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Measuring photosynthetic efficiency in brown macro algae *in situ*: a good biomarker for photosynthesis inhibitors?

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Master of Science thesis

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- The mind is not a vessel to be filled, but a fire to be kindled -

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Abstract

Photosystem II (PSII) inhibitors are ubiquitous in costal areas around the world. Changes in chlorophyll fluorescence have been seen in plants exposed to low concentrations of PSII inhibitors in laboratory experiments. Saw wrack (*Fucus serratus*), bladder wrack (*Fucus vesiculosus*) and *Fucus evanescence* are important and widespread in Norwegian coastal ecosystems, as primary producers and as structure forming perennial species. Ability to sensitively and rapidly monitor adverse effects in these species could be useful in a monitoring program. Two chlorophyll fluorescence parameters, photosynthetic efficiency (how efficient quanta is used in PSII) and quinone pool (amount of electron acceptors in PSII) were measured by Hansatech Handy Photosynthetic Efficiency Analyser. Few differences in these parameters were seen in *F. vesiculosus* was grown under different irradiances, temperatures and salinities in three laboratory studies. Photosynthetic efficiency and quinone pool in *F. serratus* and *F. vesiculosus* were adversely affected by 100 µg L^{-1} Irgarol, but not by the other biocides tested. In a field survey at 6 sites in the Oslofjord from April to December 2007, differences were seen between fluorescence parameters in samples from different sites. Photosynthetic efficiency and quinone pool were highly correlated with light intensity, temperature and salinity during the survey, but this could not explain the difference observed on all days. Analysis of Irgarol in *Fucus* tissue from different sites did not either clarify the observed differences. Measurements of photosynthetic efficiency and quinone pool in *Fucus* spp are discussed in relation to ecological relevance and other biomarker methods.

1 Introduction

Photosynthesis is the basis of all higher life and at the basis of food chains. Solar energy is used in biosynthesis and primary production. Photosynthesis consists of two processes: First light is absorbed and its energy used to generate NADPH and ATP. This energy is then used to fixate carbon from the atmosphere or water, which is used in growth and reproduction. Even though the importance of plants, they have been considered less sensitive than animals to toxic substances and thus less used in testing (Lewis 1995). To underscore the importance of including plants or algae in environmental monitoring programmes, 50 % of the substances in pre-manufacturing notices were more toxic to algae than to animals (Benenati 1990). Lewis (1995) has made a list of substances that are more toxic to freshwater algae than animals, herbicides are well represented in this list.

Herbicides and substances toxic to plants are ubiquitous in coastal areas around the world (Lytle and Lytle 2001). The sources include run-off from agriculture (Ludvigsen and Lode 2008), input by antifouling ship paint (Konstantinou and Albanis 2004), and input from industrial and municipal waste water treatment plants (Nitschke and Schussler 1998). Herbicide run-off from agriculture usually comes in pulses, as they are not constantly applied in the fields. Peaks occur when the herbicide is washed out of the soil by rainfall (Ludvigsen and Lode 2008). Biocides in modern antifouling paint have a constant leeching rate (Almeida et al. 2007) and thus even biocides with short half-life can contribute to chronic stress in a local area. Paint particles from hull cleaning increases persistence of booster biocides and can lead to high contamination around marinas (Thomas et al. 2003). One of these booster biocides is Irgarol 1051. It works by blocking the plastoquinone, Q_B, site on the D1 protein in Photosystem II (Moreland 1980). It is exclusively used as a booster biocide (Thomas et al. 2001) and thus can serve as a proxy for amount of pollution from antifouling paint. Several other herbicides have the same mechanism as Irgarol, including phenylureas (e.g. diuron and linuron), triazines (e.g. atrazine and simazine), uracils (e.g. bromacil) and bis-carbamates (Muller et al. 2008). A number of these chemicals, including linuron and simazine, have been found in high concentrations in Norwegian rivers (Ludvigsen and Lode 2008). Polycyclic aromatic hydrocarbons (Marwood et al. 2001), metals (Eklund and Kautsky 2003), and effluents from paper mills (Kautsky et al. 1992) been shown to have effect on photosynthesis. Monitoring of herbicides is presently achieved by analysing for a few key chemicals (Muller et al. 2008), and rarely done in marine environments in Norway (Langford and Thomas 2008).

Some knowledge of photosynthesis is needed to understand this paper: a short introduction to the light reactions of photosynthesis follows. Photosystem II is embedded in the thylakoid membrane in chloroplasts. It consists of two proteins called D1 and D2, flanked by two cytochrome b_{559}. Around PSII there are light harvesting complexes, which contain different pigments, such as chlorophylls and caretenoids. Light energy (photons) is absorbed by light harvesting complexes and raises one electron to an exited singlet state. This energy is transferred to the pigment P680 in PSII. One electron is then transferred from P680* to pheaophytin a, another pigment in PSII. From there the electron is transferred to the primary electron acceptor, a quinone, $Q_A \rightarrow Q_A^-$. This creates a powerful oxidant: $P680^+$, which receives an electron from a secondary donor Z, a thyrosine residue on D1. The oxidized donor, Z^+, is reduced by an electron from the oxidation of water. Now the PSII reaction centre is said to be closed: it cannot receive another electron before Q_A^- has transmitted the electron to Q_B. This is a slower reaction. After Q_B has received two electrons it binds two protons and merges into the plastoquinone/plastohydroquinone pool. The electron transport chain consists of two more reactions, but none important for this paper. More information can be found in Krause and Weis (1991) and Falkowski and Raven (2007) which this short introduction is based on.

But not all light energy is used in the photochemistry as explained above. Chlorophyll fluorescence is a widely used technique for measuring stress in plants (Maxwell and Johnson 2000). The principle is quite simple: Light energy absorbed by chlorophyll molecules is either used to drive photosynthesis by reducing electron acceptors downstream PSII; it is dissipated as heat; or it is reemitted as light at a slightly longer wavelength – fluorescence. These processes are in competition, so an increase in one process will result in a decrease in the other two. When a plant is kept in dark for some time (dependent on species) all electron acceptors, plastoquinone, Q_A, will be in oxidised form. Before transfer into light the fluorescence yield released from chl a in absence of light, Fo, can be observed. Transfer into light will give a rise in fluorescence as more reaction centres are closed (this happens on a time scale of ms). After a peak, where maximum fluorescence is measured, Fm, fluorescence yield will sink. This happens because more energy is dissipated as heat (non-photochemical quenching) and there will be a light induced activation of enzymes involved in the carbon metabolism resulting in more electrons transported away from PSII (photochemical quenching). Changes in fluorescence yield are called the Kautsky effect after the discoverer (Maxwell and Johnson 2000). To avoid non-photochemical quenching during the analysis a

high intensity, short duration flash of actinic light (light that can be used in photosynthesis) is used. This will reduce all Q_A and all reaction centres will be closed. Provided the duration of the flash is short non-photochemical quenching (heat dissipation) will be negligible. Variable fluorescence, Fv, is calculated by subtracting maximal, Fm, with initial fluorescence, Fo. The ratio Fv/Fm gives a measure of the quantum efficiency if all PSII centres were open, maximum photosynthetic efficiency, and is highly correlated with the quantum yield of net photosynthesis (Bjorkman and Demmig 1987). Change in this parameter is a rapid and sensitive measure for stress (Fai et al. 2007; Huppertz et al. 1990; Maxwell and Johnson 2000; Snel et al. 1998).

Some studies have used chlorophyll fluorescence to determine the toxicity of observed biocide concentration to plants from the same site (Lambert et al. 2006; Scarlett et al. 1997; Scarlett et al. 1999). There has been developed a toxicity equivalents (TEQ) approach to phytotoxicants, where chlorophyll fluorescence in algae grown in environmental samples was directly related to fluorescence of algae growing in known diuron concentrations (Muller et al. 2008; Nash et al. 2006). Fernandez-Alba *et al*. (2002) found synergistic effect between mixtures of phytotoxicants. A bioassay approach would reveal this, wheras analysis first and following test would not. But it is not only phytotoxicants that give a reduction of photosynthetic efficiency. Photoinhibition, a protective mechanism to prevent oxidative damage during high irradiances (Hanelt 1996), will reduce photosynthetic efficiency (Gevaert et al. 2002; Huppertz et al. 1990). Photodamage, when the D1 proteins have been damaged and must be synthesized de-novo, will also be seen as reduced photosynthetic efficiency (Gevaert et al. 2002; Huppertz et al. 1990).
Further more different types of chemicals can give different fluorescence signals which can ease the identification, or pass under the radar when only one fluorescence parameter is used (Brack and Frank 1998). While Fv/Fm were similar with controls, the time it took to reach Fm were much shorter for the triazine and urea herbicide tested (monolinuron and simazine). Thus looking at the area over the fluorescence curve between Fo and Fm can also say something about the condition of the plant. This value corresponds to the quinone pool of the plant.

Fucoid algae (Fucales, Phaeophycea) form an important part of biota of rocky shores around Europe and the North Atlantic Ocean. It provides habitat and food for aquatic life, and are important in nutrient cycling (Mann 1982). In the inner Oslofjord *F. serratus* (serrated wrack,

Norwegian: Sagtang), *F. vesiculosus* (bladder wrack, Norwegian: Blæretang), and *F. evanescens* (no English common name, Norwegian: Gjevltang) are the most common macroalgae (Magnusson 2001). *F. evanescens*, an introduced species that tolerates high turbidity and low secchi depth dominates the inner parts of the Oslofjord. *F. serratus* and *F. vesiculosus* are not found in the inner parts of the fjord, but in coastal areas bordering Vestfjorden and southward. Since they are ecological important and have a wide distribution development of a biomarker is interesting.

A definition of a biomarker is a biochemical, cellular, physiological or behavioural variations in the tissue or body fluids or at the level of whole organism that provide evidence of exposure to chemical pollutants, and may also indicate a toxic effect (English Nature 2004). The biomarker should be a rapid, simple and environmental relevant test in order to be successfully implemented in an environmental monitoring programme (Galloway et al. 2004).

The aim of this paper was to test if chlorophyll fluorescence is a suitable biomarker *in situ*. This was done by investigating the photosynthetic efficiency and quinone pool of Fucus spp. at different sites in the inner Oslofjord.
The main hypothesis of this project was:

H_0: There was no difference in photosynthetic efficiency or quinone pool between *Fucus* spp. from the different sites.

Several hypotheses were investigated in laboratory experiments:

- Photosynthetic efficiency or quinone pool in *Fucus vesiculosus* was not affected by temperature
- Photosynthetic efficiency or quinone pool in *Fucus vesiculosus* was not affected by light intensity
- Photosynthetic efficiency or quinone pool in *Fucus vesiculosus* was not affected by salinity
- Photosynthetic efficiency or quinone pool in *Fucus vesiculosus* and *Fucus serratus* was not affected by selected booster biocides.
- There was no difference in Irgarol concentration at the different sites

2 Materials and methods

Four laboratory studies and a field survey were conducted to examine the suitability of fluorescence parameters in *Fucus* spp. as biomarkers.

2.1 Laboratory studies

2.1.1 Irradiance

Bladder wrack (*Fucus vesiculosus)* were collected at Solbergstrand 7.6.07, put in plastic buckets with lids and covered with seawater and transported to the lab. Six replicates of ca. 3 cm frond tips were put in separate wells in 6 cell well plates filled with 10 ml filtered seawater. The plates were placed in a climate room with a 16 hours light: 8 hours dark regime at 17°C. They were placed in a gradient to fluorescent light strips resulting in four different irradiances; 21 µmol m^{-2} s^{-1}, 35 µmol m^{-2} s^{-1}, 43 µmol m^{-2} s^{-1} and 86 µmol m^{-2} s^{-1}. Algae were incubated for 72 hours and exposure lasted 9 days, medium was changed every three days. Photosynthetic efficiency readings were taken with Handy PEA every day the first three days and on day six and nine.

2.1.2 Temperature

Bladder wrack (*Fucus vesiculosus)* were collected at Solbergstrand 7.6.07, put in plastic buckets with lids and covered with seawater and transported to the lab. Six replicates of ca. 3 cm frond tips were put in separate wells in 6 cell well plates filled with 10 ml filtered seawater. The plates were placed in three different climate rooms with fluorescent lights (21 µmol m^{-2} s^{-1}) with a 16 hours light: 8 hours dark regime at 7°C, 12°C and 17°C. Algae were incubated for 72 hours and exposure lasted 9 days, medium was changed every three days. Photosynthetic efficiency readings were taken with Handy PEA every day the first three days and on day six and nine. °

2.1.3 Salinity

Bladder wrack (*Fucus vesiculosus)* were collected at Solbergstrand 19.4.07, put in plastic buckets with lids and covered with seawater, and transported to the lab. Three replicates of ca. 3 cm frond tips were put in separate wells in 6 cell well plates filled with 10 ml filtered seawater. Algae were exposed to filtered seawater diluted with distilled water at six different salinities: 36, 30, 24, 18, 12 and 6. The exposure lasted for 9 days. The plates were placed in a

climate room with fluorescent lights (21 µmol m^{-2} s^{-1}) with a 16 hours light: 8 hours dark regime at 12°C. Algae were incubated for 72 hours, medium was changed every day during incubation to gradually change salinity (6 ppt each day from 24 ppt). Exposure lasted 9 days, and medium was changed every second day. Photosynthetic efficiency readings were taken with Handy PEA every day the first three days and on day six and nine.

2.1.4 Booster biocides

The experiment was carried out from 14.3.08 to 19.3.08. Two species of Fucus (*F. vesiculosus* and *F. serratus)* was used. The algae were collected 14.3.08 at Solbergstrand, put in plastic buckets with lids and transported to the lab. Six replicates of 2,5 – 3 cm frond tips were put in separate wells in 6 cell well plates filled with 10 ml filtered seawater. The plates were placed in a climate room with daylight lights (ca. 65 µmol m^{-2} s^{-1}) with a 14 hours light: 10 hours dark regime at 10°C. The algae were incubated for 48 hours and exposure lasted 72 hours.

Algae was exposed to three different booster biocides, Irgarol 1051(2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) (Ciba), Zineb (((1,2-ethanediylbis(carbamodithioato))(2-) zinc) (Sigma-Aldrich), Zinc pyrithione (ZPT) (1-hydroxypyridine-2-thione zinc) (Sigma-Aldrich), at four concentrations. Stock solutions of the biocides were made up in methanol and diluted with filtered seawater to get the required concentration. A carrier control was made up to make sure there were no significant effects of the methanol used to dissolve the biocides. Medium was not changed during the exposure. The concentrations of Irgarol 1051 were 100 µg L^{-1}, 10 µg L^{-1}, 1 µg L^{-1}, 0,1 µg L^{-1}. The concentrations of Zineb and ZPT were 1 mg L^{-1}, 100 µg L^{-1}, 10 µg L^{-1}, 1 µg L^{-1}. Photosynthetic efficiency readings were taken every 24 hours with PEA.

2.2 Field survey

Field observations were carried out from April to Desember in 2007 at five sites in the Inner Oslofjord and one reference site (Solbergstrand) in the outer Oslofjord (Figure 2.1). An YSI 63 salinity meter (Rickly hydrological company, USA) measured salinity each sampling day, except on 20 June and 23 August. Temperature and light intensity (Lux) were measured at 30 minutes intervals with Onset HOBO loggers (Onset computer corporation, USA) during the whole period. The logger was placed ca. 30 cm below the surface at low tide. Yearly mean tide in the Oslo fjord for 2007 is 0.68 meters in Oslo, and 0.66 at Oscarsborg at the start of the

inner Oslo fjord. All algae samples were collected at approximately the same depth as the logger. Frond tips (not shorter than 3 cm) were cut from algae and stored in plastic buckets with lids filled with seawater before sampling. Fronds were dried on cell paper and care was taken to avoid epiphytic growth in the sample area of the frond.

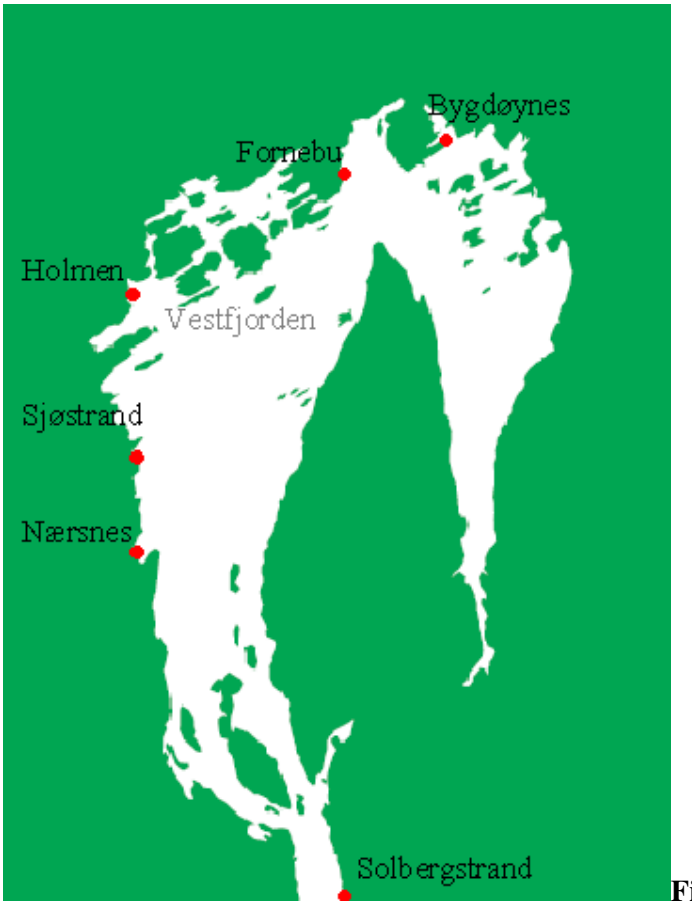

Figure 2.1 Inner Oslofjord, field stations marked.

2.2.1 Description of sites

Bygdøynes (59°54'3" N, 10°42'1" E): A small beach facing northeast. *Fucus evanescens* is growing on stones in coarse shale sediment. Possible sources of contaminants include close vicinity to marinas; Kongelig Norske Seilforening (KNS) båthavn (ca. 650 boats), Norhavn (Kongen) (ca. 250 boats), Frognerkilens båtforening (ca. 800 boats), ferry and container harbours (Hjortnes and Filipstad, Oslo Havn KF), and contaminants from rivers (Frognerelva).

Fornebu, Rolvsbukta (59°53'6" N, 10°38'1" E): A bay facing east-northeast. *Fucus evanescens* is growing on stones in soft sediment. There are no marinas close by,

possible sources of contaminants include release of sediment bound contaminants and run-off from rivers (Lysakerelva, Merradalsbekken and Hoffselva).

Holmen (59°51'2" N, 10°28'9" E): A stony shore facing east. *Fucus evanescens* and *Fucus serratus* are growing on rocky bottom. Possible sources of contaminants include close vicinity to marinas; Holmen Slipp (330 boats) and Holmenskjæret båtforening (175 boats).

Sjøstrand (59°47'8" N, 10°30'0" E): A shale stone beach facing east. *Fucus vesiculosus* and *Fucus serratus* are growing on stones and rocky bottom. Possible sources of contaminants include treated sewage water from Vestfjorden Avløpsselskap (VEAS). Treated water is released from a diffuser 900 m from land, up to 40 m below surface.

Nærsnes (59°45'7" N, 10°30'2" E): A shale stone beach facing northeast, situated in a bay with a breakwater closing approximately one third of the bay's opening. *Fucus vesiculosus* and *Fucus serratus* are growing on stones. Possible sources of contaminants include close vicinity to marinas; Nærsnes båtforening (ca. 100 boats), Promhavn Slipp (30 boats), Røyken båtforening (240 boats).

Solbergstrand (59°37'0" N, 10°39'3" E): A sandy beach facing west. *Fucus vesiculosus* and *Fucus serratus* are growing on stones. Possible sources of contaminants include release from sediment, a very small marina (ca. 10 small boats), and agricultural run-off from a small stream nearby. This site was considered unpolluted and chosen as a reference location.

