EFFECT OF CRUDE OIL ON BIOFILMS PHOTOCHEMICAL EFFICIENCY

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ABSTRACT

Four separate mesocosm experiments (A-D) were carried out over a period of four months involving impactation of microalgal mats in cores at two levels of petroleum hydrocarbons (PHC) concentrations and nutrient enrichment (using nitrate and phosphate) at three levels of concentrations in the light and in the dark. The effect of periodic variation on the photochemical efficiency at the baseline indicated an initial decrease followed by a continuous increase to the 14th day. For the non-enriched but oil-spiked control, there was increase from the 3rd day to the 7th followed by a decrease thereafter to the 14th. At low and high oil and nutrient impactation, the same pattern was shown between the 3rd and 14th day except for the moderately high nitrate and low to high phosphate enrichments. But after correction for the percent baseline, the treatments that were above 100% baseline on the 3rd day were L I Np, L I N P and D I N p which were about 130% baseline value. At higher oil and nutrient enrichment, the only treatment which was above100% baseline value on the 3rd day was L II N 3P. Photochemical efficiency increased from 110, 105% on the 3rd day respectively to 130% on the 14th day. At higher oil impactation in the light after correction to baseline value photochemical efficiency increased from 110 on the 3rd day to about 115% of baseline

value on the 14th day at moderate nitrate enrichment and maximum phosphate enrichment. The measure of the photochemical efficiency clearly indicated that the microalgal forms were negatively affected from oil impactation. This could be due to the environmental and ecological state of the sediment. There is a consensus that PHC is a veritable retardant of photosynthetic activity. As observed, at low oil input, there was dependence on increased nitrate and low phosphate enrichment but as oil input was increased, there was commensurate requirement for more nitrate and phosphate. The probable recovery cascade of the photochemical efficiency from oil impairment appears to hinge on two pivots; balanced nutrient-enrichment and compensatory light effect.

KEY WORDS: Sediment, exopolysaccharide, petroleum hydrocarbon, estuary, biofilms, Pulse amplitude modulation fluorometry.

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1.0 MATERIALS AND METHODS

1.1. Photosynthetic efficiency

One of the most recent developments in the study of photosynthetic activity in the environment, is the estimation of active fluorescence as a means of determining photochemical quantum efficiency (or photochemical efficiency) and photosynthetic rates. Many different methods are available for this purpose: Fluorescence induction, pulse amplitude modulation (PAM)

fluorometry; pump and probe fluorometry (PPF); and fast repetition rate fluorometry (FRRF). Using the PAM method:

Photochemical efficiency = Fm – Fo / Fm (or Fv / Fm).

Where Fv = Fm – Fo or variable fluorescence

Fo = Ambient fluorescence pulse of photosystem II (trap 'open');

Fm = the saturation fluorescence pulse (Aiken, 2001).

Oil impactation of rivers and estuaries (especially from urban surface runoffs, have been known to cause chronic pollution especially in the wetlands (Koon and Jahns, 1992). Changes in fluorescence resulting from oil pollution could give rise to changes in photochemical efficiency (Mathis and Rutherford, 1994) hence serves as an indicator of stress in the microphytobenthos; such responses have been measured for plants (Bolhar-Nordenkrampf *et al.*, 1989).

1.2. Photosynthetic efficiency assessment

Photosynthetic efficiency for the mesocosm experiments was determined on the fresh core samples of mesocosm sediments by the PAM fluorescence assessment, using the Hansatech FMS portable fluorometer. PAM fluorescence allowed the no-invasive measurement of the efficiency of photosystem II. The optic fibre probe was set at 2 mm from the surface of the sediment core; this allowed the fluorescence signal to be used as a proxy for surface biomass. The cores were initially kept in the dark for 15 min before fluorescence was measured for dark adaptation followed by background fluorescence (or Fo) and the saturation fluorescence pulse (or Fm) measurement (Kromkamp et al., 1998; Underwood et al., 1999).

2.0 RESULTS

Photochemical efficiency is a measure of the biofilms photosynthetic capability. It is used in this study to ascertain the *fecundity* of the estuarine biofilms. Photochemical efficiency of the

estuarine biofilms is a direct measure of the effect PHC on primary production of the ecosystem. The baseline status of each mesocosm photochemical efficiency is shown in Fig 1; the effect of periodic variation after enrichment or its absence on oil spiked biofilms photochemical efficiency in the light and in the dark is as shown in Fig 2 while the percent correction to the baseline value is shown in Fig 3.

