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Life cycle of *Praestephanos suzukii* inferred from annual changes in its cell density and size in the north basin of Lake Biwa

Megumi Saito-Kato[1, *], Kazumi Matsuoka[2, 3], Ken-Ichiro Ishii[3], Manabu Wen-Liu Tanimura[3] and Motoki Kayama[3]

[1]Department of Geology and Paleontology, National Museum of Nature and Science, 4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
[2]C/O Institute for East China Sea Research, Nagasaki University, 1551–7 Taira-machi, Nagasaki 851–2213, Japan
[3]Seed Bank Ltd., 76–1 Kitashirakawa-nishimachi, Sakyo-ku, Kyoto 606–8267, Japan
*Author for correspondence: memekato@kahaku.go.jp

Abstract An almost two-year investigation of surface water in Lake Biwa showed annual cyclic changes in cell density and size of the diatom *Praestephanos suzukii*. Cell density increased abruptly to $>10^5$ cells/L in late summer (late August or September), and gradually decreased to $<10^3$ cells/L during autumn and winter. Living cells were rarely observed during late spring to early summer. Small cells ($<15\,\mu$m diameter) were dominant during the late-summer bloom, and the modal size decreased over the following 1–2 months. Large cells ($>45\,\mu$m in diameter) were observed after the bloom (in October–December), and some had hemispherical initial valves on one or both sides. Cell size decreased during times when cell density decreased or remained low. These observations suggest that *P. suzukii* has a one-year life cycle with sexual reproduction in autumn just after the late-summer bloom. The onset of the bloom coincided with a decline in surface water temperature and the beginning of vertical mixing.
Key words: *Praestephanos suzukii*, life cycle, sexual reproduction, cell density, cell size

Introduction

Praestephanos suzukii (Tuji and Kociolek) Tuji (Tuji *et al.*, 2014) is an extant endemic diatom in Lake Biwa. It was described originally as two related species, *Stephanodiscus suzukii* and *S. pseudosuzukii* (Tuji and Kociolek, 2000), which were mainly distinguished on the basis of size (i.e., the former was large and the latter small). Before Tuji *et al.* (2014) proposed the new genus *Praestephanos*, Kato *et al.* (2003) emended the taxonomy by showing that *S. pseudosuzukii* is synonymous with *S. suzukii*, with the two groups showing continuous variation in size and valve morphology; the apparent morphological differences were interpreted as intraspecific changes in the life cycle of one species. However, these life-cycle changes were inferred from fossil specimens preserved in sediment (Kato *et al.*, 2003), and the life cycle of living specimens in Lake Biwa remains unknown.

Diatom life cycles are closely associated with changes in cell size. Cell size is reduced during each asexual cell division because daughter valves must form inside the rigid siliceous valve of the mother cell (Round *et al.*, 1990). Once the cells fall below a threshold size, sexual reproduction (which increases cell size) is induced (see Drebs, 1977). The life cycle of diatoms can therefore be traced by observing changes in cell size within a population (e.g., Jewson, 1992; Pérez-Martínez *et al.*, 1992; Jewson *et al.*, 2015; Rioual *et al.*, 2017). Despite the operation of long-term plankton monitoring programs in Lake Biwa (Ichise *et al.*, 2011), the fact that *P. suzukii* was previously considered to be two species of differing size means that its life cycle has not been adequately studied (e.g. Ichise *et al.*, 2011).

Therefore, in the present study, we conducted monthly sampling of surface water in Lake Biwa over a period of nearly two years to determine changes in the cell density and size of *P. suzukii*. Our results should be sufficient to deduce the life cycle of this species. In addition, to examine any impacts of environmental conditions on the species' life cycle, we compared the *P. suzukii* timeseries to changes in meteorological and hydrological factors. Finally, we used additional water samples collected

from a small boat to determine the horizontal and vertical distributions of *P. suzukii* in the lake to identify any relationships between lake water circulation and the growth of this species.

Material and Methods

To investigate annual changes in the cell density and size of *P. suzukii*, surface water was sampled monthly from Oura Fishing Port (located at the northern-most end of Lake Biwa; Fig. 1) from March 2021 to November 2022 by using a bucket. Oura fishing port, which opens to the west, has artificial wave barriers on the south and north sides, and surface water was sampled at the western end of the south side of the barrier, which extends farther offshore (Fig. 1C). When surface water could not be collected at Oura Fishing Port, sampling was conducted at Kaizu Osaki (ca. 6 km southwest of Oura Fishing Port). This situation occurred twice during the study period, due to abnormally low water levels on 10 November 2021 and the presence of abundant driftwood after heavy rainfall on 20 September 2022.

