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Effects of Lugol's iodine solution and formalin on cell volume of three bloom-forming dinoflagellates*

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Abstract Fixatives are traditionally used in marine ecosystem research. The bias introduced by fixatives on the dimensions of plankton cells may lead to an overestimation or underestimation of the carbon biomass. To determine the impact of traditional fixatives on dinoflagellates during short- and long-term fixation, we analyzed the degree of change in three bloom-forming dinoflagellates (*Prorocentrum micans*, *Scrippsiella trochoidea* and *Noctiluca scintillans*) brought about by Lugol's iodine solution (hereafter Lugol's) and formalin. The fixation effects were species-specific. *P. micans* cell volume showed no significant change following long-term preservation, and *S. trochoidea* swelled by approximately 8.06% in Lugol's and by 20.97% in formalin as a percentage of the live cell volume, respectively. *N. scintillans* shrank significantly in both fixatives. The volume change due to formalin in *N. scintillans* was not concentration-dependent, whereas the volume shrinkage of *N. scintillans* cells fixed with Lugol's at a concentration of 2% was nearly six-fold that in cells fixed with Lugol's at a concentration of 0.6%–0.8%. To better estimate the volume of *N. scintillans* fixed in formalin at a concentration of 5%, we suggest that the conversion relationship was as follows: volume of live cell=volume of intact fixed cell/0.61. Apart from size change, damage induced by fixatives on *N. scintillans* was obvious. Lugol's is not a suitable fixative for *N. scintillans* due to high frequency of broken cells. Accurate carbon biomass estimate of *N. scintillans* should be performed on live samples. These findings help to improve the estimate of phytoplankton cell volume and carbon biomass in marine ecosystem.

Keyword: cell volume; formalin; Lugol's; *Noctiluca scintillans*

1 INTRODUCTION

Phytoplankton blooms may cause mortality across several trophic levels (Stephen and Hockey, 2007), leading to changes in the pelagic food web. *Prorocentrum micans*, *Scrippsiella trochoidea* and *Noctiluca scintillans* are bloom-forming dinoflagellates found in coastal aquatic ecosystems of the world. Their blooms have been widely observed, e.g. in the coastal area of Korea (Lee and Lim, 2006), the coast of western South Africa (Stephen and Hockey, 2007), the coast of India (Naik et al., 2011), and in Greek coastal waters (Ignatiades and Gotsis-Skretas, 2010). *P. micans* and *S. trochoidea* blooms have an effect on the level of chlorophyll *a* and dissolved oxygen (Pybus, 1990; Hallegraeff, 1992). *N. scintillans* was reported to induce predation pressure on phytoplankton, copepods, crustacean larvae, and fish eggs and larvae (Huang and Qi, 1997). Therefore, accurate biomass estimates of these bloom-forming dinoflagellate species are required to evaluate their quantitative roles in microbial food webs and help to assess the state of marine ecosystems

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Phytoplankton biomass is traditionally estimated based on empirical relationships between carbon biomass and the cell volume of planktonic organisms (Mullin et al., 1966; Strathmann, 1967; Eppley et al., 1970; Montagnes et al., 1994; Menden-Deuer and Lessard, 2000). Besides the bias introduced from sampling procedure (e.g. where the sample was taken), counting and measuring procedure (e.g. variations in the estimates by different analysts) (Majaneva et al., 2009; Jakobsen et al., 2015), and uncertainly in the empirical conversion from biovolume to carbon (Jakobsen et al., 2015), preservation may also bring potential errors in the biomass estimate. Swelling of phytoplankton cells in fixatives will lead to overestimate of the carbon biomass based on the carbon to volume relationship and vice versa. In large-scale marine ecology surveys, plankton samples are commonly preserved using fixatives for subsequent species identification and cell size measurement. Inaccurate estimate of cell volume for each species would be one important component of the measurement uncertainty in carbon biomass. Thus, the accurate estimate of cell volume from preserved samples is essential.