2.2.2 Analysis of Irgarol 1051 in Fucus samples

Wrack (*Fucus evanescens, F. serratus* and *F. vesiculosus*) were collected at all field stations on 5.10.07, wrapped in hexane rinsed aluminium foil, transported to the lab and stored at -20°C. The frozen material was cut into smaller pieces, weighed, and freeze-dried for 48 hours (Lyovac GT2, art nr 045000). Freeze dried samples were weighed, and then homogenized (Grindomax GM200) for 30 s at 8000 rpm. Homogenized samples were weighed and put in glass tubes and samples were extracted with 30 ml dichloromethane (DCM) and shaken for three minutes and then centrifuged at 3500 rpm for 10 minutes (Heraeus Megafuge 1.0). The supernatant was collected and the pellet was extracted again with 20 ml DCM, shaken for three minutes and then centrifuged and the extracts were combined. 100 µl internal standard (ametryn) was added to each tube including a blank and a spiked control sample and then evaporated under nitrogen (Zymark Turbovap) to 2 ml. Sample extracts were cleaned up using pipettes filled with approx 3 g 5 % deactivated Al_3O_2

and rinsed with approx 3 ml DCM. Samples were then evaporated under nitrogen to approx 1 ml and extracts were analysed by gas chromatography – time of flight – mass spectrometry (GC-ToF-MS). GC-ToF-MS (Waters, Milford, USA) analysis was performed in EI positive mode (70 eV) at 8000 resolution with a source temperature of 180°C. GC separation used a 30 m x 250 µm x 0.25 µm column (DB-5ms, J&W Scientific, Agilent, Norway) with a 1 µl injection in splitless
mode at 250°C. The oven temperature was 60°C and held for 2 mins then increased at 5 ^{o}C/min to 280°C and held for 10 mins. (Analyte separation is shown in Appendix 1). Analytes were identified using 2 ions, for Irgarol, 253.1417 and 182.0540 and for GS26575, 198.0796 and 213.1044 were used (see Appendix 1). Calibration standards were run alongside samples for quantification purposes; r^2 values for both analytes were 0.99.

2.3 Hansatech Handy Photosynthetic Efficiency Analyser (PEA)

All fluorescence measurements were done with Hansatech Handy Photosynthetic Efficiency Analyser (Hansatech Instruments Ltd, Narborough Road, Pentney, King's Lynn, Norfolk, England). This system consists of a control unit connected to a sensor head with three LED lights and fluorescence detector. Data is downloaded from the control unit to Handy PEA software for further treatment and analysis. A leaf clip (figure 2.2) is clipped on a *Fucus* frond (or a leaf) and a metal shutter plate is slid in front of the measuring area to dark-adapt the sample. The sensor locates over the leaf clip so that daylight is excluded. The shutter plate in the leaf clip can then be slid open to expose the dark-adapted leaf ready for illumination and measurement by the sensor unit. PEA illuminates the frond with a focused array of ultra-bright red LED's with NIR short pass cut-off filters. The peak wavelength is 650 nm, which ensures that 95 % of the fluorescence comes from Photosystem II (PSII). Fluorescence is detected with a fast response PIN photodiode with RG9 long pass filter.

The parameters calculated from these measurements are:

Fo: The fluorescence level when the plastoquinone electron acceptor (Q_A) is fully oxidized. This value is extrapolated to time zero from a line of best fit through initial data points (4-16, 40 µsec to 160 µsec after illumination). This value is only accurate if the sample is dark-adapted.

Fm: The maximum fluorescence level measured, ideally when Q_A is fully reduced. This value is only accurate if the irradiance is fully saturating the plant and Q_A is actually fully reduced.

Fv: This is the variable component of fluorescence. It is obtained from Fm subtracted by Fo.

Fv/Fm: A ratio of the variable fluorescence divided by the maximal fluorescence. This is a ratio that has been shown to be proportional to the quantum yield of photochemistry, and shows a high degree of correlation with the quantum yield of net photosynthesis.

Area: The area above the fluorescence curve between Fo and Fm (Kautsky curve) is proportional to the pool size of the electron acceptors Qa on the reducing side of Photosystem II. If electron transfer from the reaction centers to the quinone pool is blocked such as the mode of action of a photosynthetically active herbicide, this area will be dramatically reduced.

For all three species (*F. vesiculosus, F. evanescens* and *F. serratus*) measured with PEA required dark adaptation time and irradiance was determined with a simple experiment. The fronds are dark-adapted using original Hansatech Handy PEA leaf clips (Fig 1). Five replicate samples were dark adapted for 2, 4, 6, 8, 10, 12, and 25 minutes and illuminated with maximum irradiance (3000 µmol m^{-2} s^{-1}). The mean values did not differ much and 8 minutes was chosen as dark adaptation time for all species.

A similar experiment determined irradiance. Five replicate samples of all species were dark adapted for 8 minutes and illuminated with 500, 1000, 1500, 2000, 2500 and 3000 µmol m^{-2} s^{-1}. Looking at the curve of the mean values, 2000 µmol m^{-2} s^{-1} was chosen as the irradiance to be used for all species. The same dark adaptation time, 8 minutes, and irradiance, 2000 µmol m^{-2} s^{-1}, were used in all field observations and laboratory experiments.

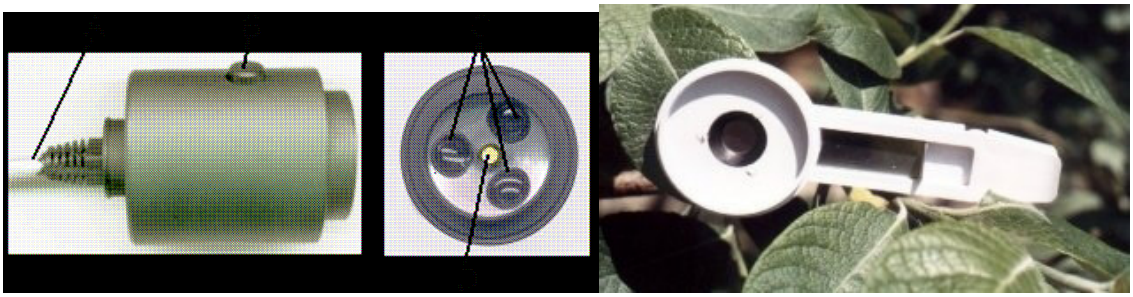

Figure 2.2 From the left: Sensor head with fiber optic data cable and trigger indicated with lines. In the middle: Sensor head seen from the front, with three LED lights and fluorescence detector in the middle indicated with lines. To the right: Leaf clip with measuring area, and metal shutter (Handy PEA manual, Hansatech Instruments Ltd 2001).

2.4 Estimation of photon flux density

Lux is a measure of illuminace and seldom used in plant physiology anymore. It has been replaced by photon flux density (PFD), which measures the number of photons indecent on a surface in a given time (irradiance) with unit mol m^{-2} s^{-1}. Lux is not easily converted to µmol $m^{-2}s^{-1}$ as all wavelengths have a luminosity factor and you would have to know the spectral composition of the light measured to get an accurate conversion. In full sun (zenith) a conversion factor of 0.0185 can be used to give an estimate (Thimijan and Heins 1982). This was done with data from Hobo loggers, so the results could be more comparable with literature data.

2.5 Statistical analysis

Statistical analyses and figures were done in R (version 2.4.0 © 2006 The R foundation for statistical computing).

Homogeneity of variance between samples was tested using Barlett's test of the null that the variances in each of the samples are the same (Sokal and Rohlf 1981) (p404). Where non-homogeneity of variance was found, the non-parametric method of Kruskal-Wallis rank sum test was applied and post-hoc testing was done with Mann-Whitney test using Bonferoni correction of significance level (Hollander and Wolfe 1999). Otherwise one-way ANOVA was applied (Sokal and Rohlf 1981) and post-hoc testing was done with Tukey Honest Significant Differences test (Yandell 1997). Relationship between fluorescence parameters and light intensity, temperature, salinity and Irgarol concentration were investigated with Spearman's rho test (Hollander and Wolfe 1999).

Significance level was set to 0.05 for rejection of H_0.

3 Results

3.1 Laboratory studies

Three studies under different growing conditions were conducted with *Fucus vesiculosus* to investigate the significance of irradiance, temperature and salinity on photosynthetic efficiency and quinone pool size. One study looked at photosynthetic efficiency and quinone pool size in *F. vesiculosus* and *F. serratus* when exposed to three biocides widely used in antifouling paint.

3.1.1 Irradiance

Fronds of *Fucus vesiculosus* were exposed to four different irradiances ranging from 21 to 85 µmol m^{-2} s^{-1} for 9 days.

The photosynthetic efficiency (Fv/Fm) varied between 0.791 and 0.662 with a mean of 0.750 (figure 3.1a). Fv/Fm decreased slightly towards the end of the experiment, with significant difference between the first and the last measurement for two highest irradiances (Tukey, $p < 0.01$ for both 43 and 86 µmol m^{-2} s^{-1}), but no significant difference for the two lowest irradiances (Tukey, p= 0.14 and p= 0.32 for respectively 21 and 35 µmol m^{-2}). There was no significant difference (ANOVA, $p>0.05$) between the different irradiances at each sampling day.

The quinone pool (area) increased significantly during the experiment for all treatments (figure 3.1b). The mean increased from 4767 the first measurement to 8658 the last measurement. There was significant difference between the treatments at day 2 (Tukey, 43 µmol $m^{-2}s^{-1}$ > 35 µmol $m^{-2}s^{-1}$, $p < 0.05$) and at day 3 (Tukey, 21µmol $m^{-2}s^{-1}$ > 35 µmol $m^{-2}s^{-1}$, $p < 0.05$), but treatments were not significantly different at any of the later measurements.

3.1.2 Temperature

Fronds of *Fucus vesiculosus* were growing in three different temperatures (7, 12 and 17 ℃) for 9 days.

The photosynthetic efficiency (Fv/Fm) varied between 0.799 and 0.669 with a mean of 0.760 (figure 3.2a). The mean decreased slightly towards the end of the experiment but there wasis no significant difference between the first and last measurement. There was no significant difference between the different temperature treatments for each sampling day.

The quinone pool (Area) mean increased significantly from the first measurement, 4433, to the last measurement, 9078 (Figure 3.2b). There was no significant difference between the treatments, apart from day 3 (Tukey, 17°C > 12 and 7°C, $p < 0.05$).

3.1.3 Salinity

Fronds of *Fucus vesiculosus* were held for nine days in six different salinities ranging from 6 to 36. The experiment was done with only three replicates, so the statistical analyses have low power (the probability of not making a type I error, accepting a false hypothesis).
Photosynthetic efficiency (Fv/Fm) varied between 0.754 and 0.579 with a mean of 0.6911 (Figure 3.3a). In the start and end of the experiment there is no significant difference between the treatments. Photosynthetic efficiency of fronds growing in salinities 6, 12, 18 and 36 stay at the same level it started until day 6 and decreases on day 9, while it decreases in fronds growing in salinities 24 and 30 until day 6 where it increases again. There was significant difference between the treatments on day 2, 3 and 6 (ANOVA, $p < 0.001$, < 0.0001 and < 0.01, respectivly). Fronds growing in salinity 6 had significantly higher photosynthetic efficiency than fronds growing in salinities 24 (Tukey, $p< 0.001$, < 0.0001 and < 0.05) and 30 (Tukey, $p< 0.01$, < 0.001, < 0.01) on these three days and fronds growing in salinity 36 on day 6 (Tukey, $p < 0.01$). Fronds growing in salinity 12 had significantly higher photosynthetic efficiency than fronds growing in salinities 24 (Tukey, $p< 0.01$, < 0.001) and 30 (Tukey, $p< 0.05$ and < 0.05) on day 2 and 3. Fronds growing in salinity 18 had significantly higher photosynthetic efficiency than fronds growing in salinity 24 on day 2 and 3 (Tukey, $p< 0.05$ and 0.001) and higher than fronds growing in salinity 30 on day 3 (Tukey, $p< 0.05$).
Quinone pool (Area) varied between 3000 and 19600 with a mean of 10931 (Figure 3.3b). Quinone pool increased slightly in fronds growing in salinities 12 and 18 until day 6, and then decreased. In fronds growing in salinities 24 and 30 quinone pool decreased slightly before increasing from day 3. None of these changes were statistical significant.
There was significant difference in quinone pool on day 2 and 3 (ANOVA, $p< 0.05$), but post-hoc test only showed significant difference on day 3: fronds growing in salinity 30 had significantly smaller quinone pool than those growing in salinity 36 (Tukey, $p < 0.05$).

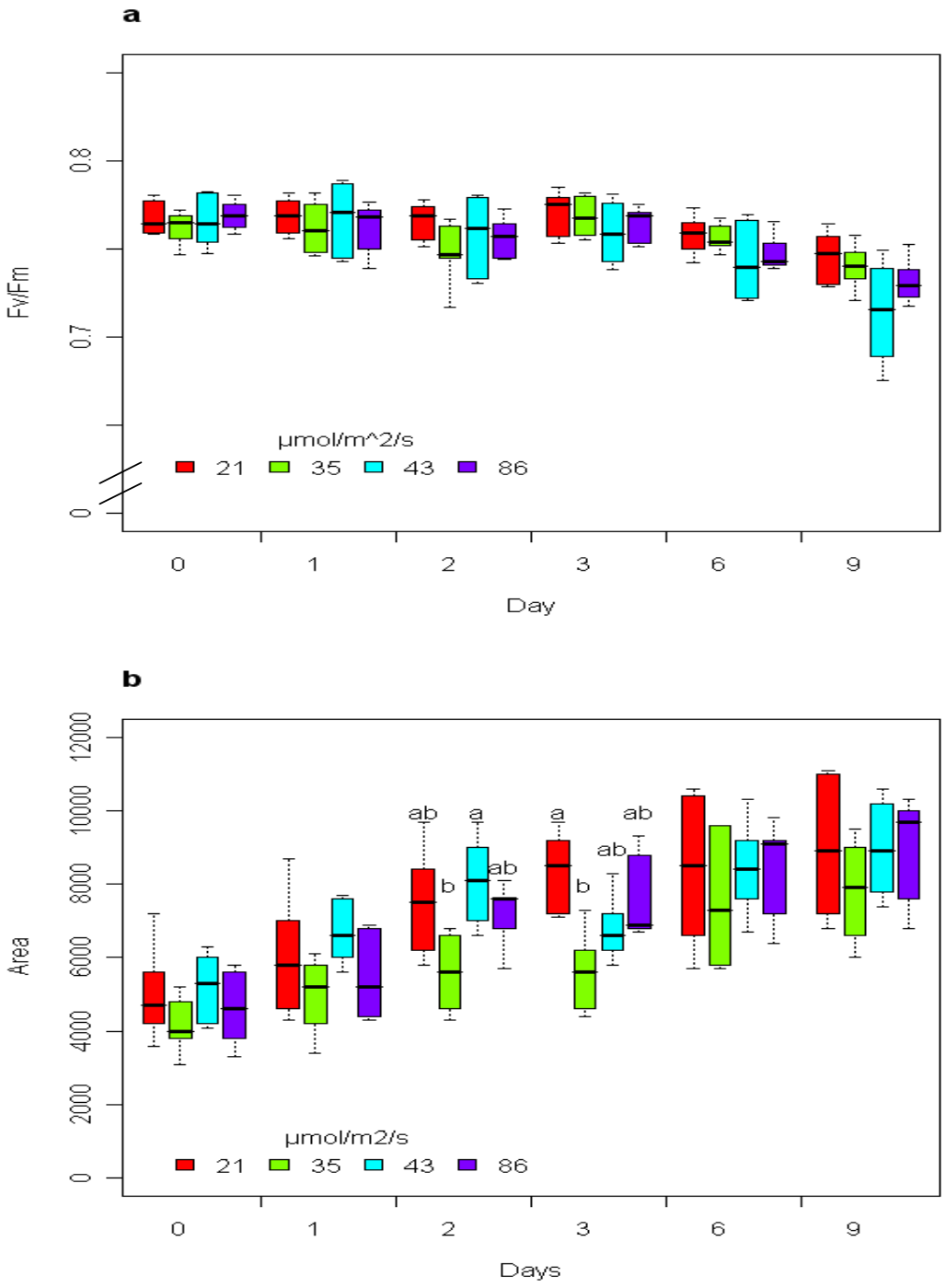

Figure 3.1 a) Photosynthetic efficiency and **b)** quinone pool in *Fucus vesiculosus* grown under four different irradiances. Note scale break on y-axis in **a)**. Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

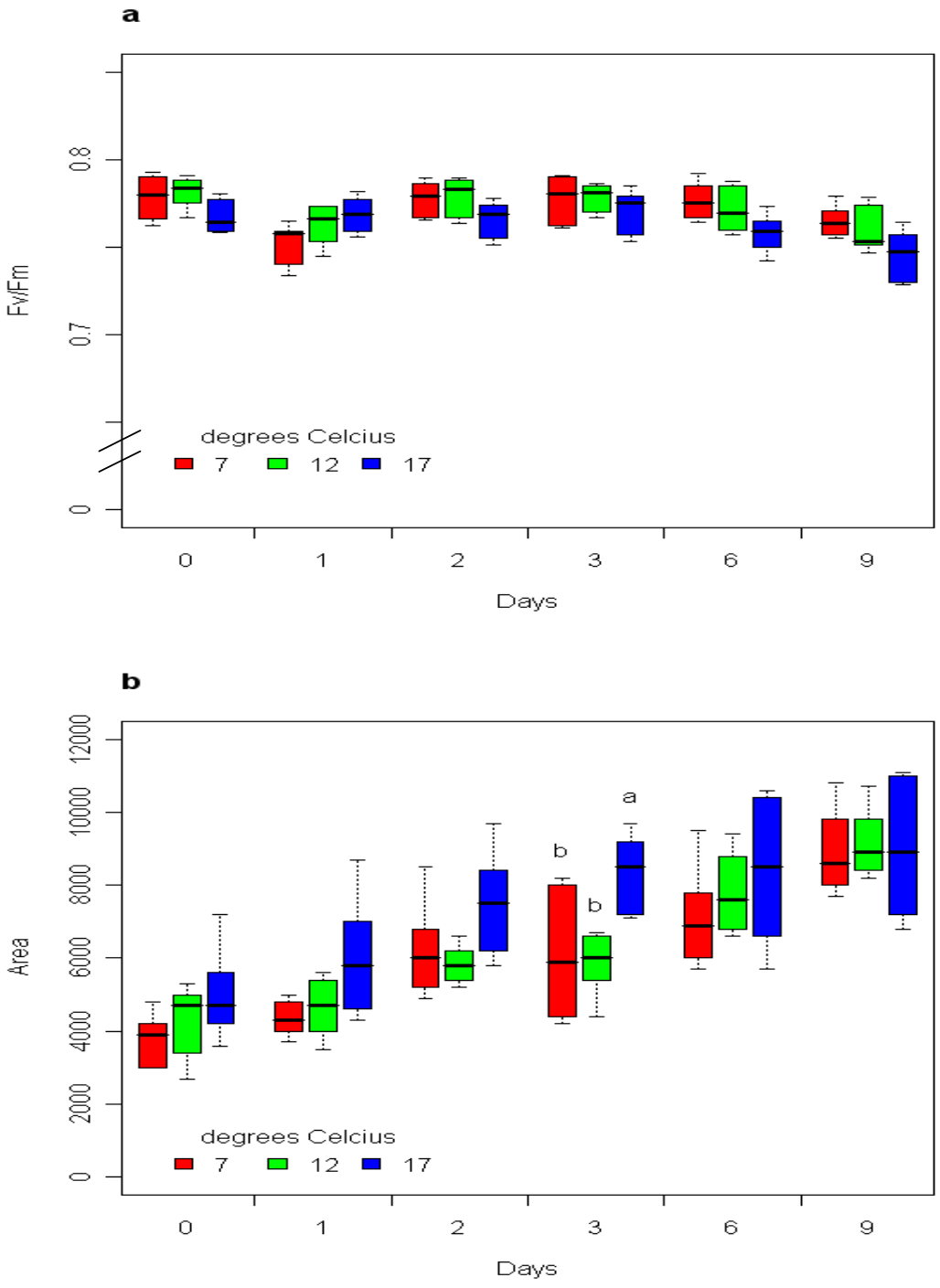

Figure 3.2 a) Photosynthetic efficiency (Fv/Fm) and **b)** quinone pool (Area) in *Fucus vesiculosus* grown under three different temperatures. Note scale break on y axis in **a)**. Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

