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Diatoms as indicators of environmental health on Korean islands

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A R T I C L E I N F O

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A B S T R A C T

Diatoms are highly sensitive to perturbations in their environment and are thus useful as bioindicators for anthropogenic impacts such as pollution. However, there is no consensus about what aspects of diatom populations to measure (e.g., diversity, physiology, or morphology) and efficient and reliable survey protocols are lacking. Here, we evaluated the ecological status of diatom communities using both traditional and relatively novel methods on two islands (Deokjeok island and Daeijak island) affected by anthropogenic activities due to extensive agricultural practices and exploitation and that are under consideration for representative touristic sites in South Korea. Dissolved concentrations of metals and metalloid (As, Cu, Cr, Cd, Ni, Hg, Pb, and Zn) were below the ecological screening and toxicity reference values in water fractions but were above these values for sediment, particularly at one island, Deokjeok. The tested methods were generally consistent in finding little evidence for disruption of diatom communities, with dominance by *Navicula* and *Gyrosigma*, relatively high diversity, and typical abundance of lipid bodies and morphological deformities. However, analysis of lipid bodies and morphological deformities suggested greater potential anthropogenic disturbance at one site in Deokjeok. Future planning is required to ensure the maintenance of the near-pristine environments present on these islands.

1. Introduction

Island ecosystems are some of the most intact but also vulnerable ecosystems in the world (Hong, 2012). Increases in anthropogenic impacts related to human population growth and tourism on island ecosystems can degrade their social, cultural, and economic value (IPCC, 2014; Leong et al., 2014). In Korea, there are more than three thousand islands (3358 officially confirmed) off the western and southern coasts, many of which attract national and international tourism (Kim, 2013). Won et al. (2017) recently reported that human disturbance around South Korea, especially sand extraction, has resulted in a loss of biodiversity and changes to the composition of microbial surface water assemblages. Other anthropogenic effects such as sea level rise and reclamation activities along the west coast of the Korean Peninsula have decreased the volume of water leaving estuaries at ebb tide and caused infilling of muddy sediments due to weakened tidal currents (Choi, 2014). Regulations require that pre- and post-construction surveys are carried out for each major construction or reclamation project to evaluate their effects on the island environment. Unfortunately, however, many effects are evident only on timescales longer than a year and are heterogeneous in space and time, requiring evaluation based heavily on model simulations (Choi, 2014). Additionally, regulatory authorities lack consensus on methods for evaluating anthropogenic effects on island health.

Surveys of organisms representative of an ecosystem's health (i.e. 'bioassays') are increasingly important ways of monitoring island ecosystems (Won et al., 2017), especially given the importance that national laws and international agreements place on biotic integrity (IPCC, 2014). Bio-indicator assessments may require less time and cost than other strategies, and provide vital information about biodiversity on impacted islands (Ryu et al., 2014).

Diatoms are model organisms for assessing the ecological health of environments affected by anthropogenic activities (e.g., polar regions, islands, and fluvial ecosystems), largely because of their short life spans and sensitivity to environmental perturbations (Pandey et al., 2017). Periphytic diatom community parameters such as cell density, biovolume, percentage relative abundance, species richness, and Shannon index are widely used for water quality monitoring. However, diatoms

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have not been used to assess the ecological impacts of disturbance on islands, although there have been sporadic surveys of environmental and ecological changes in diatom diversity (Park et al., 2012; Ryu et al., 2014).

As the standard community parameters may not be directly suitable for assessing contamination in aquatic ecosystems, there is a need for other cost-effective, reliable, and widely adoptable measures. Measurements of lipid body (LB) quantity, body size dynamics, and the presence of frustule deformities (DFs), may enhance the efficiency or reliability of diatom bioassays as a biomonitoring tool when used in combination with traditional metrics (Pandey et al., 2017).

The aim of the present study was to establish relationships between diatom community parameters and physico-chemical properties of the environment for three sites on two islands (Deokjeok 1, Deokjeok 2, and Daeijak) close to Incheon city that are under consideration as potential tourism sites. We (a) investigated diatom biodiversity in different habitat types (planktonic, periphytic and benthic) and performed physiochemical analysis, (b) quantified diatom community parameters including species diversity, species richness, community composition, life forms, and physiological status, and (c) quantified recently established biomonitoring endpoints of diatoms including LBs, frustule DFs, and size reduction. The results illustrate the potential usefulness of the newer and non-taxonomic diatom metrics, particularly LB characteristics and frustule deformities in biomonitoring, especially as early warning tools for ecotoxicity assessment.

2. Materials and methods

2.1. Surveyed sites

We surveyed disturbed sites on Deokjeok island (Deokjeok 1 and Deokjeok 2 at 37.24˚N and 126.13˚E) and on Daeijak island (at 37.18˚N and 126.25˚E) of Incheon, South Korea (Figs. S1 and S2). The survey was conducted in summer (25–28th August 2016) that had the following weather conditions: temperature (22–28 °C, average surface velocity 90–100 cm/s, rainfall 240 mm and relative humidity 76.5 %). The tidal currents flow northward or northwestward during rising tides and southward or southeastward during ebb tides, and the coastal current flows eastward along the coastline. The tidal range varies from 7 m during neap tides to 9 m during spring tides. These sites were impacted by leaching of herbicides and pesticides from agricultural activities, and pollutant discharge from boats and households in the surrounding area (Fig. S2). All sampling and field measurements were conducted according to the guidelines of the South Korean Ministry of Environment.

2.2. Sediment characteristics

The surface sediments from each island were collected in polyethylene bags upto the depth of 20 cm (Figs. S3 and S4). Immediately after collection, the samples were dried in an oven at 70 °C and then homogenized using an agate mortar. Mean grain size (Mz) analysis for fractions coarser than 63 μm used a standard dry sieving method was used (Ingram, 1971). Fractions finer than 63 μm were analyzed using a laser diffraction analyzer (Sympatec GmbH, HELOS/RODOS model), after pretreatment with H_2O_2 and wet sieving through 63 μm nylon sieve.

2.3. Analysis of environmental data

For physicochemical characterization of seawater, a 0.45 μm syringe filter was used to filter water samples, which were then analyzed for dissolved organic carbon using a 5000a Total Organic Carbon Analyzer (Shimadzu, Kyoto, Japan). Samples were also analyzed for hardness and total suspended solids (TSSs) according to the 'Standard Methods for the Examination of Water and Wastewater' (APHA, 1998), total residual chlorine using a 97–70 Residual Chlorine Analyzer (Thermo-Electron Inc., USA), ammonia concentration using a 95–12 Ammonia Electrode (Thermo-Electron Inc.), and metal and metalloid concentrations using a Vista PRO inductively coupled plasma optical emission spectrophotometer [ICP-OES] (Varian, CA, USA). Salinity were measured in the field with a Atago hand refractometer (Model: JP/ATC-S/Mill-E, Atago®, Japan). At all sites, salinity varied between 28–30 ppt. All apparatus and containers used for metal analyses were acid-cleaned prior to use. Standard solutions were prepared fresh at the time of sampling, and standard calibration curves ($r^2 > 0.995$) were plotted daily. A standard solution was analyzed after every 10 samples to ensure data quality.

2.4. Diatom community sampling and identification

Three types of diatom communities (planktonic, periphytic and benthic) were collected from each island site (Fig. S2 and S4). Planktonic sampling was initially performed using a plankton net with a mesh size <25 μm. The plankton net was held in moving water for a few minutes to collect sufficient cells. Due to regular tides this method of sampling was not possible so we choose another method for plankton collection. For plankton sampling, we pooled 30 small 30-mL samples of water collected along the coastline and filtered the pooled sample in the laboratory using small nets with a mesh size <25 μm. The filtrate was used to identify the planktonic diatoms. Only planktonic samples were considered because the percentage of tychopelagic samples was very low and unlikely to affect our results. Benthic diatom samples were collected from wet marine mud flats. Samples were collected from each site by softly scrapping the yellow colored biofilms colonizing the surface layer. Periphytic diatom assemblages were sampled from small rocky substrata (surface area, ~25 cm^2) with the aid of a toothbrush and blade (Pandey et al., 2014). Six samples were collected from each site and community in plastic vials (15 ml), and 5 ml of water was collected concurrently. All samples were analyzed within 1 week.