Lugol's iodine (hereafter referred to as Lugol's), formalin, and glutaraldehyde are traditional fixatives widely used in phytoplankton preservation. Lugol's and glutaraldehyde have been reported to induce changes in the biovolume of some species of dinoflagellates and diatoms (Verity et al., 1992; Montagnes et al., 1994; Menden-Deuer et al., 2001; Zarauz and Irigoien, 2008; Mukherjee et al., 2014), and formalin has been reported to induce changes in planktonic protozoa (Choi and Stoecker, 1989; Zinabu and Bott, 2000; Chaput and Carrias, 2002; Karayanni et al., 2004). However, few studies have estimated the long-term and short-term effect of Lugol's or formalin on *P. micans*, *S. trochoidea* and especially *N. scintillans*.

The objective of this study was to determine the degree of change in the biovolume of these three bloom-forming dinoflagellates brought about by traditional fixatives during short- and long-term fixation. We also examined the effects of fixative concentration on *N. scintillans*.

2 MATERIAL AND METHOD

P. micans is tear drop- or heart-shaped cell of medium-size (42–57 μm long and 23–33 μm wide); *S. trochoidea* is pear-shaped in relatively small size (16–36 μm long and 20–23 μm wide) (Dodge, 1975; Horner, 2002; Long et al., 2013). Both are thecate dinoflagellates with cellulosic thecal plates. *P. micans* and *S. trochoidea* were isolated from the coastal water of the East China Sea. To simulate normal natural conditions, Whatman GF/F-filtered seawater was collected where the *P. micans* and *S. trochoidea* were sampled. Cultures were kept in a 14 h:10 h light/dark cycle at 18°C. Samples were obtained from cultures in the late logarithmic growth phase. *N. scintillans* is an athecate, non-photosynthetic dinoflagellate, roughly spherical with diameter larger than 200 μm (Tada et al., 2000; Horner, 2002). *N. scintillans* was sampled using a phytoplankton net (mesh size, 76 μm) in the coastal water of the East China Sea.

Lugol's and formalin were freshly prepared in accord with China's Standard Method, the Specifications for Oceanographic Surveys, Marine Biological Survey (GB/T 12763.6-2007), which was also in accord with the method of Throndsen (1978). *P. micans* and *S. trochoidea* samples were divided into two sub-samples and fixed immediately after the initial measurements. Applying the standard methods, one sub-sample was preserved with Lugol's at a final concentration of 0.6%–0.8% and the other was preserved with formalin at a final concentration of 5%, respectively. The samples of *N. scintillans* were divided into four sub-samples after measurement, two of which were preserved with the standard methods as mentioned above. To exclude the potential impact of microbial intervention, the other two *N. scintillans* samples were preserved with higher concentrations for comparing the effects induced by fixative concentration, i.e. a final concentration of 2% for Lugol's and 10% for formalin, respectively. Both of these two concentrations were used in previous plankton preservation experiments (Graham and Sprules, 1992; Montagnes et al., 1994; Menden-Deuer et al., 2001; Coyle and Pinchuk, 2002; Palardy et al., 2006). All the samples were preserved in dark at room temperature after adding the fixatives. The dimensions of the cells in each sub-sample were measured again after 2 h, 12 h, 24 h, 72 h, 168 h, 720 h, and 2 160 h, respectively.

A VS-IV FlowCAM (Fluid Imaging Inc., Edgecomb, ME, USA) under the control of VisualSpreadsheet™ software version 2.4.8 was used to perform the analysis. Samples were analyzed in autoimage mode (Sieracki et al., 1998) following standard procedure. A ×40 magnification with a ×4 objective was used. A digital camera, which has a resolution of 1 024×768 pixels, photographs the cells. A calibration factor is used in the software to

Table 1 Size information of the live cells for three species

Species	ESD±std (μm)	CV(%) between triplicates	Distribution CV(%)	Volume±std (μm³)	CV(%) between triplicates	Distribution CV(%)	*N*
P. micans	27.73±0.09	0.34	9.09	11 440.68±77.62	0.68	27.86	1 683
S. trochoidea	23.5±0.18	0.76	8.45	6 952.33±99.88	1.44	39.67	1 188
N. scintillans	482.08±30.35	6.30	22.75	7.85e7±1.71e7	21.82	65.61	129

Note: values of ESD and volume shown are mean±standard deviation between triplicates; CV represented the coefficient of variation.

