IMPACT OF PHYTOPLANKTON COMMUNITY STRUCTURE AND FUNCTION ON MARINE PARTICULATE OPTICAL PROPERTIES

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IMPACT OF PHYTOPLANKTON COMMUNITY STRUCTURE AND FUNCTION
ON MARINE PARTICULATE OPTICAL PROPERTIES

BY

MALCOLM NEIL MCFARLAND

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
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OF

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ABSTRACT

Phytoplankton are an ecologically important and diverse group of organisms whose distribution, abundance, and population dynamics vary significantly over small spatial (cm) and temporal (minutes) scales in the coastal ocean. Our inability to observe phytoplankton community structure and function at these small scales has severely limited our understanding of the fundamental ecological and evolutionary mechanisms that drive phytoplankton growth, mortality, adaptation and speciation. The goal of this dissertation was to enhance our understanding of phytoplankton ecology by improving in situ observational techniques based on the optical properties of cells, colonies, populations, and communities. Field and laboratory studies were used to determine the effects of phytoplankton species composition, morphology, and physiology on the inherent optical properties of communities and to explore the adaptive significance of bio-optically important cellular characteristics. Initial field studies found a strong association between species composition and the relative magnitude and shape of particulate absorption ($a_p$), scattering ($b_p$), and attenuation ($c_p$) coefficient spectra. Subsequent field studies using scanning flow cytometry to directly measure optically important phytoplankton and non-algal particle characteristics demonstrated that the size and pigment content of large (>20 µm) phytoplankton cells and colonies vary significantly with the slope of particulate attenuation and absorption spectra, and with the ratio of particulate scattering to absorption. These relationships enabled visualization of phytoplankton community composition and mortality over small spatial and temporal scales derived from high resolution optical measurements acquired with an autonomous profiling system. Laboratory studies with diverse uni-
algal cultures showed that morphological and physiological characteristics of cells and colonies can account for ~30% of the optical variation observed in natural communities and that complex morphologies and low intracellular pigment concentrations minimize pigment self-shading that could otherwise limit bio-optical fitness. These results demonstrate that optical properties reveal detailed information about the distribution, abundance, morphology, and physiology of phytoplankton that can help explain their ecological dynamics over small spatial scales and the bio-optical function of diverse forms in the ocean.
ACKNOWLEDGMENTS

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PREFACE

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INTRODUCTION

Phytoplankton are a fundamental component of ocean ecosystems that account for approximately half of global primary production and are important to global-scale biogeochemical cycling of elements such as carbon and oxygen (Longhurst et al. 1995, Field et al. 1998, Falkowski et al. 1998). The first planktonic and photosynthetic microorganisms, probably similar to extant marine cyanobacteria, appeared ~2.6 billion years ago and were responsible for the oxygenation of earth’s atmosphere (Hedges et al. 2001). In the modern ocean, there are close to 25,000 described species of phytoplankton classified among eight major phylogenetic groups (Katz et al., 2004), although the number of species is likely to increase with the use of molecular genetic approaches (Amato et al. 2007). Species exhibit a wide variety of distinguishing morphological and physiological characteristics that control their distribution, population dynamics, and impact on biogeochemical processes in the pelagic environment (Smayda 2010, Smetacek et al 2004). Despite their large diversity and ecological significance, our basic understanding of phytoplankton ecology has been limited to very coarse distinctions among large taxonomic classes (e.g. diatoms, dinoflagellates, or cyanobacteria) by the difficulty of determining community structure and function in the ocean over the small spatial and temporal scales critical to their growth and mortality. Community characteristics such as the abundance of species and the processes that determine succession can vary substantially over tens of centimeters or within minutes. As a result, it is difficult to understand or predict phytoplankton community responses to environmental change at the local or global level (Falkowski & Oliver 2007).
The high diversity of phytoplankton species in a turbulent environment with little or no barriers to dispersal was described as the “paradox of the plankton” by G.E. Hutchinson in 1961. This apparent paradox stems from the principle of competitive exclusion which predicts the ultimate dominance of a single, most fit form when multiple species compete for the same resources. Of course, the ocean is not an unstructured, isotropic environment at the scales important to phytoplankton population dynamics, and all species do not compete for resources under the same environmental conditions. Consequently, the differential growth rates of forms in response to variable local conditions results in complex and heterogeneous patterns of distribution and abundance over multiple spatial and temporal scales (Longhurst 1998, Rines et al. 2010). The function of their distinctive traits is fundamental to this complex ecology (Litchman & Klausmeier 2008). Ecologists have long sought explanations for observed patterns of distribution and abundance through an understanding of the function of species traits (Gran 1912, Margalef 1978, Sournia 1982, Fogg 1991, Smayda and Reynolds 2001). Morphological traits such as the size and shape of cells can enhance nutrient acquisition rates through sinking or interaction with small scale turbulence (Smayda 1974, Margalef 1978 & 1997, Karp-Boss et al. 1996, Pahlow et al. et al. 1997, Padisak et al. 2003, Jumars et al. 2009). Size and hard cell coverings (e.g. the diatom frustule or dinoflagellate theca) can reduce grazing and defend against pathogens (Frost 1972, Smetacek 2001, Pondaven et al. 2007, Hamm & Smetacek 2007). Basic questions such as “What is this trait for?” or “Where is this trait found?” can lead to a better understanding of the ecological niches to which species are adapted and the parameters that determine community structure.
For natural phytoplankton populations, species-specific traits and their function are typically investigated by analysis of discrete samples. These analyses (e.g. identifying and counting individual species or performing bottle experiments) are often time consuming and, as a result, severely limit the resolution of observations that can be made in the ocean. Low resolution observations can lead to mischaracterization of marine ecosystem structure and function if important but small scale features are missed by sparse sample collection (Cassie 1963, Haury et al. 1978). Optical measurements provide an alternate method of determining the distribution of certain species-specific traits and their function with a high spatial resolution of ~1 cm and temporal resolution of <1 second (Cowles et al. 1998, Hanson & Donaghay 1998). Light absorption and scattering by phytoplankton cells and colonies determine, to a large extent, the particulate absorption ($a_p$), scattering ($b_p$), and attenuation ($c_p$) coefficient spectra measured by commercially available optical instrumentation. These instruments can be deployed on moored, profiling, and towed platforms to collect large, high resolution optical data sets that describe the spatial and temporal variation of bio-optical properties (Holliday et al. 2003, Babin et al. 2005, Stemmann & Boss 2012). The shape and magnitude of $a_p$, $b_p$, and $c_p$ spectra are determined by particle abundance, size, shape, and complex refractive index (Morel & Bricaud 1986), characteristics that vary substantially among phytoplankton taxa and with their physiology. Measured optical properties, therefore, can serve as a proxy for these phytoplankton characteristics and can be used to map phytoplankton community types with high resolution over small scales. Through observation of ocean ecosystems, recent studies have demonstrated the importance of phytoplankton size and pigment
characteristics on the inherent optical properties of ocean waters and established the viability of optical measurements as a proxy for phytoplankton characteristics (Babin et al. 2003, Lohrenz et al. 2003, Bricaud et al., 2004, Cetinic et al. 2012, Bowers et al. 2009). Optical data could, therefore, be used to correlate phytoplankton community characteristics with environmental gradients and hydrographic processes in the ocean to obtain a deeper understanding of the selective forces that shape phytoplankton ecology and evolution.

The interaction of phytoplankton with light facilitates the discrimination of distinct communities, but is also a critical function of cells and colonies. Light is an essential and often growth limiting resource for phytoplankton in the ocean. The absorption of light energy by pigments embedded within chloroplasts drives photosynthesis which, in turn, fuels cellular metabolism and population growth. However, light absorption varies considerably among species and natural populations due to differences in cell volume, shape and pigment content. These morphological and physiological characteristics determine the amount of pigment self-shading (i.e. the “package effect”), which limits light absorption efficiency and growth as cell size or intracellular pigment concentrations increase (Duysens 1956, Das 1967, Kirk 1975, Bricaud et al. 1988, Finkel 2001). Competition for available light among bio-optically distinct species, therefore, can play an important role in structuring marine phytoplankton communities. For example, the competitive success of species with different minimum light requirements or absorption spectra can vary with light intensity (Huisman et al. 1999) and the spectral shape of the ambient light field (Stomp et al. 2004). In addition, fluctuations in available light intensity at 3-12 day
time scales have been shown to alternately favor distinct species resulting in increased diversity through disruption of competitive exclusion (Floder et al. 2002, Litchman & Klausmeier 2001, Litchman 2003). This previous work indicates absorption of light is a critical function of phytoplankton cells that varies with cellular traits and influences patterns of distribution and abundance in the ocean. Measurement of phytoplankton optical properties can provide information about the optical function of different populations and the possible strategies they employ to compete for light in the pelagic environment. Furthermore, light absorption efficiency can have a significant impact on rates of primary production and is therefore important to ecosystem models and calculations of biogeochemical fluxes (Bricaud et al. 1995, Platt & Sathyendranath 1999).

Although the broad goal of determining phytoplankton bio-optical function and ocean ecosystem structure using optical methods is promising, such methods require an understanding of the influence of specific traits on in situ particulate optical properties. While previous studies have established a basic theoretical framework with which to interpret optical measurements, there is insufficient empirical data available to adequately determine the dominant sources of optical variation over small scales in the ocean and their relationship to phytoplankton characteristics. To address this problem, this dissertation examines the variation of optical properties with community composition and the species–specific morphological and physiological characteristics of cells. The following questions are addressed using field and laboratory based approaches. First, do inherent optical properties vary with phytoplankton species composition and, if so, how? Next, how do phytoplankton size, pigment content, and
non-algal particulate material influence inherent optical properties, and can variation of these bio-optical characteristics be visualized over small scales? Finally, to what degree can species-specific morphology and physiology account for optical variation in natural environments and what is the bio-optical function and ecological significance of these characteristics? Field measurements were made over small scales critical to phytoplankton population dynamics in hydrodynamically complex coastal ocean waters using in situ optical measurements, autonomous profiling, live microscopy, and scanning flow cytometry. Laboratory studies used size fractionated optical measurements, automated photomicrographic image analysis, and scanning flow cytometry to determine the bio-optical properties of cultures with highly divergent morphologies.

Answers to the specific questions addressed in this dissertation will advance in situ bio-optical methods capable of high resolution observations that are directly related to phytoplankton cellular characteristics and functions essential to growth. With a better understanding of the influence of species and population specific traits on particulate optical properties, such methods can provide new and ecologically important information on natural phytoplankton community structure and function over small scales. The ability to infer ecologically important characteristics of phytoplankton from high resolution optical measurements can provide powerful insights into the conditions that favor certain forms over others and the processes that govern community assembly and succession. This type of detailed understanding of phytoplankton ecology has broad implications for biological processes that operate
over local (e.g. bloom dynamics) and global (e.g. biogeochemical cycles) scales throughout the world’s oceans.

REFERENCES


OPTICAL SIGNATURES OF MARINE PHYTOPLANKTON COMMUNITIES
OVER SMALL SCALES

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ABSTRACT

The shape and relative magnitude of particulate absorption ($a_p$), scattering ($b_p$), and attenuation ($c_p$) coefficient spectra can vary with phytoplankton pigment characteristics and suspended particle size distributions. Variation of these inherent optical properties in the ocean, therefore, conveys important information about the structure and function of pelagic ecosystems. In this study, the composition of coastal phytoplankton in Monterey Bay, CA is related to particulate scatter to absorption ratios ($b_p:a_p$), backscatter ratios ($b_b:b$), and the slopes of particulate attenuation and absorption spectra. Results show these optical parameters varied with phytoplankton species composition leading to different optical signatures associated with taxonomically distinct communities. The particulate attenuation and absorption slope parameters varied most with community composition, suggesting that size distribution and pigment content of cells are bio-optically important phytoplankton characteristics that differentiate populations over small scales in the coastal ocean. Results support the hypothesis that taxon specific morphological and physiological characteristics influence the optical properties of ocean waters in a manner consistent with optical theory. Furthermore, this study demonstrates the ability of inherent optical properties to provide ecologically important information about the structure of phytoplankton communities over small scales.

INTRODUCTION

Phytoplankton communities are composed of many species with distinct morphological and physiological characteristics. The distribution and abundance of these diverse species are important to the structure and function of marine ecosystems.
For example, species specific characteristics can affect rates of photosynthesis (Platt & Jassby 1976; Geider et al. 1986; Finkel 2001), nutrient uptake (Margalef 1978, 1997; Pahlow et al. 1997), grazing rates (Frost 1972; Verity & Smetacek 1996), sinking rates (Smayda & Boleyn 1965, 1966a, 1966b; Padisak et al. 2003) and the flux of carbon to deep ocean sediments where it can be sequestered for centuries (Smetacek et al. 2004; Smetacek et al. 2012). In hydrographically complex coastal ocean environments the composition of phytoplankton communities often varies significantly over centimeter to kilometer scales (Donaghay et al. 1992; Cowles et al. 1998; Dekshenieks et al. 2001; Rines et al. 2010). The physical and biological processes that determine species composition at these scales are not well understood, largely due to the difficulty of acquiring the number of observations necessary to resolve natural patterns of variation. In situ measurements of light scatter, absorption and attenuation provide a potential solution to this problem. In many pelagic ecosystems these optical properties are determined to a large extent by the abundance and characteristics of phytoplankton cells and colonies. In addition, optical measurements can be made with centimeter scale resolution. Modern optical instruments are capable of high data acquisition rates (> 1 Hz) and can be deployed in situ on a variety of moored, towed or vertically profiling platforms (Donaghay et al. 1992, Donaghay 2003, Holliday et al. 2003, Babin et al. 2005). Used as a proxy for phytoplankton, optical properties can facilitate the analysis of communities with very high spatial and temporal resolution. The resulting data provide a detailed picture of phytoplankton distribution and abundance that cannot be achieved with the analysis of discrete samples.
High resolution, multi-spectral measurements of the particulate absorption \( (a_p) \), attenuation \( (c_p) \), scatter \( (b_p) \), and backscatter \( (b_b) \) coefficients are of particular interest since the relative magnitude and spectral shape of these inherent optical properties (IOPs) can be influenced by cellular characteristics that vary within and among species. These coefficients are affected by the size, shape, pigment content, and refractive index of phytoplankton cells and colonies (Morel and Bricaud 1986). Intracellular pigment packaging can flatten the \( a_p \) spectrum and reduce the absorption efficiency of cells (Duysens 1956; Kirk 1994; Ciotti et al. 2002). A high ratio of photoprotective to photosynthetic carotenoids may also increase the slope of \( a_p \) spectra between 488 and 532 nm (Eisner et al. 2003 & 2005). Theoretical and empirical studies have shown that the exponential slope of the \( c_p \) spectrum is related to the shape of the particle size distribution. The \( c_p \) slope is high for size distributions dominated by very small particles and closer to zero for distributions with abundant large particles such as phytoplankton cells and colonies (Kitchen et al. 1982; Boss et al. 2001). The direction of scattered light can also be influenced by cell size and refractive index. Large cells with low refractive index will scatter most light in the forward direction, while smaller cells or cells with high refractive index will scatter a larger proportion of light in the backwards direction (Twardowski et al. 2001; Sullivan et al. 2005).

Variations of these IOPs in field collected data sets, therefore, can be indicative of changes in the composition and physiological characteristics of the phytoplankton community.

Previous studies have found that a large amount of the variability of ocean water optical properties is derived from variation of the cell size distribution and pigment
packaging within phytoplankton (Babin et al. 2003; Bricaud et al. 2004). This suggests IOPs contain substantial information about the composition and characteristics of phytoplankton communities. However, previous studies have not quantified taxonomic composition, and the relationship between natural phytoplankton communities and their optical properties remains poorly understood. The ability to infer phytoplankton characteristics from in situ optical measurements, therefore, has been limited. This study tests the hypothesis that the relative abundance of morphologically and physiologically distinct taxa influences ocean water optical properties to produce unique optical signatures associated with different phytoplankton communities. To test this hypothesis discrete water samples were analyzed using manual, microscope based cell counts to determine species composition. Hierarchical cluster analysis was then used to define distinct communities based on species abundance data. For a subset of samples from each community, the size and abundance of pico- and nano-phytoplankton (2 – 20 µm in size) were also determined using image analysis of auto-fluorescent cells collected on filters. For each community we examined the relative magnitude and spectral shape of particulate attenuation, particulate absorption, and scattering coefficients; optical properties that can be influenced by the morphological and physiological characteristics of phytoplankton cells and colonies. Specifically, we measured the ratio of $b_p$ at 555 nm to $a_p$ at 676 nm, the spectral slope of $c_p$, the ratio of $b_p$ to $b$ at 532 nm, and the normalized slope of $a_p$ between 488 and 532 nm for all communities. Redundancy analysis was used to assess the variation of optical properties among all samples and determine unique combinations of optical parameter values associated with different communities.
METHODS

This study was conducted in the northeast portion of Monterey Bay, CA near the 20 m isobath (Fig. 1). Data were collected between August 20th and September 2nd in 2005, and between July 17th and July 26th in 2006. Monterey Bay is a broad, open coastal system influenced primarily by three distinct water masses with different relative importance at different times of the year. During summer months recently upwelled waters are drawn into the Bay during formation of a cyclonic gyre (see Rines et al. 2010 Fig. 8), low salinity offshore waters intrude during upwelling relaxation, and tidal pulsing pumps Monterey Canyon water up from depth (Ryan et al. 2010). These different water masses can contain different environmental conditions, ecological histories and, consequently, different phytoplankton communities.

Optical data

Optical data were collected with a profiling instrument package equipped with two WET-Labs ac-9 (2005) or ac-s (2006) multi-spectral absorption and attenuation meters (25 cm path length), a Sea-Bird Electronics SBE-25 CTD, a WET-Labs ECO VSF 532 nm scattering sensor, and a WET-Labs WETStar chlorophyll fluorometer. One ac-9 meter was fitted with a 0.2 micron filter on the intake tubing to enable measurement of absorption by colored dissolved organic matter ($a_c$). The entire instrument package was slightly negatively buoyant and allowed to descend slowly through the water column during profiles, decoupled from ship motion (Donaghay, 1992). Profiles were conducted in duplicate to ensure that the measured water column structure was accurate.
Absorption and attenuation meters were calibrated before, during and after the study with 0.2 micron filtered, de-ionized water from a Barnstead E-Pure purification system. Optical data were corrected for the effects of temperature and salinity according to the methods of Twardowski et al. (1999) and Sullivan et al. (2006). Scattering errors in the ac-9 and ac-s meters were corrected using the proportional correction algorithm of Zaneveld et al. (1994). The unfiltered ac-9 or ac-s meter was used to measure the absorption \( (a_{pg}) \) and attenuation \( (c_{pg}) \) coefficients for particulate and dissolved water constituents. The 0.2 micron filtered ac-9 meter measured the dissolved absorption coefficient \( (a_g) \). A flow sensor in line with the filtered ac meter was used to compensate for the slower flow rate through the filter. Particulate absorption \( (a_p) \) and attenuation \( (c_p) \) were calculated by difference \( (a_p = a_{pg} - a_g \text{ and } c_p = c_{pg} - a_g) \). The particulate scattering coefficient \( (b_p) \) was calculated as the difference between the particulate absorption and attenuation coefficients \( (b_p = c_p - a_p) \). Backscattering coefficients \( (b_b) \) at 532 nm were calculated from the ECO VSF data according to Moore et al. (2000).

