An approach to identifying potential surrogates of periphytic ciliate communities for monitoring water quality of coastal waters

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A B S T R A C T
For identifying the potential surrogate of periphytic ciliate communities for monitoring marine water quality, the different taxonomic resolutions/taxa as surrogates and different data transformations were studied based on two datasets of ciliate communities in Korean coastal waters. Multivariate analyses showed that: (1) a dominant Zoothamnium duplicatum significantly masked the temporal patterns of periphytic ciliate community; (2) the order level resolution maintained sufficient information to evaluate the efficient patterns of ciliate communities in response to environmental impacts; (3) the vagile-ciliate assemblage at species-level resolution was as costly as whole periphytic ciliate communities without Z. duplicatum at the order level; and (4) the severity of data transformations played a crucial role for effectiveness of surrogates, e.g., heavy transformation for species level and mild for higher. These results suggest that the use of lower taxonomic resolutions is time-efficient and would allow improving sampling strategies of large spatial/temporal scale monitoring researches in the marine ecosystem.

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1. Introduction

With easy sampling, relative immobile, increasing availability of easily used taxonomic references and allowing standardization for temporal and spatial comparisons, periphytic ciliates have widely been accepted to evaluate responses to contamination in ecological investigations (Gong et al., 2005; Norf et al., 2007, 2009; Xu et al., 2009a,b; Mieczan, 2010). So far bioassessments using ciliated protozoan communities have traditionally been based on the identification of ciliates at the lowest possible taxonomic level, usually species. However, this resolution requires a skilled taxonomic expertise and is time-consuming and expensive, and thus limits the use of ciliated protozoa for monitoring water quality, in particular by the environmental agencies that are often dealing with large scales in a limited time span (Lovell et al., 2007; Bertasi et al., 2009; Yu et al., 2010).

Taxonomic sufficiency has proved to be an effective technique for reducing the costs of ecological monitoring researches by identifying taxa to the highest possible category without losing information (Warwick, 1988; Stark et al., 2003; Bertasi et al., 2009).

Several researches on benthic communities have suggested that higher taxonomic levels may be surrogates of the whole species assemblages in response to severer anthropogenic impacts (e.g., Olsgard et al., 1998; Terlizzi et al., 2008). The other technique is to identify a single taxonomic/ecological group as a surrogate of the whole species assemblages for assessing environmental stress (Olsgard and Somerfield, 2000; Bertasi et al., 2009). Otherwise, data transformations have been known to affect the results of multivariate analyses by weighting the relative contributions to results of abundant and rare taxa (Stark et al., 2003; Anderson et al., 2005). Although the effectiveness of taxonomic/ecological surrogates has been reported on metazoan assemblages (Bertasi et al., 2009), such studies have yet to be carried out on protozoan communities for assessing marine environments (Foissner and Berger, 1996; Mieczan, 2007).

In the present study, datasets of two field surveys, which were carried out on impact of tidal currents and circulations on periphytic ciliate colonizations for monitoring water quality in Korean coastal waters, were analyzed. Our aims were (1) to determine the effects of decreasing taxonomic resolution to show differences in the structure of periphytic communities during colonizazion and succession; (2) to assess the efficiency of vagile ciliate assemblages as a surrogate to document the temporal patterns of the whole periphytic ciliate communities; and (3) to evaluate the influence of data transformation on the impact assessment.

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Fig. 1. Spearman correlation coefficient values between the original matrix (OM) including Z. duplicatum and the matrix without Z. duplicatum (NZM) at each taxonomic level and under different types of data transformations for the datasets Com-InchKr (a) and Col-InchKr (b). S, species; G, genus; F, family; O, order; C, class; NONE, untransformed; SR, square-root; FR, fourth-root; LOG, logarithmic; P/A, presence/absence.

2. Materials and methods

Two datasets of periphytic ciliate assemblages, obtained from two surveys on effects of tidal currents and circulations on periphytic ciliate colonizations for monitoring water quality in Korean waters. The polyurethane foam enveloped slide (PFES) system, a modified glass slide method, and the conventional slide (CS) method were used to host periphytic ciliates in marine ecosystems (Xu et al., 2009a,b). The study sites of the two surveys were located at the coastal waters near Incheon Harbor, Korea. It was a polluted coastal area, with a depth of about 8 m, having high turbidity due to its mud-sandy bottom. During the study periods, the water depths were usually changed at a tidal interval of about 3.5 m by the strong tide currents and circulations.

