Response of Haptera Growth to Different Frequencies of Light in Deep Water *Agarum fimbriatum* and Shallow Water *Alaria marginata*

Nirupam Nigam[1,2,3], Megan Dethier[1,3], Tom Mumford[1]

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[1]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA, 98250
[2]Department of Aquatic and Fisheries Sciences, University of Washington, Seattle, WA 98195
[3]Department of Biology, University of Washington, Seattle, WA 98195

Contact Information:
Nirupam Nigam
Department of Aquatic and Fisheries Sciences
1122 NE Boat St.
Seattle, WA 98105
njnigam@uw.edu

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Abstract

Light plays an important role in kelp ecology as an energy source and as an important kelp growth regulator. It induces a negative phototropic response in kelp haptera. Because longer wavelengths of light are available at shallower depths in additions to shorter wavelengths, it was hypothesized that kelp species generally inhabiting shallower water (e.g., *Alaria marginata*) would have a phototropic response to red (720-740 nm), green (520-540 nm), and blue (420-440 nm) light. Species generally inhabiting deeper water (*e.g., Agarum fimbriatum*) would have a stronger negative phototropic response in the blue wavelength of the light in comparison to *A. marginata* and other color treatments. Kelp haptera were isolated and exposed to unidirectional red, green, and blue light for 16 days. Controls were exposed to unidirectional sunlight or no light. The hypothesis was mostly confirmed. The blue light exposure showed the highest phototropic response in both species, but a higher relative response in *A. fimbriatum*. *Alaria marginata* haptera responded to all wavelengths of light. Green exposure produced the lowest response in all species Moreover, it was discovered that red light was responsible for greater growth of haptera in *A. marginata* than other treatments colors. The mechanism and ecological implications of red light-driven hapteral growth are opportunities for further research.

Introduction

Kelp are macroscopic, brown algae in the order Laminariales, that function as “ecosystem engineers” by creating sub-tidal kelp forest ecosystems in temperate seas (Jones et al. 1997; Steneck et al. 2002). With some species reaching lengths greater than twenty meters, kelp provide habitat for a large diversity of vertebrate and invertebrate species including pinnipeds, echinoderms, crustaceans, fish, and molluscs, as well as other algae (Steneck et al. 2002). Moreover, kelp forests are some of the most productive ecosystems in the world with primary productivity rates similar to those of tropical rainforests (Mann 1973). Much of this productivity is usually consumed in the form of detritus by sea urchins and other organisms, which are then consumed by higher trophic levels. This high productivity and habitat formation make kelp the mainstay of many fisheries and algin-based industries (Davis 2005; Leet et al. 2001).

Sunlight plays many roles in the lifecycle of kelp. It is the energy source for photosynthesis (Freeman 2014), and it regulates changes in kelp growth in the forms of polarity induction, phototaxis, and phototropism (Rico and Guiry 1996). Phototropism is a process in which plant tissue grows in response to light. It can be negative, when tissue grows away from a light source, or positive when tissue grows towards a light source. This response is initiated by photoreceptors, photo sensory proteins that regulate growth (Rockwell et a1. 2014) and occurs via the growth and elongation of meristematic tissue on one side of the plant (Iino 1990).

Phototropism is important for kelp orientation in the water column (Buggeln 1974). Negative phototropism has been demonstrated in kelp haptera, the rhizoid-like structures found in the holdfast that attach to substrate. Haptera grow away from light to find substrate (Buggeln 1974). Kelp haptera are most responsive to blue wavelengths of light (410-497 nm), moderately responsive to green (552 nm), and unresponsive to red (662 nm) (Buggeln 1974; Rico and Guiry 1996). Therefore, it is hypothesized that a blue-light photoreceptor is the trigger for phototropism in seaweeds (Rico and Guiry 1996).

However, recent genetic studies show that photoreceptors found in some kelps are able to sense reds, far-reds, oranges, and greens (Rockwell et al. 2014), suggesting that kelp may have a greater response to longer wavelengths of light than previously expected. Another genetic study by Wang et al. (2013) showed that red light is significantly more responsible for the regulation of gene tags in *Saccharina japonica* than blue light, thus playing a significant role in kelp gene expression.

