A dual dye approach to measuring sunlight in lotic systems

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Abstract

Light is an energy source that affects the metabolism of an aquatic system by providing energy for photosynthesis, the thermal structure by the transfer of light to heat energy, and the chemical make-up by providing energy for both indirect and direct photochemical reactions. This thesis considers the use of two dyes (rhodamine WT and fluorescein) as a way to measure the amount of light affecting lotic systems. These dyes are common tracers usually used in aquatic systems to measure water flow and mixing. Rhodamine WT exhibits photo-stability; while fluorescein exhibits photo-lability. Combining these two could potentially provide a Lagrangian measure of sunlight exposure in a lotic system; a previously unattainable view of the light field in such systems.

In this thesis, several lab experiments were performed to test the effects of temperature and pH on the fluorescence of the dyes and to test the effects of pH and wavelength of light on the photoresponse of the dyes upon irradiation. A correction factor for temperature was found in lab experiments that matched previously published results. When pH remained within the normal range of North Shore streams (6.99-7.54), rhodamine WT exhibited photo-stability while fluorescein exhibited photo-lability; however, changes in response were seen as a function of pH and there are indications that rhodamine WT may be photo-labile at high pH. Irradiation of the dyes showed that wavelengths at and/or above 420 nm degrade the fluorescein.

The dual-dye approach was tested in Amity Creek, a designated trout stream located on the north shore of Lake Superior. During deployment along a given reach, the dyes showed robust applicability on base flow, high irradiance days but no appreciable photoresponse on high flow days. During base flow deployments, the fluorescein to rhodamine WT ratio decreased linearly over time (with an R^2 ranging from 0.971-0.998). Overall, in-stream Lagrangian deployments showed similar degradation rates to those seen in batch samples of stream water placed on the stream bed in a fixed position in sunlight for the same period of time. This concurs with visual observations that there is no significant canopy cover down this stream reach. Although the dual-dye methodology was successful in acting as an *in situ* light monitor and is applicable to streams if temperature is recorded and pH is within a certain operating range, more work needs to be done to demonstrate if the use of fluorescein and rhodamine WT is a practical alternative to current methods of light measurement.

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1. Introduction

Light is one of the most important energy providers to aquatic systems. Light is an energy source that affects the metabolism of an aquatic system by providing energy for photosynthesis, the thermal structure by the transfer of light to heat energy, and the chemical make-up by providing energy for chemical reactions (Wetzel, 2001). This thesis considers the use of two dyes as way to measure the amount of light that affects lotic systems.

1.1 Dye use as tracers

Fluorescent dyes have been commonly used as tracers in water in a variety of aquatic systems, and their applicability has been extensively evaluated for certain types of these systems (Smart and Laidlaw, 1977; Kasnavia et al., 1999; Klonis and Sawyer, 2000; Dierberg and DeBusk, 2005; Chua et al., 2007; Upstill-Goddard, 2008). In order to be a good tracer, these dyes need to have several important characteristics. They must have detectable fluorometer ranges, high solubility, low background concentrations, and be inert to chemical and biological factors (i.e. change in pH, change in temperature, sediment sorption, and microbial uptake) within the aquatic system of interest. Most dyes have ionic functional groups, making them soluble in water; however, these functional groups are often pH sensitive and are sometimes attracted to charged particles or surfaces within a system, which may be problematic due to potential sorption and removal of dye (Kasnavia et al., 1999).

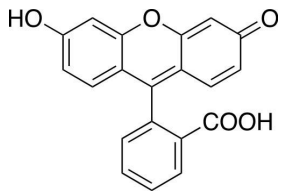
Rhodamine WT was specifically designed to be an inexpensive and effective conservative tracer for water flow (Smart and Laidlaw, 1977). Rhodamine WT appears to meet the characteristics of a good dye, and as such it is one of the most commonly used dyes for tracer studies. It has been shown to sorb to charged particles in longer-term studies (greater than 24 hours), but seems to be effective for short-term experiments (less than 24 hours) (Dierberg and DeBusk, 2005). It also has low photolytic losses (less than five percent in 11.5 days) making it an excellent surface water tracer (Upstill-Goddard, 2008).

Fluorescein is another commonly used dye. It has been shown to exhibit low sorption in brackish water aquifers and appears to be unchanged by electrical conductivity, and thus water salinity (Chua et al., 2007); however it is susceptible to photolytic losses (Smart and Laidlaw, 1977). This has made it more commonly used in groundwater or aquifer studies (Chua et al., 2007).

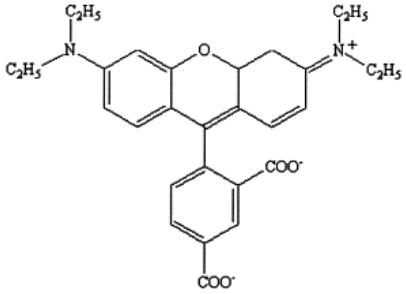
Both fluorescein and rhodamine WT have anionic functional groups at neutral pH (e.g., Figure 1b), while rhodamine WT has a positively charged functional group, as well. In acidic conditions these negatively charged functional groups become protonated, which affects the resonance of the dye, thereby decreasing fluorescence (Kasnavia et al., 1999). For example, when in basic conditions (pH>9), fluorescein has a quinoid structure which fluoresces; however, in acidic conditions (pH<5), a lactone ring is formed that does not fluoresce (Smart and Laidlaw, 1977). Fluorescein has six different structures that may occur. They include a cation, monoanion, and dianion,which are charged

species, and a quinonoid, lactone, and zwitterion, which are neutral species or have an overall neutral charge. Because of these different structures, the pK_a can be difficult to determine. Smith and Pretorius (2002) used a spectophotometric method for determining the pK_a values that included activity, temperature and absorptivity temperature corrections. They found pK_{a1} ($[H^+][H_2Fl]/[H_3Fl^+]$ where "Fl" represents a deprotonated fluorescein molecule) to be 2.22, pK_{a2} ($[H^+][HFl^-]/[H_2Fl]$) to be 4.34, and pK_{a3} ($[H^+][Fl^{2-}]/[HFl^-]$) to be 6.68 (Smith and Pretoruis, 2002). The relevant pK_a for rhodamine WT in natural waters is 5.1, which refers to the interchange between the zwitterion and the overall negatively-charged species seen in Figure 1b. (Shiau et al., 1993; Kasnavia et al., 1999; Vasudevan, 2001).

a. Fluorescein (acidic conditions)



b. Rhodamine WT (in basic conditions)



(http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---F/Fluorescein.htm accessed on 6/01/2012)	(http://www.sciencedirect.com/science/article/pii/S0925857403000053 accessed on 6/01/12)

Figure 1: The molecular structures of our dyes of interest.

Combining a photostable dye, rhodamine WT, and a photodegradable dye, fluorescein, could potentially provide a Lagrangian measure of sunlight exposure in a lotic system. This could provide information about the light field within a lotic system which has been previously unattainable. By using a Lagrangian technique, advection and other water processes can be more easily addressed, as can changes in light quality through a lotic system. Natural waters absorb and scatter light and can also be affected by shading from terrestrial objects, making light history of a water parcel difficult to quantify with traditional point measurements. Light absorption and scattering is dependant on many different factors, such as water depth, turbidity, and on the organic and particulate matter present. Light not only affects productivity and the biological composition of an aquatic system, but it also significantly impacts the chemical composition of dissolved and particulate substances (Kirk, 1994; Mayer et al., 2009).