Diatom community parameters were derived from examination of 500 frustules. Permanent slides of diatom frustules were prepared as described by Biggs and Kilroy (2000). The method involved treating diatom samples with 90 % acetone to remove cytoplasmic content followed by rigorous acid cleaning for 2 days (Pandey et al., 2018a). Diluted dried diatom samples were spread on cover slips and mounted on glass slides using Pleurax mounting media (refractive index = 1.73). Slides were permanently fixed by heating.

Diatom frustules were examined separately after cleaning. Marine diatoms were identified using the "Identification of marine diatoms and dinoflagellates" (Hasle et al., 1996). Diatom frustules were enumerated using a Spencer Bright-Line Hemocytometer at 450× magnification. Percentage relative abundance per sample was used to estimate the dominance of different diatom species from planktonic, periphytic and benthic diatom communities. However, in case of plankton (e.g., the genus *Actinoptychus*), samples likely include resuspended empty valves due to sediment mobilization associated with tidal changes.

Deformations (DFs) of diatom frustules were investigated using permanent acid-cleaned slides of diatom frustules. DFs of diatom frustules were categorized into four types; (1) valve (D_1), (2) striae (D_2), (3) raphe (D_3), and (4) mixed (Pandey et al., 2016a,b). Length of each cell was measured and variation in length was analyzed as the percent difference from longest cell measured (Pandey et al., 2018a,b).

2.5. Live diatom parameters

Live diatom samples were examined using a Zeiss Axioskop 2 microscope (Zeiss, Germany) at 450×magnification, with a higher magnification where required. Individuals were classified into motile and non-motile (pioneer, tube-dweller) life forms according to published literature (Rimet and Bouchez, 2011). The physiological status of live diatom frustules was assessed by examining the condition of the protoplast content, especially the shape of photosynthetic apparatus

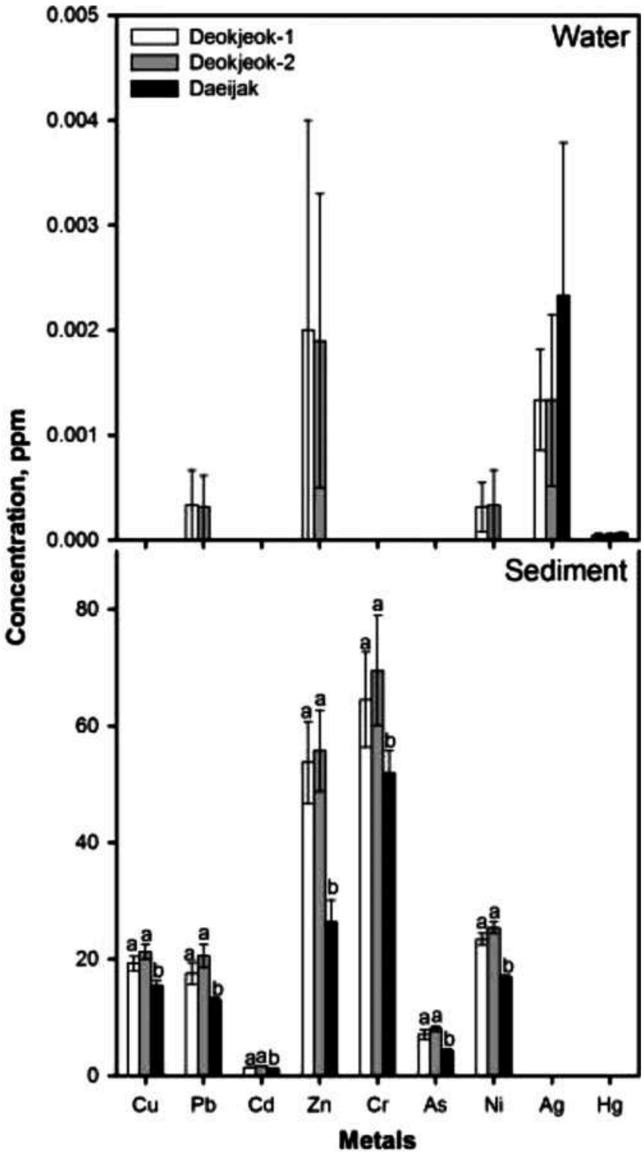

Fig. 1. Metal concentrations (mean ± standard error/SE; n = 6) in water and sediment fractions from the three survey sites. Data bearing different letters above bars are significantly different ($p < 0.05$; Tukey's HSD tests).

(Pandey et al., 2017). The size and biovolume of individual LBs were investigated (400× and 1000× magnification) in living diatom frustules as described previously by Pandey et al. (2015). Life form, physiological status (live, unhealthy, and dead frustules), and LB were quantified for a total of 500 live diatom frustules per sample. Additionally, frustule length was quantified for 300 acid-cleaned frustules of each assayed diatom species.

2.6. Statistical analyses

After testing for homogeneity of variance, data were analyzed using one-way and two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test. Community analysis based on Shannon index and species richness was carried out using the PAST software package (Natural History Museum, University of Oslo, Norway). Principal component analysis (PCA) of normalized data was carried out to identify patterns in contaminants at each survey site using XLSTAT software (Microsoft Corporation, USA).

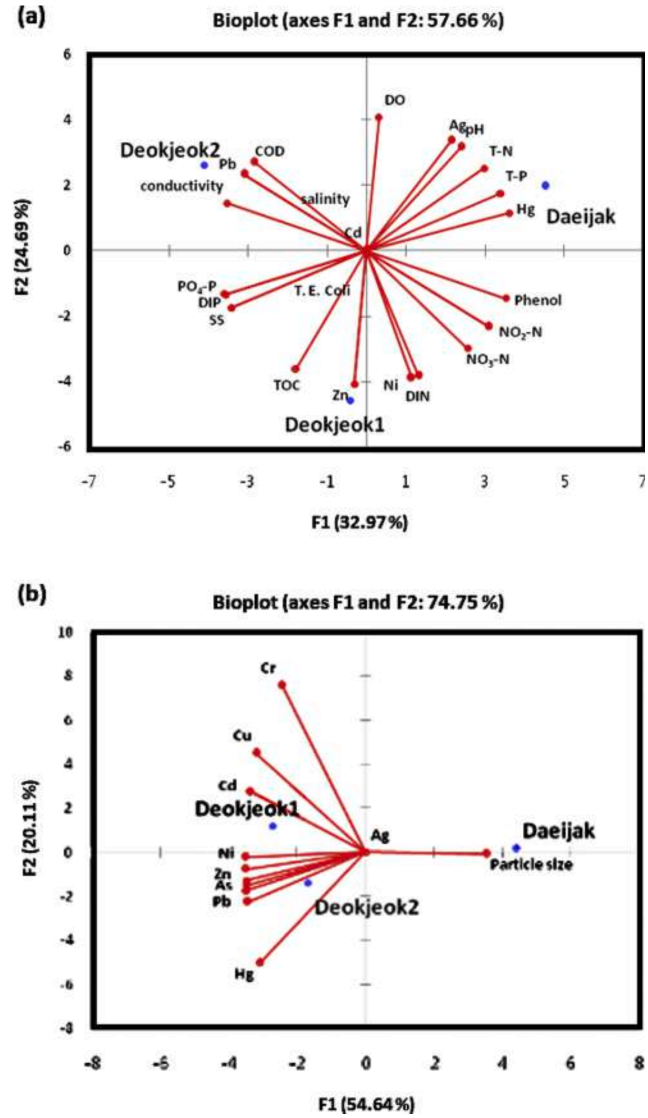

Fig. 2. Principal component analysis (PCA) of surveyed island sites and physico- chemical parameters of water (a) and sediment (b) fractions.

3. Results

3.1. Metal concentrations in water and sediment fractions

In water and sediment fractions, we detected contamination from eight metals (Ag, Cu, Cr, Cd, Ni, Hg, Pb, and Zn) and one metalloid (As). Overall, water fractions had low levels of contamination (Fig. 1), with all concentrations of metals and metalloids lower or equal to background levels reported previously (Kim, 2013). We did not detect statistical differences among sampling sites. Apart from Ag, the concentrations of all other measured metal(loid)s were lower than those reported by international agencies that cause negative (acute and chronic) effects on resident aquatic communities (Table S1). Concentrations of macronutrients in water fractions were similar (DIN = 0.30–0.33, DIP = 0.025–0.030 ppm) to levels reported previously (DIN = 0.32–0.35 and DIP = 0.022–0.030 ppm) by Kim (2013).