Four parameters that depend on the shape and relative magnitude of IOP spectra were computed for each sample. The ratio of scattering to absorption \( (b_p:a_p) \) was calculated from \( b_p \) measured at 555 nm and \( a_p \) measured at 676 nm. The wavelength for \( b_p \) was chosen to coincide with peak scattering and to avoid the major chlorophyll absorption wave bands. The wavelength for \( a_p \) was chosen to coincide with the red chlorophyll absorption band and to minimize the influence of non-algal particulate absorption. The slope of the attenuation coefficient \( (c_p) \) was computed by fitting an equation of the form \( c_p(\lambda) = s\lambda^{-\gamma} \) where \( \lambda \) is the wavelength, \( \gamma \) is the exponential slope.
of the spectrum, and $s$ is a scale factor (Twardowski et al., 2001, Boss et al., 2001).

The backscatter ratio was calculated from $bb$ and $b$, both measured at 532 nm. Finally, the negative of the $ap$ slope parameter was calculated according to Eisner et al. 2003 as:

$$ap\, slope = -\frac{ap\, 488 - ap\, 532}{ap\, 676 (488 - 532 \text{ nm})}$$

Note that Eisner et al. report $ap$ slope as a negative value and we report this slope as a positive value to maintain consistency with conventions for $cp$ slope.

**Discrete samples**

Discrete water samples were collected with a Sea-Bird Electronics SBE-32SC sub-compact rosette bottle sampler equipped with ~1L niskin bottles (~30cm height), a Sea-Bird Electronics SBE-25 CTD, and a WET Labs WETStar chlorophyll fluorometer. As with the optical package, buoyancy was adjusted to be slightly negative and profiles were conducted with the package floating freely, decoupled from ship motion. To ensure accurate and efficient sampling over the full range of optical variation throughout the water column, an adaptive sampling strategy was employed that targeted optically distinct layers, some of which were less than a meter thick.

High resolution optical profiles were conducted immediately prior to sampling (generally within 15 minutes) and depths were chosen for sampling based on in situ optical and CTD data available in real time ($apg$, $cpg$, temperature, salinity, density and chlorophyll fluorescence). Sample depths were chosen to capture the full range of optical properties throughout the water column including minimum, maximum, and various intermediate values of $apg$, $cpg$, and chlorophyll. During the sampling profile,
bottles were triggered manually at depths where temperature, salinity, density and chlorophyll signals matched the depths chosen previously during the optical profile. This strategy ensured that ecologically and optically important thin layers of phytoplankton were accurately sampled.

Ten sample profiles were obtained in 2005, and nine sample profiles were obtained in 2006. Between 5 and 10 depths were sampled for each profile. Live samples were examined immediately after collection using a compound microscope equipped with a digital video camera system. 250 mL sub-samples were preserved in 1% formaldehyde and 1% glacial acetic acid and stored for later analysis in the lab. Immediately prior to microscope cell counts, samples were gently homogenized by inverting the sample bottle ∼50 times. 1 mL sub-samples were analyzed in a Sedgewick-Rafter chamber at 100x magnification using a Nikon Eclipse E800 light microscope equipped with phase contrast optics. Phytoplankton species greater than ∼10 µm in size were identified and manually enumerated. At least 400 cells were counted for each sample. When necessary, samples with very low cell densities were gently concentrated up to 10 times their original concentration by reverse filtration with a 7 µm mesh size Nitex filter.

Small photosynthetic cyanobacteria and pico-eukaryotes between 2 and 20 µm in size are not well preserved in whole water samples and cannot be enumerated in water mounts. To determine the abundance of these small cells, 25 mL sub-samples were collected and immediately filtered through 0.2 µm pore size, 25 mm Whatman Anodisc filters. Samples were filtered to a volume of 5 mL, fixed with a 1% solution of Formaldehyde for 5 minutes, and then filtered until dry. The fixed filters were
mounted on glass slides in immersion oil, stored at -20° C, and analyzed within four weeks. Images of cells on filters were acquired with a 1600 x 1200 pixel digital color camera (Diagnostic Instruments, Spot Insight) mounted on a Nikon Eclipse E800 microscope. Cells were excited with blue light (450 - 490 nm) from a mercury arc lamp and the autofluorescence of phycoerythrin and chlorophyll was detected at wavelengths greater than 515 nm in the green and red channels of the color camera. Images collected in 2005 were acquired with a 40x objective at a resolution of 5.445 pixels µm\(^{-1}\). Images collected in 2006 were acquired with a 20x objective at a resolution of 2.734 pixels µm\(^{-1}\) to compensate for lower cell densities by maximizing image area. To extend the dynamic range of the camera and reduce noise, four exposures at 100 ms, 800 ms, 1.5 s, and 2.5 s were captured and averaged to create each image. For each sample, 4 to 6 images were acquired from different quadrants of each filter to compensate for any uneven distribution of cells.

Color images were processed, segmented, and analyzed using MATLAB (The Mathworks, Inc.). Images were processed to reduce noise and equalize intensity by applying multiple Gaussian bandpass filters. The resulting filtered images were segmented by application of a threshold. Mean Feret diameter and mean red and green fluorescence intensity were recorded for all contiguous regions with intensities above the threshold. Regions touching the edge of the image were ignored. *Synechococcus* was discriminated from *Prochlorococcus* and photosynthetic nano-eukaryotes manually for each sample based the relative intensity of red and green fluorescence. Cell concentrations were calculated from the number of cells counted, the volume filtered (25 mL), the total filter area (283.53 mm\(^2\)), and the total filter area imaged.
Data analysis

Due to the offset in time (generally less than 15 minutes) between optical profiles and sample collection, it was necessary to align the optical and biological data sets to compensate for slight changes in water column structure caused by internal waves and other short time scale hydrographic processes. For each sample, optical data were extracted from profiles at the depth where temperature, salinity and chlorophyll fluorescence matched data from the bottle sampler package. Aligned biological and optical data sets were then compared to investigate the relationship between community composition and optical properties of samples. Statistical methods employed to assess this relationship included hierarchical cluster analysis, to identify communities based on species composition, and redundancy analysis, to explore the variation of optical properties among communities.

A distance matrix based on species abundance data was computed for all samples using the Bray-Curtis distance metric. Hierarchical cluster analysis performed on this distance matrix was used to group samples according to taxonomic composition. Cluster analysis was computed with the MATLAB statistics toolbox version 8.0 (The MathWorks, Inc. 2012). The resulting dendrogram was cut at a fixed distance to produce groups of samples representing distinct phytoplankton communities. The level at which the dendrogram was cut was determined as the smallest distance above which similarity profile tests could confirm significant structure ($p < 0.05$) for all groups (Clarke et al. 2008). Similarity profile tests were performed with the fathom toolbox (Jones 2012) in MATLAB using $10^4$ iterations. To compare among communities, mean $a_p$, $b_p$, and $c_p$ spectra were calculated for each community by
averaging the spectra for all samples in each community. Likewise, mean $b_p$: $a_p$, $b_b$: $b$, $a_p$ slope and $c_p$ slope were calculated for each community by averaging these parameters for all samples in each community. To examine the relationship between species composition and the optical properties of communities, redundancy analysis was performed using the Vegan package for R (Oksanen et al., 2013).

RESULTS

The species composition of samples collected over the small spatial and temporal scales in this study varied considerably within and between years. A total of 146 distinct taxa were found in 145 collected samples (72 samples in 2005 and 73 samples in 2006). 44 taxa were unique to 2005, 41 taxa were unique to 2006, and 61 taxa were found in both 2005 and 2006. The result of hierarchical cluster analysis for all samples collected in both years is shown in the dendrogram in Fig. 2. Similarity profiles supported all divisions of the dendrogram down to a distance of 0.72 ($p < 0.05$). Cutting the dendrogram at this distance (dotted line in Fig. 2) produced a total of 9 distinct phytoplankton communities. The number of samples in each community ranged from 4 to 52 (Table 1). Three of these communities were present in 2005 (communities 1, 5, & 7) and six communities were present in 2006 (communities 2, 3, 4, 6, 8, & 9). Although there was no overlap of communities between years, in some cases similarity of community composition between years was greater than within year similarity. For example, communities 3 and 9 (present in 2006) are more similar to communities 1 and 7 (present in 2005) then they are to communities 2 and 8. This suggests some degree of year to year continuity of phytoplankton populations within Monterey Bay.
The composition of each community determined by manual cell counts is shown in Fig. 3 with a selection of the most abundant taxa highlighted. Dominant taxa within the communities present during 2005 included *Pseudo-nitzschia* sp., *Alexandrium* sp., and *Akashiwo sanguinea*. Communities 1 and 7 contained a diverse array of taxa and differed predominantly in the presence of species found at relatively low concentrations. Figure 4A shows the depth distribution of communities for the duration of the 2005 field project. Community 7 was present throughout much of the water column during the early part of the field project and then at greater depths later in time. Community 1 was more prevalent towards the end of the field project. Community 5 represents samples collected within a dense thin layer of *A. sanguinea* that was migrating vertically on a diurnal cycle (Rines et al. 2010, Sullivan et al. 2010).

During 2006, dominant taxa included *Chaetoceros debilis, Alexandrium* sp., *Chaetoceros concavicornis, Pseudo-nitzschia* sp., *Chaetoceros perpusillus, Prorocentrum gracile*, and *Alexandrium catenella* (Fig. 3). Community 4, overwhelmingly dominated by the small colonial pennate diatom *Pseudo-nitzschia*, was present throughout the water column at the beginning of the 2006 field project (Fig. 4B). Near the surface this community was replaced by communities 2 and 8, dominated by dinoflagellates in the genera *Alexandrium* and *Prorocentrum*. Community 9, with significant populations of the small unicellular diatom *C. perpusillus*, was found at various depths during the field project. Community 6, dominated by the large colonial diatom *C. concavicornis*, was present for a short time at depth and may have been introduced with an intrusion of off shore water.
Community 3, dominated by another large colonial diatom *C. debilis*, was present only near the end of the field project. These patchy distributions of taxa over depth and time suggest a dynamic physical environment that was continuously advecting distinct communities through the study area.

The abundance of *Synechococcus*, *Prochlorococcus*, and photosynthetic nano-eukaryotes was determined for a subset of 80 samples (Fig. 5). The percentage of samples analyzed from each community ranged from 41% to 100%. Small cyanobacterial cells (1 to 5 µm) were most abundant during 2005 (communities 1, 5, & 7) with consistently high concentrations of *Synechococcus*. Community 1 had somewhat higher concentrations of cells in the 5 to 15 µm size range while communities 5 and 7 contained few of these photosynthetic nano-eukaryotes. During 2006, overall concentrations of pico and nano-phytoplankton were lower. Communities 2, 3, 6 and 9 were all similar in their composition of pico- and nano-phytoplankton. The relative abundance of larger cells in the 5 to 20 µm size range was greater for communities 4 and 8.

The relative magnitude and shape of the mean $a_p$, $b_p$, and $c_p$ coefficient spectra varied considerably among communities (Fig. 6). Consequently, there was substantial variation in the $b_p:a_p$, $c_p$ slope, $b_b:b$, and $a_p$ slope parameters. The mean values and standard deviations of these optical parameters are listed for each community in Table 1. Results of the redundancy analysis (Fig. 7) show the variation of taxonomic composition and optical properties among all 145 samples from both years. A permutation test of this result was highly significant ($p = 0.005$). In the redundancy analysis biplot, symbols represent individual samples and symbol shape indicates
community membership as determined by the previous cluster analysis. Samples with similar community composition are closer together. Vectors indicate the direction of increase in value for each optical parameter. The length of a vector indicates the importance of a particular optical parameter in explaining the observed variation. The plotted result best represents the variation of the $c_p$ and $a_p$ slope parameters since these vectors are longest. The $b_p:a_p$ vector is shorter and therefore explains less of the variation shown in the biplot. Vectors representing the $b_p:a_p$ and $b_b:b$ parameters point in similar directions indicating that these parameters are correlated. Vectors representing the $c_p$ slope and $a_p$ slope parameters are perpendicular indicating these parameters are not correlated.

The distribution of samples relative to the vectors shows how optical properties varied among the 9 communities. Comparison of the communities at greatest distance from the origin most clearly demonstrates the relationship between composition and optics. For example, community 4, dominated by the colony forming pennate diatom Pseudo-nitzschia sp. (Fig. 3), was found to have a high $b_p:a_p$ ratio, a high $b_b:b$ ratio, a high $a_p$ slope, and the highest $c_p$ slopes (Fig. 7, Table 1). This community also had relatively low concentrations of pico-phytoplankton but relatively high concentrations of nano-phytoplankton (Fig. 5). In contrast, community 5, dominated by A. sanguinea (Fig. 3), had low $a_p$ slopes, intermediate $b_p:a_p$ values, low $b_b:b$ ratios, and moderate to high $c_p$ slopes (Fig. 7, Table 1). Synechococcus were abundant in community 5 but nano-phytoplankton were not (Fig. 5). Community 6 contained substantial numbers of Chaetoceros concavicorns and low concentrations of pico- and nano-phytoplankton (Figs. 2 & 4). It had low $c_p$ slopes with intermediate values of other optical
parameters. Community 8 had high concentrations of dinoflagellates in the genus *Alexandrium* (Fig. 3), low concentrations of pico-phytoplankton, and high concentrations of nano-phytoplankton (Fig. 5). This community had the lowest $c_p$ slopes, the lowest $b_p:a_p$ values, and intermediate values of $b_h:b$ and $a_p$ slope (Fig. 7, Table 1). Community 9 had the highest $a_p$ slope values, the highest $b_h:b$ values, high $b_p:a_p$ values, and intermediate $c_p$ slope values. This community was dominated by the diatoms *C. perpusillus* and *Pseudo-nitzschia sp.* (Fig. 3) and it had low concentrations of pico- and nano-phytoplankton (Fig. 5).

**DISCUSSION**

Analysis of phytoplankton community composition in Monterey Bay revealed a highly structured and dynamic ecosystem that supports a large number of diverse phytoplankton (Fig. 3). The fine-scale distribution of species and their general ecology during this study are discussed in Rines et al. (2010). Taxa were not evenly distributed throughout the bay but organized into communities with distinct spatial and temporal distributions (Fig. 4). Community composition varied considerably over the smallest spatial (meters) and temporal (days) scales that were resolved by our adaptive sampling methodology. Results clearly show that the spectral shape and relative magnitude of IOPs varied with phytoplankton composition (Fig. 7) leading to different optical signatures associated with taxonomically distinct communities. The $c_p$ slope and $a_p$ slope parameters varied most with community composition, suggesting that size distribution and pigment content of cells are bio-optically important phytoplankton characteristics that differentiate populations in the coastal ocean. These results support the hypothesis that taxon specific morphological and physiological
characteristics influence the optical properties of ocean waters and suggest that IOPs can provide detailed information about the structure of phytoplankton communities over small scales.

The optical variation among communities found in this study is generally in agreement with previous theoretical and empirical research that has examined the relationship between particle characteristics and the optical properties of particle suspensions. Previous studies that examined the relationship between $c_p$ slope and the particle size distribution have shown that large $c_p$ slopes are associated with abundant small particles and smaller $c_p$ slopes are associated with abundant larger particles (Kitchen et al. 1982, Boss et al. 2001, Sullivan et al. 2005). In this study, communities with high concentrations of the pico-phytoplankton Synechococcus (1, 5 and 7) and communities dominated by small celled diatoms such as Pseudo-nitzschia sp. and C. perpusillus (4 and 9) had moderate to high $c_p$ slopes (Fig. 7, Table 1). In contrast, communities dominated by larger cells such as C. concavicornis, C. debilis, or Alexandrium spp. with lower concentrations of pico-phytoplankton (communities 6, 3 and 8) had lower $c_p$ slopes (Fig. 7, Table 1). This suggests $c_p$ slope, on average, is a good indicator of the size distribution of phytoplankton cells, a characteristic that varies with community structure and function (Platt & Jassby 1976; Geider et al. 1986; Finkel 2001).

The $a_p$ slope parameter is affected by the ratio of photoprotective to photosynthetic carotenoids (PPC:PSC), the package effect, and the relative abundance of non-algal particles. A high $a_p$ slope is associated with a large PPC:PSC ratio typical of high light adapted cells (Eisner et al. 2003 & 2005). The $a_p$ slopes measured for
communities in this study corresponded to moderate to high PPC:PSC ratios ranging from ~.5 – 1 (Eisner et al. 2003) suggesting phytoplankton were adapted to high light levels. Package effects, on the other hand, will decrease the slope of the $a_p$ spectrum resulting in an $a_p$ slope parameter closer to zero (Duysens 1956, Kirk 1975, Ciotti et al. 2002). Non-algal particles may also increase $a_p$ slopes due to their high absorption at short wavelengths and exponentially decreasing absorption at longer wavelengths (Kishino et al. 1984, Roesler 1989). We found community 5, dominated by $A$. sanguinea, to have the lowest $a_p$ slopes (Fig. 7, Table 1). This result may be due to strong packaging and is consistent with microscope observations of high intracellular pigment concentrations within live cells (Rines et al. 2010). Community 1 also had a relatively high $a_p$ slope which may reflect low PPC:PSC ratios. Communities 4, 6 and 9 had the lowest $a_p$ slopes (Fig. 7, Table 1). These communities were all dominated by diatoms of various size and had relatively low concentrations of pico-phytoplankton (Figs. 3 & 5). These low $a_p$ slopes therefore may indicate high PPC:PSC ratios for these communities.

Previous studies have shown that $b_b$ and $b_b:b$ can vary among particle populations with different size distributions, carbon content, and refractive indexes (Vaillancourt et al. 2004, Sullivan et al. 2005). The range of mean $b_b:b$ values in this study is relatively small compared to the range of values obtained from diverse locations (Sullivan et al. 2005) and there was substantial variation within communities. In our data the $b_b:b$ parameter was highest for communities 4 and 9 dominated by small micro-phytoplankton sized cells (Figs. 3 & 7, Table 1). This is consistent with expectations for communities dominated by small cells. However, this parameter was
lowest for community 1 despite high concentrations of pico- and nano-phytoplankton (Figs. 5 & 7, Table 1). These cells may have been too small, or their refractive index may have been too low to contribute significantly to the measured scatter. Non-algal particles not measured in this study may also have influenced $b_p:b$ since they have been shown to contribute significantly to backscatter (Green et al. 2003). It is possible both the $c_p$ slope and $b_p:b$ parameters responded not only to overall cell size, but also to the morphological features of individual cells. *Pseudo-nitzschia* cells, for example, have widths of ~3 to 7 µm, but they also have numerous striations along the valve face with sizes at or near the wavelengths of visible light (~500 nm to 1 µm). Also, *C. perpusillus* and *C. debilis* have 4 siliceous setae per cell that are ~1 µm or less in width, although these species form larger cells and colonies (Rines et al. 2010 Fig. 1F & Fig. 5H). It is possible that small morphological features of cells such as striations and setae behave optically as abundant small particles.

In laboratory based studies, Stramski & Reynolds (1993) have shown $b_p:a_p$ ratios are related to the ratio of intracellular carbon to chlorophyll. Photoadaptation, which can affect intracellular pigment content, may also be important to $b_p:a_p$ ratios for natural communities (Sosik et al. 2001). Physiological differences among taxa, therefore, are likely to have contributed to variation of this parameter among communities in Monterey Bay. We found the $b_p:a_p$ parameter to be highest for communities 4, 5, 7, and 9 (Table 1) although this result is poorly represented by the redundancy analysis (Fig. 7). In the case of communities 4, 7 and 9, this parameter was likely high due to the weak absorption of *Pseudo-nitzschia* sp. and *C. perpusillus* cells which had very low amounts of chlorophyll and appeared physiologically
stressed (see Rines et al. 2010, Figs. 1C, 1D, 5A, & 5H). In the case of community 5, the relatively high $b_p:a_p$ ratio may be attributable to a strong package effect for *A. sanguinea* that reduced $a_p$ at 676 nm relative to total scatter. Microscopy of live *A. sanguinea* showed high intracellular chlorophyll concentrations for these relatively large (~40 µm) cells. *A. sanguinea* may be able to reach high cell concentrations in spite of this high package effect and reduced absorption efficiency due to its motility. Cells can vertically migrate to surface waters where high light levels may minimize any reduction in fitness associated with package effects. The $b_p:a_p$ parameter was lowest for communities 1 and 8 (Table 1). Both of these communities have somewhat higher concentrations of photosynthetic nano-eukaryotes and dinoflagellates such as *Alexandrium* spp. or *A. sanguinea* (Figs. 3 & 5) that may have had different physiological characteristics.

