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Visual Biology of Hawaiian Coral Reef Fishes. I. Ocular Transmission and Visual Pigments

G. S. Losey, W. N. McFarland, E. R. Loew, J. P. Zamzow, P. A. Nelson, and N. J. Marshall

The visual biology of Hawaiian reef fishes was explored by examining their eyes for spectral sensitivity of their visual pigments and for transmission of light through the ocular media to the retina. The spectral absorption curves for the visual pigments of 38 species of Hawaiian fish were recorded using microspectrophotometry. The peak absorption wavelength (λ_{max}) of the rods varied from 477–502 nm and the λ_{max} of individual species conformed closely to values for the same species previously reported using a whole retina extraction procedure. The visual pigments of single cone photoreceptors were categorized, dependent on their λ_{max}-values, as ultraviolet (347–376 nm), violet (398–431 nm) or blue (439–498 nm) sensitive cones. Eight species possessed ultraviolet-sensitive cones and 14 species violet-sensitive cones. Thus, 47% of the species examined displayed photosensitivity to the short-wavelength region of the spectrum. Both identical and nonidentical paired and double cones were found with blue sensitivity or green absorption peaks (> 500 nm).

Spectrophotometry of the lens, cornea, and humors for 195 species from 49 families found that the spectral composition of the light transmitted to the retina was most often limited by the lens (73% of species examined). Except for two unusual species with humor-limited eyes, *Acanthocybium solandri* (Scombridae) and the priacanthid fish, *Heteropriacanthus cruentatus*, the remainder had corneal-limited eyes. The wavelength at which 50% of the light was blocked (T50) was classified according to a system modified from Douglas and McGuigan (1989) as Type I, T50 <= 355 nm, (32 species); Type IIa, 355 < T50 <= 380 nm (30 species); Type IIb, 380 < T50 <= 405 nm (53 species) and Type III, T50 > 405 nm (84 species). Possession of UV-transmitting ocular media follows both taxonomic and functional lines and, if the ecology of the species is considered, is correlated with the short-wavelength visual pigments found in the species.

Three types of short-wavelength vision in fishes are hypothesized: UV-sensitive, UV-specialized, and violet-specialized. UV-sensitive eyes lack UV blockers (Type I and IIa) and can sense UV light with the secondary absorption peak or beta peak of their longer wavelength visual pigments but do not possess specialized UV receptor cells and, therefore, probably lack UV hue discrimination. UV-specialized eyes allow transmission of UV light to the retina (Type I and IIa) and also possess UV-sensitive cone receptors with peak absorption between 300 and 400 nm. Given the appropriate perceptual mechanisms, these species could possess true UV-color vision and hue discrimination. Violet-specialized eyes extend into Type IIb eyes and possess violet-sensitive cone cells.

UV-sensitive eyes are found throughout the fishes from at least two species of sharks to modern bony fishes. Eyes with specialized short-wavelength sensitivity are common in tropical reef fishes and must be taken into consideration when performing research involving the visual perception systems of these fishes. Because most glass and plastics are UV-opaque, great care must be taken to ensure that aquarium dividers, specimen holding containers, etc., are UV-transparent or at least to report the types of materials in use.

THE great diversity of coral reef fishes and the accompanying multitude of ecotypes they represent have provided fish biologists with a natural laboratory in which to examine adaptation (see Sale, 1991). The many colors and well-developed eyes of these species encouraged investigation of their spectral environment and visual capabilities of these species.

During the 1960s and 1970s, attention was directed to evaluating the spectral sensitivity of the visual pigments of reef fishes and relating these to general aspects of their behavior and the photic environments they inhabited (Lythgoe, 1966; Hobson, 1972; review in Loew, 1995). These earlier investigations used extraction techniques on dark-adapted fish retinae and subsequent spectrophotometry of the extracts to characterize their absorption spectrum and to establish, by template fitting, the wavelength at peak absorption or λ_{max}. Although a large number of different tropical marine fishes were characterized and several hypotheses generated, extraction spectrophotometry procedures (ESP) limited interpretations to visual pigments of the rods, because the cone pigments were either destroyed by extraction or spectrally swamped by the rod pigments (but see Munz and McFarland, 1975; McFarland and Munz, 1975a, where some evidence for the extraction of cone pigments is presented).

This limitation has been circumvented by the introduction of microspectrophotometry (MSP). With this technique, the spectral absorption of individual photoreceptors is measured by passing a focused microbeam through the outer segment of a receptor cell, scanning the beam through the spectrum and measuring the transmitted light intensity at each wavelength with a photomultipler/amplifier/computer system (see Harosi, 1981).

Prior to the early 1980s, MSP recordings from fishes routinely covered the spectral range from about 380 nm in the near ultraviolet (UV) to 750 nm in the red (see Loew and Lythgoe, 1978; Levine and MacNicol, 1979). Measurements to shorter wavelengths that might have confirmed the presence of an ultraviolet class of photoreceptors were not made partly because of instrumental limitations and also the mistaken belief that UV-radiation was so rapidly absorbed in natural waters that not enough penetrated to make vision possible (see Loew and McFarland, 1990). By the mid-1980s, however, it was becoming clear that at least several fresh water fish species were sensitive in the UV and possessed a class of cone photoreceptors containing a visual pigment that absorbed maximally in the UV (Harosi, 1981; Avery et al., 1983; review in Bowmaker, 1991). The subsequent demonstration of UV visual pigments in several euryhaline fishes (salmonids, Kunz and Bowmaker, 1986; cyprinodontids, Harosi and Fukorotami, 1986) suggested that very short-wavelength visual pigments might be widespread among fishes. This possibility should be especially true for stenohaline marine species inhabiting clear oceanic waters, as exemplified by coral reef fishes, where UV light would be relatively intense (Baker and Smith, 1982; Loew and McFarland, 1990; Loew et al., 1996).

A second source for clues as to the presence of UV-sensitive vision can be gained by examination of the light absorption properties of the ocular media (cornea, lens, and humors). These less technically demanding methods allowed survey of a wider variety of species. The critical hypothesis is that, if a portion of the spectrum is nearly totally absorbed before it reaches the retina, vision at these wavelengths is unlikely. Light-blocking pigments that absorb UV radiation have been found in a wide variety of fish species, including freshwater (Douglas and McGuigan, 1989), deep-sea (Douglas and Thorpe, 1992), pelagic (Dunlap et al., 1989), mesopelagic (Muntz, 1976; McFall-Ngai et al., 1986), and shallow marine (Thorpe et al., 1993; Siebeck and Marshall, 2000, 2001) species. When present, these compounds are generally retained in either the lens or cornea, thereby blocking the transmission of UV light through the ocular media to the retina.

In fishes, these UV-absorbing pigments consist generally of three types. Kynurenines, found in freshwater (Thorpe and Douglas, 1993) and deep-sea (Thorpe et al., 1992) fishes, are characterized by simple absorption spectra with peak absorbance between 360 and 370nm (Posner, 1998). Carotenoids, which have been found in reef fishes (Siebeck and Marshall, 2000) and mesopelagic fishes (McFall-Ngai et al., 1986), as well as in a species of deep-sea fish (Douglas and Thorpe, 1992), produce a complex absorption spectrum with four major peaks at longer wavelengths ranging from 382 to 462 nm (Posner, 1998). Mycosporine-like amino acids (MAAs) that absorb at 320–340 and about 360 nm are by far the most prevalent UV-blocking compounds and are found in a wide variety of shallow marine species, including fishes (review in Dunlap and Shick, 1998). Mason et al. (1998) demonstrated that at least one fish species, *Oryzias latipes*, cannot synthesize MAAs but must acquire them from dietary sources.

Two principle advantages to possessing these UV-blocking compounds have been hypothesized to explain their prevalence in the fish fau-

na (Siebeck and Marshall 2000, 2001; review in Douglas and Marshall, 1999). High-energy UV radiation, plentiful in relatively shallow clear waters (Smith and Baker, 1979; Frank and Widder, 1996), is damaging to the retina (Collier and Zigman, 1987; Zigman, 1995). Possession of UV-blockers would help prevent retinal damage. Short-wavelength light is also known to degrade retinal image clarity, both by scattering within the eye and through chromatic aberration or the focusing of different wavelengths at different distances from the lens (Muntz, 1976, but see McLellan et al., 2002) .

Despite the benefits gained by having UV-absorbing pigments in the ocular media, a number of species lack these compounds (Douglas and McGuigan, 1989; Thorpe et al., 1993), thereby letting potentially detrimental UV photons through the ocular media to the retina. Regardless of the peak wavelength(s) of sensitivity of their visual pigment(s), these species would achieve at least some ability to form images in the UV because all visual pigments have a secondary absorption peak at wavelengths less than 400nm (Dartnall and Lythgoe, 1965) and could, thus, show sensitivity to UV photons. Some of the species that lack short-wavelength filters are deep-sea or nocturnal species and may not possess specialized UV-sensitive cone cells. These species may be simply maximizing the number of photons reaching the retina. Alternatively, they may not possess UV-blockers because they are unable to acquire MAAs from their diet or because their environment lacks sufficient UV radiation to select for retention of UV-blockers. Other fish species that lack short-wavelength blockers, however, allow penetration of UV light to the retina and possess UV-sensitive cone cells in the retina that have their primary absorption peak in the UV, usually around 360nm (Hawryshyn and Harosi, 1991; Loew et al., 1996; Bowmaker et al., 1991).

There are many possible benefits to having UV vision (review in Losey et al., 1999), but few have been demonstrated conclusively. The imaging of objects against back-scattered UV light is one of the better-supported possible functions of UV vision (see figs. 7–8 in Losey et al., 1999). Plankton usually absorb UV light (Johnsen, 2002) and would, therefore, be imaged in the UV as dark objects against a bright back-scatter. Some small planktivorous fishes feed successfully under UV light alone (Loew et al., 1996). The feeding efficiency of trout and sunfish on plankton improved with the addition of UV radiation to the visible spectrum (Browman et al., 1994), but others have failed to find this effect with rainbow trout (Rocco et al., 2002). Further hypothetical benefits of UV vision include navigation and orientation via UV polarization patterns, analysis, and imaging of polarized UV reflectance patterns, recognition of species-specific UV color patterns, avoidance of excessive UV photo-exposure, intra- or interspecific social signaling in the UV, breaking of camouflage or crypsis, and simply extending the range of color vision.