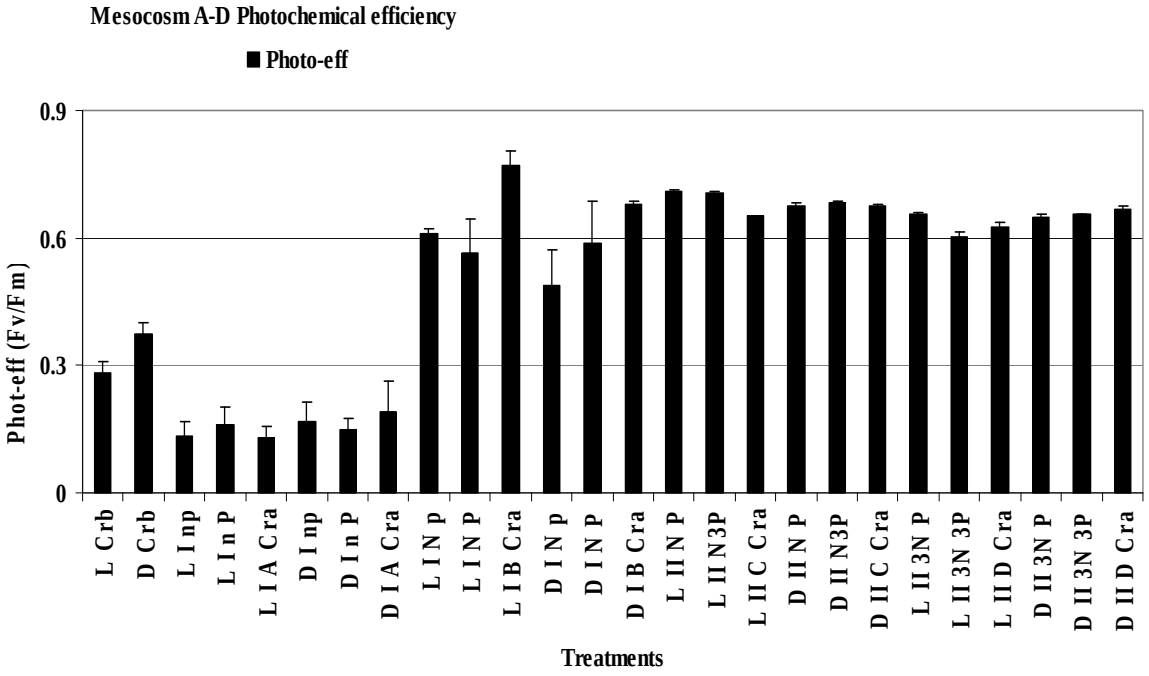

Fig 1. Baseline photochemical efficiency of mesocosms treatments

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;*

L Crb and *D Crb*: Baseline status of photochemical efficiency of mesocosms in the light and dark. (A, P=<0.05; B-D, P = <0.001); Mean ± SE, n = 3.

