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Ecological Studies on Togyo Reservoir in Chulwon, Korea. VII. The Colonization of Epilithic Algae on Artificial Substrata (Tiles) at Mesocosm

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Patterns of epilithic algal colonization on artificial substrata (unglazed ceramic tiles) were investigated from 23rd April to 3rd July 1999 at weekly intervals over a 10 weeks period outside and inside the mesocosm in Togyo reservoir within the Civilian Passage Restriction Line near Demilitarized Zone (DMZ) in Korea. The highest standing crops of epilithic algae was $1{,}798 \cdot 10^3$ cells $\cdot$ cm^{-2} outside the mesocosm on 26th June and also inside the mesocosm those was $2{,}391 \cdot 10^3$ cells $\cdot$ cm^{-2} on 26th June, 9 weeks after the experiment began. The dominants outside the mesocosm were *Achnanthes minutissima, Navicula bicephala, Oscillatoria angusta, Synedra delicastissima, S. tenuissima, S. ulna* v. *danica* and *Tabellaria flocculosa*, and those inside the mesocosm were *Achnanthes minitissima, Coenochloris polycocca, Fragilaria crotonenesis, Peridinium cinctum, Synedra delicatissima, Tabellaria flocculosa* and *Ulothrix subtilissima*. Diatoms were most abundant and *Achnanthes minutissima* was the most important species colonizing on the tiles. Chlorophyll-*a* content was highest value of 5.4 mg $\cdot$ m^{-2} on 19th June after 8 weeks growth outside the mesocosm and was 24.4 mg $\cdot$ m^{-2} on 26th June, 9 weeks after the experiment began on tiles inside the mesocosm. It was also shown that unglazed ceramic tiles were a more suitable substratum for colonization than the glass slides. Consequently the substratum selection plays an important role in the colonization by the epilithic algal community.

Key Words: artificial substrata (tiles), colonization, epilithic algae, mesocosm, Togyo reservoir

INTRODUCTION

Diatoms have been focused on as powerful indicators of environmental change (Dixit *et al.* 1992). They have been used extensively in recent studies related to ecological aspects e.g. indicator value for water quality (Van Dam *et al.* 1994), acidification of water systems (Battarbee 1994) and trophic index for monitoring eutrophication (Kelly and Whitton 1995). But with the increase of ecological studies, problems of taxonomy have arisen as already been pointed out by Fryer (1987) and also the controversing over what the 'true' epilithon is according to the collection method between the more vigorous washing procedure by Round (1993) and the less vigorous washing procedure by Kelly *et al.* (1995).

However, due to the advantage of habit and sampling of epilithon (usually epilithic diatoms), the colonization and/or establishment of the epilithic algal community both structure and composition was studied in a lentic and lotic water system. Blinn *et al.* (1980) pointed out that the substratum selection between limestone, sandstone and basalt may play only a limited role in the structure of the epilithic diatom community, with other variables such as current velocity, light energy, and nutrient budget. But some controversy on the physical structure of the hard substrata and the chemical composition of these were suggested by Antoine and Bensen-Evans (1985). They emphasized that the physical structure of the hard substrata was more important than the chemical composition. Subsequently the colonization of epilithic algae on a range of substrates was made: the establishment of epilithic algae on newly exposed stream bedrock (Stock and Ward 1989), the colonization of the periphytic microalgae on artificial substrates: wood, acryl plate and marble stone (Cho 1994), the colonization dynamics of algal communities on wood and tiles (Sabater *et al.* 1998) and the establishment of epilithic

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algae on glass slides (Lee *et al.* 1998; Lam and Lei 1999). But recently Cattaneo *et al.* (1997) pointed out the importance of substratum rather than the biomass of periphyton distribution and abundance. On fine sediments (sand and fine gravel) most algae were loosely attached to the substratum and colonial bluegreens and motile diatoms were important but in contrast on coarser substrata algae were strongly attached and adnate and filamentous forms were important. However the relationships between the colonizers and the substratum were uncertain.