Previous studies have been divided regarding whether UV vision follows (Thorpe et al., 1993) or does not follow (Dunlap et al., 1989) phylogenetic lines. Ontogenetically, the lenticular 50% short-wavelength cutoffs of the lens may increase, decrease, or remain the same (Thorpe and Douglas, 1993, Siebeck and Marshall 2001, Losey et al., 2000).

The demonstration of UV-sensitive visual pigments in cone cells from three damselfish species (McFarland and Loew, 1994), in part, provided the motivation for us to examine a wide selection of coral reef fish taxa. In this study, we measured ocular transmission both through whole eyes and the separate components of these eyes, for fish species from Hawai'i. For a smaller number of these species, we conducted MSP characterization of their visual pigments. Our primary intent was to identify types of ocular systems that may eventually be attributable to taxonomic links, ecological conditions, or both. Our second goal was to test the hypothesis that, if a fish inhabits an environment characterized by strong UV radiation and fails to block this radiation with its ocular media, it likely possesses UV-sensitive vision, the benefits of which outweigh the costs. Finally, we compare the new MSP results with prior studies of visual pigments in the same species using ESP methods.

Materials and Methods

Retinal MSP measurements.—Thirty-nine species of fishes were collected during May and June 1999 from locations surrounding the Hawaii Institute of Marine Biology (HIMB), Kaneohe Bay, Oahu, Hawaii. The species, sex, and age of fishes examined were dictated by availability and our desire to obtain a broad taxonomic sample. Fishes were transported to HIMB and maintained on a chopped squid diet for seven days or less in aquaria with running seawater on the natural light/dark cycle. Such holding times should have no effect on absorption properties of the eye. Prior to visual pigment measurement, we measured standard length (SL) and then dark-adapted the fish for a minimum of four hours. Fish were anesthetized with an overdose of MS222 (Sigma) and pithed. Under in-

frared (IR) illumination, each eye was enucleated and the retina removed in a standard Sorensen's buffer (pH 7.2) supplemented with 6% sucrose. Small patches of retina, sampled from throughout the retina, were placed on a 22 × 30 mm cover slip, chopped into small fragments, and teased with needles to release cells from the retinal mass. The preparation was sealed with an 18 × 18 mm cover slip edged with grease.

The MSP was a single-beam, computer-controlled instrument fitted with quartz and flourite optics (for details see Loew, 1994). A 100-W quartz iodine lamp provided sufficient UV radiation for absorption measurements to 350 nm. The retinal preparation was placed on the microscope stage of the MSP and examined under IR illumination. A baseline scan from 750 to 350 or 360 nm and back was taken through a clear area of the preparation. The measuring beam was then passed through an outer segment (OS) of an individual photoreceptor and the spectrum scanned and stored in the computer. Subtraction of the baseline provided a record of each cell's absorption spectrum.

Three criteria were used to establish that an absorption spectrum was from a visual pigment and not some other colored contaminant. The first criterion is based on the fact that visual pigments are photosensitive and thus are destroyed by light exposure—a process called bleaching. The second criterion rests on the fact that the fixed orientation of visual pigment molecules in the membranes of the outer segment renders them dichroic. That is, light of one plane of polarization is absorbed more strongly than orthogonally polarized light. This means that absorption spectra from the same cell measured under the two polarization conditions should have different peak amplitudes but the same shape. The third criterion is based on the fact that when visual pigment absorption spectra are plotted as normalized optical density versus normalized frequency they have a characteristic shape that is essentially the same regardless of the spectral position of the absorption maximum. This is the basis of the template-fitting routine used not only to establish the absorbing substance as a visual pigment but also to locate accurately the absorption maximum or λ_{max} (for details, see Loew, 1995; McFarland and Loew, 1994). In most instances, the best fitting template to the right-hand limb of a spectrum (i.e., the long-wavelength limb) was used to estimate the λ_{max}. When three or more similar identifiable cells (e.g., rods, single cones, etc.) were scanned the mean estimated λ_{max}-value was calculated ± 1 SD.

Ocular media transmission.—Fishes were captured by various means and either iced down on the collection boat or sacrificed within two days in the laboratory. Thorpe et al., 1993, used lenses frozen for up to 2 months with minimal effects. Our tests showed negligible effects of being iced down for up to 21 h, longer than any fish was iced down in our study. Eyes were removed with care to prevent separation of the dermal cornea that was sometimes loosely attached to the deeper scleral cornea. Our goal was to measure the spectral transmission through each of the components of the preretinal ocular filters. To measure transmission through the entire suite of filters, a slit or window was cut through the sclera, near the back of the eye and opposite the pupil, just through the retina. Following measurement of light transmission through the entire eye, the lens was dissected from the eye, rinsed in fresh water, and measured. When the dermal cornea was only loosely attached, measurement through the intact cornea was followed by separate examination of the dermal cornea. When absorption by the lens and cornea could not account for the absorption measured for the whole eye, the humors were also separately examined.

Spectral transmission was measured with an Ocean Optics S2000 spectrometer optimized for UV sensitivity with a 100 um slit. The broadbandwidth light source was an Ocean Optics DT-1000 with deuterium and tungsten bulbs. All components were designed for UV-visible light analysis. Whole eyes were mounted with the pupil facing down over a hole in an aluminum slide. A modified microscope stand with a micromanipulator stand was fitted with a 400-micron fiber optic probe held in a syringe needle. The needle was introduced into the window or slit in the eye until just submerged in the humor. Light from the DT-1000 was fed through the fiber optic probe, through the eye, into a lens below the microscope stage, and through a 400-micron fiber to the S-2000.

Because we did not use an integrating sphere to capture all light passing through the eye, it was necessary to manipulate the eye carefully under the probe to obtain transmission curves with a more or less flat long wavelength slope at about 100–150% transmission. A flat curve at maximum transmission was an important indication that the effects of any chromatic aberration had been minimized. At least two samples were taken from different locations for each preparation and percent spectral transmission for each component was standardized to 100% at the largest value obtained below 601nm. Losey et al. (2000) found that this method pro-

duced an average difference between normalized samples at each wavelength of only 2.6%. A few eyes were also analyzed by the SubSpec instrument that had been validated against an integration sphere (Siebeck and Marshall, 2000, 2001) and no critical differences were detected between the methods.

Larger lenses were placed over a hole in an aluminum slide that was just smaller than the lens, and smaller lenses, corneas, and dermal corneas were placed on top of a UV-transmitting (UVT) plastic slide. UVT transmits nearly all light down to 320 nm. Lenses were manipulated for measurement of transmission similar to the entire eye. Humor samples were placed on a UVT slide with 0.25-mm thick cover slip spacers at each end. A second UVT slide was placed over the top to flatten the humor sample to 0.25-mm thickness (see Zamzow and Losey, 2002). For a few species with extremely thick, jelly-like humors, a ''thick humor'' sample was obtained from humor samples up to about 2 mm thick prior to flattening as above.

The wavelength at which the ocular media blocked 50% of the incoming radiation (T50) was used to indicate the short-wavelength cutoff of the eye. This is the wavelength at which half of the quanta of that wavelength are blocked. In general, eyes sharply increase their blocking characteristics at the shorter wavelengths, usually below blue and often in the range of UVA radiation (320—400 nm). All of the eyes we examined blocked essentially all of the UVB radiation (< 320 nm).

Each family was characterized by the average and range of T50 values found for that family. To interpret the widely divergent ranges found, a randomization technique was used to establish the random expected range. In each of 1000 iterations of this test, our entire dataset of T50 values was sampled the appropriate number of times as determined by the number of species sampled in each family (2–19). The 975th smallest and largest value for each family sample size estimated the random expected range of T50 values.

Results

Visual pigment categories.—For the 38 species examined, the rod λ_{max} ranged from 477 nm in the damselfish, *Chromis hanui*, to 502 nm for the squirrelfish, *Neoniphon sammara* (Fig. 1, Table 1). Nearly half of the fishes examined possessed cone cells that would provide enhanced photosensitivity in the violet or UV regions of the spectrum. *Kuhlia sandvicensis* possessed unusually large UV-sensitive cone cells (OS diameter of 4–5 μm) and low noise UV absorption spectra (Fig. 2).

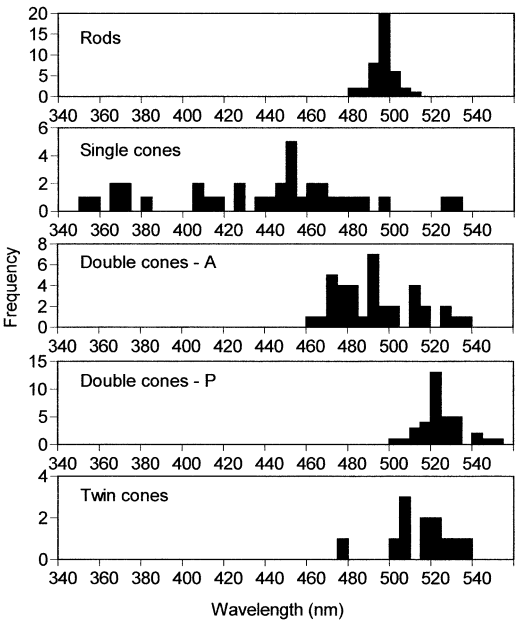

Fig. 1. Frequency distributions of the peak absorbance wavelength (λ_{max}) of the visual pigments from 39 Hawaiian coral reef fishes. Note the tight clustering for the rods around 490 nm and the short-wavelength distribution of single cones versus the longer-wavelength positions of the double cones. Visual pigment λ_{max} have been sorted into 5 nm bins.

