

**Visual Communication between the Hawaiian Cleaner Wrasse  
(*Labroides phthirophagus*) and Host Fish in Kaneohe Bay Hawaii**

by


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We accept this thesis as conforming  
to the required standard




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## ABSTRACT

The goal of this study was to identify the visual cues which attract host fish to the Hawaiian cleaner wrasse (*Labroides phthirophagus*) in order to understand how visual communication mediates behaviour between Hawaiian coral reef fishes. The central idea was that the cleaner wrasse has one or more optical signals which maximize its conspicuousness to other reef fish. Specifically, it was hypothesized that the colour pattern of the cleaner wrasse provides high contrast and that the swimming motion, known as the dance, is a distinctive behaviour. Since the visibility of optical signals not only depends on properties of the target but also photic conditions of the habitat and the sensory system of the recipient host fish, these factors were also studied.

To characterize the photic conditions, an underwater spectroradiometer was used to measure spectral and transmission properties of the water at three study sites in Kaneohe Bay, and reflectance properties of coral substrates (300-850nm). Spectral reflectance was measured from the colour patches of the fish and the amounts of luminance contrast (LC) and spectral contrast (SC) were calculated :1) between the colour patches within the pattern, 2) between the colour pattern and backwelling light and 3) between the colour pattern and coral background. The contrast provided by *L. phthirophagus* was compared to that of three other reef fish (*Chaetodon auriga*, *Thalassoma duperrey*, *Zebrasoma flavescens*). The dance behaviour was evaluated as a potential optical signal by analyzing the movements which were recorded with an underwater video camera. Finally, to examine the visual ability of host fish, spectral sensitivity of *T. duperrey* was determined with electrophysiological recordings from the optic nerve.


The two inshore sites had mean diffuse attenuation coefficients ( $K_D$ ) of 0.89 and 0.41, while the offshore site was comparatively clear ( $K_D=0.12$ ). The wavelength at which half of the photons occurred,  $\lambda_{p50}$ , for downwelling light at the 5m and 15m site were 542nm and 524nm respectively, corresponding to the green region of the spectrum. The dominant wavelength at the offshore site occurred in the blue region of the spectrum ( $\lambda_{p50} = 485\text{nm}$  for downwelling light). For coral substrates, about 8 - 20% of


downwelling incident light was reflected and the dominant wavelength occurred in the green part of the spectrum ( $\lambda_{p50} = 543 \text{ nm}$ ). Several factors were found to affect the underwater photic regime including direction, sky conditions and time of day.


The colour pattern of *L. phthiophagus* showed significantly greater inherent LC than the other fish, while *C. auriga* had greatest inherent SC. Against water and coral backgrounds, the amount of luminance and spectral contrast provided by the fish did not differ significantly between species. With respect to the dance, it was observed less frequently than expected in adult cleaner wrasses, however the body movements were distinctive and very stereotyped.


*T. duperrey* was found to possess at least two cone mechanisms. The ON response had a short wavelength sensitivity peak ( $\lambda_{\text{max}} = 460\text{nm}$ ) and a medium wavelength mechanism ( $\lambda_{\text{max}} = 555\text{nm}$ ). The OFF response lacked a short wavelength mechanism, but a medium wavelength mechanism ( $\lambda_{\text{max}} = 555\text{nm}$ ) was present. When the contrast provided by the colour pattern of *L. phthiophagus* was related to visual abilities of *T. duperrey* (by restricting the wavelength specification of the contrast calculations to correspond with the sensitivity range of *T. duperrey*), there was sufficient LC and SC to be visually detectable by this host. In conclusion, the role of the cleaner's dance as an optical signal is unclear, however the conspicuousness of *L. phthiophagus* due to luminance contrast may be an important optical signal for hosts.

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## LIST OF ABBREVIATIONS

CAP: compound action potential

DW: downwelling

HZ: horizontal

LC: luminance contrast

RGC: retinal ganglion cell

SC: spectral contrast

UP: upwelling

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## **Chapter 1. General Introduction and Background Information**

### **BIOLOGICAL COMMUNICATION**

Communication has been defined as a process whereby information is exchanged to the mutual adaptive advantage to both participants (Klopfer and Hatch, 1968). One problem with this definition is that in some cases it is possible that one party may not accrue an advantage from the signal of the other organism. Wilson (1975) defined communication as occurring when one organism alters the behavioural probability pattern of another in a manner which is adaptive to one or both of the participants. This type of broad definition includes almost every type of animal interaction, including some which may not be communicative in nature, such as predator-prey interactions. Although the preceding definitions are not incorrect, it is more useful to adopt a definition which considers the evolutionary basis of signaling systems and receptors. Therefore, biological communication occurs when an exchange of information between organisms takes place through specific signals and receptors which are favored by natural selection.

There are three fundamental components to all communication systems, namely the sender of the signal, the receiver of the signal and the signal itself (Marler, 1973). Since signals, their associated behaviours and the receptors which process sensory input are influenced by selective pressures, communication systems must be adapted to a particular function and environment.

Communicative signals can be considered to have two distinct design components. The first component is the strategy of the signal. This includes the ultimate purpose for the signal, i.e. how it increases or maintains the fitness of the sender by causing the receiver to respond to that signal. Secondly, there is the efficacy component of the signal, which refers to the probability that the signal will reach the target receiver and elicit the appropriate response (Guilford and Dawkins, 1991). Within the efficacy design component of the signal, there are three equally important stages upon which selective forces act. These include signal emission, signal transmission and signal detection. The signal should be emitted at the places, times and environmental conditions which maximize efficient communication. As well, the structure of the signal should be

such that the effects of background noise and signal distortion are minimized during transmission. Finally, the signal should evolve so as to overcome biophysical, biochemical and energetic limits of the receiver's sensory system (Endler, 1993).

Several different modes of communication exist, including chemical, auditory, tactile and visual to name a few. Visual communication typically takes place through passive and active optical signaling. For most visual signals, the variables which may be incorporated into the optical signal are intensity or brightness, wavelength or colour, degree and plane of polarization, spatial pattern and temporal frequency. Since the structure of a visual signal depends on efficient emission, transmission and detection, the main factors affecting the efficacy of visual signals are the photic conditions of the habitat and the visual capabilities of the receiver.

For diurnally active fish, many inter and intraspecific communicative behaviours have been shown to be dependent on visual stimuli. For example, visual communication mediates several courtship behaviours and reproduction. As a result of sexual selection, male fish often possess certain physical characteristics which females visually evaluate in order to choose mates (Bischoff et al., 1985; Kodric-Brown, 1985; Long and Houde, 1989; Endler, 1991). Similarly, males respond to visual stimuli which are typical of reproductive females, such as a swollen abdomen, or specific coloration and posture (Tinbergen, 1951). Visual communication is also important to schooling fish. Specific morphological characteristics, such as colored spots, are used by some schooling fish to maintain visual contact, thereby ensuring school integrity (Keenlyside, 1955; Denton and Rowe, 1994). Furthermore, disruption of normal visual processes has been shown to impair group cohesion since blinded fish cease to school (Noble and Curtis, 1939; Keenlyside, 1955; Moller et al., 1982). Aggression between conspecific males during breeding periods is often triggered by specific visual stimuli, such as colour and posture (Tinbergen, 1951, Rasa, 1968; Leong, 1969; Keenlyside, 1971; Heiligenberg et al., 1972; Stacey and Chizar, 1977). Finally foraging behaviour can be mediated by visual communication in some fish. For instance, fish which view typical feeding behaviours by other individuals will initiate feeding behaviour even if food is absent (Protasov, 1968).

Underwater environments are extremely complex and variable, yet fish are present, if not abundant, in most types of natural waters. Obviously, visual communication systems of fish have evolved so as to optimize function under particular environmental conditions. Visual communication has long been considered to be important for fishes which inhabit tropical coral reefs (Longley, 1917; Munz and McFarland, 1977; Lythgoe, 1979; McFarland, 1991). To fully understand the importance of vision in communication between coral reef fishes, several factors must be considered. These include the optical signal of the sender, the optical properties of the underwater reef habitat and the visual sensitivity of the receiving fish.

