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Replacement of Chroococcales and Nostocales by Oscillatoriales caused a significant increase in microcystin concentrations in a dam reservoir

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Abstract

A two-year study on cyanobacterial development and the dynamics of intracellular microcystins was carried out in a shallow dam reservoir. Potentially toxic cyanobacteria (*Microcystis wesenbergii*, *M. aeruginosa*, *Woronichinia naegeliana*, *Anabaena* spp., *Planktothrix agardhii*) were observed to be the main component (70-94% total biomass) of the phytoplankton community, in which species composition was unstable and was very different between the 2005 and 2006 summer seasons. Generally, total phytoplankton, cyanobacterial biomass and total microcystin (MC) concentrations in the reservoir were much higher in 2006 than in 2005. The

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highest MC concentration (173.8 µg MC-LR equivalent dm^{-3}) was seen in 2006 during *P. agardhii* (Oscillatoriales) domination (max. fresh biomass 50.3 mg dm^{-3}; above 91% of phytoplankton biomass). Positive correlations between microcystin concentrations and cyanobacterial biomass suggest that populations of *Nostocales* and *Oscillatoriales* in 2005 and *Oscillatoriales* (*P. agardhii*) in 2006 may have been the main producers of MCs in the reservoir. The strong increase in *P. agardhii* biomass concomitant with a decrease in the total biomass of *Chroococcales* and *Nostocales* was responsible for the increase in MC concentration in the Siemianówka dam reservoir.

INTRODUCTION

Microcystins (MCs) are hepatotoxic cyanobacterial metabolites (heptapeptides) that occur in about 90 different isoforms (Welker and Van Dohren 2006) and are synthesized by different taxa of cyanobacteria such as *Microcystis*, *Woronichinia*, *Anabaena* and *Planktothrix* among others (Pawlik-Skowrońska et al. 2004, Mbedi et al. 2005**,** Willame et al. 2005, Burchardt and Pawlik-Skowrońska 2005, Mazur-Marzec 2006). MCs have been most recorded in eutrophic lakes but have also been reported in dystrophic and oligotrophic ones (Lindholm et al. 2003). During cyanobacterial bloom formation MCs may or may not be detected in aquatic ecosystems (Willén and Mattsson 1997, Willame et al. 2005) depending on the presence or absence of strains that possess the ability to produce the toxins (Christiansen et al. 2006). In addition, environmental conditions that are optimal for growth of cyanobacteria may not be optimal for cyanotoxin production (Kotak et al. 2000).

The aim of this study is to examine both the mass development of potentially toxic cyanobacteria and the production of MCs in the eutrophicated Siemianówka dam reservoir.

MATERIALS AND METHODS

Study area, water sampling and analysis

The Siemianówka Dam Reservoir (SDR), which is located on the upper Narew River (NE Poland), is a shallow, eutrophic reservoir constructed in 1990 as a multifunctional impoundment. Since 1992 the development of potentially toxic cyanobacterial blooms has been a regular phenomenon in the reservoir (Grabowska 2005, 2006).

Surface water (0 – 0.5 m) samples were collected monthly from 2-3 stations on SDR from July – October 2005 and 2006.

Water temperature, pH and conductivity were measured in the field by means of a Hydrolab DataSonde 4 (USA). Transparency was determined via visibility of a Secchi Disk (SD). Physico-chemical characteristics of the water body are presented in Tables 1 and 2.

Table 1

Physico-chemical characteristics of Siemianówka dam reservoir water in 2005; range (above line) and average value (below line); n – number of samples.

Parameter	**2005**				
	11 July n=3	9 August n=2	12 September n=2	12 October n=1	**average** n=8
Temperature [°C]	23.0-24.9 24.1	18.6-19.0 18.8	21.3-21.5 21.4	13.2	**20.7**
pH	8.3-8.8 8.6	8.0-8.1 8.1	8.9 -9.0 8.9	8.4	**8.5**
Conductivity [µS cm^{-1}]	273.7-288.2 280.3	273.0-274.0 273.5	285.0-279.0 282.0	296.0	**281.0**
Transparency [m]	0.60-0.70 0.65	0.48-0.65 0.49	0.50-0.60 0.50	0.72	**0.59**

Table 2

Physico-chemical characteristics of Siemianówka dam reservoir water in 2006; range (above line) and average value (below line); n – number of samples.