determine the pixel to micron conversion into μm units. The default calibration factor for a ×4 objective is 1.362 2, i.e. each 1.362 2 pixels are used to size 1 μm in the output of the cell size. Several studies have applied FlowCAM in the size analysis of phytoplankton (Zarauz and Irigoien, 2008; Álvarez et al., 2011; Jakobsen and Carstensen, 2011). The FlowCAM in this study was calibrated using beads of known size. To further verify the measurement accuracy of the FlowCAM, we have also compared the measurement results with those of a BX-51 microscope (Olympus, Melville, NY, USA). Results showed that no significant differences existed between these two methods (Yang et al., 2016). The images were checked manually to eliminate invalid images such as bubbles, detritus, and repeated images. Damaged cells of preserved *N. scintillans* were excluded. The biovolume of each cell was calculated from the equivalent sphere diameter (ESD), which was the mean feret distance based on 36 sample measurements by VisualSpreadsheet™ software. The analysis of each live sample was conducted three times. Means and standard deviations were calculated. Each treatment sample of *P. micans* and *S. trochoidea* was measured three times and at least 300 cells in total were measured. Each treatment sample of *N. scintillans* was measured twice and at least 60 cells in total were measured.

Differences in cell size induced by fixation time were tested by one-way ANOVA. Kruskal-Wallis was used when the data failed to meet the assumption of normality or equal variance. Student's *t*-test and Wilcoxon rank sum test were used to compare the differences in cell size between the two fixation treatments. $P<0.05$ was considered statistically significant. All *P*-values reported are two-tailed. Statistical analyses were carried out using SAS software (SAS Institute Inc., Cary, NC, USA).

3 RESULT

3.1 Error of the measurement

The size information of the live cells are listed in Table 1. The coefficient of variation (CV) between triplicates reflected the measurement precision of the FlowCAM and the variation in cell size. The CVs for *P. micans* and *S. trochoidea* were rather small, indicating a small method variation between triplicates. The CVs for *N. scintillans* were relatively larger. The cell volume of *N. scintillans* had a CV of 21.82%. It was mainly induced by the variability in the natural sample of *N. scintillans* population. The distribution CV of *N. scintillans* was 65.61%. Besides, the number of observations were relatively larger for *P. micans* and *S. trochoidea*. We analyzed the short-term and long-term effects of Lugol's and formalin on *P. micans*, *S. trochoidea* and *N. scintillans* by comparing the ESD, which was equivalent to using a cube-root transformation of volume.

3.2 Effects on biovolume of short-term and long-term fixation with Lugol's and formalin

There was significant difference among fixation time for each tested species ($P<0.000\ 1$ for each treatment). We first analyzed the short-term effects of Lugol's and formalin on *P. micans*, *S. trochoidea* and *N. scintillans* by comparing the ESD of live cells and cells preserved for 2 h. The results showed that *P. micans* shrank slightly in Lugol's, but the difference was not significant (Table 2). However, *P. micans* preserved with formalin showed statistically significant shrinkage of 1.91% in ESD within 2 h compared with live cells. *S. trochoidea* was significantly swollen within 2 h with an increase of 1.32% in ESD when fixed with Lugol's and 6.64% when fixed with formalin, respectively. No statistically significant changes were found in *N. scintillans* with short-term preservation.

The ESDs of *P. micans*, *S. trochoidea*, and *N. scintillans* cells preserved for 720 h were not significantly different from those preserved for 2 160 h ($P>0.05$ for *P. micans*, *S. trochoidea*, and *N. scintillans*, respectively), which indicated that after fixation for one month, the cell volume tended to be stable. Thus, we analyzed the long-term effects of Lugol's and formalin by comparing the ESDs of live

Table 2 Comparison of the effects of short-term preservation

Species	Lugol's (0.6%–0.8%)			Formalin (5%)		
	ESD±std (μm)	Volume±std (μm^3)	*N*	ESD±std (μm)	Volume±std (μm^3)	*N*
P. micans	27.47±0.15	11 125.31±230.05	774	27.20±0.06*	10 799.24±40.12	681
S. trochoidea	23.81±0.10*	7 210.54±81.66	1 038	25.06±0.09*	8 604.84±111.71	786
N. scintillans	510.45±30.25	8.50e7±2.06e7	66	485.19±48.59	7.35e7±2.32e7	64

* indicated a significant difference of the ESD compared with live sample.

