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Color Infrared Photography Is Not a Good Predictor of Macro Invertebrate Abundance on Mudflats Used by Shorebirds

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Abstract.—The applicability of color infrared photography to indirectly predict prey abundance for shorebirds was tested by measuring the chlorophyll concentrations of primary producers on the mudflat surface at eleven migratory stopover sites of shorebirds in Georgia Strait, British Columbia during southward migration in July and August 2002. Many shorebirds are associated with regions of high coastal zone productivity, which may contribute to high prey abundance. Chlorophyll levels of primary producers contribute to the red tones of an infrared photograph. The hue of an infrared photograph was positively related to the chlorophyll concentration of the sediment surface across all sites. However, invertebrate density was not strongly related to surficial sediment chlorophyll concentration or photograph hue. The color infrared photography method is useful to quickly assess the surficial sediment concentration of the phytobenthos, but of low value to estimate invertebrate prey densities.

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Key words.— Biofilm, benthic macro invertebrate, color infrared photography, inter tidal, prey, shorebird.

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Food density has been used by many authors to explain differential habitat use by shorebirds at migratory stop over sites (Hicklin and Smith 1984; Colwell 1993; Colwell and Landrum 1993; Tsipoura and Burger 1999; Gill *et al.* 2001). Many shorebirds are associated with regions of high coastal zone primary productivity. It is likely that the factors contributing to high primary ocean productivity inducing the growth of phytoplankton, also contribute to the growth of the benthic macro invertebrate fauna consumed by shorebirds (Butler *et al.* 2001). For example, Barnes and deVilliers (2000) found that the biomass of chlorophyll-consuming and deposit-feeding macro invertebrates increased with chlorophyll-α concentration across intertidal mudflats and lagoons in Norfolk, UK.

The sediments of intertidal mudflats support an assemblage of microphytobenthos that contribute significantly to the primary productivity of these habitats (Yallop *et al.* 1994: MacIntyre *et al.* 1996). Benthic epipelic diatoms are important primary producers in intertidal mudflats (Admiraal 1984; Smith and Underwood 1998). These organisms grow in the upper few millimeters of sediments (the surficial layer) exposed to light, where they form biofilms on the sediment surface (Cognie *et al.* 2001). Biofilms are made up of the extracellular polymeric substances, mainly consisting of carbohydrates, which are produced by surficial diatoms and bacteria (Baird and Thistle 1986; Hoagland *et al.* 1993).

Color infrared photography (CIR) is sensitive in the visible green, red, and near infrared spectral regions (Greer *et al.* 1990). Chlorophyll levels of primary producers contribute significantly to red tones on a color infrared photograph (Everitt *et al.* 2001) since vegetation reflects up to ten times as much energy in the near infrared part of the spectrum as it does in the wavelengths to which natural color films are sensitive (Colwell 1984). Aerial CIR has been widely used to classify terrestrial plant communities (Holopainen and Wang 1998; Ramsey *et al.*1998; Nilsen *et al.* 1999; Ramsey *et al.* 2002).

Conventional methods of sorting and counting invertebrates from cores of mud to determine prey abundance are laborious and time consuming, especially on very large mudflats (Schneider and Harrington 1981; Hicklin and Smith 1984; Wilson and Vogel 1997; Sutherland *et al.* 2000). We tested the method of color infrared photography as an indirect measure of infaunal macro invertebrate abundance at migration stopover sites for shorebirds in Georgia Strait, British Columbia. The possibility was examined that the hue of an infrared photograph was positively related to the abundance of chlorophyll producing organisms on the mudflat surface as measured by chlorophyll concentration. It was also considered whether the infaunal macro invertebrate abundance was positively related to the concentration of chlorophyll-α on the mudflat surface.

METHODS

Study Area

The CIR method was tested at eleven sand and mudflats in the Georgia Strait in July and August 2002 (Fig. 1). Sites ranged in sediment size class from very fine to very coarse sands (0.1 to 1.4 mm) and the maximum mudflat area exposed at each site ranged from less than 1 to 60 km^2. Sites chosen were known or suspected stopover sites used by migratory shorebirds during the southward migration period in July and August.

