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ORIGINAL PAPER

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Visual pigments and optical habitats of surfperch (Embiotocidae) in the California kelp forest

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Abstract We studied the optical microhabitat use and visual pigment variation among a group of closely related teleosts (surfperch: Embiotocidae) living along the nearshore central California coast. We employed a diver-operated spectroradiometer to record the optical microhabitat use of eight surfperch species in Monterey Bay, and microspectrophotometry to measure visual pigment absorbance for nine surfperch species. Species were dichromatic with mixtures of A_1- and A_2-based visual pigments exhibiting extensive maximum absorbance (λ_{max}) variation across species: 455–482 nm for SWS cones and 527–546 nm for LWS cones. Interspecific variation in sidewelling irradiance measurements (mean $\lambda Fmax_S$) significantly accounted for 63% of the variation in surfperch LWS visual pigments and 83% of the interspecific variation in SWS visual pigments using a phylogenetically-corrected regression technique. Optimality models for maximizing relative photon capture of background radiance demonstrate that the LWS cone λ_{max} values are tuned for maximizing photon capture of the species-specific horizontal visual field, while the SWS cone λ_{max}, are well offset from the dominant background radiance. This study is one of the first to demonstrate species-specific differences in habitat usage at microhabitat scales accounting for differences in photoreceptor peak absorbance among closely related, sympatric species.

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Abbreviations *CNS* central nervous system · *LWS* long-wavelength-sensitive · *MSP* microspectrophotometry · *PBS* phosphate-buffered saline · *SWS* short-wavelength-sensitive

Introduction

For the past several decades visual ecologists have documented the variation in spectral sensitivities among aquatic organisms living in different optical waters (Wald et al. 1955; Denton and Warren 1956; Lythgoe 1972, 1979; Bowmaker et al. 1994; Lythgoe et al. 1994; McDonald and Hawryshyn 1995). This approach has documented a general pattern in which spectral sensitivity correlates with the photic environment and target detection against the optical background (Lythgoe 1966, 1968; McFarland and Munz 1975; Loew and Dartnall 1976; Loew and Lythgoe 1978; Munz and McFarland 1973, 1977; Bowmaker 1995). What has proven to be more elusive is the ability to explain the visual pigment variation observed amongst animals living at the same depth or in the same general habitat.

Variation in spectral tuning of photoreceptor sensitivity among teleosts living in similar optical water types has been a puzzle for visual scientists over the years. Hypotheses that address this variation have proposed a variety of factors that may be involved such as interspecific differences in visual tasks (Levine and Mac Nichol 1979; Lythgoe 1979; Partridge and Cummings 1999), microhabitat preferences (McFarland and Munz 1975), visual detection strategies (Lythgoe 1966, 1968; McFarland and Munz 1975; Loew and Lythgoe 1978; Lythgoe and Partridge 1991), and phylogenetic histories (Bridges 1972; Munz and McFarland 1977; Cronin et al. 1993). Considerable progress has been made to address each of these sources of variation, but rarely does a

dataset allow for control of several sources of variation simultaneously. Here we begin an investigation of a teleost group in which we are able to isolate various sources of sympatric sensory variation while exploring the relationship of visual sensitivities and photic environment on a much finer scale than previously reported.

Isolating sources of interspecific variation in visual sensitivity requires controlling for phylogenetic components of a group of related species and having an accurate account of species-specific optical microhabitat use. The surfperch (Embiotocidae), a monophyletic teleost family with 23 member species, are good candidates for this type of exploration because they meet many of these requirements. Surfperch inhabit the optically variable environment of the temperate North American coast and forage among macroalgae for small invertebrates (Tarp 1952; De Martini 1969). While largely sympatric, they have been observed to visit different microhabitats for foraging (Holbrook and Schmitt 1986, 1992). The moderately shallow depth ranges (<20 m) for many surfperch species make them an accessible group for characterizing species-specific microhabitat preferences with a diver-operated spectroradiometer; and a complete molecular phylogeny of the Embiotocidae (Bernardi and Bucciarelli 1999) allows us to control for phylogenetic variation when exploring the relationship between species-specific habitats and photoreceptor sensitivities.

The optical conditions of the California nearshore marine environment change with depth, sun angle, cloud cover, canopy cover, wave action, and phytoplankton quantity and composition (Lythgoe 1972; Gerard 1984; Dean 1985; Wing et al. 1993; Kirk 1994). Biases in microhabitat usage among nearshore surfperch species can lead to species experiencing different optical conditions and visual backgrounds. In this study we begin an investigation exploring whether variation in species-specific microhabitat use accounts for differences in visual pigment absorbances, and we begin to investigate the visual basis for the strong relationship between photoreceptor peak absorbance and background light.

Materials and methods

Characterization of optical habitat

To characterize the optical habitat of various surfperch species, over 250 SCUBA dives were made in the nearshore environment in Monterey Bay, California. Most (>90%) of these optical observation dives were made within Hopkins Marine Life Refuge, Pacific Grove, Calif., between 1995 and 1997. Species chosen for this investigation (except for *Zalembius rosaceus*) were among those most commonly observed in the nearshore environment throughout the year. On each SCUBA dive fish behavior, habitat use, depth, and measurements of optical environment (temporally sequential pairs of downwelling and sidewelling irradiance spectra) were recorded for various surfperch species. Spectral irradiance measurements were collected with a diver-operated spectroradiometer fitted into an underwater housing. Underwater light enters the housing through an exterior cosine collector (Ocean Optics CC-1), is transmitted by a UV-VIS 400 μm diameter fiber-optic cable through the housing end cap and onto a quartz detector lens at the entrance slit of the Ocean Optics PS1000 UV-VIS grating spectrometer. The spectrometer interfaces with a Gateway 486 handbook computer via a National Instruments DAQ-700 PCMCIA A/D card. Data acquisition is controlled by Ocean Optics SS-1-SC SpectraScope software that was modified to allow a diver to operate the program using single-key interactions. Underwater computer interactions are made possible by a magnetically activated solenoid device. The spectroradiometer was calibrated to photometric units ($\mu E\ m^{-2}\ s^{-1}\ nm^{-1}$) with a LICOR LI-1800-02 Optical Radiation Calibrator lamp every 6 weeks to account for changes in sensitivity with continued deployment.

Optical measurements were collected in a stratified random design in the nearshore environment. On each SCUBA dive, measurements were collected within four habitats: shallow surfgrass region (1–3 m); canopy-free boulder areas dominated by macro-algal turf (2–5 m); canopied kelp forest, *Macrocystis pyrifera* (3–20 m); and sand channel environment (1–15 m). Within each habitat, optical measurements were collected using a randomly positioned 10-m horizontal transect. Measurements were taken at 0 m, 5 m, and 10 m along the transect, as well as any location along the transect where surfperch were encountered within 1 m of the transect line. Optical measurements consist of paired downwelling, $I_D(\lambda)$, and sidewelling, $I_S(\lambda)$, irradiance spectra collected within 1 m of the species under observation, sidewelling irradiance being measured by orienting the spectroradiometer horizontally. The depth, time of day, sun angle, cloud cover, canopy cover, water column visibility, and substrate vegetation were recorded with each pair of irradiance spectra along with information on the number, gender, and behavior of surfperch present. To prevent biasing the optical characterization of a species habitat toward schooling fish, only one pair of irradiance spectra were collected whether there was 1 or 50 individuals of a species present in a given location. The observed optical range for each surfperch species was defined as all optical measurements collected in the presence of that species.

It should be noted that for both *Brachyistius frenatus* and *Cymatogaster aggregata*, the optically observed habitat range is likely to be inconsistent with their known habitat range. *C. aggregata* are common among the shallow surfgrass for most of the year; however, they are often captured by fishermen at depths greater than 50 m during the winter months (De Martini 1969). No irradiance measurements were collected deeper than 25 m for this study. Meanwhile, *B. frenatus* are commonly found within the kelp forest canopy within the first 1–2 m of the surface. Given the physiological constraints of SCUBA diving, multiple vertical transects per SCUBA dive to census this microhabitat is not advisable, so the measured optical habitat conditions of *B. frenatus* in this study are biased toward the deeper regions of their habitat.

Microspectrophotometry: preparation of retinal tissue and lens transmission measurements

Fish were captured by a number of methods including beach seining, hand netting on SCUBA and otter trawling. Fish were sacrificed for retinal dissection within 24 h of capture by dark-adaptation in aerated darkened tanks for at least 1 h before being euthanized with tricaine methanesulfonate (MS-222) and killed by approved humane methods including destruction of the CNS. Under infrared illumination (Kodak Wratten filter no. 87 C) and the aid of image-converter (Moonlight productions model MPN 35 K, 35,000× light amplification) eyes were hemisected and the cornea and lens removed and placed into phosphate-buffered saline (PBS) solution, pH 7.4, for transmission measurements. PBS solution contained ($g\ l^{-1}$): 7.725 NaCl, 1.1 sodium pyruvate, 0.1938 KCl, 0.2033 $MgCl_2$, 0.3969 $CaCl_2$, 1.554 $NaHCO_3$, and 0.384 NaH_2PO_4. Retinal tissue was removed with forceps and prepared for microspectrophotometry (MSP) examination by either light 'fixation' (Partridge et al. 1989) or 'slow-freezing' preparations.