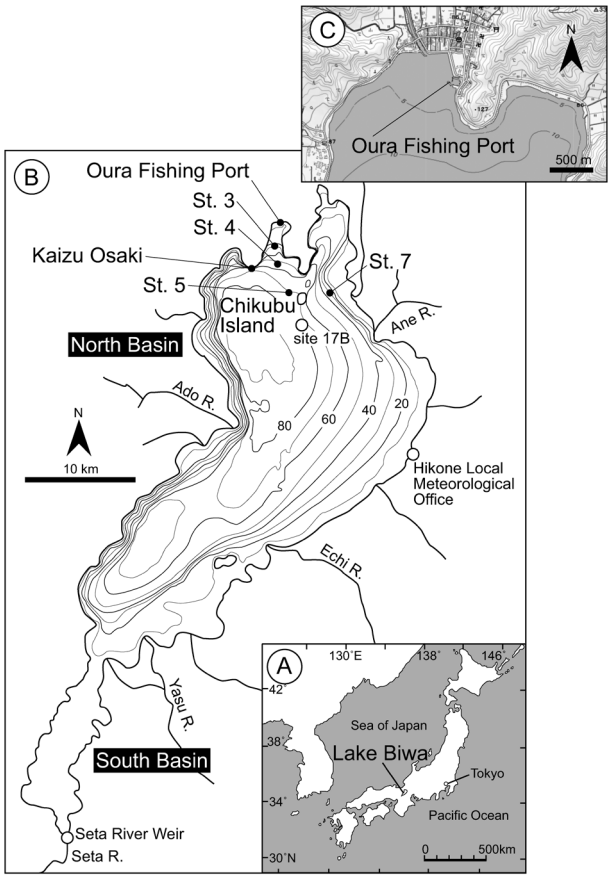

Fig. 1. Maps of (A) the study region, (B) sampling sites and (C) detailed location of Oura Fishing Port in Lake Biwa. Filled circles in (B) show Oura Fishing Port, Kaizu Osaki, St. 3, St. 4, St. 5, and St. 7. Open circles show the Hikone Local Meteorological Office, where meteorological data for the northern Lake Biwa region are collected; the Seta River Weir, where the lake level is measured and regulated; and Site 17B offshore of Imazu (known as Imazu-oki-chuo), where total N, total P, and SiO_2 concentrations are monitored by the Lake Biwa Environmental Research Institute.

In addition to the monthly surface water samples, water samples were collected from four stations (St. 3, 4, 5, and 7; Fig. 1) in the middle of the lake from aboard a boat on five days (19 April, 20 July, 11 October, and 14 December 2021, and 7 June 2022) to assess whether the Oura samples were representative of the wider north basin region of Lake Biwa. These samples were collected by using a Van Dorn water sampler (Rigo 5026-A owned by Seed Bank Co., Ltd) at depths of 5, 10, 20, 40, and 60 m and from 1 m above the lake bottom (hereafter, B-1 m). For each water sample, 2 L of surface lake water was transferred to a plastic bottle and immediately stored in the dark in a refrigerator.

Surface water temperature was measured during each water sampling. A vertical profile of water temperature was also obtained by using DEFI2-D50 and DEFI2-CT loggers (JFE Advantech Co., Ltd., Hyogo, Japan) owned by the National Museum of Nature and Science (NMNS). The loggers were slowly lowered to the bottom of the lake and then pulled up to obtain the vertical profiles. Although there was a noticeable offset between the temperatures obtained on the way down to the lake bottom and on the way up to the surface (Fig. 2), we judged the data to be sufficient for estimating the depth of the thermocline.

Once the surface lake water had been obtained, a 0.5- or 1-L subsample was filtered through a Millipore AAWP membrane filter (0.8-μm pore size; Merck, Darmstadt, Germany) by the following day at the latest. The filters were dried at room temperature, cut into twelfths, oil-mounted on glass slides covered by cover slips, and observed at 400× through an Axioplan light microscope (Carl Zeiss AG, Oberkochen, Germany) at NMNS. Living cells (i.e., those filled with chloroplasts) and dead empty cells (converted to number of cells) were counted

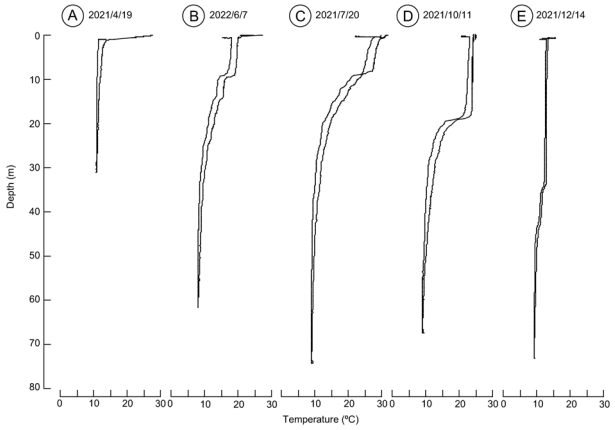

Fig. 2. Vertical profiles of water temperature measured at St. 5 (offshore of Chikubu Island) during the collection of water samples from a boat on (A) 19 April 2021, when no thermocline was observed; (B) 7 June 2022, when the thermocline was developing; (C) 20 July 2021, when a strong thermocline had developed; (D) 11 October 2021, when vertical mixing deepened from the surface; and (E) 14 December 2021, when the thermocline had almost disappeared.

separately, and area on the filter was used to estimate cell density per liter of water sample. Cell diameter was measured using an ocular micrometer with precision 1.25 μm. Diatom cells on the dried filters were also coated in gold with Quick Auto Coater (SC-701AT; Sanyu Denshi Ltd., Tokyo, Japan) and examined with a JSM-6510 scanning electron microscope (JEOL, Ltd., Tokyo, Japan). Both the scanning electron microscope and the coater were supplied by NMNS.

To identify potential environmental factors affecting the *P. suzukii* population, we compared our timeseries with weather data provided by the Hikone Local Meteorological Office, including average monthly temperature, precipitation, solar radiation and sunshine hours (available at the website of the Japan Meteorological Agency, https://www.data.jma.go.jp/gmd/risk/obsdl/index.php). Water dynamics were estimated from vertical profiles of water temperature obtained from the boat in the present study. Daily lake levels at Seta River Weir, where the lake discharges, were obtained from the monthly report of the Civil Engineering Disaster Prevention Information System of Shiga Prefecture (https://shiga-bousai.jp/report/report01.php?day=2023-06-19&time=15:40&id1=12&id2=0&id3=0&id4=0&sid=0). Total nitrogen (T-N), total phosphorus (T-P), and dissolved silica (SiO_2) concentrations at Imazu-oki Chuo (Site 17B, Fig. 1) were obtained from the database of the Lake Biwa Environmental Research Institute (https://www.lberi.jp/investigate/date).