We group cones with λ_{max}-values below 435 nm as short wavelength cones. We divide this group further into UV- ($\lambda_{max} \leq 376$ nm) and violet-sensitive (λ_{max} 398 to 435 nm) cones. Although these designations are arbitrary, such groupings serve to emphasize the peculiar properties of the violet and UV portions of the spectrum underwater, where the scattering of light is increased and the visual range of fishes is reduced (Loew and McFarland, 1990; Losey et al., 1999). Of the 38 species, eight possessed UV-sensitive cones with λ_{max} from 347 to 376 nm, and 14 species had violet-sensitive cones with λ_{max} from 398 to 435 nm. All of these short-wavelength visual pigments were present in single cones. Specific examples include the flagtail, *Kuhlia sandvicensis*, that possessed large, single UV-sensitive cones and the filefish, *Pervagor aspricaudus*, with a violet-sensitive cone λ_{max} at 404 nm (Fig. 2). Several species possessed both UV- and blue-sensitive (λ_{max} between 436 and 500 nm) cones, but no species had both UV- and violet-sensitive cones. In the 10 species having violet-sensitive cones, we detected no blue-sensitive cones. In most of the coral reef fishes with

TABLE 1. THE WAVELENGTH OF MAXIMUM ABSORBANCE FOR THE RODS AND CONES FROM 39 SPECIES OF HAWAIIAN MARINE FISHES. All values are expressed in nanometers (nm) and where appropriate as nm ± 1SD (n). Ditto marks for a species refer to additional individuals.

Scientific name	Rod by MSP	Rod by ESP	UV single	Violet single	Long wave single	Double-P	Double-A	Twin
Synodontidae								
Synodus variegatus	493 ± 1.9 (8)		368 ± 2.6 (3)			503 ± 2.1 (5)	482 ± 3.2 (7)	
Holocentridae								
Myripristis berndti	495 (1)	493			443, 453 (2)	514 ± 1.4 (4)	506 ± 2.4 (8)	
Neoniphon sammara	502 ± 0.3 (4)	502			446 (2)			512 ± 3.0 (19)
Sargocentrum xantherythrum	490 ± 1.4 (6)				447 ± 2.8 (3)	516 ± 1.0 (5)	509 ± 2.4 (5)	
Aulostomidae								
Aulostomus chinensis	494 ± 1.8 (5)	490		421 ± 2.1 (2)				473 ± 1.5 (6)
Kuhliidae								
Kuhlia sandvicensis	491 ± 2.7 (5)		362 ± 7.0 (18)		476 ± 4.5 (6)	498 ± 3.7 (1)	469 ± 4.5 (12)	
Kuhlia sandvicensis						518 ± 5.5 (5)	488 ± 3.8 (10)	
Apogonidae								
Apogon kallopterus	492 ± 3.4 (4)				441 ± 3.7 (4)	516 ± 4.3 (8)	494 ± 3.2 (8)	
Mullidae								
Mulloidichthys flavolineatus	486 (1)	484	366 ± 2.4 (4)			523 ± 2.7 (4)	480 ± 2.9 (5)	
Parupeneus multifasceatus	494 ± 1.3 (6)	491				518 ± 0.6 (4)	489 ± 2.4 (8)	515 ± 1.2 (4)
Parupeneus multifasceatus						516 ± 1.9 (13)	486 ± 2.5 (11)	
Chaetodontidae								
Chaetodon unimaculatus						530 ± 4.1 (2)	487 ± 1.0 (3)	
Chaetodon kleini	496 ± 2.6 (3)	491				530 ± 3.5 (8)	496 ± 2.6 (11)	
Forcipiger flavissimus	488 ± 1.0 (18)				431 (1)	527 ± 1.7 (4)	490 ± 3.7 (3)	
Pomacentridae								
Plectroglyphidodon johnstonianus	495 (1)		present			518 (2)	474 (2)	
Abudefduf abdominalis	492 ± 4.8 (8)	496	347 ± 3.6 (8)		464 (1)	519 ± 8.5 (6)	457 ± 2.1 (12)	
Stegastes fasciolatus	495 (1)		363 ± 1.0 (3)			528 ± 2.1 (8)	470 ± 3.8 (4)	
Chromis ovalis	492 ± 2.3 (5)			404 ± 1.1 (5)		518 ± 1.7 (8)	473 ± 3.6 (7)	
Chromis hanui	477 ± 4.0 (5)		355 ± 7.2 (7)		482, 484 (2)	514 ± 4.4 (8)	470 ± 3.5 (15)	
Chromis verater	480 ± 1.0 (4)	480		410 ± 1.4 (3)		514 ± 1.1 (6)	471 ± 2.9 (11)	
Chromis vanderbilti	498 ± 1.3 (4)					522 ± 1.3 (4)	462 ± 2.9 (5)	
Dascyllus albisella	490 ± 7.0 (3)	491	376, 359 (2)		464, 462 (2)	510 ± 3.5 (7)	467 ± 3.3 (7)	
Sphyraenidae								
Sphyraena barracuda	498 ± 2.2 (6)	498			455 ± 4.8 (9)			531 ± 7.6 (14)
Labridae								
Bodianus bilunnulatus	485 (1)	480				545 (2)	526 (2)	
Gobiidae								

TABLE 1. CONTINUED.

Scientific name	Rod by MSP	Rod by ESP	UV single	Violet single	Long wave single	Double-P	Double-A	Twin
Asteropteryx semipunctatus	498 ± 1.5 (9)					538 ± 1.0 (4)	531 ± 1.4 (4)	
Zanclidae								
Zanclus cornutus	492 ± 2.4 (3)	492			447 ± 0.7 (3)			517 ± 2.9 (12)
Acanthuridae								
Naso lituratus	497 ± 1.2 (4)	492		421 ± 0.6 (2)		521 ± 1.9 (3)	496 ± 1.8 (5)	
Naso lituratus						515 ± 0.6 (3)	488 ± 0.4 (3)	
Naso unicornis	494 (1)	492		416 ± 1.3 (5)		507 ± 2.8 (4)	486 ± 3.2 (5)	
Ctenochaetus strigosus	486 ± 3.6 (7)	492			458, 449 (2)	548 ± 0.2 (5)	521 ± 0.2 (5)	501 ± 7.7 (6)
Zebrasoma flavescens	494 ± 0.9 (2)	490			446 (2)	519 ± 1.8 (7)	510 ± 2.5 (4)	
Zebrasoma veliferum	494 (1)	492			448 ± 2.8 (4)	517 ± 3.8 (5)	511 ± 2.2 (5)	523 ± 2.7 (12)
Acanthurus triostegus	493 ± 2.9 (7)	497			458 ± 6.8 (7)			518 ± 3.7 (21)
Acanthurus nigroris	494 ± 0.9 (3)				439 ± 3.4 (10)	538 ± 3.3 (8)	523 ± 1.6 (10)	
Acanthurus nigroris	494 (1)				446 (1)	526 (1)	514 (1)	
Acanthurus achilles	496 ± 1.9 (4)							501 ± 4.4 (4)
Acanthurus achilles								532 ± 2.6 (6)
Balistidae								
Sufflamen bursa	487 ± 10 (3)			404 ± 3.2 (9)				503 ± 7.7 (4)
Monocanthidae								
Canthidermis maculatus	486 ± 2.2 (5)			407 ± 2.6 (3)	493 ± 2.7 (1)	522 ± 2.7 (1)	479 ± 8.4 (4)	
Pergavor aspricaudus	492 ± 2.4 (3)			404 ± 1.5 (5)		516 ± 3.3 (3)	476 ± 1.1 (5)	
Pergavor spilosoma	494 ± 1.3 (5)	492		398 ± 5.0 (3)		510 ± 0.8 (4)	492 ± 1.4 (5)	
Tetraodontidae								
Arothron meleagris	493 ± 3.0 (3)	495			439 ± 1.2 (5)	525 ± 5.5 (9)	507 ± 0.2 (3)	
Ostraciidae								
Ostracion meleagris	495 ± 3.3 (4)			416 ± 5.4 (6)	471.3 (2)	520 ± 3.8 (8)	479 ± 8.4 (7)	497 ± 0.8 (4)

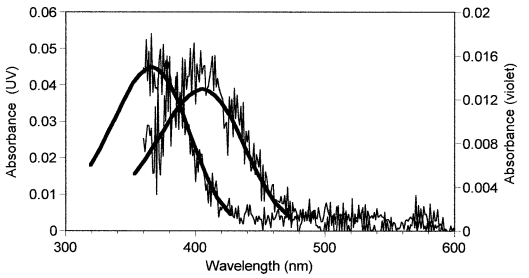

Fig. 2. Short-wavelength spectral absorbance curves from the Hawaiian Flagtail, *Kuhlia sandvicensis* (left curves), and the Orangetail Filefish, *Pergavor aspricaudus* (right curves). The noisier spectral curves are the recorded MSP values and the solid curves are the nomograms of visual pigments with absorbance maxima at 355 and 405 nm.

a violet absorbing visual pigment, the λ_{max} was close to 400 nm. Only in the trumpet fish, *Aulostomus chinensis* and surgeon fishes of the genus *Naso* was the violet pigment located closer to 420 nm.

Paired cones (double and twin) were found in all species examined. Double cones consist of a principle member (double cone; P) and a morphologically dissimilar accessory member (double cone; A). In twin cones the two members are morphologically identical. The members of double cones contain different visual pigments. Twin cone members, however, may contain the same or different visual pigments in which case they are called identical twins or nonidentical twins, respectively. In our sampling, double cones were the most common (31 of 38 species), and four species had both double and twin cones. Twenty-two species had green-blue-sensitive pairs (i.e., blue $<$ 500 nm vs green $>$ 500 nm), and nine species had unequal green-green-sensitive pairs (Table 2). Eleven species had identical twin cones, nine of which contained matched green visual pigments. Identical blue-blue-sensitive twin cones were found in the trumpet fish, *Aulostomus chinensis*, and the boxfish, *Ostracion meleagris*.