1.2 Light in aquatic systems

Light has a long and complicated journey through an aquatic medium. It first has to be transmitted across the air-water interface. Some of the incoming solar radiation will be reflected back to the atmosphere. The amount of reflectance depends on the angle of penetration and whether the water surface is flat. An incident light penetrating vertically to a flat surface reflects two percent (%) of the light; this will increase up to 100% as the beam approaches a grazing incident angle. In addition, the calmer the surface layer of the water, the easier it is for solar radiation to penetrate the system. For example, a freshly formed whitecap in the ocean reflects 55% of the incoming solar radiation (Kirk, 1994).

Once light passes the air-water interface, it can be absorbed or scattered by molecules. Absorption is when a molecule captures a photon. Whenever a photon enters a system with molecules, there is a certain probability that a molecule will absorb that photon. When a molecule captures a photon, the transition state of an electron can be changed from its ground state to an excited state. This can alter a molecule's rotational energy level, vibrational energy level and electronic energy level, depending on the energy of the photon. A photon with a longer wavelength, like in the microwave or low infrared region, has a lower energy level and therefore can only cause a transition in rotational energy. If the photon has more energy, like in the higher infrared region, it can alter the vibrational transition state to a higher energy. Photons in the photosynthetically available part of the spectrum (PAR) can cause larger energy changes in transition states (Kirk, 1994). If a molecule in a system absorbs a photon, it can alter the electronic energy level, but eventually the molecule will be returned to the ground state and the energy may be released as heat. If the photons are used by a photosynthetic reaction center in an autotroph, they can be converted into the chemical energy used to create biomass. When more energetic UV light is absorbed by a molecule, the energy may be released by the breaking of chemical bonds, otherwise known as photodegradation (Kirk, 1994).

Kirk (1994) defines several major light absorbing components. They include water, gilvin, tripton and phytoplankton. Pure water absorbs mostly in the red region, and the amount it can absorb increases as wavelength increases above 550 nm (Kirk, 1994). Kirk coins the term gilvin as yellow substances that have sufficient concentrations to attenuate photosynthetically available radiation (PAR); thus gilvin is a term generally

interchangeable with and technically a subset of chromophoric dissolved organic matter or CDOM (e.g., Boss et al., 2001). These yellow substances are generally humic substances which are part of the dissolved organic matter (DOM) pool that absorbs at the blue end of the PAR spectrum and within the ultraviolet range. Although sometimes stable, this gilvin can be photodegraded (Moran and Zepp, 1997; Kirk, 1994; Anesio et al., 2005). Tripton consists of particulate matter found in natural waters. Tripton absorbs very little at the lower energy, red end of the spectrum, but the proportion absorbed increases as you move towards the ultra violet end of the spectrum. Phytoplankton contains photosynthetic pigments and attenuates PAR. Chlorophyll generally absorbs in the range of 380-500 nm and 610-720 nm, or in the blue and red wavelength bands, respectively. Each of these variables can be added and given as an absorption coefficient for an aquatic medium. Total absorption can be quantified by summing all of the absorption coefficients at each wavelength (Kirk, 1994).

Most photons that enter an aquatic medium are absorbed; however, many of these photons undergo scattering before a molecule absorbs them. Scattering is when a photon diverges from its original path. It increases the path length a photon follows while traveling to a certain depth. It also increases the likelihood that a photon will be absorbed. There are two types of scattering that occur in aquatic media, density fluctuation scattering and particle scattering (Kirk, 1994; Wetzel, 2001).

Density fluctuation scattering occurs in all liquids. Due to the constant random motion in a liquid, microscopic density fluctuations are constantly occurring, which

causes photons to diverge from their original path. Particle scattering is when a photon encounters a particle and is either reflected or refracted by that particle. The photon can be reflected by the surface of the molecule or it can be refracted after transmission through a molecule. Another form of light scattering is through diffraction, which is more easily described by the wave characteristics of light than photon characteristics. Light travels in waves at different angles around an object. When waves hit an object at the same time, they can combine either destructively or constructively. If the waves are in-phase with one another, there is constructive interference, amplifying the resulting wave. If the waves are out-of-phase there is destructive interference, which scatters the light (Kirk, 1994).

Irradiance is a common measurement of light flux and is defined by Kirk as "the radiant flux incident on an infinitesimal element of a surface, containing the point under consideration, divided by the area of that element," where radiant flux is the time rate of flow of radiant energy (1994). Therefore, irradiance is a measure of radiant flux per unit area of a surface. One way to look at how light penetrates an aquatic medium is by looking at the downward irradiance, or E_d. Downward irradiances diminishes with depth because of absorption and scattering in the aquatic medium. The absorption spectrum determines the amount of light that is attenuated, where the amount scattered (Q_{scat}) and the amount absorbed (Q_{abs}) equal the total amount that is attenuated (Q_{att}), given by

$$Q_{att}=Q_{scat}+Q_{abs}$$

Different types of aquatic media attenuate light differently. Most fresh-water lotic systems have a high concentration of gilvin, which absorbs and attenuates blue light. In highly turbid systems, both green and blue light are attenuated. Comparatively, in non-productive open ocean waters, red light is absorbed by the water molecules while green and blue light are able to penetrate (Kirk, 1994). Along with downward irradiance, there is also upward irradiance, which consists of scattered light. Although the downward irradiance is much larger, upward irradiance can still significantly contribute to photosynthesis in a system (Kirk, 1994; Wetzel, 2001).

In a natural system, such as a stream, light is not only influenced by optical properties in the water column, but by shoreline vegetation and the geomorphic structure of the stream bed, as well. The canopy in the riparian zone of a stream and the slope of its banks or walls can influence the amount and quality of light that reaches the stream (Hauer and Lamberti, 2006). The type of vegetation, angle of the sun relative to the vegetation and the amount of cover all influence irradiance that reaches a stream bed (DeNicola and Hoagland, 1992).

Light is an important factor for the biological system in lotic environments, since it acts as the main energy provider to the system (Wetzel, 2001; Hauer and Lamberti, 2006). Availability of light can limit primary production, and in heavily shaded streams it can often be the main limiting factor (Fisher and Likens, 1973; Hill et al., 1995). Light has been shown to affect carbon uptake by both macroinvertebrates and periphyton. In a study by Hill et al., (1995) periphyton in a non-shaded environment took up 2.4 times

more carbon than periphyton in a shaded environment; however, they noted that once photosaturation was achieved, carbon uptake could be photoinhibited at high irradiances. The study also found that there was a relationship between snail growth and photon flux densities; therefore, light not only affects primary production, but also secondary production (Hill et al., 1995).

The chemical composition of a lotic system can also be affected by the amount of irradiance seen by that system. One major contributing factor is the impact of light on DOM. DOM is known to be an important reduced carbon contributor to lakes; however, DOM's journey to the lake system from the rivers and streams is not as well understood. Different sources of organic carbon are degraded differently by sunlight and microbes along their path to the lake system, altering their composition (Moran and Zepp, 1997; Anesio et al., 2005). When photoproducts are formed from parent organic molecules via photodegradation, they are often smaller and more biologically active (Moran and Zepp, 1997). Depending on the source of the parent material (whether its allochthonous or autochthonous), photodegradation of DOM can result in more or less labile by-products for use in bacterial carbon production (BCP) (Anesio et al., 2005).

Although it is known that sunlight affects both chemical and biological components of a lotic system, the amount of sunlight that impacts stream or river water on its journey down a reach can be difficult to estimate. In lotic systems, photodegradation is difficult to study since both water motion and light penetration are hard to determine (Kohler et al., 2002). By deploying the rhodamine WT and fluorescein

dyes into a lotic system, in-situ light exposure could potentially be quantified since the photostable rhodamine WT would act as a control for water motion. The ratio of the two dyes could then be compared in order to determine the amount that fluorescein was degraded by sunlight relative to its dispersion by water motion, and therefore, how much light a lotic system experiences over a period of time or distance.