Although we found that variation of mean optical properties among phytoplankton communities was related to phytoplankton morphological and physiological characteristics in agreement with our current understanding of ocean optics, there was substantial variation within communities for all optical parameters. Much of this within community variation may be due to non-algal particles that were not measured in this study. Some of the variation among communities may also be attributable to co-varying non-algal particles (e.g. bacteria, cell debris, exopolymers, and suspended sediments). These particles would likely increase $b_p:a_p$, $b_b:b$, $c_p$ slope and $a_p$ slope. However, Monterey Bay receives very little freshwater run-off during summer months and there was little evidence of significant re-suspension of sediments in our optical profiles and samples. Furthermore, $a_p$ values in the blue portion of the
spectrum for all communities are higher at 440 nm than at 400 nm (Fig. 6), characteristic of absorption by photosynthetic pigments and not by non-algal particulate material (Kishino et al. 1984, Roesler 1989). It seems likely, therefore, that non-algal particles made only small contributions to measured values of $a_p$. Also, it is important to note that the relative abundance of taxa as depicted in Fig. 3 does not take into account the projected area of cells and therefore may underestimate the contribution of large, rare taxa to the measured optical properties. Despite some methodological limitations, our data show a compelling pattern of correlation between phytoplankton community characteristics and optical properties that is in agreement with our basic understanding of the optical properties of suspended particles in ocean waters. Future work should employ methods to quantify the abundance of non-algal particles and take into account the effect of a particle size.

The results presented here build upon previous studies to show that the optical properties of phytoplankton communities can provide valuable biological information on the morphological and physiological characteristics of cells. We found evidence that cell size and intracellular pigment content can influence the shape and relative magnitude absorption, attenuation, and scattering coefficient spectra. Consequently, the measurement of optical properties can facilitate the analysis of these phytoplankton community characteristics in the ocean with very high spatial and temporal resolution. Mapping distribution patterns of phytoplankton traits over smaller scales than has traditionally been possible could greatly enhance our understanding of the processes that determine species succession, community assembly and ecosystem change.
Acknowledgements

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Table 1. Mean and standard deviations (s.d.) of $b_p:a_p$, $c_p$ slope, $b_b:b$, and $a_p$ slope for samples in each community and for all samples. The magnitude of the standard deviation indicates the range of values within each community. Also shown is the number of samples (N) for each community.

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Figure 1. Map of the northeast corner of Monterey Bay showing locations of high resolution optical profiles and water sample collection. Colors for each location correspond to samples plotted according to time and depth in figure 4.
Figure 2. Dendrogram showing results of hierarchical cluster analysis based on phytoplankton species abundance determined for all 145 samples from both years. Dotted line indicates distance (0.72, similarity profiles $p < 0.05$) at which the dendrogram was cut to produce 9 distinct communities.
Figure 3. Relative taxonomic composition for each community with most abundant taxa labelled. Hatched patterns represent the same species in all charts. Pie charts represent the relative cell concentration (cells mL$^{-1}$) of species averaged among samples grouped into communities according to cluster analysis.
Figure 4. Distribution of samples over time, depth, and location for 2005 (A) and 2006 (B) showing variation of community composition over small scales. Symbol shapes indicate community membership as determined by cluster analysis. Color indicates profile location as in figure 1.
Figure 5. Size and abundance of pico- and nano-phytoplankton determined by image analysis for a sub-set of n samples from each community. Stacked histograms show cell concentrations (cells mL$^{-1}$) in 0.5 µm mean diameter size bins between 0.5 and 20 µm.
Figure 6. Mean $a_p$, $b_p$ and $c_p$ spectra calculated for all samples in each community.
Figure 7. Results of the redundancy analysis for all samples based on community composition and optical parameters. Symbols indicate community membership of samples as determined by cluster analysis. Vectors indicate direction of increase for optical parameters.
VARIATION OF MARINE PHYTOPLANKTON COMMUNITY SIZE
STRUCTURE AND PHYSIOLOGY OVER SMALL SCALES DETERMINED
WITH IN SITU OPTICAL MEASUREMENTS

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ABSTRACT

Small scale layers and patches of phytoplankton are ubiquitous/widespread features in the coastal ocean that are fundamental to population dynamics and community structure in pelagic ecosystems. To better understand biological processes at small scales, we developed methods to assess phytoplankton community composition and physiological characteristics based on high resolution, in situ optical measurements. Scanning flow cytometry was used to determine the effects of abundance, size, and pigment content of phytoplankton cells and non-algal particles on the spectral shape and relative magnitude of particulate absorption, scatter, and backscatter. We found the slope of particulate attenuation varied with phytoplankton size and morphology, the slope of particulate absorption and the ratio of scatter to absorption varied primarily with cellular pigment content, and the backscatter ratio varied primarily with the relative abundance of non-algal particles. The ability of these relationships to predict particle and phytoplankton characteristics over small spatial and temporal scales was tested with two independent high resolution optical data sets collected from an in situ autonomous profiling system. Comparison of high resolution optical data with flow cytometric sample analyses agreed with the previously determined relationships. High resolution data revealed small scale variations in community size structure and physiology that would be difficult to visualize with discrete samples or measures of total chlorophyll concentration.
INTRODUCTION

It is well established that marine phytoplankton communities can vary considerably over very small spatial (decimeter) and temporal (minutes) scales (Cassie 1963; Haury et al. 1978; Derenbach et al. 1979; Bjørnsen and Nielsen 1991; Donaghay et al. 1992; Cowles et al. 1998; Rines et al. 2010). In the ocean, this small scale structure is often observed as horizontal patches or thin vertical layers that can contain a large proportion of chlorophyll biomass and can persist over time and space scales of critical importance to phytoplankton growth, mortality and life history (Dekshieneks et al. 2001; McManus et al. 2003; Sullivan et al. 2010b). Variations in distribution and abundance over small scales, therefore, are fundamental to the ecology of planktonic organisms and to the function of marine pelagic ecosystems. This heterogeneous distribution of organisms is a result of their interactions with physical, chemical and biological ecosystem components. Thin layers, for example, can be generated by a variety of mechanisms including shear, lateral intrusions, active migration, buoyancy, differential growth rates, and gyrotactic trapping (Durham and Stocker 2012). The physical, chemical and biological processes that occur within and around small scale structures can be important to the establishment of harmful algal blooms (Donaghay and Osborn 1997; Smayda and Reynolds 2001; Rines 2002), trophic interactions (Nielsen et al. 1990; Menden-Deuer 2008; Benoit-Bird et al. 2009; Greer et al. 2013), biochemical cycling (Sieburth and Donaghay 1993; Hanson and Donaghay 1998), sexual reproduction (Peperzak 2006), and community assembly, succession and diversity (Smayda and Reynolds 2001; Reynolds 2006).
Technological developments over the past several decades have enabled the analysis of natural phytoplankton communities with increasing resolution over decreasing spatial and temporal scales. High resolution methods targeting phytoplankton typically use in situ optical measurements of absorption, attenuation, scattering or fluorescence to detect and map small scale structure (Derenbach et al. 1979, Cowles & Desiderio 1993, Twardowski et al. 1999). Modern optical instrumentation can routinely collect data at high rates (> 1 Hz) and can be deployed on a variety of profiling, towed, moored, or autonomous platforms (Babin et al. 2005, Holliday et al. 2009, Sullivan et al. 2010a). While these data are often used to infer total phytoplankton biomass, a better understanding of ecological processes over small scales could be obtained from optical parameters that respond to variation of phytoplankton characteristics such as cell size, shape, or physiology (Stemmann & Boss 2012).

Theoretical and empirical studies have shown the shape and relative magnitude of particulate absorption ($a_p$), scattering ($b_p$), and attenuation ($c_p$) coefficient spectra are determined by particle size, morphology, and complex refractive index (Morel & Bricaud 1986, Sathyendranath et al. 1987). These particle characteristics can vary significantly among phytoplankton taxa and with their physiology (Bricaud et al. 1983, Spinrad & Yentsch 1987, Ackleson et al. 1988, Stramski & Reynolds 1993). Intracellular pigment packaging can flatten and decrease the slope of the $a_p$ spectrum while reducing the absorption efficiency of cells (Duysens 1956; Kirk 1975; Ciotti et al. 2002). The slope of $a_p$ spectra between 488 and 532 nm may also increase as the ratio of photoprotective to photosynthetic carotenoids increases (Eisner et al. 2003).
The ratio of scattering to absorption \((b_p:a_p)\) can vary among particle types and with intracellular carbon and pigment concentrations (Stramski & Morel 1990, Sosik et al. 2001). The proportion of light scattered in the backward direction \((b_b:b)\) is low for large particles and particles with low refractive index. Smaller particles or those with high refractive index have a higher \(b_b:b\) (Twardowski et al. 2001; Sullivan et al. 2005). The exponential slope of the \(c_p\) spectrum is high for suspensions dominated by very small particles such as picoplankton or suspended sediments, and closer to zero for suspensions with abundant large particles such as diatom or dinoflagellate cells (Kitchen et al. 1982; Boss et al. 2001). Because of their dependence on particle characteristics, these optical parameters convey ecologically important information about the composition and physiology of phytoplankton populations in the ocean.

Previous work in multiple different pelagic ecosystems suggests that particle characteristics are important to the bulk inherent optical properties (IOPs) of ocean waters that can be measured in situ with high resolution (Babin et al. 2003, Eisner & Cowles 2005, Bricaud et al. 2004, Sullivan et al. 2005). However, few field studies have directly measured individual phytoplankton and non-algal particle characteristics to determine their influence on bio-optical parameters and test the predictive capabilities of optical data. Iturriaga and Siegel (1989) used microspectrophotometric measurements of individual particles to show substantial variation in the absorption characteristics of different phytoplankton and non-algal particles. However, their methods were limited to approximately 50 particles for each water sample. In a study conducted in New England continental shelf waters, Green et al. (2003) used flow cytometry to show that bulk IOPs were highly dependent on the abundance, size and
complex refractive index of different plankton and particle groups. While focusing primarily on larger scales, Green et al. documented significant variation in phytoplankton optical cross sections and complex refractive indexes between spring and summer communities and with depth. A previous study in Monterey Bay, CA found that variation of phytoplankton community taxonomic composition and characteristics over small scales, determined with microscope based observations and cell counts, were associated with changes in the relative magnitude and spectral shape of $a_p$, $b_p$, and $c_p$ coefficient spectra (McFarland et al. in prep.). This study, however, clearly demonstrated the need for quantitative measurements of cell size, pigment content, and non-algal particles.

In the present study, conducted in the coastal fjord of East Sound, WA, we determined the influence of natural phytoplankton and non-algal particle characteristics on particulate IOPs, and tested the ability of in situ optical measurements to predict phytoplankton community characteristics over small spatial and temporal scales. The $b_p:a_p$, $a_p$ slope, $c_p$ slope, and $b_b:b$ parameters were used to determine the extent to which particulate optical properties vary with phytoplankton composition, morphology and physiological characteristics. We used a CytoSense (CytoBuoy b.v.) scanning flow cytometer to directly measure abundance, size, and fluorescence of phytoplankton cells and non-algal particles in discrete seawater samples. The relationships between these particle characteristics and corresponding measurements of the $b_p:a_p$, $a_p$ slope, $c_p$ slope, and $b_b:b$ parameters were determined with a redundancy analysis. Based on these relationships, we used an independent, high resolution optical data set collected with an autonomous profiler to predict
phytoplankton and non-algal particle characteristics over small scales. Finally, these predictions were tested by comparison with the particulate composition of discrete samples determined with flow cytometry. The methods employed in this study enabled visualization of phytoplankton community characteristics with high resolution and can be used to better understand the ecological dynamics of phytoplankton populations and the processes that determine the abundance of different taxa over small scales.

METHODS

High resolution optical profiles and samples were collected in East Sound, Washington between May 14\textsuperscript{th} and May 26\textsuperscript{th} in 2009 and between May 8\textsuperscript{th} and May 20\textsuperscript{th} in 2010. East Sound is a small fjord located in the northwest corner of Washington State at 48.7 degrees north latitude. It is approximately 13 km in length, 2 km wide, oriented in a north-northwest direction and open at the southern end. The sound is 30 - 40 m deep on average with narrow topographical constraints and a partial shallow sill at the southern mouth. Currents and mixing are primarily driven by wind and tides. When mixing is not intense, East Sound often develops complex layered structure. Multiple distinct water masses can be stacked on top of each other with significant differences in phytoplankton community composition (Dekshieneks et al. 2001, Rines et al. 2002, McManus et al. 2003). This layered water column structure makes East Sound an excellent system in which to capture large amounts of biological and optical variation over small scales.

Ship based optical profiles

Optical data for comparison with discrete samples was collected from multiple locations throughout the sound with a ship based profiling instrument package.
Instruments included a 0.2 µm filtered WET Labs ac-9 absorption and attenuation meter, an unfiltered WET Labs ac-9 (2009) or ac-s (2010) absorption and attenuation meter, a SeaBird Electronics SBE-25 CTD, a WET Labs WETStar chlorophyll fluorometer, and a WET Labs ECO VSF 532 nm scatter sensor. During 2010 the profiler also included a WET Labs CDOM fluorometer. The buoyancy of the optical instrument package was adjusted to be slightly negative and, during data collection, was allowed to descend freely through the water column decoupled from ship motion (Donaghay 1992). Profiles were conducted in duplicate to ensure accuracy of measurements and stability of the local water column structure.

Absorption and attenuation meters were calibrated before, during and after each field project with 0.2 µm filtered de-ionized water from a Barnstead E-pure water purification system. Optical data were corrected for the effects of temperature and salinity according to the methods of Twardowski et al. (1999) and Sullivan et al. (2006). Scattering errors in the ac-9 and ac-s meters were corrected using the proportional correction algorithm of Zaneveld et al. (1994). Particulate absorption ($a_p$) and attenuation ($c_p$) coefficients were calculated by subtracting dissolved absorption ($a_g$) measured with the 0.2 µm filtered ac-9 from total absorption ($a_{pg}$) and attenuation ($c_{pg}$) measured with the unfiltered ac-9 or ac-s meters. A flow sensor in line with the filtered ac meter was used to compensate for the slower flow rate through the filter. To match the higher spectral resolution of the ac-s meter used during 2010, the dissolved absorption spectrum was interpolated by fitting to measured values, an equation of the form $a_g(\lambda) = s\lambda^{-\gamma}$ where $\lambda$ is the wavelength, $\gamma$ is the exponential slope, and $s$ is a scale factor. The particulate scattering coefficient ($b_p$) was calculated as the difference...
between the particulate absorption and attenuation coefficients \((b_p = c_p - a_p)\).

Backscattering coefficients \((b_b)\) at 532 nm were calculated from the ECO VSF data according to Sullivan et al. (2013).

**Adaptive sampling**

Optical profiles were conducted immediately prior to water sample collection and target sample depths were selected based on total phytoplankton biomass inferred from optical data available in real time. Depths were selected to capture the maximum amount of variation of biomass throughout the water column in the most efficient manner possible. At each profiling station samples were generally collected from two or three depths where total absorption and chlorophyll fluorescence exhibited minimum and maximum values. This adaptive sampling procedure ensured that thin layers of phytoplankton were accurately sampled and the full range of phytoplankton biomass and optical variation throughout the water column were captured at each station. Samples were collected with a ~2 L, hand deployed, Ruttner type water sampler (KC Denmark) on a graduated line. Phytoplankton cells and colonies in collected samples were immediately viewed and recorded on deck with a compound microscope equipped with a video camera. Sub-samples for microscopy were gently concentrated with a 20 µm Nitex mesh. Sub-samples (~20 mL) were also acquired for immediate flow cytometric analysis. In addition, 250 mL of each sample was preserved with 1% formalin and 1% glacial acetic acid for later analysis in the lab.

**Scanning flow cytometry**

A CytoSense scanning flow cytometer (CytoBuoy b.v., http://www.cytobuoy.com) was used to measure the size, concentration, light scatter
and chlorophyll fluorescence of phytoplankton cells and non-algal particles in collected samples. This instrument has a wide flow path that can accommodate particles up to 800 µm in width. The CytoSense records a scan (0.5 µm resolution) of the light scattered and fluoresced by particles as they pass individually through the incident laser beam at a fixed speed (Fig. 1). Descriptive particle parameters such as total light scatter, total fluorescence and length are computed for each particle from scan data. Particles that are elongate in shape tend to align lengthwise along the axis of flow and length measurements therefore represent maximum particle dimensions rather than spherical equivalent diameters (Fig. 1A & B). For each sample, the volume of fluid analyzed is recorded and used to calculate particle concentrations. Sample volumes generally ranged from several hundred microliters to several milliliters depending on particle concentration. The CytoSense flow cytometer used in this study was equipped with a 488 nm blue laser and detectors for forward scatter, side scatter, and fluorescence at green (515 – 565 nm), yellow (565 – 595 nm), orange (595 – 664 nm), and red (>664 nm) wavelengths. Detector gain settings and trigger levels were adjusted using natural samples and phytoplankton cultures to ensure adequate signal strength and detection of cells as small as 2 µm (e.g. Synechococcus), and to avoid saturation by large dinoflagellates or colonial diatoms (e.g. Chaetoceros) that are common in East Sound. Gain settings and trigger levels were kept consistent for all samples within each year to ensure comparable results. Alignment and performance of detectors was checked daily by running a broad spectrum fluorescent, 2.5 µm diameter bead standard (Molecular Probes, AlignFlow beads).
The standard algorithm in the CytoBuoy CytoClus analysis software (version 3.0) calculates particle length from scan width at half maximum with a correction for particles smaller than the focused beam height. This algorithm was found to be inaccurate for large particles such as pennate diatoms or Chaetoceros spp. colonies that had low signal intensity near the ends of scans. We developed a modified algorithm to calculate length according to scan width at a fraction of scan height that was dependent on the log of total integrated forward scatter. The algorithm used a generalized logistic function of the form:

$$f = 0.594 + \frac{0.01 - 0.594}{(1 + 0.001e^{-2.92(s-3.415)})^{1000}}$$

where $f$ is the fraction of scan height at which to determine length and $s$ is the base 10 log of the integrated forward scatter scan. The parameters for this logistic function were determined by unconstrained nonlinear optimization using the fminsearch function in Matlab (The Mathworks Inc.). The procedure found parameter values that minimized the length measurement error of six bead size standards (Duke Scientific polymer microspheres) with diameters of 2.7, 9.7, 14.6, 20, 30.1, and 69.9 µm. Length measurements were then recalculated based on this optimized algorithm for scan data from all collected samples.

The accuracy of CytoSense concentration measurements was tested by comparison with manual microscope based cell counts of a sub-set of samples collected during 2009 and two uni-algal phytoplankton cultures. Field samples contained an abundant population of the large pennate diatom Haslea sp. which was easily identified in the microscope and in CytoSense data due to its consistent size and shape. A culture of this taxon, isolated from East Sound in 2011, and a culture of the
dinoflagellate *Akashiwo sanguinea*, isolated from Grays Harbor, WA were also used to test CytoSense cell concentration measurements. Manual microscope based cell counts of preserved field samples and cultures were conducted in the lab on a Nikon Eclipse E800 compound microscope using a 10X objective and phase contrast illumination. Cells were counted in a 0.1 mL Palmer-Maloney or 1mL Sedgewick-Rafter chamber depending on concentration.