A small number of fish were transported live to Bristol, England in O_2-saturated water and maintained in aquaria at the University of Bristol for fresh retinal MSP examination. Fish were dark adapted for 6 h, euthanized with an overdose of MS-222 and killed by humane methods in accordance with UK Home Office legisla-

tion. Eyes were dissected out in Dulbecco A PBS (Oxoid, Basingstoke, UK) at a concentration of 425 mosmol kg^{-1} and small pieces of retina were transferred to a solution of PBS and 10% Dextran (Sigma 250 k RMM).

Whole fish lens spectral transmission curves were measured by techniques described by Douglas and McGuigan (1989) using a Shimadzu 2101 scanning spectrophotometer fitted with an integrating sphere assembly. Whole lens absorbance measurements between 300 nm and 800 nm were made within 20 min of dissection and then converted to transmission units with all transmission spectra normalized by the 700-nm transmission (Douglas and McGuigan 1989; Douglas et al. 1995).

Preservation of retinae for MSP

Retinal tissue was preserved for MSP by two methods: light 'fixation' (Partridge et al. 1989) and 'slow freeze'(similar method used in Fritsches et al. 2000). For the 'slow freeze' preparation, a small piece (2 mm×2 mm) of retinal tissue was dissected out of the eye and placed on a 50 mm×22 mm coverslip with a drop of 'freezing medium'. The tissue was agitated with needles to detach individual photoreceptors from other retinal tissue. A smaller cover slip was placed on top and pressed down to expel excess liquid. The edges of the coverslip were sealed with clear nail varnish and the sealed coverslip was placed in a light-tight container and kept for 1 h at 4°C, followed by a transfer to a freezer at –20°C for 1–2 h, and then transferring it to a –70°C freezer until final transport to Bristol University on dry ice. The freezing medium consisted of PIPES-buffered saline (in g l^{-1}: 16.926 PIPES,10.519 NaCl, 1.416 KCl, 0.2033 $MgCl_2$, 0.147 $CaCl_2$), 10% Dextran 260,000 MW (SIGMA D-7265), 15% Dextran 12,000 MW (SIGMA D-9260).

MSP measurements

Fixed and frozen retinal tissue was transported to Bristol University for measurement by MSP. The preparation of samples for MSP measurement has been previously detailed elsewhere (Partridge et al. 1989; Hart et al. 1998). Similarly, the single-beam microspectrophotometer used to measure photoreceptor absorbance measurements is described in detail elsewhere (Partridge1989; Hart et al. 1998). Isolated individual photoreceptors were located on the sample slide and absorption spectra recorded between 350 nm and 730 nm. After a series of MSP absorbance measurements, photoreceptors were bleached for 2 min under monochromatic light from the MSP monochromator with a wavelength close to the sample's absorbance peak. Subsequent measurements of the outer segment were made to confirm that the pigment was photolabile.

Analysis of visual pigment absorption spectra

Absorption spectra recorded from individual photoreceptors were subject to selection criteria in order to exclude distorted measurements (Levine and MacNichol 1985; Hart et al. 1998). The criteria varied with species and cell type due to variation in measurement quantity and quality. In general, however, the selective criteria for useable sample absorbance included all measurements that: (1) exhibit low noise in λ_{max} estimation with standard deviations <10 nm; (2) short-wave arm absorbance falls within 25% of the template short-wave arm at 0.75 normalized absorbance; (3) exhibit flat absorbance beyond 650 nm; (4) exhibit no obvious distortions; and (5) prove to be photolabile through bleaching. All sample measurements that met the selective criteria were averaged and the average file, as well as individual scans, analyzed further.

Absorption spectra were analyzed to determine the wavelength of peak absorption (λ_{max}) by fitting rhodopsin or porphyropsin visual pigment templates to the longwave limb of the data using methods described by Partridge et al. (1989) and Hart et al. (1998). No attempt was made to create hybrid templates representing a mixture of rhodopsin and porphyropsin (Munz and Beatty 1965; Allen 1971). The polynomial coefficients for rhodopsin α-band are reported in Partridge and DeGrip (1991), and the polynomial coefficients for porphyropsin have been modified by J.C. Partridge (unpublished data):

$$\lambda_{\max} = \lambda\left(0.81475 + 0.24483(D) - 0.22381\left(D^2\right) + 0.12486\left(D^3\right)\right) \quad (1)$$

where D is the normalized absorbance at λ restricted to $0.2 \geq D \geq 0.8$. The wavelength of maximum absorbance (λ_{max}) estimate is calculated as the mean estimate from all measured points between 20% and 80% on the long-wavelength limb. The standard deviation of all λ_{max} estimates from the 20–80% longwave arm absorbance region (25–45 points depending on the λ_{max} of the visual pigment and the signal-to-noise ratio of the measurement) was also computed. For display, the estimated sample absorbance λ_{max} is then used as the α-peak in producing either a rhodopsin or porphyropsin template by incorporating the exponential function of Stavenga et al. (1993), with the linear β-peak shift suggested by Palacios et al. (1996).

The relationship between $\lambda_{\max}$ and photic environment

Visual pigment λ_{max} estimates for each species were used to investigate whether species-specific measurements of habitat parameters (habitat depth, downwelling, and sidewelling irradiance) co-vary with visual pigment absorbance. For habitat depth we use the mean depth for all optical measurements recorded with each species present. Surfperch photic environments were characterized by the mean wavelength of maximum flux, $\lambda Fmax_I$, for all irradiance measurements collected in the presence of each species. The mean wavelength of maximum flux is calculated for each optical plane giving a mean sidewelling irradiance wavelength of maximum flux, $\lambda Fmax_S$, and a mean downwelling irradiance wavelength of maximum flux, $\lambda Fmax_D$.

Statistical evaluations of the relationship between surfperch habitat parameters and cone sensitivities are more informative when phylogenetic corrections are applied because estimates of species' mean habitat depth, cone λ_{max}, $\lambda Fmax_S$, and $\lambda Fmax_D$ are not independent data points (Felsenstein 1985). We computed an autocorrelation model that partitions the variation in habitat parameters and cone sensitivities between phylogenetic and non-phylogenetic components and allows standard regression analysis on the non-phylogenetic residuals (Cheverud et al. 1985). We used mitochondrial DNA sequence divergence data from the molecular phylogeny of the embiotocids (Bernardi and Bucciarelli 1999) for the branch length and phylogenetic distance matrix required for these analyses. We used COMPARE version 3.0 (Martins 1999) to calculate the autocorrelation model (AUTOCORR program).

Relative photon capture and sensitivity to visual background

Using a single wavelength variable to describe the optical habitat of a species, such as $\lambda Fmax$, allows us to compute a simple correlation between habitat and visual sensitivity; however, it does not tell us how surfperch visual pigments are 'tuned' to their visual backgrounds. Tuning to background environment suggests a matching between the sensory sensitivity and background environment. While we might assume a direct relationship between $\lambda Fmax$ and λ_{max}, the variability in distribution of spectral irradiance make an exact match between these two wavelength parameters unlikely. To investigate whether surfperch cone sensitivities are tuned for capturing background radiance, we calculated the fraction of the background flux absorbed by a given cone class as a measure of its background sensitivity.

We estimated cone sensitivity to background radiance, S_c, by comparing cone quantum catch, Q_c, relative to the total sidewelling irradiance flux, $\Sigma I(\lambda)_S$. Cone quantum catch estimates, Q_c, for each cone class, c, were calculated across species-specific optical datasets with the following equation:

$$Q_c = \sum_{\lambda=350}^{700} I_S(\lambda)T(\lambda)A_c(\lambda) \quad (2)$$

Numerical integrations were made for all wavelengths, λ, from 350 nm to 700 nm at 2-nm intervals. $I_S(\lambda)$ represents the sidewelling irradiance spectrum, $T(\lambda)$ represents the species' whole lens transmission spectrum (corneal absorption was insignificant in this wavelength range), and $A_c(\lambda)$ represents the absorptance spectrum for cone class, c. Absorptance, $A_c(\lambda)$, represents the fraction of incident light absorbed by the photoreceptor and is calculated from the MSP absorbance measurements by the following:

$$A(\lambda) = 1 - 10^{-D(\lambda)sl} \quad (3)$$

where $D(\lambda)$ is the sample optical density as measured by MSP normalized absorbance, s is the photoreceptor specific absorbance (i.e., absorbance per μm pathlength) and l is the pathlength of photon travel, taken to be the length of the outer segment. Since MSP absorbance measurements were made transversely to the outer segment, the specific absorbance was calculated as the measured maximum absorbance divided by the diameter of the outer segment.

The cone sensitivity fraction, S_c, represents the relative proportion of the horizontal light field, $I_S(\lambda)$, absorbed by a particular cone class, c. Estimates of S_c were calculated for each sidewelling irradiance measurement with the following equation:

$$S_c = Q_c \Big/ \sum_{\lambda=350}^{700} I_S(\lambda) \quad (4)$$

where S_c describes the quantum catch relative to background sidewelling irradiance numerically integrated from 350 nm to 700 nm at 2-nm intervals. The numerically integrated sidewelling irradiance represents the total amount of quanta available in the horizontal visual field. The average cone sensitivities, $\overline{S_c}$, were computed for the entire optical habitat range of each surfperch species:

$$\overline{S_c} = \left(\sum_{j=1}^{N} S_{c,j}\right) \Big/ N \quad (5)$$

where the sensitivity for cone, c, is summed across all optical measurements, j, for $j = 1$ to N, and N is the total number of sidewelling irradiance measurements, $I_S(\lambda)$, collected with a particular surfperch species present (see Table 1 for N values).