1.3 Purpose of this study

Preliminary work by Austin et al. (2010) assessed whether or not natural DOM fluorescence and the presence of natural particles affects the dual dye system's response to irradiation. Fluorescein and rhodamine WT were added to a phosphate buffer with a pH of 7 and also to filtered and whole stream water samples from Oregon Creek in Duluth, Minnesota that had significant colored DOM (CDOM). All samples showed similar degradation rates, indicating that natural stream DOM and particles did not affect the dye degradation. This work also presented an initial stream deployment of the dual-dye mixture, in which the fluorescein to rhodamine ratio decreased during travel downstream and appeared to be responding to sunlight.

Encouraged by this preliminary work, we performed laboratory testing and calibration of the two-dye approach and also report below results from several deployments in a local lotic system, Amity Creek in Duluth, MN.

2. Methodology

2.1 Lab experiments

To assess whether fluorescein and rhodamine WT in combination can be used as an in situ light proxy (actinometer) in a lotic system, several lab experiments were performed. These experiments looked at the effect of temperature on dye fluorescence, the effect of pH on photodegradation and fluorescence, and the effect of wavelength on dye photodegradation measured as a decrease in fluorescence response.

The same stock solutions of fluorescein and rhodamine WT were used throughout all experiments and were as delivered by the manufacturer. The fluorescein used was labeled as "uranine K", manufactured by Keystone, item ID 801-073-42, lot number A208F209. The rhodamine was labeled as "rhodamine WT" and manufactured by Keystone, item ID 703-010-27, lot number A207K221. All dye samples were monitored using a Sea Point fluorometer and Onset thermister (temperature probe).

The effect of temperature on dye fluorescence was examined by an experiment measuring the dye fluorescence in a solution warming from 2 degrees Celsius (°C) to 40°C. Fluorescein and rhodamine stock solutions were diluted separately with tap water to make up a 1.30×10^{-7} milliliter stock solution per milliliter working solution (ml/ml) and a 96.10×10^{-7} ml/ml solution, consecutively. 1 liter (L) of each working solution was placed separately into a beaker and cooled in a chest freezer until it reached approximately 2° C. A stir bar was added to the both beakers, which were both placed on a hot plate. A temperature probe and a fluorescence probe were also placed into each

beaker while the temperature and fluorescence were recorded every two seconds using a data logger until the solution reached 40°C for both rhodamine and fluorescein. This data was used to determine the relationship between temperature and fluorescence.

The effect of pH on dye photodegradation was examined for pH values ranging from 5 to 9. All of the buffer solutions (see Table 1) had a molarity of 0.5 and a final volume of 1 L. The buffer solutions were mixed on a stir plate for at least one hour prior to use in the experiment.

pH	Reagents	Grams (g) or milliliters (ml)
4.89	A.C.S. Fisher Scientific sodium acetate	41.0 g
	17.4 M Fisher Scientific acetic acid, HPLC grade	20 ml
6.2	Arcos Organics ammonium phosphate	66.0 g
	17.4 M Fisher Scientific acetic acid, HPLC grade	20 ml
7.24	Fisher Scientific HPLC grade ammonium acetate	7.71 g
8.06	Fisher Scientific A.C.S. certified sodium bicarbonate	8.40 g
	Fisher Scientific A.C.S. certified 6 M HCl	small aliquots until desired pH was reached
8.26	Fisher Scientific A.C.S. certified sodium bicarbonate	8.40 g
	Fisher Scientific A.C.S. certified 6 M HCl	small aliquots until desired pH was reached
9.04	Fisher Scientific A.C.S. certified sodium bicarbonate	8.40 g
	Fisher Scientific certified A.C.S. sodium hydroxide beads	added until desired pH was reached

Table 1: The final pH, reagents used and amount of reagent used to make buffer solutions.

The effect of pH on dye photodegradation was tested using a QSun solar simulator delivering 0.56 Watts per meter squared (W/m^2) at 340 nanometers (nm) and equipped with a water bath ranging from 23°C to 27°C. The first experiment (January 27, 2012) tested buffer solutions with a pH of 5 and 9. In this experiment, 300 ml of each buffer solution were placed into two 500 ml quartz round bottom flasks and two 500 ml boro-silicate round bottom flasks. A dye solution consisting of fluorescein and rhodamine WT was added to each flask (which had a diameter of 10.9 cm). An additional mixture of the same dye solution concentrations (re-made in tap water on June 7, 2012) had a pH of 7 as determined using Whatman pH indicator paper. This mixture had maximum absorption coefficients of 0.186 m^{-1} at 492 nm for fluorescein and 0.186 m^{-1} at 559 nm for rhodamine WT when measured with a Genysis 6 Spectrophotometer (on June 14, 2012). For the photodegradation experiment, the boro-silicate flasks were covered completely in aluminum foil so that no light could pass through, while the quartz flasks were uncovered and left exposed to irradiation. All dye/pH mixtures were irradiated for 50 minutes.

For the next set of irradiations (February 6, 2012), the procedure was repeated using buffers with a pH of approximately 6, 7, 8 and 9 (see Table 1); however this experiment consisted of one light exposed and one-aluminum wrapped dark control sample. A final irradiation experiment (February 8, 2012) was performed using a buffer solution with a pH of 8.06 in two light exposed samples and two-aluminum wrapped dark control samples. All irradiated and control samples were measured using Sea Point

fluorescein and rhodamine fluorometers. Temperature and pH were also recorded at the time of the fluorometer measurement.

Another QSun solar simulator experiment was performed to determine whether the dyes' fluorescence ratio decreases along similar time scales and responds to the same wavelengths as photobleaching of stream-water dissolved organic matter (DOM). To look at the effect of wavelength on the decrease in dye ratio, 10 ml aliquots of dye mixture were added to 10 ml of a phosphate buffer solution at pH 7. To make the buffer solution, 33.0 g of Arcos Organics ammonium phosphate and 29.9 g of Fisher Scientific monobasic anhydrous sodium phosphate were dissolved in 0.5 L of distilled water. The buffer and dye mixture were placed into wide-mouthed clear glass 20 ml vials, whose openings had a diameter of 2.3 cm. The same concentration of dye solution was re-made in tap water on June 7, 2012 and had a pH that read 7 on Whatman pH indicator paper. This solution had maximum absorption coefficients of 115.56 m^{-1} at 492 nm (fluorescein) and 206.07 m^{-1} at 559 nm (rhodamine WT) when measured with a Genysis 6 Spectrophotometer on June 14, 2012.