The accuracy of CytoSense length measurements were tested for four uni-algal cell cultures by comparison with automated image analysis using a FlowCAM imaging particle analyzer. Selected species varied in size from approximately 10 µm to nearly 1 mm and included *Teleaulax amphioxeia* (Cryptomonadacea), *Akashiwo sanguinea* (Dinophyceae), *Chaetoceros socialis* (Bacillariophyceae), and *Chaetoceros eibenii* (Bacillariophyceae). Cultures were isolated from Narragansett Bay RI, East Sound (*C. eibeneii*), or Grays Harbor WA (*A. sanguinea*) and grown in L1 sea water medium (Guillard & Hargraves 1993) with a 12:12 light:dark cycle at 15°C. To promote proper morphology of large colonial diatoms and prevent settling, cells were grown in 10 L tanks equipped with stir bars to simulate moderate levels of natural turbulence. Measurements from six replicate tanks for each species were acquired while cells were in exponential growth phase.

Particle length, forward scatter, side scatter, chlorophyll fluorescence, and phycoerythrin fluorescence were used to classify particles into separate types. Classification was performed by manually defining parameter value ranges (gates) for different particle types on scatter plots of length vs. maximum red fluorescence, maximum forward scatter vs. maximum side scatter, and maximum red vs. maximum
yellow fluorescence. Identifiable particle types included non-algal particles (without chlorophyll fluorescence), picoplankton sized (~1-2 µm) *Synechococcus*, nanoplankton sized (2-20 µm) photosynthetic eukaryotes, microplankton sized (>20 µm) diatoms and dinoflagellates, nano- and microplankton sized cells with phycoerythrin (e.g. cryptomonads and *Dinophysis* sp.), and nano- and microplankton sized dead algal cells. Dead cells were distinguished from live cells and non-algal particles by having very low, residual chlorophyll fluorescence but with length and scatter characteristics similar to live cells.

**Data analysis**

For comparison with flow cytometry data, optical data were extracted from ship based profiles collected at the same location, depth and time as samples. For each sample, four optical parameters that describe the shape and relative magnitude of $a_p$, $c_p$, and $b_p$ spectra were calculated. These included the ratio of $b_p$ at 555 nm to $a_p$ at 676 nm ($b_p:a_p$), the spectral slope of the $a_p$ coefficient between 488 and 532 nm, the spectral slope of $c_p$, and the ratio of $b_b$ to $b$ at 532 nm ($b_b:b$). Wavelengths for $b_p:a_p$ were chosen to coincide with the peak scattering signal and the long wavelength chlorophyll absorption peak to minimize the influence of absorption by non-algal particles. The negative $a_p$ slope was calculated according to Eisner et al. (2003 & 2005) as

$$a_p \text{ slope} = - \frac{a_p 488 - a_p 532}{a_p 676 (488 - 532 \text{ nm})}$$

Note that Eisner et al. report $a_p$ slope as a negative value and we report this slope as a positive value to maintain consistency with conventions for $c_p$ slope. The slope of $c_p$
was determined between 412 and 650 nm by fitting an equation of the form $c_p(\lambda) = s\lambda^{-\gamma}$ where $\lambda$ is the wavelength, $\gamma$ is the exponential slope of the spectrum, and $s$ is a scale factor (Twardowski et al. 2001, Boss et al. 2001). The backscatter ratio $(b_s:b)$ at 532 nm was calculated from $b_b$ measured with the ECO VSF and $b_p$ measured with the ac-9 or ac-s, both at 532 nm.

A percent scatter distribution based on scanning flow cytometry data was used to characterize the particulate composition of samples. We used scatter, rather than particle concentration, to account for differences in particle cross sectional area that determine their impact on bulk absorption, scattering and attenuation coefficients (Bricaud and Morel 1986). For every sample, flow cytometry data for each of the six particle types was binned according to length into 50 logarithmically sized bins between 1 $\mu$m and 2 mm. This resulted in 300 categories of particles (50 sizes of each type) for which the percentage of total particle scatter was calculated. For each particle, scatter was calculated as the sum of the integrated forward scatter and side scatter scan data. Percent total scatter was calculated as the sum of scatter for all particles of a given type in a size bin normalized by the sum of total scatter for all particles in the sample.

A combination of hierarchical cluster analysis and redundancy analysis was used to determine the relationship between the particulate composition of samples and their corresponding optical properties. These analyses were conducted separately for each year due to differences in gain settings for the flow cytometer that may have affected particle detection and classification. The percentage of particle scatter by all 300 categories was used to cluster samples into four groups in each year. Hierarchical
cluster analyses (complete linkage method) were based on a Bray-Curtis distance matrix and computed with the Matlab statistics toolbox version 8.0. Dendrograms were subdivided at a fixed distance above which similarity profile tests could confirm significant structure ($p < 0.01$) for all groups (Clarke et al. 2008). This resulted in four clusters of samples in each year. Means for each cluster were determined by averaging percent scatter distributions and optical parameters among all samples in each community. Similarity profile tests were performed with the Fathom toolbox for Matlab (Jones 2012) using $10^4$ iterations. Redundancy analysis was performed with the Vegan package for the R statistical computing environment (Oksanen et al. 2013). Redundancy analyses were based on the same 300 categories of percent scatter data used for cluster analyses and measurements of the four optical parameters $b_p; a_p; a_p$ slope, $c_p$ slope, and $b_b; b$ corresponding to each sample.

**High resolution autonomous profiles**

High spatial and temporal resolution optical data were collected with an Ocean Response Coastal Analysis System (ORCAS) autonomous vertical profiler (Donaghay 2003, Babin et al. 2005, Sullivan et al. 2005, Sullivan et al. 2010) moored in the upper sound from May 20$^{th}$ through May 26$^{th}$ in 2009 and from May 11$^{th}$ through May 15$^{th}$ in 2010. This system is equipped with a programmable submersible winch anchored to the sea floor, floats to provide positive buoyancy, batteries, a data logger, and a radio system to transmit collected data to a receiving station. The instrument payload included a WET Labs ac-9 absorption and attenuation meter, a Sea-Bird SBE49 CTD, a WET Labs WETstar chlorophyll fluorometer, a WET Labs ECO VSF three angle back scatter sensor (532 nm), and a WET Labs CDOM fluorometer. The system
collected profiles at an ascent rate of ~3 cm s\(^{-1}\) resulting in data with a vertical spatial resolution of ~1 cm. Profiles were conducted hourly in 2010 and every 2 hours in 2009. The ac-9 meter was calibrated before and after deployment and corrections for temperature, salinity and backscatter errors were applied to data as with the ship based profiling system. Profiles were smoothed with a 10 point running mean after outliers greater than 3 standard deviations from the running mean were removed. Chlorophyll concentrations were calculated from absorption spectra according to the methods of Sullivan et al. (2005) using a chlorophyll specific absorption coefficient of 0.014 m\(^2\) mg\(^{-1}\) typical for East Sound. Absorption based chlorophyll concentrations were used to avoid the effects of fluorescence quenching by photo-inhibition that can happen in near surface waters.

Since the ORCAS profiler could not be equipped with a filtered ac meter, \(a_g\) was determined from measurements of CDOM fluorescence. A model of \(a_g\) based on CDOM fluorescence and depth was developed from \(a_g\) and CDOM fluorescence measurements acquired with the ship based profiling package during 2010. The value of \(a_g\) at 412 nm was found to be linearly related to CDOM fluorescence according to:

\[
a_g (412 \text{ nm}) = 0.0135 F + 0.1806
\]

where \(F\) is CDOM fluorescence. The exponential slope (\(s\)) of the \(a_g\) spectrum was found to vary with depth according to:

\[
s = 0.0008e^{(-0.2334d)} + 0.016
\]

where \(d\) is depth. These relationships determined the parameters used to calculate \(a_g\) spectra according to:

\[
a_g(\lambda) = a_g(412 \text{ nm})e^{(-s(\lambda-412))}
\]
where \( \lambda \) is wavelength and \( s \) is the spectral slope. Modeled \( a_s \) spectra were then subtracted from total attenuation spectra to determine \( a_p \) spectra, \( c_p \) spectra and \( c_p \) slope.

To test the association between optical parameters and particle characteristics, \( b_p; a_p, a_p \) slope, \( c_p \) slope, and \( b_p; b \) derived from high resolution data were compared to samples collected from three points during the time series from each year. Samples were analyzed with flow cytometry to determine particle composition and characteristics as described above.

RESULTS

CytoSense validation

Particle concentrations determined by flow cytometry and manual microscope counts were linearly related (Fig. 2A). A model II least squares linear fit to the log converted data had an \( r^2 \) value of 0.97 indicating consistent precision over a wide range of cell densities. Cell concentrations determined with the flow cytometer were generally slightly lower than cell concentrations determined manually for all samples, a result that appears exaggerated at lower cell densities on the log:log plot. Cell length measurements determined with the CytoSense flow cytometer were similar to measurements of cell length determined by automated image analysis with a FlowCAM particle analyzer (Fig. 2B). Cytosense length measurements are slightly higher than FlowCAM measurements for the smallest cells (\( T. amphioxeia \)) and slightly lower than FlowCAM measurements for the larger \( C. socialis \).
Ship based optical profiles and sample analyses

A total of 55 samples in 2009 and 61 samples in 2010 were acquired and analyzed. Microscopy of live samples collected during 2009 revealed a phytoplankton community dominated by an abundant population of a pennate diatom in the genus *Haslea* (Fig. 1A & B, see also Rines et al. in prep.). During 2010, live microscopy revealed a phytoplankton community still dominated by diatoms but with a greater diversity of species including multiple *Chaetoceros* (especially *C. socialis*, Fig. 1C) and a number of other colony forming centric and pennate diatoms such as *Dactyliosolen fragilissimus, Detonula pumila, Ditylum brightwellii, Eucampia zodiacus, Guinardia delicatula, Pseudo-nitzschia spp., Skeletonema spp., Rhizosolenia spp., Leptocylindrus danicus, and Thalassiosira spp.* These species represent a wide range of cell sizes, shapes, and modes of colony formation. Individual cells of these species could be as small as ~10 µm (e.g. *Skeletonema* spp.) while colonies *C. socialis* or single cells of *Rhizosolenia* spp. could be several hundred µm in size. The morphology of the siliceous frustules for many of these diatoms was quite complex with some species having long siliceous setae or interconnecting spines (e.g. *Chaetoceros* spp. and *Skeletonema* spp.) and others with patterns of pores or striations along the valve face (e.g. *Thalassiosira* spp., *Pseudo-nitzschia* spp., and *Haslea* sp.).

Approximately 1 mL of seawater and an average of 16,589 particles were analyzed per sample during 2009. Average sample volumes and number of particles analyzed were increased during 2010 to 1.7 mL of seawater and 58,681 particles per sample to account for a lower overall concentration of phytoplankton and a more diverse phytoplankton community. During 2009 particles were classified as non-algal,
eukaryotic cells containing phycoerythrin, live *Haslea* sp., dead *Haslea* sp., nanophytoplankton, or microphytoplankton. Despite initial efforts to calibrate detector gain settings and trigger levels prior to sample analyses, signal strength from picoplankton sized cells was insufficient for accurate enumeration in most samples. However, despite their high abundance, these small cells accounted for a very small proportion of total scatter or fluorescence most likely due to their small size and low refractive index. Low contributions of picophytoplankton sized cells to total scatter were also found by Green et al. (2003). During 2010, gain settings were increased and trigger levels were decreased to ensure efficient detection of *Synechococcus*. Flow cytometer data collected during 2010 were classified as non-algal, eukaryotic cells with phycoerythrin, *Synechococcus*, nanophytoplankton, microphytoplankton, and dead cells.

Cluster analyses based on the particulate composition of samples identified four distinct communities in each year (Fig. 3). Simprof analyses confirmed the significance of all clusters (*p* < 0.01) and produced cutoff distances of 0.52 and 0.49 for 2009 and 2010, respectively. The mean composition of these communities is shown in Fig. 4 as stacked histograms representing percent scatter for each particle type. In 2009 (Fig. 4A – D), community 3 was overwhelmingly dominated by live *Haslea* sp. cells (Fig. 4C). Community 2 was also dominated by live *Haslea* sp. but had a higher proportion of scatter by other particle types (Fig. 4B). Community 1 had a large proportion of scatter from dead *Haslea* sp. cells (Fig. 4A) and community 4 had a more even mixture of scatter by different particle types (Fig. 4D). During 2010 phytoplankton communities were not dominated by a single taxon but contained a far
more diverse complement of particles with a range of sizes and shapes (Fig. 4E - F). Community 5, although consisting only of 3 samples, was distinctive in composition due to a very high proportion of scatter by dead cells (Fig. 4E). Community 7 contained a high proportion of scatter by large (> 100 m) phytoplankton cells and colonies (Fig. 4G). Community 6 was dominated by non-algal particles (Fig. 4F) and community 8 had a more even mixture of scatter by all particle types (Fig. 4H).

Mean values and standard deviations of $b_p\cdot a_p$, $c_p$ slope, $a_p$ slope, and $b_b\cdot b$ for each community are shown in Table 1. Results of redundancy analyses for each year (Figs. 5 & 6) show the variation of these optical parameters among communities. Permutation tests found results of both redundancy analyses to be highly significant ($p < 0.005$). In the resulting biplots (Figs. 5 & 6), symbols represent samples and symbol shape indicates community membership determined by cluster analyses as in Fig. 3. The labeled vectors extending from the origin indicate the direction of increase for each optical parameter among all samples. In both years, the $a_p$ slope was correlated with $b_p\cdot a_p$, and $c_p$ slope was correlated with $b_b\cdot b$. These correlations are most evident in the 2010 data. Vector length indicates how well the plotted result represents variation of a particular parameter. In Fig. 6, for example, the vector representing $c_p$ slope is relatively short indicating that the variation of this parameter is not represented as well as other optical parameters in the biplot. In 2009 community 1, dominated by dead Haslea sp., was found to have high values of $b_p\cdot a_p$ and $a_p$ slope, intermediate $c_p$ slopes, and low values of $b_b\cdot b$ (Fig. 5, Table 1). Samples from community 2 spanned a range of moderate to high $c_p$ slopes, moderate to high $b_b\cdot b$, and had intermediate values of $a_p$ slope and $b_p\cdot a_p$. Community 3, dominated by live
Haslea sp. cells, had low values of $c_p$ slope and $a_p$ slope, low values of $b_p:a_p$ and moderate values of $b_h:b$. Community 4 had high $c_p$ and $a_p$ slopes, high $b_p:a_p$, and moderate to high values of $b_h:b$. During 2010, samples in community 5 had the highest values of $b_p:a_p$, and $a_p$ slopes, low $c_p$ slopes, and low $b_h:b$ (Fig. 6, Table 1). Community 6 had high values of $b_h:b$, high values of $c_p$ slope, and intermediate values of $b_p:a_p$ and $a_p$ slope. Community 7 had moderate to low values of $c_p$ slope and $a_p$ slope, low $b_p:a_p$, and intermediate values of $b_h:b$. Community 8 spanned a range of intermediate values of all optical parameters.

Comparison of community composition (Fig. 3) with results of redundancy analyses (Figs. 5 & 6) and mean community optical parameters (Table 1) shows the influence of particle types on particulate IOPs. During 2009 $c_p$ slopes decreased with the relative amount of scatter by live or dead Haslea sp. cells. Also during 2009, the lowest $a_p$ slopes were associated with live Haslea sp. cells (community 3) and the highest $a_p$ slopes were associated with dead Haslea sp. cells (community 1). Conversely, high $b_p:a_p$ was associated with dead Haslea sp. cells (community 1), and low $b_p:a_p$ was associated with live Haslea sp. cells (community 3). However, the $b_p:a_p$ parameter may also have been affected by non-algal particles since the highest mean values were associated with community 4 (Table 1). High values of $b_h:b$ were associated with non-algal particles and low $b_h:b$ was associated with dead Haslea sp. cells. Lower $b_h:b$ for community 1 compared to community 2 may be attributed to dead Haslea sp. cells since these communities are otherwise similar. Higher $b_h:b$ for community 2 compared to community 3 is likely due to non-algal particles since these communities are otherwise similar. During 2010 high $c_p$ slopes were associated with
scatter from small non-algal particles (community 6) and $c_p$ slopes decreased in association with scatter by large (> 100 µm) phytoplankton cells and colonies (communities 7 & 8). However, the lowest mean $c_p$ slopes were associated with the high scatter from dead cells in community 5 (Table 1). As in 2009, high $b_p:a_p$ and high $a_p$ slopes were associated with dead cells (community 5) while low $b_p:a_p$ and $a_p$ slopes were associated with large, healthy phytoplankton (community 7). Also similar to 2009, low values of $b_b:b$ were associated with dead cells and high values of $b_b:b$ were associated with scatter from non-algal particles.

Results from both years support three general conclusions for East Sound waters. First, communities dominated by large phytoplankton cells and colonies are generally associated with low $c_p$ slope values. This is consistent with previous studies that show $c_p$ slope is a function of the slope of the particle size distribution (Kitchen et al. 1982; Boss et al. 2001). However, the low $c_p$ slope values associated with dead cells during 2010 represent a deviation from this general trend within the context of this study. Second, communities dominated by non-algal particles tend to have higher $b_b:b$, communities dominated by live phytoplankton cells have low $b_b:b$, and communities dominated by dead cells have the lowest $b_b:b$ values. Third, communities with abundant concentrations of dead phytoplankton are associated with high $b_p:a_p$ ratios and high $a_p$ slopes.

**High resolution autonomous profiles**

High resolution optical data collected with an ORCAS autonomous profiling system was used to infer phytoplankton community characteristics over small scales based on results of discrete sample analyses (Figs. 7 & 8). During both years the
ORCAS profiler captured a transition from high chlorophyll, bloom conditions to waters with low chlorophyll concentrations. In 2009, chlorophyll concentrations were highest below the pycnocline at the start of the high resolution time series and decreased over time (Fig. 7A). The $b_p:a_p$ ratio was highest near the surface at the beginning of the time series (Fig. 7B). Low $b_p:a_p$ values were found below the pycnocline through the beginning and middle of the time series. The lowest $b_p:a_p$ values were found associated with the pycnocline between 5 and 10 m depth during the middle of the time series. The $b_p:a_p$ parameter was of intermediate value and uniform throughout the water column at the end of the time series. The $a_p$ slope parameter was highest in near surface waters at the beginning of the time series and decreased with depth below the pycnocline (Fig. 7C). This parameter was lowest throughout the water column near the end of the time series (Fig. 7C). The $c_p$ slope parameter was lowest below the pycnocline and of intermediate value in surface waters at the beginning of the time series (Fig. 7D). Values of $c_p$ slope were high throughout the water column at the end of the time series. The $b_p:b$ data collected by the autonomous profiler during 2009 was of poor quality and is not shown.

High resolution optical data collected in 2010 showed high chlorophyll concentrations in surface waters near the beginning of the time series that decreased over time to form a subsurface maximum between 5 and 10 m depth (Fig. 8A). The $b_p:a_p$ ratio was initially low where chlorophyll concentrations were high, and increased in near surface waters at the end of the time series (Fig. 8B). Elevated $b_p:a_p$ values in thin layers were associated with the pycnocline between 15 and 20 m on May 12th and between 10 and 15 m on May 13th. The $a_p$ slope parameter was lowest at depth at the
start of the time series, moderately high where chlorophyll concentrations were highest, and generally increased for most of the water column over time with some lower values remaining in layers between 10 and 20 meters (Fig. 8C). The $c_p$ slope parameter was lowest near the surface throughout the time series (Fig. 8D) with strong gradients located along the pycnocline. The $b_p:b$ ratio was consistently higher at depth throughout the time series (Fig. 8E).