Spectral transmission categories.—The T50 for the entire eye ranged from 337 to 455 nm for the 185 species and 49 families for which the entire eye was sampled (Appendix 1). The T50 for the lens alone ranged over slightly shorter wavelengths (322 to 441 nm) since the blocking effects of the cornea, and in some cases, the humors, were lacking. Douglas and McGuigan (1989) suggested three categories for ocular lens T50 cutoffs: type I ($<$ 355 nm), type II or ''colorless'' (355 $<$ T50 $<$ 405 nm) and type III or ''yellow'' ($>$ 405 nm). They suggested that, considering a typical UV-sensitive visual pigment with maximum absorption at 360 nm, only fish with type I lenses were likely to perceive UV radiation. Two findings suggest modification of their scheme. First, we have indicated UV-sensitive visual pigments at longer wavelengths in Hawaiian fishes. Second, Thorpe et al. (1993) indicated that the goldfish, *Carassius auratus*, known to possess UV color vision, could have a T50 as high as 391 nm. In recognition that fishes with UV vision may have longer wavelength T50 values, we organized our results into four categories of ocular transmission that expand those of Douglas and McGuigan (1989) as type I has T50 $<=$ 355 nm, type IIa has 355 $<$ T50 $<=$ 380 nm, type IIb has 380 $<$ T50 $<=$ 405 nm, and type III has T50 $>$ 405 nm.

If we take the lens transmission measurement as a substitute for the 10 species for which we lack a T50 estimate for the entire eye, 195 species can be classed as to ocular transmission category. Thirty-two species were in class I suggested by Douglas and McGuigan (1989) as likely to have UV vision. Of the remaining species, 80 were classified as transmitting short-wavelength light to the retina with 30 as class IIa and 50 in

Table 2. Cone Visual Pigments of Coral Reef Fishes that Contained Nonidentical Green Double Photoreceptors. None of these species possessed violet or UV single cones.

Species	Paired cone λ_{max}-values	Blue cone λ_{max}-values	Deviations between double cones (nm)
Myripristes berndti	515–506	448	9
Sargocentrum xantherythrum	516–509	447	7
Bodianus bilunulatus	545–526	not found	19
Asterropteryx semipunctatus	538–531	not found	7
Ctenochaetus strigosus	548–521	458	27
Zebrasoma flavescens	519–510	447	9
Zebrasoma veliferum	517–511	448	6
Acanthurus nigrosis	538–523	440	15
Arothron meleagris	525–507	439	18

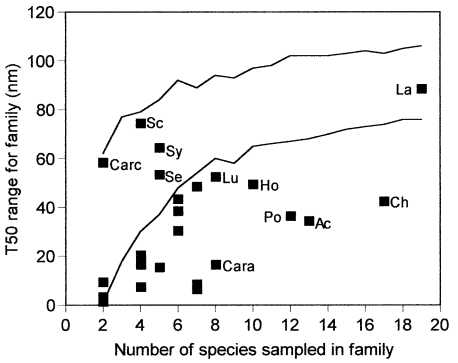

Fig. 3. The range of T50 values found in each family versus the number of species sampled. The solid lines represent the 97.5th percentile upper and lower confidence limits by randomization test. Adequately sampled outlier families with unexpectedly small ranges are indicated as "Ac," Acanthuridae; "Cara," Carangidae "Ch," Chaetodontidae; "Ho," Holocentridae; "Lu," Lutjanidae; and "Po," Pomacentridae. Families with randomly expected range sizes are indicated as "Carc," Carcharhinidae; "La," Labridae; "Sc," Scombridae; "Se," Serranidae; and "Sy," Synodontidae.

class IIb. The 83 remaining species were in class III.

Taxonomic distribution of T50 values.—The range of T50 values within a family was generally lower than expected (Appendix 1, Fig. 3). Only five of the 25 families with more than one species sampled fell clearly within the 95% confidence limits (randomization test): Carcharhinidae, Scombridae, Synodontidae, Serranidae, and Labridae. Other than sharing a predatory lifestyle, there is little in common among these five families.

A few families had both an adequate sampling of species (≥ 8) and genera and an unexpectedly low range of T50 values: Chaetodontidae, Acanthuridae, Pomacentridae, Holocentridae, Lutjanidae, and Carangidae. Their family average T50 values were diverse and ranged from 350 to 410 nm. In terms of their ecology, the species have little in common and range from nocturnal to diurnal activity and from pursuit predators to herbivores.

The phylogenetic order of the families taken from Nelson (1994), with a few modifications as suggested by D. Greenfield (pers. comm.; (Table 3), was used to indicate their relative phylogenetic position. Lacking knowledge of the relative phylogenetic distance between families, they were simply assigned an order number from 1 to 49. Linear regression of the family average T50 value on taxonomic position (Fig.

Table 3. Families Sampled and Their Averages T50 For the Entire Eye in Order of Their Assumed Phylogenetic Position.

Family	Mean T50
Squalidae	405
Carcharhinidae	371
Sphyrnidae	385
Albulidae	374
Muraenidae	384
Congridae	376
Clupeidae	385
Synodontidae	363
Ophidiidae	353
Moridae	383
Antennariidae	408
Mugilidae	396
Hemiramphidae	366
Holocentridae	365
Caproidae	353
Fistulariidae	410
Aulostomidae	409
Scorpaenidae	381
Dactylopteridae	402
Caracanthidae	355
Serranidae	401
Priacanthidae	392
Apogonidae	349
Coryphaenidae	443
Carangidae	410
Lutjanidae	386
Polynemidae	374
Mullidae	358
Chaetodontidae	393
Pomacanthidae	403
Kyphosidae	406
Kuhliidae	363
Cirrhitidae	408
Pomacentridae	350
Labridae	420
Scaridae	426
Pinguipedidae	423
Blenniidae	422
Gobiidae	384
Zanclidae	420
Acanthuridae	408
Sphyraenidae	410
Scombridae	396
Istiophoridae	429
Bothidae	420
Balistidae	419
Monacanthidae	406
Tetraodontidae	408
Ostraciidae	405

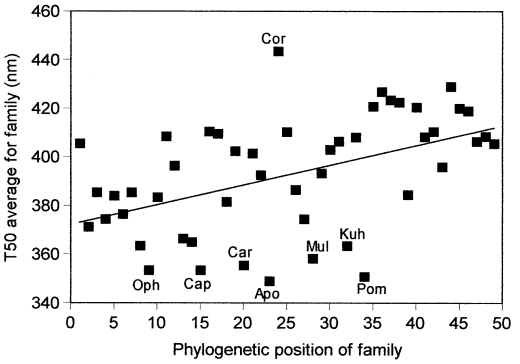

Fig. 4. The average T50 for each family is shown against the relative taxonomic order position (1–50) of the family as shown in Table 1. The linear regression slope is greater than zero ($r^2 = 0.23$, $n = 49$, $P < 0.01$). Outlier families are identified as "Apo," Apogonidae; "Cap," Caproidae; "Car," Caracanthidae; "Cor," Coryphaenidae; "Kuh," Kuhliidae; "Mul," Mullidae; "Oph," Ophidiidae; and "Pom," Pomacentridae.

4) indicates that more phylogenetically advanced families are more likely to have short wavelength ocular blockers and lack UV vision. The scatter is, however, extremely wide such that phylogenetic position alone cannot indicate the short-wavelength properties of their eyes. Much of the correlation depends on the 15 most phylogenetically advanced families past the Pomacentridae (Labridae–Ostraciidae, Table 3). If these families are removed, the correlation is lost ($r^2 = 0.01$, $n = 34$, $P > 0.1$).

The frequency distribution of the T50 values is clearly bimodal (Fig. 5). As an initial attempt to discover the source for the bimodality, we separated out all families in our sample that are diurnally active, mobile and closely associated with the coral reef as opposed to sand flats, etc. These were Acanthuridae, Balistidae, Chaetodontidae, Labridae, Monocanthidae, Pomacanthidae, Pomacentridae, Scaridae, and Tetraodontidae. These families had an arguably unimodal distribution of T50 values and the remaining families remained bimodal but shifted to shorter wavelengths.

Transmission blocker location categories.—The anatomical location of the radiation blockers varies between species (Fig. 6). Here we use the anatomical location of the transmission blocking effect that results in the longest-wavelength blocking. When the difference between the T50 for the lens and that for the entire eye was less than 6 nm, this was assumed to be caused by a measurement error, and the lens was assumed to be the critical or "limiting" blocking agent. When the difference was within 15 nm but the shapes of the curves were identical, the lens was again assumed to be the limiting element. The differences were likely caused by either a measurement error or the cumulative effect of adding the blockers from the cornea and humors that were similar to those in the lens. In other cases, the lens was not classed as the limiting blocking agent, and, when possible, the location of the limiting blockers was noted as either corneal or, in a few cases, humoral.

We characterized slope of the spectral transmission curve for the whole eye similar to Siebeck and Marshall (2000, 2001) as: "Steep" or "Class I": less than 30 nm between the 20 and 80% cutoffs (T20 and T80); "Gradual" or "Class II": less steep (> 30 nm difference between T20 and T80) with a gently curving slope but never having intermediate maxima; "Vari-

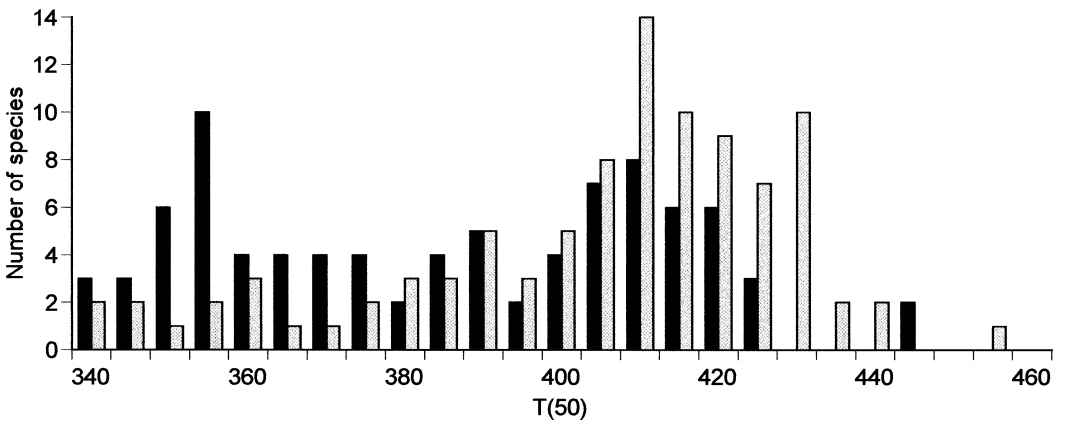

Fig. 5. The frequency distribution of T50 wavelengths for largely diurnal families closely associated with coral reefs (shaded bars, see text) versus all others (black bars).