Results

The vertical distribution of *P. suzukii* cell density (as estimated from water samples taken from the boat) varied considerably among sampling dates (Fig. 3). On 19 April 2021 (when no thermocline was present), both living and dead cells were distributed almost homogenously across sampling stations and depths. On 20 July 2021, cell density was very low (less than 1000 cells/L), and samples obtained from below the thermocline (ca. 8–9-m depth) mostly contained dead cells, although a high proportion of living cells was observed at a depth of 20 m at St. 5 and at 10 m at St. 7. On 11 October 2021, more than 10^5 cells/L were observed at every depth and site, with living cells accounting for over three-quarters of cells above the thermocline (ca. 20-m depth). On 14 December 2021, cell densities in surface water were lower than on 11 October at every site but remained high at depths greater than 5 m at eastern stations (St. 5, St. 7), and the ratio of living to dead cells was small below the thermocline (ca. 20–30-m depth). On 7 June 2022, fewer than 10^4 cells/L were observed at every site and depth and the ratio of living to dead cells was high below the thermocline (ca. 10-m depth).

Cell densities in the surface water of Oura Fishing Port were compared against those at other sites and depths (Fig. 4). On most sampling days, cell densities in the surface water were similar across all stations. The one exception was on 7 June 2022, when cell density in the surface water was considerably lower at St. 4 and St. 7, stations in the middle of the lake, but higher cell densities at these stations were observed below the thermocline.

At Oura Fishing Port, apparent blooms of *P. suzukii* were observed during late summer to early autumn (Fig. 5). The mode of cell size was 12.5–15.0 μm on 23 August 2021, when cell density was increasing rapidly. Subsequently, cell density declined and the mode of cell size also fell to 7.5–10.0 μm. In 2022, a similar increase in cell density was observed during late summer to early autumn,

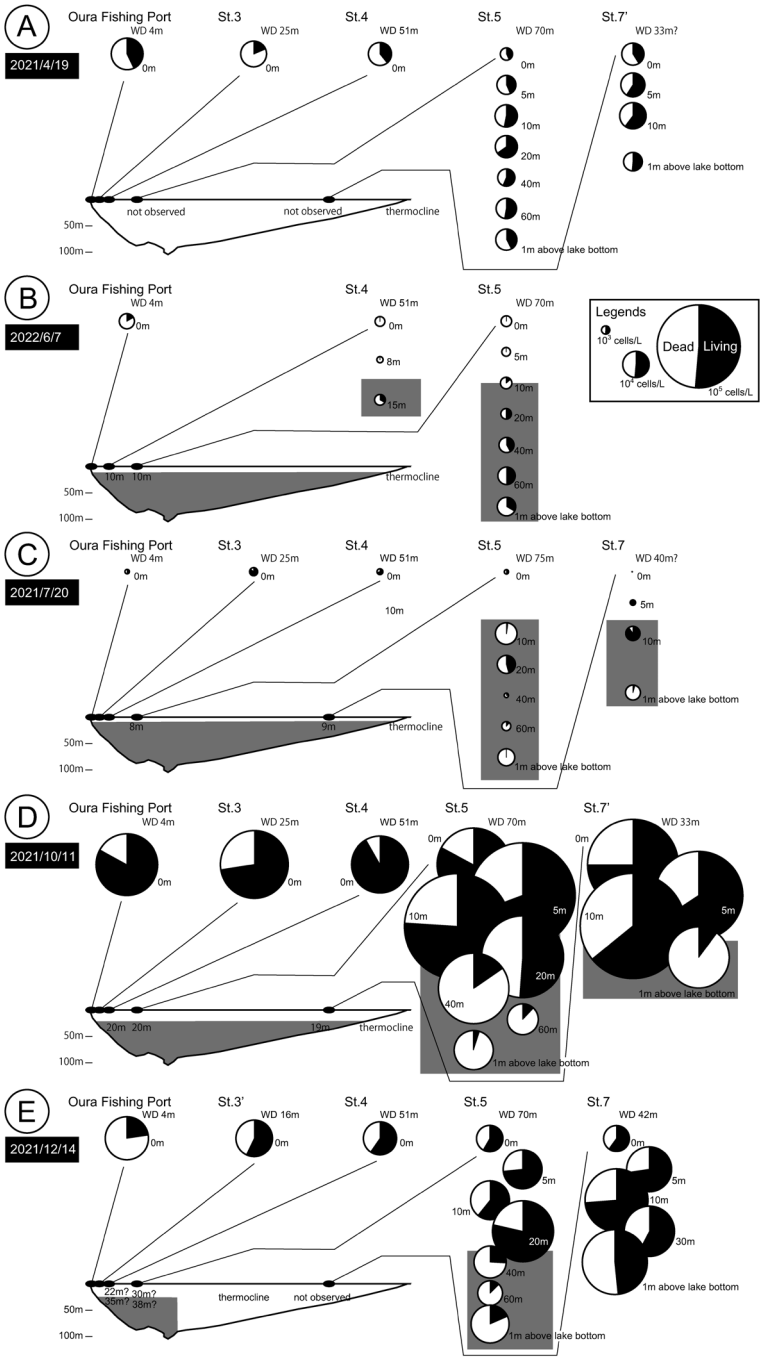

Fig. 3. Abundances of living and dead cells at various sampling sites and depths on (A) 19 April 2021, (B) 7 June 2022, (C) 20 July 2021, (D) 11 October 2021, and (E) 14 December 2021. The diameter of each pie diagram indicates cell density (living and dead), and the ratio of the number of living to dead cells is shown by the white and black regions. WD, water depth. Gray shading indicates depths below the thermocline at each sampling site and on a vertical cross section of the lake.

and this increase was accompanied by a reduction in cell size. The highest cell density was recorded on 20 September of that year, when the mode of cell size was 7.5–10.0 μm. The small cells observed at this time appeared to be undergoing rapid growth, as several cells were connected to each other (Fig. 6A) by mucus threads secreted from fultoportulae surrounding the valve margin (Fig. 6B).