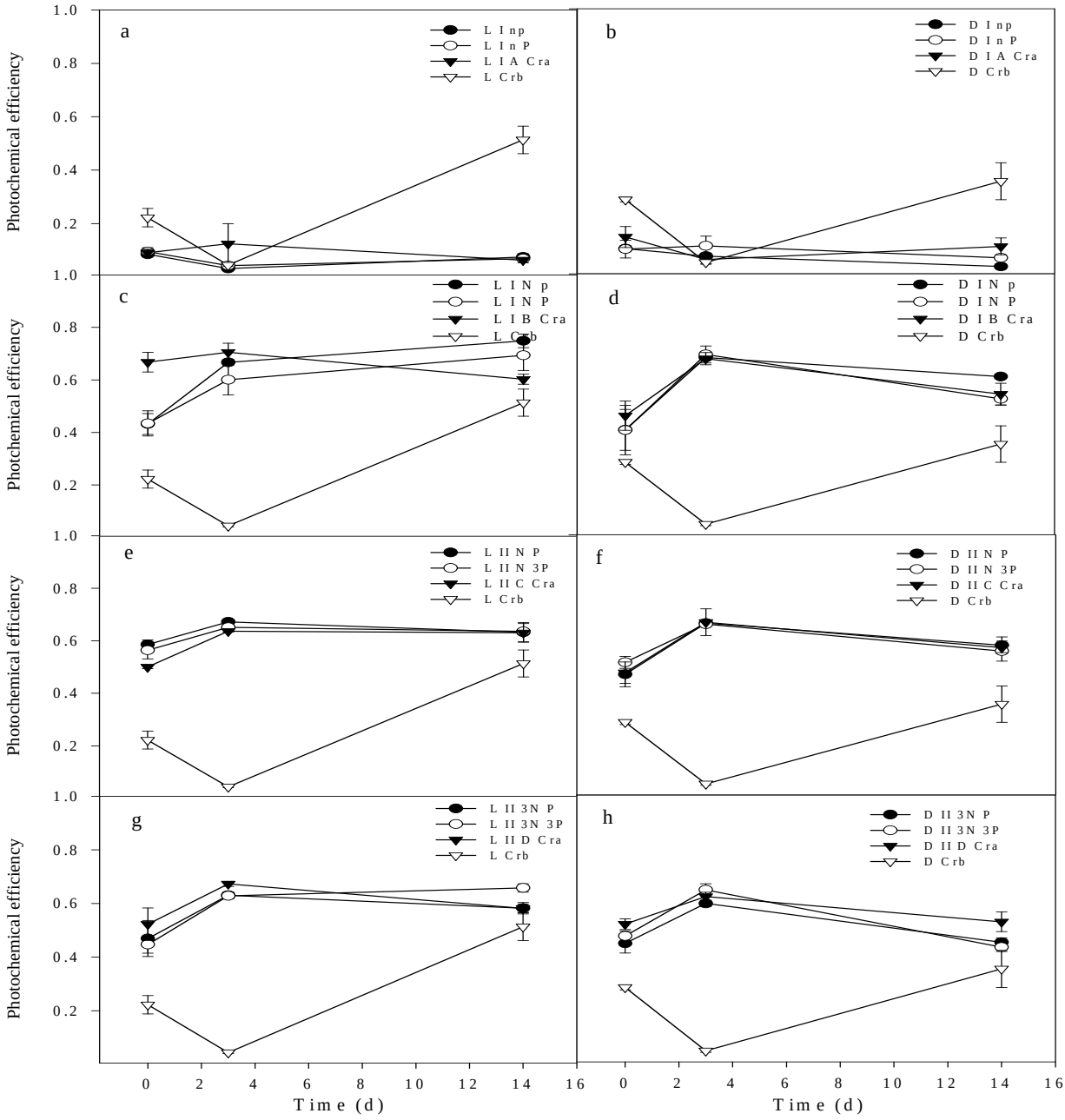

Fig 2. Effect of periodic variation on PHC effect on biofilm photochemical efficiency.

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;* Mean (± S.E; n = 3).