The aim of this study is to elucidate the patterns of colonization of the epilithic algal community on artificial substrata (unglazed ceramic tiles) in the enclosed mesocosm on Togyo reservoir within the Civilian Passage Restriction Line close to Demilitarized Zone (DMZ), which has not been influenced by human activities or agricultural and industrial wastes from surrounding areas. Also the different patterns of growth both outside and inside the mesocosm, and also the differences between unglazed ceramic tiles and glass slides was investigated.

MATERIALS AND METHODS

The measurement of general environmental factors (i.e., air temperature, water temperature and pH) were made *in situ* according to Lee *et al.* (1996). The variations in the epilithic algal community structure were investigated from 23rd April to 3rd July 1999 at weekly intervals during 10 weeks at the mesocosm on Togyo reservoir (Han *et al.* 1995). Outside and inside the mesocosm respectively, 170 unglazed ceramic tiles (each one is 76 × 24 × 3.5 mm) were attached on a vinyl sheet (1.5 × 1.0 m).

Samples were scraped from the surface of the tiles, washed with distilled water, transferred into glass vials, fixed with neutral formalin, and counted in a Sedgwick-Rafter counting chamber under a light microscope at × 100 magnification. Cleaning was by nitric acid or hydrogen peroxide, and mounting was with Naphrax. Identification was made at × 1,000 magnification. The identification of diatoms followed Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) and that of bluegreens and greens followed Prescott (1962) mainly. The algal populations were also estimated indirectly by chlorophyll-a content (Lorenzen, 1967).

Table 1. The physicochemical factors for Togyo reservoir from 23rd April to 3rd July 1999

Date and Time	pH		Temperature(°C)		
	outside	inside	Air	Water outside	Water inside
23rd April 11:30	8.6	8.3	21.0	16.8	15.5
1st May 10:30	8.6	7.8	18.0	17.0	16.8
8th May 11:00	8.3	8.2	20.5	18.8	19.0
16th May 10:30	8.9	9.5	24.5	23.8	23.8
21st May 12:15	8.4	9.9	23.5	21.2	21.0
29th May 11:00	8.5	8.5	22.6	19.4	20.0
5th June 10:30	8.6	8.5	26.5	23.4	24.0
12th June 11:10	8.6	9.2	27.5	26.6	26.2
19th June 11:05	8.8	9.5	23.0	25.0	24.0
26th June 10:40	8.8	9.7	26.5	27.4	26.8
3rd July 10:25	8.8	9.6	30.2	27.5	27.2

RESULTS

General environmental factors

Water temperature varied from 16.8°C on 23rd April to 27.5°C on 3rd July outside the mesocosm and from 15.5°C on 23rd April to 27.2°C on 3rd July inside the mesocosm. pH varied from 8.3 on 8th May to 8.9 on 16th May outside the mesocosm and from 7.8 on 1st May to 9.9 on 21st May inside the mesocosm (Table 1). Outside the mesocosm there was a gradual decrease of pH from 23rd April to 8th May and increased until 16th May and decreased on 21st May, and since then there was a gradual increase of pH to the end of experiment. On the inside of the mesocosm there was greater fluctuation of pH than on the outside, but the variation pattern was almost same on both side of the mesocosm.

Standing crops of epilithic algae and chlorophyll-*a* content

Outside the mesocosm, the standing crops of epilithon (artificial substrata: unglazed ceramic tiles) showed the highest value of $1{,}798 \cdot 10^3$ cells $\cdot$ cm^{-2} on 26th June, 9 weeks after the experiment began (Fig. 1), but that on another substrata (glass slides) had a highest value of $306 \cdot 10^3$ cells $\cdot$ cm^{-2} in September, 4 months after the experiment began in the same mesocosm of the same reservoir in 1996 (Lee *et al.* 1998). Also it was in April that the phytoplankton standing crops under natural conditions in the reservoir reached the highest value of $773 \cdot 10^3$ cells $\cdot$ l^{-1} (Lee *et al.* 1996). After the initial colonization, the epilithic algal community grew slowly