Based on results of the redundancy analyses, $c_p$ slope values suggest that waters below the pycnocline near the beginning of the time series in 2009 were dominated by large Haslea sp. cells. Low $b_p:a_p$ and $a_p$ slopes suggest a low proportion of dead cells in this high chlorophyll region. In surface waters, moderate $c_p$ slopes, high $b_p:a_p$, and high $a_p$ slopes suggest abundant dead Haslea sp. cells and more abundant small particles. Towards the end of the 2009 time series, high $c_p$ slope and low $a_p$ slope values suggest smaller average particle sizes and a smaller proportion of dead cells. Flow cytometric analyses of samples collected at three points during the time series support these optical predictions of particle composition (Fig. 7). A sample collected from high chlorophyll waters below the pycnocline on May 20$^{th}$ was dominated by live Haslea sp. (Fig. 9A) with a low proportion of scatter by dead cells (3.03%). There was a greater proportion of scatter by dead cells (10.1%) in near surface waters on May 21$^{st}$ where $b_p:a_p$ and $a_p$ slope were high (Fig. 9B). There was also considerably more scatter by small non-algal particles ($<10$ $\mu$m) in these surface waters as predicted by moderate $c_p$ slopes. The sample collected on May 25$^{th}$ near the end of the time series contained a higher proportion of scatter by cells in the 10 to 100 $\mu$m size range and a lower proportion of scatter (5.5%) by dead Haslea sp. cells (Fig. 9C).
The spatial and temporal distribution of $c_p$ slopes in 2010 high resolution data (Fig. 8D) suggest that large particles dominated in much of the upper portion of the water column throughout the time series, especially after May 13$^{th}$ when chlorophyll concentrations were low. Also throughout the time series, $b_p:b$ is higher in near bottom waters suggesting non-algal particles, possibly re-suspended sediments, are more abundant at depth. High $b_p:a_p$ and $a_p$ slope in near surface waters at the end of the time series suggest a high proportion of dead cells. Samples collected close to the ORCAS profiler in 2010 partially support these predictions. A near surface sample collected on May 12$^{th}$ from the region of high chlorophyll, low $b_p:a_p$, and moderate $a_p$ slope showed scatter was dominated by large (> 100 µm) phytoplankton cells and colonies (Fig. 9D). A sample collected from ~20 m depth on May 14$^{th}$ showed scatter dominated by non-algal particles (Fig. 9E). A sample collected near the surface at the end of the time series showed considerable scatter from dead cells (Fig. 9F). Although $c_p$ slope for waters dominated by phytoplankton colonies > 100 µm in size was lower than $c_p$ slope for waters dominated by small non-algal particles, the lowest $c_p$ slopes were associated with abundant dead cells in the 10 to 100 µm size range. Higher $b_p:a_p$ and $a_p$ slopes in surface waters also suggest that surface waters after May 13$^{th}$ are dominated by dead cells. This is contrary to the normal positive correlation between $c_p$ slope and the relative abundance of large particles (Kitchen et al. 1982; Boss et al. 2001) but in agreement with results from previous sample analyses that showed low $c_p$ slopes associated with dead cells (community 5, Table 1). For the large diatom colonies that were found in East Sound during the study in 2010, morphology may
play an important role in determining their scattering characteristics and the $c_p$ slope parameter.

DISCUSSION

This study compared the phytoplankton and non-algal particle composition of ocean waters with in situ measurements of particulate IOPs over small scales. Results show a strong influence of phytoplankton morphological and physiological characteristics on the spectral shape and relative magnitude of $a_p$, $c_p$, and $b_p$, coefficients. Variation in phytoplankton community composition, primarily driven by the relative abundance of large diatom cells and colonies, determined $c_p$ slope. However, it appears the complex morphology of some large colonies resulted in higher $c_p$ slopes than would be expected based on their size as measured with the Cytosense scanning flow cytometer. Phytoplankton cell death and subsequent loss of chlorophyll increased $b_p:a_p$ and $a_p$ slopes. In addition, the relative abundance of non-algal particles primarily determined $b_p:b$. These results demonstrate the ability of optical parameters to reveal variation of phytoplankton community characteristics with high resolution over ecologically critical, small spatial and temporal scales in the ocean.

Results were similar for two very different field collected data sets. In the first year, suspended particles in East Sound were dominated by a single diatom taxon in the genus *Haslea*. It’s relative abundance throughout the water column over time determined variation of the $c_p$ slope parameter. The death of this population caused dramatic changes in $b_p:a_p$ and $a_p$ slope as cellular pigment content changed and the proportion of empty frustules increased. Increases in $b_p:a_p$ are likely due to a decrease
in absorption by dead cells through loss of chlorophyll. Increases in $a_p$ slope may be associated with a relative increase in absorption by photoprotective carotenoids, or with changes in pigment packaging as cells die. In Monterey Bay, high $a_p$ slopes were associated with large cells with high pigment packaging (McFarland et al. in prep.) while in East Sound it seems more likely that changes in pigment ratios as cells died determined changes in $a_p$ slope. Although the phytoplankton community contained very different taxa and was far more diverse in the second year, similar results were found. Large colonial diatoms (C. socialis in particular) drove changes in $c_p$ slope. As these cells died $b_p:a_p$ and $a_p$ slope increased. In this case, dead and dying cells covered a range of sizes as colonies fell apart into single cells or short chains. Contrary to expectations, these smaller dead cells were associated with the highest $c_p$ slopes. The complex morphology of these large intact diatom colonies may have resulted in lower $c_p$ slopes than would be expected from theoretical predictions based on the assumption that particles are spheres or ellipsoids (e.g. Mie theory). Diatoms such as C. socialis form colonies several hundred µm in size, composed of curving intertwined chains of cells, each with multiple long thin siliceous setae (Fig. 1C). This highly non-spherical colony may scatter light similar to a population of smaller particles resulting in lower than expected $c_p$ slopes. Disruption of this structure as cells die and possible aggregation of colony fragments may result in particle populations with $c_p$ slopes higher than for healthy intact colonies.

In both years, optical data proved to be an effective tool for determining changes in phytoplankton community characteristics with high resolution over small spatial and temporal scales. Autonomous in situ measurements from an ORCAS profiler
enabled visualization of small scale features and important ecological processes that would have been otherwise very difficult to detect. During 2009, dead Haslea cells accumulated near the surface rather than sinking. This was visually confirmed by a distinctive surface slick seen throughout the sound at the termination of the bloom. Dead Haslea sp. cells may have been positively buoyant due to intracellular accumulation of lipids (Rines et al. in prep.). Also during 2009, the depth of the pycnocline corresponded to a minimum in $b_p:a_p$ values and high $c_p$ slopes (Fig. 7).

This suggests that the transition region between low and high density waters contained very few dead cells or large particles. During 2010, the depth of the pycnocline at times corresponded to elevated $b_p:a_p$ values. This suggests that dying cells were settling out of surface waters and accumulating at the interface between low and high density waters (Alldredge et al. 2002), a process that may have implications for biogeochemical cycling on a global scale. These small scale features would not have been visible by looking only at measures of total phytoplankton abundance such as chlorophyll absorption or fluorescence.

Previous work conducted in Monterey Bay showed that particulate optical properties varied among phytoplankton communities composed of taxa with very different morphological and physiological characteristics (McFarland et al. in prep.). The methods employed in the present study used direct flow cytometric measurements of these taxon specific characteristics to provide further evidence that phytoplankton morphological and physiological characteristics can be derived from in situ optical measurements to determine ecological patterns and processes in the ocean.
Currently the ecological dynamics of phytoplankton communities over small spatial and temporal scales in the ocean are poorly understood. Processes at these scales, however, are critical to the ecology of individual species and populations. Use of optical techniques as demonstrated here, can provide the tools necessary to greatly enhance our understanding of phytoplankton ecology in the pelagic ocean environment. Results show the particulate optical properties of natural phytoplankton communities clearly vary with biologically important morphological and physiological characteristics of cells and colonies. However, the relationship between cellular characteristics and optical properties can be influenced by multiple factors, and optical data may be difficult to interpret. Simultaneous biological or chemical analyses of discrete samples, therefore, may be required to accurately determine ecological patterns and processes from in situ optical measurements.

Acknowledgements

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Hanson AK, Donaghay PL (1998) Micro-to fine-scale chemical gradients and layers in stratified coastal waters. Oceanography 11:10–17


### Table 1. Number of samples (N) and the mean and standard deviations (s.d.) of optical parameters for samples in each community.

<table>
<thead>
<tr>
<th>community</th>
<th>N</th>
<th>$b_p:a_p$ mean</th>
<th>$b_p:a_p$ s.d.</th>
<th>$c_p$ slope mean</th>
<th>$c_p$ slope s.d.</th>
<th>$b_b:b$ mean</th>
<th>$b_b:b$ s.d.</th>
<th>$a_p$ slope mean</th>
<th>$a_p$ slope s.d.</th>
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<tr>
<td>2009</td>
<td>1</td>
<td>8</td>
<td>16.82 5.33</td>
<td>0.410 0.061</td>
<td>0.0096 0.0012</td>
<td>0.0218 0.0049</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>10.35 1.88</td>
<td>0.502 0.132</td>
<td>0.0160 0.0023</td>
<td>0.0131 0.0016</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>27</td>
<td>6.44 0.86</td>
<td>0.281 0.054</td>
<td>0.0112 0.0027</td>
<td>0.0109 0.0012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12</td>
<td>18.78 8.78</td>
<td>0.637 0.202</td>
<td>0.0131 0.0032</td>
<td>0.0187 0.0062</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>5</td>
<td>3</td>
<td>20.90 4.32</td>
<td>0.091 0.166</td>
<td>0.0070 0.0004</td>
<td>0.0176 0.0037</td>
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<td>13</td>
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<td>0.211 0.157</td>
<td>0.0084 0.0020</td>
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<td>10.41 4.89</td>
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<td>0.0122 0.0033</td>
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Figure 1. Examples of several phytoplankton cells and corresponding scan data from the Cytosense flow cytometer. (A) Image of live *Haslea* sp. cell above corresponding Cytosense scan. (B) Image and scan of empty *Haslea* sp. frustule. Note lack of chlorophyll fluorescence. (C) Image and corresponding scan of live *Chaetoceros socialis* colony.
Figure 2. (A) Comparison of phytoplankton cell concentrations determined by flow cytometry and manual, microscope based cell counts. (B) Comparison of mean phytoplankton cell length measurements determined by scanning flow cytometry and automated image analysis for four different uni-algal cultures. Standard error bars are smaller than most symbols.
Figure 3. Dendrograms showing results of cluster analyses for 2009 (A) and 2010 (B) based on particle size-scatter distributions of samples determined with scanning flow cytometry (See Fig. 4). Dashed lines indicate cutoff levels of 0.52 (2009) and 0.49 (2010) determined with similarity profiles ($p < 0.01$).
Figure 4. Mean particulate composition of communities determined with scanning flow cytometry for 2009 (A – D) and 2010 (E – H). Stacked histograms show percentage of total scatter as a function of particle type and length (µm).
Figure 5. Biplots showing results of redundancy analysis for 2009. Symbols indicate community membership of samples as determined by hierarchical cluster analyses (Fig. 3). Vectors indicate direction of increase for optical parameter values.
Figure 6. Biplots showing results of redundancy analysis for 2010. Symbols indicate community membership of samples as determined by hierarchical cluster analyses (Fig. 3). Vectors indicate direction of increase for optical parameter values.
Figure 7. High resolution optical data collected during 2009 with the ORCAS autonomous profiler. Symbols indicate location of samples shown in Fig. 9A – C. Black line indicates depth of the pycnocline.
Figure 8. High resolution optical data collected during 2010 with the ORCAS autonomous profiler. Symbols indicate location of samples shown in Fig. 9D – F. Black line indicates depth of the pycnocline.
Figure 9. Composition of individual samples collected near the ORCAS autonomous profiler during 2009 (A – C) and 2010 (D – F) shown as percent scatter determined by scanning flow cytometry. Symbols correspond to sample locations in Figs. 7 & 8 and to the communities they most resemble as in Figs. 5 & 6. Colors represent particle types as in Fig. 4.
THE EFFECTS OF SIZE, SHAPE AND PIGMENT CONTENT ON LIGHT ABSORPTION AND SCATTERING FOR FIVE MARINE PHYTOPLANKTON SPECIES

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ABSTRACT

The optical properties of phytoplankton determine the light harvesting efficiency of cells and influence light absorption and scattering in the ocean. To improve our understanding of the effects of morphology and physiology on the optical properties of cells and colonies we measured light absorption and scattering by 5 uni-algal cultures with distinct size, shape, and pigment characteristics. Light absorption and scattering efficiency factors, the ratio of scattering and absorption coefficients, and the shape of the absorption coefficient spectra all varied among cultures during exponential growth and after senescence. Results suggest that the size and intracellular pigment content of diverse species are ecologically important traits of cells that compete for available light in the marine pelagic environment. The low intracellular pigment concentrations and complex morphology of large forms appear to minimize the negative impact on fitness caused by self-shading of pigments (i.e. package effects). Results also suggest that optical properties measured in situ may be used to infer the morphological and physiological characteristics of large phytoplankton cells and colonies to better understand natural community structure and function.

INTRODUCTION

For more than 100 years ecologists have sought to explain the function of the many diverse forms of phytoplankton found in the marine pelagic environment (Gran 1912, Sournia 1982, Fogg 1991). Acquisition of light to fuel photosynthesis is one of the critical functions of these autotrophic organisms influenced by species-specific traits (Raven 1984). Phytoplankton morphological and physiological characteristics such as size, shape, structure, and pigment content determine how cells and colonies...
absorb and scatter light (Morel & Bricaud 1986, Johnsen et al. 1994, Ciotti et al. 2002). These characteristics vary considerably among the tens of thousands of described species and eight major phylogenetic groups of phytoplankton (Katz et al. 2004). Distinct taxa have different photoprotective and photosynthetic accessory pigments with characteristic absorption spectra (Hoepfner & Sathyendranath 1991). Cells and colonies of different species range in size over 4 orders of magnitude from less than 1 µm to greater than 1 mm. Taxa also exhibit a wide variety of morphologies ranging from simple spheres to complex three-dimensional structures. Despite these differences, all phytoplankton rely on the absorption of light to fuel photosynthesis and growth. Bio-optically important cellular characteristics, therefore, influence the ability of cells to compete for available light, and may determine the fitness of different forms in the marine pelagic environment where light is often a limiting resource (Huisman et al. 1999a, Huisman et al. 1999b, Finkel 2001, Litchman 2003, Litchman et al. 2004, Stomp 2007, Huisman et al. 2004).

In many ocean waters, absorption and scattering of light by phytoplankton cells and colonies determine, to a large extent, the magnitude and shape of particulate absorption ($a_p$) and scattering ($b_p$) coefficient spectra (Lorenzen 1972, Itturiaga & Siegel 1989, Stramski et al. 2001). These inherent optical properties (IOPs) are functions of phytoplankton and non-algal particle abundance, their projected area (i.e. the shadow cast on a 2 dimensional plane representing the amount of light intercepted), and the efficiency with which particles absorb or scatter the light they intercept. With modern optical and oceanographic instrumentation, $a_p$ and $b_p$ can be measured in situ with high resolution to reveal detailed patterns of variation over a
wide range of spatial and temporal scales (Cowles et al. 1998, Babin et al. 2005, Churnside & Donaghay 2009). Optical measurements made at very high spatial or temporal frequencies (less than 10 cm, greater than 1 Hz) can resolve small scale bio-optical variation critical to the ecological processes that determine phytoplankton community structure and function (Holliday et al. 2003, Rines et al. 2010, McFarland et al. in prep. b). IOPs, therefore, represent a valuable tool in the study of pelagic marine ecology that can be used to investigate ecosystem structure and the interactions between light and phytoplankton that affect growth rates of different species.

that often dominate coastal phytoplankton blooms. Consequently, the extent to which in situ optical variability can be attributed to ecologically relevant morphological and physiological differences among populations is not well known. Previous studies employing microscopy or flow cytometry to directly examine individual cells and colonies demonstrated that large phytoplankton can contribute substantially to particulate IOPs and that physiological changes affecting pigment content can have a significant impact on the relative magnitude and shape of $a_p$ and $b_p$ spectra (McFarland et al. in prep. a & b). With a better understanding of the optical effects of cellular morphology and physiology, in situ measurements of $a_p$ and $b_p$ may reveal the biological function of optically important phytoplankton characteristics and help explain ecological processes within natural phytoplankton communities.

In this study, the effects of morphological and physiological characteristics on ecologically important optical properties are analyzed for five distinct uni-algal phytoplankton cultures representing 3 taxonomic classes. We selected species that have been observed to vary with in situ measurements of $a_p$ and $b_p$ in the field (Rines et al. 2010, McFarland et al. in prep. a & b). The selected taxa ranged from ~10 µm long single cells with simple shapes to complex, 3-dimensional colonies greater than 700 µm in length. Cultured species included *Teleaulax amphioxeia* (Cryptophyceae), *Akashiwo sanguinea* (Dinophyceae), *Haslea* sp. (Bacillariophyceae), *Chaetoceros socialis* (Bacillariophyceae), and *Chaetoceros eibenii* (Bacillariophyceae). We measured the phytoplankton specific absorption and scattering coefficients ($a_{ph}$ and $b_{ph}$) for each species by size fractionation. Automated image analysis was used to measure the concentration and projected area of phytoplankton cells or colonies and
calculate their mean absorption and scattering efficiencies ($Q_a$ and $Q_b$). We also examined the slope of $a_{ph}$ between 440 nm and 532 nm, and the ratio of $b_p$ at 555 nm to $a_p$ at 676 nm ($b_{ph}:a_{ph}$) as biologically sensitive optical parameters that can be measured with high resolution in the ocean to study natural phytoplankton community structure and ecological processes. In addition, we used scanning flow cytometry to determine the particulate composition of cultures and quantify the contribution of cells, colonies, and non-algal particles to measured optical parameters. Measurements were obtained during exponential growth and after cells had settled from suspensions to evaluate the optical effects of physiological changes associated with senescence.

**METHODS**

For each species, six replicate sets of optical measurements, image analysis, and flow cytometry data were obtained during exponential growth. Four sets of these measurements were obtained for each species after cultures had settled out of suspension. Comparison among species during exponential growth allowed for assessment of the effects of species-specific characteristics on optical properties. Comparison between exponential growth phase and settled cultures allowed for assessment of the effects of physiology on the optical properties of each species.

**Culture isolation and growth**

Uni-algal cultures were obtained from field samples collected with a 64 µm mesh size plankton net or from un-concentrated sea water samples. *T. amphioxeia* was isolated from a Narragansett Bay, RI water sample. *C. socialis* was isolated from a water sample collected from Falmouth, ME. *Haslea* sp. and *C. eibenii* were isolated from samples collected in East Sound, WA. *A. sanguinea* was isolated from a sample
collected in Grays Harbor, WA. Single cells were identified and isolated by hand using an inverted compound microscope and a drawn glass pipet. Cells were washed 4 to 5 times by transferring into clean 0.2 \( \mu \text{m} \) filtered sea water and then placed into glass tubes with 10 mL of L1 sea water medium (Guillard & Hargraves 1993). \( A. \) sanguinea and \( T. \) amphioxeia were grown in L1/2 strength medium without enrichment of Si. Diatoms were grown in L1/10 strength medium. All cultures were maintained at 15\(^\circ\)C with a 12:12 light:dark cycle and transferred into fresh medium every two weeks.