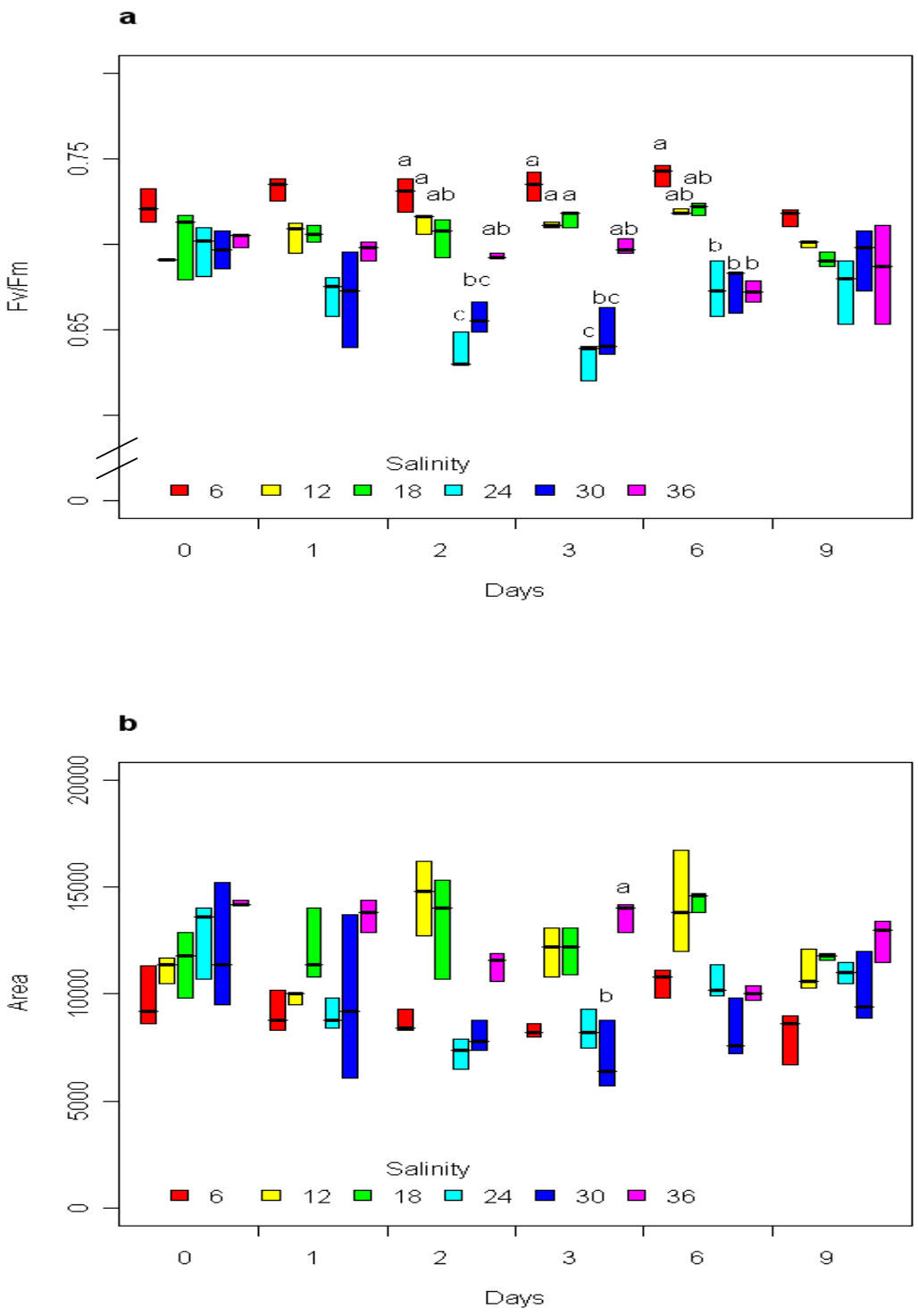

Figure 3.3 a) Photosynthetic efficiency (Fv/Fm) and **b)** quinone pool in *Fucus vesiculosus* grown under different salinities. Note scale break on y axis in **a)**. Median and quartiles, since n= 3 there are no error bars. No or same symbol indicates no significant difference between treatments.

3.1.4 Booster biocides

Fronds of *Fucus serratus* and *F. vesiculosus* were exposed to 0.1 µg L^{-1} to 100 µg L^{-1} Irgarol 1051 and 1 µg l^{-1} to 1000 µg L^{-1} Zineb and Zinc pyrithione for 72 hours. Photosynthetic efficiency (Fv/Fm) and quinone pool (Area) measurements were taken every 24 hours.

Irgarol

Photosynthetic efficiency (Fv/Fm) in algae exposed to 100µg L^{-1} Irgarol were significant difference from and all other concentrations and seawater and carrier control at 24, 48 and 72 hours for both species (Tukey, $p< 0.0001$ on all days)(Figure 3.4). For *F. serratus* at 72 hours there was significant difference between concentrations 0.1 and 1 µg L^{-1} and concentration 10 µg L^{-1}, but not between filtered seawater and carrier control and 10 µg L^{-1} (Figure 3.4a). There was no significant difference between the days for all treatments, except concentrations 10 and 100 µg L^{-1} in which all days are different from day 0. For *F. vesiculosus* there was significant difference between 0 hours and 72 hours for all treatments, except concentration 1 µg L^{-1} where there was no significant difference between sampling days (Figure 3.4b). There was significant difference in quinone pool between algae exposed for 100 µg L^{-1} and all other concentrations and seawater and carrier control at 24, 48 and 72 hours for both species (Figure 3.5). There was no significant difference between the other concentrations of Irgarol compared to controls. In *F. serratus* samples the quinone pool increased during the experiment (figure 3.5a), but only significantly for algae exposed to 1 µg L^{-1} (Tukey, $72 > 0$ hours, $p< 0.05$). In *F. vesiculosus* samples the quinone pool decreased during the experiment (Figure 3.5b), but only significantly for seawater control (Tukey, $72 < 0$ hours, $p< 0.05$).

Zineb

There was no significant difference in photosynthetic efficiency (Fv/Fm) between the treatments at each sampling for either species (Figure 3.5). For *F. serratus* there is a significant decrease from 0 hours to 72 hours for 100 µg L^{-1} (Tukey, $p< 0.05$) and 1000 µg L^{-1} (Tukey, $p< 001$). For *F. vesiculosus* there is a significant decrease in mean for seawater control, and concentrations 10 µg L^{-1} (Tukey, $p< 0.01$) and 100 µg L^{-1} (Tukey, $p< 0.05$) from 0 hours to 72 hours.

There was no significant difference in quinone pool (area) between the treatments at each sampling for either species (Figure 3.6). The quinone pool increased during experiment with *F. serratus* but only significant between 0 hours and 72 hours for seawater (Tukey, $p< 0.01$)

and carrier control (Mann-Whitney, $p < 0.05$). In the experiment with *F.vesiculosus* there was no significant change in mean quinone pool during the experiment.

Zinc pyrithione (ZPT)

There was no significant difference in photosynthetic efficiency (Fv/Fm) between algae exposed to different concentrations of ZPT or controls at each sampling for either species (Figure 3.7). For *F. serratus* there was a significant decrease in mean from 0 to 72 hours for filtered seawater (Tukey, $p < 0.05$), carrier control (Tukey, $p = 0.01$), 1 µg L^{-1} (Mann-Whitney, $p = 0.01$), 10 µg L^{-1} (Mann-Whitney, $p = 0.01$) and 100µg L^{-1} (Tukey, $p < 0.01$). There is no significant difference between the samplings of 1000 µg L^{-1} (Tukey, $p = 0.13$). For *F. vesiculosus* there was a significant decrease in mean from 0 to 72 hours for seawater (Tukey, $p < 0.01$) carrier control (Tukey, $p < 0.05$), 1 µg L^{-1} (Mann-Whitney, $p < 0.01$), 10 µg L^{-1} (Tukey, $p < 0.05$) and 100µg L^{-1} (Tukey, $p < 0.05$). There was no significant difference between the samplings of 1000µg L^{-1} (Tukey, $p=0.13$).

There was no significant difference in quinone pool (Area) between algae exposed to different concentrations of ZPT or controls at each sampling for *F. serratus* (Figure 3.8b). For *F. vesiculosus* at 48 hours the seawater control is significantly lower than carrier control (Tukey, $p < 0.05$), but there was no significant difference between the treatments at 24 hours or 72 hours. For *F. serratus* there was a small increase in mean from 0 hours to 72 hours although not significant. For *F. vesiculosus* there was no significant change from 0 hours to 72 hours.

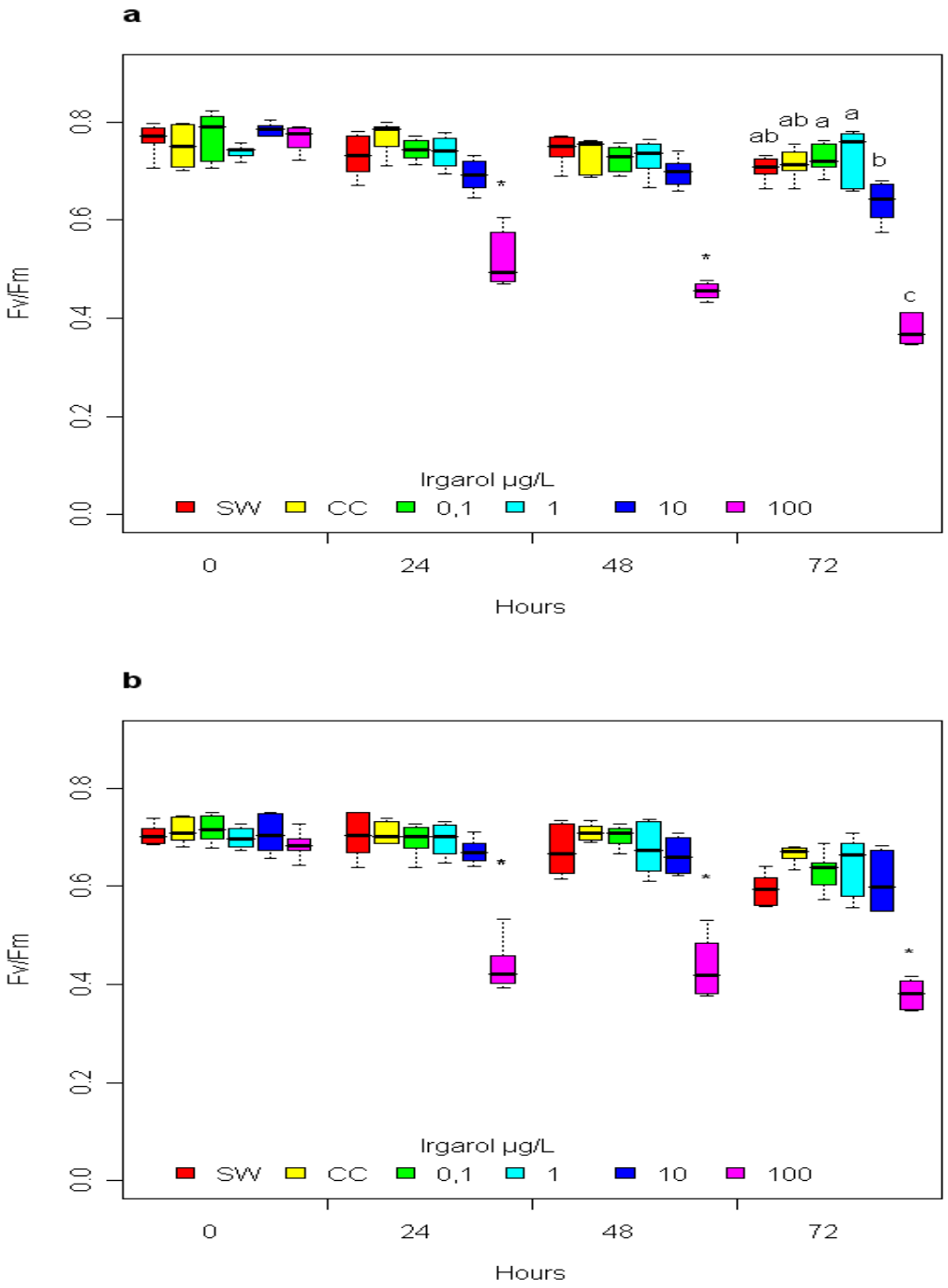

Figure 3.4 Photosynthetic efficiency of **a)** *F. serratus* and **b)** *F. vesiculosus* exposed to Irgarol 1051, seawater (SW) and carrier (CC) controls for 72 hours with measurements every 24 hours. Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

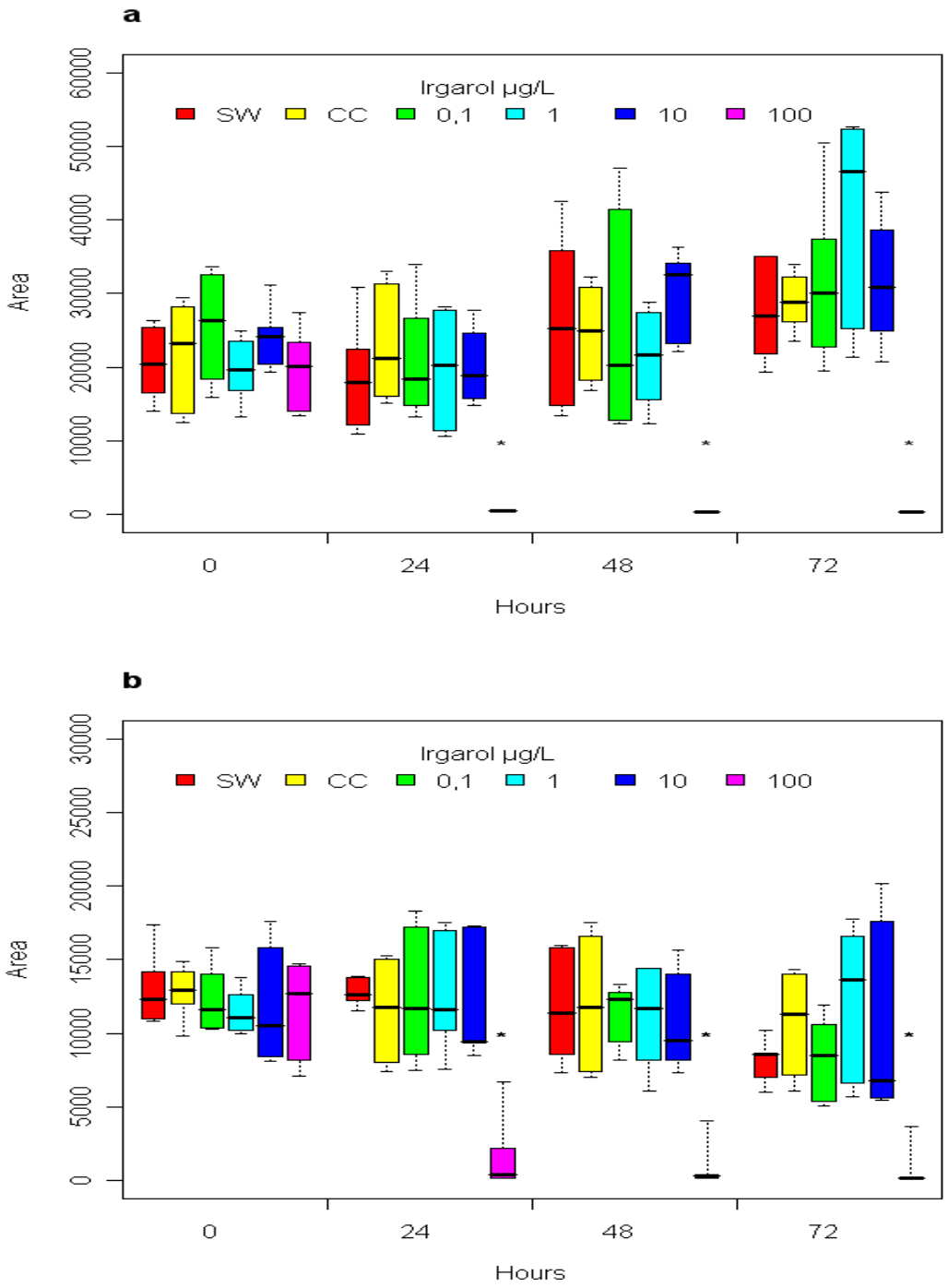

Figure 3.5 Quinone pool (Area) of **a)** *F. serratus* and **b)** *F. vesiculosus* exposed to Irgarol 1051, seawater (SW) and carrier (CC) controls for 72 hours with measurements every 24 hours. Note the scale on y-axis in b) is different from a). Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

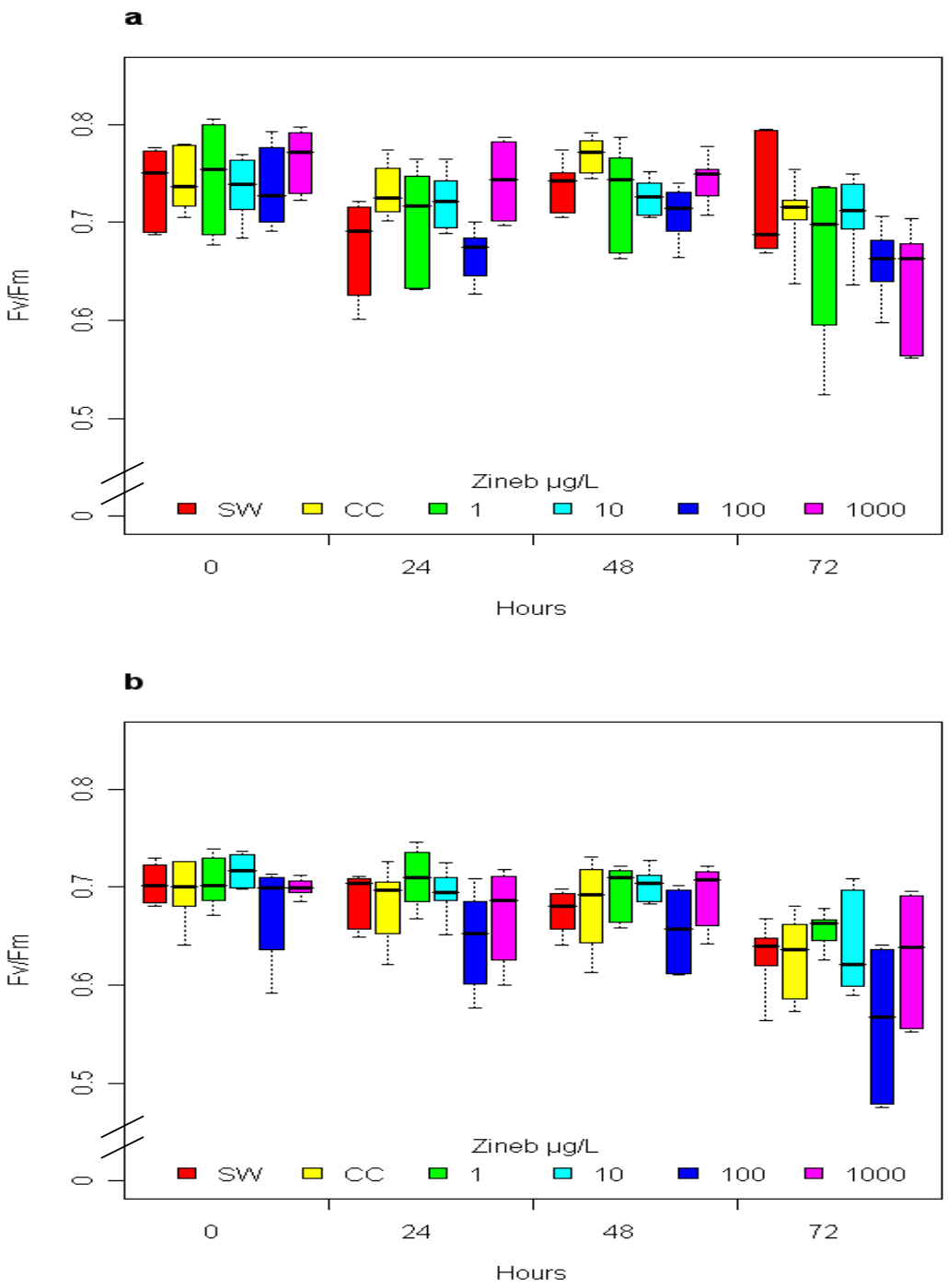

Figure 3.6 Photosynthetic efficiency of **a)** *Fucus serratus* and **b)** *F. vesiculosus* exposed to Zineb, seawater (SW) and carrier (CC) controls for 72 hours with PEA measurements every 24 hours. Note scale break on y-axis. Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