Because water differentially absorbs light at depth, in clear water red wavelengths of light are only available down to 10 meters, whereas blue wavelengths are available down to 200 m (Kettle and Merchant 2008). This effect is compounded in the Strait of Georgia where the euphotic zone only ranges from 20-35 m of depth and light attenuation is significant (Masson and Pena 2009). This occurs due to the abundance of phytoplankton in the water column (Masson and Pena 2009). Thus longer wavelengths of light are lost at even shallower depths. I hypothesize that there could be a difference in negative phototropic responses to different wavelengths of light by haptera of kelps that are able to inhabit deeper ranges in the photic zone versus kelps that are able to inhabit shallow ranges. These differences could provide insight concerning the type or mix of photoreceptors used in phototropic responses by haptera. Specifically, I hypothesize that deeper-dwelling species are more likely to have a greater phototropic response to blue light in comparison with green or red light than shallow water species. Red light will cause little to no response in deeper kelps, but it will cause some response in shallow water kelps due to the availability of red light at shallow depths. Green light will cause an intermediate response in both types of kelp. These hypotheses were tested by exposing the haptera of *Alaria marginata* (a shallow subtidal and intertidal species) and *Agarum* *fimbriatum* (a deep subtidal species) to different wavelengths of light and measuring the phototropic response.

Materials and Methods

The experiment was conducted in two sea tables at the Friday Harbor Laboratories in Friday Harbor, WA during mid to late May, 2014. The design consisted of three experimental treatments and two control treatments for both *A. marginata* and *A. fimbriatum*. In the experimental treatments, the kelp haptera were exposed to unidirectional red (720-740 nm), green (520-540 nm) and blue (420-440 nm) light. The target wavelengths were achieved by filtering ambient sunlight. The haptera were exposed to ambient sunlight in one control group and no light in another control group. Each experimental treatment had three replicates from each species. Both of the control groups had one replicate from each species. Exposure lasted for 16 days.

Eleven specimens of *A. marginata* were collected from the tires on the floating dock of the Friday Harbor Laboratories in Friday Harbor, Washington in approximately 30 cm of depth. Because they were attached to a floating structure, the water depth remained constant on a tide cycle. Eleven specimens of *A. frimbriatum* were collected by scuba divers at approximately 12 m of depth MLLW at Brown Island, WA. *A. marginata* lives from the low intertidal zone to approximately 8 m of depth, and *A. fimbriatum* inhabits the subtidal to 20 m (Lamb and Hanby 2005). The blade of each specimen of *A. marginata* was cut to the dimensions of 20 cm x 6 cm for standardization. The blade of each specimen of *A. fimbriatum* was cut to the dimensions of 24 cm x 18 cm.

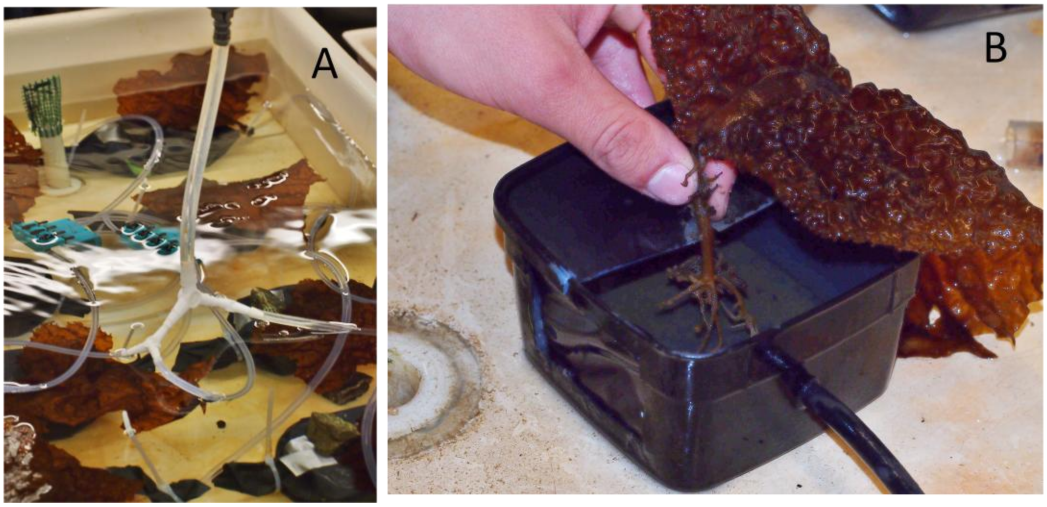

Figure 1. A. Dark boxes with *A. fimbriatum* in indoor sea table. B. Dark box (without black plastic) with *A. fimbriatum* after treatment