In both study years, large cells (including initial valves; Fig. 6C) were commonly observed in October once blooms had subsided (Fig. 5). Although at least some of the small cells produced during the bloom are presumed to have differentiated into gametes, no gametes were identified in our observations. The sizes of living cells observed in October and November of both years were broadly distrib-

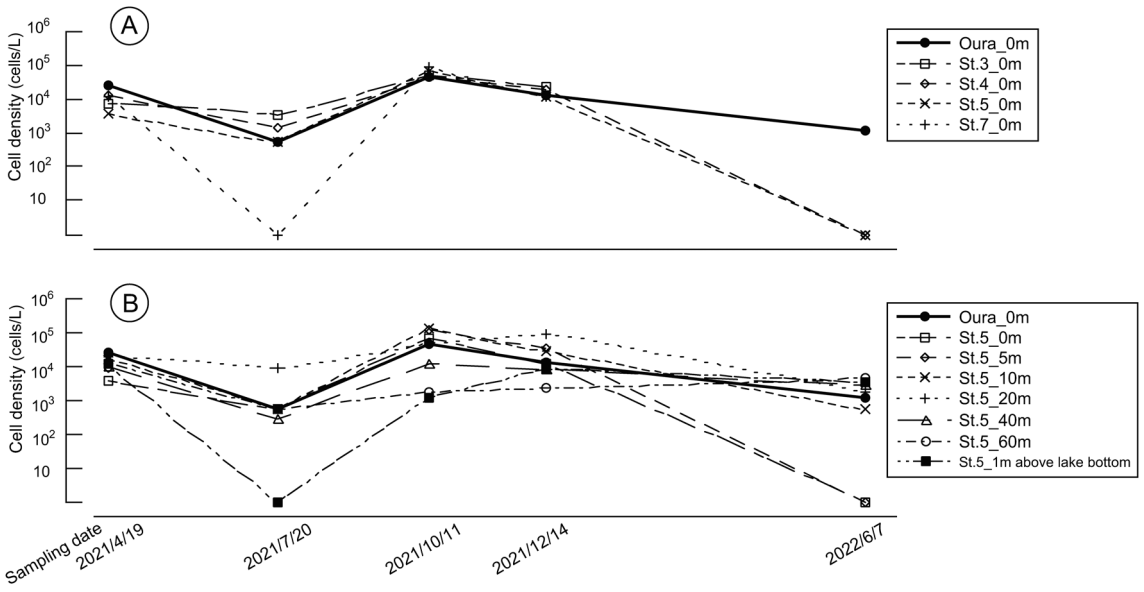

Fig. 4. Timeseries of living cell density at various sampling sites and water depths. (A) Cell densities in surface waters across five sampling sites. (B) Cell densities at the surface (0 m) at Oura Fishing Port (labeled "Oura" in the legend) and at St. 5 at depths of 0, 5, 10, 20, 40, and 60 m and at 1 m above the lake bottom.