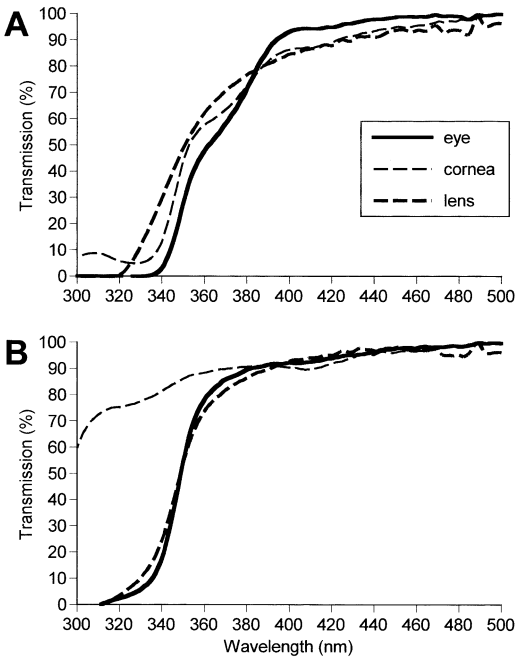

Fig. 6. Spectral transmission curves for (A) a short-wavelength, corneal-limited eye as found in an adult *Dascyllus albisella* and (B) a short-wavelength, lens-limited eye as found in an adult *Saurida flamma*. Ocular elements are entire eye (solid line), lens (thick dashed line), and cornea (thin dashed line).

able'' or ''Class III'': changes in slope result in intermediate maxima.

The lens was the limiting filter in 73% of the species, but this varied with the species' spectral transmission category. Steep lens transmission curves were most common in eyes where the lens was the limiting filter (Chi-square = 30.4, df = 1, $P < 0.001$) and in species that blocked most short wavelengths (Chi-square = 33.0, df = 1, $P < 0.001$). As expected, vision likelihood categories least likely to possess UV vision (see below) were most likely to use the lens as the limiting filter (Table 4). Twenty-four of the 49 families had at least one species in which the lens was not the limiting filter. Only two families, Priacanthidae and Scombridae, included the unusual combination that employed the humors as the limiting filter. There was no obvious correlation for any of these features with taxonomic position or ecological group.

Vision likelihood categories.—The diurnal habits of each species were assigned a category as (1) diurnally active and exposed to full ambient light, (2) nocturnal but exposed to full ambient light during the day, and (3) hidden from daylight. We hypothesized that fish exposed to full daylight that had the adaptation to block short wavelengths were least likely to be adapted for UV vision. Five ''vision likelihood'' categories were created as (1) highly likely to possess true UV color vision (transmission category I, daylight exposure category 1 or 2); (2) likely to possess true UV color vision (transmission category IIa, daylight exposure category 1 or 2); (3) likely to have violet visual sensitivity, but unlikely to have true UV color vision (transmission category IIb, daylight exposure category 1 or 2); (4) sensitive to short wavelength light, but not adapted for UV color vision (transmission category I, IIa, or IIb, daylight exposure category 3); and (5) unlikely to have short wavelength vision (transmission category III, any daylight exposure category).

Table 4. Count of the Number of Species in the Five Vision Likelihood Categories in which the Lens or Other ocular Element Was the Limiting Filter. The lens was more often the primary filter for higher classes that are unlikely to have short-wavelength vision (Chi-square = 12.8, df = 4, $P < 0.025$).

Transmission category	Other	Lens
1. UV color vision highly likely	6	9
2. UV color vision likely	7	13
3. Violet color vision likely	9	24
4. Short wavelength sensitivity likely but no UV color vision	14	16
5. No short wavelength sensitivity	14	72

Thirty-eight of the 195 species were found in categories 1–2 in which UV vision is likely. Of the 26 families that had at least two species in our samples, 11 had at least one representative in categories 1–2 that are strong candidates for UV vision. For those 24 species for which we have both T50 and MSP data for single cones, there appears to be a strong correlation between the shortest wavelength absorption maximum for their single cone visual pigment and both T50 ($r^2 = 0.76$, $P < 0.01$, excluding the four nocturnal species) and vision category ($r^2 = 0.62$, $P < 0.01$; Fig. 7).

Discussion

Types of short-wavelength vision.—Visual systems, taken in the context of stimulation of the retina by short-wavelength radiation, can be of three general types. The simplest type lacks specialized UV receptors and merely fails to have UV blockers in the eye that function below 400 nm. At least some UV radiation would be absorbed by receptor cells that have their maximum sensitivity in the visible range but also have consid-

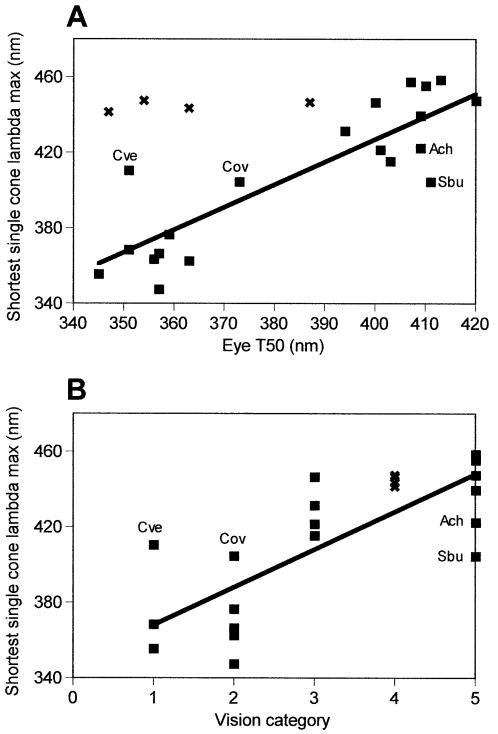

Fig. 7. Absorption maximum for the shortest wavelength visual pigment from single cone cells with regression lines as a function of the T50 for the ocular media (A) and the visual category to which the species was assigned (B). Squares are species from ecological categories 1 and 2 that are exposed to full daylight, and "x"s are nocturnal species that avoid full exposure to sunlight. Species that deviate from the norm are indicated as "Ach," *Aulostomus chinensis;* "Cov," *Chromis ovalis*; "Cve," *Chromis verater*; and "Sbu," *Sufflamen bursa*. The regression line in (A) excludes the nocturnal species.

erable short-wavelength sensitivity in a secondary absorption peak (beta peak) in the UV (Dartnall and Lythgoe, 1965). We will refer to this type of eye as "UV-sensitive." Fish with UV-sensitive eyes likely lack true UV color vision or hue discrimination because the beta peaks for different visual pigments are similarly placed and have far less sensitivity than the primary absorption peak or λ_{max}. Such eyes will act to gather as much light as possible but will result in a lowered color constancy (perception of a certain pigment color as the same regardless of changes in the incident illumination) that could be improved by filtering out the short-wavelength light (Dyer, 2001). These eyes may also indicate a lack of selection pressure to avoid the detrimental effects of UV exposure in habitats with little UV radiation (see below).

"UV-specialized" eyes must fail to block at least some of the UV spectrum and possess receptor cells that are maximally responsive to UV radiation. The visual pigments in these cells absorbed maximally at about 360 nm, similar to a variety of other vertebrates (reviews in Bowmaker, 1990; Losey et al., 1999; Locket, 1999). Such eyes might, but do not necessarily, have true color vision that recognizes a UV component as a different color. Alternatively, the UV-sensitive cone cells could serve separate perceptual functions such as prey detection. At least in the goldfish, UV-sensitive cone cells serve the function of true color vision and hue discrimination (Neumeyer, 1992).

"Violet-specialized" eyes must fail to block at least some radiation below 435 nm and possess receptor cells that are maximally sensitive to radiation from about 400—435 nm. Such eyes are similar to UV-sensitive eyes but should have increased sensitivity in deeper water where UV radiation is scarce compared with violet radiation and be subject to less damage because of harmful UV radiation. These eyes would also achieve some advantages of improved color constancy and focusing ability (Dyer, 2001; Muntz, 1976) and still retain some of the advantages of UV-specialized vision such as detecting short-wavelength opaque plankton against a bright scattering background.

Selection for and against short-wavelength vision.—Ultraviolet-sensitive visual pigments occur in primitive fish families and likely occurred in fishes ancestral to all present day species (Bowmaker, 1991). We must then ask why UV-specialized vision does not occur throughout the fishes. Selection against short-wavelength vision can result in both a lack of visual pigments with the primary absorption peak or λ_{max} at short wavelengths and blocking of short wavelengths by the ocular media.

The bimodal distribution of ocular media blocking wavelengths (T50s, Fig. 5) suggests that shallow, diurnal and active coral reef fishes, in general, have been selected to block somewhat more short-wavelength light than the remainder of the Hawaiian species studied. These families also have a lower than expected range of T50 values within each family (Fig. 3) even though many are ecologically diverse. This group of fishes does, however, include species with UV-specialized vision even though, for some, much of the short-wavelength radiation is absorbed prior to reaching the retina. Siebeck and Marshall (2001) found a similar bimodal distribution of T50 values for Australian reef fishes. This might be expected because both studies were conducted on tropical coral reefs

with similar environmental light conditions and presumably similar selection pressures. Hawai'i, however, has a comparatively depauperate fauna with 24.3% endemism and is marked by a lack of many tropical families and large shallow reef predators such as the groupers. Most of the families sampled in Hawai'i were included in Siebeck and Marshall (2001), but many families sampled in Australia are not found in Hawai'i. Similar selection pressures must be responsible for this agreement regardless of the phylogenetic differences.