Table 3 Comparison of the effects of long-term preservation

Species	Lugol's (0.6%–0.8%)			Formalin (5%)		
	ESD±std (μm)	Volume±std (μm^3)	*N*	ESD±std (μm)	Volume±std (μm^3)	*N*
P. micans	27.73±0.20	11 511.85±247.41	717	28.05±0.23	12 055.27±305.49	393
S. trochoidea	24.10±0.08*	7 512.90±79.48	852	24.98±0.18*	8 413.47±187.14	723
N. scintillans	466.18±19.32*	5.67e7±1.94e7	69	423.00±18.55*	5.00e7±3.82e7	72

* indicated a significant difference of the ESD compared with live sample.

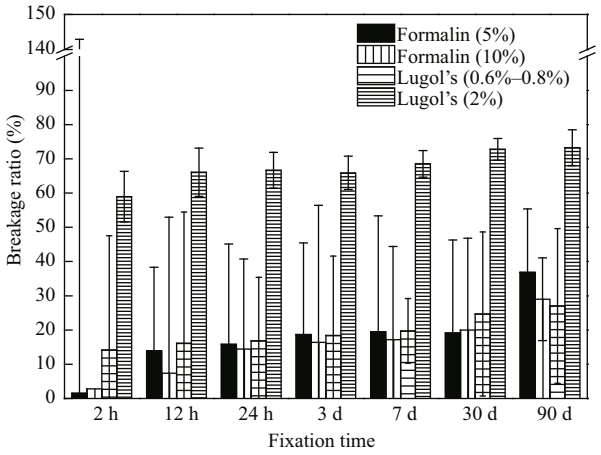

Fig.1 The breakage percentage of *N. scintillans* in different fixatives

cells and cells in the presence of fixatives for 2 160 h (Table 3). These results showed that long-term preservation made no significant change on the ESD of *P. micans*. However, *S. trochoidea* cells were significantly swollen in both fixatives and the swelling percentages were 2.55% with Lugol's and 6.30% with formalin, respectively. In contrast, *N. scintillans* shrank significantly in both fixatives, i.e. 3.30% with Lugol's and 12.26% with formalin. According to these results, the long-term effects of these fixatives were species-specific.

3.3 Effects of fixative concentration on *N. scintillans* cell volume

Higher concentration of fixatives was applied for long-term preservation to avoid microbial intervention. For *N. scintillans*, the ESD of cells preserved for 720 h was not significantly different to that of cells preserved for 2 160 h (P>0.05) in all the treatments. The differences between different concentrations were assessed following fixation for 2 160 h. The results showed that *N. scintillans* preserved with formalin at a concentration of 10% was not significantly different to that with the traditionally used concentration of 5% (P>0.05). However, *N. scintillans* preserved with 2% Lugol's shrank by 28.27%, which was significantly different to that with the traditionally used concentration of 0.6%–0.8% with a shrinkage of 3.30% (P<0.000 1).

3.4 The breakage ratio of *N. scintillans* in fixatives

Apart from size change, broken *N. scintillans* was also found during the fixation experiments. Damage induced by Lugol's was obvious during short-term fixation (Fig.1). The concentration of fixative had a greater effect on *N. scintillans* cell breakage ratio than fixation time. Lugol's at a concentration of 2% caused the greatest damage to *N. scintillans*. The breakage ratio caused by Lugol's at a concentration of 2% was 58.95% when preserved for 2 h and 73.29% when preserved for 2 160 h, almost three-fold that at a concentration of 0.6%–0.8%, which caused a 14.16% breakage when preserved for 2 h and a 27.05% breakage when preserved for 2 160 h. These results indicated that Lugol's (2%) was not suitable for the preservation of *N. scintillans*. The breakage ratio when preserved for 2 h were 1.52% and 2.78% for formalin at a concentration of 5% and 10%, respectively. Cell damage in the first 2 hours' preservation caused by formalin was less obvious