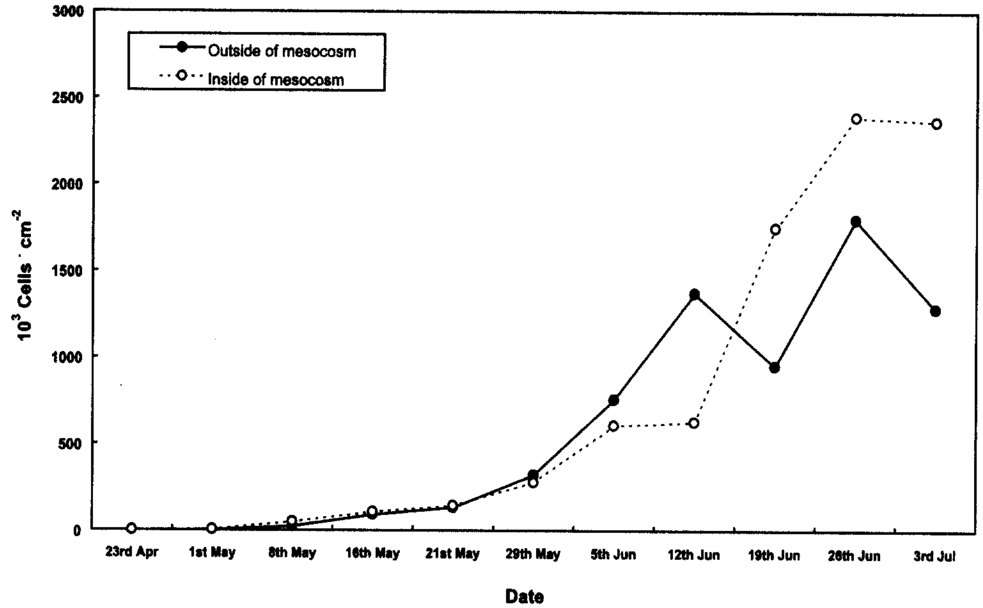

Fig. 1. The weekly variations of standing crops of epilithic algae in Togyo reservoir from 23rd April to 3rd July 1999 (□: outside the mesocosm, ○: inside the mesocosm).

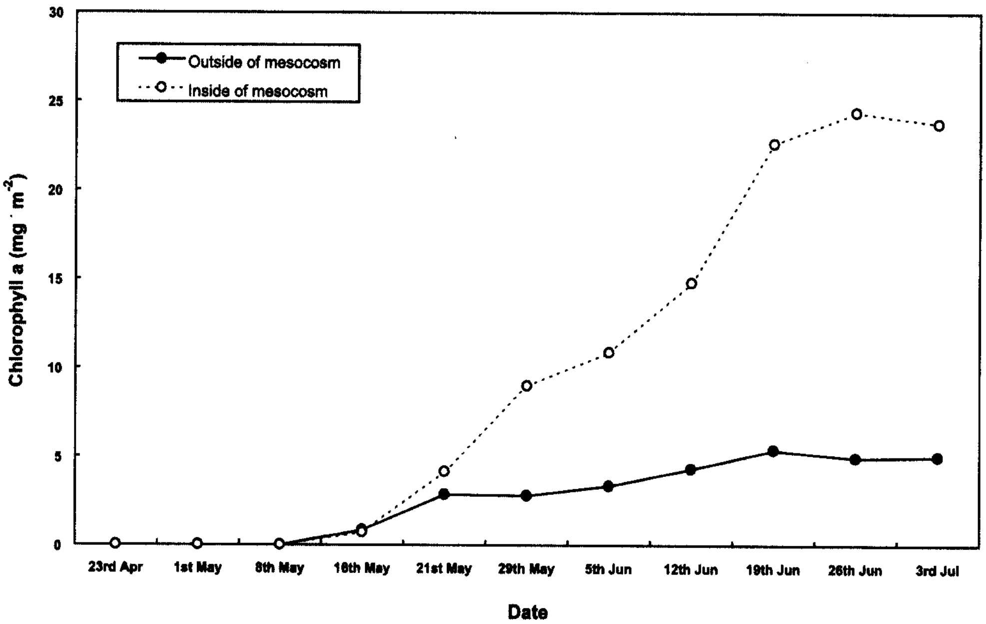

Fig. 2. The weekly variations of chlorophyll-a contents in Togyo reservoir from 23rd April to 3rd July 1999 (□: outside the mesocosm, ○: inside themesocosm).

and after 5 weeks they attained $1{,}373 \cdot 10^3$ cells · cm^{-2} and decreased to some extent due to the heavy rain in summer flood and increased again rapidly and reached the highest values ($1{,}798 \cdot 10^3$ cells · cm^{-2}) on 26th June and then decreased.