Optimal photopic pigment for maximizing sensitivity

To evaluate whether surfperch visual pigments provide the maximum possible photon capture of their specific habitat backgrounds, we compared estimates of background sensitivity of each species' measured visual pigments to optimal estimates from model visual pigments. Model absorbance curves were created by modifying the α-peak of the absorbance template of the species' mean absorbance spectrum. The peak absorbance of these normalized absorbance templates varied by 2-nm increments across a 100-nm range (± 50 nm of the species measured average λ_{max}). The same template algorithms from above were employed to produce the model absorbance curves. Model absorbance curves were transformed to absorptance using the species-specific mean measurements of photoreceptor length, diameter, and maximum corrected MSP absorbance. We then calculated mean relative photon capture, $\overline{S_c}$, across all species-specific optical observations for each of the 50 template absorptance curves. The model visual pigment with the highest mean relative photon capture, $\max \overline{S}$ (the visual pigment with λ_{max} that resulted in the highest mean percent of sidewelling irradiance absorbed), represents the optimal visual pigment for maximizing sensitivity to the background light for each species-specific optical dataset. Each model visual pigment was then scaled

Table 1 Surfperch habitat characteristics

Species	Mean depth (m) [range][a]	*n* (obs)[b]	Observed habitats[c]	Vegetative association[d]
Micrometrus aurora (reef surfperch)	1.0 ± 0.5 [0.5–1.5]	71	Surfgrass zone****	*Phy*****; *Rhod**
Cymatogaster aggregata (shiner surfperch)	1.3 ± 0.9 [0.5–2.5]	93	Surfgrass zone****	*Phy*****; *Rhod**
M. minimus (dwarf surfperch)	1.6 ± 1.7 [0.5–5]	26	Surfgrass zone**** Canopied kelp forest*	*Phy***** *Rhod**; *Phae**
Embiotoca jacksoni (black surfperch)	5.8 ± 3.6 [1–20]	411	Canopied kelp forest*** Surfgrass zone** Turf-covered boulders**	*Rhod**** *Phy*** *Phae***
Damalichthys vacca (pile surfperch)	6.1 ± 3.5 [1–20]	401	Canopied kelp forest*** Sand channel** Surfgrass zone*	*Rhod**** *Phae*** *Phy**
E. lateralis (striped surfperch)	6.5 ± 3.3 [1–20]	579	Turf-covered boulders*** Canopied kelp forest** Surfgrass/sand channel*	*Rhod**** *Phae*** *Phy**
Brachyistius frenatus (kelp surfperch)	6.8 ± 3.1 [0.5–15]	75	Canopied kelp forest**** Surfgrass zone* Sand channel*	*Phae***** *Rhod*** *Phy**
Hypsurus caryi (rainbow surfperch)	7.9 ± 3.1 [1–20]	185	Canopied kelp forest*** Edge of kelp forest** Sand channel**	*Phae**** *Rhod**** *Phy**
Zalembius rosaceus (pink surfperch)	> 20	0	Pelagic	*n.a.*

[a]Mean depth (m) ± 1 standard deviation for all optical observations, with observed range given in brackets

[b]Total number of optical observations (paired irradiance measurements)

[c]Habitats in which optical observations were collected

[d]Vegetation present during optical observations: *Phy* = *Phyllospadix* sp. (green surfgrass), *Rhod* = Rhodophyta (red macroalgae), and *Phae* = Phaeophyta (brown macroalgae, including the giant kelp *Macrocystis pyrifera*). Asterisks indicate the percentage of all observations in which a species was found in a particular habitat or with a particular type of vegetation present: * < 25%; ** > 25%; *** > 50%; **** > 75% of all observations recorded for that species. (*n.a.* not available)

relative to this maximum value to produce a range of visual pigment performance for background sensitivity proportional to the maximum, *pmaxS* :

$$p\max S = \frac{\overline{S_c}}{\max \overline{S}} \quad (6)$$

Results

Over 4,000 measurements of underwater irradiance spectra coupled with depth, time of day, habitat, and surfperch behavior were collected over a 3-year period. The optical habitat for each surfperch species was then defined as the set of all irradiance spectra collected in the presence of each species. The surfperch observed in this study exhibited differences in depth and habitat associations (Table 1). These interspecific differences in microhabitat use resulted in differences in average downwelling [$I_D(\lambda)$] and sidewelling [$I_S(\lambda)$] spectral irradiances (Fig. 1). While these average irradiance spectra do not represent actual irradiance measurements, they do provide information on some general

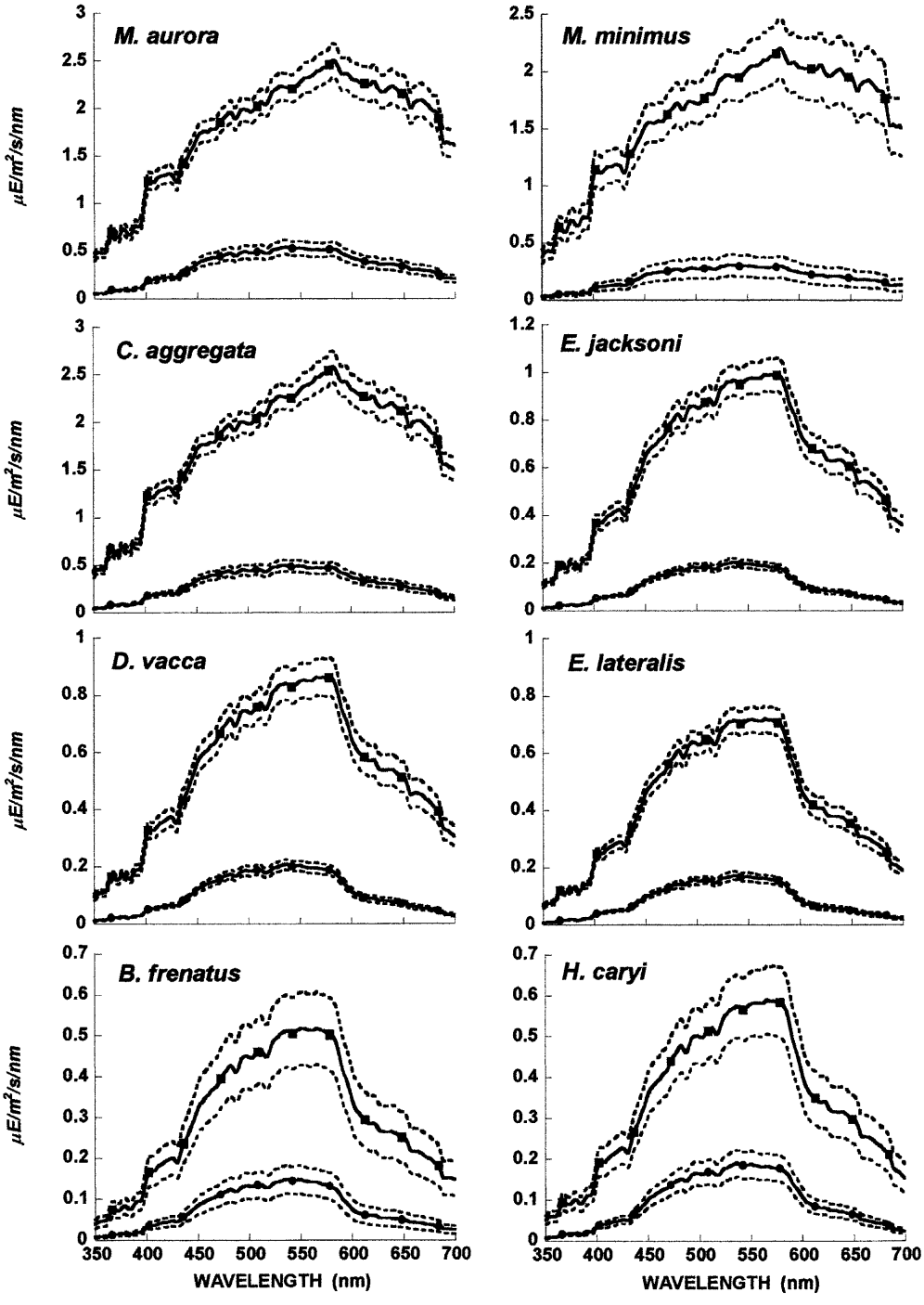

Fig. 1 Mean (±1 standard error) downwelling ($I_D(\lambda)$, *filled squares*) and sidewelling ($I_S(\lambda)$, *filled circles*) irradiance spectra for eight surfperch species observed within the near-shore environment of Monterey Bay from 1995 to 1997. Mean spectral irradiances are calculated from all optical measurements collected within a 1-m radius of each species. Numbers of observations are presented in Table 1. Sidewelling irradiance, representing the horizontal visual field, is measured directly following each downwelling measurement by orienting the spectroradiometer in the horizontal plane

habitat characteristics of the different species. The average spectral irradiance spectra differ in a predictable manner with depth: on average species with the shallowest depth ranges observed in this study (e.g., *Micrometrus aurora*) experience broader spectral irradiance in their habitats than species with deeper depth ranges (e.g., *Hypsurus caryi*).