There is, however, a clear phylogenetic trend toward increased blocking of short wavelength radiation (Fig. 4) as suggested by Douglas and McGuigan (1989) and Thorpe et al. (1993). The remaining variability, however, is high as noted by Dunlap et al. (1989) and must be variable because of selection pressures. Siebeck and Marshall (2001) found that UV-transmitting species from Australian reefs were restricted to the Acanthopterygii but noted that the Rajiformes are quite diverse, and UV transmission in the Rajidae had been reported by Thorpe and Douglas (1993). We can now extend the transmission of UV and probably possession of UV-sensitive vision to at least one species each of carcharhinid and sphyrnid sharks.

It is difficult, however, to find firm evidence for specific ecological effects in our data. Families with unusually low T50 values (Fig. 4) are of various ecological types: Ophidiidae and Caproidae include species that are sheltered within the structure of the reef. The Caracanthidae often sit on coral branches and are exposed to full sunlight. The Pomacentridae are active above the reef and are ecologically diverse, and members range from benthic herbivores to zooplanktivores. The Coryphaenidae, another distinct outlier with a high T50, is notable for their near-surface, UV-rich habitat.

Selection pressure on species must also vary within families. Of the five families in which at least four species were sampled by MSP (Holocentridae, Chaetodontidae, Pomacentridae, Acanthuridae), none had short-wavelength visual pigments present in all of the species.

More detailed understanding of the effects of phylogeny demands comparison at the species and age class level with detailed ecological information. For example, without considering details of their ecology, the relatively long wavelength visual pigments found in the pomacentrid planktivores, *Chromis verater* and *Chromis ovalis*, appear to be at odds with their placement in vision likelihood categories 1 and 2, respectively, that are expected to have UV-specialized vision (Fig. 7B). Other planktivorous pomacentrids (*Chromis hanui* and *Dascyllus albisella*) have shorter wavelength visual pigments as predicted by studies that show improved detection of zooplankton in the presence of UV light (Browman et al., 1994). *Chromis verator* and *C. ovalis* inhabit deeper reaches of coral reefs than do the other species (Gosline and Brock, 1965; Tinker, 1991). The reduction in available UV-light relative to the longer wavelengths with increased depth caused by filtering by the water would favor a visual pigment centered more toward the bluer or longer wavelength regions of the spectrum (McFarland, 1986; Loew and McFarland, 1990; Frank and Widder, 1996).

Can ocular media transmission categories predict visual pigment sensitivities?—One goal of this study is to determine whether we can place any faith in our ability to predict visual pigment sensitivity based on spectral transmission data and vision likelihood categories. Spectral sensitivity MSP studies are extremely demanding, whereas determination of T50 values can be accomplished with minimal instrumentation and in a short time period.

Our sample size for species with both ocular transmission and MSP data is small, but the results are encouraging. So long as we remove nocturnal species from consideration, the correlation with ocular media transmission is good (Fig. 7A). Our vision likelihood categories also have a good correlation with visual pigment sensitivity (Fig. 7B) that can be improved with admittedly post hoc consideration of additional ecological variables such as the habitat depth for *Chromis* spp.

For two other species that have visual pigments at unexpectedly short wavelengths, explanation escapes us. *Aulostomus chinensis* is a stalking predator that uses several guises to approach and remain close to prey and moves slowly about the reef. It feeds on fishes and shrimps and, although feeding during both day and night, appears to be most successful during twilight (Hobson, 1974). The presence of UV and/or violet absorbing visual pigments accompanied by blue-sensitive cones will increase the relative short-wavelength photosensitivity of each predator. Other predators, however, such as carangids lack short-wavelength sensitivity and it is doubtful that long-range detection of prey is of much value to any of these species. In clear water, predatory success is largely a function of detecting a defensive error by prey that are already at close range (Hobson, 1974; Landeau and Terborgh, 1986; Parrish, 1989). For *Sufflamen bursa*, a triggerfish that feeds on algae and various benthic invertebrates, we are simi-

larly at a loss to explain their short wavelength visual pigment.

Visual pigments: Rods.—The frequency distribution of the visual pigments from the 38 species emphasize the differences in spectral photosensitivity among rods, single and double cones (Fig. 1, also see Marshall et al., 2003b).

The wavelength of maximum sensitivity (λ_{max}) of the rods, which are centered tightly around 490 nm, agree for individual species with the earlier findings of Munz and McFarland (1973) with an average difference of only 2.8 nm between the methods. Rods in this region of the spectrum maximize photosensitivity during twilight and at night when the near-surface underwater spectrum is shifted further toward the blue-wavebands (for review, see Loew 1995). Inter- and/or intraspecific differences in rod pigment λ_{max} may be adaptations to behavioral pressures (e.g., predation) but are also constrained by the photic conditions (Munz and McFarland, 1973; Lythgoe, 1984; Loew, 1995). Differences of 5 to 10 nm, when related to the photic regime, can enhance photosensitivity and, therefore, visibility.

Do the λ_{max}-values actually represent ecologically meaningful differences in the spectral locations of the rod pigments for each species, or are they merely an expression of variance in the MSP technique? Two independent results suggest the former. First, dispersion of the rod λ_{max}-values are relatively small (mean SD = 3.1, n = 33) for all species except *Dascyllus albisella* and *Sufflamen bursa*. For these two species, the number of records was small (n = 3), but this was also true for many of the other species. Even though the dispersion was typical of what we often obtain with MSP for most vertebrate visual pigments (i.e., < 3–4 nm), the higher dispersion for the rod values for *D. albisella* and *S. bursa* and for the cone visual pigments of several species (see Table 1) cloud the issue. Second, the test of the MSP technique is to make multiple recordings from the same cell along its length. This is done as a control for all instrumental variables. In cases where there is no evidence of multiple pigments in the same cell, such controls show variation of no more than ± 1 nm.

Visual pigments: Cones.—Single cones that are typically involved in color vision were limited to short and blue wavelengths less than 500 nm. This agrees with prior findings that visual pigments in tropical marine fishes are of shorter wavelength than many of those found in freshwater species and agree with the available environmental light (Munz and McFarland, 1977; McFarland, 1986). Given our methods and sample size, both typical for MSP studies, if other visual pigments do exist they are in a very small number of cells, found only in other developmental stages or restricted to a very small retinal area that we, by chance, failed to sample. One probable exception is our failure to find any type of single cone cell in *Parupeneus multifasciatus*, that is as likely a sampling problem as a real absence of a short-wavelength cone cell. Our results for mullids generally agree with Shand (1993) except that she found no UV-sensitive cone cells.

Of the five families in which at least three species were sampled, none had short-wavelength visual pigments present in all of the species. UV and violet single cones were never found together but have been found to coexist in shiners (W. N. McFarland, unpubl. data) and in the atherinid, *Menidia menidia* (Novales-Flamarique and Harosi, 1999).

All 38 species possessed double and/or twin cones with the following three pairings of visual pigments: blue-green doubles (540–548 and 457–496 nm), identical twin cones (green 501–532 and blue 473 nm), and nonidentical green doubles (506–545 nm). It is doubtful that any of the cells identified as single cones could have been disassociated from a double cone. Our blue single cones looked different than the accessory members of the double cones and were certainly different than twin cone members.

Blue-green doubles were found in nine of the 16 families examined, and 20 of the 26 species within these families also had an UV-, violet- or blue-sensitive single cone, or combinations of a blue- and UV-sensitive single cones. Blue-green-sensitive doubles, in combination with short-wavelength-sensitive single cones, will enhance photosensitivity to the photic habitat around reefs (McFarland and Munz, 1975b; Marshall et al., 2003b).

Six of the 12 species that had identical twin cones lacked nonidentical blue-green doubles. Each species had either a blue-sensitive single cone, as in the squirrelfish, *Neoniphon sammara*, barracuda, *Sphyraena barracuda*, moorish idol, *Zanclus cornutus*, and surgeonfish, *Acanthurus triostegus*, or a violet-sensitive single cone as in the triggerfish, *Sufflamen bursa*. The significance of these visual pigment combinations is not clear, but as is the case for all species containing multiple cone spectral classes, the answer may lay in the relationship between visual pigment spectral location and hue discrimination (Marshall et al., 2003b).

Species with nonidentical green-sensitive dou-

ble cones, especially the surgeonfishes, will have enhanced photosensitivity in the green rather than the short-wave regions of the spectrum. Differences between the two green absorbing pigments averaged about 14 nm. Except for *Bodianus bilunulatus* and *Asterropteryx semipunctatus*, blue singles also were recorded from these species. The surgeonfishes we sampled are herbivores (Randall, 2002; Tinker, 1961; Jones, 1968), although some species are planktivorous (Hobson, 1974). These fishes could possess an increase in hue discrimination in the green region of the spectrum or merely a broader sensitivity to green light.

Anatomical location of short-wavelength blockers.—When determining the short wavelength absorption or T50 for a species, it is important to consider all of the components of the eye (Fig. 6) and not just the lens (Douglas and Thorpe, 1992) or even lens plus cornea (Douglas and McGuigan, 1989) as was common practice in earlier studies. The ''limiting filter'' (Siebeck and Marshall, 2000, 2001) of the eye is that component with the longest wavelength cutoff. ''Lens-Limited'' eyes are the simplest and most common type found and the lens determines practically all of their spectral transmission properties. Our finding that 73% of the samples have lens-limited eyes agrees well with Siebeck and Marshall's (2001) finding of 80% in Australian coral reef fishes. Lens-limited eyes also tend to have the steepest transmission curve cutoff slopes and longer wavelength T50 values.