#### THE PHOTIC ENVIRONMENT

The optical conditions of underwater environments are more restrictive to vision than conditions in terrestrial habitats. Both air and water scatter, absorb and refract light, however, these effects are considerably greater in water (Lythgoe, 1988). Consequently, light undergoes profound changes in intensity and spectral quality as it penetrates water. The resultant underwater photic conditions provide the medium through which visual signals are transmitted as well as the background against which the signals are viewed. Thus, the optical properties of the underwater environment set broad operational limits on animals for efficient generation and reception of visual signals.

The amount of light which penetrates a body of water depends on atmospheric conditions and time of day. Part of the light which reaches the water's surface arrives directly from the sun while the remainder, referred to as diffuse sky light, has a complex angular distribution, resulting from scattering by atmospheric particles (Sathyendranth and Platt, 1990). Under clear sky conditions, the amount of atmospheric scattering is low, so light transmission is determined mainly by the zenith angle of the sun, which is a function of latitude, date and local time. When small loosely distributed clouds are present, the amount of light scattered in the direction of the water may increase slightly, however, thick clouds act to reduce the surface flux (Sathyendranth and Platt, 1990).

The light which penetrates the water's surface is modified by refraction and reflection at the air-water interface. The amount of reflection typically remains quite low,

approximately 2%, for angles of incidence up to  $45^\circ$  (Sathyendranth and Platt, 1990) which is referred to as Snell's window. Internal reflection increases steadily with greater angles of incidence until total internal reflection occurs and the underside of the water surface acts as a mirror. The effect of refraction is to change the angular distribution of light underwater. When the water's surface is flat, refraction limits the light to a cone of  $48^\circ$ . The extent of both reflection and refraction is a function of surface roughness.

In general, the amount of downwelling irradiance, i.e. the light from the direction of the sun, decreases as depth increases. The rate of decrease of downwelling irradiance in water can be determined with the attenuation coefficient ( $K_D$ ), which describes the loss of light with increasing depth due to absorption and scattering. Scattering refers to any process where the direction of photons is altered. Absorption on the other hand includes all of the thermodynamically irreversible processes by which the nature of photon energy is changed (Duntley, 1963). In addition to an overall decrease in the amount of light, the spectral bandwidth narrows and shifts as depth increases, the extent and direction of which depends on the nature of the water. This has led several scientists to develop classification schemes for natural waters based on their optical properties (see Jerlov, 1976; Baker and Smith, 1982)

The colour of ambient light underwater is due to four major components of water; two of these are an inherent property of pure water and the others are of biological origin. Firstly, scattering of short wavelength light occurs in the upper depths. Two types of scatter are commonly recognized. Rayleigh scatter, which is proportional to  $1/\lambda^4$ , occurs due to the water molecules themselves (Jerlov, 1976). An important property of this type of scatter is that forward scatter equals backward scatter; it is symmetrical about the plane of incidence. The second type of scatter is Mie scatter which is caused by small particles suspended in the water. In this case, the scattering is strongly asymmetrical in that the degree of forward scatter is much greater than the amount of backward scatter.

Another factor which contributes to the colour of natural waters is related to the characteristic transmittance and absorption bands of pure distilled water. Pure water is most transparent in the blue region of the visible spectrum (460 - 475nm), a property

which is unaffected by dissolved salts (Morel, 1974). Absorption increases rapidly as wavelength increases, beginning at 575nm for pure water.

The other components to give colour to water are biotic in nature. The amount of chlorophyll in water, which is directly related to the abundance of phytoplankton, influences water colour since it has maximum transmission at 650-750 nm. Finally, yellow substance, often referred to as Gelbstoffe, is the fourth component which adds colour to water. It is composed of humus-like substances associated with organic decay and it causes absorption of short wavelength light. The net effect of these factors is that each body of water has specific optical characteristics which result from a particular combination of these four components.

In most underwater situations, vision is not limited by the reduction in light, but rather the effects of scatter. As image-forming radiance travels from the target towards the observer, photons are scattered out of the path which reduces brightness. At the same time, multiple scatter in the path reduces image forming light. Finally, non-image forming "veiling" light is scattered into the direction of view (Lythgoe, 1979; Partridge, 1990) and the contrast of the image is degraded.

Contrast, which is defined as the proportional difference between target radiance and background radiance (Lythgoe, 1979), is critical to target detection underwater. An object is visible only when the contrast between it and the background is large enough to be detected. For example, as the distance between target and viewer increases, the background radiance remains constant but the effects of veiling light increase (Lythgoe and Partridge, 1991). The radiance from the target becomes increasingly similar to that of the background spacelight until the two radiances are too similar to be distinguished. At this point, the photocontrast between target and background is too low to be detected and the target is invisible.

Thus, there are various optical factors within the habitat which can influence visual communication between aquatic organisms. Indeed, these environmental factors exert selective pressures on the animals which inhabit the underwater world and drive the evolution of appropriate mechanisms for efficient visual communication.

## CLEANING SYMBIOSIS

Cleaning symbiosis involves interspecific associations whereby one organism, the cleaner, removes ectoparasites, fungi, dead tissue and other particles from a host individual (Limbaugh, 1961; Feder, 1966). Many types of animals are known to act as cleaners but the most common include birds, shrimp and fish. In coral reef habitats, there is a wide variety of cleaning organisms and there has been a great deal of speculation as to their role on the reef. At present, the ecological importance of cleaning to the overall health of reef animals remains unclear. Initially, it appeared that removal of all cleaning organisms caused a decrease in the number of fish and an increase in infection of the remaining fish (Limbaugh, 1961). Although there were several problems with the experimental design of this work, it is often cited (Feder, 1966) as evidence for the importance of cleaners to reef ecosystems. Two subsequent studies (Youngbluth, 1968; Losey, 1972a) cast some doubt on this idea. These removal experiments showed that when the number of cleaners was reduced, the remaining cleaners spent more time cleaning, however this did not appear to be caused by an increase in infection. In addition, there is some evidence which suggests that cleaning may increase the rate of wound healing in host fish (Foster, 1985).

Past research indicates that a complex series of communicative interactions occurs during cleaning encounters between coral reef fish (Youngbluth, 1968; Losey, 1971; Potts, 1973; Lemaire and Maigret, 1987). There are three phases involved in cleaning, the approach phase, the contact phase and the parting phase (Potts, 1973). Each phase is characterized by specific behaviours between the cleaner and host, however these behaviours do not always occur in the same order and not all are present in each cleaning interaction.

The approach phase can be initiated by either the cleaner or the host. During the approach phase, the cleaner may closely pursue the host as it swims by. Alternatively, the host may adopt a pose position near the cleaner, which is characterized by rigid body posture and lack of fin movements. In some cases, the host may orient its body at an irregular angle relative to the substrate, such as head-down or head-up, or undergo a colour change. Cleaning activity occurs during the contact phase when the cleaner

inspects the host by swimming close to it, orienting itself alongside the host and touching its body with its fins. Often the host may open its mouth or flare its opercula to allow the cleaner to inspect and clean these areas (Losey, 1972b). At this point, the cleaner picks at the body surface of the host fish with its mouth. The last phase is the parting phase where either the cleaner leaves the body of the host or the host fish swims away.

Previous research has confirmed that these behaviours involve communication. For example, inspecting by the cleaner has been shown to increase the duration of posing by the host. Along the same lines, posing serves as a cleaning invitation signal since its occurrence alters the probability that the cleaner will inspect and clean the host (Losey, 1971). In some host species, the pose posture is consistent, while in others it is highly variable. Nevertheless, an increase in the amount of posing was found to be the behavior which correlated most strongly with an increase in inspection by cleaners (Losey, 1971).

At any point in the cleaning sequence, the cleaner may swim in a distinctive fashion, known as the dance (Feder, 1966; Youngbluth, 1968). This is characterized by a dorso-lateral oscillation of the body, with slow jerking of the caudal area while the caudal fin is flared. The rate of sculling by the pectoral fins is reduced so forward movement is slow (Youngbluth, 1968). This dance has been suggested to act as a means of rendering the cleaner more conspicuous or as a cleaner recognition signal. As well, it may serve as an appeasement behaviour towards aggressive hosts or conspecific cleaners (Losey, 1971).