Infrared Photography

An infrared photograph was taken of approximately 0.75 m^2 of the sediment prior to extracting invertebrate, sediment and biofilm cores from the photographed area. Photographs were taken using a Nikon F401 camera with a yellow filter and Kodak Ektachrome Professional Infrared CIR slide film. The film was kept cool before and after use. The film was opened, loaded, and unloaded in total darkness, and developed using E-6 processing (see Greer *et al.* 1990).

Digitized images of each photograph were analyzed with Adobe Photoshop™ v. 6.0 (Adobe Systems, Inc., San Jose, California, USA). In each photograph, average hue was calculated using Photoshop's histogram function applied to an area of the mudflat outlined with the rectangular marquee. Photoshop assigns hue onto a 360° color wheel with pure red at 0° (or equivalently 360°) and pure green is at 180°. Hue is the color reflected from an object, and corresponds strongly with the wavelength of the reflected light (Dale 2000). In order to control for variation in ambient lighting conditions across different photographs, the hue of a color-standard that was present in each photograph (Eastman Kodak Company 1997) was scored. Standardized hue values for the mud surface were calculated as the residuals of mudflat hue regressed onto color-standard hue (see also Dale 2000; Kilner and Davies 1998).

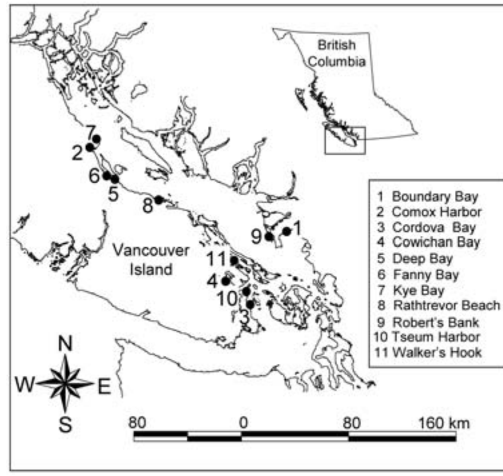

Figure 1. Sample locations in Georgia Strait, British Columbia, Canada.

Core Sampling

Immediately after each photograph was taken, we took invertebrate, biofilm, and sediment core samples, using a modified 60 cc syringe with a 2.6 cm inner diameter (Sutherland *et al.* 2000). Invertebrate and sediment core samples were taken to a depth of 4 cm. Wolf (2001) shows that the size of invertebrates ingested by the Western Sandpiper (*Calidris mauri*) are effectively sampled by this core size, since the size of the amphipod *Corophium* were recovered from sandpiper feces were within the size range of those recovered from the core samples.

To assess the abundance of primary producers we measured the concentration of chlorophyll-α produced by the diatoms in the upper millimeters of sediment that contribute to the surficial biofilm layer. Biofilm samples were taken by slicing approximately 2 mm off the mud surface with a lifter, using the 2.6 cm core sampler to cut out a standard sediment surface area. All cores were taken within 1.5 h of the mudflat being exposed by a falling tide, and were taken within 500 m of the high water mark. Table 1 summarizes the number of macro invertebrate, sediment, and biofilm cores extracted at each site.

Macro invertebrates were quantified using the conventional method of mud core sampling. Invertebrate cores were frozen within one hour of sampling. The cores were later thawed and rinsed through a 0.5 mm mesh sieve to retain the macro invertebrate fauna following Sutherland *et al.* (2000). The material on the sieve was preserved in vials containing 85% ethanol. Invertebrates in each core were identified and counted, using a dissecting microscope. The ash-free dry weight

Table 1. Sample sizes and dates of cores extracted at each site.