Explaining the differences caused by the mesocosm itself, samples were taken from inside of mesocosm. Here the standing crops of epilithon (artificial substrata: unglazed ceramic tiles) showed the highest value of $2{,}391 \cdot 10^3$ cells · cm^{-2} on 26th June, 9 weeks after the experiment began (Fig. 1), but those of another epilithon (artificial substrata: glass slides) had the highest value ($166 \cdot 10^3$ cells · cm^{-2}) in September, 4 months after the experiment began (Lee *et al.* 1998). At the beginning of colonization, there was a slow increase until 12th June. After then it showed a rapid increase and reached the highest ($2{,}391 \cdot 10^3$ cells · cm^{-2}) on 26th June, and then it remained in a stationary state for one week before

Table 2. The dominants in epilithic algal community outside the mesocosm in Togyo reservoir from 23rd April to 3rd July 1999

Date / Dominant species	April	May					June				July
	23rd	1st	8th	16th	21st	29th	5th	12th	19th	26th	3rd
Achnanthes minutissima					64.1 (48.9%)	227.2 (70.9%)	324.5 (42.9%)	508.9 (37.1%)	425.1 (44.7%)	1153.0 (64.1%)	885.0 (68.9%)
Navicula bicephala				9.0 (10.0%)		31.5 (9.8%)	81.8 (10.8%)	489.1 (35.6%)	133.7 (14.1%)		189.3 (14.7%)
Oscillatoria angusta									94.2 (9.9%)		
Synedra delicatissima		0.2 (12.5%)		26.3 (29.2%)	42.2 (32.2%)		126.6 (16.7%)	189.3 (13.8%)			
S. tenuissima		0.5 (31.3%)	12.2 (52.8%)	23.7 (26.3%)							
S. ulna v. *danica*			2.3 (10.0%)								
Tabellaria fenestrata		0.5 (31.3%)									
Total standing crops (10^3 cells · cm^{-2})	0	1.6	23.1	90.2	131.1	320.5	757.1	1372.7	951.0	1798.2	1284.1

decreasing. As on the outside of the mesocosm, the size of standing crops on unglazed ceramic tiles was higher than that at glass slides, and there was an initial time lapse. It was shorter than that required for the glass slides.

On the other hand, chlorophyll-*a* content outside the mesocosm had the highest value of 5.37 mg · m^{-2} on 19th June, 8 weeks after the experiment began and inside the mesocosm it reached the highest value of 24.40 mg · m^{-2} on 26th June, 9 weeks after the experiment began (Fig. 2).

Outside the mesocosm two major algal groups were dominant: Bacillariophyceae (diatoms) and Cyanophyta (bluegreen algae, mainly filamentous forms). The six diatom dominants outside the mesocosm were *Achnanthes minutissima, Navicula bicephala, Synedra delicatissima, Synedra tenuissima, Synedra ulna* v. *danica* and *Tabellaria fenestrata*, together with 1 cyanophyte species (*Oscillatoria angusta*). At the beginning of colonization outside the mesocosm, the araphid diatom *Synedra tenuissima* (31.3%) and *Tabellaria fenestrata* (31.3%) and *Synedra delicatissima* (12.5%) were major dominants. Subsquently *Synedra delicatissima* (29.2%) and *Synedra tenuissima* (26.3%) increased and also *Navicula bicephala* (10.0%) appeared. After this the monoraphid *Achnanthes minutissima* (48.9%) appeared and was co-dominant until the end of the experiment (Table 2). After the initial colonization, just *Achnanthes minutissima* (64.1%) was the major dominant, before that *Synedra tenuissima, Synedra delicatissima* and *Tabellaria fenestrata* were the most important species. In the later stages, *Achnanthes minutissima* and *Navicula bicephala* were the most important species (Fig. 3). Outside the mesocosm the diatoms still played an important role in the colonization of the substrata. However the dominants outside the mesocosm on glass slides at the same investigated site (Lee *et al.* 1998) were different. On glass slides, from the beginning to the end of experiment *Achnanthes minutissima* was the major dominant and at the initial colonization, *Oscillatoria* sp. was also abundant and also at the time of the highest standing crops *Cymbella japonica* was a major dominant.