Cleaning symbiosis is traditionally thought to be mutualistic since the cleaner gains food while the host is relieved of unwanted and potentially harmful material (Feder, 1966; Poulin, 1993). Consequently, there are several evolutionary implications to the communication which occurs as part of the cleaning relationship. The cleaner wrasse would be at a selective advantage to be able to attract many host fish in order to maximize food intake. Similarly, host fish species would benefit from recognizing and visiting cleaners frequently so they could reduce ectoparasitic and fungal infections. Previous research indicates that cleaners occasionally ingest healthy host tissue, such as gill tissue and fin membranes (Losey, 1972a), which is an obvious disadvantage for the host fish. In fact, hosts will leave the cleaner fish immediately after a "painful" bite has occurred

(Losey, 1971). Since the cleaner fish acts as a parasite in some cases, it must be able to attract as many hosts as possible so as to minimize negative conditioning in hosts. As long as the cleaner's activity is relatively advantageous to both the cleaner and the host, the relationship will persist. Indeed, cleaners and hosts respond to communicative signals, such as inspecting and posing, in a manner which not only initiates the association but acts to maintain it. Furthermore, both parties generate signals which are specific to cleaning symbiosis, i.e. inspecting, posing and dancing.

There are two species of cleaner wrasses in the Indo-Pacific Ocean. *Labroides dimidiatus* is the most common species of tropical cleaner fish but *Labroides phthirophagus*, which is endemic to Hawaii, was studied in this research. The Hawaiian cleaner wrasse is the most specialized, obligate cleaner inhabiting Hawaiian reefs (Randall, 1958; Youngbluth 1968). This means that it depends almost exclusively on cleaning for food. Both species of Indo-Pacific cleaner wrasses have a dark blue/black horizontal stripe along the length of the body with lighter portions above and below the stripe. The distinguishing features of the Hawaiian cleaner include a yellow head and pink on the tail. (Feder, 1966; Youngbluth, 1968). This distinctive striped pattern is present on many types of cleaner fish (*L. dimidiatus*, *L. bicolor*, *L. pectoralis*, *L. rubrolabiatus*) and it may serve as a guild sign indicative of cleaners (Eible-Eibesfeldt, 1955).

Many types of hosts visit cleaner wrasses, from small planktivorous species to those which are piscivorous. Usually visual communication involves a trade-off between maximizing information transfer and minimizing visibility to predators (Marler, 1973). However, in the case of the cleaner wrasse, predation does not seem to be a major factor influencing the communication system. There are two lines of evidence which support this idea. Firstly, there are very few accounts of a cleaner being eaten by a host in the wild (Lobel, 1976; Herald, 1964). Secondly, a blenny exists which mimics *Labroides dimidiatus* in coloration and swimming motion (Wickler, 1968). This fish (*Aspidontus taeniatus*) has large teeth and bites host fish which solicit cleaning. Presumably, the mimic gains some immunity from predation by resembling a cleaner wrasse.

Reduced predation of cleaner wrasses is thought to be due to two factors or a combination thereof. The cleaner may be able to recognize certain hosts as potential predators and cleaning activity is avoided or perhaps predatory fish simply do not regard the cleaner as a potential food item (Hobson, 1971). If the latter case is true, then perhaps cleaners are not at risk because they are able to appease predatory fish in some way. That is, the cleaner is able to reduce host aggression during the approach or contact phases.

Obligate cleaner fish often occupy a discrete spatial area on the reef known as the cleaning station where the majority of cleaning interactions take place (Feder, 1966). These stations are stable over time in that the cleaning individuals may change, but the physical location of the station remains the same (Youngbluth, 1968). In fact, hosts have been known to pose at stations even if the cleaner is not present (Losey, 1971; Potts, 1973) which indicates that hosts learn the location of cleaning stations. However, hosts tend to approach the station more frequently when a cleaner is present (Potts, 1973), suggesting that it is the cleaner itself which provides a positive stimulus to attract hosts.

Cleaning symbiosis provides a model system to study the importance of vision in communicative behaviour among coral reef fish. The signal which initially attracts the host fish to the cleaner is unknown. One hypothesis states that the proximate mechanism which attracts host fish to cleaners is the tactile stimulation provided by the cleaner (Losey, 1987). Although there is some evidence that contact between the cleaner and host is important for host response (Lemaire and Maigret, 1987), this does not account for the initial recognition of cleaners by hosts. Tactile stimulation is undoubtedly important in cleaning interactions, but it probably serves as positive reinforcement for the symbiotic relationship. Since a large number of species are known to respond to the cleaner wrasse (Youngbluth, 1968), the initial response most likely has a visual basis. In other words, the wrasse must be displaying a universal visual signal.

The hypothesis of this thesis research was that the visibility of the cleaner to other fish is maximized through specific conspicuous optical signals. The basic idea of conspicuousness refers to colour patterns which deviate from background characteristics with respect to brightness, hue, shape and motion to name a few (Endler, 1978). In the case of the Hawaiian cleaner wrasse, colour pattern and swimming motion are thought to

be the specific optical signals which identify the wrasse as a cleaner and attract host fish. As in all visual communication systems, the visibility of the cleaner is influenced by several factors such as the ambient light conditions at the cleaning stations, the properties of the cleaner's optical signal, the behaviour of the cleaner and the visual sensitivity of host fish.

## VISUAL BIOLOGY OF CORAL REEF FISH

Teleost fish generally possess well-developed vision which is based on complex sensory receptors and neuronal processes. These visual responses are initiated by the photoreceptor cells which are located in the retina. There are two types of photoreceptors in the retina of most teleost fishes, namely rods and cones. Rods function under nocturnal or dim conditions, while cones operate under bright conditions.

As light reaches the retina, individual photons are captured by the visual pigments which are located in the outer segments of the photoreceptors. Photoabsorption of light causes structural changes in the visual pigment molecules, known as photoisomerization. At this point, the photoreceptor cell hyperpolarizes which eventually leads to the conduction of an action potential to the brain via the optic nerve.

There are several types of visual pigments which have a similar broad absorbance spectrum, differing in the wavelength which is maximally absorbed ( $\lambda_{\max}$ ). Past research has indicated that the cone  $\lambda_{\max}$  of fish is related to the spectral distribution of ambient light underwater (Munz and McFarland, 1977; Loew and Lythgoe, 1978; Levine and MacNichol, 1979; Lythgoe and Partridge, 1989; Partridge, 1990; Lythgoe et al., 1994). For example, there is a general trend for absorbance maxima of fish cone pigments to be similar to the dominant wavelength specific to the depth and water type in which the fish live (Lythgoe, 1988). This is thought to be a mechanism whereby visual sensitivity is maximized. That is, the cones are most sensitive to the wavelength of light which is most abundant in the environment.

The reef environment is spectrally complex, due to reflection from sand, coral and coral rubble. Coral reef fish appear to be highly dependent on vision (Munz and McFarland, 1975) and presumably the visual biology of reef fish is adapted for the coral

reef habitat. The clarity of the water and intense solar radiation on the reef, make it unlikely that rod pigments are important for shallow reef fish during the day. Indeed, it has been estimated that rods would only function efficiently at depths greater than 280 meters in tropical seas at midday (McFarland, 1991).

Many diurnal reef fish have evolved a visual system which is capable of wavelength discrimination, that is colour vision. Colour vision is a diurnal phenomenon which is based on cone photoreceptors and their visual pigments. In order for hue discrimination to be possible, two conditions must be met. Firstly, there must be more than one spectral class of cones in the retina and secondly the output from the different cone classes must be compared.

For a fish to see a target during the day, sufficient light must be present to stimulate the photoreceptors, i.e. the cones, and there must be enough visual contrast between the target and background so that the target can be distinguished from the background. This means that sensitivity and contrast are the two main parameters affecting vision (Munz and McFarland, 1977). During the day in clear tropical seas, light intensity remains high to considerable depths. Consequently, photosensitivity is not a major visual constraint for target detection in the diurnal photic environment. Instead, enhancement of photocontrast presents a greater problem. The advantages associated with high visual acuity and maximal contrast detection under various photic conditions probably favored the evolution of colour vision (Lythgoe, 1979).

The overall goal of this research was to understand how vision mediates behaviour in coral reef fish. In order to do this, the visual communication system between the Hawaiian cleaner wrasse and its host fish was studied. Specifically, this research attempted to identify the visual cues which host fish use to recognize and respond to the cleaner wrasse in nature. The objectives were therefore:

- 1) To characterize the photic conditions of Hawaiian reefs in terms of transmission and spectral properties.
- 2) To evaluate the colour pattern and dance behaviour of the Hawaiian cleaner wrasse as potential optical signals.
- 3) To analyze the visual sensitivity of host fish.