Sediment fractions from the three sites were contaminated with eight metals and one metalloid (As, Ag, Cu, Cr, Cd, Ni, Hg, Pb, and Zn; Fig. 1). Levels of metal contamination at Deokjeok 1 and Deokjeok 2 were significantly higher ($p < 0.05$) than at Daeijak. Kim (2013), reported particularly high sediment concentrations of Cr (25–60 mg kg^{-1}) and As (4–6 mg kg^{-1}) for this area. By contrast, we observed appreciably higher

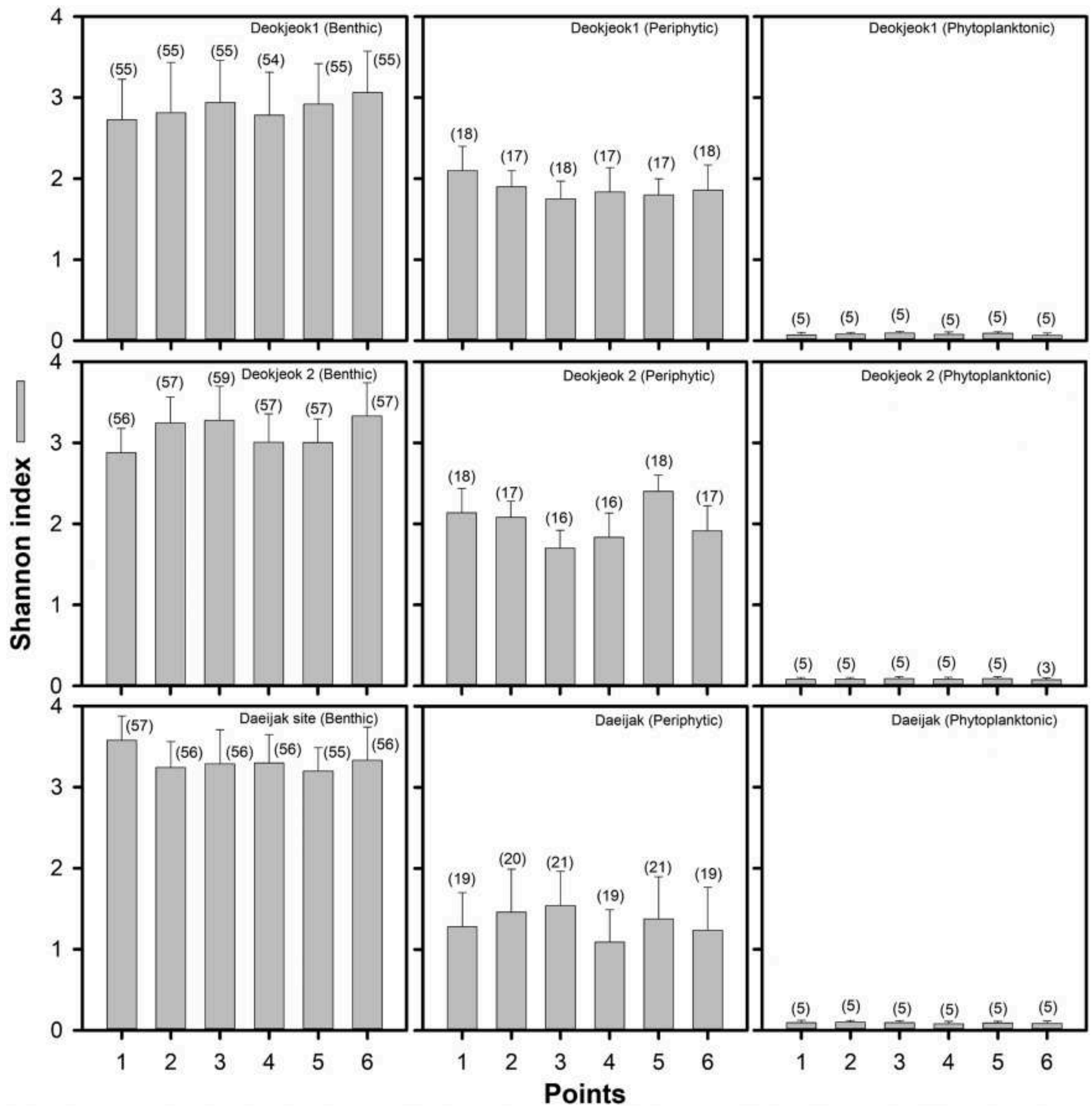

Fig. 3. Shannon index (bars) and species richness (numbers above error bars) (mean ± standard error/SE; n = 6) for different diatom communities in the three survey sites.

concentrations of Cd (1.60–1.75 mg kg^{-1}), Cr (35–94 mg kg^{-1}), and As (7–8 mg kg^{-1}) in sediments on Deokjeok island. Sediment quality guidelines do not exist in Korea (Kim et al., 2011). However, based on reports from various international agencies, the concentrations of five metals (Cd, Cr, Pb, Ni, and Zn) and As in sediment on Deokjeok island (more specifically Deokjeok 2) have the potential to cause detrimental effects on aquatic communities. Compared to ecological screening values (ESVs) and toxicity reference values (TRVs) for metals and metalloids (Table S2) (USEPA, 1995, 1999), Hg and Zn concentrations at the three sites were much lower than either standard. Levels of macronutrients in sediment fractions were similar to values reported previously by Kim (2013) (i.e., TN = 0.15–0.2 mg g^{-1} and TP = 0.12−0.21 mg g^{-1}).

The characteristics of the grain size of surface sediments are summarized in Table S3. The surface sediments were mainly composed of silt followed by clay with a small proportion of sand. Clay, slit and sand percentages were similar among the sites. Deokjeok 2 site had a slightly higher percentage of finer fractions (slit and clay) than the Deokjeok 1 and Daeijak sites. The similarity in sediment composition was reflected in the similarity of the mean grain sizes at each site (i.e., 3.4, 3.3 and 3.5 for Deokjeok 1, Deokjeok 2 and Daeijak island sites, respectively).

3.2. *Principal component analysis*

Physicochemical parameters in water and sediment fractions differed among the three sites (PCA; Fig. 2a). The PCA biplot for water fraction data explained 57.66 % of the variation (the F1 axis contributed 32.97 % and the F2 axis contributed 24.69 %) in the data. Deokjeok 1 tended to be associated with greater Pb concentrations, Deokjeok 2 with Zn and Ni, and Daeijak was associated with enriched Ag and Hg. Deokjeok 1 and Daeijak sites were associated with nutrients (TP, TN, NO_2, NO_3, NH_3, and DIN) while water samples from Deokjeok 2 were associated with PO_4 and DIP. The PCA biplot for sediment fraction physiochemistry explained 74.75 % of the variation (the F1 axis contributed 54.64 % and the F2 axis contributed 20.11 %) in the data (Fig. 2b). Sediments at Deokjeok 1 were associated with Cu and Cr, Deokjeok 2 was associated with Cd, Ni, Hg, Pb, Zn, and As, and Daeijak was associated with Ag.

3.3. *Relative abundance*

A total of 76 diatom species were identified from the three sites (Tables S4 and S5). In benthic communities, Naviculoids forms (*Navicula*