Parameter	**2006**				
	10 July n=2	11 August n=3	28 September n=2	20 October n=2	**average** n=9
Temperature [C]	28.0-28.8 28.4	22.6-23.5 23.1	19.5-20.6 20.1	10.3-10.5 10.4	**20.8**
pH	9.0-9.1 9.1	7.2-7.7 7.5	7.7-7.9 7.8	8.3-8.4 8.4	**8.1**
Conductivity [µS cm^{-1}]	224.3-229.0 226.7	235.0-250.0 242.5	213.0-250.0 231.5	274.0-282.0 278.0	**244.6**
Transparency [m]	0.60-0.73 0.67	0.25-0.40 0.33	0.40-0.50 0.45	0.45 0.45	**0.46**

Cyanobacterial species were identified and counted using a Fuchs-Rosenthal chamber and an Olympus BX-50 microscope. The biomass of the algae was determined using a method of calculating the volume of cells based on the author's own measurements. Dry weight of phytoplankton samples was determined on Whatman GF/C filters dried overnight at 90ºC.

Determination of intracellular microcystins

Due to the high variety of different forms of MCs (Carmichael 1992) their total concentration was determined using gas chromatography/mass spectrometry (GC/MS, Varian) according to Kaya and Sano (1999). Phytoplankton from the surface water samples (400 cm^3) were fixed with NaN_3 and concentrated on Whatman GF/C filters to a volume of 2 cm^3 by means of vacuum pump. The concentrated samples were kept at –20°C prior to extraction. Extracts of the phytoplankton, in 75% methanol (pure *p.a.*), were prepared using ultrasonication (3-times for 5 min., 50W, ultrasonic homogenizer Sonoplus, Bandelin). Sub-samples of the obtained extracts were evaporated under vacuum at 40°C, oxidized for 4 hours with 99.8% $NaIO_4$ and 0.024 M $KMnO_4$, extracted with n-hexane and derivatized with 12% BF_3 according to Kaya and Sano (1999**)** and Tsuji et al. (2001). As a modification phenylbutyric acid was used as internal standard after the oxidation step. For MC identification electronimpact ionization mode was used (scan mass range 60-245). Quantification was based on 91, 131 and 190 m/z ions, characteristic for Adda derivatives. Adda is a specific amino acid that is characteristic of all MC iso-forms. The calibration curve was made with standard MC-LR (Calbiochem). Total MC concentrations were expressed as µg equivalent MC-LR either per dm^3 of lake water, per g dry weight of phytoplankton (DW) or per mg of cyanobacterial fresh biomass (CB).

RESULTS

Environmental conditions influence the development of phytoplankton in surface waters. In the SDR the physico-chemical characteristics of the water were similar across the studied periods except for the water temperature, which in July-August 2006 was approximately by 4-5ºC higher than in 2005 (Tables 1 and 2). Total phytoplankton biomass (PB) in the reservoir was usually above 30 mg dm^3 (Tables 3 and 4). Potentially toxic cyanobacteria were the main component of the phytoplankton community, and their average shares in the biomass were 78.6% and 87.4% in 2005 and 2006 respectively. Cyanobacteria from three orders: *Chroococcales*, *Oscillatoriales* and *Nostocales* were the major components of the PB (Fig. 1). Both the total biomass of phytoplankton and that of potentially toxic cyanobacteria were much higher in 2006 than in 2005 (Tables 3 and 4), although the species composition of cyanobacterial phytoplankton was very variable.

In July - October 2005 the cyanobacteria had only a relatively low biomass (Fig. 1, Table 3). From July to August 2005, distinct domination by *Chroococcales* (*Microcystis* spp., *Woronichinia naegeliana* (Ung.) Elenk.) was observed (Fig. 1), however, in September of that year a rapid increase in the abundance of *P. agardhii* (Gom.) Anagn. et Kom. (*Oscillatoriales*), concomitant with some increase in the abundance of *Nostocales*, occurred. From July to October 2006, members of *Oscillatoriales* dominated the PB, with

Table 3

Seasonal changes in phytoplankton biomass and total microcystin (MC) concentrations in Siemianówka dam reservoir in 2005; DW – dry weight of phytoplankton (g); CB – cyanobacteria fresh biomass (mg dm^{-3}); range (above line) and average value (below line); n – number of samples; n.d. – not determined.