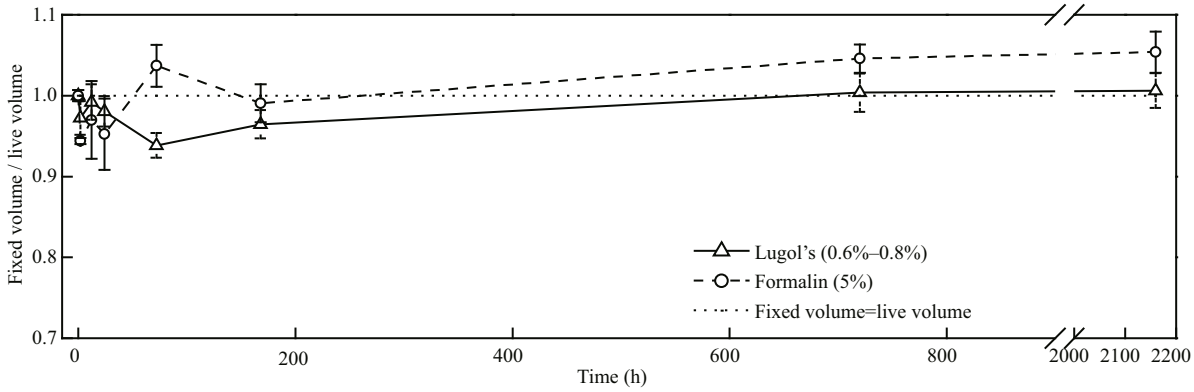

Fig.2 Effects of the fixatives on *P. micans* over time

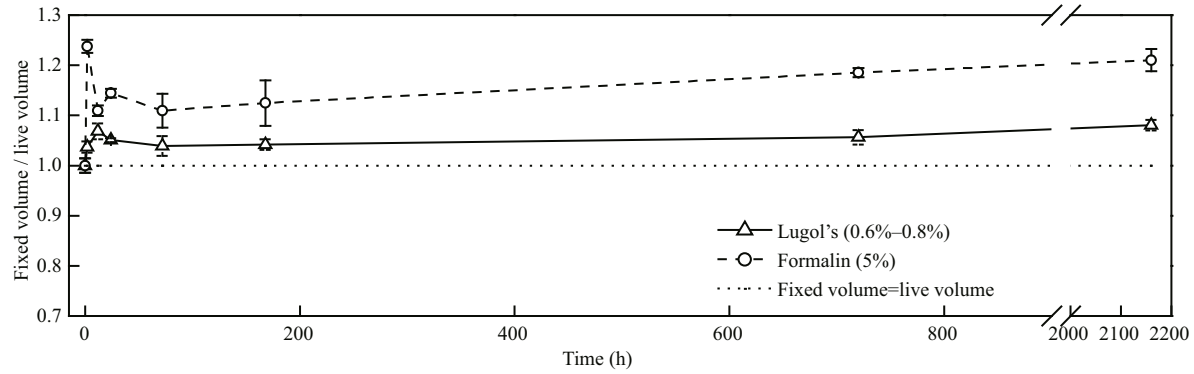

Fig.3 Effects of the fixatives on *S. trochoidea* over time

than that caused by Lugol's. The breakage ratio increased with the fixation time, and small differences were found in different concentrations of formalin. The final breakage ratio was nearly 30% for *N. scintillans* preserved with formalin.

4 DISCUSSION

4.1 Effects of the fixatives over time

4.1.1 For *P. micans* and *S. trochoidea*

Fixation time is an important factor that impacts the volume changes of preserved cells. In our study, *P. micans* preserved with Lugol's showed no obvious changes in the first 2 h, but then showed shrinkage (Fig.2). The maximum percentage decrease in *P. micans* volume was 6.16% after preservation for 72 h. After that, the cells regained their volume and no differences were found after 720 h when the equilibrium was reached, compared with live cells. When *P. micans* was preserved in formalin, the cells initially shrank and then regained their original volume. Long-term fixation resulted in no obvious size changes in *P. micans*.

Preserved with Lugol's, *S. trochoidea* swelled immediately and the maximum percentage increase in cell volume was 6.84% after preservation for 12 h (Fig.3). The final increase in percentage was 8.06% after preservation for 2 160 h. When preserved with formalin, *S. trochoidea* cells swelled within the first 2 h with a maximum percentage increase of 23.77%, and was 20.97% after reaching a steady state. When preserved with the fixatives, *S. trochoidea* cells quickly swelled initially and then swelled at a much lower rate. Thus, we recommend rectifying the volume of a single *S. trochoidea* cell during long-term preservation according to the following formula:

For Lugol's at a concentration of 0.6%–0.8%,

volume of live cell=volume of fixed cell/1.08.