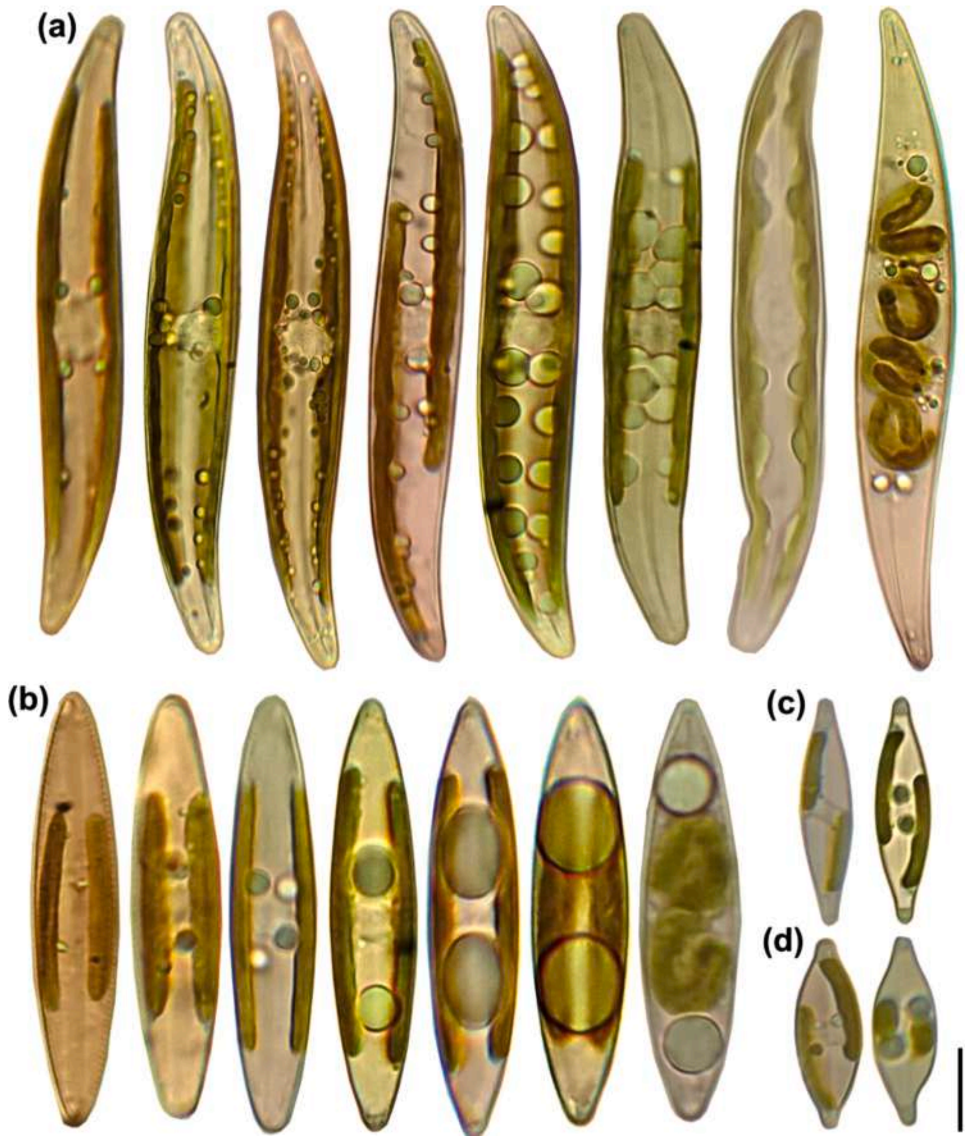

Fig. 4. Status of lipid bodies (LBs) in four live diatom frustules of (a) *P. normanii*, (b) *N. directa*, (c) *N. distans*, and (d) *N. transitrans* from Deokjeok 1, Deokjeok 2 and Daeijak islands. Scale bar =10 μm.

directa and *N. distans*) were dominant (totaling > 50 %, relative abundance). At Deokjeok 1, *N. directa*, *N. distans*, *N. leptostriata* and *N. normaloides* were dominant (totalling > 60 % of relative abundance) while at Deokjeok 2, dominant diatom species were *N. directa*, *N. distans*, *N. incertata*, *N. cryptotenella* and *N. lanceolata* were dominant (totalling > 65 % of relative abundance). At Daeijak the dominant species were *N. directa* and *N. distans* (totalling >70 % of relative abundance) (Fig. S5). In the periphytic community, Deokjeok 1 and 2 site was dominated by *Tryblionella apiculatum*, *Delphineis surirella*, *Rhaphoneis amphiceros* and *Adoneis pacifica* (totalling > 45 % of relative abundance). The Daeijak site was dominated by *R. amphiceros*, *D. surirella*, *A. pacifica* and *T. apiculatum* (totaling > 60 % of relative abundance) (Fig. S6). In planktonic communities, all three sites were dominated by *Cyclotella meneghiniana*, *Actinoptychus splendens* and *Actinoptychus normanii* (totalling > 70 % of relative abundance) (Fig. S7).

3.4. Life form

Motile and non-motile (including attached, pioneer, and tube-dwellers) life forms were compared among the three types of diatom communities (planktonic, periphytic and benthic) from the three sites (Fig. S8; Table S5). Motile forms were most common (>70 %) in benthic communities. The percentages of motile and non-motile forms were similar in planktonic and periphytic communities.

3.5. Physiological status

We categorized frustules into three categories: healthy (live frustules), unhealthy (distorted photosynthetic apparatus), and dead (empty cells; Fig. S9). Benthic communities were dominated by healthy cells, while in planktonic and periphytic communities were dominated by unhealthy and dead cells. These patterns were consistent across sites.

3.6. Shannon index and species richness

Benthic communities were characterized by higher average Shannon index and species richness values than both periphytic and planktonic communities (Fig. 3). Shannon index values at the combined Deokjeok sites and the Daeijak site averaged 2.98 ± 0.72 and 2.92 ± 0.42 for benthic communities, 1.8 ± 0.21 and 1.2 ± 0.21 for periphytic communities, and 0.2 ± 0.1 and 0.2 ± 0.21 for planktonic communities, respectively. Species richness values at the combined Deokjeok sites and

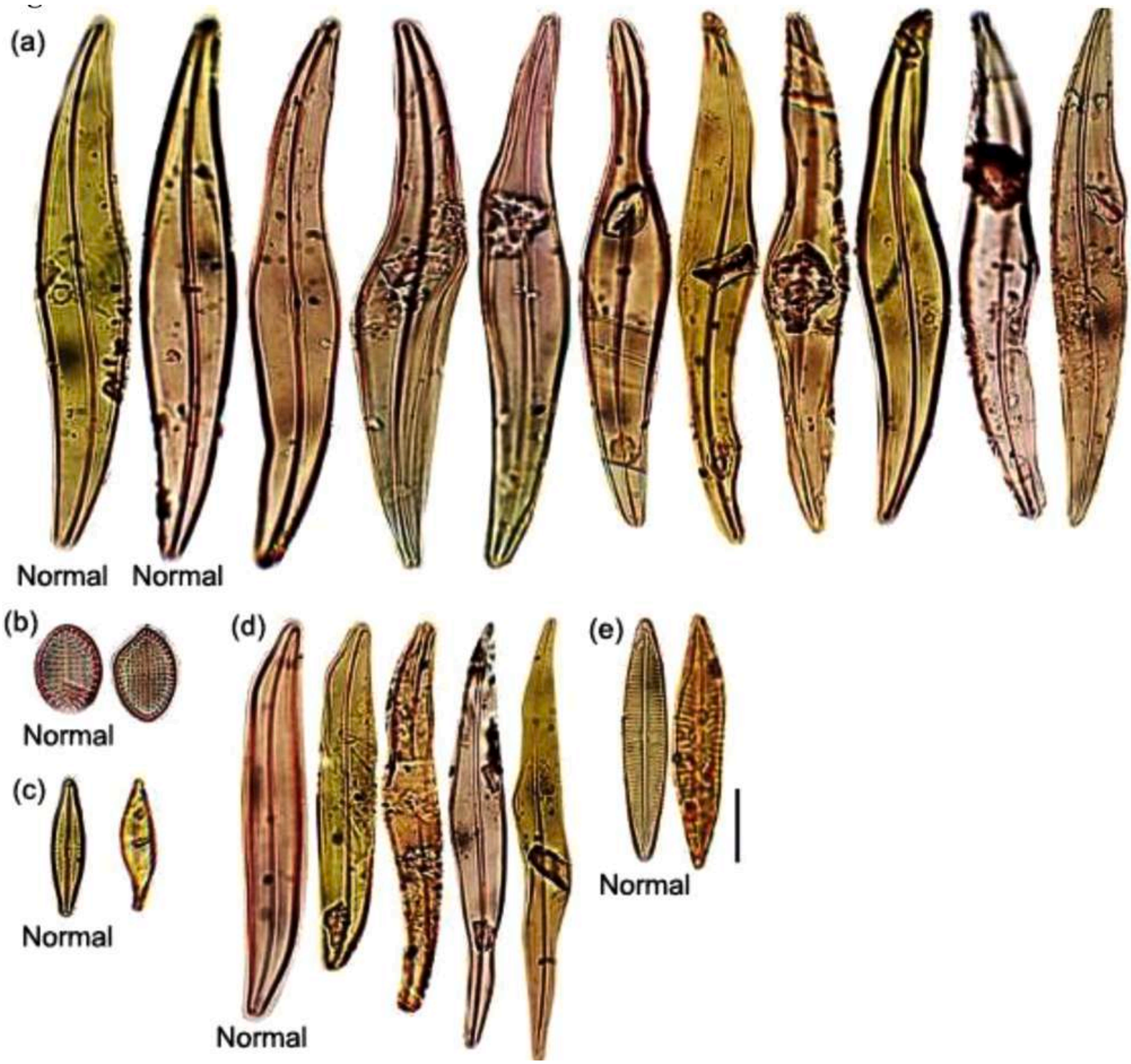

Fig. 5. Photographic documentation of deformities observed in five diatom species of (a) *P. normanii*, (b) *C. placentula*, (c) *N. transitrans*, (d) *P. normanii*, and (e) *N. directa* from Deokjeok 1, Deokjeok 2 and Daeijak islands. Scale bar =10 μm.

the Daeijak site averaged 54 ± 4 and 56 ± 1 for benthic communities, 17 ± 2 and 19 ± 2 for periphytic communities, and 5 ± 1 and 5 ± 1 for planktonic communities, respectively.