Inside the mesocosm 7 species were important: 4 diatom species (*Achnanthes minutissima, Fragilaria crotonensis, Synedra delicatissima* and *Tabellaria flocculosa*), the green algae (*Coenochloris polycocca* and *Ulothrix subtilissima*) and the dinoflagellate (*Peridinium cinctum*). At the beginning of colonization, *Peridinium cinctum* (47.4%) *Tabellaria flocculosa* (21.1%) and *Synedra delicatissima* (13.2%) were the major dominants. After that, *Achnanthes minutissima* (22.3%) and *Coenochloris polycocca* (15.2%) appeared and at the same time, *Peridinium cinctum* and *Tabellaria flocculosa* disappeared (Table 3). The standing crops of *Synedra delicatissima* decreased steadily during the sampling period. On 5th June, 6 weeks after the experiment began, *Peridinium cinctum, Synedra delicatissima* and *Tabellaria flocculosa* had disappeared and their place was taken by *Achnanthes minutissima* and *Coenochloris polycocca* as the major

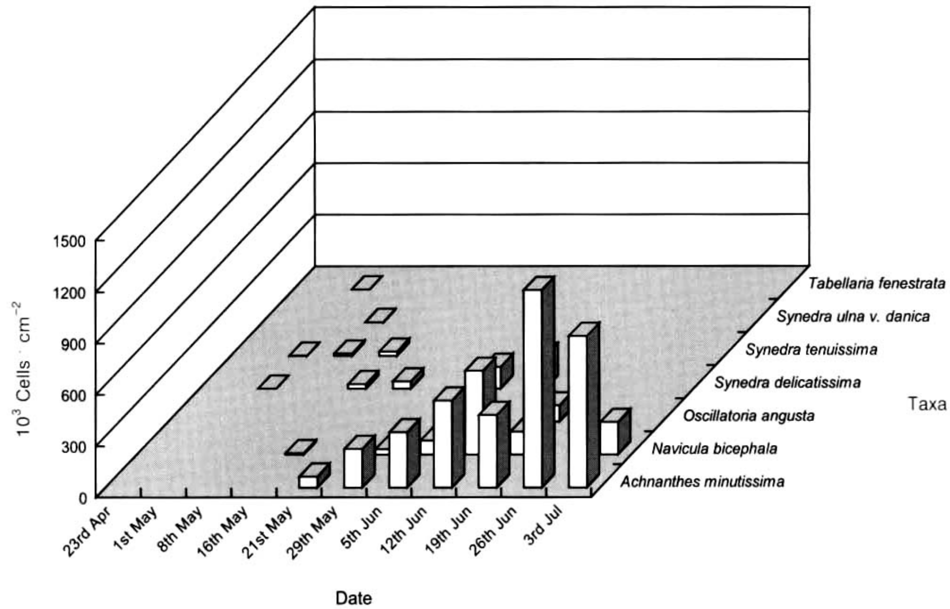

Fig. 3. The weekly variations of standing crops of dominants outside the mesocosm in Togyo reservoir from 23rd April to 3rd July 1999.