## Chapter 2. The Underwater Photic Conditions of Kaneohe Bay, Hawaii

### INTRODUCTION

Visually mediated behaviour is ultimately influenced by the photic environment in which the organisms live. The optical properties of an animal's habitat are critical to visual abilities and performance. For fishes, the underwater photic environment is affected by incident solar radiation on the water's surface, transmission of light through the water column and reflectance from substrates. The photic conditions are important to visual communication because water not only influences the transmission of visual signals but it also provides the background against which the signals are viewed.

The photic environment of tropical reefs and its importance to the visual ecology of reef fish was previously discussed by McFarland and Munz (1975) and a number of key findings were outlined. For example, during the day the number of photons between 450 and 700 nm incident on the surface is virtually constant in tropical seas (McFarland and Munz, 1975). The amount of particles and dissolved substances is minimal in tropical oceans so the attenuation of light results primarily from absorption and scattering by water molecules. As light penetrates tropical water, longer wavelengths are attenuated in the upper depths which effectively narrows the downwelling light spectrum. The wavelengths which are maximally transmitted tend to fall between 450 and 475 nm, resulting in the characteristic blue appearance of tropical ocean water.

There are three principal directions for underwater light, downwelling, upwelling and horizontal, and each is affected by a unique suite of factors. There are two features of downwelling light in tropical areas which are important to vision. Firstly, although the spectral distribution is monochromatic blue at relatively shallow depths, the overall intensity does not diminish rapidly. Thus, there is abundant light for vision down to 100m (Munz and McFarland, 1977). Secondly, the spectrum of downwelling light at a given point in the water column depends on the depth below the surface, not distance from the bottom. This means that a target below the surface will be struck by the same downwelling light in the shallow reef as in the pelagic zone and the target contrast should be similar for both locations.

Light in the horizontal direction mostly results from scattering of downwelling and upwelling light. The spectral distribution of horizontal light is independent of depth and distance from the bottom. Horizontal light often serves as the background against which targets are viewed and its spectral composition is relatively constant except in very shallow areas with reef substrate in the background.

The properties of upwelling light are due to reflection from the bottom and upward scattering from other directions. Upwelling light tends to be less intense than downwelling and in deep areas, it tends to be very blue. In shallow reef areas, light in the upwelling direction results from reflection from fine coral sand, coral and coral rubble. This spectral reflectance can affect the appearance of background spacelight as well as objects themselves. Although this is undoubtedly significant to vision, there are no data available for spectral reflectance of natural targets in the coral reef (Munz and McFarland, 1977).

This previous research focused only on the “visual” part of the spectrum (400-750nm). However, there is increasing emphasis on the importance of short wavelength light (300-400nm) since UV vision has been confirmed in several species of fish (Neumeyer, 1984; Harosi, 1986; Loew and Wahl, 1991; Hawryshyn and Harosi, 1991).

The purpose of this work is to characterize the photic environment of the natural habitat of the Hawaiian cleaner wrasse, from 300-850 nm, including spectral and transmission properties of the water and spectral reflectance from coral substrates. This allows several important parameters of visual communication systems to be assessed, namely the background against which the cleaner wrasse is viewed by host fish, the incident light which strikes the cleaner wrasse and the clarity of the water through which the host detects the cleaner wrasse.

## MATERIALS AND METHODS

This study took place during August 1994 in Kaneohe Bay, Hawaii (21° 28' N, 157° 48' W) which is located on the windward side of the island of Oahu. The bay consists of two general areas, inshore and offshore regions which are separated by a sandflat and a barrier reef (Fig. 2.1). The optical properties were characterized for 2

components of the reef habitat namely 1) the water column and 2) coral heads and other substrates. All measurements were obtained with an underwater spectroradiometer (model LI1800-UW, LiCor instruments, Lincoln, Nebraska) interfaced to a computer terminal (LiCor model LI1800-14) which was operated from a boat. The spectroradiometer was set to measure photon flux ( $\text{mMol photon}/(\text{m}^2 \cdot \text{s} \cdot \text{nm})$ ) at 5 nm intervals from 300 to 850 nm. Before starting this study, the spectroradiometer was calibrated with a LI-COR optical radiation calibrator (LiCor model LI1800-02).

### **Photic conditions in the water column**

Two types of measurements were completed to characterize light in the water column, i.e. irradiance and radiance. Irradiance measurements quantify light incident on  $180^\circ$  of a flat surface which includes light traveling in a direct path from the surface and light scattered from its original path. Irradiance represents the light which comprises the background spacelight and light which impinges upon targets (Lythgoe, 1988). Radiance measurements encompass a narrower angle of light. It includes light that travels directly from the object into the eye as well as a small portion of the background spacelight which directly surrounds the target (Lythgoe, 1988).

Irradiance was measured at three sampling sites. Two sites were located inshore, one with a maximum depth of 5m and another with a maximum depth of 15m. The third site was located offshore with a maximum depth of approximately 35m. The spectroradiometer was attached to a system of ropes and lowered into the water from a boat. The instrument was lowered from the side of the boat which faced the sun and held about 1m away from the boat to reduce effects due to shadow. During each sampling period, the sun's angle was measured with a sextant and the amount of cloud cover was estimated by eye. The amount of cloud cover was recorded as either none, moderate, when clouds were thin and shadows could be seen and high when clouds were thick and no shadows could be seen.

For the two inshore sites, 3 surface readings were obtained at 0m, 0.5m and 1m in depth. The remaining readings were taken every meter until the maximum depth was reached. By adjusting the attachment of the ropes on the spectroradiometer, the orientation of the sensor in the water column was changed with respect to the sun's

position. For the inshore sites, light was measured in three directions namely, downwelling, horizontal and upwelling. At the offshore site, downwelling light was measured from 1m to 24 m at 3m intervals. Upwelling light was measured from 1 m to 24m at 5m intervals.

Irradiance was also measured during the twilight period. The spectroradiometer was placed on the substrate at an inshore site with the sensor submerged about 1m. It was programmed to take a reading every three minutes from 18:36 hrs to 21:03 hrs (Local Hawaii Time). Sunset occurred at approximately 20:00 hrs.

To measure radiance, a 12° cone (0.4 sr) was fitted over the cosine collector on the spectroradiometer. These readings were obtained at the two inshore sites for three directions as described for the irradiance measurements. No radiance measurements were obtained from the offshore site.

### **Spectral reflectance from coral and other substrates**

To measure the spectral reflectance from coral and other substrates, the spectroradiometer was brought underwater by means of SCUBA diving. Two types of radiance measures were obtained. Firstly, a 68° (2.267 sr) fiber optic probe (Oriol) was attached to the spectroradiometer. The other method involved using the 12° cone which was described previously. The probe/cone was pointed at a particular substrate so that the aperture was positioned 5-10 cm from the substrate. For each type of substrate, spectral readings were obtained from 4 directions: downwelling ambient light, upwelling light reflected from the substrate, horizontal light reflected from the substrate and ambient horizontal light. Each reading consisted of an average of three scans. The procedure was recorded with an underwater video camera (Hitachi model VMH-38) to provide a record of the type of substrate and the direction of measurement. The types of substrates that were measured included the following: two coral species, *Montipora verrucosa* and *Porites compressa* (brown and green colour morphs), coral rubble and coral sand. These substrates were chosen because they were very common on the reef and present in most cleaning stations.

## Analysis of light data

Each data set was evaluated and negative values were removed as invalid data, since negative light is meaningless. Irradiance data from the same site were averaged and converted to log photons/(m<sup>2</sup> · s · nm) and plotted against wavelength. Radiance data was averaged and converted to log photons/(m<sup>2</sup> · s · nm).

To determine the dominant wavelength, or colour, of the water the  $\lambda_{p50}$  index was calculated. This index represents the wavelength at which half of the total photons occur (Munz and McFarland, 1973; McFarland and Munz, 1975). Since the visual process is statistical in nature, the  $\lambda_{p50}$  is useful because it represents the spectral position of prevailing ambient quanta, i.e. the wavelength where quanta are most abundant (McFarland and Munz, 1975). This index was appropriate for these data since the spectra were not multi-modal in shape.

Attenuation of light was evaluated by computing diffuse attenuation coefficients ( $K_D$ ) at each site with the following equation (Lythgoe, 1979):

$$K_D = \frac{2.3(\log I_o - \log I_z)}{z}$$

where  $I_z$  is irradiance at depth  $z$  and  $I_o$  is irradiance at surface.