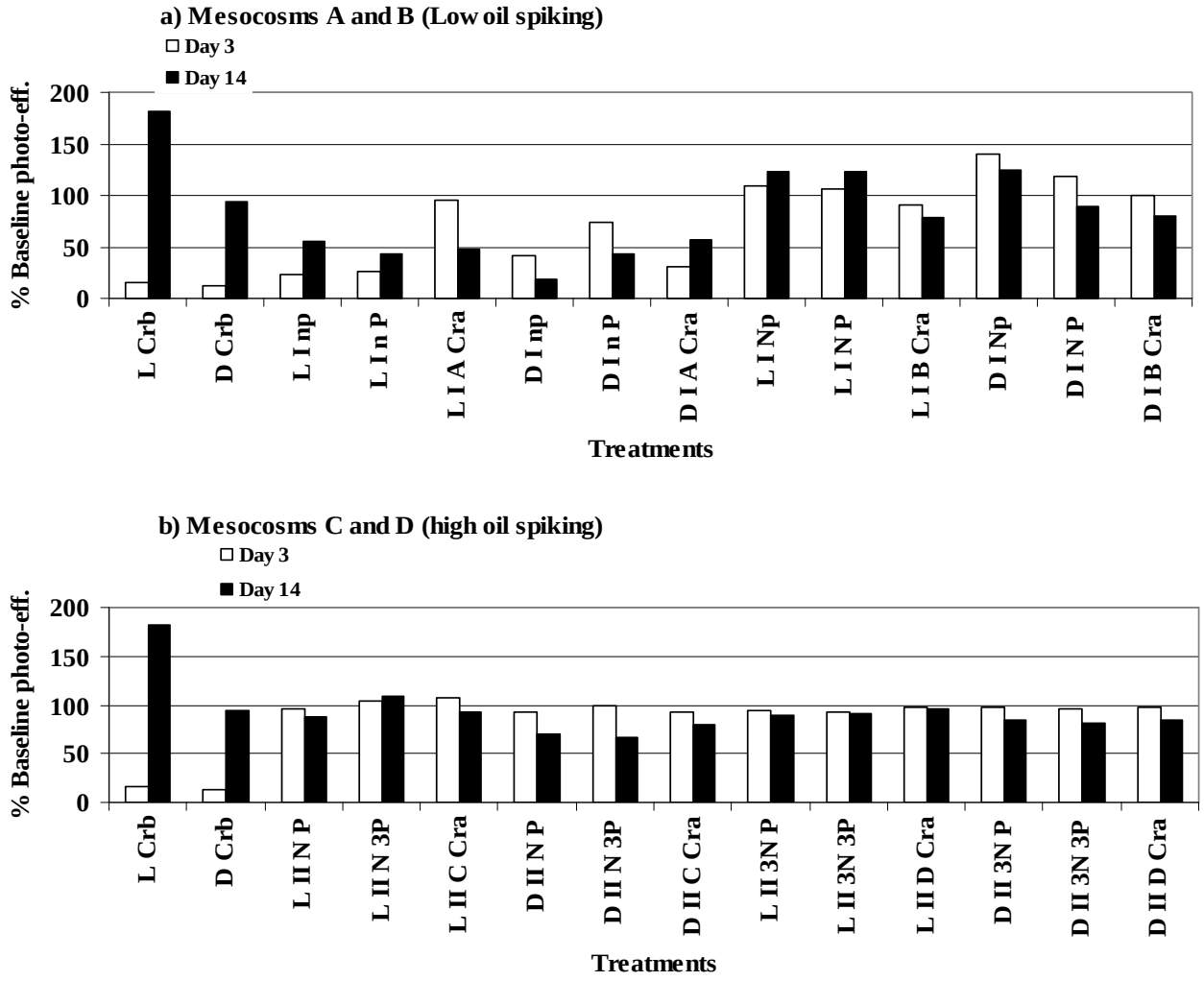

Fig 3. Percent baseline photochemical efficiency at a) low oil and b) high oil spiking:

Experiment A: *LInp; LInP; LIACra; DInp; DInP; DIACra;*

Experiment B: *LINp; LINP; LIBCra; DINp; DINP; DIBCra;*

Experiment C: *LIINP; LIIN3P; LIICCra; DIINP; DIIN3P; DIICCra;*

Experiment D: *LII3NP; LII3N3P; LIID Cra; DII3NP; DII3N3P; DIICCra;* Mean (± S.E; n = 3). Values were expressed as % photochemical efficiency corrected to the baseline for each treatment.

2.1. Mesocosm based Experiments were set up in four batches viz. A, B, C and D.

Treatments of low and high oil spiking (A & B; C & D respectively) and enrichment using nitrate and phosphate in three increasing regimes as shown in Table1. The baseline value of the mesocosms indicated that there were significant differences in the baseline photochemical efficiency in all the mesocosms (A, P =< 0.05; B-D, P = <0.001) as shown in Fig 1, thus the results were corrected as percentages of the baseline values (Fig 3).

2.2. Biofilms Response I & II (Crb & Cra)

The effect of periodic variation at low oil-spiking in the light and dark treatments in Experiment A (Fig 2 a & b), showed clear departure from the other experiments as the control, *Crb* was higher among the treatments from 0 hour to day 14 (P = <0.001). There was increase in photochemical efficiency of (0.045) from day 3 for the treatment L I A Cra in the light and for the treatment D I n p in the dark to (0.065) on day 7. From day 7, there was decrease to (0.02) photochemical efficiency to day 14. The photochemical efficiency in Experiment B (also of low oil spiking but with increased enrichment compared to Experiment A) was higher in all the other treatments in the light and in the dark than in the control *Crb* (ranging from 0.044 to 0.75 units); indicating increase from 0 h to day 3; but from day 7 to 14 there was gradual decrease to day 14 as shown in Fig 2 (c - h). While the baseline control *Crb* indicated the reverse in which there was increase in photochemical efficiency from day 7 to 14.

3.3. Biofilms Response III

After correction to the baseline value; the treatments *LINp, LINP* in the light, photochemical efficiency increased from 110, 105% on the 3rd day respectively for the treatments to 130% on the 14th day and the treatment *DINp,* decreased from 140 to 130% baseline value on the 14th day; photochemical efficiency ranging from 0.044 to 0.75 units. In all the mesocosms at day 3, there were turning points that marked the onset of decline in photochemical efficiency. At higher oil

impactation in the light the treatment *LIIN3P* (in mesocosm Fig 2 g) was significantly higher at day 14 after correction to baseline value. Photochemical efficiency increased from 110 on the 3rd day to about 115% of baseline value on the 14th day in this treatment.

4.0 DISCUSION

Mesocosm based Experiments were set up in four batches viz. A, B, C and D. Treatments of low and high oil spiking (A & B; C & D respectively) and enrichment using nitrate and phosphate in three increasing regimes. The result in Experiment A (Fig 2 a & b), showed clear departure from the other experiments as the control, *Crb* was higher among the treatments at 0 hour to day 14 ($P = <0.001$).