The lenses of most surfperch have an UV filter with a 50% cut-off wavelength at 412 nm (Fig. 2). The two exceptions to this trend among the measured species are *C. aggregata* with a 50% cut-off wavelength at 402 nm, and *Z. rosaceus* having a 50% cut-off at 384 nm. While *Z. rosaceus* is found at great depths throughout the year (30–100 m), *C. aggregata* is found in the shallow surfgrass for most of the year and frequents deeper regions during the winter season.

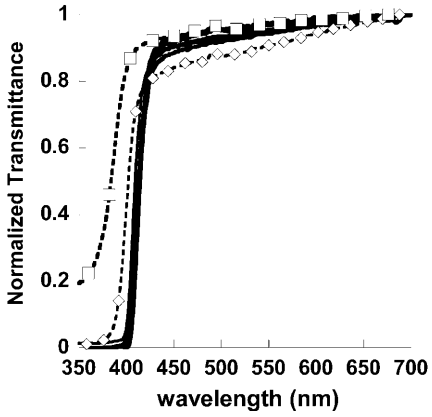

Fig. 2 Average whole lens transmission spectra of surfperch species, $T(\lambda)$. All lens transmission spectra were normalized by transmission at 700 nm: (*solid line*) *M. minimus*, *M. aurora*, *H. caryi*, *E. lateralis*, and *E. jacksoni*; (*open diamonds*) *C. aggregata*; and (*open squares*) *Z. rosaceus*

Cone types

All surfperch species that we measured had two types of retinal cone, readily distinguished by their visual pigments. The fish are thus potentially dichromats. Long-wavelength-sensitive (LWS) cones occurred in greater frequency than short-wavelength-sensitive (SWS) cones. The only exception to dichromacy was *B. frenatus*. We were only able to examine a very small amount of *B. frenatus* retinal tissue due to sample decomposition, and thus it is likely that SWS cone types will be found with further MSP examination.

Rods

All rods were distinguishable in their uniformly thin, cylindrical morphology from the thicker, tapering morphology of the cone photoreceptors. Rods across all species measured approximately 1.5–2.5 μm in diameter, 5–7 μm in length, and were often found in large clusters in MSP preparations.

SWS cones

SWS cones were single cones found in isolation among rod regions of the retina and sometimes amid LWS double-cone areas, a pattern reported in other Perciformes (Ali and Anctil 1985). SWS cone dimensions varied among species, but the diameters were on average 0.5–1.5 μm thinner in diameter than the LWS cones and ranged from 2 μm to 3 μm in diameter. Mean (±SD) SWS cone outer segment width for the photoreceptors used in the mean absorbance spectra for each species include: 2.0 ± 0 μm *M. aurora*; 2.5 ± 0 μm *C. aggregata*; 2.7 ± 0.27 μm *Micrometrus minimus*; 2.5 ± 0.58 μm *Embiotoca jacksoni*; 2 ± 0 μm *Damalichthys vacca;* 2.0 ± 0 μm *Embiotoca lateralis*; 2.56 ± 0.49 μm *H. caryi* ; and 2.75 ± 0.43 μm *Z. rosaceus*. Mean (±SD) SWS cone outer segment lengths for the photoreceptors used in the mean absorbance spectra for each species are: 6.0 ± 1 μm *M. aurora*; 12.5 ± 2.1 μm *C. aggregata*; 6.8 ± 1.64 μm *M. minimus*; 8.25 ± 2.2 μm *E. jacksoni*; 8.0 ± 0 μm *D. vacca*; 18.5 ± 4.9 μm *E. lateralis*; 12.0 ± 1.4 μm *H. caryi*; and 6.7 ± 0.57 μm *Z. rosaceus*.

LWS cones

The majority of LWS cones were double cones while approximately 45% of LWS cones measured were non-paired. It is possible that many of these 'single cones' are in fact members of the double cone assemblage and separated during the process of MSP preparation. LWS cone dimensions varied across species with the range extending from 2 μm to 5 μm in diameter. Mean (±SD) LWS single and paired cone outer segment width for the photoreceptors used in the mean absorbance spectra for each species are: 2.5 ± 0.59 μm *M. aurora*; 3.1 ± 0.55 μm *C. aggregata*; 2.6 ± 0.49 μm *M. minimus*; 3.5 ± 0.95 μm *E. jacksoni*; 2.3 ± 0.35 μm *D. vacca*; 2.7 ± 0.74 μm *E. lateralis*; 2.2 ± 0.24 μm *B. frenatus*; 3.1 ± 0.60 μm *H. caryi*; and 4.25 ± 1.1 μm *Z. rosaceus*. Mean (±SD) LWS cone outer segment lengths for the photoreceptors used in the mean absorbance spectra for each species are: 4.6 ± 0.75 μm *M. aurora*; 11.0 ± 3.76 μm *C. aggregata*; 11.5 ± 4.0 μm *M. minimus*; 6.7 ± 2.2 μm *E. jacksoni*; 8.2 ± 5.81 μm *D. vacca*; 9.7 ± 3.63 μm *E. lateralis*; 5.5 ± 0.71 μm *B. frenatus*; 9.4 ± 2.6 μm *H. caryi* ; and 12.5 ± 5 μm.2 *Z. rosaceus*.

MSP measurements

While MSP measurements were made from retinal tissue prepared using different techniques, most species λ_{max} estimates are from only a single preparation type: (1) *C. aggregata*, *E. lateralis, D. vacca*, and *H. caryi* (light 'fixation' preparation); (2) *E. jacksoni*, and *B. frenatus* ('slow-freeze' preparation); and (3) *M. aurora* (freshly

dissected retina prepared in PBS and Dextran solution). Only two species had measurements made on both types of preparations, the differences were significant for the LWS cone in *Z. rosaceus* but not for *M. minimus*. Difference between sample LWS cone λ_{max} values from fixed verses frozen MSP preparations were: *Z. rosaceus*: fixed 524.3 nm; frozen 527.3 nm [$t_{(11)} = -2.668$, $P = 0.02$]; and *M. minimus*: fixed 545.7 nm; frozen 547.6 nm [$t_{(32)} = 0.992$, $P = 0.33$]. The slight difference may be due to the fixed samples experiencing a greater degree of short-wavelength scattering than the frozen samples.

There were no significant differences between the LWS cone λ_{max} values between paired and single cone outer segments: *M. aurora* $t_{(15)} = 1.122$, $P = 0.28$; *C. aggregata* $t_{(42)} = -0.276$, $P = 0.78$; *M. minimus* $t_{(32)} = 0.905$, $P = 0.37$; *E. jacksoni* $t_{(20)} = -0.823$, $P = 0.42$; *D. vacca* $t_{(16)} = 1.79$, $P = 0.09$; *E. lateralis* $t_{(19)} = -0.173$, $P = 0.86$; *H. caryi* $t_{(25)} = -0.110$, $P = 0.91$; *B. frenatus* $t_{(6)} = 0.033$, $P = 0.97$; and *Z. rosaceus* $t_{(27)} = 0.038$, $P = 0.70$. Frequency distributions of all sample λ_{max} estimates from all preparation methods used to create surfperch average absorbance spectra are shown in Fig. 3. Sample MSP files from both single and double LWS cones, and all MSP preparations, were then pooled to make a LWS cone mean absorbance spectrum for each species.

Several sample MSP absorbance curves exhibited considerable deviation from the template long-wave arm indicating a possible mixture of A_1 and A_2 within the same visual pigment (Fig. 4). As a group, the surfperch have mixed chromophoric retinas (13 of 25 rod and cone photoreceptors fit A_2 templates better). The majority of species have one cone visual pigment MSP absorbance curves exhibiting a better fit to the rhodopsin template while the other cone class showing a better fit with the porphyropsin template. In general, surfperch LWS cones exhibited a better fit to the porphyropsin template, while most species' rod photoreceptors fit a rhodopsin template much better than porphyropsin (Table 2). Notable exceptions include *M. minimus*, *M. aurora*, and *D. vacca*. The longer wavelength λ_{max} associated with both *M. minimus* and *M. aurora* possibly indicates that surfperch rod opsin is shared across species, with changes in peak sensitivity due to changes in prosthetic group varying from A_2 to A_1 (Dartnall and Lythgoe 1965).

Only two surfperch species exhibited homogenous retinas with both rods and cones containing the same chromophore. *M. minimus* contained rods and cones with mainly A_2 as their prosthetic group, while *C. aggregata* exhibited an A_1-homogenous retina. While both of these species occupy the same shallow surfgrass habitat, *C. aggregata* can also be encountered at great depths (> 100 m) during winter. In general, however, habitat depth does not appear to explain the pattern of chromophore associations in surfperch.