For comparison with the irradiated samples, two initial samples were taken before the experiment was performed and wrapped in aluminum foil and placed in a refrigerator until the experiment was completed. The rest of the sample vials were then wrapped in aluminum foil, leaving the top exposed to light. Four different long-pass filters (345, 360, 400, and 420 nanometers nm) were placed over the exposed surface. To make replicate exposures at each wavelength, each filter was placed over two sample vials which were

taped together. Two sample vials were left open to act as full-light controls and two sample vials were capped and covered in aluminum foil to act as dark controls. The sample vials were then placed in the QSun Solar Simulator, which produced 0.56 W/m^2 at 340 nm for 50 minutes while cooled with a water bath set at 27°C. To compare dye exposure with natural DOM response, the irradiation experiment was replicated with the same filters and vial set-up using 20 ml of Amity Creek water sampled on September 1, 2011. The sample was glass fiber filtered through a Whatman GF/F filter and then 0.2 micron filtered using a Masterflex peristaltic pump and a Whatman polycap aqueous solution filter capsule immediately after sampling and then stored in a refrigerator. It was filtered again through a 0.2 μm filter Masterflex peristaltic pump and a Whatman polycap aqueous solution filter capsule on January 11, 2011 less than 24 hours before irradiation. The stream water samples were measured for UV-Visible absorbance using a Genysis 6 uv-vis Spectrophotometer. Dye samples were measured with Sea Point fluorometers and an Onset temperature probe, with voltages recorded with Onset 4-channel datalogger. The raw fluorescence voltages were corrected for background fluorescence values and converted to dye concentration (ml manufacturer solution/ml sample plus dye solution) using the temperature data and the temperature calibration described previously**.**

In order to further assess the effect of irradiation wavelength on dye photodegradation, an initial dye mixture was serially diluted until the dye was no longer visible. An additional solution made later with the same concentrations of dye had maximum absorption coefficients of 2.21 m^{-1} at 492 nm (fluorescein) and 3.81 m^{-1} at 559 nm (rhodamine WT) as determined on a Genysis 6 spectrophotometer and a pH of 7 as

measured with Whatman pH indicator paper. Using the same long-pass filters, these sample vials were then irradiated for three hours in the QSun Solar Simulator (set at 0.56 W/m^2 at 340 nm) while cooled with a water bath set at 27°C. The samples were then analyzed as before.

2.2 Field Experiments

Amity Creek (in Duluth, Minnesota) is a designated trout stream located on the north shore of Lake Superior and is the location for this study's in-situ dye deployment tests, performed 6 times across a recorded water flow range of 0.77-13.2 m^3/s (See Table 2). It is one of the least urbanized streams located within the Duluth city limits, with 2% considered urban and 71% considered forested (www.lakesuperiorstreams.org). The dye deployment reach was upstream of the confluence of the Amity Creek and Lester River, whose combined flow then drains into Lake Superior. Lester River and Amity Creek have a combined watershed area of 134.8 square kilometers. Like other streams on Lake Superior's north shore, Amity Creek is flashy, exhibiting highly different characteristics in low vs high flow periods. It is also has a federal/state listing as impaired, due to turbidity from excess sediment loading (www.lakesuperiorstreams.org). Conveniently, Amity Creek flows along Seven Bridges Road, allowing for easy sampling sites along the stream (note multiple bridge crossings).

Figure 2: Dye deployment area along Seven Bridges Road (Duluth, MN) made in Google Earth.

To assess whether fluorescein and rhodamine can be combined to act as an in situ light proxy (actinometer) in a lotic system, multiple field experiments were performed. These experiments looked at the fluorescence ratio of the dyes along a reach in Amity Creek in relation to the amount of solar radiation in watts per meter squared multiplied by

the number of hours the dye was in the stream [(W/m^2)*hrs]; the solar radiation data was taken from a Lake Superior buoy deployed at 46.864°N 91.929°W.

A mixture of 11 ml of rhodamine stock solution, 39 ml of fluorescein stock solution and 450 ml of tap water were deployed into the stream at 46.8608°N, 92.0132°W, located below bridge 6 in Figure 2. The same fluorometers used in lab tests were deployed, along with a temperature probe, at 46.8608°N, 92.0132°W, 41 meters (m) downstream of the initial dye deployment, in order to read initial dye concentrations in the stream, and recorded voltage every 2 seconds to a data logger. After the initial dye mixture passed, the fluorometers were taken to 46.85816°N, 92.0131°W (bridge 4) and then to 46.8538°N, 92.0113°W (bridge 2), where they were placed in the stream until the dyes passed. The total measured reach distance of the deployment was 1.70 km.

During each dye deployment, two controls were also monitored. These controls consisted of 4 L of creek water and 1 ml of the pre-mixed dye working solution in matching open-topped plastic containers (33 cm x 13.5cm x 12 cm). A dye mixture mixed to the same concentration had maximum absorption coefficients of 115.56 m^{-1} at 492 nm indicating fluorescein and 206.07 m^{-1} at 559 nm indicating rhodamine WT when measured with a Genysis 6 Spectrophotometer. One container was left exposed to ambient sun, acting as a light control (LC). The other container was placed in the shade, covered, and wrapped with a black plastic bag. This container acted as a dark control (DC). The control containers were sampled two to three times throughout the deployment time, using 500 ml plastic bottles. The first sample was taken at the beginning of the

deployment, a second was taken partway through the deployment, and the last sample was taken at the end of the deployment. These samples were brought back to the lab where they were analyzed using the field fluorometers and temperature probe. Data were then temperature and background corrected, as well as converted from voltage to dye concentration based upon fluorescence to concentration calibration curves.

For statistical analyses the dye concentration ratios were normalized by the initial ratio of the dyes at the beginning of the deployment. Three time points from each dye pass were taken, one at half the peak height on the rising limb, one at peak height, and one at half of the peak height on the falling limb of the fluorescence response. These were then plotted over time and solar radiation in (W/m^2)*hrs and compared to the LC samples.

Date	Start Time	Average Discharge (m^3/s)	Total Deployment Time (min)	Solar Radiation (W/m^2)*hr	Conditions
9/30/2010	13:45	NM	92.9	1205.3	Sunny with Intermittent Clouds
6/17/2011	10:00	2.5	180.8	932.5	Cloudy
6/24/2011	10:00	13.2	42	821.1	Sunny day after large rain event
7/6/2011	10:40	1.58	223.6	2789.2	Sunny
7/13/2011	9:30	0.77	282.5	3685.4	Sunny
7/29/2011	13:40	1.07	268.6	3593.2	Sunny

Table 2: The deployment date, time, average discharge, solar radiation and recorded notebook cloud conditions. Discharge data is from lakesuperiorstreams.org (accessed on February 28, 2012). Lake Superior solar radiation data was taken from a buoy deployed at 46.864°N 91.929°W and was averaged over the deployment time and multiplied by the time length of the deployment (hrs). NM indicates that the discharge was not measured; visual inspection showed that the stream flow was high during this period.

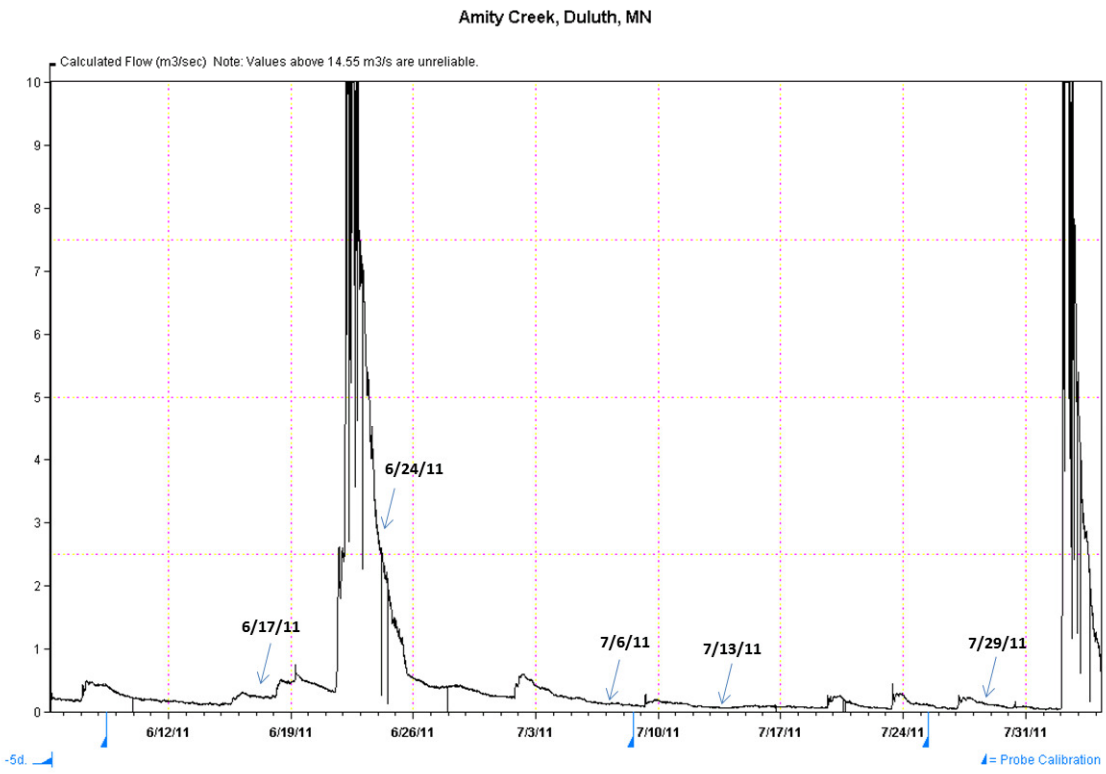


Figure 3. A dataview plot of discharge (m^3/s) over the sampling period in 2011 from www.lakesuperiorstreams.org (accessed on 7/11/12) with stream deployment dates.