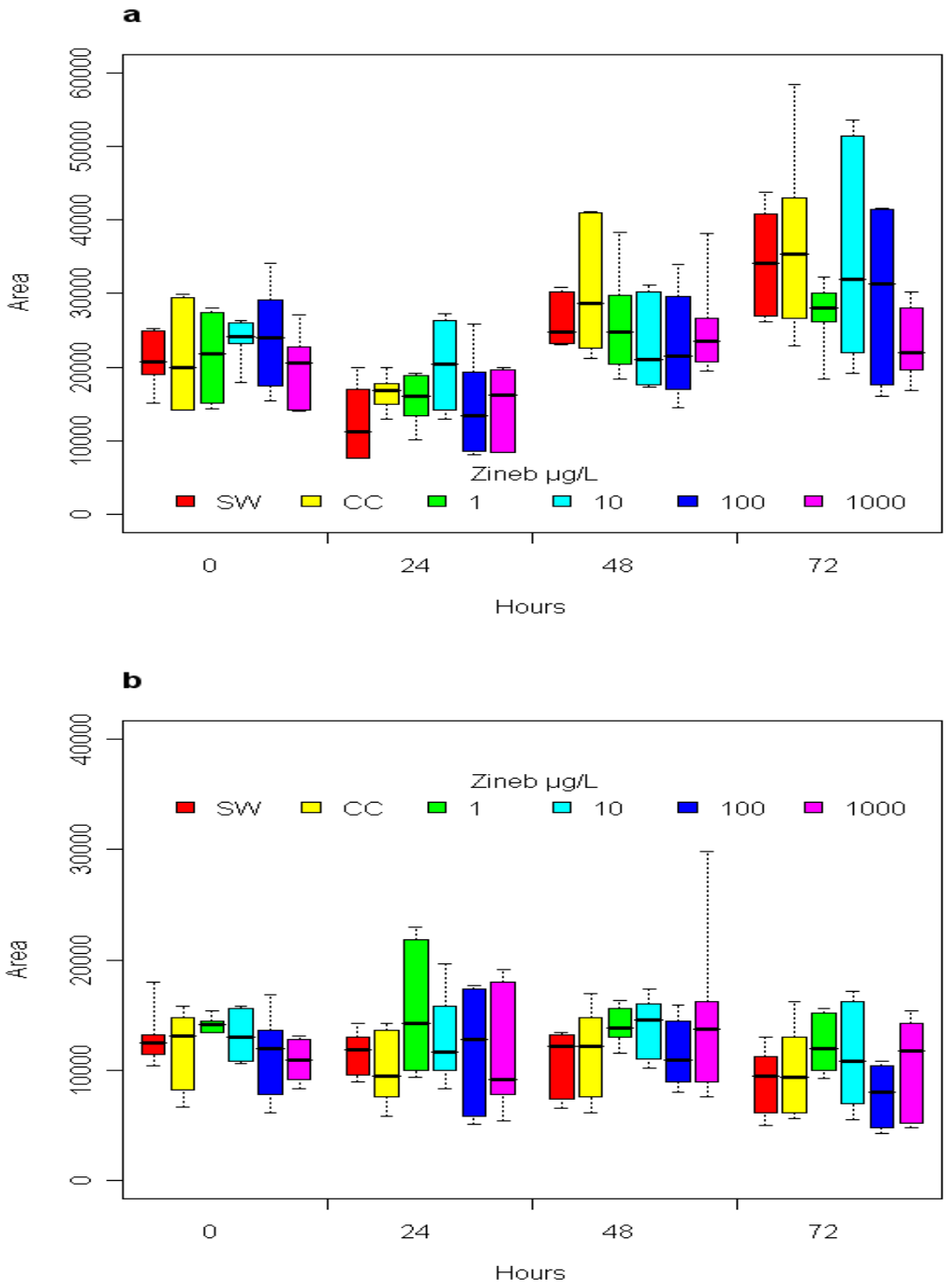

Figure 3.7 The response in quinone pool area of **a)** *Fucus serratus* and **b)** *F. vesiculosus* exposed to Zineb, seawater (SW) and carrier (CC) controls for 72 hours with PEA measurements every 24 hours. Note the scale on y-axis in b) is different from a). Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

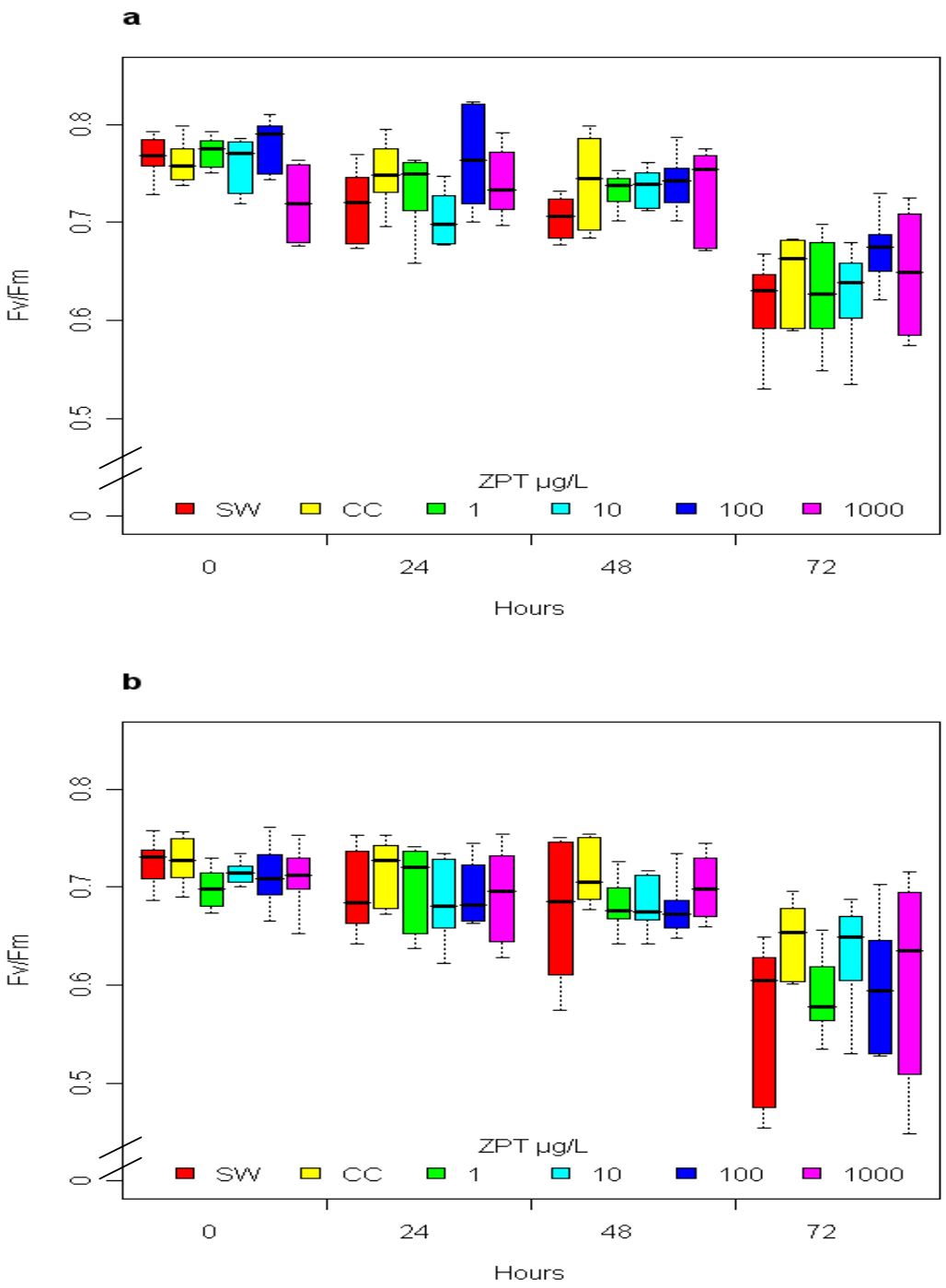

Figure 3.8 Photosynthetic efficiency of **a)** *Fucus serratus* and **b)** *F. vesiculosus* exposed to zinc pyrithione (ZPT), seawater (SW) and carrier (CC) controls for 72 hours with measurements every 24 hours. Note scale break on y-axis. Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

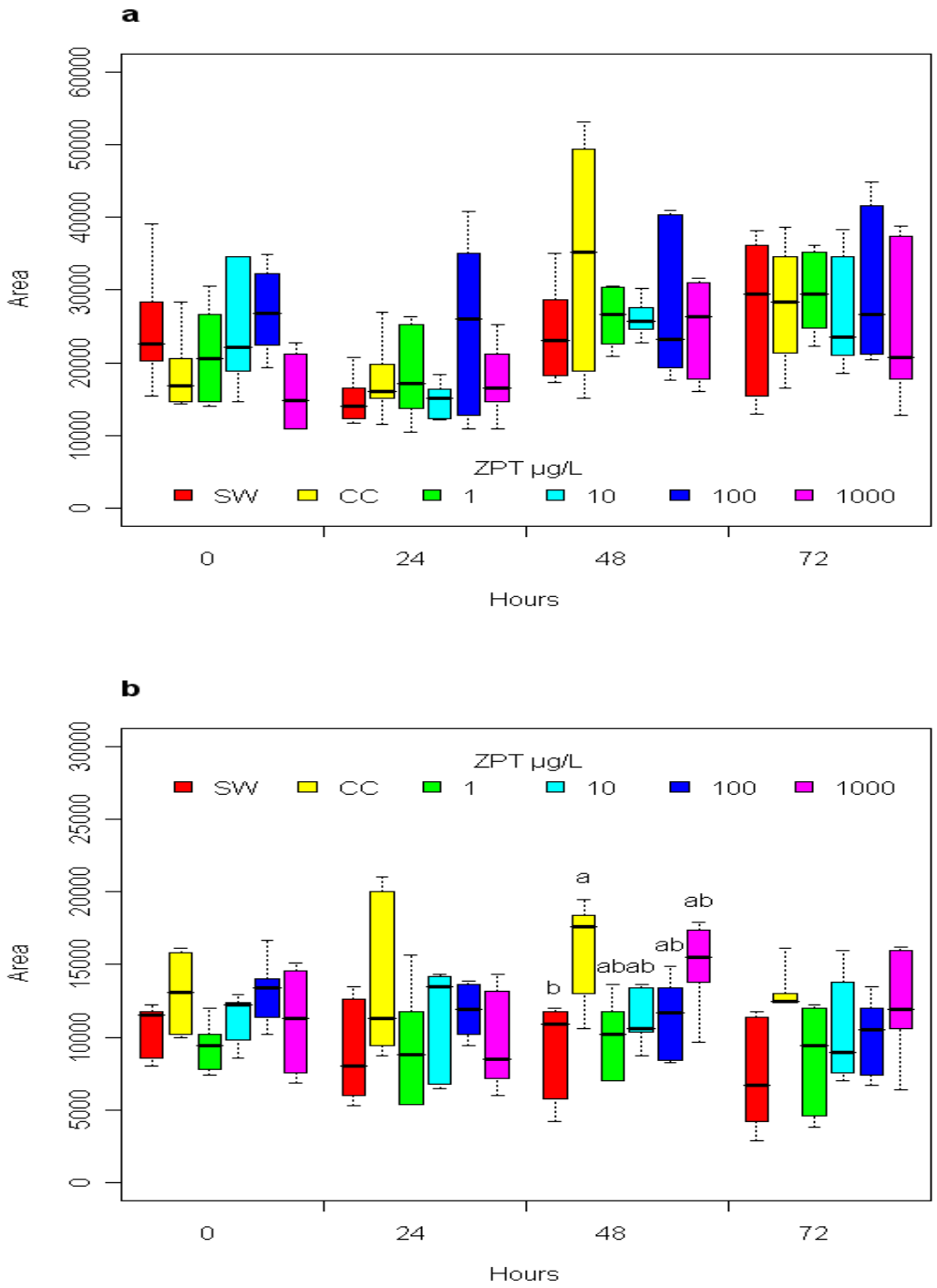

Figure 3.9 Quinone pool in **a)** *Fucus serratus* and **b)** *F. vesiculosus* exposed to zinc pyrithione (ZPT), seawater (SW) and carrier (CC) controls for 72 hours with PEA measurements every 24 hours. Note the scale on y-axis in b) is different from a). Median, quartiles and 10/90 percentiles. No or same symbol indicates no significant difference between treatments.

3.2 Field survey

A field survey was conducted at six sites in the Oslofjord from April to December in 2007. Light and temperature measurements were registered continuously by Onset Hobo loggers, fluorescence parameters and salinity were measured on 14 field days, from two to four weeks apart. On one field day (5 October), *Fucus* samples were taken and later analysed for Irgarol 1051 and its metabolite.

3.2.1 Light intensity, temperature and salinity

14 days integral light intensity, temperature and salinity measured during the field observation period are presented in figure 3.10. Light intensity was highest in spring and early summer, and stayed at approximately the same level from 10 July to 5 October. It was decreasing the three last measurements. Light intensity was highest at Nærsnes compared to the other stations from 11 May to 23 August, at Sjøstrand from 7 September to 5 October, and at Holmen from 2 November to 12 December. The difference between highest and lowest measurement on sites ranged from 1.33 to 3 times as high. The sea temperature was around 13 ^{o}C when measurements started in April, but increased to around 20 ^{o}C where it stayed from 7 June to 23 August. It decreased almost linearly to 4 ^{o}C on the last measurement 12 December. Highest temperatures were taken at Sjøstrand and Nærsnes during the summer. Solbergstrand had higher temperatures than the other stations during autumn. Difference between the sites ranged from 1.1 to 4.7 ^{o}C with a mean of 2.6 ^{o}C between highest and lowest measurement. Mean salinity was 20.0 when measurements started in April. Lowest mean was 15.6 on 10 July and from there salinity increased. Highest mean, 27.2, was measured 2 November. Salinities at Solbergstrand were generally higher than at the other sites. Difference between the sites ranged from 1.7 to 8.8 with a mean of 5 between highest and lowest measured salinity. Due to unavailable salinity meter measurement was not taken 20 June and 23 August.

3.2.2 Concentration of Irgarol 1051 measured in *Fucus* samples

The concentration of Irgarol in Fucus samples collected 5 October was determined by GC-Tof-MS (figure 3.11). The samples from Solbergstrand had the lowest concentration of Irgarol 1051,in both *F. vesiculosus* (51 ng g^{-1} dw.) and *F. serratus* (48 ng g^{-1} dw.). Highest concentration was measured in the *F. serratus* sample from Nærsnes (165 ng g^{-1} dw), the concentration in the *F. vesiculosus* sample from the same site was much lower (60 ng g^{-1} dw.). Highest concentration in *F.evanescens* was from Bygdøynes (133 ng g^{-1} dw.). The other

samples had concentrations around 100 ng g^{-1} dw. There was little difference in concentration between the species. The metabolite of Irgarol, GS26575, was not found in the samples.

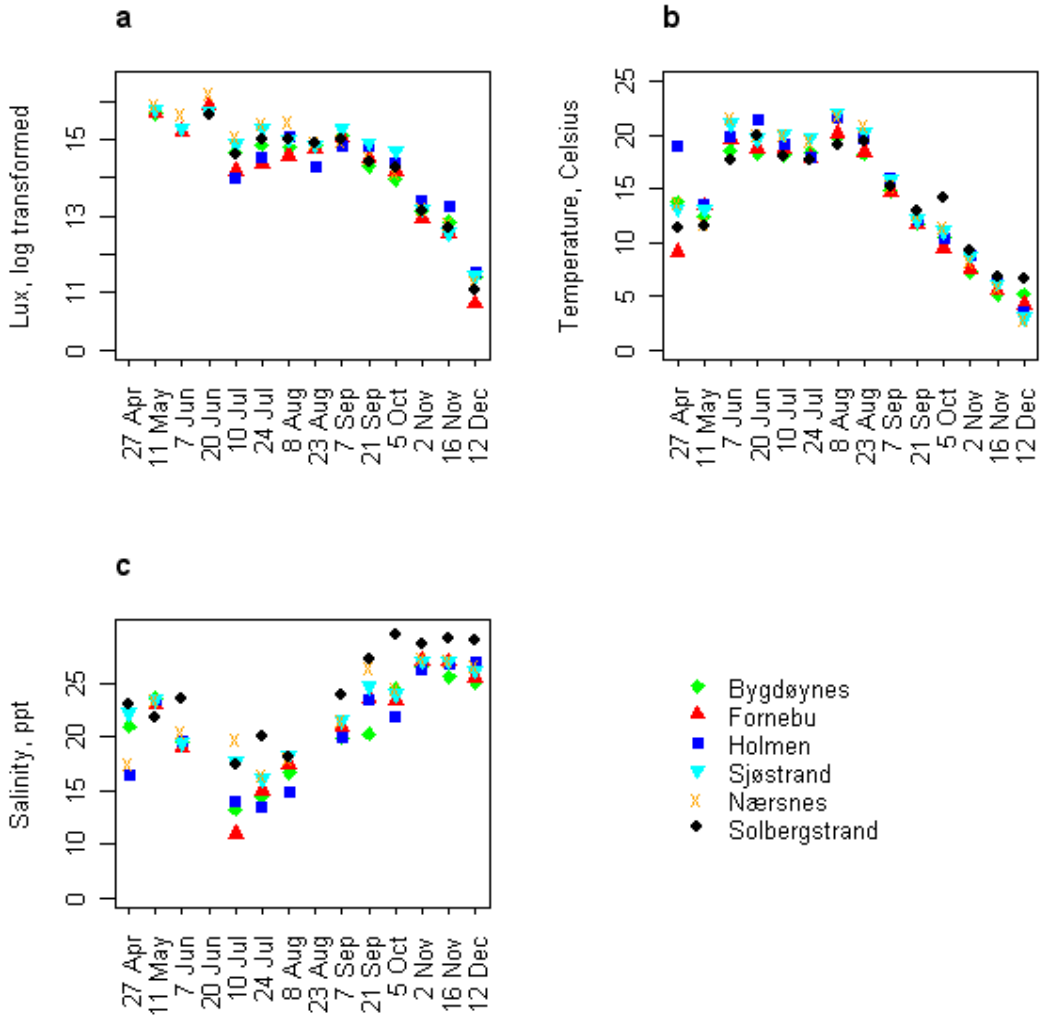

Figure 3.10 a) Integrated light intensity (Lux) from the 14 days prior to sampling. Note natural log-scale and scale break on y-axis. **b)** Measured temperature at each sampling day. **c)** Measured salinity at each sampling day, except 20 June and 23 August. Note scale break on y-axis.

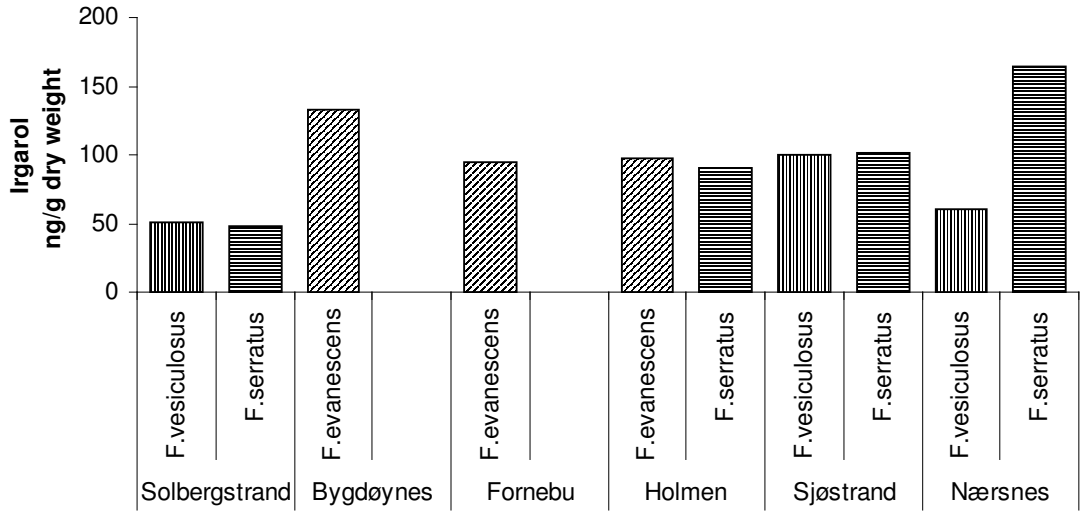

Figure 3.11 The concentration of Irgarol 1051, ng g^{-1} dry weight, in samples of *Fucus vesiculosus, F. evanescens and F. serratus* from the six field sites. The samples were collected 5 October.