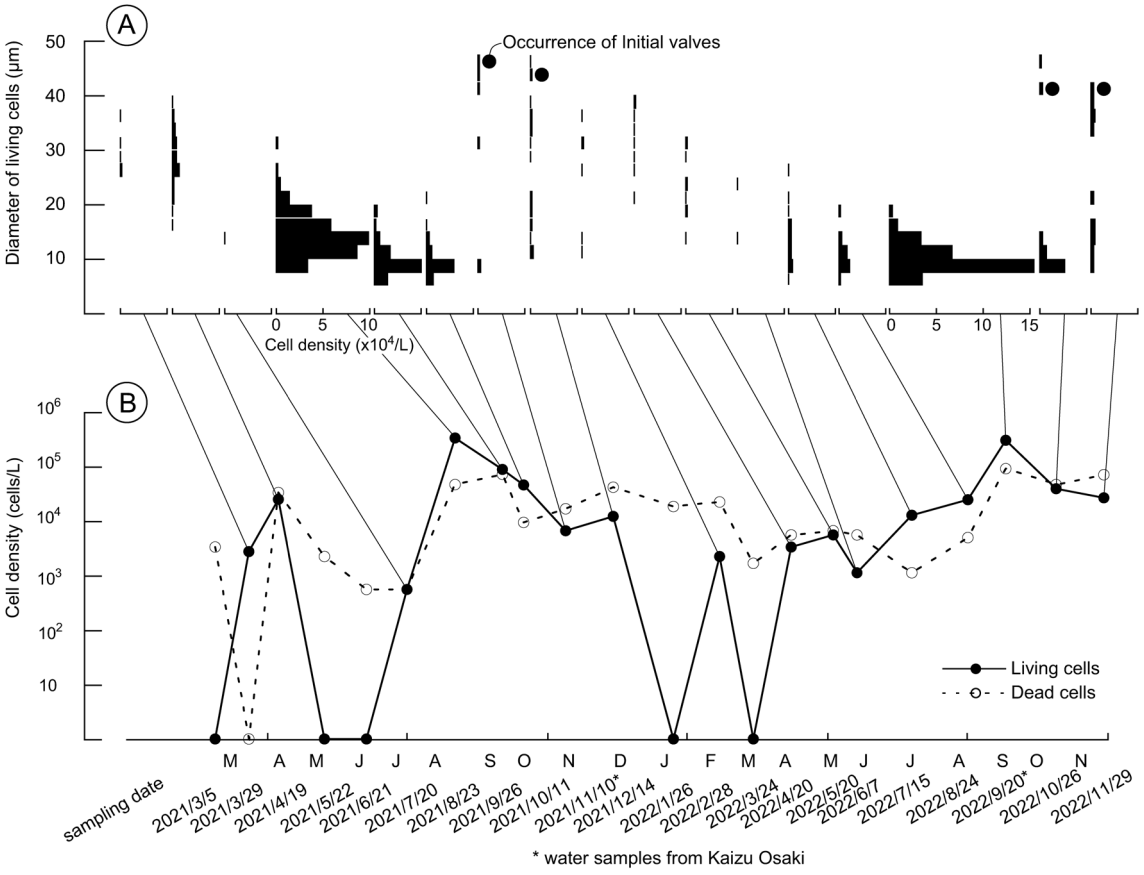

Fig. 5. Timelines of (A) size distributions of living cells in surface waters and (B) cell densities of living and dead cells. All samples were collected at Oura Fishing Port, except for those from 11 November 2021 and 20 September 2022, which were collected at Kaizu Osaki. Filled circles indicate the presence of initial valves. *X*-axis labels in (A) show the density of cells of each size class. Where labels are omitted, cell densities ranged from 0 to 5×10^4/L.

uted from 7.5–47.5 μm (Fig. 5), which corresponds to the entire size range of *P. suzukii* (see diagnostics of *S. suzukii* in Saito-Kato *et al.*, 2015). Subsequently, living cells smaller than 20 μm were observed until February in 2022 but were absent in April (Fig. 5; no living cells of any size were

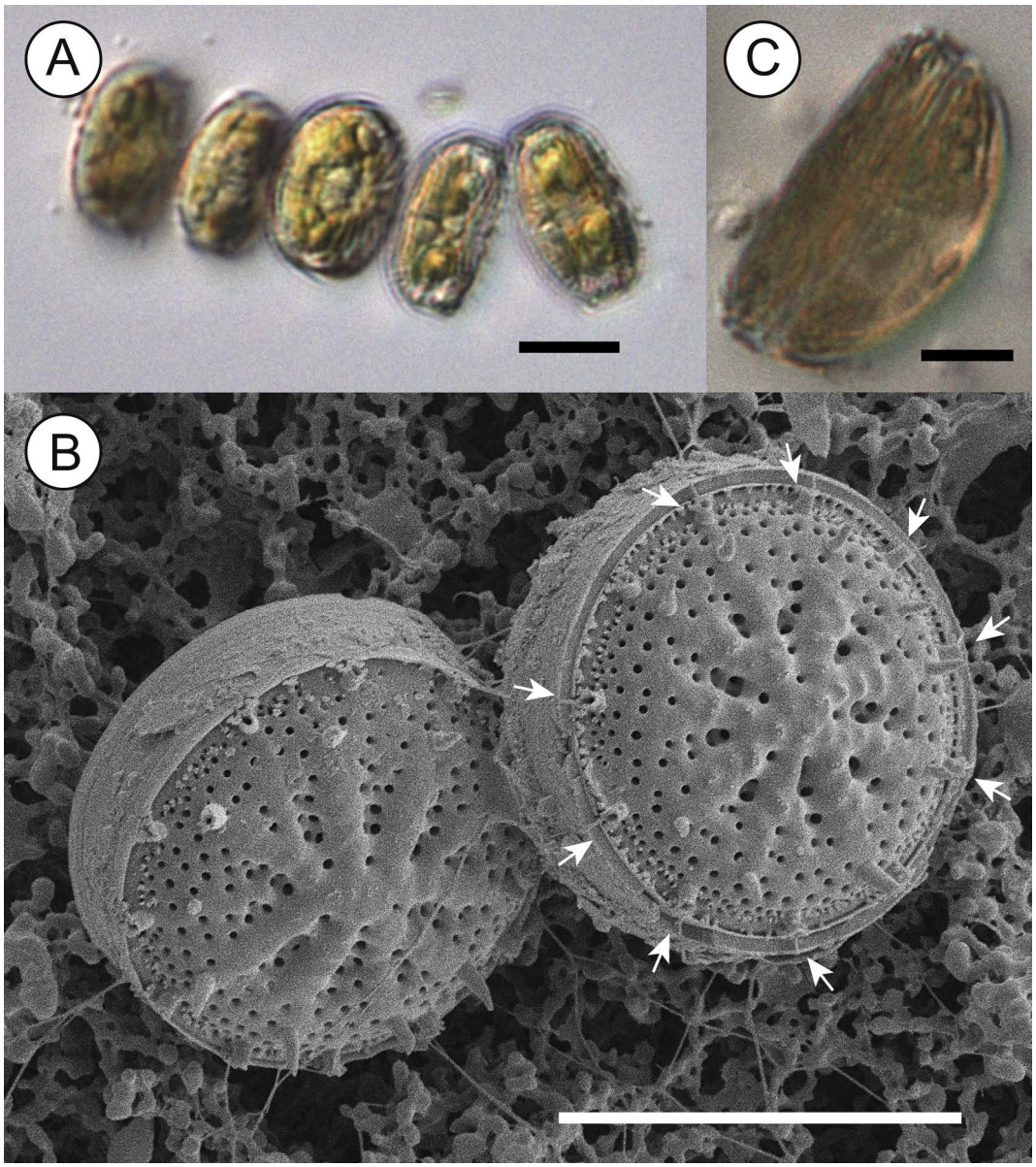

Fig. 6. Images of living cells. (A) Light-microscope image of living cells connected in a chain in a surface water sample from Oura Fishing Port collected on 23 August 2021. (B) Scanning electron microscope image of cells secreting mucus threads (arrows) from fultoportula tubes on the valve margin in a surface water sample from Oura Fishing Port collected on 23 August 2021. (C) Light-microscope image of a living cell with a hemispherical initial valve and a discoidal vegetative valve in a surface plankton net sample from St. 7 collected on 11 October 2021. Scale bars are 10 μm.

observed in March). During the spring and the summer, cell densities were too low to identify the modal size of living cells in surface water. However, the overall distribution showed a gradual decrease in cell size during this period (Fig. 5).