Parameter	2005				
	11 July n=3	9 August n=2	12 September n=2	12 October n=1	**average** n=7 or 8
Total phytoplankton biomass [mg dm^{-3}]	18.16-40.06 30.80	26.17-35.37 30.77	22.02-49.07 35.55	19.30	**30.54**
Cyanobacterial biomass [mg dm^{-3}]	15.98-31.01 25.65	20.25-23.5 21.88	20.25-39.61 29.93	11.56	**24.02**
MC concentration in reservoir water [µg equiv. MC-LR dm^{-3}]	6.5-8.4 7.2	8.2-10.9 9.6	8.2-11.9 11.6	n.d.	**9.1**
MC content in phytoplankton biomass [µg equiv. MC-LR g^{-1}DW]	354-639 463	572-576 574	433-971 702	n.d.	**563**
MC content in cyanobacteria biomass [µg equiv. MC-LR mg^{-1}CB]	0.210-0.413 0.301	0.405-0.464 0.435	0.283-0.588 0.436	n.d.	**0.377**

Table 4

Seasonal changes in phytoplankton biomass and total microcystin (MC) concentrations in Siemianówka dam reservoir in 2006; DW – dry weight of phytoplankton (g); CB – cyanobacteria fresh biomass (mg dm^{-3}); range (above line) and average value (below line); n – number of samples.

Parameter	2006				
	10 July n=2	11 August n=3	28 September n=2	20 October n=2	**average** n=9
Total phytoplankton biomass [mg dm^{-3}]	41.51-45.67 43.59	39.44-65.67 54.32	50.35-62.51 56.43	55.03-57.02 56.03	**52.78**
Cyanobacterial biomass [mg dm^{-3}]	33.43-42.71 38.07	28.10-52.58 43.62	44.12-57.35 50.74	51.95-54.56 53.26	**46.11**
MC concentration in reservoir water [µg equiv. MC-LR dm^{-3}]	4.3-9.0 6.7	28.9-39.8 34.3	129.0-138.8 133.9	156.5-173.8 165.1	**79.4**
MC content in phytoplankton biomass [µg equiv. MC-LR g^{-1}DW]	213-600 407	1072-1447 1268	4626-4691 4658	6940-7827 7384	**3189**
MC content in cyanobacteria biomass [µg equiv. MC-LR mg^{-1}CB]	0.101-0.269 0.185	0.550-1.416 0.882	2.420-2.924 2.672	3.013-3.185 3.099	**1.618**

P. agardhii being the most abundant species (61.7% PB) (Fig. 1, Tab. 4). *Limnothrix redekei* (Van Goor) Meffert and *Pseudanabaena* spp. were less abundant (data not shown). In 2006 the total biomass of *Oscillatoriales* was eight times higher than that in 2005. The *Nostocales*, such as *Aphanizomenon* spp., *Anabaena flos-aquae* (Lyngb.) Breb. ex Born. et Flah., *A. affinis* Lemm.,