For formalin at a concentration of 5%,

volume of live cell=volume of fixed cell/1.21.

4.1.2 For *N. scintillans*

N. scintillans shrank following preservation with the fixatives (Fig.4). Shrinkage began to stabilize after 168 h, longer than the previous report by Børsheim and Bratbak (1987) who found on average an 85% change in the volume of preserved samples during the first 2 h of storage. The marine ciliate,

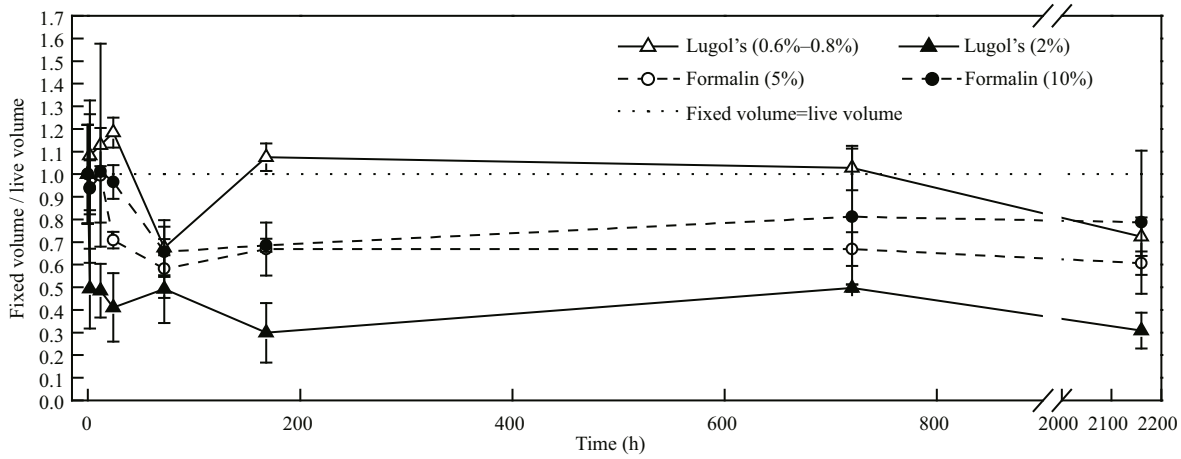

Fig.4 Effects of the fixatives on *N. scintillans* over time

Strombidium sp., were also reported to shrink immediately and then relatively small changes occurred after 24 h in 2% Lugol's (Ohman and Snyder, 1991). During long-term preservation, *N. scintillans* cell volume showed a decrease of approximately 39.36% in formalin at a concentration of 5% and a decrease of 27.59% in Lugol's at a concentration of 0.6%–0.8%, respectively. Thus, we recommend rectifying the volume of a single *N. scintillans* cell according to the following formula:

volume of live cell=volume of intact fixed cell/0.72 for Lugol's,

volume of live cell=volume of intact fixed cell/0.61 for formalin, respectively.

The high rate of cell breakage should be considered. Because the accurate volume of ruptured cell could not be available, we recommend using the average volume of intact *N. scintillans* cells combined with all the cell counts, including the ruptured ones, to estimate the total biomass of *N. scintillans*.

Thus, we summarized the uncertainty associated with the biovolume estimates of the three dinoflagellates in the present study: a) uncertainty arising from the number of cells measured. More cells measured would decrease this error. In the present study, the counting error of *N. scintillans* was potentially large since only 120 cells were analyzed, while for *P. micans* and *S. trochoidea* the data sets were relatively larger; b) the variation of FlowCAM. The FlowCAM was of high measurement precision since the CVs between triplicates were rather small (Table 1); c) the natural variation of a population. The magnitude of population variation was 27.86% for *P. micans* and 39.67% for *S. trochoidea*, respectively. The magnitude of *N. scintillans* population variation, 65.61%, was relatively larger (Table 1); d) fixation effects on the volume change. The magnitude for cell swell of fixed *S. trochoidea* ranged from 3.71% to 23.77% (Fig.3). The magnitude of cell shrinkage of *N. scintillans* due to long-term fixation ranged from 21.24% to 69.11% (Fig.4); e) breakage of fixed *N. scintillans*. Except for the short-term fixation effects of formalin, the breakage of *N. scintillans* in fixatives was rather obvious. The magnitude of errors induced by Lugol's with a concentration of 0.6%–0.8% ranged from 14.16% to 27.05% and those induced by formalin with a concentration of 5% ranged from 1.52% to 36.83% (Fig.1); f) error from cell shapes. Dinoflagellate species are of irregular shapes, thus variations existed between the real cell volume and the volume calculated from the commonly used ESD which based on two-dimensional images.