Rod λ_{max} values exhibit a blue-shifting pattern with increasing habitat depth. The shallow species have rod λ_{max} values of 518 nm, changing to around 501–506 nm for the moderate-littoral kelp forest species, and 495 nm for the deepest species (Table 2). The cone λ_{max} across these species, however, does not vary in a similar manner. Both SWS and LWS cones show great variation in mean maximum sensitivity. There is a 27 nm range in SWS cone λ_{max} values and a 19 nm range across LWS cone λ_{max} values. However, differences in the cone peak sensitivities do not correspond with differences in average depth of habitat observed across these species.

Habitat and visual sensitivity

The microhabitat associations recorded for the surfperch species in this study exhibit two different groups (Table 1): species that are commonly observed amidst the shallow surfgrass environment (*M. aurora, M. minimus*, and *C. aggregata*); and a group of species with a broader habitat range that includes the surfgrass along with other habitat regions such as the kelp forest (*E. lateralis*, *E. jacksoni*, *D. vacca*, *H. caryi*, and *B. frenatus*). The differences in microhabitat associations between these two groups can be observed in differences in mean habitat depth (Table 1) with corresponding differences in the mean downwelling irradiance wavelength of maximum flux ($\lambda Fmax_D$; see Fig. 5a, b).

Mean sidewelling irradiance wavelength of maximum photon flux, $\lambda Fmax_S$, is independent of mean habitat depth. For example, optical measurements were collected in the presence of *D. vacca* and *M. minimus* at very different depths in the near-shore marine environment (mean observed depth of 6.1 m and 1.6 m, respectively), yet the mean horizontal light field for each species has the same wavelength of maximum flux (*D. vacca*: mean $\lambda Fmax_S = 534.22$ nm; *M. minimus*: mean $\lambda Fmax_S = 534.17$ nm , see Fig. 5c, d). These two species share similar SWS and LWS cone λ_{max} values. Across the surfperch as a whole, there is a general linear trend between the maximum transmission wavelength of sidewelling light and SWS and LWS maximum sensitivity (Fig. 5c, d).

A regression on the residuals of Cheverud's autoregressive model, representing the non-phylogenetic variation in the dataset, confirmed a significant relationship between photopic pigment variation and background light measured in each species observed range. Cheverud's autoregressive model, $y = \rho Wy + \epsilon$, uses maximum likelihood techniques to describe the variation among species' traits as a combination of the phylogenetic component, ρWy, and the non-phylogenetic residuals, ϵ. The estimated autocorrelation coefficient, ρ, describes the amount of variation in the LWS or SWS cone λ_{max} values explained by the phylogenetic hypothesis. Positive values of this autocorrelation coefficient suggest that the closely related species have similar λ_{max} values, while negative values of ρ suggest that closely related species do not share similar λ_{max} values. Autocorrelation coefficients for surfperch LWS cone λ_{max} ($\rho = -0.600$), SWS cone λ_{max} ($\rho = -0.610$), and mean wavelength of maximum flux of sidewelling irradiance $\lambda Fmax_S$ ($\rho = -0.790$) were all negative, suggesting forces other than phylogeny are

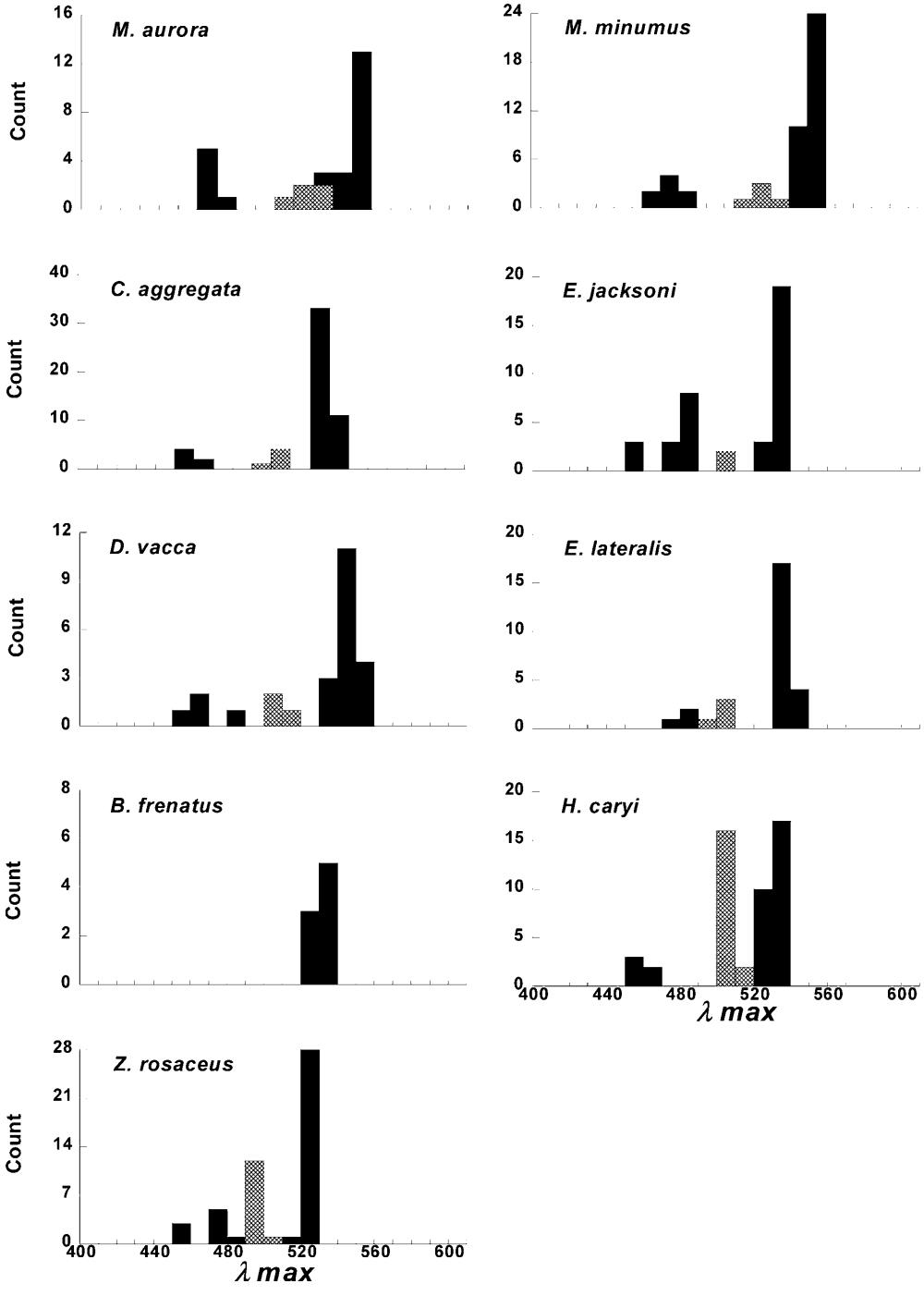

Fig. 3 Frequency histogram of all microspectrophotometry (MSP) sample λ_{max} estimates included in the average absorbance files for each surfperch species. Histogram includes MSP measurements of rod (*hatched*) and cone (*filled*) outer segments

driving the variation across surfperch optical habitat use and cone spectral sensitivities.

The regression of the non-phylogenetic residuals of surfperch SWS cone λ_{max} values on mean $\lambda Fmax_S$ from the autocorrelation model was highly significant [$r^2 = 0.827$, $b = -1.235$, $t_{(5)} = -4.891$, $P = 0.0019$], with optical habitat explaining 83% of the non-phylogenetic variation in SWS cone λ_{max} measurements (Fig. 5f). Similar results were obtained for the relationship between surfperch LWS cone λ_{max} values and mean $\lambda Fmax_S$. Cheverud's autocorrelation model regression of the non-phylogenetic residuals of LWS cone λ_{max} on mean $\lambda Fmax_S$ was significant [$r^2 = 0.629$, $b = -1.0581$, $t_{(6)} = -3.189$, $P = 0.012$], with optical habitat explaining 63% of the variation (Fig. 5e). These results were confirmed using another phylogenetic-correction technique of Independent Contrast (Felsenstein 1985; results not shown).