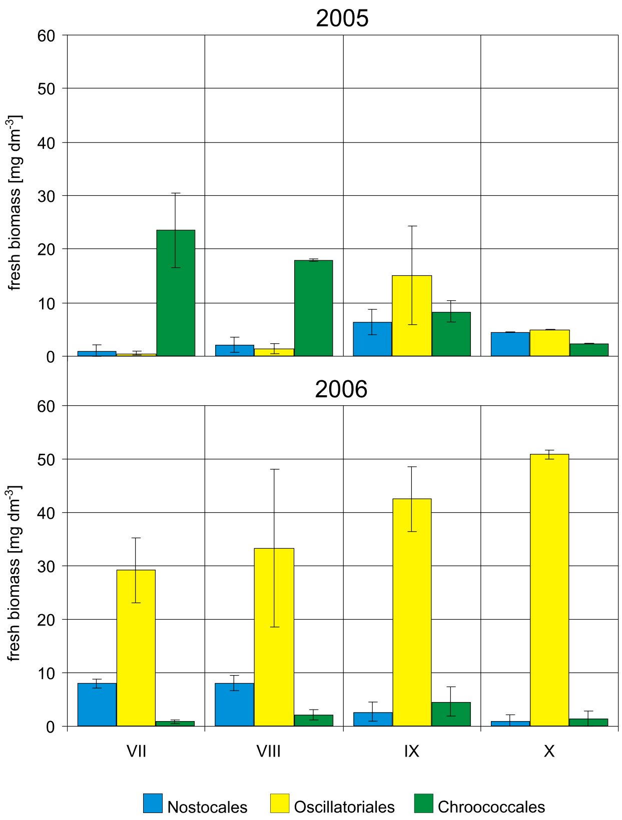

Fig. 1. Seasonal changes in biomass of dominant cyanobacteria in Siemianówka dam reservoir in 2005 and 2006.

A. spiroides Kleb. and *A. solitaria* Kleb., were observed in lower concentrations than *Oscillatoriales* in both years (Fig. 1). The potentially toxic *Microcystis* (*M. aeruginosa* (Kütz.) Kütz., *M. flos-aquae* (Wittr.) Kirchn., *Microcystis wesenbergii* (Kom.) Kom. in Kondr. and *Woronichinia naegeliana* were the main representatives of *Chroococcales* observed, but in 2006 their biomass was seven times lower than in 2005 (Fig. 1).

Cyanobacteria present in the SDR produced toxic MCs. Intracellular MCs were detected in the study seasons of both years in all analyzed water samples (Tables 3 and 4). Their total concentrations per unit water volume were distinctly lower in 2005 (average 9.1 μg MC-LR equiv. dm^{-3}) especially during *Chroococcales* domination, than in 2006 (average 79.4 μg MC-LR equiv. dm^{-3}) (Fig. 1 and 2, Tables 3 and 4). Also the MC content per unit dry mass of phytoplankton and fresh biomass of cyanobacteria were several times lower in 2005 than in 2006 (Tables 3 and 4). The highest positive correlations between total MC concentrations in the reservoir water and cyanobacterial biomass were observed in 2005 (Fig. 2) in the case of *Nostocales* (R^2 = 0.56) and *Oscillatoriales* (R^2 = 0.44), whereas in 2006 (Fig. 3) for *Oscillatoriales* (R^2 = 0.49) and especially with *P. agardhii* (R^2 = 0.63). The highest MC concentrations per unit water volume (up to 173.8 μg MC-LR equiv. dm^{-3}) and phytoplankton and cyanobacterial biomass (Table 4) were observed in October 2006 at water temperatures lower than 11°C (Table 2), when *P. agardhii* comprised more than 91% of phytoplankton biomass in the surface waters.