4.2 The effects of different fixatives

In this study, the effects of different fixatives were species-specific. When preserved with Lugol's and formalin, respectively, *P. micans* showed no significant change, *S. trochoidea* swelled significantly, and *N. scintillans* significantly shrank (Table 3). Species showed the same responses to different fixatives. However, extent of the changes was different. For *S. trochoidea*, the swelling induced by formalin was statistically different from that induced by Lugol's (t=5.45, P<0.01), and the effect on the former was greater (Fig.3). *N. scintillans* is relatively larger in size and has a large vesicle. For *N. scintillans*, after preserved for 72 h, the shrinkage induced by 0.6%–0.8% Lugol's was weaker than that induced by formalin. Lugol's at a concentration of 2% induced

Table 4 Percentage change in cell volume induced by different concentrations of Lugol's

Species	Lugol's (0.5%)	Lugol's (2%)	Reference
Diatoms			
Coscinodiscus sp.	5.0	6.0	Menden-Deuer et al. (2001)
Ditylum brightwellii	-6.1	-13.3	Menden-Deuer et al. (2001)
Thalassiosira sp.	-18.5	-3.7	Menden-Deuer et al. (2001)
Thalassiosira weissflogii	-39.5	-45.5	Montagnes et al. (1994)
Dinoflagellates			
Amphidinium carterae	-18.2	13.6	Menden-Deuer et al. (2001)
Ceratium fusus	21.7	8.7	Menden-Deuer et al. (2001)
Gymnodinium sanguineum	-11.1	-18.1	Menden-Deuer et al. (2001)
Noctiluca scintillans	-24.4	-68.0	This study
Chlorophyte			
Chlamydomonas	-35.0	-40.5	Montagnes et al. (1994)
Cryptomonad			
Chroomonas salina	-58.0	-57.0	Montagnes et al. (1994)
Chrysophyte			
Isochrysis galbana	-46.5	-49.5	Montagnes et al. (1994)
Ciliate			
Strombidium spiralis	-42.0	-10.0	Jerome et al. (1993)

The values listed here were calculated based on the following formula: percentage change=(fixed volume–live volume)/live volume×100%.

the greatest shrinkage. Despite this, *N. scintillans* was prone to damage due to Lugol's as mentioned above.

4.3 The effect of fixative concentration

Cell shrinkage varied with the concentration of fixative (Verity et al., 1992). In this study, the concentration of formalin used for fixation had little effect on *N. scintillans* cell shrinkage while *N. scintillans* cells preserved using a higher concentration of Lugol's were significantly smaller than those preserved using a lower concentration.

Different effects on cell volume induced by fixative concentration were reported to be less important (Montagnes et al., 1994; Mukherjee et al., 2014), i.e. compared with the volume differences induced by fixative concentrations, the differences between fixed cell volume and live cell volume were far greater. In addition, a specific fixative is not suitable for all plankton species. We reviewed previous work that carried out on the concentration effect of Lugol's on plankton (Table 4). It revealed that most significant effect of Lugol's concentration was on dinoflagellates and ciliate and least on chlorophytes. In the this study, Lugol's at a concentration of 0.6%–0.8% reduced *N. scintillans* volume by 8.34%, while Lugol's at a concentration of 2% shrank *N. scintillans* by 50.60% after preservation for 2 h (almost six-fold greater). Preserved for 2 160 h, shrinkage was 27.59% and 69.11% for Lugol's 0.6%–0.8% and 2%, respectively. Therefore, difference induced by fixative concentration cannot be ignored when considering dinoflagellates, especially *N. scintillans*. The effect of Lugol's concentration was species-specific as *Thalassiosira* sp. shrank more in 0.5% Lugol's than in 2% Lugol's (Table 3), while *Thalassiosira weissflogii* shrank a little more in 2% Lugol's than in 0.5% Lugol's. Apart from its effect on biovolume, Lugol's with a concentration of 3.5% or higher has been reported to cause frustule rupture or cellular disintegration in diatoms and blue-green algal cells over a period of 7 to 14 days (Mukherjee et al., 2014). The former has siliceous frustules that may limit the volume change if frustules were not ruptured. We also found broken *N. scintillans* during fixation. A high concentration of Lugol's induced obvious shrinkage of *N. scintillans*, which indicated that the fixative had a significant effect on *N. scintillans* volume and the measurement of *N. scintillans* volume should be carried out during early sampling. The results showed that Lugol's was not suitable for the preservation of *N. scintillans*. With fixation longer than 2 h, the breakage caused by formalin could not be ignored either, suggesting that live *N. scintillans* cells should be measured to avoid errors from breakage and shrinkage.