Regressions computed with the non-phylogenetic residuals of surfperch cone λ_{max} values on habitat depth were not significant. Hence, mean habitat depth can not explain a significant portion of the interspecific variation in maximum sensitivity of photopic pigments [residuals

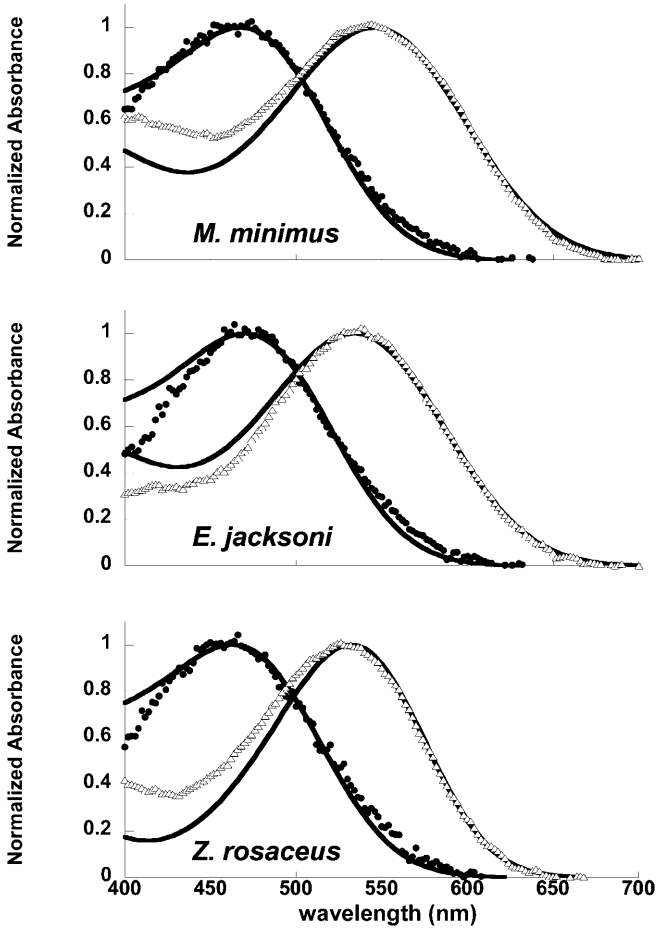

Fig. 4 Average normalized cone absorbance and template spectra for three surfperch species representing different depth zones: short-wavelength-sensitive (SWS) cones (*filled circles*), long-wavelength-sensitive (LWS) cones (*open triangles*), and rhodopsin or porphyropsin template (*solid line*). *M. minimus* occupies the shallow surfgrass zone (1–3 m); *E. jacksoni* occupies a number of sublittoral habitats (1–20 m); and *Z. rosaceus* is found predominantly at greater depths (> 20 m)

of LWS cone λ_{max} and mean depth: $r^2 = 0.012$, $b = -0.201$, $t_{(6)} = -0.265$, $P = 0.367$; and residuals of SWS cone λ_{max} and mean depth: $r^2 = 0.043$, $b = -0.381$, $t_{(5)} = -0.477$, $P = 0.332$]. Similarly, regressions computed with the non-phylogenetic residuals of surfperch cone λ_{max} values on surfperch habitat's mean wavelength of maximum flux of downwelling light ($\lambda Fmax_D$) were non-significant. The mean wavelength of maximum flux for the downwelling light ($\lambda Fmax_D$) measured within each surfperch habitat contributed insignificantly to LWS and SWS cone λ_{max} interspecific variation [residuals of LWS cone λ_{max} and $\lambda Fmax_D$: $r^2 = 0.029$, $b = 0.357$, $t_{(6)} = 0.419$, $P = 0.346$; and residuals of SWS cone λ_{max} and $\lambda Fmax_D$: $r^2 = 0.011$, $b = -0.208$, $t_{(5)} = -0.236$, $P = 0.367$].

Maximizing photon capture of background radiance

While the linear relationships between surfperch mean $\lambda Fmax_S$ and cone λ_{max} are significant, the direction of change does not show an exact match between surfperch LWS cone λ_{max} and mean $\lambda Fmax_S$ (Fig. 5c–f). If the cone λ_{max} values vary in a direction that matches habitat $\lambda Fmax_S$, then we would expect to see a positive relationship between cone peak sensitivity and background maximum flux. Comparing cone λ_{max} values to habitat $\lambda Fmax$ values, however, provides only a crude estimation of how species-specific differences in cone sensitivity vary with differences in habitat irradiances because it isolates the comparison to single wavelengths. We can assess in a more quantitative way whether visual pigments are tuned for maximizing photon capture of a species' background by using the entire cone absorptance and sidewelling irradiance spectra in modeling photoreceptor photon capture of background irradiance (Fig. 6). The modeling results show the cone λ_{max} values that provide the maximum level of photon capture of background radiances are not the same as the mean $\lambda Fmax_S$ measured within each surfperch species' habitat (Fig. 5, 6). Furthermore, most surfperch LWS cone λ_{max} values are closer to their habitat-specific model optima than to the measured mean $\lambda Fmax_S$ in their habitats.

The majority of surfperch in this study have LWS cone λ_{max} values that are at, or very near (< 6 nm), the optimal visual pigment sensitivity for maximizing absorbance for background radiance (Fig. 6). *E. jacksoni* and *E. lateralis* have measured LWS cone λ_{max} values that match the model optimum for their habitats (e.g., $pmaxS = 1.0$), while *M. minimus*, *H. caryi, B. frenatus*, and *D. vacca* all have measured LWS cone λ_{max} values that are within 1% of the model optimum in background sensitivity performance ($pmaxS > 0.99$). The LWS cones of *M. aurora* and *C. aggregata* also exhibit high levels of background sensitivity for their measured habitat range ($pmaxS > 0.93$).

Surfperch LWS cones provide much greater sensitivity to the background light (sidewelling irradiance) than the SWS cones (Fig. 6). Estimates of cone absorptance by the SWS cones are much more variable than the LWS cones and exhibit much less sensitivity to the background radiance (range 0.15–0.64). The significantly lower absorptance performance of the SWS cones, even at same wavelengths as LWS cones, is due to cone class differences in outer segment dimensions and MSP specific absorbance measurements used to convert absorbance to absorptance (see Eq. 2).

Discussion

Visual pigments and habitat depth

Our measurements of surfperch rod and cone visual pigments are comparable to those reported for other coastal dichromatic teleosts (Loew and Lythgoe 1978; Levine and MacNichol 1979). The range of interspecific cone peak sensitivity (λ_{max}) is rather broad for a relatively narrow depth gradient across these species (27 nm for SWS, 19 nm for LWS), and is unlikely to be

Table 2 Surfperch visual pigment and habitat characteristics. Wavelengths of maximum sensitivity (λ_{max}) are calculated from the best-fitting visual pigment template as described in the text. *Subscript numbers* represent the retinal (A_1 or A_2) template of best fit. For each species, the first line of data gives the λ_{max} (nm) of the mean absorbance spectrum ± 1 standard deviation (SD). The SD in this first line refers to the variance in the long-wave arm between template and mean sample absorbance spectra. The second line of data gives the mean λ_{max} ± 1 SD of all the sample absorbance λ_{max} used in producing the mean absorbance spectrum. The SD in this second row represents the variance in λ_{max} estimates across the individual sample spectra. The third line reports the number of photoreceptor cells used to calculate the average spectrum, with the number of MSP measurements used for the average in parentheses. Species' mean observation depth (m) ± 1 SD is reported (*SWS* short-wavelength-sensitive; *LWS* long-wavelength-sensitive; *n.a.* not available)

Species	Rod λ_{max} (nm)	SWS cone λ_{max} (nm)	LWS cone λ_{max} (nm)	Depth (m)
Micrometrus aurora (reef surfperch)	518.62 ± 3.8 518.02 ± 6.5 5 (5)	$465.7_1 \pm 3.7$ $464.8_1 \pm 4.8$ 3 (6)	$538.1_2 \pm 2.7$ $541.5_2 \pm 5.1$ 7 (17)	1.0 ± 0.5
Cymatogaster aggregata (shiner surfperch)	$499.9_1 \pm 0.8$ $500.3_1 \pm 1.9$ 5 (5)	$456.1_1 \pm 1.0$ $456.1_1 \pm 4.7$ 2 (6)	$529.0_1 \pm 1.0$ $529.0_1 \pm 1.7$ 26 (44)	1.3 ± 0.9
M. minimus (dwarf surfperch)	$517.7_2 \pm 5.5$ $519.4_2 \pm 6.5$ 4 (5)	$470.0_2 \pm 2.8$ $469.5_2 \pm 5.8$ 4 (8)	$545.5_2 \pm 1.6$ $545.9_2 \pm 3.7$ 22 (34)	1.6 ± 1.7
Embiotoca jacksoni (black surfperch)	$505.1_1 \pm 1.9$ $504.9_1 \pm 0.4$ 2 (2)	$476.7_2 \pm 3.8$ $476.9_2 \pm 12.9$ 5 (14)	$533.6_2 \pm 1.3$ $533.0_2 \pm 4.2$ 12 (22)	5.8 ± 3.6
Damalichthys vacca (pile surfperch)	$509.3_1 \pm 2.0$ $508.4_1 \pm 3.8$ 3 (3)	$472.8_1 \pm 6.9$ $465.8_1 \pm 11.9$ 4 (4)	$544.4_2 \pm 2.1$ $545.0_2 \pm 5.9$ 8 (18)	6.1 ± 3.5
E. lateralis (striped surfperch)	$500.8_1 \pm 2.1$ $500.2_1 \pm 0.4$ 4 (4)	$482.1_1 \pm 5.1$ $482.5_1 \pm 5.3$ 2(3)	$535.6_2 \pm 2.0$ $536.4_2 \pm 3.2$ 15 (21)	6.5 ± 3.3
Brachyistius frenatus (kelp surfperch)	n.a.	n.a.	$531.3_2 \pm 5.5$ $530.8_2 \pm 3.0$ 3 (8)	6.8 ± 3.1
Hypsurus caryi (rainbow surfperch)	$505.7_1 \pm 0.7$ $506.4_1 \pm 2.4$ 12 (18)	$455.1_2 \pm 5.8$ $456.4_2 \pm 5.2$ 2 (6)	$531.5_2 \pm 3.6$ $531.8_2 \pm 2.7$ 17 (27)	7.9 ± 3.1
Zalembius rosaceus (pink surfperch)	$494.2_1 \pm 2.9$ $494.6_1 \pm 2.1$ 9 (12)	$470.4_2 \pm 4.5$ $470.1_2 \pm 10.3$ 3 (8)	$526.5_1 \pm 0.8$ $525.5_1 \pm 2.7$ 13 (29)	> 20

accounted for by sampling error alone. The majority of surfperch co-exist in the same moderate-littoral depth zone, and only a few species such as *M. aurora*, *M. minimus*, and *Z. rosaceus*, have restricted depth zones at opposite extremes. The interspecific variation in cone λ_{max} values does not follow a clear trend with habitat depth, while surfperch lens transmission and rod λ_{max} reveals some depth-related patterns.

