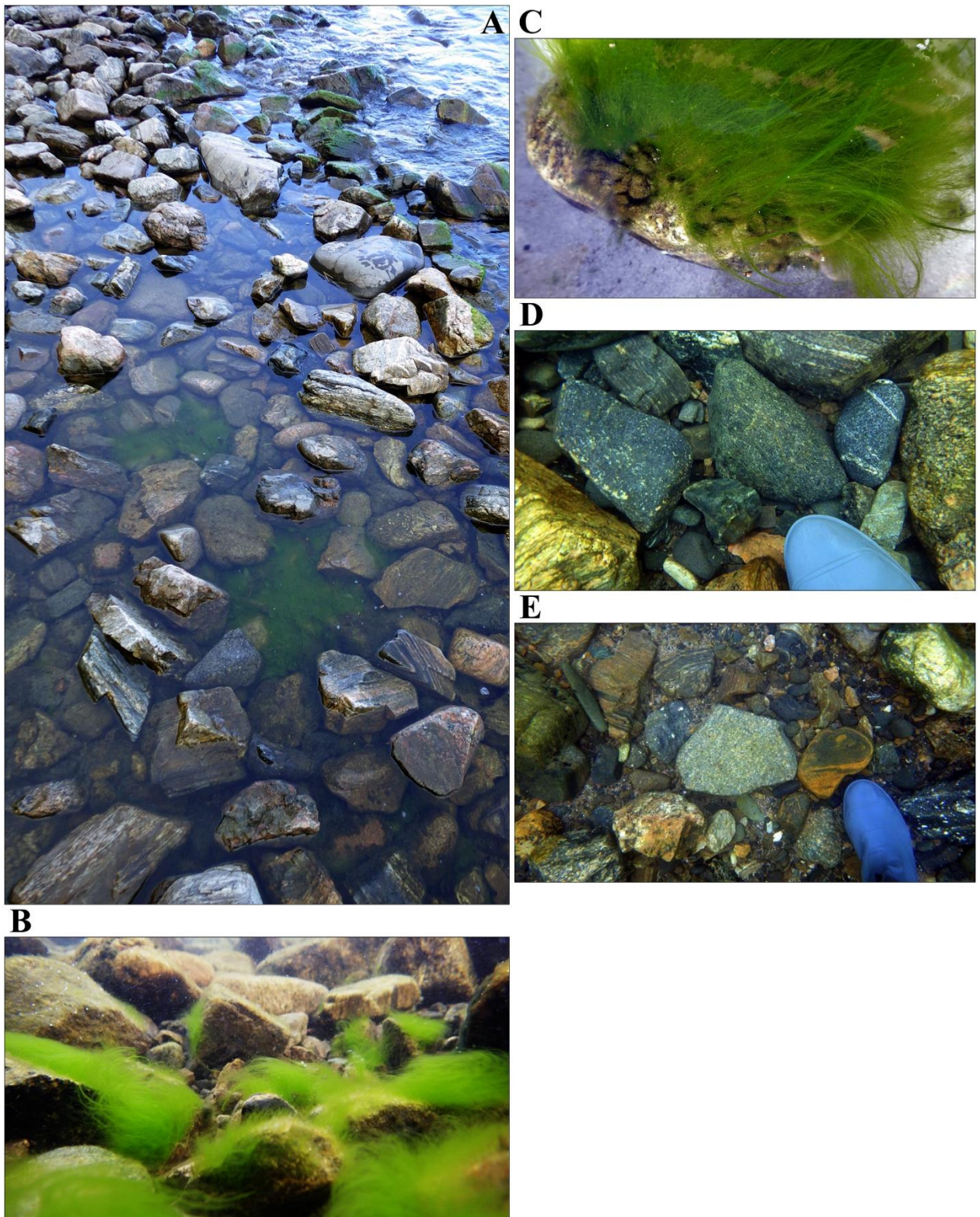


Figure S8. Patches of *Spirogyra* “morphotype 1” developed in puddles above the shoreline in Emelyanikha Bay, western coast of South Baikal (Fig. 1). A–C — aerial and underwater view, 0.2-m depth. D, E — substrate (stones beyond algal patch, 0.5 and 1.5-m depth, respectively) within the lake at the same site. September 15, 2015.



Figure S9. *Spirogyra* "morphotype 1" patch ("LFS patch"), October 29, 2015. This is the same patch featured in Figures 6 and S2, however, the lake level has now dropped. Frame size: 50 X 50 cm. See text for additional details.

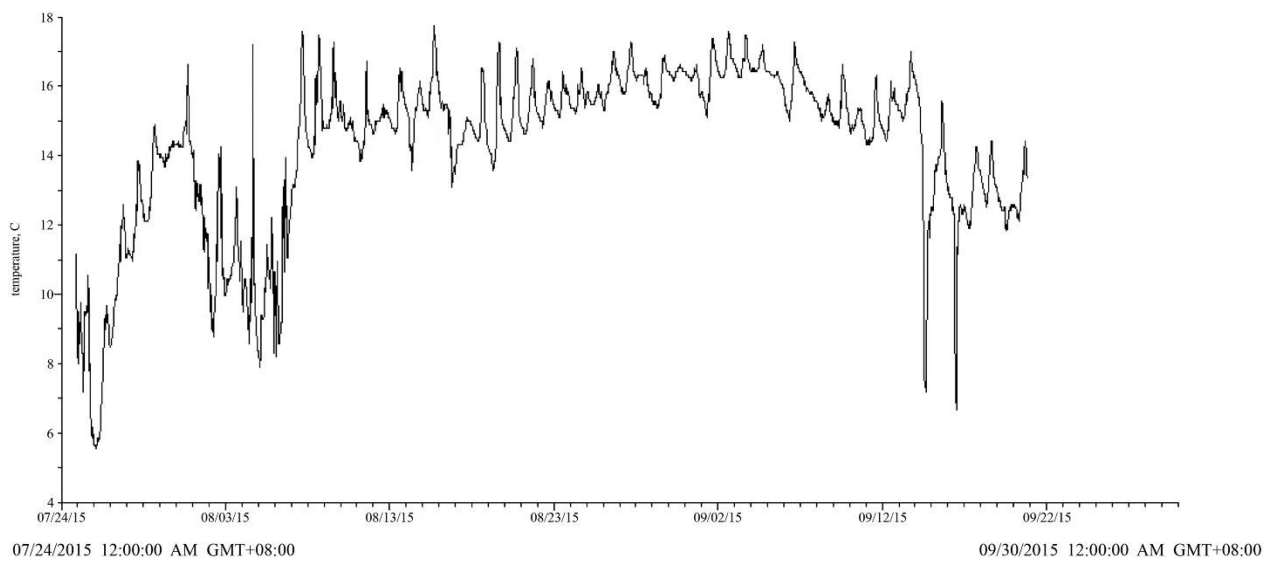


Figure S10. Variation in near-bottom water temperatures (°C) at a depth of 3 m (typical depth of *Spirogyra* mass development) in Bol'shie Koty Bay, Lake Baikal over a 30-day period in summer, 2015. Temperature logger was placed near dock A, see Figure 4.