For all measurements, replicate cultures were grown in 6 polycarbonate tanks (25 cm width, 10 cm depth, and 60 cm height) containing 10 L of sea water medium. Tanks were housed in a walk-in incubator maintained at 15\(^\circ\)C (±1\(^\circ\)C) and illuminated with cool white fluorescent lights on a 12:12 light:dark cycle. To prevent settling and enhance growth by simulating natural turbulence, cultures were continuously stirred with a horizontal 2.54 cm diameter polycarbonate rod that was raised and lowered through the tank by an electric motor at a rate of twice per minute.

**Automated Image Analysis**

A FlowCAM imaging particle analyzer (Fluid Imaging Technologies, Inc.) was used to determine the projected area and concentrations of cells, colonies (Fig. 1) and non-algal particles. The FlowCAM uses flow cells and objective lenses of various size and magnification to capture images of suspended particles in a known fluid sample volume. We used a 10x objective and a 100 \( \mu \text{m} \) width flow cell to image \( T. \) amphioxeia at a resolution of 0.553 \( \mu \text{m} \) pixel\(^{-1}\). A 2x objective and an 800 \( \mu \text{m} \) width flow cell were used to image \( C. \) eibenii at 2.94 \( \mu \text{m} \) pixel\(^{-1}\). Other cultures were imaged
at a resolution of 1.36 µm pixel⁻¹ with a 4x objective and a 300 µm width flow cell. FlowCAM software (Visual Spreadsheet version 3.2) automatically segments images (i.e. divides images into subject and background regions) by applying a threshold and calculates particle area and concentrations along with other particle shape dependent parameters such as length and width. Threshold levels were set manually for each species by visually inspecting segmented images to ensure accurate detection of particle edges. Cells and colonies were discriminated from non-algal particulate material based on their Mahalanobis distance from the mean of a sub-set of manually identified images of cells or colonies for each species. This distance metric takes into account correlation among parameters within the data set (Mahalanobis, 1936). Twenty-six descriptive particle parameters calculated by Visual Spreadsheet were used to compute the Mahalanobis distance. The maximum distance allowed for classification of particles as cells or colonies was set as the maximum distance from the mean within the sub-set of manually identified images. Mean values of particle area and concentration for particles classified as as cells, colonies or non-algal particles were computed for each replicate tank. Mean area for each species was calculated as the mean of mean areas determined for each replicate tank.

**Scanning Flow Cytometry**

A CytoSense (CytoBuoy b.v.) scanning flow cytometer was used to determine the particulate composition of cultures and the chlorophyll fluorescence of cells and colonies (Dubelaar et al. 2004). This instrument has a large sample flow path that can accommodate particles up to 800 µm in width. The CytoSense records scans of particles as they pass individually through a focused laser beam. Particles travel at a
fixed speed and the intensity of scattered and fluoresced light is measured at a fixed interval corresponding to 0.5 µm along the length of the particle (see also McFarland et al. in prep. b). Elongate particles tend to align lengthwise in the flow and the length of scans therefore represents maximum particle dimensions. The instrument used here was equipped with a blue laser (488 nm). Detectors for forward scatter, side scatter, yellow fluorescence (565 to 595 nm) and red fluorescence (>664 nm) were used to detect populations of cells, colonies and non-algal particles. Scans were triggered by scatter and fluorescence detectors to ensure accurate detection of all optically important particles within cultures. Detector gain and trigger levels were consistent for all measurements and set to ensure accurate detection of particles as small as ~2.5 µm. Cell, colony, and non-algal particle populations were discriminated by gating based on their length and maximum chlorophyll fluorescence. The total light scattered by particles was determined by integrating forward and sideward light scatter scans for each particle. The relative percentage of light scatter was determined by dividing total light scatter for each particle type by the total light scattered by all particles in a sample. Similarly, the total chlorophyll fluorescence of cells or colonies was determined by integrating red fluorescence scans. Mean chlorophyll fluorescence was calculated for all cells or colonies analyzed in each tank. Mean chlorophyll fluorescence for each species was calculated as the mean of mean values for all replicate tanks.

Optical Measurements

The $a_{ph}$ and $b_{ph}$ coefficients of each culture were determined with a WET Labs ac-s hyperspectral absorption and attenuation meter used in a bench-top mode. The ac-s
measures absorption and attenuation coefficients at >80 wavelengths between 400 and 730 nm. Absorption is measured in a reflective flow tube designed to capture most scattered light. A correction is made for light scattered in the backward direction. The instrument was mounted vertically and fitted with blacked-out tubing attached to the inlet and outlet ports of each flow cell. Measurements were conducted within the walk-in incubator chamber at the ambient culture temperature (15°C) to minimize errors induced by changes in sample temperature during measurements. For each of 3 replicate sub-samples, flow cells and attached tubing were filled with approximately 200 mL of culture. Samples were gently agitated during measurements to avoid particle settling by raising and lowering the inlet tube. For each measurement, 60 seconds of data acquired at a rate of 4 Hz was recorded for each sub-sample and subsequently averaged. Mean values for each tank were calculated as the mean of these averaged sub-sample measurements.

Total absorption ($a_t$) and attenuation ($c_t$) were measured using unfiltered culture containing both particulate and dissolved components. Absorption by dissolved substances ($a_g$) was determined by measuring a ~600 mL sub-sample of culture filtered through a 0.2 µm pore size filter. Further size fractionation was used to partition total absorption and attenuation into non-algal and phytoplankton specific parameters. Cells or colonies of all species except $T. amphioxeia$ were separated from non-algal particulate material in cultures by gentle reverse filtration through 10 (for $A. sanguinea$ only) or 20 µm size Nitex mesh. Optical data were corrected for the effects of temperature and salinity according to the methods of Twardowski et al. (1999) and Sullivan et al. (2006). Particulate IOPs ($a_p$ & $c_p$) were determined by subtracting $a_g$
from $a_t$ and $c_t$. Scattering errors in $a_p$ due to scatter in the backward direction were corrected for using the proportional algorithm of Zaneveld et al. (1994). The scattering coefficient ($b_p$) was computed for each tank by difference according to $b_p = c_p - a_p$.

Measurements of $a_p$ and $b_p$ for Nitex mesh filtered samples were subtracted from particulate IOPs to obtain $a_{ph}$ and $b_{ph}$ of cells and colonies in each tank during exponential growth. For settled cultures, non-algal particles larger than the Nitex mesh screen size could not be physically separated from cells and colonies and were therefore included in measurements of $a_p$ and $b_p$. The abundance of this large non-algal particulate material was also quantified by flow cytometry.

**Data Analysis**

To compare absorption and scattering spectra among species while accounting for cell or colony density and projected area, we computed the mean absorption and scattering efficiencies ($Q_a$ and $Q_b$) for each replicate tank according to:

$$Q_a(\lambda) = \frac{a_{ph}(\lambda)}{N\sigma} \quad Q_b(\lambda) = \frac{b_{ph}(\lambda)}{N\sigma}$$

Where $\lambda$ represents wavelength, $N$ represents cell or colony concentration in number m$^{-3}$ and $\sigma$ represents their mean projected area in m$^2$ (Morel & Bricaud 1986). $N$ and $\sigma$ for all particles larger than the Nitex mesh screen size were determined by automated image analysis with the FlowCAM. $Q_a$ and $Q_b$ describe the proportion of light absorbed or scattered by particles relative to their total projected area, and are functions of particle size, shape and complex refractive index. Values of $Q_a$ can range from 0 to 1 with a value of 1 indicating all light intercepted by the particle is absorbed. Values of $Q_b$ can be larger than 1 due to interference effects, especially for particles near visible wavelengths in size. For comparison with measured values, $Q_a$ and $Q_b$
were modeled according to Mie theory using the computer code of Bohren and Huffman (1998). $Q_a$ was modeled over a range of imaginary parts of the complex refractive index for 10 µm diameter homogeneous spheres with a real part of the refractive index equal to 1.045 (Ackleson & Spinrad 1988, Stramski & Reynolds 1993, Aas 1996, Green et al. 2003). $Q_b$ was modeled for spheres up to 250 µm in diameter with a complex refractive index of 1.045-0.001i.

We used the mean total chlorophyll fluorescence of cells or colonies measured with the flow cytometer and normalized by total projected area to determine a relative measure of intracellular pigment concentration ($C_i$) for each replicate. The ratio of phytoplankton specific scattering to absorption ($b_{ph}:a_{ph}$) was computed for each replicate using $b_{ph}$ at 555 nm and $a_{ph}$ at 676 nm to minimize the impact of absorption by non-algal particles. Non-algal particulate absorption decreases exponentially with increasing wavelength (Kishino et al. 1984, Roesler et al. 1989) and should, therefore, have minimal impact on absorption measurements at the long wavelength chlorophyll absorption peak (676 nm). The $b_{ph}:a_{ph}$ ratio is related to the carbon:chlorophyll ratio of cells (Stramski & Reynolds 1993) and is therefore indicative of changes in cellular composition. This parameter may also vary with $Q_b$ among particles of different size and shape.

The shape of phytoplankton absorption spectra varies significantly with intracellular pigment content and cell size especially between 450 and 550 nm (Kirk 1975, Hoepffner & Sathyendranath 1991, Johnsen et al. 1994, Ciotti et al. 2002). To quantify this variation among cultures we used the negative normalized slope of $a_p$ between 488 and 532 nm calculated for each replicate according to Eisner et al. (2003)
Higher values of this parameter correspond to steeper slopes which are associated with weak package effects (Duysens 1956, Das 1967) and higher ratios of photoprotective to photosynthetic carotenoids (Eisner et al. 2003). Differences in pigment content, \( b_{ph}: a_{ph} \) and \( a_{ph} \) slope among species were tested with a Kruskall-Wallis nonparametric analysis of variance (Kruskal & Wallis 1952) and multiple comparisons using the Tukey-Kramer method (Tukey 1953, Kramer 1956). Differences in \( b_{ph}: a_{ph} \) and \( a_{ph} \) slope between cultures in exponential growth phase and after settling were tested with a Wilcoxon rank sum test (Wilcoxon 1945). Statistical tests were performed with the MATLAB statistics toolbox (version 7.14.0.739, the MathWorks, Inc.).

RESULTS

Cultured species differed considerably in their morphological characteristics (Table 1, Fig. 1). The mean projected area of cells or colonies of different species ranged over three orders of magnitude from 26.9±5.1 µm\(^2\) for \( T.\) amphioxeia to 36073±9368 µm\(^2\) for \( C.\) eibenii (Table 1). Flagellates (\( T.\) amphioxeia and \( A.\) sanguinea) had relatively simple shapes, Haslea sp. was fusiform and highly elongate, and colonial diatoms had more complicated structures with numerous siliceous setae (Fig. 1). \( C.\) socialis formed hollow, spherical super colonies composed of multiple chains of cells. The thick, hollow setae of \( C.\) eibenii (subgenus Phaeoceros) contained numerous chloroplasts while the thin, hair-like setae of \( C.\) socialis (subgenus Hyalochaete) did not. Portions of diatom cells that did not contain pigments were not
detected by image analysis due to their low contrast in FlowCAM images. The thin ends of *Haslea* sp. cells, the setae of *C. socialis*, and some setae of *C. eibenii* in particular did not exceed threshold levels used to segment images (Fig. 1). As a result, measurements of projected area for these species accurately represent the photosynthetically active portions of cells but slightly underestimate their total area.

Measurements made during exponential growth were used to compute mean $Q_a$ and $Q_b$ spectra for each species (Fig. 2). $Q_a$ was highest for *A. sanguinea* followed by *T. amphioxeia*, *C. socialis*, *C. eibenii*, and *Haslea* sp. Values for *A. sanguinea* were likely overestimated and were larger than 1 at wavelengths shorter than ~500 nm due to the presence of non-algal particles not accurately accounted for by automated image analysis. The $Q_a$ spectrum for *T. amphioxeia* shows enhanced absorption at ~560 nm due to the photosynthetic accessory pigment phycoerythrin. The absorption efficiency of diatoms was lower than that of flagellates throughout most of the spectrum, except for *C. socialis* which was similar to *T. amphioxeia* at the long wavelength chlorophyll absorption peak (~676 nm). The $Q_b$ spectrum was highest for *T. amphioxeia* followed by *A. sanguinea*, *C. socialis*, *C. eibenii*, and *Haslea* sp. The shape of $Q_b$ spectra for *Haslea* sp. and *C. eibenii* was flatter than for other species. As for $Q_a$, the $Q_b$ spectrum for *A. sanguinea* was likely overestimated due to the presence of non-algal particles not detected in FlowCAM images.

The $b_{ph}:a_{ph}$ ratio varied significantly among species (Kruskal-Wallis test, $p = 0.00017$, $H = 19.98$, df = 3) during exponential growth but was not correlated with particle area or $C_i$ (Fig. 3, Table 1). Values of $b_{ph}:a_{ph}$ were highest for *T. amphioxeia* (6.73±0.34), the species with the smallest projected area per particle. This value was
significantly higher than $b_{ph}:a_{ph}$ for haslea sp., A. sanguinea, and C. socialis ($p < 0.05$). Values of $b_{ph}:a_{ph}$ were also relatively high for C. eibenii (5.38±0.18), the species that formed colonies with the largest projected area per particle. The $b_{ph}:a_{ph}$ ratio for C. eibenii was significantly higher than that of A. sanguinea ($p < 0.05$) but not significantly different from other species. Variation of the $b_{ph}:a_{ph}$ ratio among haslea sp., A. sanguinea, and C. socialis was noticeable but not significant ($p > 0.05$).

The normalized slope of $a_{ph}$ between 488 and 532 nm varied significantly among species (Kruskal-Wallis test, $p = 0.011$, $H = 11.13$, df = 3) and was not correlated with particle size or $C_i$ (Table 1, Fig. 3). A. sanguinea had the highest $a_{ph}$ slopes (0.0146±0.002). Values were significantly higher than the $a_{ph}$ slopes for T. amphioxeia, C. socialis and C. eibenii ($p < 0.05$, Fig. 3, Table 1). Haslea sp. also had a relatively high $a_{ph}$ slope (0.0108±0.0014) although values were not significantly different from other species ($p > 0.05$, Fig. 3, Table 1). Values of $a_{ph}$ slope for T. amphioxeia, C. socialis, and C. eibenii during exponential growth were all similar (~0.008) and not significantly different (Fig. 3, Table 1).

The mean relative chlorophyll content of cells or colonies ($C_i$) also varied significantly among species during exponential growth (Kruskal-Wallis test, $p = 0.001$, $H = 16.23$, df = 3) but was not correlated with cell or colony area (Table 1, Fig. 3). Haslea sp. had the lowest $C_i$ values while A. sanguinea had the highest (Fig. 3). $C_i$ for Haslea sp. was significantly lower than values measured for T. amphioxeia and A. sanguinea ($p < 0.05$). $C_i$ values for C. eibenii were also significantly lower than values for A. sanguinea ($p < 0.05$). Values of $C_i$ for C. socialis were not significantly different from other species.
During exponential growth, \( Q_a \) at 676 nm increased with the intracellular pigment concentration of cells (Fig. 4). Estimates of \( Q_a \) for \textit{A. sanguinea} are not shown due to interference from non-algal particles. These values were similar to modeled \( Q_a \) values for 10 µm spheres with imaginary parts of the complex refractive index ranging from 0.0001 to \( \sim \)0.003. \( Q_b \) at 555 nm was highest for \textit{T. amphioxeia} and lowest for \textit{Haslea} sp. (Fig. 4). Values are shown for projected areas converted to an area equivalent spherical diameter. Measured values of \( Q_b \) for \textit{T. amphioxeia}, \textit{C. socialis}, and \textit{C. eibenii} were similar to modeled values for spheres ranging in diameter from 1 to 250 µm. Measured \( Q_b \) values for \textit{Haslea} sp. were much lower than modeled \( Q_b \) for spheres with equivalent spherical diameter (see Discussion section). As for \( Q_a \), \( Q_b \) for \textit{A. sanguinea} could not be reliably calculated due to the presence of non-algal particles and is not shown.

After 8 to 45 days of growth, depending on species, most cells and colonies had settled to the bottom of tanks and visibly changed in appearance and color. These changes were generally associated with increases in \( b_{ph}:a_{ph} \) ratios and \( a_{ph} \) slopes (Table 1, Fig. 5). \textit{T. amphioxeia} had the largest increase and the highest values of \( b_{ph}:a_{ph} \) after settling (23.8). The \( b_{ph}:a_{ph} \) ratio for \textit{Haslea} sp., \textit{A. sanguinea}, and \textit{C. socialis} also increased while \( b_{ph}:a_{ph} \) for \textit{C. eibenii} decreased. Changes in \( b_{ph}:a_{ph} \) were significant for \textit{T. amphioxeia} (\( p = 0.0095 \), rank sum = 34), \textit{Haslea} sp. (\( p = 0.035 \), rank sum = 32), \textit{C. socialis} (\( p = 0.019 \), rank sum = 33), and \textit{C. eibenii} (\( p = 0.0095 \), rank sum = 10). \textit{T amphioxeia} also had the largest increase in \( a_{ph} \) slope. The \( a_{ph} \) slopes for \textit{A. sanguinea} changed little over time while \( a_{ph} \) slopes for \textit{Haslea} sp., \textit{C. socialis} and \textit{C. eibenii} increased. Increases in \( a_{ph} \) slope were significant for \textit{T. amphioxeia} (\( p = 0.0095 \), rank
sum = 34), C. socialis (0.0095, rank sum = 34), and C. eibenii (p = 0.019, rank sum = 33). For T. amphioxeia, changes in optical properties were likely influenced by very small non-algal particles (e.g. bacteria and detritus) that could not be separated from cells by filtration.

Scanning flow cytometry was used to determine the particulate composition of cultures based on the relative contribution of algal and non-algal particles to total light scatter (Figs. 6 & 7). For T. amphioxeia cultures during exponential growth, light scatter measured for all particles larger than the detection limit (~2.5 µm) was dominated by cells (Fig. 6). For Haslea sp. and C. socialis cultures, light scatter by particles larger than 20 µm was dominated by cells or colonies (Fig. 6). For C. eibenii cultures, light scatter by particles larger than 20 µm was dominated by colonies and detached setae (Fig. 6). The projected area of detached setae measured by the FlowCAM was included in calculations of $Q_a$ and $Q_b$. During exponential growth, non-algal particles generally accounted for less than 5% of the total light scattered by particulate material in all cultures except A. sanguinea. In A. sanguinea cultures, both cells and non-algal particulate material made substantial contributions to total light scattered by particles larger than the nitex mesh screen size (10 µm). This large non-algal particulate material appeared to consist of aggregated transparent exopolymeric compounds excreted by cells (Passow 2002).

After settling, large non-algal particles were abundant in all cultures except C. socialis. These particles made significant contributions to total light scatter as measured by the flow cytometer (Fig. 7). Light microscopy suggested that large non-algal particles were primarily composed of dead cells or colony fragments, aggregates
of cell debris, and excreted exopolymeric compounds. For *T. amphioxeia*, very small non-algal particles less than ~10 µm were responsible for the majority of light scatter. These small particles likely included heterotrophic bacteria. For *Haslea* sp. and *A. sanguinea*, many non-algal particles were larger than healthy cells (Fig. 7). After settling, light scatter by particles larger than 20 µm in *C. socialis* cultures continued to be dominated by algal particles. However, colonies were reduced in size and smaller colony fragments with low chlorophyll fluorescence were abundant.