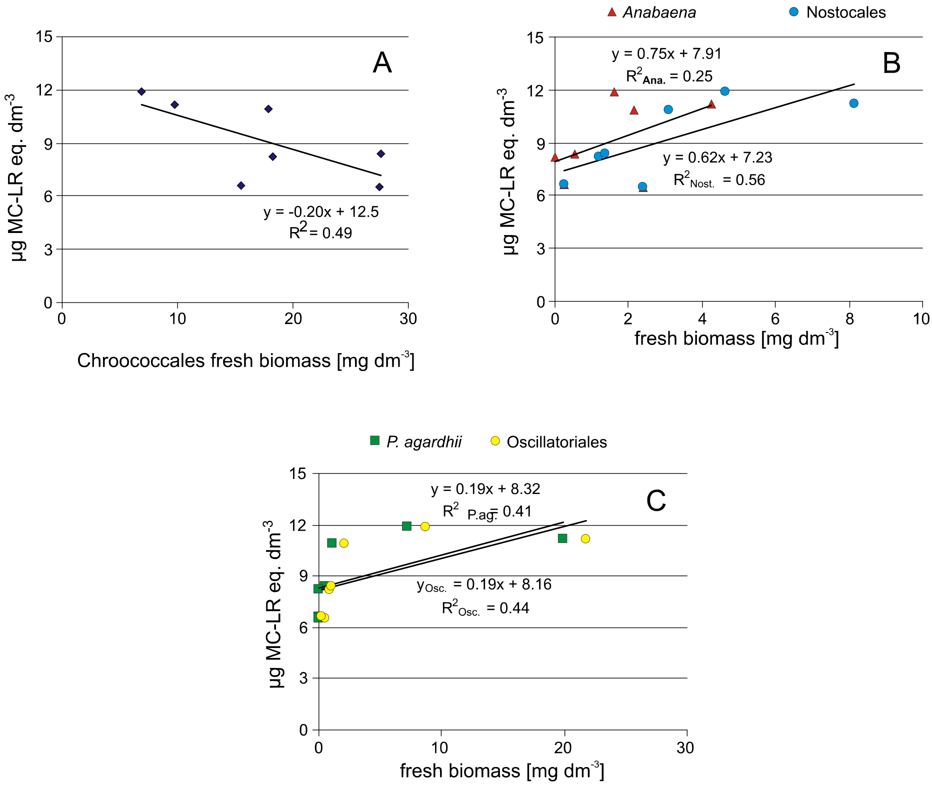

Fig. 2. Correlation between total microcystin concentration and fresh biomass of: *Chroococcales* (A), *Nostocales* (B), *Oscillatoriales* and *Planktothrix agardhii* (C), in Siemianówka dam reservoir in 2005.

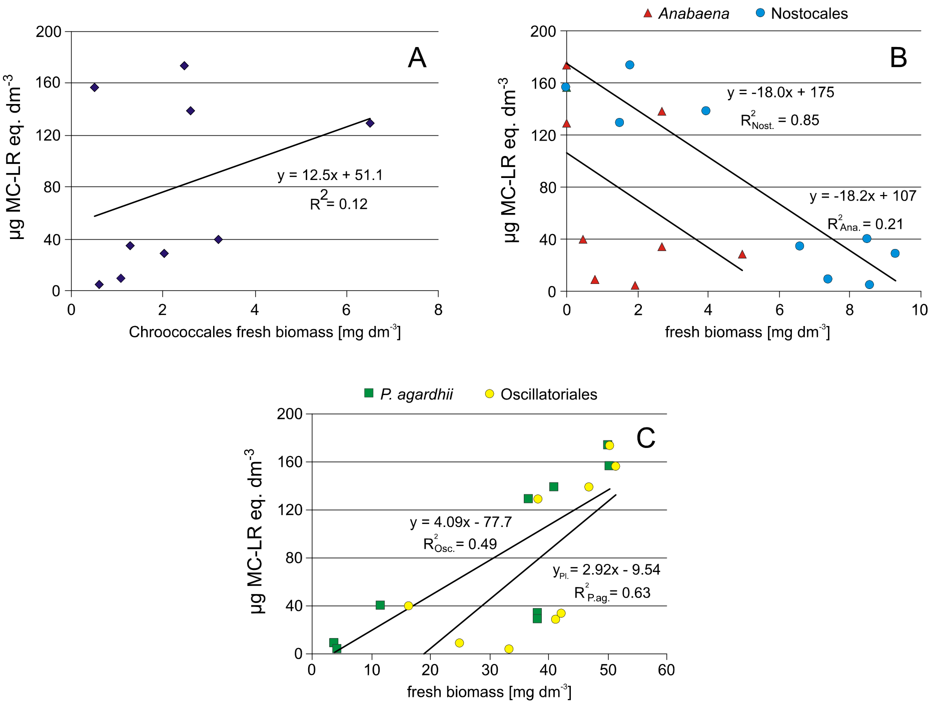

Fig. 3. Correlation between total microcystin concentration and fresh biomass of: *Chroococcales* (A), *Nostocales* (B), *Oscillatoriales* and *Planktothrix agardhii* (C) in Siemianówka dam reservoir in 2006.