4.4 Potential reasons for the shrinkage of *N. scintillans* cells in fixatives

N. scintillans differs from the other two species in several aspects. *N. scintillans* is relatively larger in size, approximately 200–750 μm in diameter in this study. To verify whether the species size was the major cause of cell shrinkage, we extracted data from the study by Menden-Deuer et al. (2001) and calculated the change ratio of each species under the same treatment (Table 5). The results indicated that the change ratio did not regularly change with the increase in cell volume. Thus, volume is likely not the main cause leading to different levels of shrinkage. *N. scintillans* lacks armor plates, thus is more fragile during preservation. The nutritional state and the size of ingested prey were reported to be the factors that impacted cell shrinkage in a study of fixed protozoa (Choi and Stoecker, 1989). *N. scintillans* is heterotrophic and has a phagotrophic food vacuole that often contains prey organisms, such as diatoms

Table 5 Percentage change in cell volume induced by Lugol's at a concentration of 2% in different species

Species	Volume of live cells (μm³)	Volume of fixed cells (μm³)	Percentage change (%)
Diatoms			
Leptocylindrus danicus	37	25	32.43
Thalassiosira sp.	66	53	19.70
Chaetoceros didymus	1 062	1 091	2.73
Ditylum brightwellii	9 713	7 291	24.94
Thalassiosira rotula	14 273	13 170	7.73
Lithodesmium undulatum	16 917	16 367	3.25
Stephanopyxis palmeriana	69 517	79 832	14.84
Coscinodiscus sp.	280 697	282 077	0.49
Dinoflagellates			
Gymnodinium simplex	209	218	4.31
Amphidinium carterae	1 018	1 072	5.30
Prorocentrum micans	1 795	1 914	6.63
Scrippsiella trochoidea	4 408	4 873	10.55
Glenodinium foliaceum	5 275	5 605	6.26
Ceratium fusus	44 619	54 697	22.59
Gymnodinium sanguineum	71 859	55 045	23.40

and ciliates (Horner, 2002), so the size of a live *N. scintillans* cell (and the degree of shrinkage) may also be affected by what the cell has previously ingested. Thus, the reaction of cells to a fixative may be influenced by a combination of factors and *N. scintillans* should be discriminated from the other dinoflagellates.

5 CONCLUSION

The effect of fixatives on dinoflagellates is species-specific. *S. trochoidea* tended to swell when preserved, whereas *P. micans* showed no significant change. *N. scintillans* significantly shrank in both Lugol's and formalin. Different fixatives had different levels of effect on cell volume. The traditional used concentration of Lugol's (0.6%–0.8%) resulted in a relatively small volume change. However, *N. scintillans* was more prone to damage due to fixation by Lugol's or formalin, which may introduce notable measurement errors. In order to accurately estimate the volume of fixed samples, we recommend the application of conversion relationships during long-term preservation of *S. trochoidea* according to the following formula:

for Lugol's at a concentration of 0.6%–0.8%,

volume of live cell=volume of fixed cell/1.08;

and for formalin at a concentration of 5%,

volume of live cell=volume of fixed cell/1.21.

Apart from size change, damage induced by fixatives on *N. scintillans* is obvious. Therefore, the measurement of *N. scintillans* is recommend to conduct on live cells during early sampling. As the effects of fixatives on plankton are complex and species-specific, new approaches such as FlowCAM, CytoSence and Phyto-PAM that permit rapid analysis of live specimens would provide more reliable and accurate estimate of biovolume.

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