A shallow water environment with a high degree of scattering matter, such as the temperate littoral zone, can produce visually obscuring veiling light dominated by short wavelengths (Lythgoe 1988; Partridge 1990). A short-wave absorbing lens is thought to improve visual resolution in such environments (Douglas and McGuigan 1989; Douglas et al. 1995). The majority of the littoral surfperch have a UV-filtering lens (surfperch average 50% transmittance cut-off is at 412 nm). Only the lens of the pelagic pink surfperch (*Z. rosaceus*) exhibits a relaxation of this UV-filtering with a 50% cut-off at 384 nm; the shiner surfperch (*C. aggregata*), a seasonal visitor to the deep, exhibits an intermediate 50% cut-off (402 nm). The variation in rod λ_{max} values also follows a depth gradient. Surfperch rods exhibit a blue-shift in λ_{max} values with increasing average depth range (Table 2). The transition from 518 nm to 494 nm in peak rod absorbance from the shallow to deeper species has been documented in other fish assemblages covering similar depth gradients (Muntz 1976; Bowmaker et al. 1994). However, unlike some of these other species flocks, there is no parallel blue-shift in surfperch cone photoreceptor λ_{max} values (Bowmaker et al. 1994; Lythgoe et al. 1994).

Mixed chromophoric group

In general, on the basis of template matching, surfperch retinas have a mixture of rhodopsin and porphyropsin as chromophore units, with the majority of LWS cones favoring a porphyropsin template (Fig. 4, Table 2). Although mixed chromophore retinas are more common among freshwater and euryhaline teleosts (Bridges

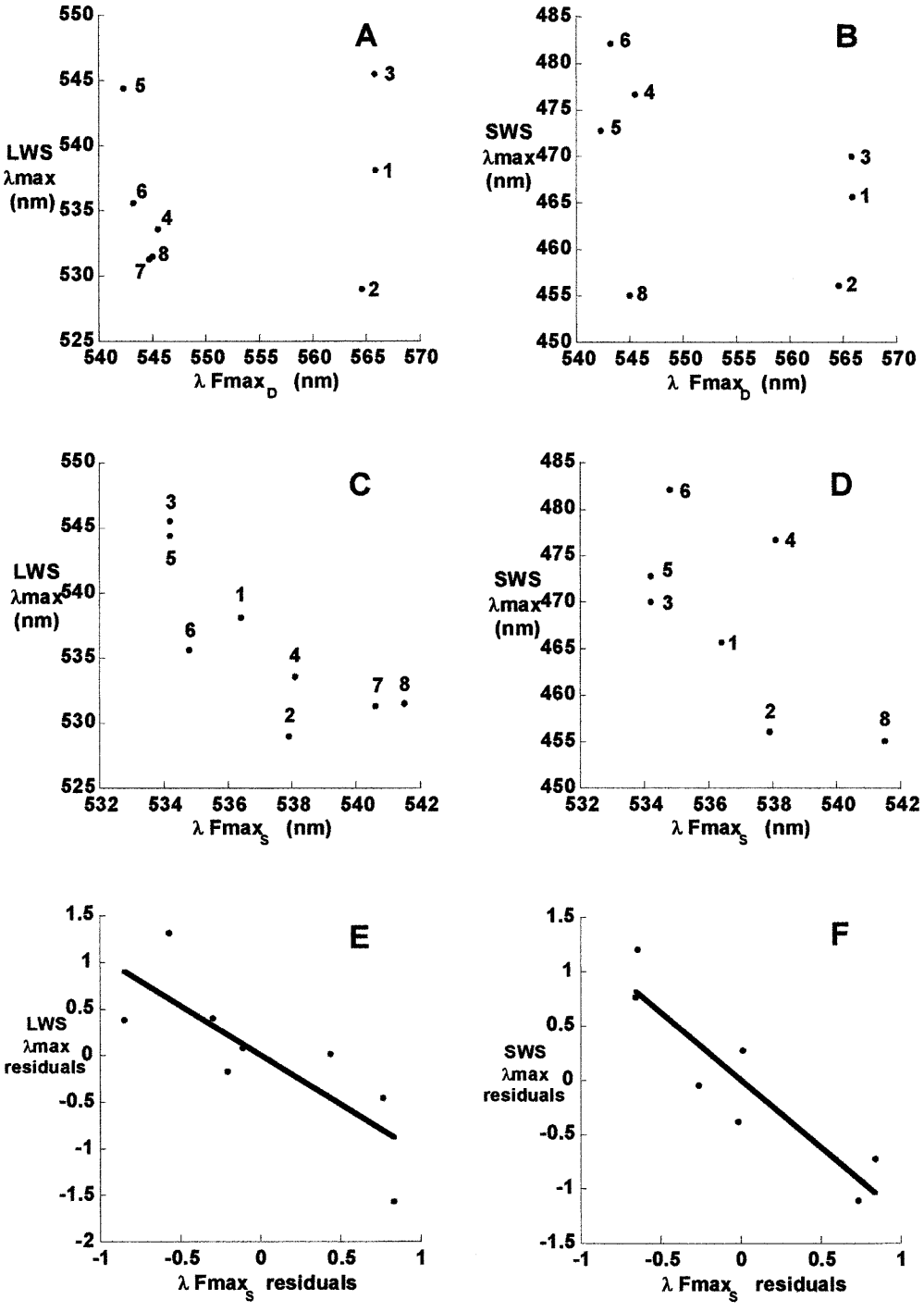

Fig. 5 Average wavelength of maximum photon flux ($\lambda Fmax_I$) in species-specific optical ranges versus surfperch photoreceptor peak absorbance (λ_{max}) (**a**–**d**), and phylogenetically corrected regression on the residuals of peak absorbance and background wavelengths of maximum photon flux (**e**, **f**). Numbers represent species in order of mean habitat depth: *1 M. aurora*, *2 C. aggregata* , *3 M. minimus*, *4 E. jacksoni*, *5 D. vacca, 6 E. lateralis*, *7 B. frenatus* (only LWS cone measurements available), and *8 H. caryi*. **a** LWS cone λ_{max} and habitat mean $\lambda Fmax_D$. **b** SWS cone λ_{max} and habitat mean $\lambda Fmax_D$. **c** LWS cone λ_{max} and habitat mean $\lambda Fmax_S$. **d** SWS cone λ_{max} and habitat mean $\lambda Fmax_S$. **e** Non-phylogenetic residuals of LWS cone λ_{max} and habitat mean $\lambda Fmax_S$; the regression line is described by $y = -1.0581x$, $r^2 = 0.63$ ($P = 0.012$). **f** Non-phylogenetic residuals of SWS cone λ_{max} and habitat $\lambda Fmax_S$; regression line is described by $y = -1.2346x$, $r^2 = 0.83$ ($P = 0.0019$)

1972), some coral reef labrids and scarids also share such a dichotomous retina (Munz and McFarland 1977). Without an investigation of the A_1/A_2 ratios in the various cell types, it is difficult to determine whether the variation within cone class λ_{max} observed across species is due to differences in cone opsins, or differences in proportions of A_1/A_2 as the prosthetic group. The visual function of a mixed retina is still not fully understood. However, species that vary their A_1/A_2 proportion with subsequent variation in photoreceptor peak absorbance do so in response to changes in ambient light conditions (Dartnall et al. 1961; Bridges 1965, 1972; Munz and Beatty 1965; Allen 1971; Loew and Dartnall 1976; Levine and MacNichol 1979; Muntz and Mouat 1984).