To isolate the haptera and expose them to unidirectional light, each was mounted in a “dark” box (Fig. 1). The stipe and blade of each thallus was exposed to normal ambient conditions in the sea tables. The primary structure of each box was an 8.5 cm by 6.5 cm Tupperware box. A hole was drilled in the center of each lid to secure the kelp stipes directly above the haptera. The lids were then cut in half to facilitate opening and closing the boxes. The boxes were plumbed to allow for seawater flow through each. A 5.5 by 4.5 cm rectangular color filter was inserted into a hole cut on a third side of each box except for the two no-light control boxes. The filters used were Roscolux #27 for the red light treatment (720-740 nm), Roscolux #90 for the green light treatment (520-540 nm), and Roscolux #381 for the blue light treatment (420-440 nm) (Rosco USA, Stamford, CT). Two clear, plastic rectangles were cut and glued to cover the rectangular holes in the two sunlight controls. Each box, lid, and outflow tube was painted black and wrapped in black plastic to prevent light penetration. A hole was cut in the black plastic overlaying the light filter to allow for the entry of unidirectional light into the box.

Each specimen was photographed before being placed in a box, and individual haptera tips were counted from photos. Each box containing *A. marginata* haptera was arranged randomly in an outdoor sea table. The *A. fimbriatum* boxes were randomly placed in an indoor sea table after all the *A. fimbriatum* died in an outdoor sea table (Fig. 1). The filters in every box were oriented to face south. Due to lack of flow in the outdoor sea table, water temperature ranged from 11 ^{o}C to 16 ^{o}C. Water flow through the indoor sea table kept the water temperature consistent at approximately 9-10 ^{o}C. The lighting in the indoor tables was natural light with the occasional overhead fluorescent lights turned on.

After the treatment, each specimen was removed and photographed. The holdfasts were removed from the blade and preserved in 70% ethanol. The orientation of the haptera in the box in relation of the window was noted when opening each box. In order to calculate the "% turn of haptera" for each specimen, the number and orientation of haptera apical tips after treatment was counted. Pre-treatment photos were analyzed in the same manner. The percent increase in haptera was determined for each specimen using the final and initial haptera numbers.

A log-linear regression was used to test the correlation between new haptera growth and the final percentage of negative phototropic curvature. Two-way ANOVAs were used to analyze the effect of color, species, and both color and species on the final percentage of haptera exhibiting phototropic curvature as well as the percent increase in haptera. One-way ANOVAs were used to test the significance between color treatments in the two separate species.

Results

Each specimen of *A. marginata* and *A. frimbriatum* exhibited new haptera growth for each of the experimental treatments and controls. Some positive correlation existed between the percent increase in number of haptera and the final percent of the haptera that exhibited a negative phototropic curvature. A log-linear regression test showed that 35% of the variation in the final percentage of the haptera exhibiting curvature could be explained by the variation in the percent increase in the number of haptera when both species were combined (P = 0.0091).

Figure 2. *Alaria marginata* (A-E) and *Agarum fimbriatum* haptera (F-J) after treatment. The negative phototropic orientation is to the right side of the image with treatment light exposures coming from the left. A. red light B. green light C. blue light D. sunlight E. no light F. red light G. green light H. blue light I. sunlight J. no light

Table 1. Results from two-way ANOVAs of final percentage of haptera exhibiting phototropism and the percent increase in the number of haptera

	Source	**Degrees of Freedom**	**F-Ratio**	**P-value**
Final % of Haptera Exhibiting Negative Phototropism	Species	1	515.90	<0.0001
	Color	2	6.82	0.0105
	Species * Color	2	1.60	0.2429
% Increase in # of Haptera	Species	1	10.59	0.0069
	Color	2	5.13	0.0245
	Species * Color	2	6.36	0.0131

Negative Phototropic Curvature

Negative phototropic curvature (orientation of haptera away from the light source) was observed in every specimen to varying degrees (Fig. 2). The percentage of haptera turned away from the light source before the treatment was compared with the percentage after the treatment with a one-way analysis of variance. The final percentage of haptera showing negative phototropic curvature was significantly greater than the initial percentage in *A. marginata* ($P < 0.05$). The final and initial percentages were not significantly different for *A. fimbriatum* ($P > 0.05$).