3.2.3 Fucus evanescens

Photosynthetic efficiency (Fv/Fm) and quinone pool were measured in *Fucus evanescens* at Bygdøynes, Fornebu and Holmen from 27 April to 12 December, but due to instrument malfunction Bygdøynes data was omitted on 12 December. Photosynthetic efficiency varied from 0.82, measured 2 November, to 0.15, measured 8 August (figure 3.12a). Values were generally higher in samples from Bygdøynes compared to the other two sites and were significantly higher than one or both other sites at all dates, except 7 June, 10 July, 7 September and 2 and 16 November (Table 3.1). Samples at Fornebu were higher than at Holmen on two dates, 27 April and 20 June, and on 12 December photosynthetic efficiency was significantly higher at Holmen than at Fornebu. Samples from different sites did however exhibit some similarities. Photosynthetic efficiency (median Fv/Fm values in parentheses) was high at Bygdøynes (0.74) and Fornebu (0.69) at the first measurement, but decreased to 0.57 and 0.50 respectively on 7 June. Values at Holmen were 0.57 and 0.53 on these dates. A peak in photosynthetic efficiency on 10 July at Fornebu (0.70) and Holmen (0.64) was followed by the lowest values (0.36 and 0.28 respectively) during the whole survey on 8 August. At Bygdøynes the highest value during summer was measured on 24 July (0.75), and although there was a decrease on 8 August (0.68) it did not match the values at Fornebu and Holmen. Lowest value at Bygdøynes was measured on 7 September (0.48), but it returned to

0.71 already 21 October. Photosynthetic efficiency increased at all sites during autumn before reaching a plateau on 2 November with values around 0.8 at all sites.
Quinone pool values varied from 0 (on several dates) to 67000 on 12 December and follow some of the same trends as photosynthetic efficiency during the survey (figure 3.12b), though there were fewer dates with significant difference between quinone pool samples from different sites compared to photosynthetic efficiency (Table 3.1). Samples from Bygdøynes were generally higher than the other sites. The two fluorescence parameters correlate well (Spearman's rho= 0.86, 0.84 and 0.85 for Bygdøynes, Fornebu and Holmen respectively). Quinone pool in samples (median value in parentheses) from Bygdøynes and Fornebu started high (9300 and 8400 respectively) while in samples from Holmen quinone pool were low (2300). The values were low at the sites over the next three sampling dates before an increase 10 July and a peak 24 July. The peak was higher at Bygdøynes (14100) than at Fornebu (4400) and Holmen (5800). On 8 August quinone pool were once again low, though samples from Bygdøynes (2400) were not as low as from Fornebu (400) or Holmen (300). Samples at Holmen increased 23 August (3100) and stayed on that level the next three measurements, and on the last three measurements quinone pool increased to 23200 on 12 December. Quinone pool in Fornebu samples increased stepwise during the autumn: on 23 August (2300), 21 September (5700), 2 November (16300) and 12 December (25000). The same stepwise increase was seen in Bygdøynes samples, though quinone pool was significantly higher 21 September (10600). Data from Bygdøynes on 12 December was omitted due to instrument malfunction. The plateau seen in photosynthetic efficiency on the last three dates was not seen in quinone pool samples.

3.2.2 Fucus vesiculosus

Photosynthetic efficiency and quinone pool were measured in *Fucus vesiculosus* at Sjøstrand, Nærsnes and Solbergstrand from 27 April to 12 December. Photosynthetic efficiency (Fv/Fm) varied from 0.16 measured at Nærsnes on 27 April to 0.84 measured at Solbergstrand on 16 November (Figure 3.13a). There was significant difference in photosynthetic efficiency between the sites on ten dates (table 3.2). Solbergstrand samples were significantly higher than one or both other sites on nine of those dates, but on 7 June photosynthetic efficiency was significantly higher in samples from Sjøstrand and Nærsnes compared to samples from Solbergstrand.
On 27 April and 11 May photosynthetic efficiency (median Fv/Fm values in parantheses) were high in samples from Solbergstrand (0.67 and 0.66 respectively), it decreased to the

lowest point for Solbergstrand samples on 7 June (0.41), but values were high already the next sampling date (0.68). On the same dates did samples from Sjøstrand exhibit the exact opposite response in photosynthetic efficiency: low on 27 April (0.43) and 11 May (0.40), high on 7 June (0.62) and low again on 20 June (0.42). In samples from Nærsnes photosynthetic efficiency was low on 27 April (0.43) but increased to 0.62 on 11 May, before it gradually decreased to 0.54 on 20 June. Photosynthetic efficiency in samples from all sites increased to a peak (0.69, 0.66 and 0.76 at Sjøstrand, Nærsnes and Solbergstrand respectively) on 10 July. In samples from Sjøstrand and Nærsnes photosynthetic efficiency decreased over the three next dates, reaching a bottom 23 August (0.52 and 0.45 respectively). At Solbergstrand photosynthetic efficiency dropped on 24 July (0.50), but increased 8 August (0.71), before dropping again on 23 August (0.58). Photosynthetic efficiency increased in samples from all sites 7 September (0.73, 0.71 and 0.73 for Sjøstrand, Nærsnes and Solbergstrand respectively) and decreased only slightly to 5 October. On 2 November photosynthetic efficiency increased again and stayed at a level around 0.75 for Sjøstrand and Nærsnes samples and around 0.80 for Solbergstrand samples.

Quinone pool varied from 0 measured at all sites on several dates to 40400 measured at Nærsnes on 12 December (Figure 3.13b). Samples from Solbergstrand generally had higher values than at the other sites and were significantly higher than one or both other sites on six dates (Table 3.2). Sjøstrand and Nærsnes samples were significantly different on four dates, samples from Sjøstrand were significantly higher 10 July and 8 August, while samples from Nærsnes were significantly higher 11 May and 20 June.

Quinone pool (median values in parentheses) in samples from Solbergstrand had the same saw-toothed pattern during the survey as samples of photosynthetic efficiency, increasing and decreasing on the same dates, except that the quinone pool did not reach a plateau on the three last dates, but continued to increase (20800 on 12 December). Peaks where values were higher than previous or next measurement, on 27 April (6000), 10 July (4500), 8 August (3500) and 21 September (12100). Bottoms where values were lower than the previous and next measurement, on 7 June (200), 24 July (500), 23 August (1300) and 5 October (6200). Sjøstrand and Nærsnes samples had low quinone pool values from 27 April to 23 August (median between 200 – 1800 at Sjøstrand and 200 – 1700 at Nærsnes), except a peak at Sjøstrand on 10 July (6800) and a peak at Nærsnes on 11 May (4200). Quinone pool in samples was high from 7 September (8800 and 6600) to 5 October (8300 and 7300), before an increase over the three next measurements to 26500 and 24100 for samples at Sjøstrand and Nærsnes respectively.

Quinone pool in *Fucus vesiculosus* samples was highly correlated with photosynthetic efficiency at all sites (Spearman's rho= 0.89, 0.86 and 0.85 for Sjøstrand, Nærsnes and Solbergstrand samples respectively).

3.2.3 Fucus serratus

Photosynthetic efficiency and quinone pool was measured in *Fucus serratus* at Holmen, Sjøstrand, Nærsnes and Solbergstrand from 27 April to 12 December. On 11May and 10 July no samples were taken at Holmen due to high tide and poor visibility in the water. Photosynthetic efficiency varied from 0.19 measured at Nærsnes on 8 August to 0.83 measured at all sites on several dates (figure 3.14a). There was significant difference in photosynthetic efficiency between samples from different sites on all dates, except 16 November (Table 3.3). Photosynthetic efficiency was generally higher in samples from Solbergstrand and Holmen than in samples from Sjøstrand and Nærsnes. On 7 June, 24 July and 23 August photosynthetic efficiency were significantly higher in samples from Holmen than samples from Solbergstrand, while it was significantly higher at Solbergstrand 21 September. Photosynthetic efficiency (median Fv/Fm values in parentheses) in samples from Holmen on 27 April (0.56) was the lowest during the survey at the site. From 7 June to 5 October samples from Holmen varied between 0.73 (on 24 July and 5 October) and 0.63 (on 20 June and 21 September). The measurements at Solbergstrand varied more: From high values on 27 April (0.65) and 11 May (0.70), photosynthetic efficiency dropped on 7 June (0.38). From there it increased again, peaking 10 July (0.77). A new low was recorded 23 August (0.53) before returning to high values from 7 September (0.73) to 5 October (0.74). On 27 April photosynthetic efficiency in samples from Sjøstrand (0.27) and Nærsnes (0.56) were significantly different, and on 11 May it increased at Sjøstrand (0.53), but decreased at Nærsnes (0.38). The rest of the survey samples from Sjøstrand and Nærsnes followed the same pattern: A peak at 10 July (0.69 and 0.59 at Sjøstrand and Nærsnes respectively) was followed by a decrease to 8 August (0.45) at Nærsnes and to 23 August at Sjøstrand (0.40). Photosynthetic efficiency at the two sites increased to 21 September (0.73 and 0.70 Nærsnes and Sjøstrand respectively), followed by a decrease on 5 October (0.53 and 0.47 at Nærsnes and Sjøstrand respectively). The three last measurements photosynthetic efficiency in samples from all sites were around 0.8.

Quinone pool values varied from 0 measured in samples from Solbergstrand, Nærsnes and Sjøstrand on several dates, to 123400 measured in a sample from Holmen on 12 December (Figure 3.14b). There was significant difference between samples from the different sites on

ten of fourteen dates (Table 3.3). Quinone pool in samples from Holmen was significantly higher than samples from other sites on 6 dates, and significantly lower in none. Solbergstrand samples were significantly higher than samples from other sites on 7 dates and significantly lower on 3 dates (one of these dates overlap). Sjøstrand samples were significantly lower than samples from other sites on 10 dates, but on 4 of these dates also significantly higher than one other site. Nærsnes samples were significantly lower than samples from other sites on 9 dates, but on 3 of these dates also significantly higher than one other site. Compared to measurements of photosynthetic efficiency, the quinone pool values in samples from Holmen vary quite a bit during the survey. The lowest value measured at Holmen was on 27 April (5900), but on 7 June it was high (47400) before it dropped back on 20 June (7400). Quinone pool values were again high in samples from Holmen on 24 July (36900), and varied between 15800 and 23600 the next four dates, before returning to high values (> 40000) the rest of the survey. Quinone pool in samples from Sjøstrand, Nærsnes and Solbergstrand had a similar pattern during the survey, apart from high values on 11 May at Solbergstrand (19600) followed by very low values on 7 June (100) when values were low at Sjøstrand (2150 and 4300) and Nærsnes (900 and 4500). There was peak in quinone values on 10 July (44000, 35000 and 13100 at Solbergstrand, Sjøstrand and Nærsnes respectively), followed decrease to 8 august at Nærsnes (500) and to 23 August at Solbergstrand (7700) and Sjøstrand (3100). There was an increase towards values above 30000 at Nærsnes and above 40000 for Sjøstrand and Solbergstrand from 2 November, interrupted by a decrease on 5 October more pronounced on Sjøstrand (5000) and Nærsnes (14700) than Solbergstrand (23200). Quinone pool values in samples from Sjøstrand decreased on 12 December (33300). Quinone pool in *Fucus serratus* was highly correlated with photosynthetic efficiency at all sites (Spearman's rho= 0.72, 0.90, 0.89 and 0.77 in samples from Holmen, Sjøstrand, Nærsnes and Solbergstrand respectively).

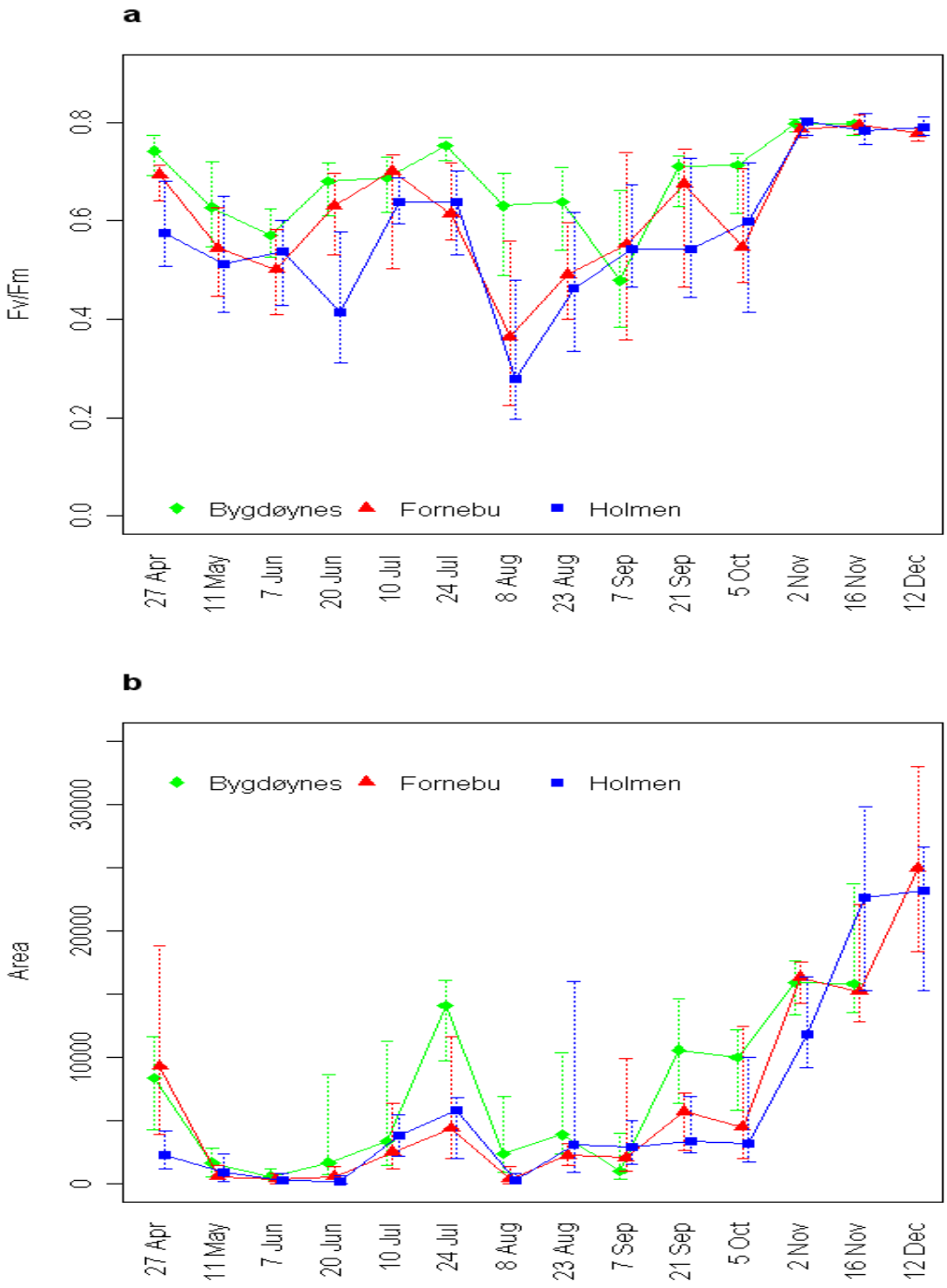

Figure 3.12 a) Mean photosynthetic efficiency and **b)** mean quinone pool for *Fucus evanescens* at Bygdøynes, Fornebu and Holmen from all sampling dates. Median and 10-90 percentiles. Data from Bygdøynes were omitted on 12 December.

Table 3.1 Statistical analysis used to test H_0, *p*-value, power of the test and sites as analysed by Tukey test for ANOVA or Mann-Whitney rank sum test for Kruskal-Wallis. Not mentioned site was not significantly different from other sites. Byg = Bygdøynes, For = Fornebu, Hol = Holmen. Photosynthetic efficiency on top half, quinone pool on bottom half. Statistically significant values in bold ($p < 0.05$).

Date	Statistical analysis	*p*	Anova test power	Tukey/Mann-Whitney
Photosynthetic efficiency				
27 Apr	Kruskal-Wallis	**< 0.0001**		**Byg > For > Hol**
11 May	ANOVA	**< 0.05**	0.61	**Byg > Hol**
7 Jun	ANOVA	0.12	0.40	
20 Jun	ANOVA	**< 0.0001**	0.96	**Byg, For > Hol**
10 Jul	ANOVA	0.49	0.66	
24 Jul	Kruskal-Wallis	**< 0.001**		**Byg > For, Hol**
8 Aug	ANOVA	**< 0.0001**	0.94	**Byg > For, Hol**
23 Aug	ANOVA	**< 0.01**	0.79	**Byg > For, Hol**
7 Sep	ANOVA	0.49	0.16	
21 Sep	Kruskal-Wallis	**< 0.05**		**Byg > Hol**
5 Oct	Kruskal-Wallis	**< 0.05**		**Byg > For, Hol**
2 Nov	ANOVA	0.14	0.37	
16 Nov	ANOVA	0.59	0.13	
12 Dec	Kruskal-Wallis	**< 0.0001**		**Hol > For**
Quinone pool				
27 Apr	Kruskal-Wallis	**< 0.001**		**Byg, For > Hol**
11 May	Kruskal-Wallis	0.08		
7 Jun	ANOVA	0.1	0.42	
20 Jun	Kruskal-Wallis	**< 0.001**		**Byg > For > Hol**
10 Jul	Kruskal-Wallis	0.52		
24 Jul	ANOVA	**< 0.0001**	0.94	**Byg > For, Hol**
8 Aug	Kruskal-Wallis	**<0.001**		**Byg > For, Hol**
23 Aug	Kruskal-Wallis	0.09		
7 Sep	Kruskal-Wallis	0.07		
21 Sep	ANOVA	**< 0.0001**	0.93	**Byg > For, Hol**
5 Oct	ANOVA	0.08	0.45	
2 Nov	ANOVA	**< 0.01**	0.71	**Byg, For > Hol**
16 Nov	ANOVA	**< 0.05**	0.52	Hol > For (p = 0.06)
12 Dec	Kruskal-Wallis	**< 0.0001**		**For, Hol > Byg**

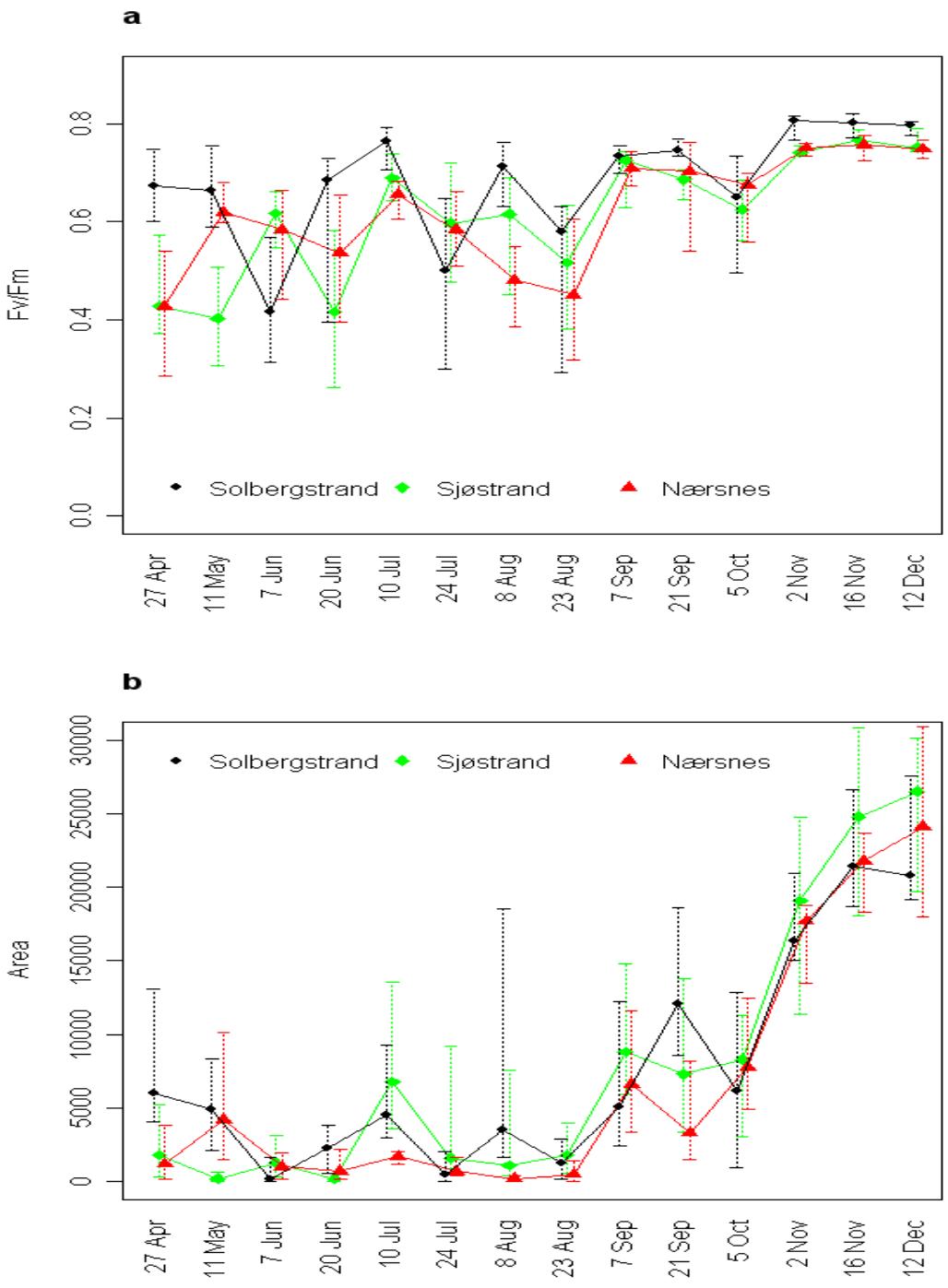

Figure 3.13 a) Mean photosynthetic efficiency (Fv/Fm) and **b**) mean quinone pool for *Fucus vesiculosus* at Solbergstrand, Sjøstrand and Nærsnes on all sampling dates. Stapled lines are 10-90 percentile values.