2.1. First dataset: Com-InchKr

This survey was carried out for detecting the temporal variations of 10-day ciliate communities during the period of August–November, 2007 (Xu et al., 2009a). A total of 8 samplings were performed using both the PFES and CS systems. In each sampling, 10 slides were collected every 10 days, followed by a next 10-day exposure of the frame holding 10 sheets of slides. For both sampling systems, 10 glass slides (2.5 cm × 7.5 cm) were clipped to a polyvinyl chloride (PVC) frame (14 cm × 8 cm × 7 cm) in back-to-back pairs, and immersed in water at a depth of 1 m below the water surface (Xu et al., 2009a).

2.2. Second dataset: Col-InchKr

This survey for evaluating the temporal dynamics of periphytic ciliate assemblages during their 20-day colonization was performed during the period of April, 2007. A total of 10 samplings were carried out for 19 days, using both sampling systems with 8 slides collected every two days. In each system, a total of 40 glass slides (2.5 cm × 7.5 cm) were used as artificial substrates for hosting periphytic ciliates from a depth of 1 m below the water surface. The glass slides were placed vertically in PVC frames (14 cm × 8 cm × 7 cm) as back-to-back pairs (Xu et al., 2009b).

2.3. Data analysis

The periphytic ciliate communities in Korean costal waters are characterized by the extreme abundance of the colonial peritrich Zoanthamnium duplicatum, which represented high temporal variations (Xu et al., 2009a,b). In the two datasets, this peritrich ranged from 70% to 98% of the total number of individuals and often masked the community patterns of the entire periphytic ciliate assemblages (Xu et al., 2009a,b). In order to evaluate the disturbance of the peritrich on the results of multivariate analyses, each dataset was given rise to two matrices: an original matrix (OM) including Z. duplicatum, and a matrix without Z. duplicatum (NZM). For both OM and NZM, species abundance data were aggregated to the levels of genus, family, order and class. In addition, in each dataset the vagile ciliate assemblage comprising several taxonomic groups (e.g., heterotrichids, pleurostomatids, heterotrichids and cyrtophorids) was the most dominant ecological assemblage. For assessing the efficiency of this ecological assemblage as a surrogate of the whole species assemblages, the matrices (VM) including abundances of this assemblage were separately computed. Each of these was also analyzed after aggregating abundances at the levels of genus, family, order and class. The taxonomic scheme used was according to Lynn (2008). The designation of species as being sessile or vagile was made in terms of their mobility and the ecological niches they occupied.

A total of 30 abundance matrices have been constructed. Bray-Curtis similarities of samples were computed based on data subjected to a sequence of transformations (gradually downweighting the dominant taxa): none, square-root, fourth-root, logarithmic and presence/absence (Clarke and Gorley, 2006). The relationships between pairs of similarity matrices and matching correlations with the temporal seriations of samples were analyzed using the Spearman rank correlation coefficients (ρ values) which were computed by the submodule RELATE (Somerfield and Clark, 1995; Clarke and Gorley, 2006). The second-stage MDS ordinations and cluster analyses were performed to summarize the related-

### Table 1

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Com-InchKr</th>
<th>CS</th>
<th>Total</th>
<th>Col-InchKr</th>
<th>CS</th>
<th>Total</th>
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<td>(a) All periphytic ciliates</td>
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<td>Class</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
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</tr>
<tr>
<td>(b) Vagile ciliates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
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<td>34</td>
<td>35</td>
<td>23</td>
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<td>Genus</td>
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<td>15</td>
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<tr>
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<td>11</td>
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<tr>
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<td>5</td>
<td>5</td>
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<td>5</td>
</tr>
</tbody>
</table>

Z. duplicatum, which represented high temporal variations (Xu et al., 2009a,b). In the two datasets, this peritrich ranged from 70% to 98% of the total number of individuals and often masked the community patterns of the entire periphytic ciliate assemblages (Xu et al., 2009a,b). In order to evaluate the disturbance of the peritrich on the results of multivariate analyses, each dataset was given rise to two matrices: an original matrix (OM) including Z. duplicatum, and a matrix without Z. duplicatum (NZM). For both OM and NZM, species abundance data were aggregated to the levels of genus, family, order and class. In addition, in each dataset the vagile ciliate assemblage comprising several taxonomic groups (e.g., heterotrichids, pleurostomatids, heterotrichids and cyrtophorids) was the most dominant ecological assemblage. For assessing the efficiency of this ecological assemblage as a surrogate of the whole species assemblages, the matrices (VM) including abundances of this assemblage were separately computed. Each of these was also analyzed after aggregating abundances at the levels of genus, family, order and class. The taxonomic scheme used was according to Lynn (2008). The designation of species as being sessile or vagile was made in terms of their mobility and the ecological niches they occupied.
ness of the NZM similarity matrices for species and those for both higher taxonomic levels and vagile ciliate assemblages at increasing taxonomic levels under all types of data transformations for both datasets. All analyses were carried out using the PRIMER package version 6.1 (Clarke and Gorley, 2006).