Environmental factors, such as air temperature, precipitation, water level and nutrient contents were shown in Fig. 7. Surface water temperature at Oura Fishing Port rose and fell following the air temperatures (Fig. 7A). The highest and lowest water temperatures lagged behind the air temperature by about a month (Fig. 7A). Precipitation patterns were different in 2021 and 2022 (Fig. 7C). In 2021, precipitation in August is the highest caused by 211.5 mm of precipitation during 12–15 August (Fig. 7C). Then, water levels in the lake increased by ca. 0.5 m shortly thereafter showing prominent increase during this investigation (Fig. 7D), and this coincided with a spike in T-P and T-N concentrations (Fig. 7E). However, in 2022 precipitation in July is the highest (Fig. 7C), but no striking heavy rain in short time occurred. Water level was rather stable during that time (Fig. 7D). T-N and SiO_2 concentrations in 2022 were high in winter and low in

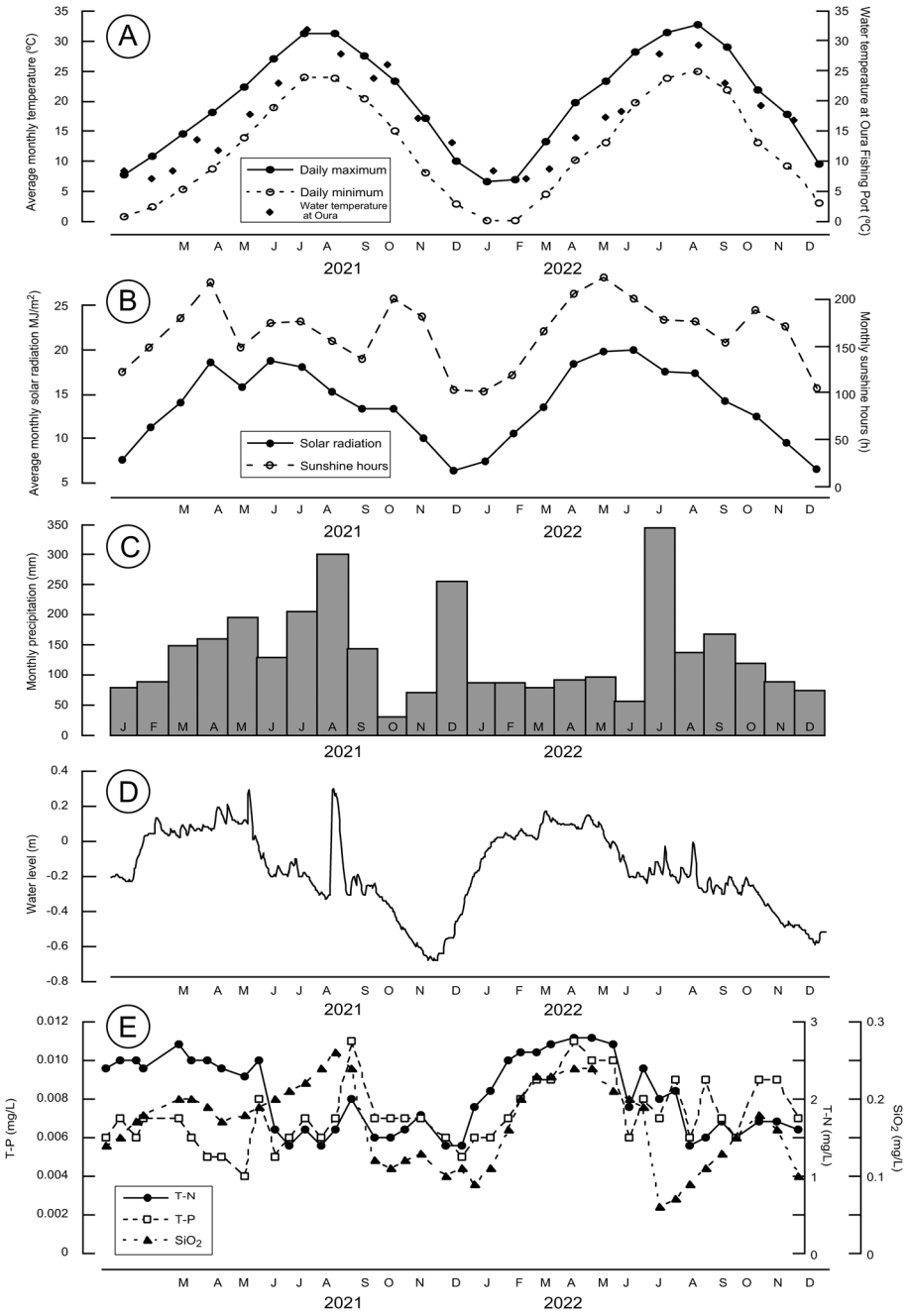

Fig. 7. Environmental conditions around the sampling site at Oura Fishing Port. Average monthly (A) temperature, (B) average monthly solar radiation and monthly sunshine hours and (C) precipitation at the Hikone Local Meteorological Office, (D) daily lake level at the Seta River Weir, and (E) concentrations of total nitrogen (T-N), total phosphorus (T-P), and dissolved silica (SiO_2) at Site 17B (offshore of Imazu).

summer, but T-P seems to have rose and fell irregularly during this year (Fig. 7E). The light conditions, average monthly solar radiation and monthly sunshine hours, roughly changed almost simultaneously, raised in spring and fell in autumn (Fig. 7B). The sunshine hours showed more obvious decline in May–September 2021 and in June–September 2022 (Fig. 7B). The decline in the sunshine hours seems to be related with the monthly precipitation (Fig. 7C).