Table 3.2 Statistical analysis used to test H_0, *p*-value, power of the test and sites as analysed by Tukey test for ANOVA or Mann-Whitney rank sum test for Kruskal-Wallis. Not mentioned site was not significantly different from other sites. Sol = Solbergstrand, Sjø = Sjøstrand, Nær = Nærsnes. Photosynthetic efficiency on top half, quinone pool on bottom half. Statistically significant values in bold ($p < 0.05$).

Date	Statistical analysis	*p*-value	Anova test power	Tukey/Mann-Whitney
Photosynthetic efficiency				
27 Apr	ANOVA	**< 0.0001**	0.96	**Sol > Sjø, Nær**
11 May	Kruskal-Wallis	**< 0.0001**		**Sol, Nær > Sjø**
7 Jun	Kruskal-Wallis	**< 0.01**		**Sjø, Nær > Sol**
20 Jun	ANOVA	**< 0.05**	0.58	**Sol > Sjø**
10 Jul	ANOVA	**< 0.0001**	0.94	**Sol > Sjø, Nær**
24 Jul	ANOVA	0.07	0.47	
8 Aug	ANOVA	**< 0.001**	0.89	**Sol, Sjø > Nær**
23 Aug	ANOVA	0.59	0.13	
7 Sep	ANOVA	0.35	0.22	
21 Sep	Kruskal-Wallis	**< 0.01**		**Sol > Sjø, Nær**
5 Oct	ANOVA	0.70	0.10	
2 Nov	Kruskal-Wallis	**< 0.0001**		**Sol > Sjø, Nær**
16 Nov	ANOVA	**< 0.001**	0.89	**Sol > Sjø, Nær**
12 Dec	ANOVA	**< 0.0001**	0.93	**Sol > Sjø, Nær**
Quinone pool				
27 Apr	Kruskal-Wallis	**< 0.01**		**Sol > Sjø, Nær**
11 May	Kruskal-Wallis	**< 0.0001**		**Sol, Nær > Sjø**
7 Jun	Kruskal-Wallis	0.11		
20 Jun	Kruskal-Wallis	**< 0.01**		**Sol, Nær > Sjø**
10 Jul	Kruskal-Wallis	**< 0.0001**		**Sol, Sjø > Nær**
24 Jul	Kruskal-Wallis	0.06		
8 Aug	Kruskal-Wallis	**< 0.05**		**Sol, Sjø > Nær**
23 Aug	ANOVA	0.12	0.40	
7 Sep	ANOVA	0.15	0.36	
21 Sep	ANOVA	**< 0.001**	0.86	**Sol > Nær**
5 Oct	ANOVA	0.43	0.19	
2 Nov	Kruskal-Wallis	0.67		
16 Nov	ANOVA	0.32	0.23	
12 Dec	ANOVA	0.28	0.26	

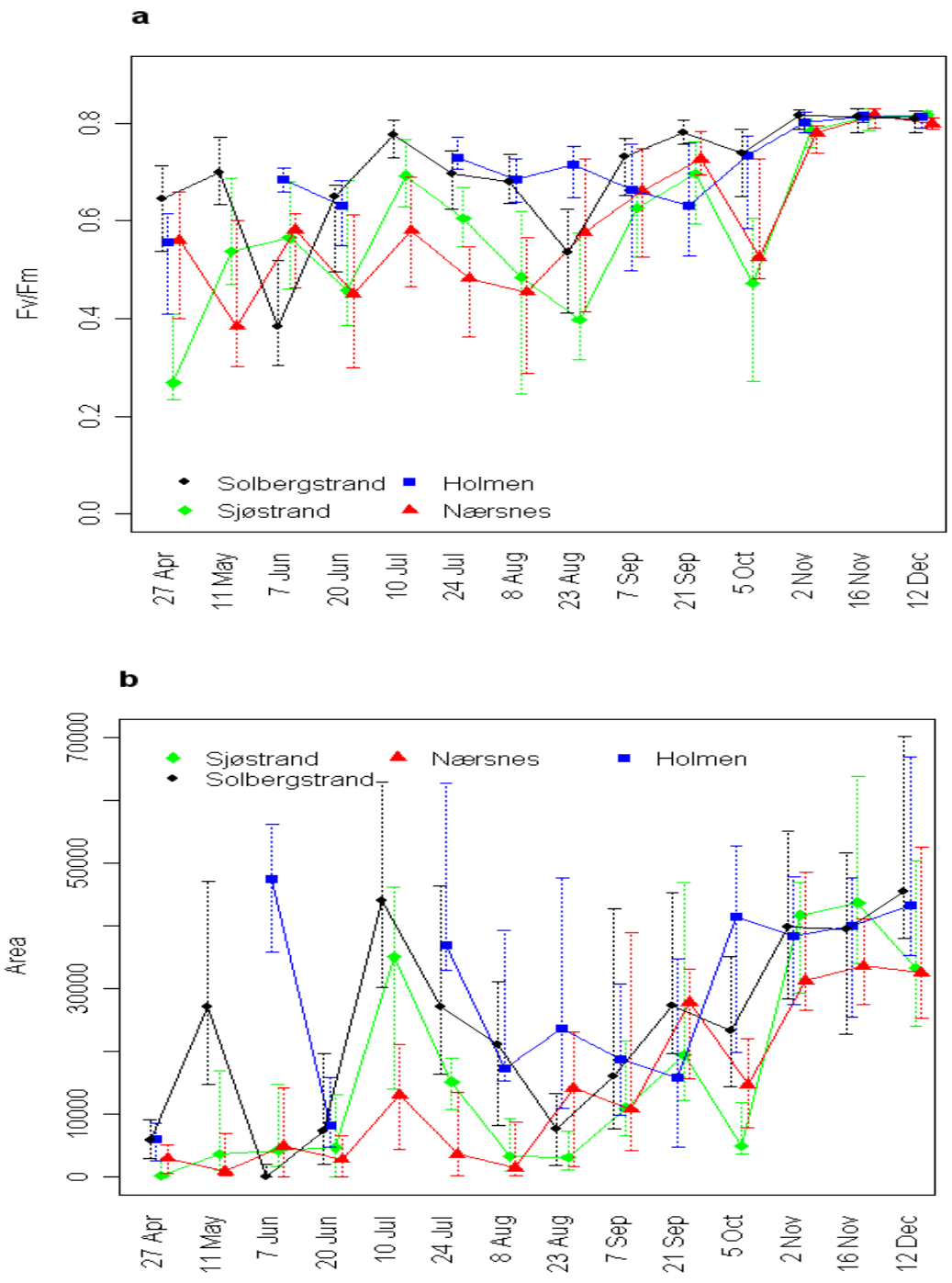

Figure 3.14 a) Mean photosynthetic efficiency, Fv/Fm, and **b)** mean quinone pool (Area) for *Fucus serratus* at Solbergstrand, Holmen, Sjøstrand and Nærsnes on all sampling dates. Stapled lines are 10-90 percentile values. Data are missing for Holmen on 11 May and 10 July.

Table 3.3 Statistical analysis used to test H_0, *p*-value, power of the test and sites as analysed by Tukey test for ANOVA or Mann-Whitney rank sum test for Kruskal-Wallis. Not mentioned site was not significantly different from other sites. Sol = Solbergstrand, Sjø = Sjøstrand, Nær = Nærsnes, Hol= Holmen. Photosynthetic efficiency on top half, quinone pool on bottom half. Statistically significant values in bold ($p < 0.05$).

Date	Statistical analysis	*p*-value	Anova test power	Tukey/Mann-Whitney
Photosynthetic efficiency				
27 Apr	ANOVA	**< 0.0001**	0.97	**Sol, Hol, Nær > Sjø**
11 May	ANOVA	**< 0.0001**	0.95	**Sol > Sjø > Nær**
7 Jun	ANOVA	**< 0.0001**	0.98	**Hol > Nær > Sol and Sjø > Sol**
20 Jun	ANOVA	**< 0.01**	0.81	**Sol, Hol > Nær**
10 Jul	Kruskal-Wallis	**< 0.0001**		**Sol, Sjø > Nær**
24 Jul	Kruskal-Wallis	**< 0.0001**		**Hol > Sol > Sjø > Nær**
8 Aug	Kruskal-Wallis	**< 0.0001**		**Sol, Hol > Sjø, Nær**
23 Aug	Kruskal-Wallis	**< 0.001**		**Hol > Nær > Sjø and Hol > Sol**
7 Sep	ANOVA	**< 0.05**	0.58	**Sol > Sjø**
21 Sep	Kruskal-Wallis	**< 0.001**		**Sol > Nær > Hol and Sol > Sjø**
5 Oct	ANOVA	**< 0.0001**	0.97	**Sol, Hol > Sjø, Nær**
2 Nov	ANOVA	**< 0.01**	0.79	**Sol, Hol > Nær**
16 Nov	ANOVA	0.94	0.07	
12 Dec	Kruskal-Wallis	**< 0.05**		**Sjø > Nær**
Quinon pool				
27 Apr	ANOVA	**< 0.001**	0.93	**Sol > Nær, Sjø and Hol > Sjø**
11 May	Kruskal-Wallis	**< 0.001**		**Sol > Sjø > Nær**
7 Jun	Kruskal-Wallis	**< 0.001**		**Hol > Sjø, Nær > Sol**
20 Jun	Kruskal-Wallis	**< 0.05**		**Hol > Sjø, Nær**
10 Jul	ANOVA	**< 0.0001**	0.93	**Sol > Sjø > Nær**
24 Jul	Kruskal-Wallis	**< 0.0001**		**Hol > Sol > Sjø > Nær**
8 Aug	Kruskal-Wallis	**< 0.0001**		**Sol, Hol > Sjø, Nær**
23 Aug	Kruskal-Wallis	**< 0.01**		**Hol > Sjø, Sol and Nær > Sjø**
7 Sep	ANOVA	0.44	0.23	
21 Sep	ANOVA	0.19	0.38	
5 Oct	Kruskal-Wallis	**< 0.0001**		**Sol, Hol > Nær > Sjø**
2 Nov	ANOVA	0.53	0.20	
16 Nov	ANOVA	**0.05**	0.57	**Sjø > Nær**
12 Dec	Kruskal-Wallis	**< 0.05**		**Sol > Sjø, Nær**

3.2.4 Relationship between fluorescence parameters and abiotic factors

Concentration of Irgarol 1051 were analysed for samples taken on 5 October, but there was little relationship between photosynthetic efficiency or quinone pool and concentration of Irgarol in the samples (Figure 3.15). Spearman's rho was 0.44 for *F. evanescence*, -0.18 for *F. vesiculosus* (not significant $p > 0.05$) and -0.62 for *F. serratus*.

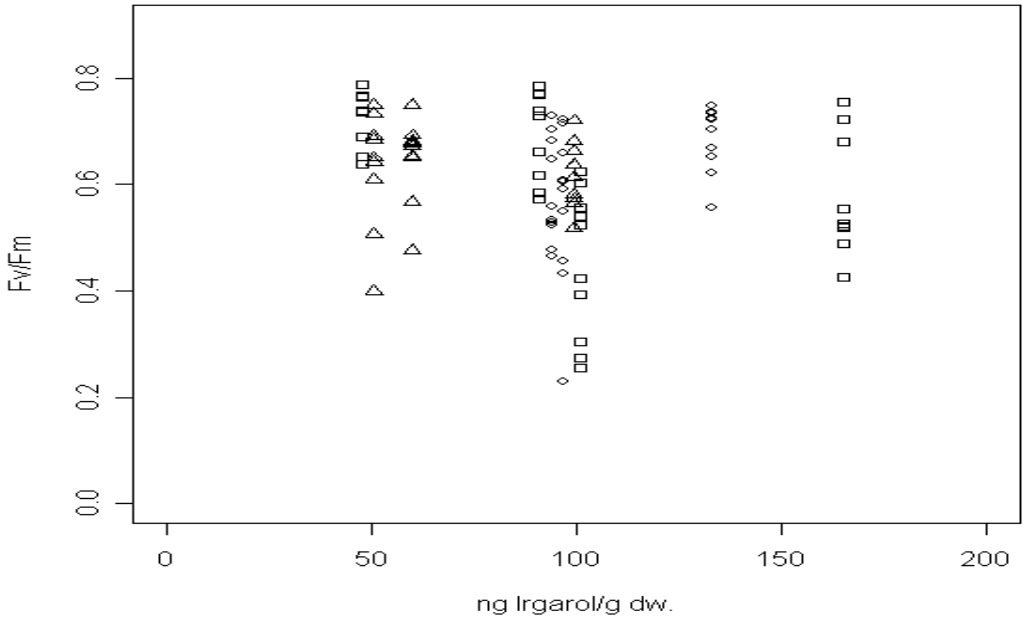

Figure 3.15 Relationship between photosynthetic efficiency (Fv/Fm) on 5 October and the concentration of Irgarol in *F. evanescens* (Circles), *F. serratus* (Squares), and *F. vesiculosus* (Triangles).

Relationships between photosynthetic efficiency and light intensity, temperature and salinity in *F. evanescens*, *F. serratus* and *F. vesiculosus* are presented in figure 3.16. Samples were negatively correlated with light intensity parameters and temperature, and positively correlated with salinity (table 3.4). Photosynthetic efficiency in *F. evanescens* was most correlated with salinity at Bygdøynes, temperature at Fornebu, and equally high with temperature and 14 days integral of light intensity at Holmen. Quinone pool were highest correlated with 14 days integral of light intensity at all these sites. In *F. serratus* photosynthetic efficiency was most correlated with 14 days integral of light intensity at Holmen and Nærsnes, and with average light intensity at Solbergstrand. Quinone pool was most correlated with average light intensity at all sites, though it was equally high with light intensity at Holmen. Photosynthetic efficiency In *F. vesiculosus* was most correlated with light intensity at Sjøstrand, salinity at Nærsnes and average light intensity at Solbergstrand. Quinone pool was most correlated with 14 days integral of light intensity at Sjøstrand,

temperature at Nærsnes and average light intensity at Solbergstrand. Correlation with salinity and temperature varied quite much between the sites, while correlation with light intensity parameters, except light intensity prior to measurement, were more similar.

Relationship between estimated photon flux density (PFD) and photosynthetic efficiency for each species on every date is presented in Appendix 2. There was significant negative correlation between PFD and photosynthetic efficiency in *F. serratus* on 27 April, 20 June, 24 July, 8 August, 23 August and 5 October. In *F. vesiculosus* on 27 April, 11 May and 8 August and in *F. evanescens* on 21 September. But this corresponds to observed relationship between sites only on 20 June, 23 August and 5 October for *F. serratus* (Table 3.3) and on 8 August for *F. vesiculosus* (Table 3.2).

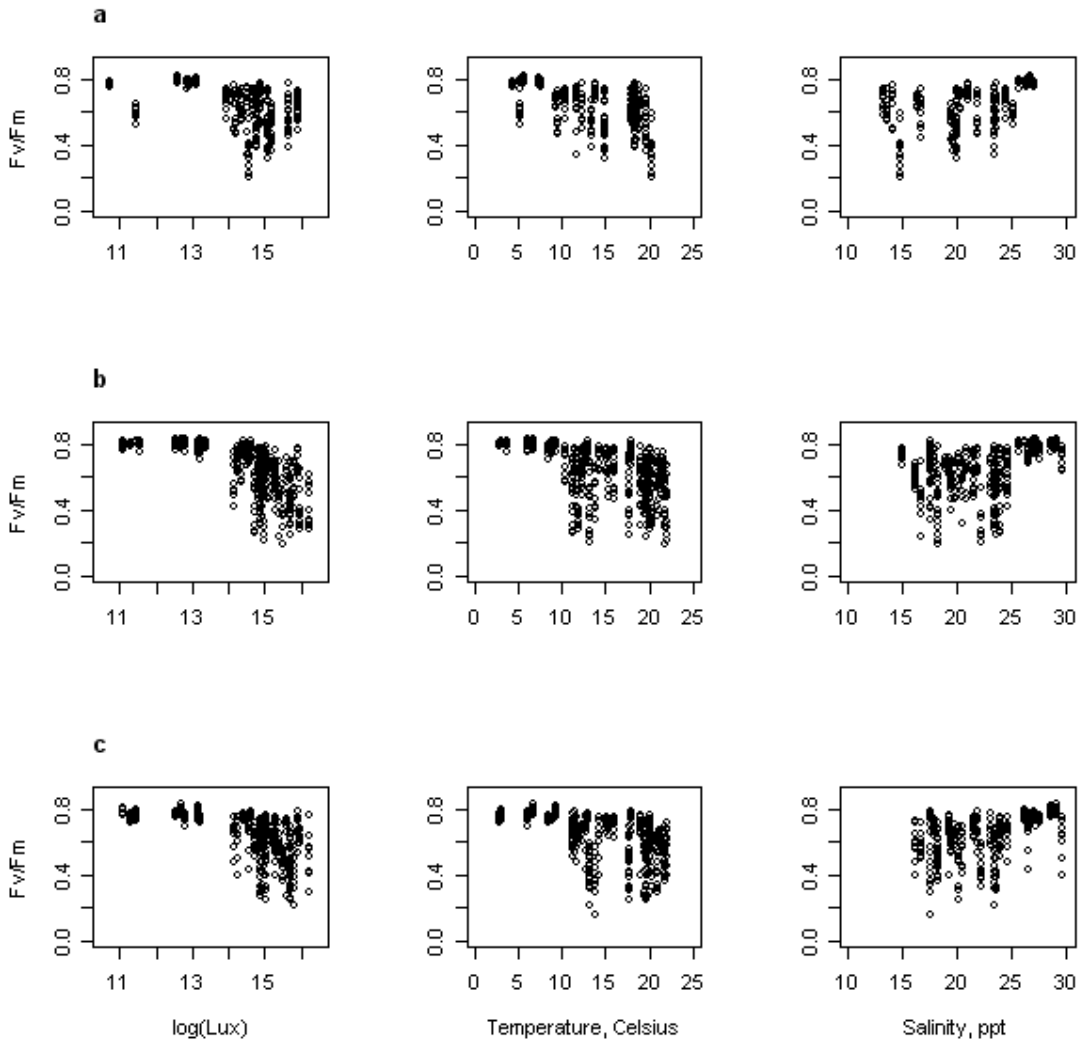

Figure 3.16 Relationship between photosynthetic efficiency and from left to right log transformed integral light intensity from 14 days prior to the measurement, temperature and salinity in a) *F. Evanescens*, b) *F.serratus* and c) *F. vesiculosus.*

Table 3.4 Relationship, expressed as Spearman's rho, between various abiotic factors and photosynthetic efficiency and quinone pool (in parentheses)

Site, Species	Light intensity	Average light intensity last 3 hours	Integral light intensity day of measurement	Integral light intensity last 14 days	Temperature	Salinity
F. Evanescens						
Bygdøy	-0.47 (-0.65)	-0.45 (-0.66)	-0.43 (-0.63)	-0.55 (-0.68)	-0.48 (-0.52)	0.61 (0.64)
Fornebu	-0.48 (-0.60)	-0.46 (-0.58)	-0.47 (-0.62)	-0.64 (-0.80)	-0.68 (-0.82)	0.53 (0.53)
Holmen	-0.53 (-0.67)	-0.51 (-0.70)	-0.50 (-0.70)	-0.69 (-0.83)	-0.69 (-0.77)	0.48 (0.53)
F. Serratus						
Holmen	-0.69 (-0.48)	-0.68 (-0.48)	-0.60 (-0.38)	-0.71 (-0.39)	-0.63 (-0.42)	0.69 (0.36)
Sjøstrand	.0.64 (-0.69)	-0.65 (-0.70)	-0.57 (-0.61)	-0.51 (-0.55)	-0.53 (-0.50)	0.51 (0.43)
Nærsnes	-0.52 (-0.59)	-0.70 (-0.77)	-0.69 (-0.75)	-0.75 (-0.79)	-0.58 (-0.57)	0.71 (0.72)
Solbergstrand	-0.49 (-0.39)	-0.84 (-0.74)	-0.81 (-0.70)	-0.72 (-0.52)	-0.62 (-0.42)	0.45 (0.13)
F. Vesiculosus						
Sjøstrand	-0.77 (-0.72)	-0.73 (-0.71)	-0.68 (-0.70)	-0.70 (-0.79)	-0.49 (-0.61)	0.49 (0.60)
Nærsnes	-0.59 (-0.60)	-0.75 (-0.74)	-0.68 (-0.68)	-0.65 (-0.71)	-0.67 (-0.84)	0.77 (0.81)
Solbergstrand	-0.49 (-0.46)	-0.73 (-0.69)	-0.63 (-0.68)	-0.64 (-0.67)	-0.58 (-0.68)	0.34 (0.51)

4 Discussion

The purpose of the experiments, the toxicity experiments and the field observations was to determine if measurements of photosynthetic efficiency are a good indicator of pollutants harmful to the photosynthetic apparatus.