A cost/benefit ($C/B$) ratio was calculated for each dataset in order to objectively select the taxonomic level with the minimal loss of information and the least taxonomic effort according to the equation:

$$CBL_L = \frac{(1 - r_L)}{[S - t_L] / S}$$

where $CBL_L$ is the cost/benefit ratio at taxonomic level $L$; $r_L$, the Spearman correlation coefficient between taxonomic level $L$ and species level; $t_L$, the number of taxa at taxonomic level $L$; $S$, the number of species (Karakassiss and Hatziyanni, 2000).

The cost/benefit ratio ranges between 0 and 1. Values equal to 0 show a high correlation between the species level and any of the other groups, which means that the loss of information is the lowest.

3. Results

3.1. Colonial peritrich and taxonomic aggregation

The taxonomic composition of the two datasets in terms of numbers of species, genera, families, orders and classes are listed in Table 1a. The Spearman correlation coefficients between the OMs and the NZMs similarity matrices at each taxonomic level and under a series of data transformations are summarized in Fig. 1. It was shown that the $\rho$ values increased with the increased severity of data transformations, but represented different trend (i.e., increasing in the dataset Com-InchKr, but decreasing in the dataset Col-InchKr) with taxonomic aggregation in both datasets (Fig. 1). High rank correlations were found for fourth-root and presence/absence transformed data up to order level in the dataset Com-InchKr. It should be noted that the matching coefficients presented higher values than 0.75 at the taxonomic resolutions up to only order level under fourth-root and presence/absence transformations for the dataset Com-InchKr (Fig. 1a).

The matching correlations with the temporal seriations of periphytic ciliate colonization in both systems PFES and CS based on similarity matrices from different transformed OM and NZM data at various taxonomic levels in each dataset are summarized in Table 2. The results showed that the matching correlation coefficients for the PFES system were comparatively higher after removing the ciliate *Z. duplicatum* than before, while the events for CS system represent a reverse case, i.e., this colonial peritrich largely reduce the contributions of the other species to the community structure, and thus significantly masked the temporal patterns of periphytic ciliate assemblages for both datasets (Table 2). It was also shown that for NZM the correlation coefficients of ciliates colonization in the PFES system were definitely high compared with those in the CS system, i.e., the NZMs presented the temporal patterns of periphytic ciliate colonization in response to the tidal disturbance for both datasets (Table 2).

The following analyses were carried out using the NZM matrices without the ciliate *Z. duplicatum* due to the reason above. Correlations between the species matrix and the matrices from the taxon-abundance data at higher taxonomic levels, under all types of data transformation, were computed by the submodule RELATE (Fig. 2a and b). Otherwise, the second-stage MDS ordinations and cluster analyses were used to compare the matching correlations between the multivariate patterns of the NZM similarity matrices at different hierarchical taxonomic resolutions and with different data transformations (Fig. 3a and b).
### Table 2

Results of matching (RELATE) analyses for temporal seriations of periphytic ciliate colonization in both PFES and CS systems based on similarity matrices from untransformed, square-root, fourth-root, logarithmic and presence/absence transformed OM and NZM data at various taxonomic levels.