Mean final percentages of haptera that exhibited negative curvature differed significantly by species and by color of treatment. The interaction of species and color was not significant, indicating that the phototropic response to the treatments were consistent and comparable between species (Table 1). There was a higher mean percentage of haptera exhibiting negative curvature in *A. marginata* than in *A. fimbriatum* in the red, green, and blue treatments ($P < 0.0001$ for all color treatments).

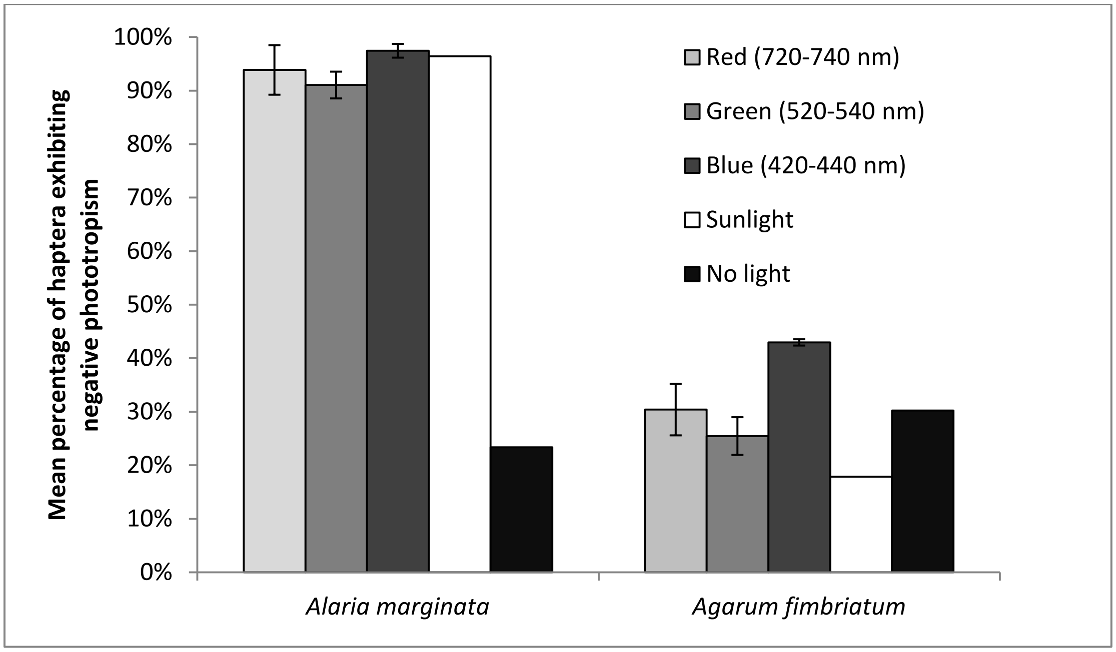

Figure 3. The mean percentage of haptera exhibiting negative phototropic curvature by treatment and species

Both species exhibited similar responses to the color treatments (Fig. 3). Haptera exposed to blue light responded the most, those exposed to green light responded the least, and those exposed to red light responded intermediately. The sunlight control had a greater response than the no light control in *A. marginata* haptera, but the reverse happened in *A. fimbriatum*. A one way analysis of variance showed that phototropic response to blue was significantly higher than green (P = 0.0028) and red (P = 0.0197) in *A. fimbriatum*.