Spectral reflectance from coral and other substrates was calculated as the ratio of light reflected in the upwelling and horizontal direction from the substrate relative to the downwelling light incident on the substrate.

## RESULTS

### Irradiance measurements

The spectral distribution of downwelling light at the 3 study sites was relatively broad and flat at the surface (Fig. 2.2). The amount of surface light was about 18 to 18.5 log photons/(m<sup>2</sup> · s · nm) for each site. As depth increased, the total amount of light decreased and the spectral distribution of downwelling light narrowed. At all sites, the amount of light between 700-800nm was reduced by half in the first 3m. The amount of UV-A and UV-B light, 300-350nm, decreased with depth, however ultraviolet light did penetrate to maximum depth at all sites. Approximately 0.3% of surface incident UV

light was present at 15m depth inshore while 34% of surface incident UV light was present at 25m depth offshore. From 400 to 600nm, the amount of light diminished the most at the 15m site, by about 1.5 log units, whereas the amount of light was reduced by about 0.5 log units at the 5m and 25m site.

The spectral characteristics of the downwelling light differed between sites (Table 2.1). At the 5m and 15m sites,  $\lambda_{p50}$  was 536nm and 525nm respectively, which corresponds to green light. The  $\lambda_{p50}$  of downwelling light at the 25m site was 485nm which indicates blue light. In other words, downwelling spectra from the offshore site were shifted to shorter wavelengths with respect to the two inshore sites. In a few situations  $\lambda_{p50}$  values showed an increase as depth increased. For instance,  $\lambda_{p50}$  increased at the 15m site for depths greater than 10m in the horizontal and upwelling directions, and at the 25m site for depths greater than 15m in the upwelling direction. This may be due to reflection caused by proximity to the bottom or other substrates.

In the upwelling direction, irradiance remained narrowly distributed at all depths (Fig. 2.3). The abundance of upwelling light decreased with increasing depth by about 0.5 log units from the surface to the maximum depth at the 3 sampling sites. At the maximum depth, the amount of upwelling UV light was greater than that of longer wavelength light.

The  $\lambda_{p50}$  indices for the upwelling direction were 505nm, 514nm and 461nm for the 5, 15 and 25m sites respectively (Table 2.1). Therefore, upwelling light at the offshore site tended to be of shorter wavelengths than light at the inshore sites. In general, the colour of light in the upwelling direction was shifted to shorter wavelengths than light in the downwelling direction.

Surface reflectance, which refers to the ratio of upwelling irradiance relative to downwelling, also indicated the dominant colour of the water for each site (Fig. 2.4). Again, both inshore sites were spectrally similar but the water at the offshore site was blue-shifted.

The dominant wavelength of light, i.e.  $\lambda_{p50}$ , was dependent on the line of sight and depth in the water column (Fig. 2.5). At the 5m site, the surface upward and horizontal light was blue-green (505nm-510nm) while the downwelling light was more

green (530nm). Close to substrate, the horizontal and upwelling light was similar (505nm) while the downwelling light was shifted slightly longer (520nm). At the 15m site, upward and horizontal light were similar (505-510nm) and downwelling wavelengths were longer (545nm) at the surface. Near the substrate, the dominant wavelength of horizontal and upwelling light corresponded to the green region of the spectrum (525-530) while the downwelling light was shorter in wavelength (515nm). At the offshore site, the upwelling light was more blue-shifted (455nm) than the downwelling (520nm) near the surface, but at greater depths upwelling and downwelling light became spectrally similar at 470 and 465nm respectively. No data were obtained for horizontal light at the offshore site.

### **Radiance measurements**

The radiance data showed the same basic trends as the irradiance data. That is, the downwelling light was relatively broad and flat at the surface and narrowed with depth so that medium wavelength light was maximally transmitted (Fig. 2.6). The upwelling radiance was narrowly distributed at all depths (Fig. 2.7). Radiance data were proportionately less than irradiance due to the smaller collecting surface of the light meter. For downwelling light, the  $\lambda_{p50}$  index was 547nm at the 5m site and 523nm at the 15m site. In the upwelling direction, the  $\lambda_{p50}$  was 507 at the 5m site and 514nm at the 15m site (Table 2.2). These results are fairly similar to the irradiance data, however the downwelling  $\lambda_{p50}$  calculated from irradiance spectra was about 10 nm less than that obtained with radiance values. Nevertheless, the predominant wavelength of light at both of the inshore sites corresponded to the green region of the spectrum in the downwelling direction and slightly more blue-shifted in the upwelling direction.

### **Light attenuation**

The overall light attenuation (Fig. 2.8) was greatest at the 5m site ( $K_D=0.89$ ) and least at the 25m site ( $K_D=0.12$ ) with the 15m site showing an intermediate attenuation ( $K_D=0.41$ ). This means that light penetrated to the greatest extent at the offshore site, while light diminished much more rapidly at the inshore sites. The attenuation curves show that light attenuation is least between 350 and 600nm for all sites. Therefore, the

water is most transmissive to light between 350 and 600nm and least transmissive to light of wavelengths greater than 600nm.

### **Effects of sky conditions**

For downwelling light, the spectral distribution remained quite similar under sunny and cloudy conditions, however the amount of light decreased when the sky was cloudy (Fig. 2.9). At the surface, the reduction in both downwelling and upwelling light due to clouds occurred in the same proportion across the entire spectrum. In total, the amount of downwelling light was reduced by approximately 45% and the upwelling light was decreased by about 61% due to clouds. At the maximum depth, cloud cover caused a proportionately greater loss of light between 450-550nm and 620-700 nm compared to other regions of the spectrum. This may be due to greater degree of attenuation of these wavelengths relative to shorter wavelengths.

### **Effects of time of day**

The abundance and spectral properties of downwelling light are affected by the sun's angle which is related to the time of day (Table 2.3). Downwelling light is most intense when the sun's angle is highest. In Kaneohe Bay in the month of August, this occurred at around 12:30hrs, local Hawaii time. Therefore, the amount of light in the morning (10:30) and in mid-afternoon (15:30) was slightly less than the amount of light at 13:00 (Fig. 2.10). At 18:36, the amount of light had decreased substantially (93%) relative to 13:00 hrs. As sunset approached, the amount of light decreased very rapidly, that is, after 18:36 hours, 1.5% of the remaining light was lost per minute. Furthermore, the spectral quality of light in the sky changed at sunset so as to become shifted to longer wavelengths. This is reflected in the underwater spectral curves which show an increase in the absorbance of wavelengths between 700-750nm due to the increased amount of incident red light.

### **Reflectance from reef substrates**

For all substrates, the amount of reflected light in the upward direction was less than that in the horizontal direction. Sand reflected a maximum of 9% in the upward direction with a peak reflectance between 500-600nm (Fig. 2.11). The dominant reflected colour was represented by a  $\lambda_{p50}$  of 550nm. No data were obtained for horizontal

reflectance from sand. The spectral reflectance of *Montipora verrucosa* was quite broad and flat with most light reflected (8%) between 550-600nm and the  $\lambda_{p50}$  was 542nm (Fig. 2.12). Coral rubble had the highest amount of reflectance of all the substrates (20%) with a large peak between 530 and 640 nm and  $\lambda_{p50}$  was 541nm (Fig. 2.13). *Porites compressa* occurred as 2 colour morphs, referred to as brown and green (Fig. 2.14). Both colour morphs had a similar amount of reflectance of 15%. The brown morph had a peak reflectance shifted to longer wavelengths, that is 560-620nm. The  $\lambda_{p50}$  for the brown morph was dependent on direction. In the upward direction, the  $\lambda_{p50}$  was 551nm while the  $\lambda_{p50}$  for the horizontal reflectance was 538nm. The green morph showed a narrower peak reflectance at 540-600nm and a  $\lambda_{p50}$  of 546nm.

**Table 2.1.**  $\lambda_{p50}$  values (nm) according to depth for each direction (DW = downwelling, HZ = horizontal, UP = upwelling) of irradiance at the 3 study sites. Mean values for all depths are shown in bold.