3.7. Lipid bodies

The number of LBs, diameter, and biovolume were examined for dominant diatom species from planktonic, periphytic and benthic communities at the three sites (Figs. 4, S10 and S11; Tables S6, S7, S8, S9, S10 and S11). LB characteristics were investigated in seven diatom species (*G. spencerii*, *P. aestuarii*, *N. palea*, *N. gregaria*, *N. distans*, *N. cryptocephala*, and *N. directa*) from Deokjeok 1 and four diatom species (*P. normanii*, *N. cryptocephala*, *N. directa* and *N. distans*) from Deokjeok 2. Of these two sites, Deokjeok 2 displayed higher LB induction (in terms of LB number, diameter, and biovolume) than Deokjeok 1. LB characteristics were examined in 12 diatom species from Daeijak (*N. gregaria*, *N. distans*, *N. cryptocephala*, *N. transitrans*, *N. capitatoradiata*, *N. notha*, *N. densilineolata*, *Nitzschia palea* var. *debalis*, *N. acidoclinata*, *N. filiformis*, *P. rigidum* and *G. exilis*). Compared with the Deokjeok sites, Daeijak displayed lower LB induction (in terms of LB number, diameter, and biovolume).

3.8. Morphological deformities

Morphological abnormalities were examined in dominant diatom species from planktonic, periphytic and benthic communities at the three island sites (Fig. 5). Deformities were examined in five commonly occurring diatoms (*P. normanii*, *R. amphiceros*, *N. transitrans*, *Pleurosigma* sp., and *N. distans*). The percentage of deformed frustules in the benthic community was significantly ($p < 0.05$) higher at Deokjeok 2 (0.34 ± 0.045) than at Deokjeok 1 (0.16 ± 0.043) and Daeijak (0.11 ± 0.047; Fig. 6). There were no significant differences in diatoms from periphytic and planktonic communities at the three sites. The percentage of deformed frustules was significantly ($p < 0.05$) higher in benthic communities than in both periphytic and planktonic communities (Fig. 6). Only type 1 (deformed valve) deformities were observed in all examined communities collected from the three sites (Table S12).

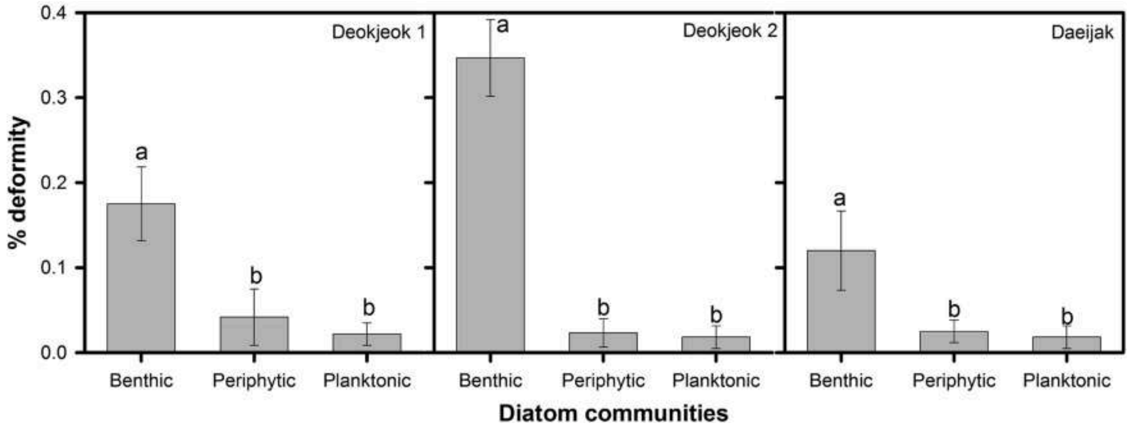

Fig. 6. Quantitative estimation (in % after 500 frustules count) of morphological deformities in diatom communities (planktonic, periphytic and benthic). Two-way analysis of variance (ANOVA) reveals effects for sites and diatom communities at a significance level of $p < 0.05$. Different letters indicate significant differences. Bars represent means and error bars represent ± SE (n = 6).

Table 1
Common diatom species (benthic and periphytic) showing size reduction (% difference in length) collected from Deokjeok 1, Deokjeok 2, and Daeijak island sites in Incheon, South Korea. For each species 300 frustules were examined. See Fig. 7 (Plates 1–3Plate 1).

	Deokjeok 1, Deokjeok 2 and Daeijak sites			
	Benthic species	% difference in length	Periphytic species	% difference in length
1	*Navicula recens*	0–50	*Diploneis stroemii*	0–45
2	*N. salinarum*	0–50	*Tryblionella coarctata*	0–55
3	*N. directa*	0–40	*Delphineis surirella*	0–90
4	*N. distans*	0–25	*Rhaphoneis amphiceros*	0–90
5	*N. cryptotenella*	0–12	*Adoneis pacifica*	0–90
6	*N. capitatoradiata*	0–15	*Delphineis surirella*	0–10
7	*N. cf. hoffmanniae*	0–8	*Surirella lacrimula*	0–50
8	*N. gregaria*	0–3	*Surirella stalagma*	0–70
9	*N. cryptocephala*	0–7	*Diploneis pseudovalis*	0–90
10	*N. densilineolata*	0–5	*Cocconeis placentula*	0–15
11	*Gyrosigma spencerii*	0–7	*Encyonema silesiacum*	0–90
12	*Pleurosigma inflatum*	0–8	*Surirella gemma*	0–50
13	*P. naviculaceum*	0–9	*Lacustriella reimeri*	0–25

3.9. Size variability

All diatom species exhibited size variability (percent difference in frustule length or diameter in centric diatoms relative to the longest measured individual), that is a natural phenomenon in diatoms. This relative size difference was examined in the commonly occurring benthic and periphytic diatoms collected from the three island sites (Table 1). Benthic diatoms showed appreciably higher size reduction in the four genera of *Navicula* (*N. recens*, *N. salinarum*, *N. directa* and *N. distans*) whereas in the other genera this percentage was low. In comparison to benthic diatoms, periphytic diatoms showed relatively higher size diminution. For example, genera *Delphineis*, *Rhaphoneis*, *Encyonema* and *Adoneis* showed a size reduction of approximately 90 % size reduction, whereas other genera showed 0–50 % size reduction (Fig. 7, Plates 1–3Plate 1).

4. Discussion

We observed high concentrations of metals in the sediments from study sites, but not in the water column. Grain size is one of the main factors influencing the heavy metal concentrations in sediments (Lin et al., 2002); Specifically, finer sediments may contain more heavy metals than coarser sediments. The main reason for this difference are that smaller grain size particles have a larger surface-to volume ratio, and usually have a relatively higher organic matter content and contain more Fe–Mn oxides than larger grain size particles (Martincic et al., 1990; Williams et al., 1994; Rae, 1997). The relationship between grain size and concentrations of other metals have also been reported (Cho et al., 1999; Rubio et al., 2000). In the present study, the sediment grain size at the three island sites was similar, a finding that was in agreement with previously published study (Cho et al., 1999). The Deokjeok 2 site had a slightly higher percentage of finer particles (silt and clay; 90 %) than the other two sites (80 % and 84 %), which may indicate a higher bioavailability of metals for the diatom community at this site. This site also had higher sediment concentrations of metals (Cd, Cr, and As) than the other sites, probably because the sediment fraction showed higher proportions of finer particles (clay and silt), which likely increased the bioavailability of these metals to the residing diatom community.

In diatom cells, bioaccumulation of metals is facilitated by nutrient uptake, and can result in a cascade of physiological changes including membrane depolarization, cytoplasmic acidification, and disruption to metal homeostasis (Pinto et al., 2003). Metal–protein complexes can also form and disrupt enzymatic systems, growth, photosynthesis, respiration, reproduction, nutrient assimilation, and molecular synthesis (Falasco et al., 2009a). These cellular consequences of metal contamination (possibly in combination with nutrients) are likely associated with some of the variables we observed, including LB induction, morphological deformity, and reduced frustules size. Overall, we obtained marginal evidence of these effects, particularly at the Deokjeok sites.