Haptera Growth

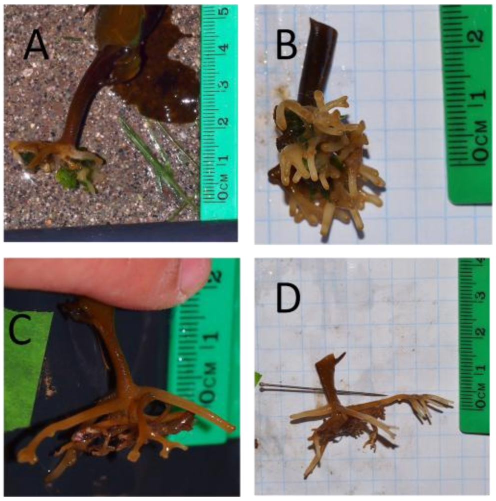

Figure 4. The increase in haptera number before and after treatment. The negative phototropic orientation is to the right side of the image with treatment light exposures coming from the left. A. *Alaria marginata* before red treatment B. *Alaria marginata* after treatment C. *Agarum fimbriatum* before red treatment D. *Agarum fimbriatum* after red treatment

An increase in the number of haptera was observed in each specimen (Fig. 4). A one-way ANOVA indicated that there was significant increase of the number of haptera in *A. marginata* ($P < 0.05$) but not in *A. fibriatum* ($P > 0.05$). The percent increase in number of haptera differed significantly by species and by color of treatment. The species and color interactions were significant, thus species must be analyzed separately (Table 1).

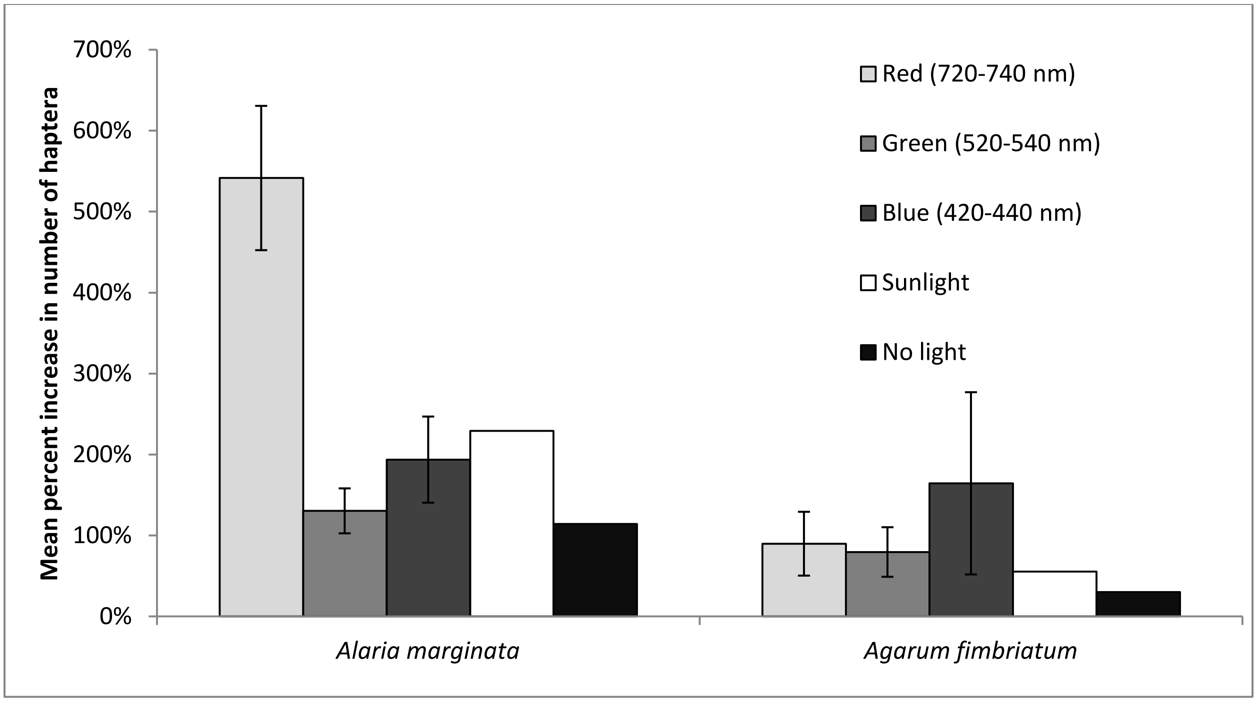

Figure 5. The mean percent increase in the number of haptera by treatment and species

In *A. marginata*, the percent increase in haptera in the red treatment is significantly larger than the percent increase in the green treatment (P = 0.0009) and the blue treatment (P = 0.0031). The sunlight control has the second highest percent increase, and the increase in haptera is slightly larger in blue than green. In *A. fimbriatum*, there is a slightly larger percent increase in the growth of haptera in blue compared to green and red, which had similar increases in growth (Fig. 5).