Statistical analysis was performed using the computer program SPSS (see appendix for statistical graphs). A univariate analysis of variance (ANOVA) was used to compare multiple variables, while a simple t-test was done to compare the light and dark controls. Significance was determined with a probability value (p-value) of $p<0.05$.

3. Results and Discussion

3.1 Lab Experiments

Preliminary lab tests suggested that the dyes could be a robust way of measuring the amount of sunlight exposure experienced by a given stream reach. Temperature calibrations for both rhodamine WT and fluorescein showed a linear relationship between dye fluorescence and temperature (as had been reported earlier by Smart and Laidlaw, 1977). By using a known concentration of the dyes, a calibration was made in order to correct measured fluorescence for temperature affects. The temperature exponent (n) for fluorescein was 0.0036, which agreed with Smart and Laidlaw's (1997) value using the equation

$$F_s = Fe^{n(t_s - t)} \qquad (1)$$

where F_s is the fluorescence at standard temperature (t_s) and F is fluorescence at sample temperature. The rhodamine WT correction factor was 0.026, which was comparable to Smart and Laidlaw's (1997) value of 0.027 using the same equation.

Lab tests show that the fluorescence of fluorescein is affected by pH, while the fluorescence of rhodamine WT is unaffected over the tested pH range of 4.89-9.04; which is consistent with Smart and Laidlaw's (1977) findings that rhodamine WT fluorescence is only affected significantly below a pH of 5.0. Fluorescein's fluorescence directly increased by over a factor of 2 with pH over the experimental range of 4.89-9.04 with samples that were unexposed to light. As long as the pH does not vary significantly

down the stream reach, the pH change in fluorescence response would not affect in situ work if the values are normalized to initial in-stream values.

Photo-lability of both fluorescein and rhodamine were affected by pH over the range of 4.89-9.04. Fluorescein showed maximum photo-lability at a pH of 7.24, but was photo-labile across the entire pH range. Rhodamine WT was photo-stable over a pH range from 4.89-8.06, as its concentration (estimated from fluorescence) did not change after photo-exposure; however, at pH values ranging from 8.26-9.04, rhodamine WT exhibited photo-lability. Considering the normal range of pH in Amity Creek, 6.99-7.54 for the summer of 2012 (www.lakesuperiorstreams.org), the photo-lability of fluorescein should be exhibited, as well as the photo-stability of rhodamine WT.

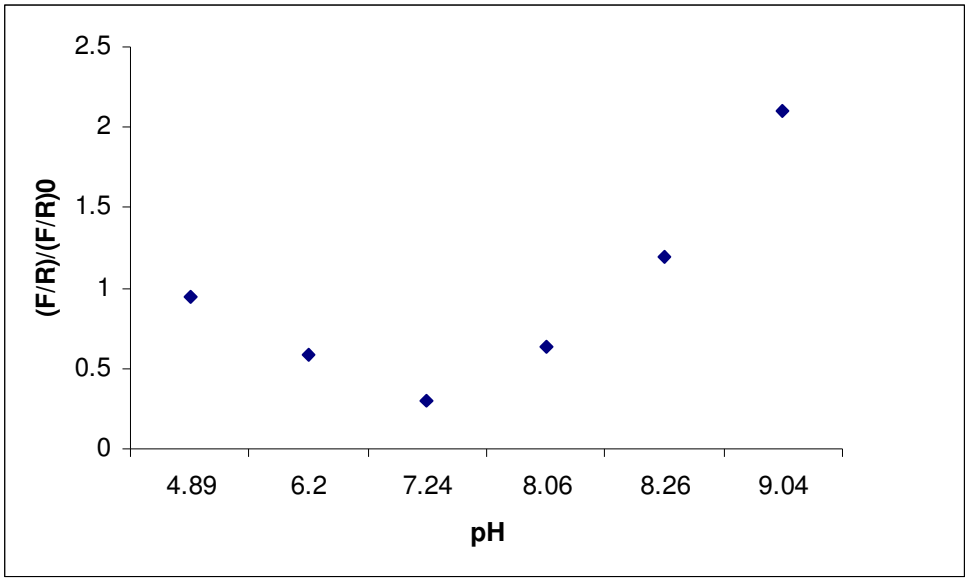


Fig. 4. Corrected dye ratio ($(F/R)/(F/R)_0$) versus pH value after irradiation for 50 minutes in a solar simulator.

If the pH of a lotic system is within the operating range of the dual dye response (pH 6-8, Fig. 4), pH should not be an issue of concern. If the dyes are outside of that range, they could still potentially be used; however, precautions would need to be taken to make sure the ratio is still effective. If the pH is between 5 and 8, fluorescein is photo-labile and rhodamine WT is relatively photo-stable. At a pH of 5, fluorescein did not exhibit photo-lability over the time period tested. If the pH is above 8, this particular mixture of dyes may not be desirable since rhodamine WT seems photo-labile. This apparent photo-lability, however, may be due to the buffer system chosen for the higher pH range in the lab experiments; a carbonate buffer was used, which may have led to the creation of carbonate radicals from interaction with photochemically produced OH•, and thus indirect photolysis of rhodamine WT (e.g., Schwartzenbach et al, 2003), rather than a strong direct photochemical response from the rhodamine WT molecules. Further studies are needed to evaluate the high pH-response of rhodamine WT.

Upon irradiation in the QSun Solar Simulator, both Amity Creek water samples and dye mixture samples containing rhodamine and fluorescein mixed in a phosphate buffer exhibited photodegradation (photobleaching of UV-Visible absorbance for Amity Creek samples and decrease in fluorescein fluorescence for the dyes). However, the dyes and CDOM differed in the wavelengths of maximum response when using long-pass filters (Figures 5-7). Amity Creek CDOM exhibited maximum photodegradation at wavelengths ranging from 345 nm to 400 nm. At wavelengths at and/or above 400 nm,

irradiated CDOM showed absorbance similar to that of the initial (non-irradiated) samples.