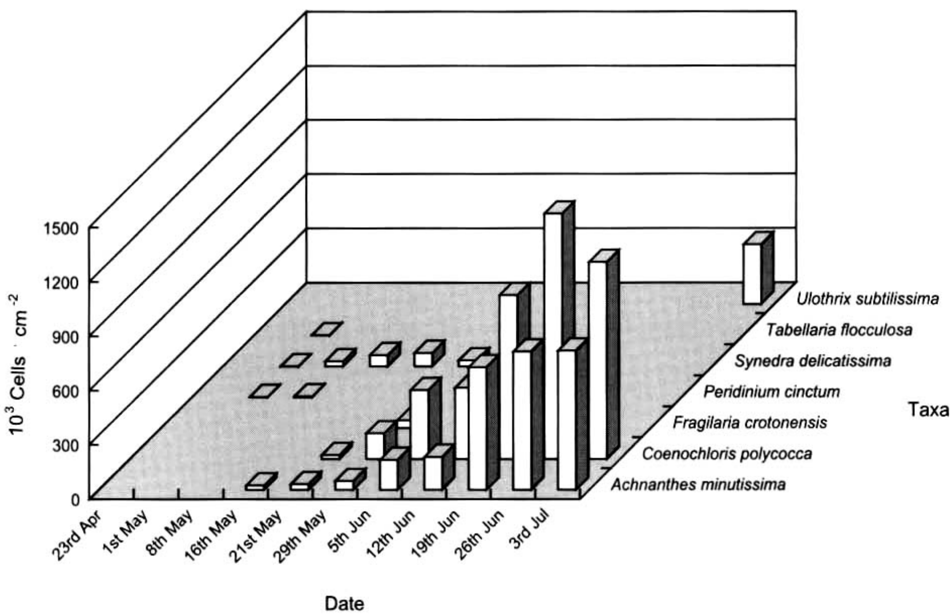

Fig. 4. The weekly variations of standing crops of dominants inside the mesocosm in Togyo reservoir from 23rd April to 3rd July 1999.

dominants until the end of the experiment (Fig. 4). However, the dominants inside of the mesocosm on glass slides at the same investigated site (Lee *et al.* 1998) were different. On glass slides at the initial colonization, *Asterionella formosa* and *Synedra fasciculata* played a role but at the time of colonization, *Coenochloris polycocca* and *Oedogonium species* were major dominants. Consequently substratum selection has played an important role in the structure of the epilithic algal community on both sides of mesocosm.

At the initial colonization, *Synedra tenuissima, Tabellaria fenestrata* and *Synedra delicatissima* played an important role outside the mesocosm but *Peridinium cinctum, Tabellaria flocculosa* and *Synedra tenuissima* occurred inside. At the time of initial colonization, *Achnanthes minutissima* and *Navicula bicephala* played an important role outside the mesocosm, but inside *Achnanthes minutissima* and *Coenochloris polycocca* were the important colonizers.

Table 3. The dominants in epilithic algal community inside the mesocosm in Togyo reservoir from 23rd April to 3rd July 1999

Date / Dominant species	April	May					June				July
	23rd	1st	8th	16th	21st	29th	5th	12th	19th	26th	3rd
Achnanthes minutissima					64.1	227.2	324.5	508.9	425.1	1153.0	885.0
Achnanthes minutissima				23.6 (22.3%)	31.3 (21.9%)	49.3 (17.7%)	165.6 (27.3%)	182.3 (29.1%)	678.0 (38.8%)	765.4 (32.0%)	769.0 (32.5%)
Coenochloris polycocca					21.7 (15.2%)	141.5 (50.9%)	381.0 (62.9%)	393.6 (62.8%)	904.0 (51.7%)	1353.6 (56.6%)	1087.5 (45.9%)
Fragilaria crotonensis						42.3	(15.2%)				
Peridinium cinctum		1.8 (47.4%)	5.0 (10.2%)								
Synedra delicatissima		0.5 (13.2%)	27.4 (56.0%)	61.5 (58.2%)	73.8 (51.7%)	32.6 (11.7%)					
Tabellaria flocculosa		0.8 (21.1%)									
Ulothrix subtilissima											330.1 (13.9%)
Total standing crops (10^3 cells·cm^{-2})	0	3.8	48.9	105.6	142.7	278.2	606.2	626.4	1749.5	2391.4	2367.3