Depth (m)	5m Site			15m Site			25m	Site
	DW	HZ	UP	DW	HZ	UP	DW	UP
0	590	705	505	580	530	510		
0.5	550	530	505	550	510	510		
1	530	510	505	545	510	505	520	455
2	520	505	505	530	510	510		
3	520	505	505	530	510	510	500	
4	520	505	505	515	510	510		
5	520	505	505	515	510	510		455
6				515	510	510	485	
7				515	510	510		
8				515	510	510		
9				515	510	510	485	
10				515	515	515		460
11				515	515	520		
12				515	515	520	480	
13				515	520	525		
14				515	525	530		
15							475	465
16								
17								
18							475	
19								
20								465
21							475	
22								
23								
24							470	465
<b>MEAN</b>	<b>536</b>	<b>536</b>	<b>505</b>	<b>525</b>	<b>514</b>	<b>514</b>	<b>485</b>	<b>461</b>

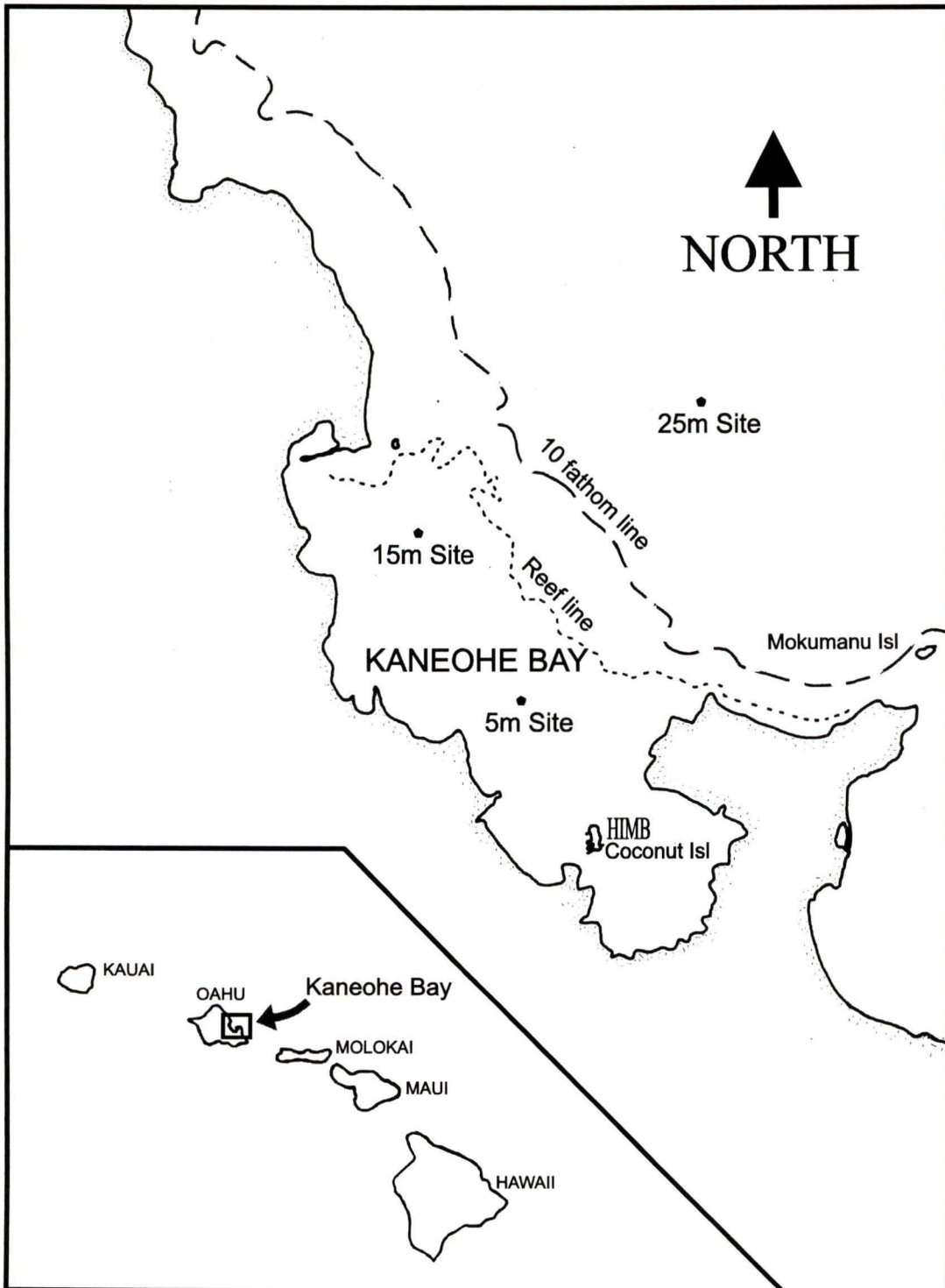
**Table 2.2.**  $\lambda_{p50}$  values (nm) according to depth for each direction (DW = downwelling, HZ = horizontal, UP = upwelling) of radiance at the 3 study sites. Mean values for all depths are shown in bold.

Depth (m)	5m Site			15m Site		
	DW	HZ	UP	DW	HZ	UP
0	615	510	505	590	515	518
0.5	535	495	505	538	505	510
1	575	510	505	528	508	505
2	540	500	505	538	510	508
3	520	500	510	520	508	508
4	520	505	510	515	510	510
5	520	510	510	510	510	513
6				508	508	513
7				520	510	513
8				513	513	515
9				515	513	518
10				513	513	518
11				515	513	518
12				515	515	520
13				518	518	523
14				518	520	530
<b>MEAN</b>	<b>546</b>	<b>504</b>	<b>507</b>	<b>523</b>	<b>512</b>	<b>514</b>

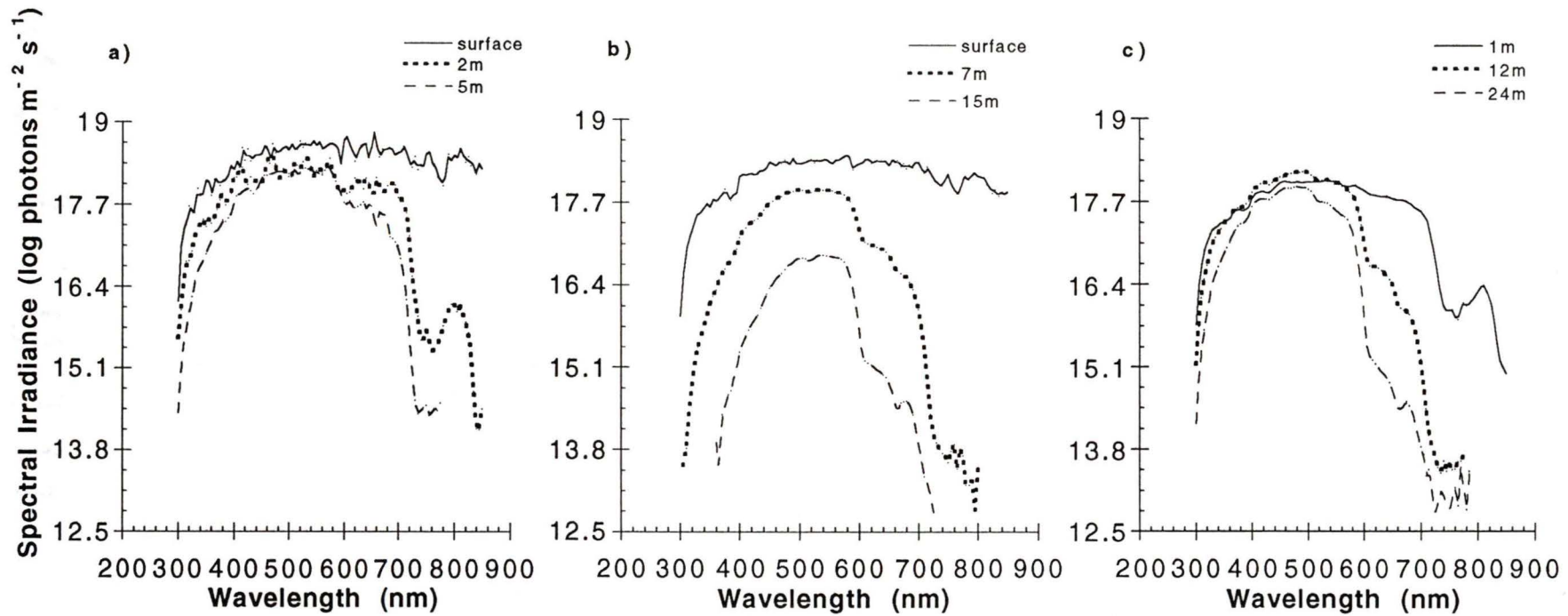
**Table 2.3.** Sun's elevation in degrees determined with a sextant for various times of day (Local Hawaii time) during August 1994. The sun is at its highest elevation as the angle approaches  $90^{\circ}$ .