If mixed A_1/A_2 retinas allow for labile photoreceptor tuning to a fish's optical habitat, then a plastic chromophore response may be an advantage in the optically variable habitat of the Californian near-shore environment. Due to changes in depth, kelp canopy cover, phytoplankton abundance and composition, the

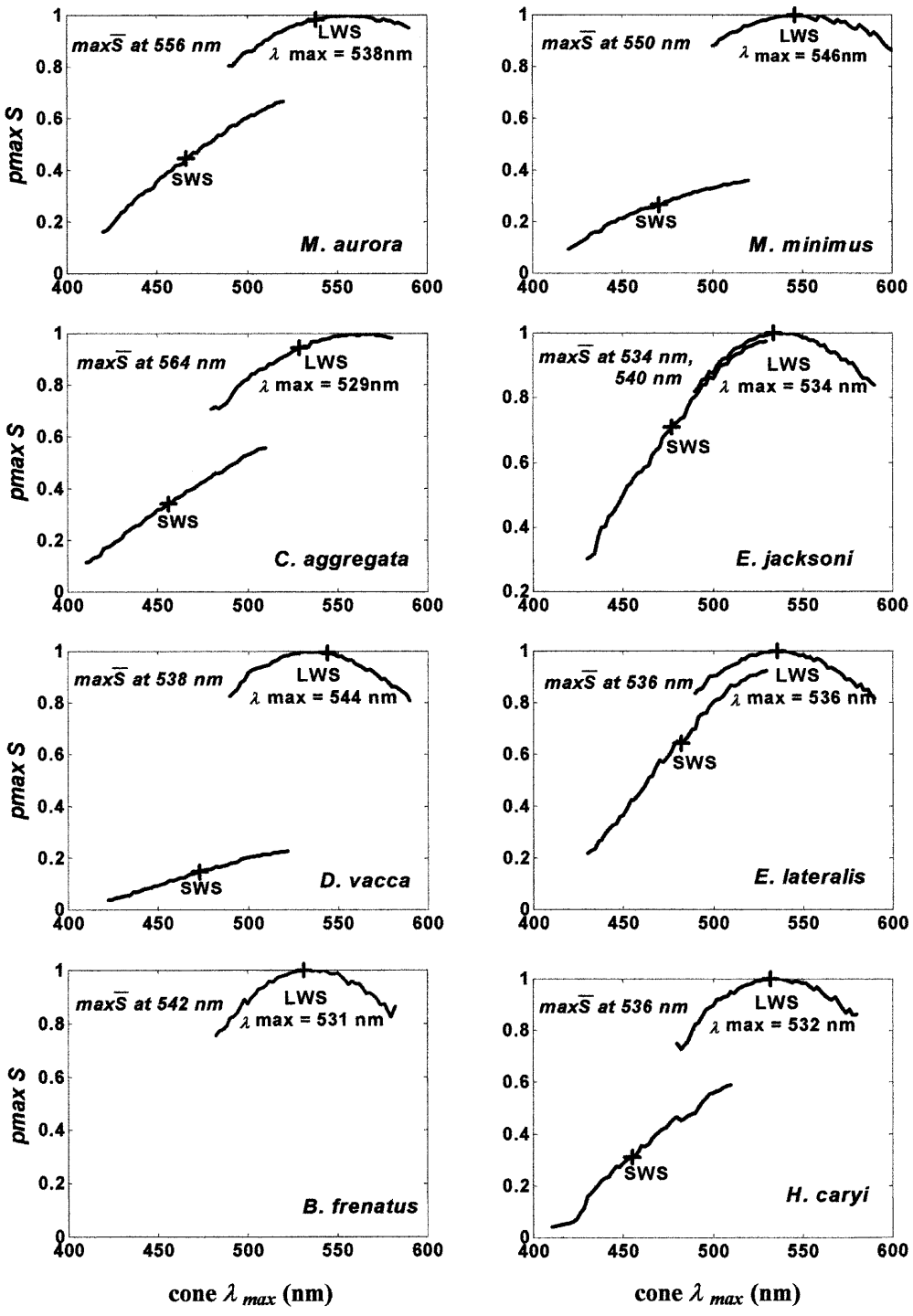

Fig. 6 Habitat-specific optimal background sensitivity curves, $p\max \overline{S_c}$, for LWS and SWS cones. The $\max \overline{S}$ values represents visual pigment with λ_{max} providing the greatest mean photon capture of sidewelling irradiance measured within each species-specific habitat. The measured SWS and LWS cone λ_{max} values are indicated by +. The SWS and LWS $p\max \overline{S_c}$ curves differ dramatically due to differences in measured specific absorbance and cell dimensions involved in converting absorbance to absorptance (see Eq. 2)

spectral environment of the surfperch near-shore temperate environment is more variable than most freshwater lake environments (Gerard 1984; Dean 1985; Wing et al. 1993; Kirk 1994). Variation in surfperch microhabitat preferences within this heterogeneous environment has resulted in species with similar depth ranges experiencing different optical conditions (Fig. 1). Can these species-specific differences in optical habitat explain the variation in surfperch cone λ_{max} values?

Photopic pigments and photic environment

Species that live in disparate depth zones or water types, often exhibit differences in cone peak absorbances that correlate to their photic habitats (Lythgoe 1972, 1979; Levine and MacNichol 1979; Lythgoe et al.1994; Bowmaker et al. 1994; McDonald and Hawryshyn 1995; Jutte et al. 1998). Surfperch species, however, have largely overlapping habitat ranges and we find no correlation between surfperch depth or mean wavelength of

maximum flux of their downwelling irradiance measurements with cone peak sensitivity [depth: $r^2 = 0.01$ (LWS) and $r^2 = 0.04$ (SWS); $\lambda Fmax_D$: $r^2 = 0.03$ (LWS) and $r^2 = 0.01$ (SWS)].

The habitat feature that explains most of the variation across visual sensitivities is the mean wavelength of maximum flux of the sidewelling irradiance, the horizontal visual field. Both photopic pigment λ_{max} values exhibit a linear pattern with changes in the sidewelling irradiance across surfperch habitats and this relationship is statistically significant even after removing the potentially confounding variable of phylogenetic relatedness (Fig. 5e, f: $r^2 = 0.63$, $P = 0.012$ for regression using the non-phylogenetic residuals of LWS-cone λ_{max} on $\lambda Fmax_S$ residuals; and $r^2 = 0.83$, $P = 0.0019$ for SWS-cone λ_{max} residuals on $\lambda Fmax_S$ residuals). This indicates that the variation observed across surfperch cone pigments is as variable amongst sister species as it is between more distantly related surfperch in this study, suggesting differences in cone sensitivity are independent of historical legacy. Microhabitat differences in optical backgrounds, rather than phylogeny, account for most of the variation in peak sensitivity of both cone classes among surfperch species in this study where species with similar optical backgrounds have similar cone λ_{max} values.

Relative photon capture of background radiance

Surfperch LWS cones are well positioned spectrally to maximize the photon capture of the horizontal visual field (Fig. 6). This is particularly true for many of the kelp forest species, such as *E. jacksoni*, *E. lateralis, H. caryi*, *B. frenatus*, and *D. vacca*. The near-optimality trend suggests that the interspecific variation in LWS cones relates to tuning for maximal sensitivity to their different optical backgrounds. Surfperch SWS cones are far less effective at capturing background radiance (Fig. 6) than the LWS cones, yet the interspecific variation across species also shows a significant relationship with changes in habitat background (e.g., $\lambda Fmax_S$, Fig. 5).

If the LWS cone class functions to maximize photon capture of the background light, then we might expect a large part of the variation ($r^2 = 0.63$, $P = 0.012$) of interspecific LWS cone λ_{max} measurements to exhibit a strong relationship to the mean sidewelling irradiance maximum for each surfperch habitat. However, it is perhaps surprising that there is even a stronger relationship between the SWS cone λ_{max} and $\lambda Fmax_S$ ($r^2 = 0.83$, $P = 0.0019$) given that the SWS-cone λ_{max} values are offset to the sidewelling irradiance maximum by 53–86 nm (Fig. 5d). Why would the SWS cone λ_{max} values track the sidewelling maximum so tightly if they are not serving a sensitivity function?

Offset visual pigments have been hypothesized to work as contrast detectors of brightly reflecting targets against a darker background (Lythgoe 1966, 1968, 1972; McFarland and Munz 1975; Munz and McFarland 1977; Loew and Lythgoe 1978). If their primary function is for capturing reflectance from targets rather than background light, then we might not expect a strong correlation to the background transmission maximum. If, however, they play some role in chromatic processing of the background light, then a relationship with the optical background is to be expected (Lythgoe 1979; Lythgoe and Partridge 1991; McFarland and Munz 1975; Munz and McFarland 1977).

To understand why the SWS cones vary linearly with changes in optical habitat background requires further modeling using dichromatic vision models (Lythgoe and Partridge 1991; Osorio and Vorobyev 1996). By exploring how both cone types contribute to the visual process of background tuning, as well as by incorporating visual tasks into the model, we can develop a better understanding of why teleost visual pigment peak sensitivities vary even on fine, microhabitat scales as observed here among surfperch.

Summary

Here we show that the interspecific surfperch variation in cone absorbance correlates significantly with species-specific optical backgrounds at a fine, microhabitat scale. Species that share the same depth zone, experience different optical backgrounds, and the variation in cone maximum absorbance is driven by these differences in the horizontal visual field. Specifically, we have shown that the spectral position of the LWS cone λ_{max} values are tuned for maximizing photon capture of background radiance, while the SWS cone λ_{max} values are considerably offset from the dominant background waveband. Our results confirm earlier hypotheses that interspecific variation in sensory sensitivities is related to differences in microhabitat preferences (Levine and MacNichol 1979; Lythgoe 1979); and the importance of the horizontal visual field as a selective force in the evolutionary divergence of fish visual pigments (Lythgoe 1966, 1968; McFarland and Munz 1975). By comparing the species-specific optical habitat range, phylogenetic relatedness, and photopic pigment λ_{max} values, we identified divergence in species-specific visual sensitivities correlating with changes in species-specific photic environments.

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