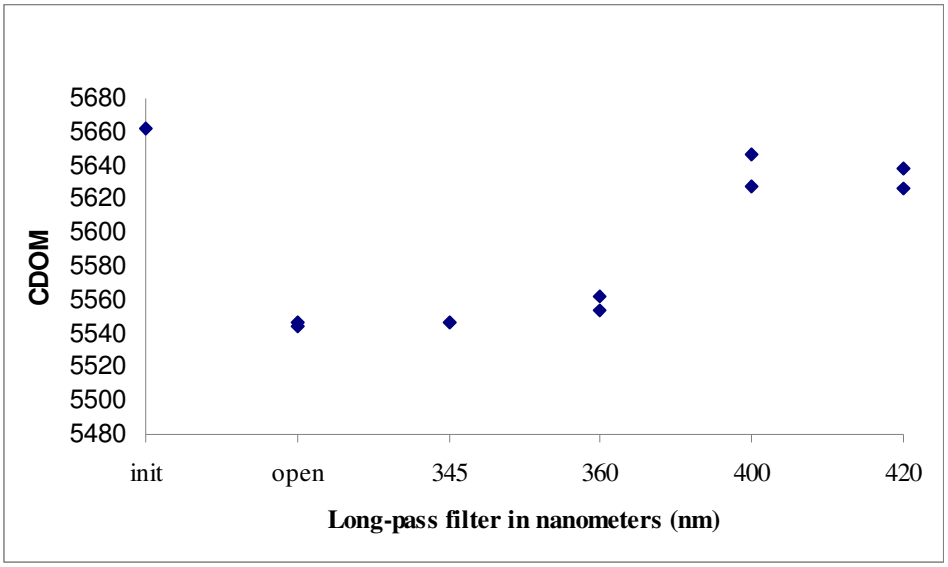


Fig. 5. Solar simulator irradiations (performed on January 11, 2011) of 0.2 micron filtered Amity Creek water (sampled on October 1, 2012) for 50 minutes using long-pass filters (345, 360, 400, and 420 nm). The colored dissolved organic matter was measured using summed absorbance coefficients from 250-400 nm.

The dye solution (which had maximum absorption coefficients of 115.56 m^{-1} at 492 nm indicating fluorescein and 206.07 m^{-1} at 559 nm indicating rhodamine WT) showed similar degradation using all long-pass filters (345, 360, 400, and 420 nm) when irradiated for 50 minutes in the QSun Solar Simulator, indicating that wavelengths at and/or above 420 nm degrade the fluorescein (Figure 6). A similar wavelength dependence was shown when a more dilute solution of the dyes (which had maximum absorption coefficients of 2.21 m^{-1} at 492 nm and 3.81 m^{-1} at 559 nm) was irradiated for

three hours in the QSun solar simulator (Figure 7). Again the wavelengths at and/or above 420 nm were responsible for the decrease in the fluorescein.

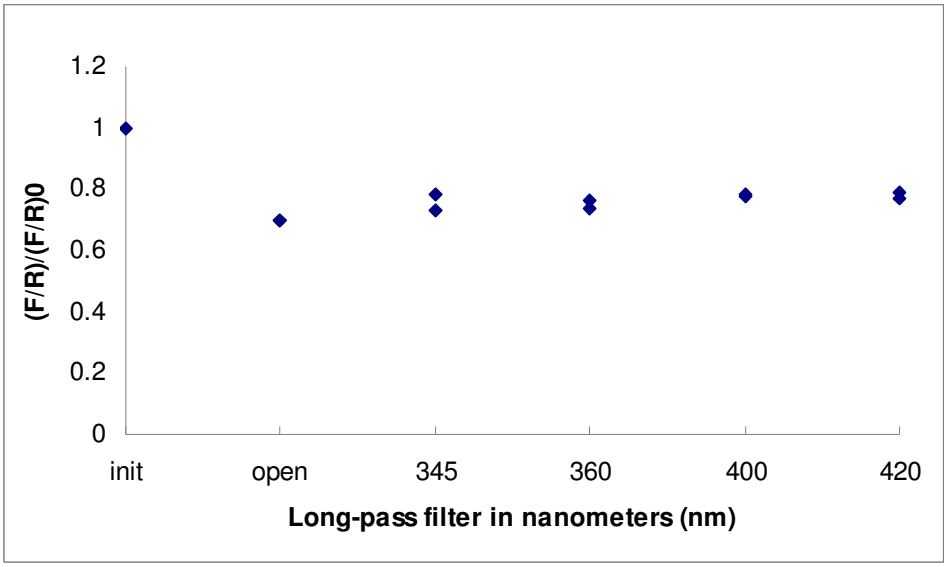


Fig. 6. The ratio of buffer-solution (pH 7) dye concentrations normalized to their initial ratio [$(F/R)/(F/R)_0$] after 50 minutes of irradiation. Long-pass filters (345, 360, 400, 420 nm) were used to determine the wavelength ranges responsible for maximum dye response.

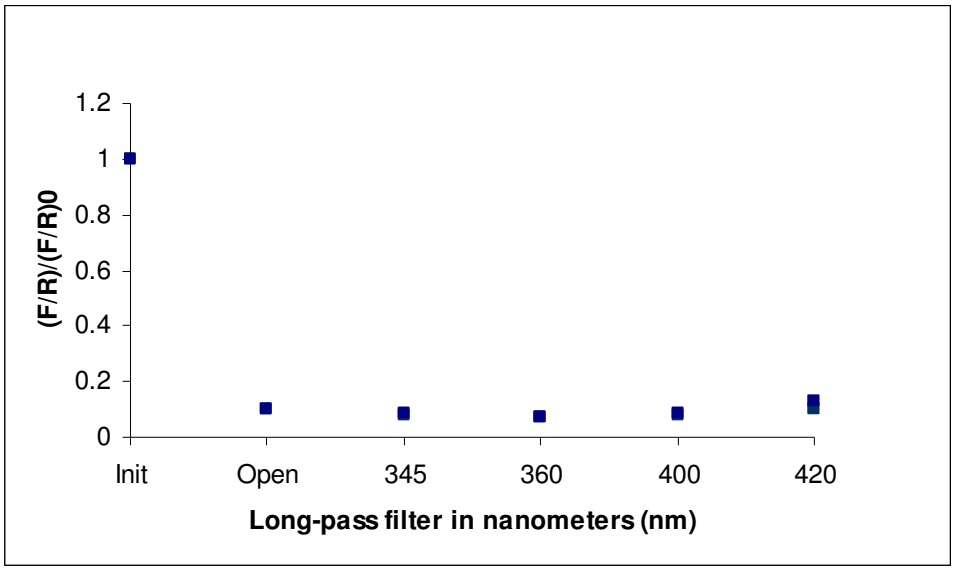


Fig. 7. Dye solution response [$(F/R)/(F/R)_0$] after a three hours of solar simulator irradiation. Fluorescein and rhodamine WT were in a phosphate buffer solution with a pH of 7. Irradiation was performed on replicate aliquots using long-pass filters (345, 360, 400, 420 nm) to determine wavelength ranges of maximum response.

Although both CDOM and the dyes were measurably affected over the same timescales of exposure, the same wavelengths were not responsible for the degradation. Therefore, the dyes may not be a suitable tracer for directly studying effects of irradiation on CDOM. More work would need to be done to relate the amount of irradiance occurring above the 420 nm region to the region that is known to affect CDOM, which preferentially absorb in the lower wavelengths of the spectrum (Kirk, 1994). Because fluorescein photodegrades above 420 nm, the dual-dye approach could be a useful proxy for exposure to photosynthetically active radiation (PAR).

3.2 Field Experiments

During dye deployments in Amity Creek, the dyes showed robust applicability on base flow, high irradiance days (Figure 8). The corresponding grouped dark controls (DC) and the light controls (LC) for the base flow, high irradiance deployments were significantly different from each other when using a t-test ($p<0.001$). The high flow, low irradiance LC and DC showed no significant difference ($p<0.348$), however, indicating that the time of sunlight exposure during flow down the reach was too short for significant photochemical response.