Site	Date sampled	Infrared photograph (N)	Invertebrate cores (N)	Sediment cores (N)	Biofilm cores (N)
Boundary Bay	14-23 Jul	45	45	43	34
Comox Harbor	2 Aug	15	15	14	13
Cordova Bay	29 Jul	14	14	14	14
Cowichan Bay	30 Jul	14	14	11	14
Deep Bay	1 Aug	11	11	14	11
Fanny Bay	1 Aug	15	15	15	11
Kye Bay	1 Aug	15	15	15	11
Rathtrevor Beach	31 Jul	15	15	14	15
Robert's Bank	19 Aug	14	14	14	13
Tseum Harbor	29 Jul	15	15	14	14
Walker's Hook	30 Jul	13	13	13	13
Total		186	186	181	163

(AFDW) of invertebrates was determined from 35 randomly selected invertebrate cores by drying in an oven at 70°C for 24 h to obtain dry weights, and then incinerated for 3 h at 550°C to determine AFDW (deBoer and Prins 2002). Sediment cores were frozen, thawed, and dried for 24 h at 65°C for analysis of sediment color. The hue of an infrared photograph of any sediment may be affected by substrate color, because of the semi-transparent nature of the diatom biofilm layer. To correct for this effect, infrared photographs were also taken of the sediment cores after they had been dried and placed in weigh boats. The hue of the photograph of the mud surface was standardized to that of the sediment hue.

Chlorophyll Analysis

The methods outlined in Sutherland *et al.* (1998) were followed to analyze biofilm samples for chlorophyll-α concentration. A sub-sample of sediment from each biofilm sample was placed in 10 ml of 90% acetone:water solution, contained in scintillation vials and stored for 24 h in a dark refrigerator to extract the chlorophyll from the sediment. The amount of chlorophyll in the supernatant was determined fluorometrically (Parsons *et al.* 1984). Chlorophyll levels were then divided by the mass of sediment that had been sub-sampled, giving chlorophyll concentrations as the amount of chlorophyll per unit mass of sediment ($\mu g_{chl}/g_{sed}$).

Statistics

Variables for invertebrate density (invertebrates/core), biomass (g) and chlorophyll concentration ($\mu g_{chl}/g_{sed}$) were transformed by log(x+1) prior to analysis to satisfy normality assumptions. Linear regression was used to determine the relationship between variables.

Results

There was a significant positive relationship between the hue of each infrared photograph and the chlorophyll concentration of the sediment surface (Fig. 2). Photograph hue explained 21% of the variation in chlorophyll concentration (μg/g; $r^2_{161} = 0.21$, $P < 0.001$).

There was a positive and significant relationship between chlorophyll concentration and invertebrate density; however, chlorophyll concentration explained very little of the variation in invertebrate density ($r^2_{161} = 0.04$). There was not a significant relationship between the hue of each infrared photograph and invertebrate density ($r^2_{184} = 0.001$, n.s.). There was not a significant rela-

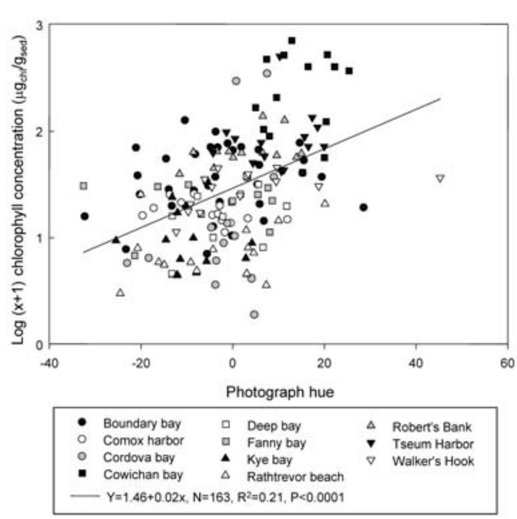

Figure 2. Photograph hue vs. chlorophyll concentration of surficial sediments on intertidal mudflats at migratory stopover sites for shorebirds in the Georgia Strait, British Columbia, Canada. Chlorophyll concentrations ($\mu g_{chl}/g_{sed}$) were transformed by log(x+1) prior to analysis (see Methods).

tionship between chlorophyll concentration and macro invertebrate biomass ($r^2_{33} = 0.02$, n.s.) or between the hue of each infrared photograph and macro invertebrate biomass ($r^2_{33} = 0.002$, n.s.).