Time (hrs)	Sun's Elevation (degrees)
0930	45
1030	66
1125	66
1215	84
1330	76
1430	71
1530	55
1615	45
1700	26

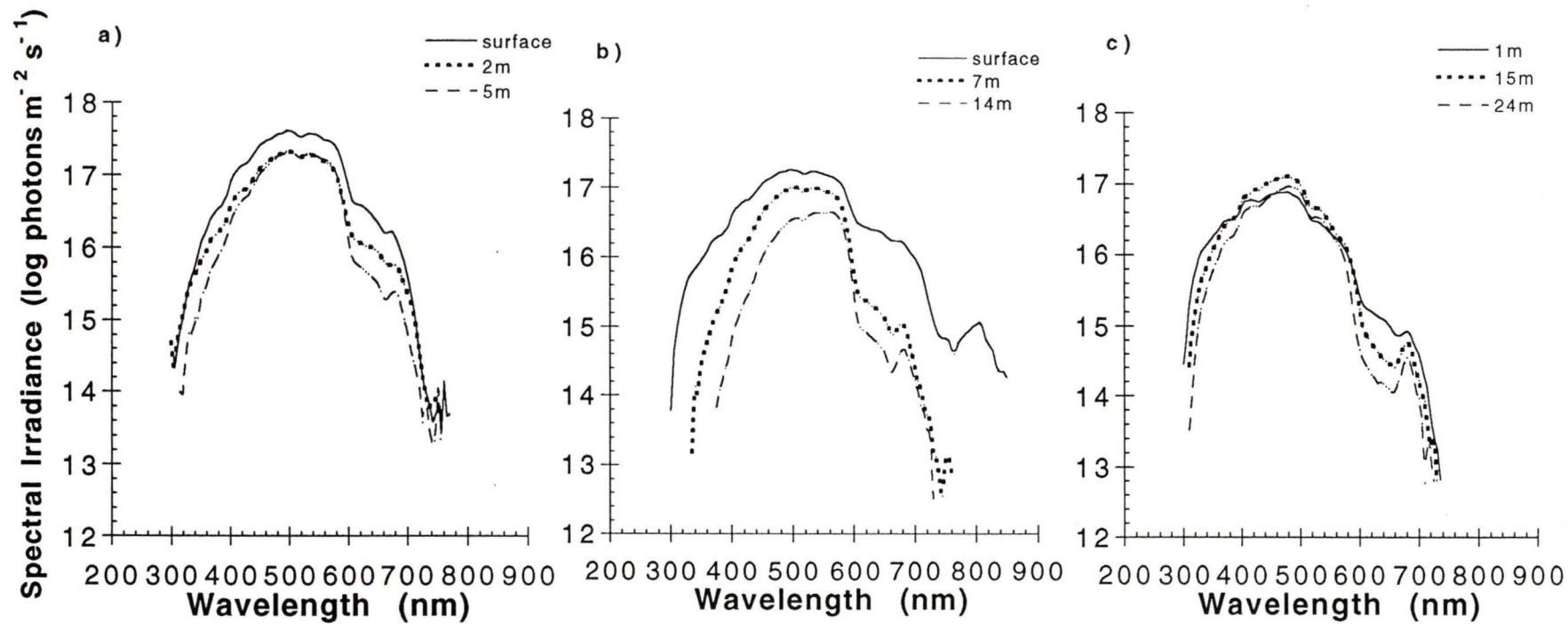
**Figure 2.1.** Map of the Hawaiian islands showing the location of Kaneohe Bay on the northwest coast of Oahu (insert). Detail of Kaneohe bay showing the location of reef line and the 3 study sites.



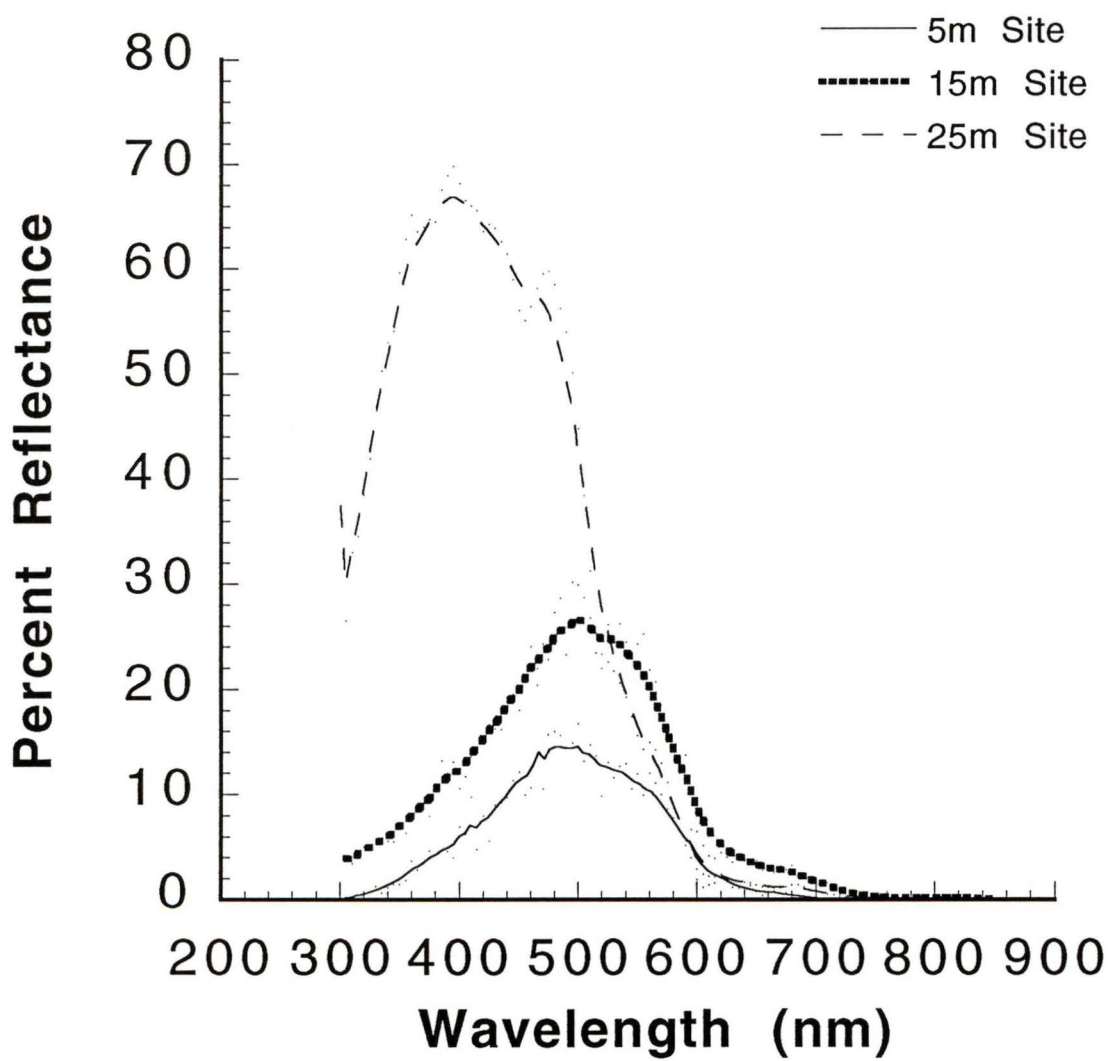
**Figure 2.2.** Depth profile of downwelling irradiance for a) 5m site (n=1), b) 15m site (n=2) and c) 25m site (n=1). At all sites, irradiance decreased and the spectral distribution narrowed as depth increased. At the surface the spectral distribution was 300-850nm but at the maximum depth it narrowed to 320-720nm. For clarity, only curves are shown for surface, midwater and the maximum depth at each site.