''Corneal-limited'' eyes have a lens with a cutoff wavelength far shorter than the cutoff of the entire eye and additional filtration is provided in the cornea (review in Douglas and Marshall, 1999). Corneal limitation provides greater flexibility in the variation of T50 values on a seasonal or even daily basis (Kondrashev and Khodtsev, 1984; Siebeck and Marshall, 2000, 2001) without necessitating resorption of blockers from the lens. Corneal filters appear to be of two types. (1) Yellow and other colors, probably formed by carotenoid pigments, may be distributed unevenly around the cornea and may change on a daily cycle are especially common in the wrasses (Siebeck and Marshall, 2000) and block nearly all UV radiation. (2) Short-wavelength UV filters in an otherwise clear cornea may combine a shallow-sloped corneal transmission curve with a shallow-sloped lens transmission curve (*Dascyllus albisella*; Fig. 6) and reduce the amount of short-wavelength radiation that strikes the retina. These are also found throughout the species sampled and are common in fishes with UV-specialized vision such as some of the damselfish (Losey et al., 2000).

''Humor limited'' eyes are rare. Douglas and Marshall (1999) attributed the only two earlier reports of filtration by the aqueous or vitreous humor to post mortem artifacts. Siebeck and Marshall (2001) found two instances in which neither the lens nor the cornea could explain the high T50 and suggested that the humors could be responsible. Humoral limitation has now been found in repeated samples of two fishes, *Acanthocybium solandri* (Scombridae), the Wahoo or Ono (Nelson et al. 2001), and the priacanthid fish, *Heteropriacanthus cruentatus*. Both their lens and cornea are relatively transparent to longer-wavelength UV radiation, and the humors have a strong absorption maximum at approximately 395–410 nm and 375–380 nm, respectively, and a steep T50 at 400–418 nm and 373–396 nm, respectively (Appendix 1). Without the absorption of UV by the humors, much shorter-wavelength radiation would reach the retina. The chemical components of the short-wavelength filters of the eye pass through the aqueous humor of the eye and are taken up by the lens and then diffuse into the vitreous humor (Posner, 1998). It is unknown why the lenses of these two species fail to incorporate some of these UV-blocking components found in the humors.

General conclusions and future studies.—Examples of species with short-wavelength sensitivity are common, but not the majority, in tropical reef fish families. The spectral transmission properties of the eye are largely conserved within most families, but exceptions that currently fail to match predictions based on ecological or behavioral characteristics are not unusual. Students of fish behavior and ecology should take caution in interpreting the results of research that involves visual perception systems of species in families such as the Pomacentridae that are highly likely to have UV vision. Presentation of a ''model bottle'' (e.g., Myrberg and Thresher, 1974) or use of aquarium dividers made of glass or Plexiglas of most types will alter the UV component of coloration and must be avoided in species that have UV vision. At the very least, one must report the short-wavelength transmission properties of model bottles, aquarium dividers, etc., and the incident illumination of the aquarium room. Species whose eyes can be shown to be UV-sensitive as opposed to UV-specialized still pose a problem. For example, if portions of the coloration of a species differ in their UV reflectance (Marshall et al., 2003a),

these differences in brightness or luminance will be lost behind the UV-opaque barrier.

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APPENDIX 1. OCCULAR TRANSMISSION DATA FOR VARIOUS HAWAIIAN FISHES. Families are sorted by the average cutoff wavelength for the family. Given are the vision likelihood category, the limiting filter (lens, cornea, or humors), the classification for the slope of the cutoff for the entire eye, and the T50 wavelengths for the entire eye and the lens alone (nm).

Family	Genus	Species	Vision likelihood category	Limiting filter	Cutoff slope	T50-eye	T50-lens
Apogonidae	*Apogon*	*coccineus*	4	lens	2	353	357
Apogonidae	*Apogon*	*erythrinus*	4	?	?	?	347
Apogonidae	*Apogon*	*kallopterus*	4	cornea	2	347	339
Apogonidae	*Apogon*	*maculiferus*	4	cornea	2	352	343
Apogonidae	*Apogon*	*menesemus*	4	lens	2	338	339
Apogonidae	*Apogonichthys*	*perdix*	4	lens	3	349	344
Apogonidae	*Foa*	*brachygramma*	4	lens	2	352	359
Pomacentridae	*Abudefduf*	*abdominalis*	2	cornea	2	357	323
Pomacentridae	*Abudefduf*	*sordidus*	1	cornea	2	345	338
Pomacentridae	*Chromis*	*agilis*	1	lens	2	337	335
Pomacentridae	*Chromis*	*hanui*	1	cornea	2	345	322
Pomacentridae	*Chromis*	*ovalis*	2	lens	1	373	373
Pomacentridae	*Chromis*	*punctipinnis*	1	lens	2	347	351
Pomacentridae	*Chromis*	*vanderbilti*	1	?	?	?	342
Pomacentridae	*Chromis*	*verater*	1	lens	1	351	342
Pomacentridae	*Dascyllus*	*albisella*	2	cornea	2	359	350
Pomacentridae	*Plectroglyphidodon*	*imparipennis*	1	cornea	2	352	334
Pomacentridae	*Plectroglyphidodon*	*johnstonianus*	1	cornea	2	337	328
Pomacentridae	*Stegastes*	*fasciolatus*	2	cornea	4	356	347
Caproidae	*Antigonia*	sp.	5	lens	2	353	358
Ophidiidae	*Brotula*	*multibarbata*	4	cornea	2	353	327
Caracanthidae	*Caracanthus*	sp.	5	lens	1	355	355
Caracanthidae	*Caracanthus*	*typicus*	5	?	?	?	358
Mullidae	*Mulloidichthys*	*flavolineatus*	2	cornea	2	357	344
Mullidae	*Mulloidichthys*	*vanicolensis*	1	lens	4	349	347
Mullidae	*Parupeneus*	*bifasciatus*	1	lens	2	354	352
Mullidae	*Parupeneus*	*cyclostomus*	2	cornea	2	374	346
Mullidae	*Parupeneus*	*multifasciatus*	1	?	2	342	?
Mullidae	*Parupeneus*	*porphyreus*	2	cornea	2	372	349
Kuhliidae	*Kuhlia*	*sandvicensis*	2	cornea	2	363	350
Synodontidae	*Saurida*	*flamma*	1	lens	1	349	349
Synodontidae	*Saurida*	*gracilis*	1	cornea	1	355	344
Synodontidae	*Saurida*	*nebulosa*	1	lens	2	348	347
Synodontidae	*Synodus*	*ulae*	5	lens	1	412	411
Synodontidae	*Synodus*	*variegatus*	1	cornea	1	351	344
Holocentridae	*Myripristis*	*amaena*	4	cornea	2	366	351
Holocentridae	*Myripristis*	*berndti*	4	cornea	2	363	353
Holocentridae	*Myripristis*	*vittata*	4	cornea	4	386	359
Holocentridae	*Neoniphon*	*sammara*	4	cornea	3	387	352
Holocentridae	*Plectrypops*	*lima*	4	lens	2	340	341
Holocentridae	*Sargocentron*	*diadema*	4	lens	2	338	345
Holocentridae	*Sargocentron*	*punctatissimum*	4	lens	2	341	346
Holocentridae	*Sargocentron*	*spiniferum*	4	cornea	2	387	374
Holocentridae	*Sargocentron*	*tiere*	4	cornea	2	384	369
Holocentridae	*Sargocentron*	*xantherythrum*	4	cornea	4	354	340
Hemiramphidae	*Hyporhamphus*	*acutus*	2	lens	1	366	363
Carcharhinidae	*Carcharhinus*	*amblyrhynchos*	3	lens	2	400	393
Carcharhinidae	*Carcharhinus*	*plumbeus*	1	lens	2	342	340
Albulidae	*Albula*	*glossodonta*	2	lens	2	374	353
Polynemidae	*Polydactylus*	*sexfilis*	2	lens	3	374	376
Congridae	*Conger*	*oligoporous*	4	cornea	2	376	352
Scorpaenidae	*Dendrochirus*	*barberi*	4	lens	1	400	397

Appendix 1. Continued.