Maximum dye degradation occurred on days where the dyes received the highest solar radiation in (W/m^2)*hrs, which coincided with base flow deployments. During base flow deployments, the fluorescein to rhodamine WT ratio decreased linearly over time, as seen in Figure 8 (with an R^2 ranging from 0.9705-0.9975). Days with high flow and thus lower overall solar radiation exposure had low to no degradation of the fluorescein dye when compared to the rhodamine concentrations, as shown in Table 3. When the high flow days were grouped compared to the base flow days, there was a significant difference in $(F/R)/(F/R)_0$ over the entire deployment as determined by univariate analysis of variance (ANOVA) when looking at the overall change for each deployment ($p<0.0001$). There was no significant difference in the dye ratio using an ANOVA when the overall LC ratio decreases were grouped and compared to the overall deployment ratio decreases ($p<0.783$ for high flow deployments and $p<0.905$ for base flow deployments).

To give an indication of experimental precision, the DC samples (n=5) (which were not exposed to sunlight) exhibited a standard deviation in dye ratio $(F/R)/(F/R)_0$ of 0.09. For the high flow dye deployment on June 17, 2011 the downstream ratio variation was within this range of 0.09, so any decrease in dye ratio was negligible, i.e., within the error of the experiment. For June 24, 2012, the variation was 0.099, just outside of the experimental precision range. These three deployments (Figure 8, in red) had significantly higher ending dye ratios, i.e. less photodegradation of the fluorescein dye, when compared to the base flow deployments as determined by ANOVA ($p<0.0001$).

The decrease in ratio of the grouped high flow deployments was not significantly different from that of the grouped high flow DC using an ANOVA ($p<0.372$ for June 16, 2011 and $p<0.659$ for June 24, 2011) for dates when both deployment and control data were available. When the dye ratio decrease was compared over solar radiation levels in W/m^2*hrs, similar results were found for the controls and the dye deployments, as seen in Table 4.

Date	Time	Discharge (m^3/sec)	W/m^2*hr	$1-(F/R)/(F/R)_0$
6/17/2011*	10:00-12:54	2.5	932.5	0.188
6/24/2011*	10:00-10:38	13.2	821.1	0
7/6/2011	10:40-13:22	1.58	2789.2	0.64
7/29/2011	13:40-18:08	1.07	3685.4	0.635
7/13/2011	9:30-14:15	0.77	3593.2	0.681

Table 3. The date of dye deployment, time the dye was deployed, the average discharge over the deployment period, the W/m^2*hr over they dye deployment, and the photoresponse of the dye pair. High flow days are indicated with a "*."

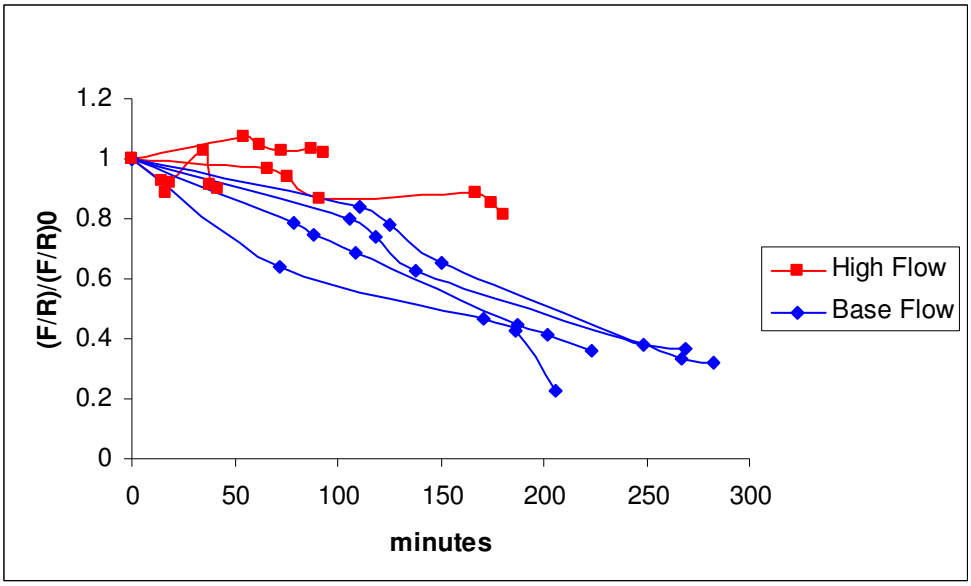


Fig. 8. The F/R ratio (normalized to the initial F/R ratio) over the dye deployment period in minutes. Square symbols indicate high flow and diamond symbols indicate low flow.

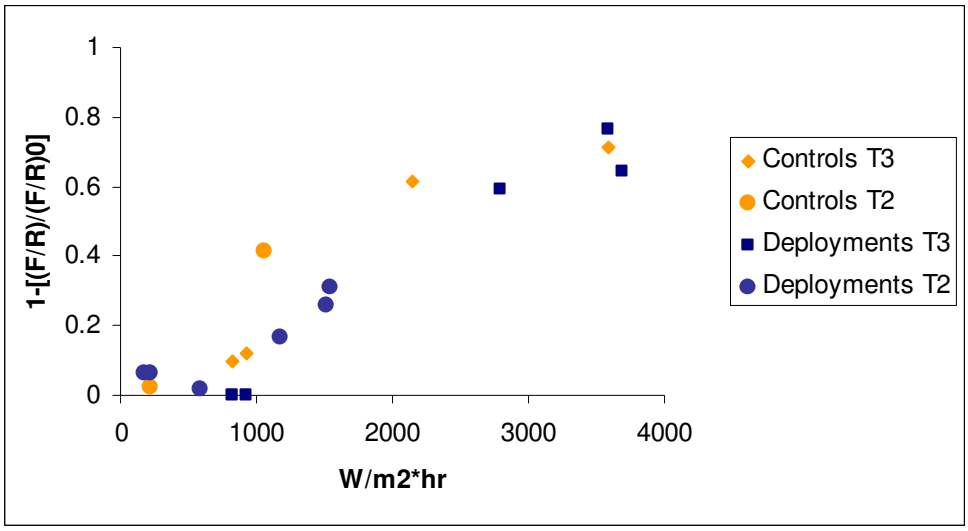


Fig. 9. The loss of normalized fluorescence response (F/R), i.e. fluorescence "photobleaching" versus solar radiation in W/m^2*hrs for the light control samples taken at the mid-point sample time (T2, indicated by a light circle symbol) and end-point sample time (T3, indicated by a diamond symbol) and the deployment measurements taken at the mid-point sample location (T2, indicated by a dark circle symbol) and end-point sample location (T3, indicated by a dark square symbol.) The R^2 values for the controls and deployments were 0.861 and 0.921**,** respectively.

Date	Control	Deployment
7/15/2010	N/A	0.775
6/17/2011*	0.12	0.188
6/24/2011*	0.099	0
7/6/2011	N/A	0.64
7/13/2011	0.613	0.681
7/29/2011	0.714	0.635

Table 4. The decrease in the F/R ratio for the light control samples and the dye deployment for the different deployment dates. High flow days are indicated with an asterisk (*).

Overall, the LC samples and the stream deployments seemed to show similar degradation rates, which concurs with visual observations that there is no serious canopy cover down this stream reach. There was no significant difference in overall change of the $(F/R)/(F/R)_0$ ratio between the deployments and their controls, with a $p<0.783$ (t-test comparison) when all of the high flow LC samples were compared to all of the high flow deployments. Likewise, when the base flow LC samples (as a group) were compared to the base flow deployments (as a group) there was no significant difference in the daily $(F/R)/(F/R)_0$ ratios with a p-value of $p<0.905$ determined by ANOVA. Table 4 shows that the insignificant variation between the light control and deployment data fell within 0.09 margin of error for all dates that had both data available, except for June 24, 2011, which varied by 0.099, just outside of the error range. This is shown in Figure 10, where standard deviation found in the DC (0.09) was used.