The measured photochemical efficiency in the first experimental setup clearly indicated a collapsed response in which the microalgal forms were grossly negatively affected by previous contamination due probably to environmental and ecological effects on the sediment which was collected for the first experimental setup. All the other experimental setups in the light and in the dark compared to the baseline, indicated higher overall photochemical efficiency from day 3 to day 14 (ranging from 0.044 to 0.75 units; $P = <0.001$). This was a clear indication that photosynthetic activity was progressively increased by increasing nutrient enrichment. Nevertheless, while all the other experimental setups in the light and in the dark compared to the baseline indicated gradual decline to day 14, the baseline assessment indicated the reverse i.e. increasing from day 3 to day 14. This indicated that in the baseline assessment the biofilms were showing recovery from the pristine contamination. After correction to percent baseline value; the treatments in the light of low oil impactation at moderate nitrate and minimum to moderate phosphate enrichment (i.e. *LINp, LINP)* indicated photochemical efficiency increases of 110, 105% on the 3rd day respectively to 130% on the 14th day. While the treatment of moderate nitrate and minimal phosphate in the dark decreased from 140 to 130% baseline value on the 14th day; photochemical efficiency ranging from 0.044 to

0.75 units. It was interesting to observe that the recorded increase on photochemical efficiency was only apparent in the dark in which photochemical efficiency unlike in the light was on the decrease from the 3rd to the 14th day.

At higher oil impactation in the light after correction to baseline value, the enrichment treatment of moderate nitrate and high phosphate indicated significantly higher photochemical efficiency at day 14. Photochemical efficiency increased from 110 on the 3rd day to about 115% of baseline value on the 14th day. From this point of view, it could be suggested that there was significant enhancement of photochemical efficiency probably only in the light.

There is a consensus that PHC is a veritable retardant of photosynthetic activity (Pritchard *et al.*, 1992; Wanchun and Kunshan, 2009). Moustakas and Ouzounidou (1994) made the observation in plants accordingly, that there were five pigment-associated levels in the photosynthetic apparatus (viz. the primary light reactions, the thylakoid electron-transport reactions, dark enzymatic stroma reactions and the regulatory feedback processes) at which point PHC interfered with chlorophyll fluorescence and caused changes in the photochemical efficiency. Borhar-Nordenkampf et al., (1989) indicated that the variable part of chlorophyll fluorescence occurs mainly in the photosystem-II and that excitation transfer to photosystem-I, may merely serve as an additional competitive pathway for de-excitation. The present study using microalgal forms showed that photochemical efficiency was progressively reduced as PHC concentration increased.

Despite the general decrease in the photochemical efficiency resulting from the spiked oil, there are clear indications that this very important physiological variable for the primary producers in the wetlands could be significantly enhanced in oil-polluted sites through *in situ* bioremediation. As observed, at low oil input, there was dependence on increased nitrate and low phosphate enrichment but as oil input was increased, there was commensurate requirement for more nitrate and much more phosphate.

The probable recovery cascade from added oil impairment appears to hinge on two pivots; balanced nutrient-enrichment and compensatory light effect. It was not clear if the observed laboratory results could be directly applicable in the field. There are a number of factors that could be responsible for the enhanced photochemical efficiency observed in the laboratory. Foremost is the effect of translocation of the cores of sediment from the site to the mesocosms in the greenhouse. This could result from increased fluorescence in the greenhouse in contrast to the estuarine ecosystem in which factors such as muddiness, shading from algal over-growth, turbulence from waves, variation in moisture, temperature, irradiation etc. could reduce fluorescence at the site. PHC, its composition, toxicity etc. together with its bioavailability in wetlands (Clark et al., 1997; Alexander, 1999) are complex issues. Since there was steady decrease in photochemical efficiency later at day 14, it was a clear indication that the initial increase at day 3 did not occlude the subsequent toxicity arising from PHC. A comparison with the non-oil impacted baseline treatment makes this to be clearer as there was a significant and continuous increase in photochemical efficiency even at 14^{th} day in the control. It was speculated that from this point of view, that there was the need for a further increase in the enrichment regime; provided it was still below amounts that could cause exacerbated eutrophication.

A summary of the effect of PHC on biofilms photochemical efficiency appear to suggest the following:

1. *That photochemical efficiency assessment showed the detrimental effect of PHC on photosynthetic state of the estuarine biofilms*
2. *That the effect of nutrient enrichment (especially of phosphate) in improving the photochemical efficiency was demonstrated.*
3. *That photochemical efficiency was more enhanced in the light than in the dark.*

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