4.1 Laboratory experiments

In three experiments fronds of *Fucus vesiculosus* was exposed to different irradiances, salinities, and temperatures. Photosynthetic efficiency was not affected by the irradiances chosen in the experiment, though it decreased slightly during the experiment in fronds exposed to the two highest irradiances, but not in fronds exposed to the two lowest. The lamps used gave of some heat, and higher temperature and higher salinity due to evaporation might have affected the samples. *Fucus serratus* fronds follow hyperbolic photosynthesis-irradiances curves, reaching saturation below 160 µmol m^{-2} s^{-1} (Binzer and Middelboe 2005) and similar values are reported for *F. vesiculosus* in King and Schramm (1976).
Photoinhibition occurs where irradiances are greater than the photosynthetic saturation. The irradiances used (highest 86 µmol m^{-2} s^{-1}) were far below the reported saturation rate, and thus photoinhibition with all probability did not occur. This makes photoinhibition a little plausible cause for differences observed in the other lab studies, but the irradiances were too low to say anything about field conditions.
The salinity experiment was done with only three replicates and thus the results should be viewed with caution. Photosynthetic efficiency was higher in low salinities than high. But the results does not show a dose-response relationship, fronds growing in salinity 24 were lower than those growing in 36, which were similar to those growing in 12 and 18. Literature suggest optimum salinity for photosynthesis between 12 – 34 for atlantic fucoids (Chapman 1995).
Quinone pool increased in the irradiance and temperature experiment, this could be an adaption to lower irradiances as the bladder wrack was harvested in June (*in situ* quinone pool measurements were very low). In the booster biocide experiments quinone pool also increased, but only in the *F. serratus* samples.
There were no irradiance-response relationship between the differences observed in quinone pool in the irradiance experiment, and the difference could be explained as a coincident and would probably have disappeared with more replicates. Compared to the increase in quinone pool in the temperature experiment, quinone pool reached high values quicker in the high

temperature. Synthesis of proteins is usually accelerated at higher temperatures (Falkowski and Raven 2007). For quinone pool measurements to be useful in experiments longer acclimation is needed compared to photosynthetic efficiency.

Fronds of *Fucus vesiculosus* and *F. serratus* were exposed to Irgarol 1051, zinc pyrithione and Zineb for 72 hours. Both photosynthetic efficiency and quinone pool in fronds exposed to the highest concentration Irgarol was significant different from the controls and the other treatments for both species. Photosynthetic efficiency in *F. vesiculosus* fronds exposed to the second highest concentration 10 µg L^{-1} was significant lower than the other Irgarol treatments, but not significant lower than the controls. There was no significant difference between quinone pool measurements for fronds of both species exposed to lower concentrations. Brack and Frank (1998) investigated the fluorescent pattern of a triazine herbicide (Simazine) and found that fluorescent emission quickly rose to the same plateau as the control, which would result in little difference in photosynthetic efficiency, but indicates reduced quinone pool. In this experiment the photosynthetic efficiency of fronds exposed to highest Irgarol concentration did not go below 50% of the control, but the quinone pool was reduced 100 fold compared to the control. Surprisingly was reduction of quinone pool not seen at the second lowest concentration of Irgarol. The quinone pool increased in all treatments during the experiment in *F. vesiculosus*, but not in *F. serratus*. In a similar unpublished experiment with *Fucus vesiculosus* (Thomas, unpublished) photosynthetic efficiency effects were seen after 72 hours at 10 µg L^{-1} Irgarol, and after 14 days at 3.3 µg L^{-1}. Diuron, metabolites of diuron and Irgarol, and a fungicide, TCMTB, was also tested in this experiment, with toxicity to photosynthetic efficiency being Irgarol > Diuron > metabolites > TCMTB. Scarlett *et al.* (1999) observed significant effects in marine seagrass, *Zostera marina*, at 0.18 µg L^{-1} and calculated a 10-day EC_{50} value of 2.5 µg L^{-1}. Photosynthetic efficiency was negatively reduced by Irgarol in green macroalgae, *Enteromorpha intestinalis*, with a 72 hour EC_{50} of 2.5 µg L^{-1} (Scarlett et al. 1997). Photosynthetic efficiency of a freshwater macrophyte, *Chara vulgaris*, was very sensitive to Irgarol, 14d EC_{50} of 17 ng L^{-1} and NOEC less than 0.05 ng L^{-1} (Lambert et al. 2006). In the same study the photosynthetic efficiency of two other freshwater plants were largely unresponsive to both Irgarol and diuron, and growth rate measurements were more sensitive to toxicity. No effect concentrations of Irgarol to photosynthetic efficiency in *F. serratus* and *F. vesiculosus* were much higher than any observed environmental concentration (Konstantinou and Albanis 2004). The diuron NOEC in *F.*

vesiculosus from Thomas (unpublished) was higher than the greatest reported phytoxicity of Thames river, 180 ng L^{-1} (Nash et al. 2006).

Photosynthetic efficiency was not affected by the two other booster biocides tested. There was a decrease in photosynthetic efficiency for all treatments on day 3 compared to day 0, though only significant for some. This was surprising as the fronds were kept under same conditions as the fronds exposed to Irgarol, where there was no such decrease in photosynthetic efficiency. Most likely there were differences in irradiance, although this was not the case on day 0, but not checked later in the experiment.

There is little phytotoxicity data for Zineb, and perhaps of no surprise that photosynthetic efficiency or quinone pool is not affected. It is however one of the most widely used biocides, together with zinc pyrithione, in modern antifouling paint (Almeida et al. 2007). ZPT is quickly broken down in sunlight, with a $T_{1/2}$ of < 1 hour (Thomas et al. 2001), and a differently designed experiment, with measurement quickly after exposure, would perhaps have seen a reduction in photosynthetic efficiency.

4.2 Field survey

A survey of 6 sites in the Oslo fjord was conducted from late April to medio December in 2007. The different species show some similarity in response during the sampling period, photosynthetic efficiency were quite low during summer, except a peak in around 10 or 24 July, and photosynthetic efficiency gradually rose to a plateau in November. Quinone pool measurements were correlated with photosynthetic efficiency measurements. But at some sites photosynthetic efficiency were almost consistently significantly higher. The quite large variation in photosynthetic efficiency and quinone pool can be due to the method of sampling, shade adapted plants have higher photosynthetic efficiency and quinone pool compared to light adapted (Chapman 1995). Both photosynthetic efficiency and quinone pool in *Fucus evanescence* were often higher at Bygdøynes compared to Fornebu and Holmen. In *Fucus vesiculosus* they were often higher at Solbergstrand compared to Sjøstrand and Nærsnes. In *Fucus serratus* photosynthetic efficiency and quinone pool were often higher at Holmen, and to a lesser degree at Solbergstrand compared to Sjøstrand and Nærsnes. Why were there significant differences between samples from different sites, and why were this difference quite consistent?

Pooled data for each site were compared to four different estimations of irradiance, temperature and salinity. These parameters were quite highly correlated with photosynthetic

efficiency and quinone pool at most sites, but no single parameter was highest correlated at all sites.

The temperature experiment was carried out in temperatures observed during the field study. Significant difference between the treatments in the temperature experiment, and thus the correlation seen with temperature and photosynthetic efficiency in the field experiment can probably be attributed to the fact that temperature and 14 days integral light intensity is also highly correlated. One exception might be the increasing photosynthetic efficiency during winter as in low temperatures there tends to be a reduction in light absorption capacity and increase in photosynthetic capacity (Davison 1991). This was not observed in the temperature experiment, but the temperatures used were perhaps not low enough.

Fucus vesiculosus from the Irish Sea, adapted to salinities around 35 did not recover from photoinhibition when kept in salinities < 10. At salinities > 20 there were almost 100 % recovery in photosynthetic efficiency (Nygard and Dring 2008). *F. vesiculosus* from the Baltic sea, adapted to low salinities had a rate of recovery around 80 % in both high and low salinity. Temperature and salinity can affect photosynthetic efficiency, but probably not alone, as indicated by references above and laboratory experiments.

High light intensity over time is perhaps a more likely reason why photosynthetic efficiency changes from measurement to measurement. In a simulated diurnal cycle with sugar kelp (*Laminiaria saccharina*) significant differences in photosynthetic efficiency were seen already at 116 µmol $m^{-2}s^{-1}$ and at the highest irradiance photosynthetic efficiency was reduced by almost 70 % compared to pre dawn values (Gevaert et al. 2002). The photosynthetic efficiency did not fully recover to pre dawn values after a gradual decrease in irradiance and a 12-hour dark period. Lack of recovery in photosynthetic efficiency after photoinhibition can be a sign of photodamage. In this survey an integral of light intensity the last 14 days was calculated, as this uses all the data between measurements. Low irradiances seen prior to 10 and 24 July can explain the peak in photosynthetic efficiency and quinone pool seen for most sites. But the differences between the sites are not accounted for, there was no consistent relationship between sites having the highest light intensity over time and lower photosynthetic efficiency. Photoinhibition is detected rapidly as a drop in photosynthetic efficiency after high irradiances, but almost immediately after irradiance is reduced the photosynthetic efficiency recovers (Huppertz et al. 1990; Raven and Samuelsson 1988). We should expect a strong negative correlation with irradiance measured just before the sampling Still there was a clear negative correlation between irradiance and photosynthetic efficiency

only on a few dates. Some dates with high irradiances (over 160 µmol m^{-2} s^{-1}) even had a positive correlation between irradiance and photosynthetic efficiency.
Other factors which might affect photosynthetic efficiency such as nitrogen limitation during summer or changes in physiology due to life cycle (Chapman 1995) have not been investigated.

Is photosynthesis inhibitors the reason for the observed differences between the sites? If Irgarol is used as a proxy of biocide pollution from antifouling paint it is clear that the relative cleanness was Solbergstrand > Sjøstrand > Fornebu, Holmen, Sjøstrand > Nærsnes >Bygdøynes. This corresponds with the relative photosynthetic efficiency between sites for *F. vesiculosus*, but not for *F. evanescens* and only to a lesser degree for *F. serratus*. Even though Irgarol is persistent (Thomas 2001) and bioconcentrates (Scarlett et al. 1999), the concentrations of Irgarol itself in seawater is probably low, but probably not below toxicity thresholds for sensitive species (Scarlett et al. 1997; Thomas et al. 2001) and thus the concentration could be of toxicological relevance close to marinas (van Wezel 2004). Pulse exposure of herbicides, which is normal in rivers after rainfall (Ludvigsen and Lode 2008), can perhaps explain why photosynthetic efficiency sank in *Fucus* from Solbergstrand on 7 June, but increased at other sites. But ecological relevance of such a drop is small as photosynthetic efficiency quickly recovers after the stressor is removed or diluted (Snel et al. 1998; Vallotton et al. 2008).

While photosynthetic efficiency (Fv/Fm) is a measure of maximum quantum efficiency, it does necessarily say much about growth in a plant. Many other factors more important in realized biomass like desiccation, grazing, wave exposure and ice scouring (Mann 1982). But small changes in photosynthetic efficiency can have significant changes on community structure. Dahl and Blanck (1996) observed changes in community structure and photosynthetic activity at the same concentration of Irgarol, while biomass changes was seen at higher concentration. However, in a benthic microalgae and meiofauna community study by Alsterberg *et al.* (2007) light utilization efficiency (proportion of light actively used in photosynthesis) was reduced by booster biocide copper pyrithione, but had no effect on community structure or biomass. Scarlett *et al.* (1999) were concerned that marine mammal dugong and green turtle, who's diet mainly consists of marine seagrass (*Zoestera marina*), might be at risk from concentrations of Irgarol that lowers photosynthetic efficiency in *Z. marina* by 15 %. Other stages in the life cycle of *Fucus* spp. are more sensitive to pollutants

(Braithwaite and Fletcher 2005; Eklund and Kautsky 2003; Scanlan and Wilkinson 1987) and if pollution concentration peaks around fertilization or germination recruitment can be adversely affected (Kautsky et al. 1992). Recently a rapid PSII inhibitor assay were developed. Photosynthetic efficiency analysis in microalgae exposed to water samples from the Brisbane river could account for almost all PSII inhibitors in the river. This was confirmed by water analysis for selected herbicides and detailed dose-response experiments with the same herbicides. Detection limit was 2.3 ng L^{-1} diuron, and highest detected concentrations were 190 ng L^{-1} diuron equivalents (Nash et al. 2006).

5 Conclusion

Measuring photosynthetic efficiency in brown macro algae *in situ* is probably not a good marker for photosynthetic inhibitors. Neither photosynthetic efficiency or quinone pool in *Fucus* was very sensitive to ecological relevant concentrations of Irgarol, a common biocide. Irradiance, temperature and salinity affected photosynthetic efficiency and quinone pool in *Fucus* in field samples, but not so much in laboratory conditions. The interaction between these abiotic factors on fluorescence would have to be modeled before *in situ* fluorescence measurements can say anything about pollution levels. Most differences observed in photosynthetic efficiency were also observed in quinone pool.

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7 Appendix

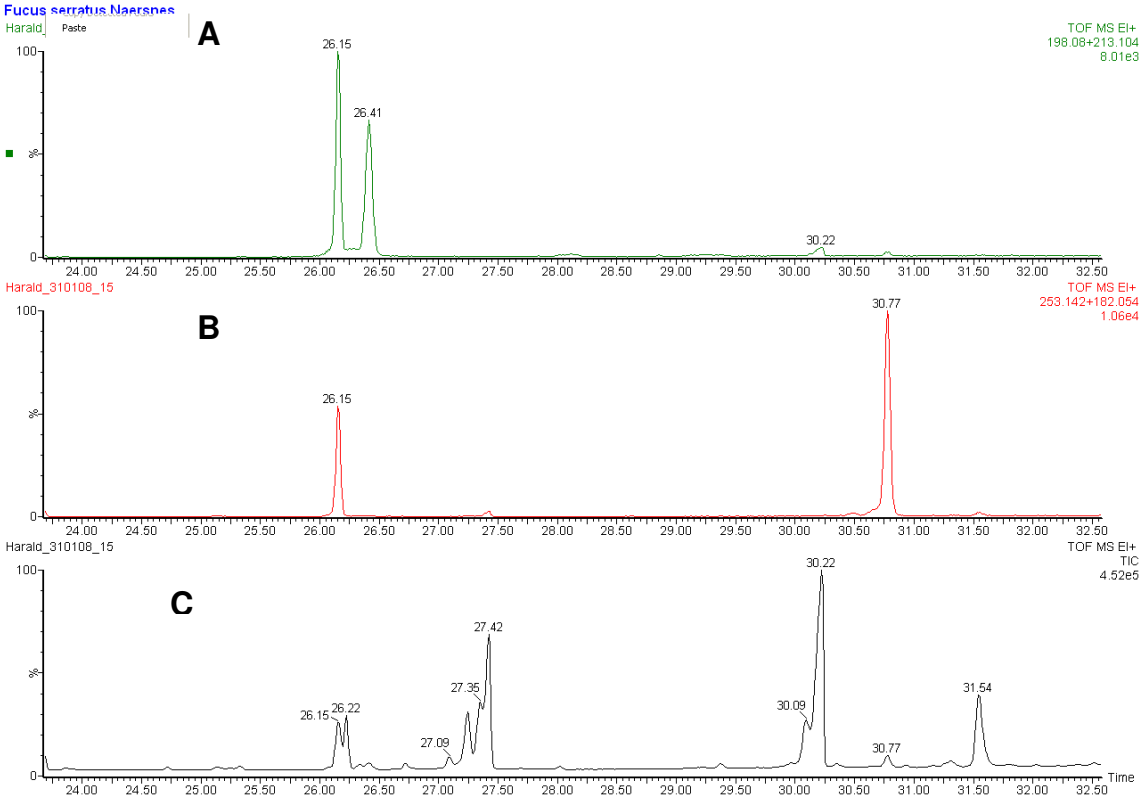


Figure 7.1 Chromatogram of extracted *F. serratus* from Nærsnes. A. GS26575, B. Irgarol and C. total ion chromatogram.