Epilithic algal assemblages

The algal assemblages were dominated by diatoms, green and bluegreen algae. The total number of species observed was 114 species outside and inside the mesocosm (Lee and Yoon 2003). Throughout the investigation periods, *Achnanthes minutissima, Cymbella cymbiformis, Cymbella gracilis, Gomphonema acuminatum* and *Navicula bicephala* appeared very frequently outside the mesocosm: araphid diatoms were most diverse. Inside the mesocosm, *Coenochloris polycocca, Fragilaria crotonensis, Oedogonium* sp. and *Ulothrix subtilissima* were more common: colonial and filamentous forms of green algae were abundant rather than araphid diatoms. At the same investigation site, 125 species of the epipelic species were recorded (Lee *et al.* 1997) under natural sediment conditions and the epilithon on the glass slides was composed of 104 species outside the mesocosm and 92 species inside (Lee *et al.* 1998). Lam and Lei (1999) also reported 81 species on glass slides. Also in the Naktong River, Cho (1994) reported 109 species as epilithic diatoms at wood and acryl plate, and Eulin and Le Cohu (1998) reported 132 species growing on artificial substrate conditions in a river. Comparison between all the studies reveals little differences in species number observed. However, as compared with Lee *et al.* (1998), glass slides mainly supported loosely attached algae and colonial bluegreen and motile diatoms occurred frequently but on unglazed ceramic tiles epilithic algae were strongly attached as were adnate and filamentous forms as commented on by Cattaneo *et al.* (1997).

DISCUSSION

Clearly there are small differences depending on the choice of substrata. The size of standing crops and the time lapse for colonization differs between two; unglazed ceramic tiles were more suitable for colonization than glass slides. This confirms Antoine and Bensen-Evans (1985) finding that a rough surface was particularly favorable for algal colonization. Consequently substratum selection may have played not only a limited role in the structure of the epilithic algal community in differences with Blinn *et al.* (1980).

The seasonal variations of standing crops on unglazed ceramic tiles and glass slides was quite similar but ceramic tiles yielded larger population, and the time lapse needed for colonization was shorter on tiles. However, the time lapse on glass slides was 4 weeks (Lam and Lei 1999), the same time as regarded for the recovery of algal colonization on new rock surface (Stock and Ward 1989). Blinn *et al.* (1980), Sabater *et al.* (1998) and Cho (1994) recorded time lapse for on various substratas ranging between 3 and 5 weeks.

The results of this investigation confirm Stevenson and Peterson's (1989) proposal that araphid diatoms with relatively large size were good pioneer colonists, while monoraphid and biraphid diatoms tend to be more successful during later stages of colonization. Algae are not the initial colonizers as reported by Stock and Ward (1989). Primary colonization is by bacteria and this is followed by the development of a monolayer of adnate diatoms, and after the diatom monolayer, filamentous algae develop. Cho (1994) also reported *Melosira italica* and *Achnanthes minutissima* as major dominants and Blinn *et al.* (1980) recorded *Nitzschia frustulum* and *Epithemia sorex* as major dominants.

Achnanthes minutissima is a major taxon in this community as already reported by Kim *et al.* (1992) and Stock and Ward (1989) on natural substrates in the stream bed. On artificial substrata, Korte and Blinn (1983) recorded *Achnanthes minutissima* and *Cocconeis placentula* v. *euglypta* as early colonizers and plexiglass was more suitable for colonization than aluminum stubs. On the other hand, Cho (1994) reported yellow brown unicellular algae and filamentous bluegreens (*Lyngbya* sp. and *Phormidium* sp.) as the initial colonizers on artificial substrata, and Acs and Kiss (1993) also reported relatively large araphid and biraphid diatoms as initial colonizers on sand blasted glass slides. *Achnanthes* spp. were also reported as the most abundant taxonomic group on Douglas fir wood and artificial rock tile substrata by Sabater *et al.* (1988) and by Eulin and Le Cohu (1998) on artificial substrata and natural substrata. But *Coenochloris polycocca* was the most abundant species inside the mesocosm.

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