**DISCUSSION**

The species examined in this study varied considerably in their morphological (Fig. 1), physiological and, consequently, their optical properties (Table 1, Figs. 2 & 3). Flow cytometry showed that the composition of the large size fraction for all cultures except *A. sanguinea* was overwhelmingly dominated by algal cells or colonies (Fig. 6). For *T. amphioxeia*, *Haslea* sp., *C. socialis*, and *C. eibenii* cultures, therefore, size fractionated optical measurements of $a_{ph}$, $b_{ph}$, $Q_a$, and $Q_b$ can be reliably attributed to algal particles. Absorption and scattering efficiency factors ($Q_a$ and $Q_b$) represent optical characteristics of species dependent on the morphology and physiology of cells and colonies. Unlike most previous studies (Bricaud et al. 1988, Agusti 1991, Green et al. 2003, Zhou et al. 2012), automated image analysis was used to measure the projected area of cells or colonies and determine $Q_a$ and $Q_b$. Using this method, particles were not assumed to be spherical and the optical properties of large phytoplankton with complex shape were more accurately determined.

Differences in $Q_a$ among cells and colonies may influence photosynthesis, growth rates, and competition for light within natural phytoplankton communities. $Q_a$ for
species tested here did not follow simple relationships with respect to cell or colony size. Rather, absorption efficiency was primarily a function of intracellular pigment concentrations (Fig. 4). While high $C_i$ and $Q_a$ ensure most of the light intercepted by cells is absorbed, this also results in a strong pigment packaging effect and a reduction in light absorbed per unit pigment (Duysens 1956, Das et al. 1967, Kirk 1975). This package effect alters the allometric scaling of metabolic rates and can result in lower growth rates for large cells when intracellular pigment concentrations are high (Finkel & Irwin 2000, Finkel 2001). Small cell size or low $C_i$, therefore, is advantageous in low light environments such as deep chlorophyll maxima or in turbid coastal waters.

$C_i$ and $Q_a$ were highest for the dinoflagellate *A. sanguinea* (Fig. 2). This is consistent with microscopic observations of large cells containing many chloroplasts. However, the presence of large non-algal particles in cultures interfered with measurements of $a_{ph}$ for this species and $Q_a$ and $Q_b$ are, therefore, overestimated by an unknown amount. Since absorption by non-algal particulate material generally decreases exponentially with increasing wavelength (Kishino et al. 1984, Roesler et al. 1989), $Q_a$ values should be more accurate at long wavelengths (e.g. 676 nm). Non-algal material was most likely aggregated exopolymeric compounds excreted by cells (Passow 2002). Interestingly, these results demonstrate that excreted exopolymeric compounds may be an optically important component of *A. sanguinea* blooms in the ocean. In Monterey Bay, CA these blooms have been associated with copious excreted material (Rines et al. 2010) and have been implicated in the death of sea birds through production of proteinaceous surfactants that damage feather waterproofing (Jessup et al. 2009). Despite problems with measurement of $a_{ph}$ and $Q_a$, estimates of $C_i$ suggest a
strong package effect for this species. As a motile cell, however, *A. sanguinea* may be able to reduce package effects by continually changing its orientation relative to incident light and by swimming to surface waters where light intensities are higher (Kamykowski et al. 1998). In fact, diurnal vertical migratory behavior that may mitigate package effects is commonly observed for this species (Kiefer & Lasker, 1975, Cullen & Horrigan 1981, Rines et al. 2010).

In contrast to *A. sanguinea*, $C_i$ for *T. amphioxeia, Haslea* sp., *C. socialis*, and *C. eibenii* were considerably lower (Fig. 3). $Q_a$ for these species increased with increasing $C_i$ regardless of cell or colony projected area (Fig. 4). Although the diatoms *C. socialis* and *C. eibenii* formed large colonies, lower $C_i$ for these species also resulted in low $Q_a$ which should minimize pigment package effects that could limit growth rates. The long thin morphology of *Haslea* sp. cells and the colony structure of *C. socialis* and *C. eibenii* may also have contributed to low absorption efficiency by minimizing optical path lengths through cells and reducing self-shading of pigments. For *Haslea* sp., path length would depend on cellular orientation relative to incident light, but in many cases would be similar to cell width (~10 µm). For *C. socialis*, although the total colony area is large, the path length through which light passed over this area was generally only 10 – 30 µm, or the depth of one or two cells, due to the arrangement of cells in the colony (Fig. 1). For *C. eibenii*, optical path length through colonies depended on orientation relative to incident light for the central chain of cells, but for many orientations nearly perpendicular to incident light, optical path lengths were the depth of a single cell (~30 – 40 µm). In addition, a substantial amount of photosynthetic pigment was located within thin, hollow setae (Fig. 1). Setae were less
than ~5 \( \mu m \) thick and extended several hundred micrometers from cell corners. These morphological features should greatly reduce self-shading for chloroplasts within setae and decrease the overall \( Q_a \) of colonies leading to more efficient light absorption and photosynthesis. As a result, \( C. eibenii \) in particular seems well adapted to low light environments despite its exceptionally large size. For similar species in the sub-genus \( Phaeoceros \), all of which have setae containing chloroplasts, the amount of light necessary to saturate photosynthesis is several times lower than for most other coastal diatoms (Harrison et al. 1993). \( Phaeoceros \) can also move chloroplasts within setae and has been observed to move chloroplasts towards the base of setae under high light intensities (Pickett-Heaps et al. 1994). In doing so, a colony may change its effective projected area, \( C_i \), \( Q_a \), and ultimately its capacity for photosynthesis and growth in order to adapt to different light environments. The low \( C_i \) and \( Q_a \) values found for large colonial forms in the present study suggest that the complex morphology of these species helps minimize pigment package effects and may increase fitness with respect to light acquisition.

Despite very different particle morphologies, scattering efficiencies at 555 nm for \( T. amphioxeia \), \( C. socialis \), and \( C. eibenii \) decreased with increasing cell or colony projected area similar to modeled values for spheres with a complex refractive index of 1.045-0.001i (Fig. 4). Particle shape or structure, therefore, seems to have little impact on scattering efficiencies for these species. Alternatively, the various components of colonies (e.g. valve, organelles, setae) combine to produce a mean particle \( Q_b \) that is closely approximated by Mie theory. \( Q_b \) values for \( C. socialis \) may be slightly overestimated since setae were not resolved in FlowCAM images and were,
therefore, not included in measurements of projected area. Setae for this species, however, are <1 µm thick (thinner than C. eibenii setae) and should, therefore, be very inefficient at scattering light as evidenced by their low contrast in acquired images. Haslea sp. had much lower values of $Q_b$ than would be expected from Mie theory based on their projected area. This discrepancy could be the result of measurement error caused by alignment of cells within the ac-9 or FlowCAM flow cells. Despite efforts to agitate cells during optical measurements, alignment of cells parallel to the incident irradiance during optical measurements could have resulted in the minimum possible projected areas and underestimation of $b_{ph}$ and $Q_b$. Perhaps more likely, due to flow induced shear fields, alignment of cells perpendicular to the camera within the FlowCAM flow cell could have resulted in overestimation of projected areas and underestimation of $Q_b$. If deviations from Mie theory are in fact the result of cell alignment, our results suggest that cellular orientation of elongate species may have a significant impact on scattering by natural populations in ocean waters. Orientation of phytoplankton cells and colonies has been observed for natural populations through the use of in situ holographic imaging techniques (Malkiel et al. 1999, Talapatra et al. 2013). Measurements of $Q_a$ and $Q_b$ in the present study are within the range of other published values, which generally report $Q_a$ at ~676 nm between 0.1 and 0.5, and $Q_b$ at ~555 nm between 1.2 and 2.8 for nano- and microphytoplankton size cells (Bricaud et al. 1988, Iturriaga & Siegel 1989, Agusti 1991, Stramski & Reynolds 1993, Zhou et al. 2012). Much of the variation of $Q_a$ and $Q_b$ within species observed here (Fig. 4) may be attributable to physiological variation among replicate tanks analyzed at different times. Measurements were generally conducted during the day over the
course of ~8 hours, and changes in cellular composition within this time period could account for much of the observed variation within species (Stramski & Reynolds 1993). However, there was no indication in flow cytometry data or by microscopy that cell division was synchronous or in phase with light:dark cycles for any of our cultures.

Within the context of optical theory, the capacity of a particle to absorb light is described by the imaginary part of its complex refractive index. This parameter in combination with the real part of the refractive index and particle size determine $Q_a$, which can be exactly calculated for a sphere by applying Mie theory (Bohren & Huffman 1998). Modeled $Q_a$ values for 20 µm diameter spheres that were similar to measured values had imaginary refractive indexes ($n'$) generally below 0.002, and an $n'$ of 0.001 produced a rate of decrease in $Q_b$ with increasing size that closely matched measured values. This suggests lower $n'$ for species studied here compared to other published values in the range of 0.003 to 0.01 (Stramski & Reynolds 1993, Green et al. 2003, Zhou et al. 2012) and is further evidence that large phytoplankton minimize package effects by maintaining low $C_i$.

Scattering to absorption ratios $(b_{ph}:a_{ph})$ for each species appeared to be determined by both projected area and $C_i$. In the case of *T. amphioxeia*, $b_{ph}:a_{ph}$ was likely high due to the high $Q_b$ for this species. Alternatively, the relatively high $b_{ph}:a_{ph}$ for *C. eibenii* was most likely due to the low $C_i$ for this species. *A. sanguinea* had the lowest $b_{ph}:a_{ph}$ ratios as would be expected from its high $C_i$, although overestimation of $a_{ph}$ due to non-algal particles may have influenced values for this species. Stramski & Reynolds (1993) showed carbon and chlorophyll content of *Thalassiosira pseudonana*
influence $b_{ph}:a_{ph}$ ratios over a similar range as measured here. This ratio, therefore is an indicator of both morphological and physiological characteristics of phytoplankton. The difference in $b_{ph}:a_{ph}$ ratios among species during exponential growth determined here can account for ~25 – 30% of the variability measured in the coastal ocean where values have been measured up to ~20, although at different wavelengths in some cases (Sosik et al. 2001, McFarland et al. in prep. a & b).

The normalized $a_{ph}$ slope can be determined by ratios of photoprotective to photosynthetic carotenoids (PPC:PSC) within cells or by pigment package effects that can flatten absorption spectra (Eisner et al. 2003). PPC:PSC ratios reflect the adaptation of cells to different light levels. In this study, $a_{ph}$ slope was highest for $A. sanguinea$ most likely due to absorption by non-algal particulate material. Only Haslea sp. showed elevated $a_{ph}$ slopes that could be attributed to pigment ratios, although differences could not be considered significant by multiple comparison tests. Differences were significant ($p < 0.05$), however, if $A. sanguinea$ was omitted from the analysis. $T. amphioxeia$, $C. socialis$, and $C. eibenii$ all had similar $a_{ph}$ slopes indicative of PPC:PSC ratios less than 0.25 (Eisner et al. 2003). These values are low compared to in situ measurements of $a_{ph}$ slope for natural phytoplankton communities (Eisner & Cowles 2005, McFarland et al. in prep. a & b) possibly due to the relatively low light intensities under which cultures were grown. However, $a_{ph}$ slopes for Haslea sp. suggests that species-specific differences may be important and $a_{ph}$ slopes measured for natural communities may vary with species composition as well as light adaptation. The low $a_{ph}$ slope of $T. amphioxeia$ is somewhat surprising given its unique pigment composition, including sufficient phycoerythrin to visibly affect the
color of cultures. For this species, weak package effects which might increase $a_{ph}$ slope may be counteracted by enhanced phycoerythrin absorption at 532 nm which would decrease $a_{ph}$ slope.

Increases in $b_{ph}:a_{ph}$ and $a_{ph}$ slope for *T. amphioxeia*, *Haslea* sp., *A. sanguinea*, and *C. eibenii* after cultures settled (Fig. 5) were likely due to the increase in abundance of non-algal particles (Fig. 7). Non-algal particles generally have decreasing absorption with increasing wavelength (Kishino et al. 1984, Roesler 1989) and an increase in their relative abundance would increase $b_{ph}:a_{ph}$ and $a_{ph}$ slopes. However, $b_{ph}:a_{ph}$ and $a_{ph}$ slope also increased for *C. socialis* despite a negligible contribution of non-algal particulate material to total light scatter in settled cultures (Fig. 7). This suggests physiological changes in phytoplankton particle characteristics, possibly associated with abundant colony fragments in the case of *C. socialis*, can also be important in determining $b_{ph}:a_{ph}$ and $a_{ph}$ slope. Increases were greatest for *T. amphioxeia* and values for settled cultures exceeded maximum values measured for natural communities (Sosik 2001, Eisner & Cowles 2005, McFarland et al. in prep. a & b). Increases for other species were more modest and covered only a portion of the range seen for in situ measurements. Since *T. amphioxeia* cultures were not size fractionated, this suggests much of the variation in $b_{ph}:a_{ph}$ and $a_{ph}$ slope in natural communities may be associated with the relative abundance of small non-algal particulate material. Unlike other species, the $b_{ph}:a_{ph}$ ratio for *C. eibenii* increased after cultures settled. This incongruous result may reflect changes in the contribution of setae to $b_{ph}$. Setae appeared to lose much of their chlorophyll after cultures settled and, devoid of
chloroplasts, their refractive index and scattering efficiency may have been greatly reduced.

As a whole, results demonstrate that differences among species and changes associated with senescence are both important to the optical properties of phytoplankton and the IOPs of ocean waters. The distribution of chlorophyll within colonies and low $C_i$ of large colonial forms suggest that these species are morphologically and physiologically adapted to minimize pigment package effects that would otherwise limit light absorption, photosynthesis and growth (Raven 1984, 1986, 1998). Although disadvantageous for efficient light absorption due to the package effect, large size can protect species from grazers (Tillman 2004 and references therein) and facilitate interaction with small scale turbulence to promote nutrient uptake (Margalef 1978, Karp-Boss et al. 1996). In the natural environment, the bio-optical characteristics of phytoplankton are one of many factors that determine growth rates of species and community structure. Variation in light intensity or spectral distribution in combination with turbulent mixing can create optical niches which favor certain forms over others and provide opportunities for differentiation and coexistence of functionally distinct species (Margalef 1978). This variable optical niche structure coupled with the distinct bio-optical properties of species represents a possible solution to Hutchinson’s classic “paradox of the plankton” (Hutchinson 1961, Huisman & Weissing 1999, Huisman et al. 2001).

In natural phytoplankton communities, measurements of particulate absorption and scattering will reflect the combined characteristics of all suspended particulate material. Results of this study, however, indicate that cell or colony size and
physiology can be important factors that determine ocean water IOPs. Particulate absorption and scattering can be measured in the ocean with centimeter scale spatial resolution and second scale temporal resolution (Cowles et al. 1998, Rines et al. 2002, McManus et al. 2003, Holliday et al. 2003, Babin et al. 2005). Optical measurements therefore can resolve patterns of variability in natural phytoplankton communities at scales critical to population dynamics and ecosystem structure. Although the FlowCAM instrument used here is limited to analysis of discrete samples, determination of cell and colony projected areas in situ are possible with techniques such as digital in-line holography (Talapatra et al. 2013). Future field work, therefore, could determine the detailed bio-optical characteristics of natural communities in the ocean to better understand the processes that control marine pelagic ecosystem structure and function. Such a bio-optical trait based approach could provide an improved mechanistic understanding of phytoplankton diversity, distribution, and abundance in the ocean (Litchman & Klausmeier 2008, Edwards et al. 2013).

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Table 1. Mean and standard deviation (s.d.) of morphological and optical properties for each species during exponential growth phase and senescence (settled). Mean areas were determined with the FlowCam. Mean lengths were determined with the CytoSense scanning flow cytometer.

<table>
<thead>
<tr>
<th>Exponential</th>
<th>Teleaulax amphioxeia</th>
<th>Haslea sp.</th>
<th>Akashiwo sanguinea</th>
<th>Chaetoceros socialis</th>
<th>Chaetoceros eibeni</th>
</tr>
</thead>
<tbody>
<tr>
<td>area (µm²)</td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
<td>s.d.</td>
<td>mean</td>
</tr>
<tr>
<td>length (µm)</td>
<td>10.28</td>
<td>1.05</td>
<td>177.2</td>
<td>1.31</td>
<td>48.73</td>
</tr>
<tr>
<td>Qa 676 nm</td>
<td>0.39</td>
<td>0.04</td>
<td>0.12</td>
<td>0.01</td>
<td>0.79</td>
</tr>
<tr>
<td>Qb 555 nm</td>
<td>2.63</td>
<td>0.16</td>
<td>0.47</td>
<td>0.14</td>
<td>2.54</td>
</tr>
<tr>
<td>baph·aph</td>
<td>6.73</td>
<td>0.34</td>
<td>4.04</td>
<td>0.92</td>
<td>3.14</td>
</tr>
<tr>
<td>aaph slope</td>
<td>0.0079</td>
<td>0.0012</td>
<td>0.0108</td>
<td>0.0014</td>
<td>0.0154</td>
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<tr>
<td>Ci</td>
<td>44.5</td>
<td>6.9</td>
<td>20.7</td>
<td>6.3</td>
<td>116.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Settled</th>
<th>Teleaulax amphioxeia</th>
<th>Haslea sp.</th>
<th>Akashiwo sanguinea</th>
<th>Chaetoceros socialis</th>
<th>Chaetoceros eibeni</th>
</tr>
</thead>
<tbody>
<tr>
<td>baph·aph</td>
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<td>9.58</td>
<td>8.03</td>
<td>3.24</td>
<td>5.01</td>
</tr>
<tr>
<td>aaph slope</td>
<td>0.0566</td>
<td>0.0228</td>
<td>0.0154</td>
<td>0.0046</td>
<td>0.0160</td>
</tr>
</tbody>
</table>
Figure 1. Example images acquired by the FlowCAM particle analyzer for each phytoplankton species. Black scale bars represent 20 µm. *A. sanguinea*, *Haslea* sp. and *C. socialis* are shown at the same scale. Scales for *T. amphioxeia* and *C. eibeni* are different. Note that setae were not resolved for *C. socialis*. 
Figure 2. Mean absorption and scattering efficiency for each culture during exponential growth.
Figure 3. Mean $b_{ph}:a_{ph}$ ratios, $a_{ph}$ slopes, and chlorophyll fluorescence per cell or colony during exponential growth for *T. amphioxeia* (amp), *Haslea* sp. (has), *A. sanguinea* (san), *C. socialis* (soc), and *C. eibenii* (eib). Species are ordered from left to right by increasing projected area (see Table 1).
Figure 4. Absorption efficiency at 676 nm of species as a function of intracellular chlorophyll concentrations ($C_i$) and scattering efficiency at 555 nm of species as a function of area equivalent spherical diameter (ESD). Lines indicate efficiency factors modeled using Mie theory. Modeled $Q_a$ values were computed by varying the imaginary part of the refractive index ($n'$). Modeled $Q_b$ values were computed by varying the diameter.
Figure 5. Mean $b_{ph}$:$a_{ph}$ ratio and $a_{ph}$ slopes during exponential growth and after settling for *T. amphioxeia* (amp), *Haslea* sp. (has), *A. sanguinea* (san), *C. socialis* (soe), and *C. eibenii* (eib).
Figure 6. Mean particulate composition of cultures during exponential growth determined by the relative contribution of particle types to total light scatter measured with the scanning flow cytometer. Only particles larger than the Nitex mesh screen size are shown for all species except *T. amphioxeta*. 
Figure 7. Mean particulate composition of settled cultures determined by the relative contribution of particle types to total light scatter measured with the scanning flow cytometer. Only particles larger than the Nitex mesh screen size are shown for all species except *T. amphioxea*. 
CONCLUSIONS

The fundamental goal of this dissertation was to improve observations of natural phytoplankton communities in the ocean. To achieve this goal, optical methods to determine community structure and bio-optical characteristics of cells and colonies over small spatial and temporal scales were developed and tested. Results show optical parameters that can be measured in situ with high resolution such as the $c_p$ slope, $a_p$ slope, and $b_p:a_p$ ratio can be indicative of phytoplankton cell or colony size and pigment content, characteristics that vary with species composition, physiology, and bio-optical fitness. Non-algal particles such as bacteria, sediments, and exopolymers which may also influence optical properties, varied predominantly with $b_p:b$ ratios. The combination of these optical parameters, which reflect the shape and relative magnitude of particulate absorption and scattering spectra, facilitate analysis of phytoplankton composition and characteristics with high resolution over small scales in the ocean.