<table>
<thead>
<tr>
<th></th>
<th>OMCom-InchKr</th>
<th>NZMCom-InchKr</th>
<th>OMCol-InchKr</th>
<th>NZMCol-InchKr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFES CS</td>
<td>PFES CS</td>
<td>PFES CS</td>
<td>PFES CS</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.227 0.518</td>
<td>0.472 0.440</td>
<td>0.836 0.643</td>
<td>0.826 0.427</td>
</tr>
<tr>
<td>G</td>
<td>0.218 0.373</td>
<td>0.474 0.358</td>
<td>0.862 0.643</td>
<td>0.805 0.423</td>
</tr>
<tr>
<td>F</td>
<td>0.197 0.373</td>
<td>0.375 0.334</td>
<td>0.875 0.643</td>
<td>0.774 0.419</td>
</tr>
<tr>
<td>C</td>
<td>0.192 0.373</td>
<td>0.162 0.290</td>
<td>0.889 0.652</td>
<td>0.746 0.529</td>
</tr>
<tr>
<td>S</td>
<td>0.314 0.516</td>
<td>0.452 0.408</td>
<td>0.805 0.608</td>
<td>0.828 0.433</td>
</tr>
<tr>
<td>G</td>
<td>0.267 0.432</td>
<td>0.432 0.399</td>
<td>0.823 0.606</td>
<td>0.825 0.427</td>
</tr>
<tr>
<td>FR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.481 0.584</td>
<td>0.441 0.422</td>
<td>0.781 0.557</td>
<td>0.796 0.435</td>
</tr>
<tr>
<td>G</td>
<td>0.501 0.421</td>
<td>0.397 0.296</td>
<td>0.774 0.553</td>
<td>0.814 0.410</td>
</tr>
<tr>
<td>LOG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.363 0.466</td>
<td>0.350 0.478</td>
<td>0.870 0.553</td>
<td>0.819 0.431</td>
</tr>
<tr>
<td>G</td>
<td>0.351 0.397</td>
<td>0.376 0.446</td>
<td>0.791 0.549</td>
<td>0.811 0.429</td>
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<td>P/A</td>
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<td></td>
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<tr>
<td>S</td>
<td>0.376 0.245</td>
<td>0.353 0.219</td>
<td>0.717 0.439</td>
<td>0.749 0.377</td>
</tr>
<tr>
<td>G</td>
<td>0.121 0.260</td>
<td>0.121 0.263</td>
<td>0.617 0.414</td>
<td>0.576 0.353</td>
</tr>
<tr>
<td>C</td>
<td>0.387 0.172</td>
<td>0.405 0.172</td>
<td>0.731 0.387</td>
<td>0.721 0.431</td>
</tr>
</tbody>
</table>

Values are the matching coefficient $r$, significant tests ($P < 0.05$) are in bold. NONE, untransformed; SR, square-root; FR, fourth-root; LOG, logarithmic; P/A, presence/absence; S, species; G, genus; F, family; O, order; C, class. OM, original matrix including $Z. duplicatum$; NZM, matrix without $Z. duplicatum$; for Com-InchKr and Col-InchKr, see text.

For the dataset Com-InchKr, the correlation coefficients remained higher than 0.75 up to the order level, except for the patterns at the taxonomic levels higher than genus with fourth-root and presence/absence data transformations (Fig. 2a). The MDS-ordination-based clustering analyses resulted in the NZM-species matrices falling into the group I with the matrices at the lower taxonomic resolutions up to order at a 0.75 Spearman correlation level (Fig. 3a). It should noted that group I was assigned into three subgroups (Ia, Ib and Ic) at a 0.85 matching correlation level, in subgroup Ib of which the NZM-species matrix from untransformed data was aggregated with the matrices at genus- and family-level resolutions.
from untransformed, square-root and fourth-root transformed data.

For the dataset Col-InchKr, all matching coefficients showed higher values than 0.85 up to the class level under all types of data transformations (Fig. 2b). The MDS-ordination-based clustering analyses showed a similar pattern to the dataset Com-InchKr: the NZM-species similarity matrices were aggregated with those of VM at the taxonomic resolutions up to order level, except for the case from untransformed data (Fig. 3b). It was also showed that the high matching correlations between species and higher taxonomic resolutions were maintained at a $\rho$ value of 0.95 up to order level under square-root and fourth-root transformations, but family level under P/A transformation (Fig. 3b: subgroups Ia and Ib).

### 3.2. Vagile ciliates as surrogate

The taxonomic composition of vagile ciliate assemblages in terms of the numbers of species, genera, families, order and class for each dataset are summarized in Table 1b. The matching correlations between the NZM-species similarity matrices and those for VM at different taxonomic aggregation levels are shown in Fig. 2c and d. The second-stage MDS ordination and group-average clustering analyses were carried out to compare these matching relationships (Fig. 3c and d).

For the dataset Col-InchKr, all matching correlations showed higher values than 0.85 up to the class level under all types of data transformations but the class-level resolution with no transformation (Fig. 2d). The second-stage MDS ordination with clustering analyses determined that the matching correlations between NZM-species matrix and different taxonomic resolutions of VM were maintained at the level of $\rho > 0.95$ up to family level under square-root and fourth-root transformations (Fig. 3b: subgroup Ib).

The matching correlations with the temporal seriations of samples from each sampling system for VM similarity matrices from different transformed data at all taxonomic levels in each dataset are given in Table 3. It was demonstrated that the temporal seriations for matrices of the PFES samples represented definitely higher $\rho$ values than those of CS samples, especially for the dataset Col-InchKr, i.e., the PFES system may effectively protect the vagile ciliate colonizations from disturbances of the tidal currents and circulations (Table 3).