In the present study, the diatom communities we observed did not show strong signs of abnormalities resulting from anthropogenic disturbance. Pandey et al. (2018b) reported that marine diatoms appear to be more resistant to metal pollution than those in estuarine and freshwater systems, possibly because they must tolerate osmotic stress caused by extreme salinity (35 ppt). This osmotic stress tolerance may also assist marine diatoms in countering osmotic effects of intracellular accumulation of contaminants, particularly heavy metals and nutrients. In addition, the dispersal and chelation of contaminants by tides and currents may dilute impacts on diatom communities in island

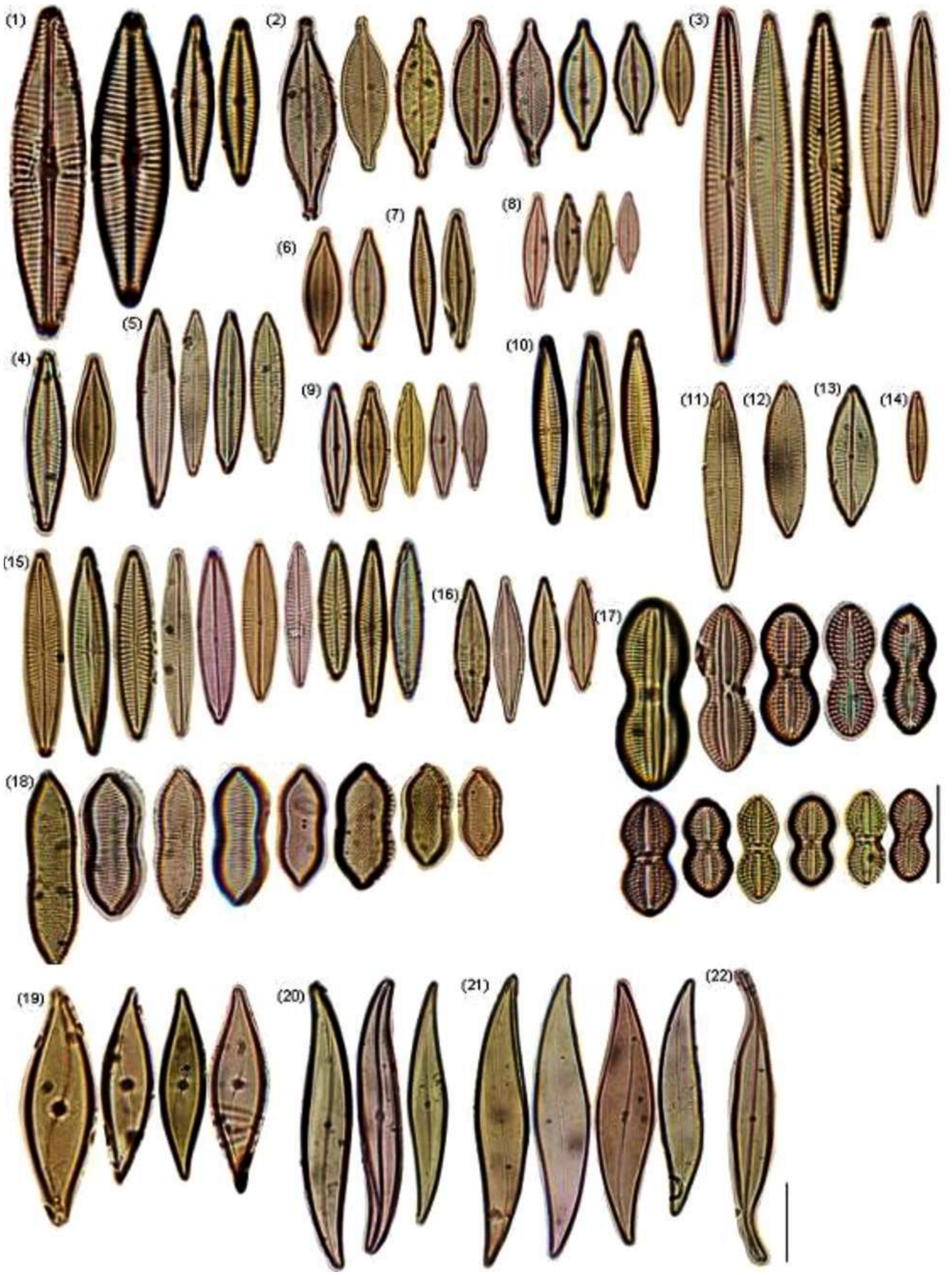

Fig. 7. Photographic documentation (Plates 1–3Plate 1) of common diatom species (periphytic and benthic) showing size reduction collected from Deokjeok 1, Deokjeok 2 and Daeijak island sites in Incheon, South Korea. See Table 1 for more details.

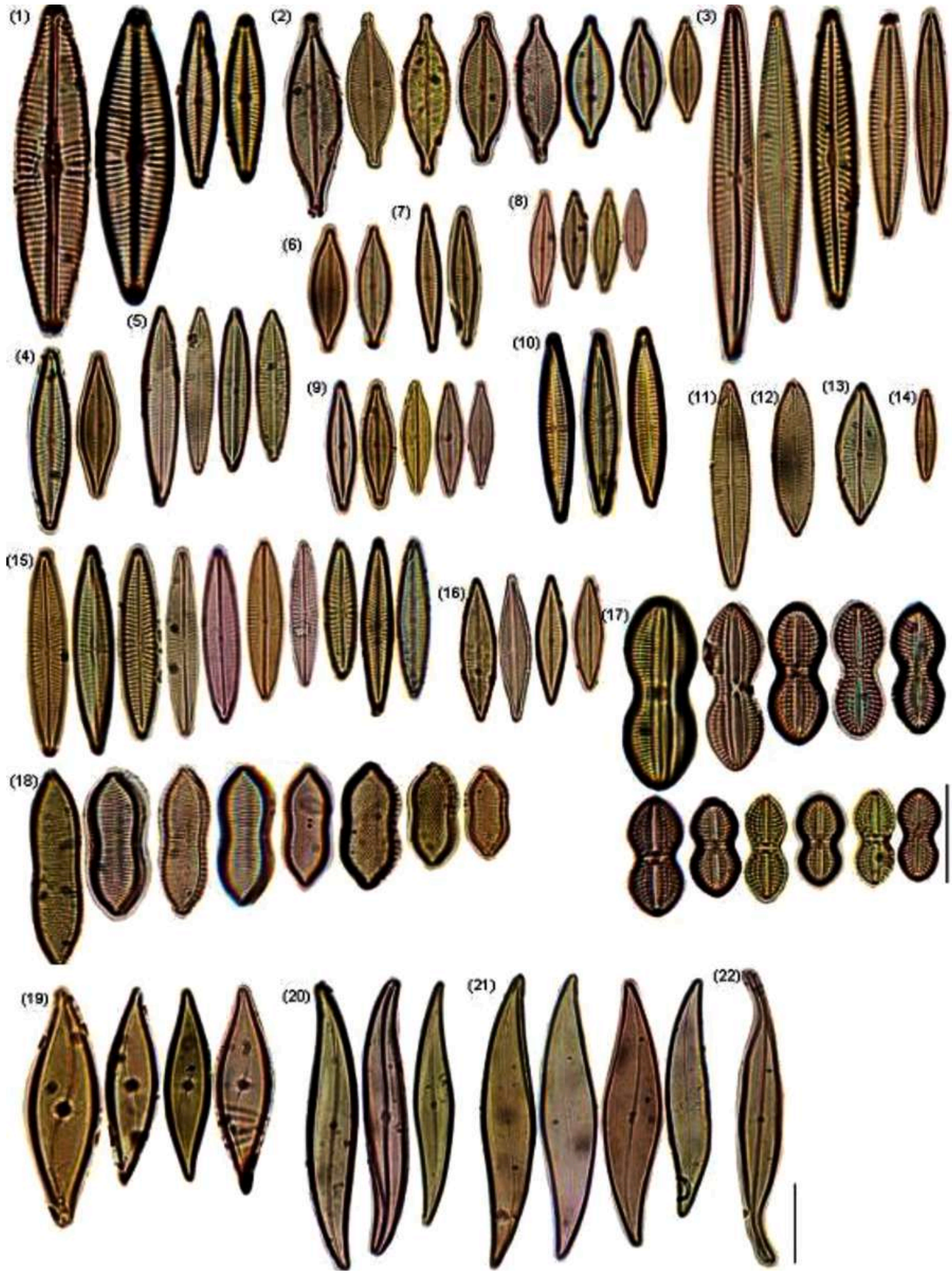

Plate 1. Frustules of 1-*N. recens*, 2-*N. salinarium*, 3- *N. directa*, 4- *N. lanceolata*, 5- *N. cryptotenella*, 6- *N. gregaria*, 7- *N. leptostriata*, 8- *N. capitatoradiata*, 9-*N.* cf. *hoffmanniae*, 10- *N. densilineolata*, 11- *N. parabilis*, 12- *N. incertata*, 13- *N. salinarum* var. *minima*, 14- *N. notha*, 15- *N. distans*, 16- *N. cryptocephala*, 17-*D. stroemii*, 18-*T. coarctata*, 19-*P. naviculaceum*, 20-*G. spencerii*, 21- *P. inflatum*, and 22-*G. fasciola*. Scale bar =10 µm.

ecosystems.