The studies conducted here advance techniques necessary to achieve a better understanding of phytoplankton population dynamics, responses to environmental conditions, interactions among species, and impacts on biogeochemical cycles. Optical methods revealed detailed information about the distribution, abundance, morphology and physiology of phytoplankton that can lead to a better understanding of their ecology and the function of diverse forms in the ocean. Such methods provide a dramatic improvement in resolution over discrete sampling techniques and offer a new perspective on the ecology of planktonic organisms. The major findings of the three components of this research are summarized below.
The primary goal of this study was to determine the extent to which phytoplankton species composition is correlated with inherent optical properties (IOPs) over small scales in hydrographically complex coastal ocean waters. Although such a correlation does not necessarily imply phytoplankton are responsible for measured optical values, the lack of any correlation would indicate in situ optical data, as measured here, cannot be used to determine community characteristics over small scales. This first study, therefore, established whether further development of optical methods as ecological tools was warranted.

Results show a strong association between distinct phytoplankton communities and the shape and relative magnitude of the particulate absorption ($a_p$), scattering ($b_p$), and attenuation ($c_p$) coefficient spectra. Furthermore, optical properties varied with the morphological and physiological characteristics of species within different communities in agreement with previous theoretical and empirical work. Specifically, the $c_p$ slope decreased with increasing mean cell size and the $a_p$ slope decreased with intracellular pigment concentrations. The $b_p:a_p$ ratio also increased with decreasing cell size or pigment content, although to a lesser degree.

These results confirmed that in situ optical data can relate information about the distribution and composition of phytoplankton communities over small scales in the coastal ocean. Cell size and pigment content appeared to be important cellular characteristics responsible for the bio-optical differentiation of species assemblages. However, this study did not directly measure these cellular characteristics and did not account for suspended non-algal particulate material. The next study, therefore, was
designed to quantify the size and pigment content of cells, colonies, and non-algal particulate material.

**Manuscript 2**

Based on results of the first study, the primary goal of the second field study was to determine the effects of phytoplankton cell or colony size, pigment content, and non-algal particles on IOPs over small scales in coastal waters. Scanning flow cytometry was used to measure the size distribution and pigment content of phytoplankton and non-algal particles. These data provided a more robust and quantitative method of relating optical properties to the diverse characteristics of phytoplankton species and populations by directly measuring optically important particle features.

Results indicate large (>20 µm) and morphologically variable phytoplankton can dominate optical signals in dense bloom conditions or concentrated thin layers of cells that are common in the coastal ocean. As observed in previous studies, the size and physiological characteristics of phytoplankton varied with the shape and relative magnitude of $a_p$, $b_p$, and $c_p$ coefficient spectra. Optical parameters such as the $b_p:a_p$ ratio and $a_p$ slope increased dramatically with the relative abundance of dead and dying cells, identified by their weak chlorophyll fluorescence. The $c_p$ slope increased with the relative abundance of large phytoplankton cells or colonies while the $b_b:b$ ratio increased with the relative abundance of suspended sediment particles. High resolution optical data collected with an autonomous profiler enabled visualization of these phytoplankton community characteristics over centimeter vertical scales and hourly temporal scales.
The strong variation of $c_p$ slope with phytoplankton size distributions demonstrates that optical data can be used to discriminate among communities dominated by species of different size. However, particle morphology and the method of determining particle size may be important since large morphologically complex colonies of *Chaetoceros socialis* had lower than expected $c_p$ slopes. These optically important morphological characteristics may have adaptive significance, and may help certain forms compete for light by increasing light harvesting efficiency. The strong variation of $b_p:a_p$ and $a_p$ slope with the pigment characteristics of phytoplankton demonstrates that optical properties can be used to determine the physiological state of cells and the ecologically important process of mortality in natural communities. The relative abundance and size distribution of non-algal particles such as heterotrophic bacteria and suspended sediments are also important to bulk optical properties and must be taken into account when interpreting in situ measurements. Fortunately, high refractive index non-algal particles such as suspended sediments have a disproportionately strong impact on $b_p:b$ and this parameter, therefore, can serve to indicate when their abundance is optically significant.

High resolution data were effective in visualizing phytoplankton community characteristics and ecological processes over very small scales. This facilitated localization of transitions in phytoplankton community composition and accumulations of senescent cells. In one case, senescent cells were observed to aggregate in a near surface layer while in another case, aggregation of senescent cells was observed along the pycnocline. These observations would not have been possible through the use of discrete sampling techniques.
Results of this study demonstrate the use of in situ optical properties to identify characteristics of natural phytoplankton communities that help explain their function, distribution, abundance and dynamics in the ocean. Measurements were made for whole species assemblages whose natural range of variation is determined by populations of many different particles. To better understand how individual species and their characteristics contribute to this natural variation, a series of laboratory measurements on monospecific cultures were conducted.

**Manuscript 3**

The goal of this study was to determine variation of optical properties among live and senescent cells of five morphologically distinct species including large complex colonial forms that have not been previously studied yet can dominate optical signals in coastal ocean waters. Results were compared to field measurements to determine the amount of natural variation that could be attributed to either morphological or physiological differences in natural communities. Measured optical properties were also used to better understand the optical function of species specific traits and their effects on bio-optical fitness and adaptation to life in the pelagic environment.

As seen in field studies, variation in the size, shape, and pigment content of species resulted in significant differences in bio-optical properties. The $b_p:a_p$ ratio increased for small cells due to high scattering efficiency ($Q_b$) and for large colonies due to low absorption efficiencies ($Q_a$) and intracellular pigment concentrations. Large diatoms had very low $Q_a$ values compared to other published results which may enhance bio-optical fitness by minimizing pigment package effects. Orientation of highly elongate *Haslea* sp. cells appeared to cause underestimation of $Q_b$ values and
suggests that orientation within natural communities may have important implications for the optical properties and light harvesting capabilities of certain taxa. Among cultures in exponential growth phase, the magnitude of variation of $b_p:a_p$ accounted for ~30% of variation observed in natural communities while the magnitude of variation of $a_p$ slope could account for nearly all observed natural variation. However, $a_p$ slope variation could be attributed to exopolymeric material excreted by cells, pigment package effects, or changes in photoprotective:photosynthetic pigment ratios. For all but one species, senescent cultures showed increases in $b_p:a_p$ and $a_p$ slope due to loss of pigment and accumulating cell debris. Loss of chlorophyll from hollow setae in senescent *Chaetoceros eibenii* cultures resulted in a decrease of $b_p:a_p$ demonstrating that species specific characteristics can be optically important, may impact bio-optical fitness, and may have adaptive significance. For large particles, isolated by size fractionation, the changes in $b_p:a_p$ and $a_p$ slope after onset of senescence accounted for ~25% of the variation seen in natural communities.

Results of this laboratory based study demonstrate that the unique morphological and physiological characteristics of different species can account for a substantial proportion of the variation of optical properties measured for natural communities over small scales in the coastal ocean. Therefore, optical properties can be used to visualize community structure and function in the ocean with high resolution over small spatial scales. However, other particle types such as heterotrophic bacteria and cell debris are likely to be optically important for natural suspended particle assemblages but these particles are likely to covary with phytoplankton ecological processes such as mortality. Furthermore, the low intracellular pigment concentrations
and complex morphology of colonial diatom species tested here suggests that these species are adapted to minimize self-shading of chloroplasts which can limit light harvesting efficiency, growth, and fitness.

**Implications**

The results of this work have important implications for ocean observation techniques upon which our understanding of natural phytoplankton community structure and function is built. In situ observation systems can easily incorporate the types of optical measurements made here to better understand changes in phytoplankton community composition, size structure, and pigment content over longer time scales. Such measurements could provide a continuous record of phytoplankton structure and population dynamics through all types of environmental conditions, data sets that have been impossible to obtain with ship based and discrete sampling methods. Remote observation systems such as airborne LIDAR and satellite based sensors can provide synoptic optical measurements over regional to global scales. For these types of optical data, a better understanding of absorption and scattering by phytoplankton can help improve estimates of biomass and primary production that are often derived from remote measurements. Ultimately, the relationships among phytoplankton cellular characteristics and high resolution optical data revealed by this dissertation can be used to better understand natural phytoplankton community structure and function in the ocean and the selective processes that drive phytoplankton evolution.

Integration of phytoplankton bio-optical dynamics into optical and ecological ocean models could greatly improve their accuracy and their ability to predict
response to changing environmental conditions. Inverse optical models strive to
determine characteristics of dissolved and particulate material from optical
measurements. Although unique solutions to inverse models may still be unattainable,
it should be possible to derive useful biological information with a better
understanding of the effects of phytoplankton characteristics on optical variation in the
ocean. Results presented here show how optical properties vary with phytoplankton
size, shape, and pigment content, and suggest that these characteristics could be
derived from optical measurements provided we can account for the influence of non-
algal particles. Current ecological models often assume constant biomass specific
absorption and reflectance, yet results of this study indicate that such assumptions are
not valid for dynamic coastal ecosystems where phytoplankton biomass and rates of
primary production are often very high and extremely variable. Better model
parameterization that captures the variable bio-optical characteristics of phytoplankton
will improve their ability to predict local phenomena such as harmful algal blooms and
long term, global scale responses to climate change. Results of this dissertation
suggest that ecological models should incorporate phytoplankton size and pigment
content as variables that influence their distribution and function.

In the long term, a better understanding of ecological pattern and process in
natural phytoplankton communities is only possible with better in situ observational
techniques that can resolve the small scale biological features important to population
dynamics. Although the function of marine ecosystems is critical to the global
biosphere, our ability to measure and model their structure and function at all but the
coarsest scales has been severely limited. Improved observational capabilities based
on optical measurements will undoubtedly help explain the distribution of phytoplankton species in space and time, diversification over their 2.6 billion year history, the functioning of earth’s biological systems, and may even be applied to the search for life in other parts of the solar system.

**Future directions**

The results of work conducted in this dissertation suggest several lines of possible future research. A logical next step would be to compare phytoplankton optical properties and their patterns of variation in different ocean ecosystems such as central ocean gyres, upwelling regions, or permanently stratified tropical ocean waters. Optical data may reveal unique community structure and function in these globally important ocean environments that reflect the distinct conditions to which diverse species are adapted. Further investigation with a larger number of morphologically and physiologically diverse uni-algal cultures would also improve our understanding of the effects of species-specific characteristics on the optical properties of cells, colonies and ocean waters.

Different methods of acquiring optical data may also further enhance our understanding of distinct phytoplankton communities. For example, sensor platforms that can follow phytoplankton communities over time could provide a detailed picture of community level processes such as differential growth, mortality, competition, and succession. Linking these patterns and processes to environmental conditions and hydrographic features would provide a new and more detailed understanding of biological organization in the ocean.
In situ observations might be further improved by combining optical data with other existing and emerging technologies. Automated particle imaging and analysis techniques, as used in the laboratory studies presented here, applied to tens of thousands of individual cells, colonies, and non-algal particles could facilitate determination of optical efficiency factors and bio-optical function for natural phytoplankton populations. Techniques such as digital inline holography, capable of imaging particles in an undisturbed volume of sea water, could be used to quantify the effects of orientation of cells or colonies on inherent optical properties and population dynamics. Quantitative imaging techniques could combine absorption or attenuation measurements with morphological measurements into a single sensor. Fast repetition rate fluorometry could provide information on the photophysiology of communities identified by their optical signatures. Molecular and genetic methods adapted for in situ measurements through the development of microfluidic technologies and used in combination with high resolution optical data could address a large number of biological questions ranging from community diversity to gene expression. In fact, the high resolution optical methods developed here can be used to guide any number of in situ measurement techniques or sampling strategies that might target different phytoplankton communities.

In short, there is much work remaining to be done before we fully understand the complex structure and function of planktonic communities or the evolutionary processes that have shaped phytoplankton species. What we currently know is compiled from sparse observations, scattered in space and time. These form an incomplete picture that is incapable of accurately representing the dynamic ecology of
pelagic marine ecosystems. Future research must overcome these limitations by focusing on methods that can quantify community characteristics over the small scales critical to population growth and mortality. This dissertation has provided a step in that direction by improving our ability to infer phytoplankton characteristics from high resolution optical data. By changing how we observe phytoplankton communities we can achieve a deeper understanding of their structure and function.
APPENDICES

A. GLOSSARY

\(a\) – absorption coefficient (m\(^{-1}\))

\(a_s\) – dissolved absorption coefficient (m\(^{-1}\))

\(a_p\) – particulate absorption coefficient (m\(^{-1}\))

\(a_{pg}\) – particulate plus dissolved absorption coefficient (m\(^{-1}\))

\(a_{ph}\) – phytoplankton absorption coefficient (m\(^{-1}\))

\(b\) – scattering coefficient (m\(^{-1}\))

\(b_b:b\) – backscatter ratio

\(b_p\) – particulate scattering coefficient (m\(^{-1}\))

\(b_p:a_p\) – particulate scattering to absorption ratio

\(b_{ph}\) – phytoplankton scattering coefficient (m\(^{-1}\))

\(c\) – attenuation coefficient (m\(^{-1}\))

CDOM – colored dissolved organic matter

\(C_i\) – relative intracellular chlorophyll concentration

\(c_p\) – particulate attenuation coefficient (m\(^{-1}\))

\(c_{pg}\) – particulate plus dissolved attenuation coefficient (m\(^{-1}\))

ESD – equivalent spherical diameter (\(\mu\)m)

IOP – inherent optical property

\(\lambda\) – wavelength (nm)

\(n'\) – imaginary part of the complex refractive index

PPC:PPS – ratio of photoprotective to photosynthetic carotenoids

\(Q_a\) – absorption efficiency factor
\( Q_b \) – scattering efficiency factor

\( \sigma \) – particle projected area (m\(^2\))
OCEAN OPTICS AND PHYTOPLANKTON ECOLOGY: OPTICAL PROPERTIES OF CELLS AND COLONIES PROVIDE NEW INSIGHT INTO COMMUNITY STRUCTURE AND FUNCTION

Malcolm McFarland
Graduate School of Oceanography
University of Rhode Island

Paradox of the Plankton

“The problem that is presented by the phytoplankton is essentially how it is possible for a number of species to coexist in a relatively isotropic or unstructured environment all competing for the same sorts of materials.”

- Hutchinson (1961)

How is diversity created and maintained despite competitive exclusion?
The ocean is NOT isotropic or unstructured!

The problem and a potential solution

Problem: inadequate resolution
  – Determination of phytoplankton distribution, abundance and characteristics over small scales is extremely difficult!

Potential solution: optical properties
  – High resolution in situ measurements
  – Sensitive to abundance, size, shape, and pigments
  – Direct measure of light harvesting
Can optics resolve phytoplankton composition and characteristics over small scales?

1. Monterey Bay
   – Do optical properties vary with species composition?

2. East Sound
   – How do cellular characteristics (size and pigment content) affect optical properties?
   – What’s the significance of non-algal particles?

3. Laboratory cultures
   – Can morphological and physiological differences account for observed variation?

---

### Inherent Optical Properties (IOP)

\[ a + b = c \]

- Dissolved and particulate components:
  \[ a_g + a_p = a_{total} \]

- Ratios affected by particle characteristics:
  \[ b_p : a_p \quad b_b : b \]
Normalized IOP spectra

Monterey Bay
Questions

- Are communities associated with distinct optical signatures?
- How do optical properties vary among communities?
Communities

- Hierarchical cluster analysis
- Based on manual count data
- 9 communities
Dominant cell types

Community 4

Community 5

Pico & nanophytoplankton

Cells collected on filters, imaged with epifluorescence microscopy

Cells classified, counted and sized using image analysis

- Synechococcus
- Prochlorococcus & nanoeukaryotes

![Graph showing size distribution of cells](image)
Redundancy Analysis

4 – abundant but stressed *Pseudo-nitzschia* and abundant nano-eukaryotes

5 – abundant *Akashiwo sanguinea* and abundant *Synechococcus*

6 – abundant *Chaetoceros concavicornis* and few pico and nanoplanckton

Conclusions (Monterey Bay)

- Shape and relative magnitude of $a_p$ and $b_p$ vary among distinct communities
- Cell size and pigment content appear to be optically important characteristics

- Next steps:
  - Measure cellular characteristics
  - Measure non-algal particles
East Sound

Questions

• Do phytoplankton size and pigment content vary with optical properties?

• What is the influence of non-algal particles?

• Can we visualize phytoplankton characteristics over small scales with high resolution optical data?
Optical profiles and sample collection

Scanning Flow Cytometry

A. *Havica* sp. (live cell)

B. *Havica* sp. (dead cell)

C. *Chromococcus socialis*
Scanning Flow Cytometry

Communities

- Hierarchical cluster analysis
- Based on flow cytometry data
- 4 communities in each year
Community composition

Redundancy analysis 2009

**Community 1** – abundant dead *Haslea* cells

**Community 3** – dominated by live *Haslea*

**Community 4** – abundant non-algal particles and smaller phytoplankton
Redundancy analysis 2010

Community 5 – abundant dead cells

Community 6 – abundant non-algal particles

Community 7 – abundant large colonial diatoms

Results of multivariate analyses

- $b_p : a_p$ and $a_p$ slope increase with the relative abundance of dead cells
- $c_p$ slope decreases with the relative abundance of large cells and colonies
- $b_p : b$ and $c_p$ slope increase with the relative abundance of non-algal particles
- Can we apply this to high resolution optical data?
High resolution data 2009

High resolution data 2010
Conclusions (East Sound)

- Phytoplankton size and pigment content influence optical properties
- Non-algal particles are associated with high $b_b:b$ and $c_p$ slope
- Can visualize processes (e.g. mortality) and small scale distributions
- Morphology may be optically important characteristics
Laboratory studies: questions

- Can morphological and physiological variation account for optical variation observed in the field?

- Do morphological and physiological traits have a bio-optical function?

Cultures

Optical properties: $b_p$, $a_p$, and $a_p$ slope

FlowCam: projected area of cells and colonies ($\sigma$)

Flow Cytometry: intracellular pigment concentrations ($C_i$)
**Effects on light harvesting**

- Absorption efficiency factors:
  \[ Q_a = \frac{a_p}{\sigma} \]
- \( \sigma \) = total projected area of particles from FlowCam
- \( Q_a \) is typically large for large particles
- Measured values are low compared to other published studies

**Conclusions (lab studies)**

- Morphological or physiological differences account for ~25-30% of observed variability in \( b_p; a_p \) and \( a_p \) slope
  - Other particles (e.g. bacteria) must also contribute to observed variation
- Morphology is important, not just size
- Low intracellular chlorophyll concentrations and complex morphology of large forms minimize pigment self shading
Overall Conclusions

• Morphological and physiological characteristics of cells and colonies determine the optical properties of phytoplankton communities

• High resolution optical data reveals detailed information about the distribution, composition and characteristics of phytoplankton in the ocean

Future Directions

• Wider application
• Ocean observing systems
• Robust inverse models
• Population tracking
• Complementary observation techniques
  – In situ imaging: FlowCam, holography
  – Molecular approaches: Environmental Sample Processor
  – Satellite, LIDAR
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