Discussion

Phytoplankton growth generally follows an annual cycle (Reynolds, 2006). Annual climate cycles for a variety of factors, such as light availability, water temperature, and nutrient discharge from rivers, all directly or indirectly impact phytoplankton growth (Reynolds, 2006). Additionally, climate-driven water dynamics, such as turbulence, vertical mixing, and thermal stratification, strongly affect the sinking loss of phytoplankton from the

surface photic zone (Reynolds, 2006). The annual cycles of *P. suzukii* cell density and size observed in the present study could be related to these environmental factors.

Maximum cell density was observed in late August in 2021 and in late September in 2022 (Fig. 5). During these periods, thermal stratification is inferred to have weakened, allowing vertical mixing to thicken the epilimnion and deepen the thermocline (Fig. 2). In 2021, heavy rain fall during 12–15 August (Fig. 7C). Water levels in the lake increased by ca. 0.5 m shortly thereafter (Fig. 7D), and this coincided with a spike in T-P and T-N concentrations (Fig. 7E). The cell density of *P. suzukii* then reached a maximum on 23 August (Fig. 5), possibly in response to the increased availability of P and N. Concentrations of T-N, T-P, and SiO_2 declined after 23 August, perhaps because of consumption by *P. suzukii* and other phytoplankton. By contrast, in 2022 there was a summer peak in SiO_2 but not in T-N, and T-P was variable. Cell density declined in early summer and then increased gradually to a maximum in September (Fig. 5). Overall, our two-year timeseries suggests that summer rainfall events carried terrestrial nutrients into the lake, where blooms of phytoplankton including *P. suzukii* consumed the nutrients in the surface mixed layer. However, our data do not show whether rises in nutrient concentrations were the trigger for *P. suzukii* blooms. In fact, the onset of vertical mixing coincided more closely to the timing of the blooms, and could have been the triggering event.

One or two months after the cell density maximum, the modal cell diameter decreased to less than 10 μm (Fig. 5). The subsequent appearance of cells possessing initial valves (Figs. 5, 6C), which are the first two siliceous valves covering the auxospore and are observed after sexual reproduction, indicates that sexual reproduction might have occurred. Sexual reproduction can be induced in diatoms by external cues such as light intensity, day length, and salinity (in brackish environments), in addition to intrinsic factors such as a decrease in cell size (Drebs, 1977; Chepurnov *et al.*, 2004). Additionally, some studies have shown a link between sexual reproduction in diatoms and nutrient stress (i.e., low nutrient levels), although this only occurs in diatoms producing a resistant stage after auxosporulation (Chepurnov *et al.*, 2004). By contrast, the freshwater diatoms *Stephanodiscus neoastraea* (*Stephanodiscus* sp. of Jewson [1992]) and *Cyclotella ocellata*, which do not form apparent resting spores, have been suggested to reproduce sexually in response to high nutrient levels (Jewson 1992; Pérez-Martínez *et al.*, 1992). Despite the lack of experimental culture data to support this assertion (Chepurnov *et al.*, 2004), the species examined by Jewson (1992) and Pérez-Martínez *et al.* (1992) are phylogenetically related to *P. suzukii*, and the possibility of a similar response in our study species should be considered.

Jewson (1992) reported that *Stephanodiscus* sp. sensu Jewson reached maximum cell density in Lough Neagh, Northern Ireland in spring, and sexual reproduction occurred in autumn at a time when growth rates remained low. Similarly, Pérez-Martínez *et al.* (1992) reported that *Cyclotella ocellata* in Bermejales Reservoir of southern Spain appeared to undergo sexual reproduction in September or later in autumn, when the cell density was too low to observe, and maximum cell density for this species was observed in January when the population included large cells. Although the occurrence of sexual reproduction during low-growth seasons in these two species differs from our results suggesting sexual reproduction after blooms in *P. suzukii*, all three species appear to undergo sexual reproduction during autumn when light intensity and day length decrease and vertical mixing is intensified. Therefore, all three studies are consistent with the possibility that light conditions provide the trigger for sexual reproduction. Differences in the timings of growth and sexual reproduction among the species probably reflect differences in environmental conditions among the studied lakes. For example, Lake Biwa is a monomictic lake without ice cover. Bermejales Reservoir is also monomictic and has a maximum depth of 31 m (Pérez-Martínez *et al.*, 1922). Lough Neagh is shallow (maximum depth 14.5 m) and polymictic (Bunting *et al.*, 2007), with wind-driven turbulence and variable ice-cover duration (Jewson, 1992). In addition to differences in circulation, the three lakes also differ in trophic condition and phytoplankton and grazer communities.

Although observations from related species are sparse, the late-summer and early-autumn growth

periods for *P. suzukii* observed in the present study do not appear to fall outside the normal range for species in the closely related family Stephanodiscaceae. Rioual *et al.* (2017) described a new species of the genus *Lindavia* (family Stephanodiscaceae) from Lake Tuofengling Tianchi, a crater lake in Inner Mongolia. The highest flux of this *Lindavia* species in sediment traps occurred in autumn, when summer thermal stratification breaks down and other species are similarly abundant (Rioual *et al.*, 2017). However, several studies mentioned in Rioual *et al.* (2017) have reported that *Lindavia* species are also associated with the spring circulation period, and growth during the summer stratification period has been observed in Lake Yamanaka, Japan (Inokuchi and Maruyama, 1990). More field observations of sexual reproduction are needed to identify links between growth season and the timing of sexual reproduction for various species.