### 3.3. Cost/benefit analysis

The cost/benefit (C/B) ratios for the two datasets are summarized in Fig. 4. For each dataset, the NZM at species level was compared to the matrices of higher taxonomic levels and those of species, genera, family, order and class of vagile ciliate assemblages, respectively. The C/B ratios for the two datasets represented minimal values at the level of genus due to the high correlation coefficients with the species level, but the decrease of the taxon numbers was only 26.8% and 27.6% compared with the number of species for the datasets Com-InchKr and Col-InchKr, respectively (Fig. 4 and Table 1a). At the level of family, the numbers of taxa correspondingly decreased of 41.5% and 37.9%, but the C/B ratios were acceptably low for both datasets respectively (Fig. 4 and Table 1a).

However, it should be noted that the severity of the data transformation played a significant role in determining the analyzing results. The lower values were obtained from the untransformed, square-root and log-transformed data at the levels of species genus
and order for both datasets, as well as the data of vagile ciliate assemblages at the levels of genus and family for the dataset Com-InchKr (Fig. 4a). It should also be addressed that the C/B ratios were acceptably low for vagile ciliate species assemblages under the fourth-root and P/A transformations for both datasets, in particular for the dataset Col-InchKr (Fig. 4b).

4. Discussion

Although many investigations on colonization, using artificial substrate methods, have conducted on freshwater protozoa (Cairns and Yongue, 1968; Cairns and Henebry, 1982; Xu et al., 2005; Mieczan, 2010), few have carried out in marine waters (Cairns and Yongue, 1968; Xu et al., 2002; Gong et al., 2005; Mieczan, 2007). Our previous studies have demonstrated that the tidal conditions can also distinctly influence the both species diversities and species colonization parameters of periphytic ciliate communities on the naked glass slides (Xu et al., 2009a,b). This was mainly due to the fragile ciliates were removed by rough tidal conditions during the colonization process (Xu et al., 2009b).

We demonstrate that information loss with the taxonomic aggregations primarily due to the dominant ciliate Z. duplicatum, which represented a patchy temporal variation in abundance and significantly masked the patterns of the whole periphytic ciliate communities. In addition, our analyses showed that the aggregation to higher taxonomic levels did not reduce the effect of the significant dominance of Z. duplicatum, whereas the removal of this ciliate from the species matrices may increase the effectiveness in detecting the temporal seriations of ciliate colonization. In particular, the abundance of Z. duplicatum did not comparatively affect the temporal patterns of ciliate communities under the heavy data transformations for both datasets (Bertasi et al., 2009). These suggest that the taxa with high variations in abundance but without response to disturbance might be ignored for reducing costs and saving time when analyzing the temporal/spatial patterns of a community in response to disturbance.

For all datasets after removal of the ciliate Z. duplicatum from the two datasets, the analyses demonstrated the application of higher taxonomic levels to evaluate the temporal patterns of periphytic ciliate communities. Lovell et al. (2007) proposed that a $\rho$ value of greater than 0.75 should be used as minimal level of congruency for an optimal surrogate. In this study, the $\rho$ values at species level remained higher than 0.75 at order level for the dataset Com-InchKr, even at class level for the dataset Col-InchKr. Notably, for the community dataset the Spearman values dropped below 0.75 at the order level under severer data transformations. With regard to the cost/benefit ratios, the order level and the mild data transformation may obtain the best results for both datasets. This suggests that the order level might be sufficient in evaluating the temporal/spatial patterns of periphytic ciliate communities.

In this study, the vagile ciliate assemblage represented higher correlations ($\rho$ value $>0.75$) to the patterns of NZM-species matrices even at family level, even up to the order level for the dataset Col-InchKr. The cost/benefit ratios showed significant disturbances of the severity of data transformations to the effectiveness of the surrogates, i.e., the best results may be obtained under the fourth-root and/or logarithmic transformation at species level. Furthermore, almost all cost/benefit ratios for vagile ciliate taxa were comparatively low compared to the NZM taxa for all datasets. It should be noted that the vagile ciliate assemblage represented lowest C/B ratio values at the species level under P/A data transformation, which was as costly as the order level of whole periphytic ciliate communities without Z. duplicatum, for both datasets (Col-InchKr in particular). This might suggest that the vagile ciliate assemblage is a good surrogate for periphytic ciliate communities in detecting their functional parameters by the species presence/absence data (Xu et al., 2009b).

In summary, the present study has demonstrated that the taxonomic resolution even at order level and the vagile ciliate assemblages maintain sufficient information for evaluating the patterns of periphytic ciliate communities, and the use of the time-efficient taxonomic resolution could improve sampling strategies of monitoring researches that involves long time-scales and large numbers of sampling sites. However, it should be addressed that species identification is still indispensable for studies on evaluating the responses of ciliate species to environmental impacts and/or selecting species-indicator of water quality.

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