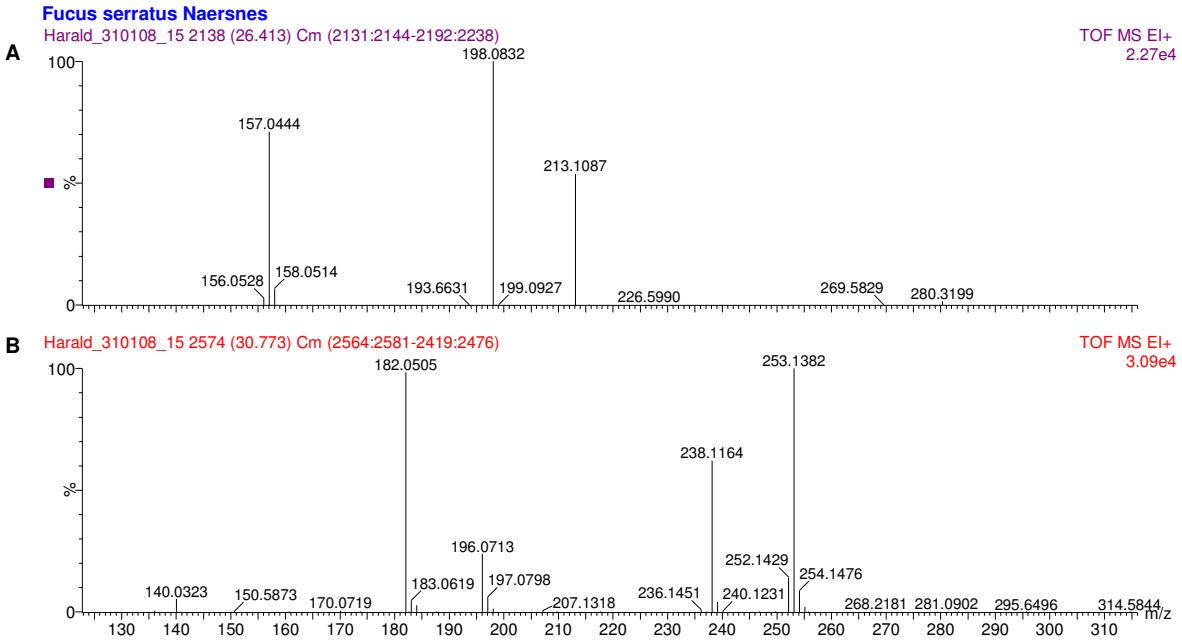
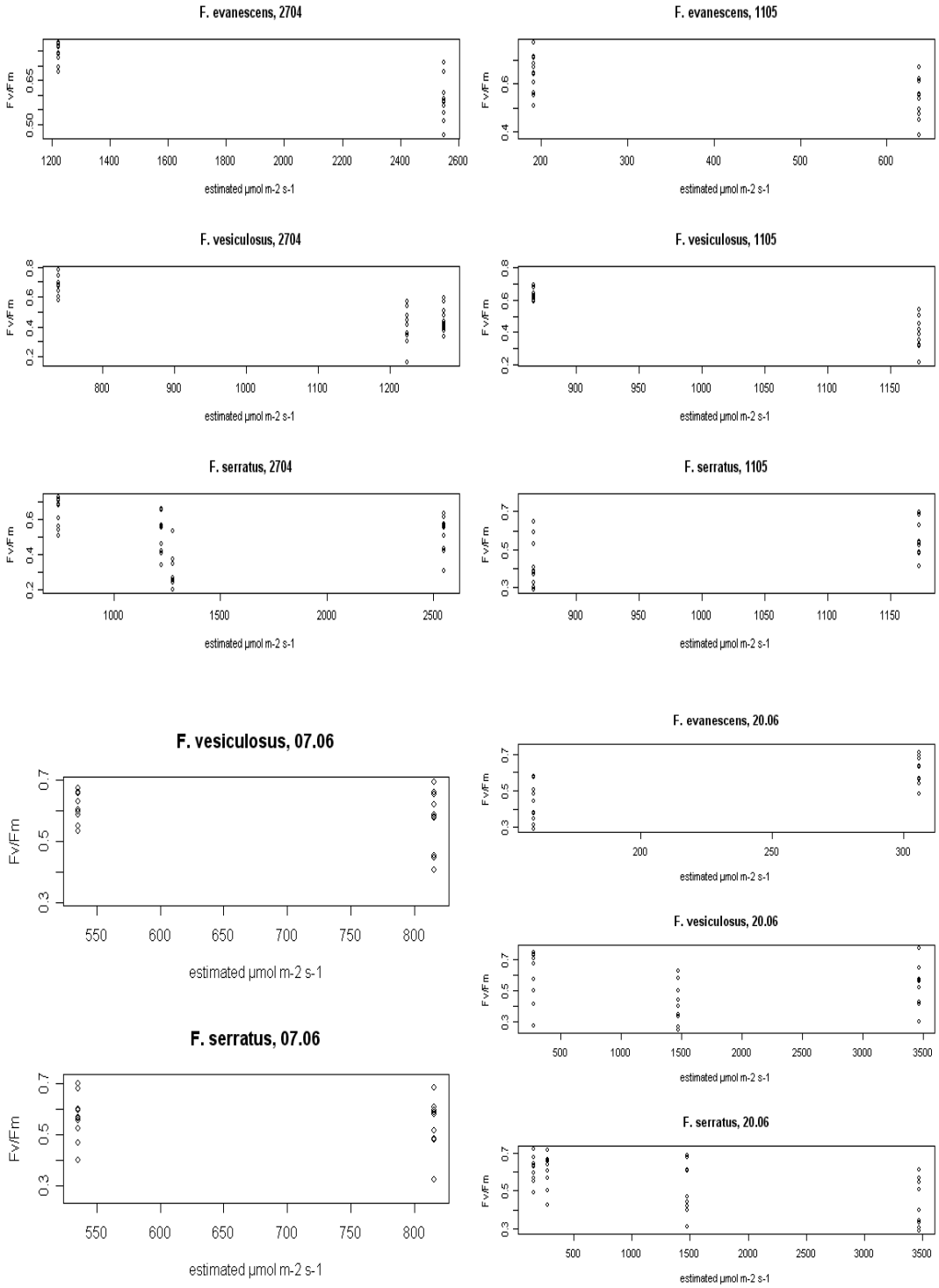
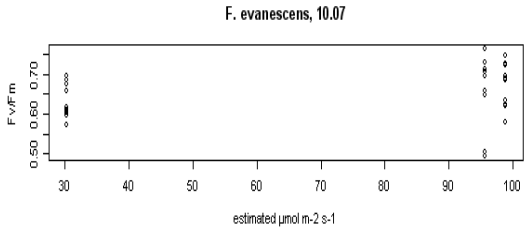


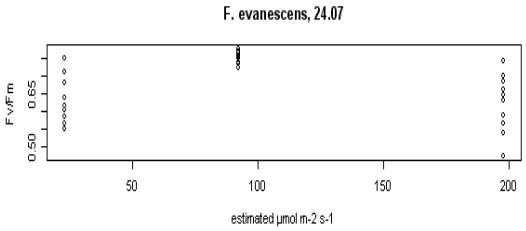
Figure 7.2 Accurate mass spectrum of *F. serratus* from Nærsnes. A. GS26575, B. Irgarol.

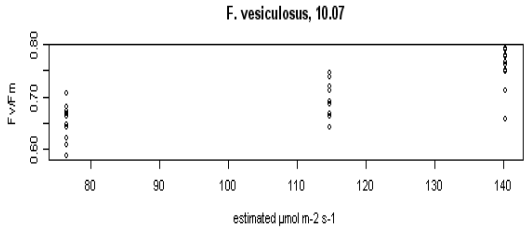
Appendix 2

Estimated photon flux density (µmol m^{-2} s^{-1} = Lux * 0.0185) compared to photosynthetic efficiency. Due to problems with loggers F. vesiculosus and F. serratus samples are only complete from 20 June and on 27 April, and F. evanescens samples are only complete from 10 July.




F. evanescens, 10.07
Fv/Fm
estimated µmol m-2 s-1


F. evanescens, 24.07
Fv/Fm
estimated µmol m-2 s-1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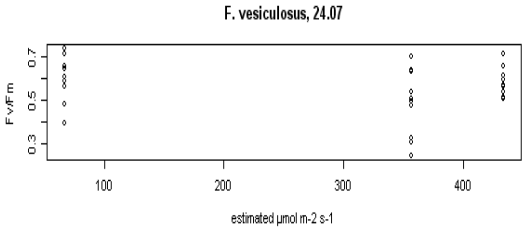
Fv/Fm
estimated µmol m-2 s-1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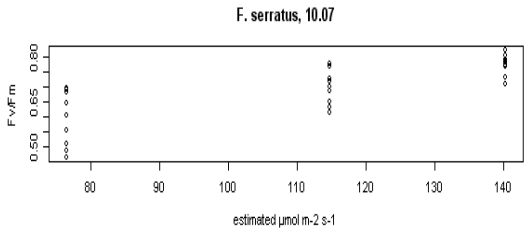
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Fv/Fm
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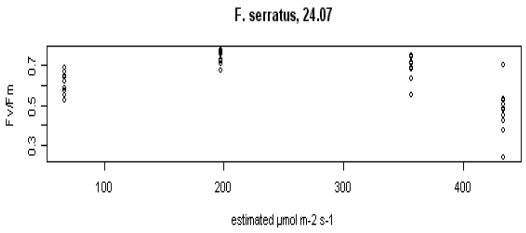
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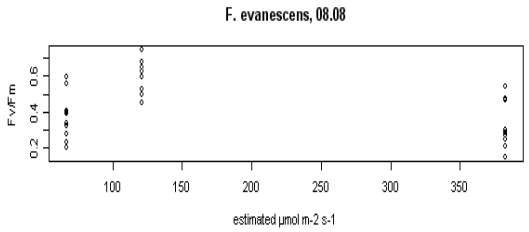
Fv/Fm
estimated µmol m-2 s-1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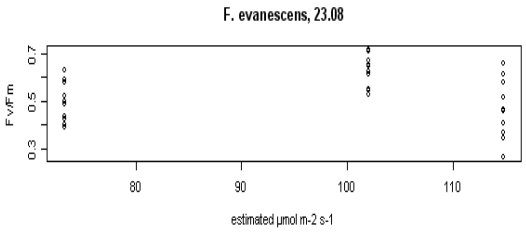
Fv/Fm
estimated µmol m-2 s-1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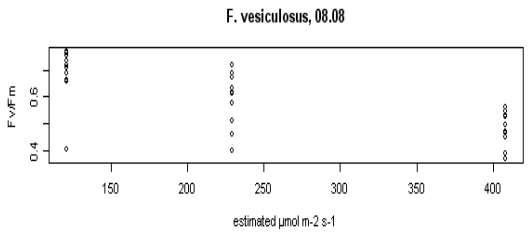
Fv/Fm
estimated µmol m-2 s-1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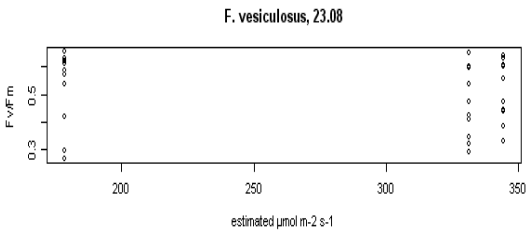
Fv/Fm
estimated µmol m-2 s-1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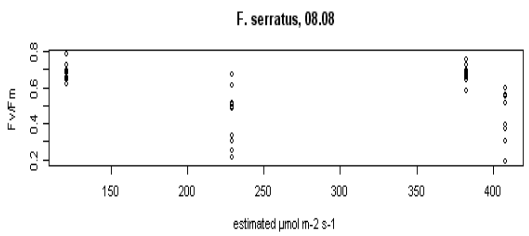
Fv/Fm
estimated µmol m-2 s-1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Fv/Fm
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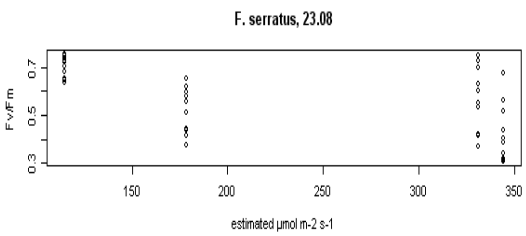
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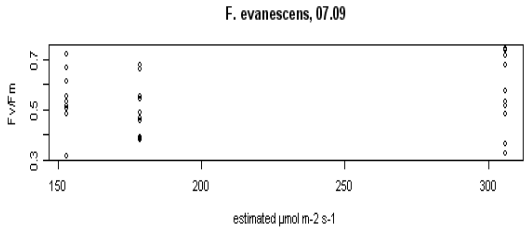
Fv/Fm
estimated µmol m-2 s-1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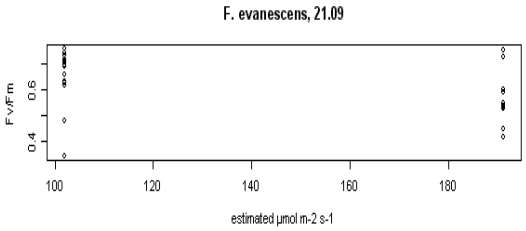
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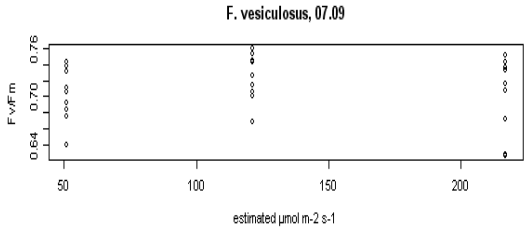
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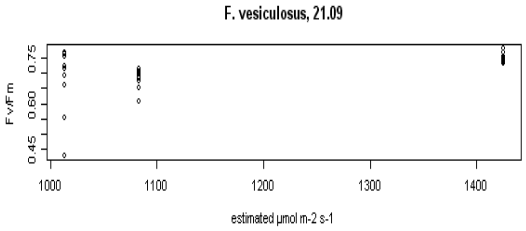
Fv/Fm
estimated µmol m-2 s-1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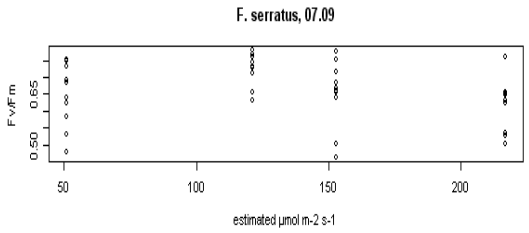
Fv/Fm
estimated µmol m-2 s-1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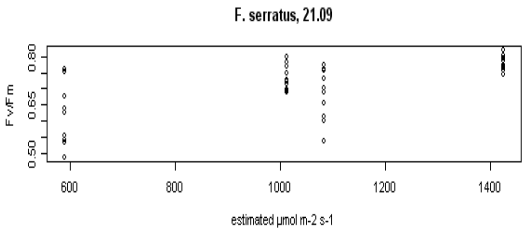
Fv/Fm
estimated µmol m-2 s-1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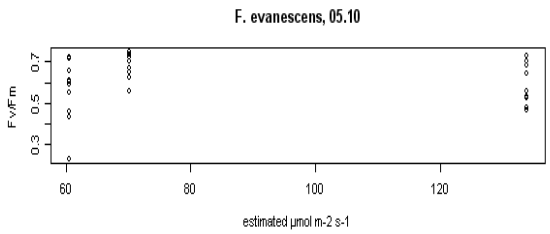
Fv/Fm
estimated µmol m-2 s-1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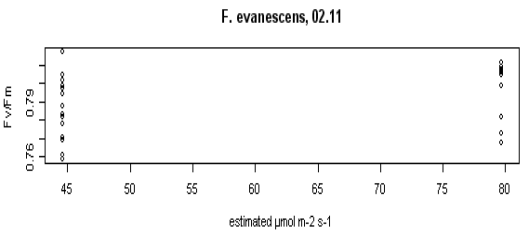
Fv/Fm
estimated µmol m-2 s-1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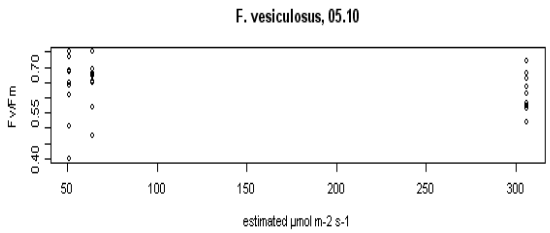
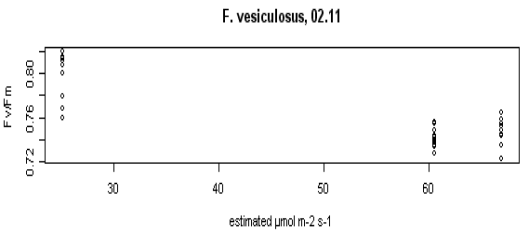
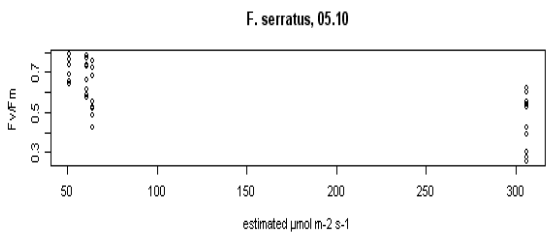
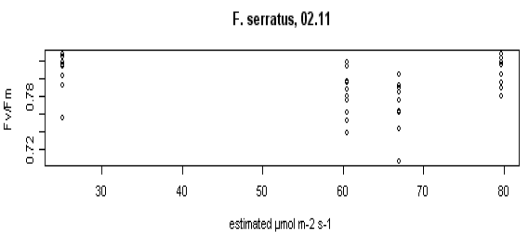
Fv/Fm
estimated µmol m-2 s-1


F. vesiculosus, 05.10
Fv/Fm
estimated µmol m-2 s-1


F. vesiculosus, 02.11
Fv/Fm
estimated µmol m-2 s-1


F. serratus, 05.10
Fv/Fm
estimated µmol m-2 s-1


F. serratus, 02.11
Fv/Fm
estimated µmol m-2 s-1

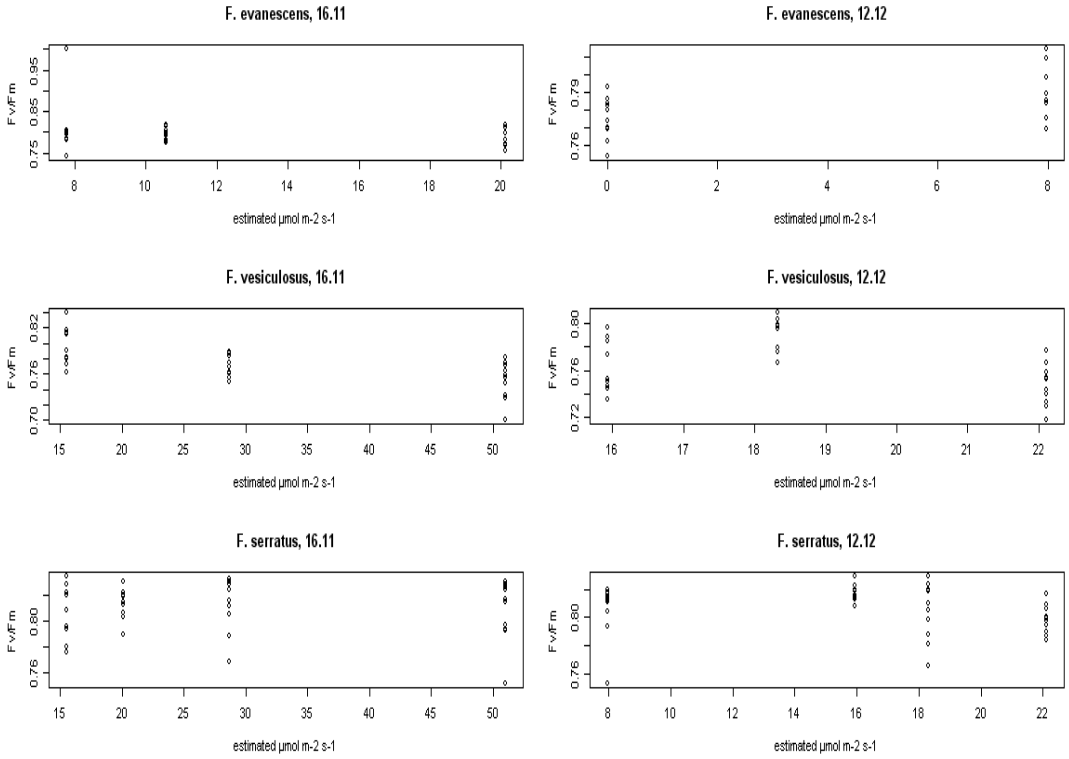


Figure 8.1 Relationship between estimated photon flux density (µmol m^{-2} s^{-1}) and photosynthetic efficiency (Fv/Fm) for *F. evanescens*, *F. vesiculosus*, and *F. serratus* on all dates of the field survey. Please notice scales are different in most figures. Scale break on both x and y axis.

Table 8.1 Spearman's rho between estimated photon flux density and photosynthetic efficiency for *F. evanescens*, *F. vesiculosus*, and *F. serratus* on all dates of the field survey. Data is missing for *F. evanescens* on 7 June since only one site had a working logger.

Date	*F. evanescens*	*F. vesiculosus*	*F. serratus*
27 Apr	**-0.83**	**-0.67**	**-0.40**
11 May	**-0.56**	**-0.87**	**0.55**
7 Jun		-0.22	-0.05
20 Jun	**0.69**	-0.23	**-0.50**
10 Jul	**0.81**	**0.73**	**0.84**
24 Jul	-0.05	-0.05	**-0.32**
8 Aug	-0.12	**-0.70**	**-0.34**
23 Aug	-0.07	-0.03	**-0.66**
7 Sep	0.06	0.03	-0.23
21 Sep	**-0.42**	**0.44**	**0.54**
5 Oct	0.03	-0.18	**-0.74**
2 Nov	0.32	**-0.58**	-0.11
16 Nov	-0.17	**-0.66**	0.03
12 Dec	0.52	-0.21	**-0.33**