The mean number of invertebrates was 76 individuals per core (N = 186, range; 1-462). The major taxa recovered were polychaetes, nematodes, amphipods, tanaids, ostracods, copepods, foraminiferans, insect larvae, and small mollusks (gastropods and bivalves). The mean density of each invertebrate taxa collected at each sampling site is shown in Appendix 1. Each taxa sampled has been shown to be ingested intentionally (Wolf 2001 and references therein) or unintentionally (Sutherland *et al.* 2000) by the Western Sandpiper, a small calidrine shorebird.

Surficial chlorophyll concentration between sites was compared by examining the data at site level. The relationship between the site means of the infrared photograph hue and chlorophyll concentration was highly significant (Fig. 3). Furthermore, photograph hue explained 84% of the variation in chlorophyll concentration ($r^2_9 = 0.84$, $P < 0.001$). However, there was no significant relationship between the mean infrared photograph hue at each site and the mean invertebrate density ($r^2_9 = 0.02$, n.s.) or the mean chlorophyll concentration at each site and mean site invertebrate density ($r^2_9 = 0.01$, n.s.).

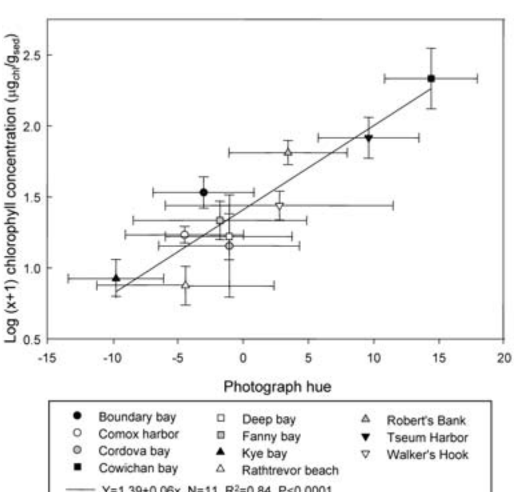

Figure 3. The mean photograph hue at each site is a significant predictor of the mean site chlorophyll concentration. Chlorophyll concentrations ($\mu g_{chl}/g_{sed}$) were transformed by log(x+1) prior to analysis (see methods). Vertical and horizontal lines represent 95% confidence limits.

DISCUSSION

The aim of this study was to test the applicability of color infrared photography as an indicator of the abundance of primary producers and subsequently macro invertebrate abundance at intertidal mudflats. The hue of an infrared photograph was positively related to chlorophyll concentration across all sites and between sites when using site means. However contrary to our expectation, invertebrate density was not significantly related to chlorophyll concentration, and therefore was not related to photograph hue.

Across sites, photograph hue was a poor predictor of chlorophyll concentration and explained only 21% of the variation in chlorophyll concentration. It is likely that the significant positive relationship between these two variables was driven by the relationship between photograph hue and chlorophyll concentrations between sites. The relationship improved when site means for photograph hue and chlorophyll concentration were compared ($r^2 = 0.84$), indicating that CIR as a technique may be most valuable to compare the surficial sediment concentration of benthic epipelic diatoms, between sampling sites.

The relationship between chlorophyll concentration and invertebrate density had nearly no predictive power ($r^2 = 0.04$). There are several possible reasons for this poor relationship. The abundance of chlorophyll producing organisms varies with season and the presence of grazers, providing inconsistent results (Underwood 1984; MacLulich 1987; Anderson 1995; Hillebrand *et al.* 2002). Furthermore, while dense biofilm assemblages may be able to support high densities of grazers, the presence of macrograzers, such as gastropods and crustaceans, can significantly decrease the biomass of biofilm assemblages and algae (Anderson 1995; Hillebrand *et al.* 2002).

In intertidal ecosystems, benthic diatoms, bacterial mats, and their associated exopolymers provide food for filter-feeding bivalves (Newell *et al.* 1989; Cognie *et al.* 2001), deposit-feeding holothurians (sea cucumbers) and deposit feeders and grazing organisms in general (Baird and Thistle 1986). Biofilm layers on intertidal surfaces may also provide food sources for organisms in higher trophic levels such as juvenile Dungeness Crabs (Stevens *et al.* 1982; Jensen and Asplen 1998). In systems where the relationship between chlorophyll and macro invertebrate biomass is known (Barnes and deVilliers 2000), relative macro invertebrate biomass may be estimated using CIR.