Discussion

The observation of significant negative phototropism in the haptera of both *A. marginata* and *A. fimbriatum* is consistent with Buggeln's (1974) findings of negative phototropism in *A. marginata*. However, *A. marginata* showed a significant increase in the percent of haptera that exhibited negative curvature at all color treatments, contradicting the findings of Buggeln’s study that suggested a lack of response to red treatment. It is possible that Buggeln did not conduct his study for a long enough time to show a definitive red response. However, these results confirm the findings of the recent genetic study by Rockwell et. al (2014) that showed the presence of multiple color photoreceptors in marine algae. Furthermore, there was no significant difference in the final percent of negative curvature between color treatments in *A. marginata* (Fig. 3), although there was a trend towards a greater response with blue light. Use of more replicates might have clarified these comparisons. The new growth of the haptera was responsible for some of the variance in the final percent of negative curvature. This suggests that new growth exhibits phototropic affects as well and renders comparisons between initial percent of curvature and final percent of curvature difficult.

The blue treatment in *A. fimbriatum* had a significantly higher percent of negative curvature in comparison to the red and green treatments, supporting the hypothesized response of deep-dwelling kelp. The blue treatment in *A. fimbriatum* may have had the largest phototropic effect due to the increased importance of blue photoreceptors in deep-dwelling kelp species. This increased importance would be caused by the lack of longer wavelengths of light at deeper depths. Likewise, the equal importance of all color treatments in *A. marginata* could indicate that red, green, and blue light have equal importance in inducing phototropic response due to the availability of longer wavelengths of light at shallower depths. Curiously, green light induced the smallest phototropic response, suggesting a smaller availability of green light photoreceptors in haptera (Fig. 3).

The deaths of the A. fimbriatum treatments due to light-stress in the outdoor sea table as well as the smaller percentage of negative curvature may be explained by the drastic decrease in light intensity at depth. Light measurements made in mid June of 2010 by the summer marine botany class off the dock at FHL showed that at -11 to -12m MLLW there was about 1.3% of surface light intensity (T. Mumford, pers. com). The depth of the water in the outdoor sea table was approximately 30 cm, which had significantly more light than at natural habitat depths. The indoor sea tables had lower light intensities, but this may have caused slower curvature and growth rates in *A. fimbriatum*.

Unexpected results arose from the analysis of haptera growth rates, providing novel insight into the mechanisms driving haptera growth. Although there was no significant increase in the number of haptera in *A. fimbriatum*, there was a significant increase in *A. marginata*. This could be a product of *A. marginata* growing quicker due to a higher accessibility of light in the outdoor sea tables as well as a difference in the growth rates between the two species. The increase of haptera number in specimens of *A. marginata* undergoing red light treatment was significantly greater than other color treatments (Fig. 5). Thus, red light could play an important role of instigating the growth of haptera in kelps living at shallow depths. *A. marginata* may have evolved this growth response as a result of the relatively large presence of red light at shallow depths. This would be beneficial to fitness because the presence of more haptera could increase the strength of attachment to the substrate. The genetic study by Wang et al. (2013) supports the hypothesis of the existence of a red light driven mechanism for morphological change by showing that red light is a significant inducer of kelp gene expression. The lack of growth response to red light in *A. fimbriatum* suggests the absence of the mechanism. This is expected as deep water kelps do not live in the presence of red light and would not have a red light driven mechanism. The red light driven mechanism could have ecological effects on kelp forests if the effect of light attenuation fluctuates. For example, a recent unpublished study by Besso (2014) of the Elwha river plume showed that river plumes attenuate light and are partially responsible for the decrease in kelp density around plumes. If the availability of red light decreases at shallow depths in response to river plumes, the growth of haptera and kelp attachment could be affected. A study should be conducted on the affects of plumes on haptera growth to understand these ecological implications.

This study would have benefited from more replicates, especially in the controls. Moreover, phototropic response in a larger variety of kelp species and wavelengths of light may be tested. Further study is needed to explore the mechanism, availability, and ecological function of red light driven haptera growth.

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