4.1. Life form

It was somewhat surprising that the percentages of motile and non-motile frustule forms were similar in periphytic and planktonic communities. We expected that solid surfaces would favor attached forms for periphyton and non-motile forms in the water column for plankton. Other studies have similarly observed more motile diatoms on soft than hard substrates (Bergey, 2008; Potapova and Charles, 2005). Passy (2007) reported the dominance of motile forms under nutrient enrichment, whereas non-motile pioneer forms were more frequent in

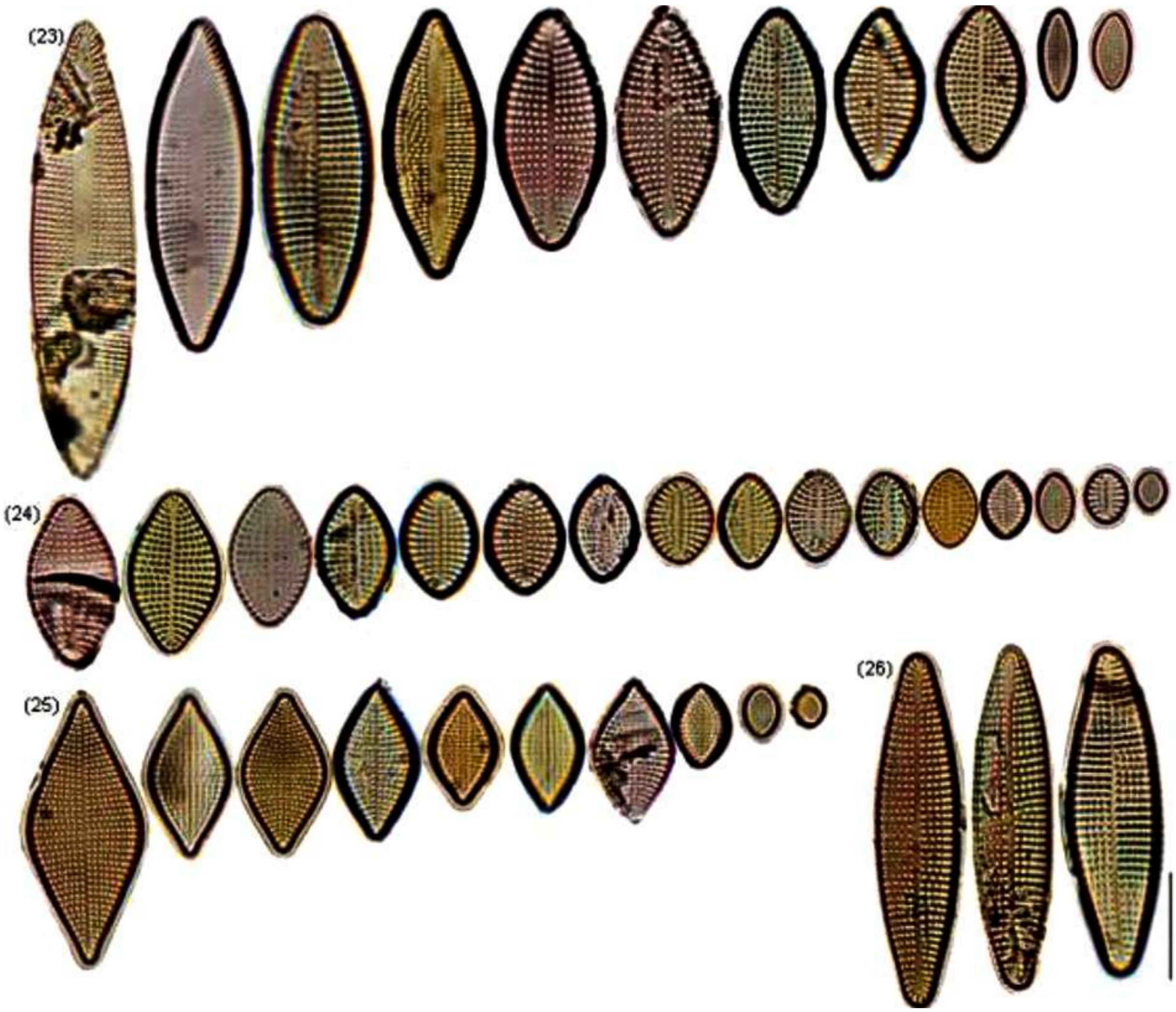

Plate 2. Photographic documentation of size variability in 3 commonly occurring diatom species. **23-**Frustules of *D. surirella*, 24-*R. amphiceros*, 25-*A. pacifica*, and 26-*D. surirella*. Scale bar =8 μm. See Table 1 for more details.

nutrient-poor conditions. The high abundance of motile diatoms at our three sites is consistent with the sites' nutrient enrichment.

There are several potential mechanisms by which motile diatoms would be favored under enrichment by nutrients and other pollutants. First, motile diatoms can secrete extracellular enzymes, which enables them to use macromolecules adsorbed on hard substrata or sediments (Pringle, 1990). Second, motile diatoms are often bigger than sessile diatoms, which enables them to store more nutrients. Third, motile diatoms may also move rapidly from nutrient-poor microenvironments to nutrient rich locations (Johnson et al., 1997). The abundance of motile life forms we observed in the periphytic diatom community could thus be linked to herbicide and runoff.

4.2. Physiological status

Spatial and temporal patterns of diatom physiological status, assessed from cellular content, can relate to environmental and anthropogenic perturbations (Gillett et al., 2009, 2011; Pandey et al., 2017). For example, Gillet et al. (2011) observed a lower percentage of live diatoms at impacted streams vs. reference, demonstrating the potential for using cell health for monitoring human disturbance (Gillett et al., 2011). More recently, Pandey et al. (2018a) reported fewer live or healthy diatom frustules in periphytic diatom communities of severely impacted (metals and nutrients) sites compared with less impacted waterbodies in South Korea. Thus, the relatively high abundance of live diatom cells we observed at the studied sites is indicative of low environmental/anthropogenic impact.

4.3. Community measures

Diversity indices for benthic and periphytic diatom communities at Deokjeok and Daeijak islands were greater than that of planktonic communities. The composition of benthic and periphytic diatoms is more likely to reflect local conditions than that of planktonic diatoms. The Shannon diversity indices across sites ranged between 1.2 and 2.9 which is comparable to other studies of diatom diversity in the region. We also observed a predominance of large, motile diatom species at both island sites which we interpret as a signal of ecological integrity.