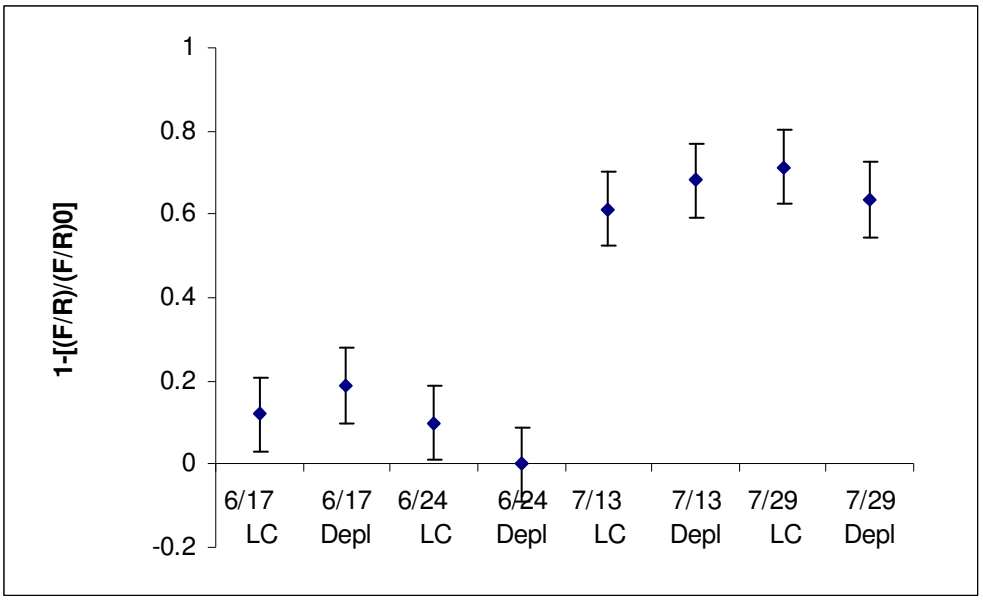


Figure 10: The decrease in F/R for the light controls as compared to the deployments using the standard deviation of the dark control samples to indicate experimental error.

4. Conclusions

Although the dual-dye methodology was successful in acting as an *in situ* light monitor and is applicable to streams if temperature is recorded and pH is within a certain operating range, more work needs to be done to demonstrate if the use of fluorescein and rhodamine WT is a practical alternative to current methods of light measurement. When all of the dye runs were compared to all of the light controls, they were not significantly different from one another ($p<0.783$; $p<0.0905$). This implies that at base flow a light sensor set it in direct sunlight on the stream's shore in this lotic system, coupled with flow information, could provide an accurate measure of how much sunlight was available along this stream reach. In a stream reach that was more shaded or over a longer reach, the dye response may differ between the *in situ* and control conditions. If the length of

the stream reach was increased for high flow days, thereby increasing the amount of time the dye is in the reach, there would be an increase in the amount of solar radiation exposure for the fluorescein, perhaps giving more information about light exposure on high flow days. The time of year, canopy cover, chemical composition, stream color, turbidity and size and length of reach are all factors that could make the dye more or less useful as a proxy for *in situ* light measurements for lotic systems. More intermediate flow days would be useful to test in the future.

For a stream reach with minimal canopy cover and sufficiently low flow, a stream bank measurement of light may appropriate for providing information about *in situ* lotic light exposure; however, in a more shaded stream reach or for a faster flowing or deeper stream, a stream bank measurement may not be adequate. In this situation, a dual dye approach using fluorescein and rhodamine WT would provide more information about the light affecting the lotic system. Another issue of concern is the length of the stream reach. The dye needs enough exposure to sunlight in order to provide a measurement, but the dyes must also still be within detectable ranges. For high flow days and more shaded reaches, an increase in stream reach length could potentially give more information about the sunlight affecting the stream reach since the dye would have more exposure to sunlight; however, care would need to be taken in order to assess the correct amount of dye to use.

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6. Appendix

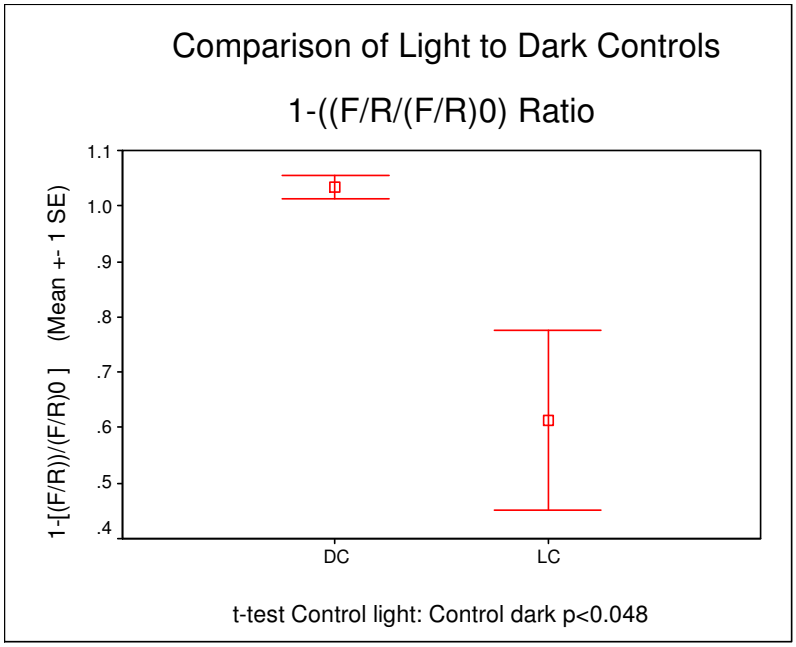

Figure 11: A comparison of all light control samples to all dark controls for all deployment dates using a t-test.

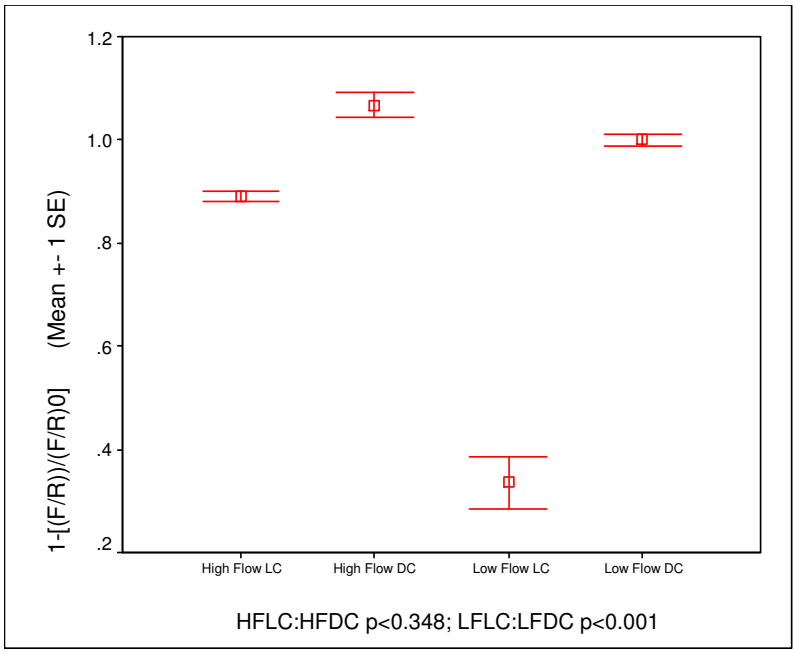

Figure 12: A comparison of high and low flow dark controls to light controls using ANOVA.