The utility of CIR has been applied to studies of the distribution and health of plants (Field and Philipp 2000; Weber and Dunno 2001; Ramsey *et al.* 2002). The CIR method was useful for comparing the surficial chlorophyll concentration of mudflats between sites in our study, but of no use to predict macro invertebrate abundance.

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Appendix 1. The mean density per m² of invertebrate taxa collected at each sampling site (standard error shown in brackets).

Phylum	Class	Order		Site: Boundary Bay (N = 45)	Cordova Bay (N = 14)	Comox Harbor (N = 15)	Cowichan Bay (N = 14)	Deep Bay (N = 11)
Sarcomastigophora	Granuloreticulosea	Foraminifera		962 (297)	403 (403)	125 (125)	10224 (5569)	0 (0)
Nematoda	Adenophorea			44952 (5703)	13318 (3265)	8538 (2118)	7668 (4115)	17807 (6634)
Mollusca	Bivalvia			42 (42)	0 (0)	502 (222)	135 (135)	342 (229)
	Gastropoda			841 (59)	134 (134)	0 (0)	0 (0)	171 (171)
Annelida	Polychaeta			215345 (31837)	403 (291)	5273 (885)	1614 (587)	1198 (730)
	Branchiopoda	Cladocera		460 (379)	0 (0)	0 (0)	0 (0)	0 (0)
Arthropoda	Ostracoda			24443 (3960)	0 (0)	1255 (397)	807 (548)	1712 (593)
	Copepoda			10087 (2482)	2287 (1875)	8161 (1431)	1345 (800)	3253 (883)
	Malacostraca	Amphipoda	*Corophium*	18500 (3617)	0 (0)	13937 (3256)	403 (403)	1883 (803)
			Gammaridea	84 (59)	0 (0)	0 (0)	0 (0)	342 (342)
		Cumacea		42 (41)	0 (0)	502 (288)	0 (0)	171 (171)
		Tanaidacea		24359 (5560)	0 (0)	19462 (4948)	0 (0)	0 (0)
	Insecta	Diptera		841 (59)	0 (0)	502 (388)	14529 (3578)	171 (171)

Phylum	Class	Order		Site: Fanny Bay (N = 15)	Kye Bay (N = 15)	Rathtrevor Beach (N =15)	Robert's Bank (N = 14)	Tseum Harbor (N = 15)	Walker's Hook (N = 13)
Sarcomastigophoa	Granuloreticulosea	Foraminifera		0 (0)	0 (0)	0 (0)	1749 (574)	1256 (750)	8983 (2798)
Nematoda	Adenophorea			10924 (2522)	80990 (18483)	34028 (8812)	27580 (3867)	80111 (12224)	91856 (19480)
Mollusca	Bivalvia			251 (171)	251 (171)	1005 (516)	135 (135)	0 (0)	1594 (764)
	Gastropoda			628 (237)	0 (0)	1632 (798)	0 (0)	0 (0)	0 (0)
Annelida	Polychaeta			2888 (1601)	43320 (10626)	7785 (1021)	212296 (26169)	2637 (731)	6230 (1657)
	Branchiopoda	Cladocera		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Arthropoda	Ostracoda			1005 (445)	0 (0)	5148 (2673)	269 (269)	502 (289)	3477 (1184)
	Copepoda			1381 (430)	9920 (3831)	4269 (2299)	2691 (548)	3014 (1053)	6810 (2492)
	Malacostraca	Amphipoda	*Corophium*	5776 (1719)	0 (0)	24109 (6301)	228 (1356)	753 (443)	21443 (4828)
			Gammaridea	0 (0)	126 (126)	377 (201)	0 (0)	0 (0)	724 (724)
		Cumacea		0 (0)	0 (0)	377 (201)	0 (0)	126 (126)	290 (196)
		Tanaidacea		0 (0)	0 (0)	0 (0)	6727 (1881)	0 (0)	724 (340)
	Insecta	Diptera		126 (126)	0 (0)	0 (0)	269 (269)	126 (126)	290 (196)