Family	Genus	Species	Vision likelihood category	Limiting filter	Cutoff slope	T50-eye	T50-lens
Scorpaenidae	*Pontinus*	*macrocephalus*	4	lens	2	382	394
Scorpaenidae	*Scorpaenodes*	*kelloggi*	4	cornea	1	365	357
Scorpaenidae	*Scorpaenodes*	*parvipinnis*	4	lens	1	356	352
Scorpaenidae	*Scorpaenopsis*	*brevifrons*	4	lens	1	404	404
Scorpaenidae	*Scorpaenopsis*	*cacopsis*	4	lens	1	403	403
Scorpaenidae	*Sebastapistes*	*coniorta*	4	lens	1	359	356
Moridae	?	?	5	cornea	2	383	358
Muraenidae	?? young		4	lens	1	389	387
Muraenidae	*Gymnothorax*	*eurostus*	5	lens	1	407	404
Muraenidae	*Gymnothorax*	*flavimarginatus*	4	?	?	?	356
Muraenidae	*Gymnothorax*	*nuttingi*	4	?	2	369	?
Muraenidae	*Gymnothorax*	*undulates*	4	cornea	4	387	341
Muraenidae	*Gymnothorax*	*ypsilon*	4	lens	2	366	373
Gobiidae	*Priolepis*	sp.	3	?	?	?	384
Clupeidae	*Herklotsichthys*	*quadrimaculatus*	3	?	?	?	385
Sphyrnidae	*Sphyrna*	*lewini*	3	cornea	2	385	339
Lutjanidae	*Aphareus*	*rutilans*	3	lens	1	403	399
Lutjanidae	*Aprion*	*virescens*	5	lens	1	409	407
Lutjanidae	*Etelis*	*carbunculus*	2	lens	2	376	374
Lutjanidae	*Lutjanus*	*kasmira*	2	lens	2	357	358
Lutjanidae	*Pristipomoides*	*filamentosus*	3	lens	1	399	399
Lutjanidae	*Pristipomoides*	*sieboldii*	2	lens	1	364	367
Lutjanidae	*Pristipomoides*	*zonatus*	3	cornea	2	394	375
Priacanthidae	*Heteropriacanthus*	*cruentatus*	4	humors	4	383	357
Priacanthidae	*Priacanthus*	*arenatus*	4	lens	3	401	402
Priacanthidae	*Priacanthus*	*meeki*	4	lens	4	392	391
Chaetodontidae	*Chaetodon*	*auriga*	5	lens	1	415	413
Chaetodontidae	*Chaetodon*	*citrinellus*	3	lens	1	400	397
Chaetodontidae	*Chaetodon*	*ephippium*	5	lens	1	413	412
Chaetodontidae	*Chaetodon*	*fremblii*	3	lens	1	389	385
Chaetodontidae	*Chaetodon*	*kleinii*	2	lens	1	373	372
Chaetodontidae	*Chaetodon*	*lunula*	5	lens	1	407	408
Chaetodontidae	*Chaetodon*	*miliaris*	3	cornea	1	387	374
Chaetodontidae	*Chaetodon*	*multicinctus*	3	cornea	1	383	374
Chaetodontidae	*Chaetodon*	*ornatissimus*	3	lens	1	398	396
Chaetodontidae	*Chaetodon*	*quadrimaculatus*	3	lens	1	384	380
Chaetodontidae	*Chaetodon*	*tinkeri*	2	lens	1	378	376
Chaetodontidae	*Chaetodon*	*trifascialis*	5	lens	1	406	404
Chaetodontidae	*Chaetodon*	*unimaculatus*	3	lens	1	384	377
Chaetodontidae	*Forcipiger*	*flavissimus*	3	cornea	1	394	383
Chaetodontidae	*Hemitaurichthys*	*polylepis*	3	cornea	3	390	371
Chaetodontidae	*Hemitaurichthys*	*thompsoni*	3	lens	1	390	381
Chaetodontidae	*Heniochus*	*diphreutes*	3	lens	2	387	373
Scombridae	*Acanthocybium*	*solandri*	5	humors	1	420	401
Scombridae	*Euthynnus*	*affinis*	3	cornea	4	401	349
Scombridae	*Katsuwonus*	*pelamis*	1	lens	1	346	344
Scombridae	*Thunnus*	*albacares*	5	lens	1	415	413
Mugilidae	*Mugil*	*cephalus*	3	lens	1	396	397
Serranidae	*Cephalopholis*	*argus*	5	lens	1	412	412
Serranidae	*Epinephelus*	*quernus*	3	lens	1	405	403
Serranidae	*Pseudanthias*	*bicolor*	5	lens	1	416	417
Serranidae	*Pseudanthias*	*thompsoni*	5	lens	1	408	405
Serranide	*Pseudogramma*	*polyacanthum*	2	lens	1	363	362
Dactylopteridae	*Dactyloptena*	*orientalis*	3	lens	1	402	400
Pomacanthidae	*Centropyge*	*flavissimus*	5	lens	1	410	412

Appendix 1. Continued.

Family	Genus	Species	Vision likelihood category	Limiting filter	Cutoff slope	T50-eye	T50-lens
Pomacanthidae	*Centropyge*	*loriculus*	3	lens	1	394	393
Pomacanthidae	*Centropyge*	*potteri*	3	lens	2	404	402
Pomacanthidae	*Holacanthus*	*arcuatus*	3	lens	1	402	403
Ostraciidae	*Ostracion*	*meleagris*	3	?	1	405	?
Squalidae	*Squalus*	*mitsukurii*	5	lens	1	405	408
Kyphosidae	*Kyphosus*	*bigibbus*	5	lens	1	406	405
Monacanthidae	*Aluterus*	*scriptus*	5	cornea	1	419	409
Monacanthidae	*Cantherhines*	*dumerili*	5	lens	1	421	417
Monacanthidae	*Cantherhines*	*sandwichiensis*	5	lens	1	414	409
Monacanthidae	*Cantherhines*	*verecundus*	5	cornea	3	417	397
Monacanthidae	*Pervagor*	*aspricaudus*	5	cornea	1	413	382
Monacanthidae	*Pervagor*	*spilosoma*	5	cornea	1	413	397
Cirrhitidae	*Amblycirrhitus*	*bimacula*	5	lens	1	419	418
Cirrhitidae	*Cirrhitops*	*fasciatus*	2	lens	1	377	373
Cirrhitidae	*Cirrhitus*	*pinnulatus*	5	lens	1	409	408
Cirrhitidae	*Oxycirrhites*	*typus*	3	lens	1	402	402
Cirrhitidae	*Paracirrhites*	*arcatus*	5	lens	1	420	417
Cirrhitidae	*Paracirrhites*	*forsteri*	5	lens	1	419	421
Acanthuridae	*Acanthurus*	*achilles*	5	lens	1	412	412
Acanthuridae	*Acanthurus*	*blochii*	5	lens	1	411	409
Acanthuridae	*Acanthurus*	*dussumieri*	5	lens	1	408	405
Acanthuridae	*Acanthurus*	*nigrofuscus*	5	lens	1	411	410
Acanthuridae	*Acanthurus*	*nigroris*	5	lens	1	409	408
Acanthuridae	*Acanthurus*	*thompsoni*	3	lens	1	401	400
Acanthuridae	*Acanthurus*	*triostegus*	5	lens	1	413	411
Acanthuridae	*Ctenochaetus*	*strigosus*	5	lens	3	407	406
Acanthuridae	*Naso*	*caesius*	3	cornea	1	396	384
Acanthuridae	*Naso*	*hexacanthus*	5	cornea	3	430	377
Acanthuridae	*Naso*	*lituratus*	3	lens	1	401	400
Acanthuridae	*Naso*	*unicornis*	3	lens	1	403	404
Acanthuridae	*Zebrasoma*	*flavescens*	3	lens	1	400	400
Antennariidae	*Histrio*	*histrio*	5	lens	1	408	406
Tetraodontidae	*Arothron*	*hispidus*	5	lens	3	408	408
Tetraodontidae	*Arothron*	*meleagris*	5	lens	1	411	410
Tetraodontidae	*Canthigaster*	*coronata*	3	lens	3	399	398
Tetraodontidae	*Canthigaster*	*jactator*	5	lens	3	414	411
Aulostomadae	*Aulostomus*	*chinensis*	5	lens	1	409	409
Carangidae	*Alectis*	*ciliaris*	5	lens	1	408	412
Carangidae	*Carangoides*	*orthogrammus*	5	lens	1	411	418
Carangidae	*Caranx*	*ignobilis*	5	lens	1	407	406
Carangidae	*Caranx*	*melampygus*	3	?	?	?	404
Carangidae	*Gnathanodon*	*speciosus*	5	lens	1	408	406
Carangidae	*Scomberoides*	*lysan*	5	lens	1	407	404
Carangidae	*Selar*	*crumenophthalmus*	5	lens	1	422	419
Carangidae	*Seriola*	*dumerili*	5	lens	1	406	404
Fistulariidae	*Fistularia*	*commersonii*	5	lens	1	410	411
Sphyranenidae	*Sphyraena*	*barracuda*	5	lens	2	410	408
Balistidae	*Canthidermis*	*maculatus*	1	?	?	?	347
Balistidae	*Melichthys*	*niger*	5	lens	1	416	410
Balistidae	*Melichthys*	*vidua*	5	cornea	1	416	409
Balistidae	*Rhinecanthus*	*aculeatus*	5	cornea	3	431	402
Balistidae	*Sufflamen*	*bursa*	5	lens	1	411	408
Bothidae	*Bothus*	*mancus*	5	lens	1	419	414
Bothidae	*Bothus*	*pantherinus*	5	lens	1	420	419
Zanclidae	*Zanclus*	*cornutus*	5	lens	2	420	422

Appendix 1. Continued.

Family	Genus	Species	Vision likelihood category	Limiting filter	Cutoff slope	T50-eye	T50-lens
Labridae	*Anampses*	*cuvier*	5	lens	3	435	430
Labridae	*Bodianus*	*bilunulatus*	3	cornea	3	394	378
Labridae	*Cheilio*	*inermis*	5	lens	3	426	422
Labridae	*Coris*	*flavovittata*	5	lens	3	430	427
Labridae	*Coris*	*venusta*	5	lens	3	425	424
Labridae	*Cymolutes*	*lecluse*	5	lens	1	421	419
Labridae	*Epibulus*	*insidiator*	5	lens	3	430	426
Labridae	*Gomphosus*	*varius*	5	lens	3	455	425
Labridae	*Halichoeres*	*ornatissimus*	5	lens	3	407	406
Labridae	*Labroides*	*phthirophagus*	5	lens	1	421	419
Labridae	*Macropharyngodon*	*geoffroy*	5	cornea	3	439	423
Labridae	*Oxycheilinus*	*unifasciatus*	5	lens	1	438	431
Labridae	*Pseudocheilinus*	*octotaenia*	2	lens	3	378	374
Labridae	*Pseudocheilinus*	*tetrataenia*	2	lens	3	367	368
Labridae	*Pseudojuloides*	*cerasinus*	5	lens	3	421	419
Labridae	*Stethojulis*	*balteata*	5	lens	3	425	420
labridae	*Thalassoma*	*ballieui*	5	lens	1	427	427
labridae	*Thalassoma*	*duperrey*	5	lens	1	429	427
Labridae	*Xyrichthys*	*umbrilatus*	5	lens	3	420	421
Blenniidae	*Cirripectes*	*vanderbilti*	5	lens	1	418	416
Blenniidae	*Exallias*	*brevis*	5	lens	1	422	418
Blenniidae	*Istiblennius*	*zebra*	5	?	?	?	409
Blenniidae	*Omobranchus*	*rotundiceps*	5	cornea	3	426	420
Pinguipedidae	*Parapercis*	*schauinslandii*	5	lens	1	423	424
Scaridae	*Calotomus*	*carolinus*	5	lens	1	422	412
Scaridae	*Chlorurus*	*perspicillatus*	5	lens	1	429	428
Scaridae	*Chlorurus*	*sordidus*	5	lens	2	426	426
Scaridae	*Scarus*	*dubius*	5	lens	1	429	422
Istiophoridae	*Makaira*	*mazara*	5	cornea	1	441	418
Istiophoridae	*Tetrapturus*	*audax*	5	lens	1	416	416
Istiophoridae	*Tetrapturus*	*angustirostris*	5	?	1	?	414
Coryphaenidae	*Coryphaena*	*hippurus*	5	lens	1	443	441