After reaching peak cell density in August and September, the distribution of living *P. suzukii* cells shifted into deeper water during winter and spring (Fig. 3), albeit at lower cell densities (Fig. 5). In surface waters, cell densities of *P. suzukii* remained low until late summer/early autumn (Fig. 5). On 7 June 2022, cell density was much lower offshore than at Oura Fishing Port (Fig. 4), and the proportion of living cells was higher below the thermocline than in the surface water (Fig. 3). This pattern suggests that living cells sank below the thermocline earlier in the central part of the lake than near the coast, where individuals were able to survive for longer. Living cells that sink too deep may soon die, because living cells were only observed at depths less than 20 m on 20 July 2021.

Annual changes in the size distribution of *P. suzukii* (Fig. 5) indirectly suggest that the species has a one-year life cycle. The presence of initial valves of the largest size in October in both study years (Fig. 5) indicates that sexual reproduction (and the consequent restoration of cell size) occurred just prior to this time, with the smallest cells likely undergoing sexual reproduction to reach the maximum cell size. The remaining cells in the smallest size range disappeared by March in both 2021 and 2022 (Fig. 5). Although the number of living cells in the surface water was too low during that time to identify any modes of cell size, the distribution of cell size appears to have been broad (Fig. 5). Despite some skipped observations during the spring and the summer, our results show that cell size during these seasons underwent a gradual decrease of 5–7.5 μm per month across the entire size distribution. When a bloom occurred (in late summer–early autumn), the rate of decline in cell size was similar (5 μm per month) in 2021 to the rate observed in the spring and summer and smaller in 2022 (Fig. 5). During the period of increasing cell density (i.e., the growth period), the range of cell sizes gradually narrowed and a distinct mode was recognized.

These observations differ from previous reports of a perennial life cycle in *Stephanodiscus* sp. (Jewson, 1992). Jewson (1992) estimated a life cycle length of 4 years by extrapolation from a trendline of decreasing cell size, and reported the presence of at least two cell-size modes, suggesting the presence of multiple cohorts. These cohorts were inferred to have originated from sexual reproduction observed every year (Jewson, 1992). Similar estimates have been reported for one of two co-existing *Cyclotella* species in Lake Baikal (Jewson *et al.*, 2015). The cell density of *Cyclotella minuta* increased in spring and autumn, and sexual reproduction occurred in February–March (Jewson *et al.*, 2015). A 3–4-year life cycle was estimated on the basis of the growth rate and rate of size reduction of this species during the growth period; however, this could have been an overestimate if the rate of size reduction during the non-growth period was faster than during the growth period (Jewson *et al.*, 2015). Jewson *et al.* (2015) were unable to estimate the life-cycle length of the other Baikal *Cyclotella* species, *C. baicalensis*, because its cell density was too low to identify a mode of cell size. The occurrence of sexual reproduction in June–July after the blooming period in spring in this species is similar to our observations of *P. suzukii*, which also undergoes sexual reproduction just after a bloom (Fig. 5).

Cell densities of *P. suzukii* during the late summer–early autumn growth period differed by less than 1000 times from those in winter–spring, with cell densities during the former ranging from 10^5 to 10^6 cells/L and during the latter from 10^3 to 10^4 cells/L (Fig. 5). In combination with differences in cell size, this finding indicates that there is likely to be a

moderate difference in carbon biomass between the two periods. Cell size affects estimates of carbon biomass because large diatom cells generally have low carbon content per volume (Strathmann, 1967). An analysis of a 30-year phytoplankton monitoring dataset collected offshore of Imazu (Site 17B) estimated that *S. suzukii* (corresponding to the large *P. suzukii* cells in the present study) accounted on average for 0.5% of total phytoplankton carbon biomass and *S. pseudosuzukii* (corresponding to the small *P. suzukii* cells in the present study) accounted for 0.8% (Ichise *et al.*, 2011), assuming cell volumes of 4200 μm^3 for the former and 480 μm^3 for the latter. These relative biomasses are consistent with our data on large and small *P. suzukii* cells in the present study. More estimates of biomass and productivity are needed to improve understanding of the ecology and natural history of this species.

The size distribution of *P. suzukii* preserved in sediments can be used to estimate its life cycle in the past. The sediment record shows clear fluctuations in *P. suzukii* size distribution (Saito-Kato *et al.*, 2019), with a mode of 7.5–10.0 μm during the Holocene, 10.0–15.0 μm during the latter part of the last glacial period (the latest Pleistocene), 17.5–20.0 μm at ca. 24 ka, and 10.0–12.5 μm during the early part of the last glacial period. Generally, the size distribution of *P. suzukii* in sediments is right-skewed (i.e., the median is smaller than the mean; Kato *et al.*, 2003; Saito-Kato *et al.*, 2019). Because of sediment mixing by macro- and meiobenthos, each distribution is thought to reflect the size distribution of sinking cells averaged over several tens or hundreds of years. By contrast, the size distribution of living cells in surface water samples reflects that of the standing crop at a given point in time. This complicates direct comparisons to the sediment record, but the presence of a right-skewed distribution with modes within the range 7.5–12.5 μm (apart from ca. 24 ka) can be more easily compared to present-day observations. The mode may reflect the cell size that was observed most frequently during blooms in this study. If this assumption is correct, the larger mode during the last glacial period would indicate that some process such as an earlier initiation of vertical mixing or weaker summer thermal stratification caused an increased prevalence of large cells during blooms in this period. The modes in the sedimentary record appear to jump between discrete values (Saito-Kato *et al.*, 2019), suggesting the presence of discrete ecologically stable states that may have been associated with particular (e.g., monomictic or dimictic) circulation states. Ecological observations of *P. suzukii* in the field are not sufficient to test this hypothesis; culture experiments in which diatoms are exposed to conditions beyond the range of interannual environmental fluctuations should be conducted to address this in the future.

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