4.4. Lipid body characteristics

In the present study, LB induction was more prevalent in commonly occurring naviculoid (*N. gregaria*, *N. distans*) and sigmoid (*P. normanii*) forms at Deokjeok 2. This site was associated with appreciably higher concentrations (compared with background) of Cd, Cr, and As. In diatoms, LBs and chrysolaminarin are the main food reserves (Pandey et al., 2017). Under metal stress, LBs typically increase in number and, more characteristically, in size (Pandey et al., 2015,2018a; Pandey and

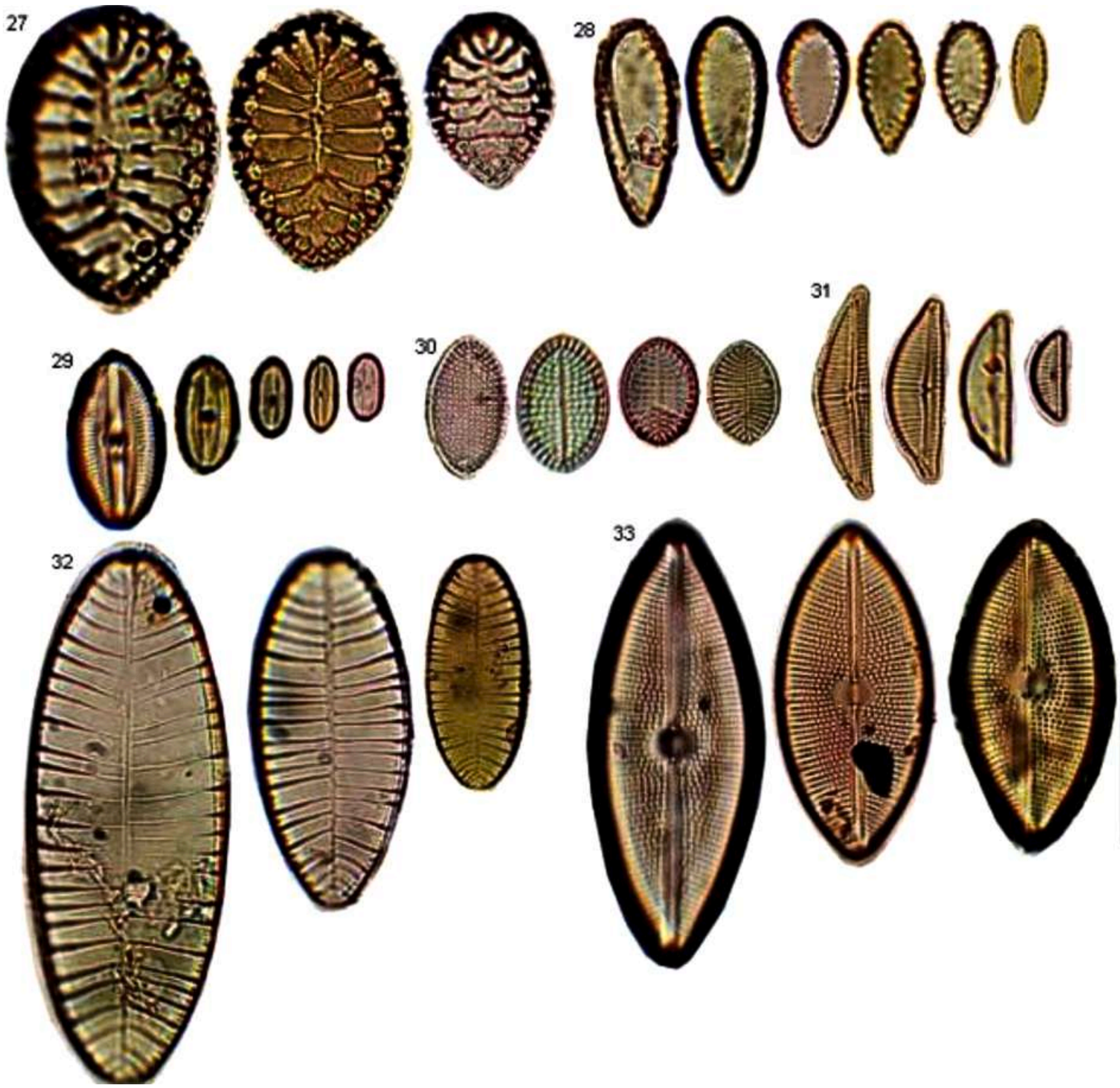

Plate 3. Photographic documentation of size variability in 7 commonly occurring diatom species. Frustules of 27-*S. lacrimula*, 28-*S. stalagma*, 29-*D. pseudovalis*, 30-*C. placentula*, 31-*E. sileasiacum*, 32-*S. gemma*, and 33-*L. reimeri*. Scale bar =10 µm. See Table 1 for more details.

Bergey, 2018; Pandey, 2020). Although the exact mechanism of LB induction in diatom cells is not clearly understood, it is reported that metal stress can result in membrane depolarization and cytoplasmic acidification due to intracellular accumulation of metals, leading to the disruption of cellular homeostasis (Pinto et al., 2003). This cytoplasmic imbalance caused by metals may increase diatom sinking rates (Pandey et al., 2015; Park et al., 2020) and impact light and nutrient conditions that are necessary for growth. Increased intracellular lipid content through the formation of LBs increases buoyancy and reduces diatom sinking rates (Ramachandra et al., 2009).

4.5. Morphological deformities

In the present study, the ranking among sites for frustules deformation rates and anthropogenic contamination (nutrients and metals) were the same (Deokjeok 2 > Deokjeok 1 > Daeijak). In general, stressful conditions tend to generate asymmetrical morphologies across taxa, but particularly so in diatoms (Clarson et al., 2009; Dickman, 1998). Measuring the frequency of diatom frustule deformities is a common biomonitoring practice (Clarson et al., 2009; Dziengo-Czaja et al., 2008; Falasco et al., 2009b; Morin et al., 2012; Pandey et al., 2014,2015, 2016a). Significantly higher percentages of deformed frustules have been documented in laboratory and field studies under metal stress conditions (Gautam et al., 2017; Pandey et al., 2014,2015,2016a). Metal toxicity can vary with, among other factors, water pH and the presence of organic matter (Roig et al., 2007). As a consequence, toxicity and hence the frequency of deformities is likely to be more variable under field conditions than under controlled laboratory conditions (Pandey et al., 2017).

Of the four deformity types we recorded, only type 1 (deformed valves) was prevalent at all three sites. Under stressed conditions, all four deformities (deformed valves, striae, raphes, and more than one deformity type) are often reported (Falasco et al., 2009a, 2009b; Pandey et al., 2014,2016a; Rimet and Bouchez, 2011). Thus, the occurrence of only type 1 deformities may correspond to relatively healthy ecological conditions at the examined sites. Frustule deformities are more prevalent with metal contamination, particularly in areas with acid mine drainage (AMD), sewage, and municipal and industrial wastewaters (Cantonati et al., 2014; Pandey et al., 2016a). Nutrients facilitate the entry of metals and metalloids into cells, including those non-essential for growth (especially Cd, As, Cr, and Hg) (Behra et al., 2002; Holding et al., 2003; Wang and Dei, 2001). These metal ions can cause microtubular poisoning, which disturbs the transportation of silica to silicon transport vesicles, and ultimately results in frustule deformities (Kim et al., 2011; Pandey et al., 2015).

4.6. Size variability

In the present study there was little variability in the size of diatoms in the planktonic samples, but pronounced variation in benthic and periphytic diatoms from both islands. The low number of total plankton diatoms observed may have contributed to this lack of size variation. Reduction in frustule size is one common response to environmental stress among diatoms (Daufresne et al., 2009), and metal stress has been associated with size reduction in diatom frustules (Cantonati et al., 2014; Pandey et al., 2016a,b). However, diatom frustule size varies naturally over time, often slowly declining over time due to the formation of new valves within the parental thecae. Thus without comparisons over time, it is not possible to determine the degree to which size variation observed in this study may be related to environmental factors. Future investigations using this metric would need to establish the natural size range of each species, their normal rate of decline, and seasonal sampling to determine the timing of size regeneration.

5. Conclusions

We investigated both traditional (percentage relative abundance, Shannon index, species richness) and more novel metrics (life form, physiological status, LBs, deformity rates, and size variation) of diatom population status across plankton, periphytic, and benthic diatom communities. We did not find strong evidence of disturbance to diatom communities at our study sites despite low-level anthropogenic activities and relatively high metal concentrations in sediment. However, the higher induction of LBs and deformities in diatom frustules at the Deokjeok 2 site than at Deokjeok 1 or Daeijak suggests that this site could be more affected by anthropogenic disturbance. This site also had higher sediment concentrations of metals (Cd, Cr, and As), probably because sediment fraction showed higher proportions of finer particles (clay and silt) making the easy bioavailability of these metals to the residing diatom community. A more detailed investigation of this area is required. Finally, we conclude that the newer diatom metrics, particularly LB characteristics and frustule deformities are relatively rapid, simple, cheap, reproducible, and based on accepted protocols. Furthermore, they allow for direct comparison between sites over large geographical scales (countries, continents, etc.) because they are independent of taxonomic specificity or endemism.

CRediT authorship contribution statement

Jihae Park: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing, Project administration. **Elizabeth A. Bergey:** Methodology, Writing - review & editing. **Taejun Han:** Methodology, Writing - review & editing. **Lalit K. Pandey:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquatox.2020.105594.

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