DISCUSSION

Total concentrations of intracellular microcystins (max. 173.8 µg MC-LR equiv. dm^{-3} water; 3.185 µg MC-LR equiv. mg^{-1}CB or 7827 µg MC-LR equiv. g^{-1} DW) observed in the Siemianówka dam reservoir in 2005-6 were extremely high, much higher than those observed in the same reservoir in 1999 (i.e. max. 1062 µg g^{-1} DW), when *Microcystis wesenbergii* was the dominant potentially toxic cyanobacterium (Grabowska 2006). *M. wesenbergii* was previously reported as being a non-producer of MCs in Europe (Via-Ordorika et al. 2004). The concentrations of MCs observed in the SDR in 2005-6 are also higher than the values reported in other water-bodies. For example, Pawlik-Skowrońska et al. (2004) found 5.41 µg MC-LR equiv. dm^{-3} (in filtered water and seston biomass) in the Zemborzycki dam reservoir during cyanobacterial blooms dominated by *Nostocales* or *Oscillatoriales*, whereas Kabziński et al. (2000)

reported a maximum 595 μg MC-LR g^{-1} DW from three other dam reservoirs in Poland. In lakes from southern Belgium and Luxembourg MC presence was mainly assigned to *Microcystis* dominance, but the highest MC concentration, of 2231 μg g^{-1} seston DW, was recorded in a sample dominated by *Woronichinia naegeliana* (Willame et al. 2005). In contrast, in many eutrophic lakes with high abundances of cyanobacteria no MCs were seen (Lindholm et al. 2003). It is well known that different species of *Microcystis*, *Anabaena* and *Planktothrix* may exist both as MC producers, or non-producers if they lack the *mcy* genes encoding MC production (Kurmayer et al. 2004, Welker et al. 2004, Via-Ordorika et al. 2004, Mbedi et al. 2005). In populations of *P. agardhii* both the MC–producing and non-producing strains can coexist, and may be morphologically identical (Mbedi et al. 2005).

Our results suggest that in the SDR *P. agardhii*, the population of which increased considerably from 2005 to 2006, was the most likely and most prolific MC-producer. A similar situation has been observed in lakes in northern Germany where this species was the dominant taxon in water samples containing MCs (Willame et al. 2005) and in a hypertrophic lake (Poland) with perennial blooms of *P. agardhii* (Wiśniewska et al. 2007, Pawlik-Skowrońska et al. 2008). However, Willame et al. (2005) showed that other cyanobacterial species may also produce very high quantities of MCs. Not only different species of *Microcystis*, *Planktothrix* but also of *Anabaena* are often reported as prolific MC producers (Willén and Mattsson 1997, Lindholm et al. 2003). In this study in 2005 the highest positive correlation ($R^2 = 0.56$) was seen between MCs and the biomass of *Nostocales* in the SDR.

Increasing MC concentrations were observed from July to October, with the highest toxin concentrations being recorded in the SDR in the autumn, at lower water temperatures (between 10-20°C). A similar phenomenon was reported by Kotak et al. (2000) in Canadian lakes, and is in agreement with work by Sivonen (1990) showing that the most effective MC production by *P. agardhii* occurred at 15-25°C. It seems that at temperatures as low as 10-15°C neither MC synthesis is inhibited nor are *P. agardhii* blooms negatively impacted. For example, in a French eutrophic lake the maximum density of *P. agardhii* (4.5 10^6 trichomes dm^{-3}) was observed in winter (Briand et al. 2002). In our studies the highest MC content in cyanobacterial biomass (3185 μg mg^{-1} CB) was seen in October 2006. In comparison, *Anabaena* cell death reportedly already occurs in late summer (Sigee et al. 2007). The observed replacement, in 2006, of the dominant *Nostocales* by *Oscillatoriales* (mainly *P. agardhii*), increase in cyanobacterial biomass and parallel strong increase in MC concentration in the reservoir suggest that bloom-forming strains of *P. agardhii*, growing at low water temperatures, were the most effective MCs producers.

Despite the high concentrations of MCs present inside cyanobacterial cells, it is not obvious that equally high concentrations will be released into water, because some component of the cyanobacterial population may over-winter on lake sediments (Verspagen et al. 2004). MCs can be released into water after bloom decay but no evidence for losses from the intracellular MC pool could be found in intact cells of *Microcystis aeruginosa* (Rohrlack and Hyestrand 2006). A similar phenomenon may be true for *P. agardhii*. The results obtained suggest that *P. agardhii*, which dominated over other cyanobacteria in the second year of the study, is responsible for the observed increase in the intracellular MC concentration in the reservoir.

The seasonal fast and fundamental changes in the phytoplankton community composition observed in the Siemianówka dam reservoir appear to be disadvantageous, not only because the bloom decreases water quality, but also as a result of the increase in concentration of cyanotoxins that are harmful for living organisms.

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