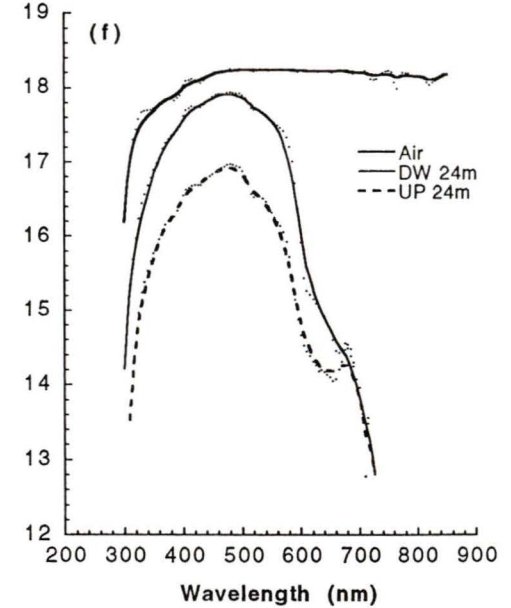
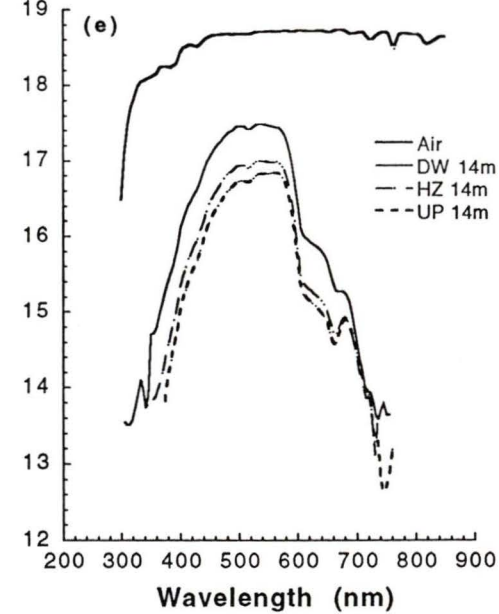
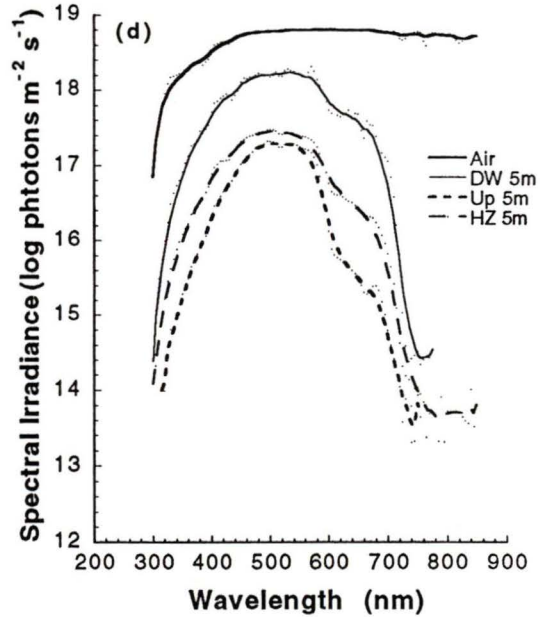
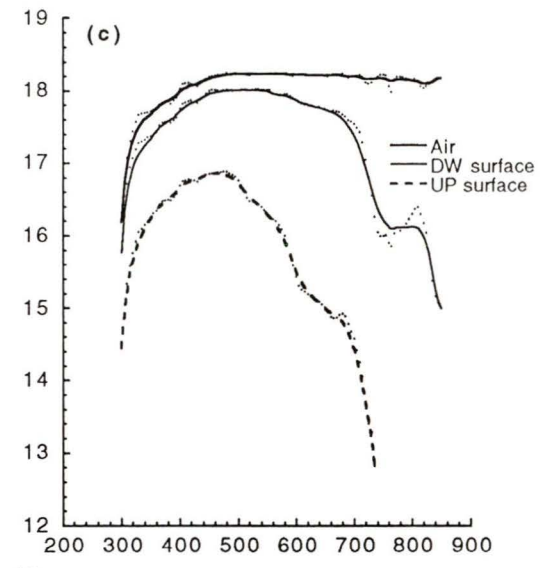
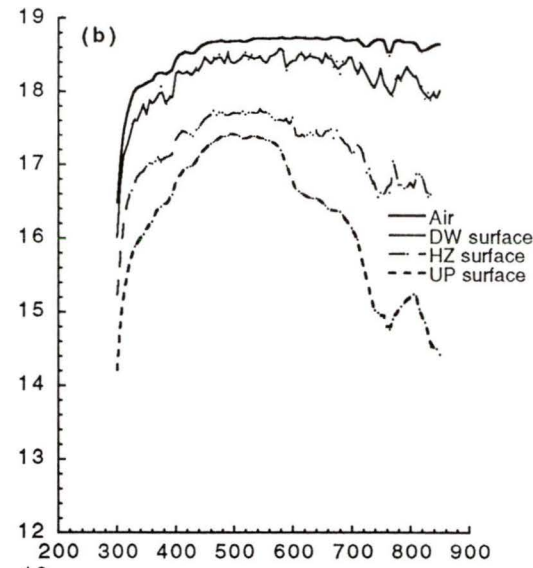
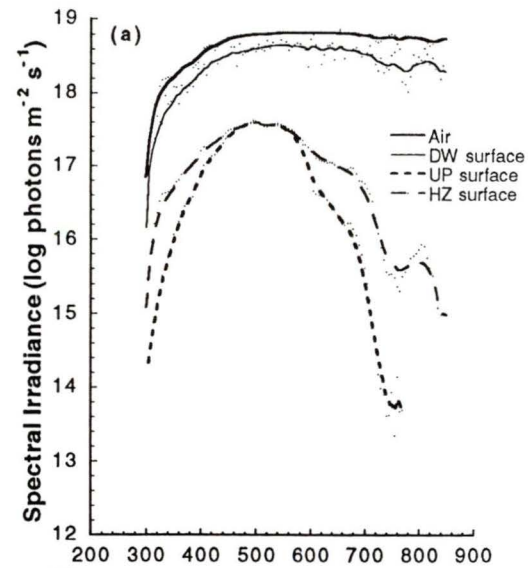
**Figure 2.3.** Depth profile of upwelling irradiance for a) 5m site (n=1), b) 15m site (n=2) and c) 25m site (n=1). At all sites, irradiance decreased and the spectral distribution narrowed as depth increased. At the surface the distribution was 300-850nm whereas the maximum depth had a range of 330-750nm. For clarity, only curves are shown for surface, midwater and the maximum depth at each site.



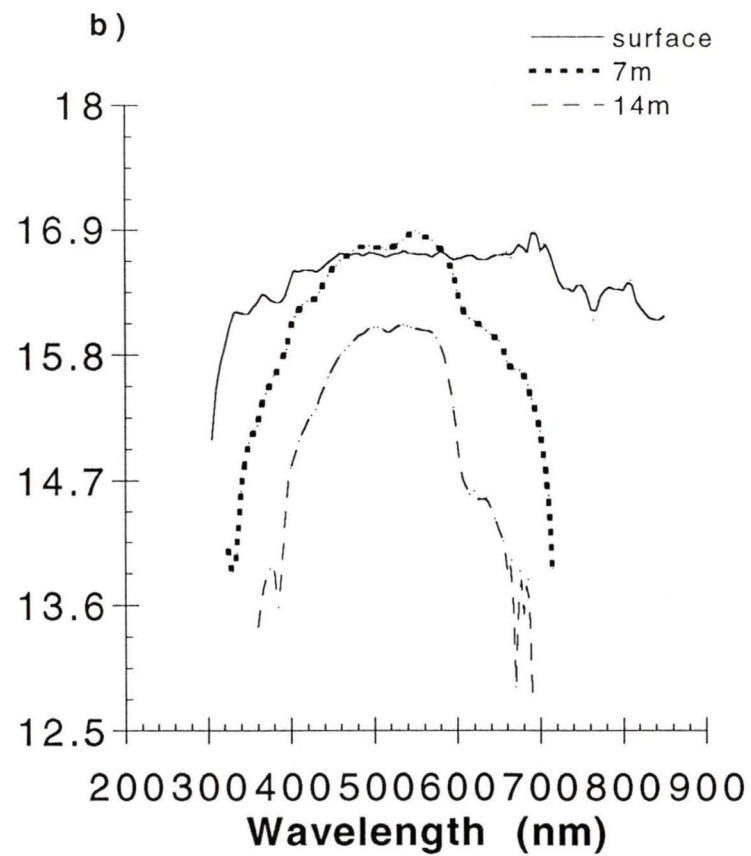
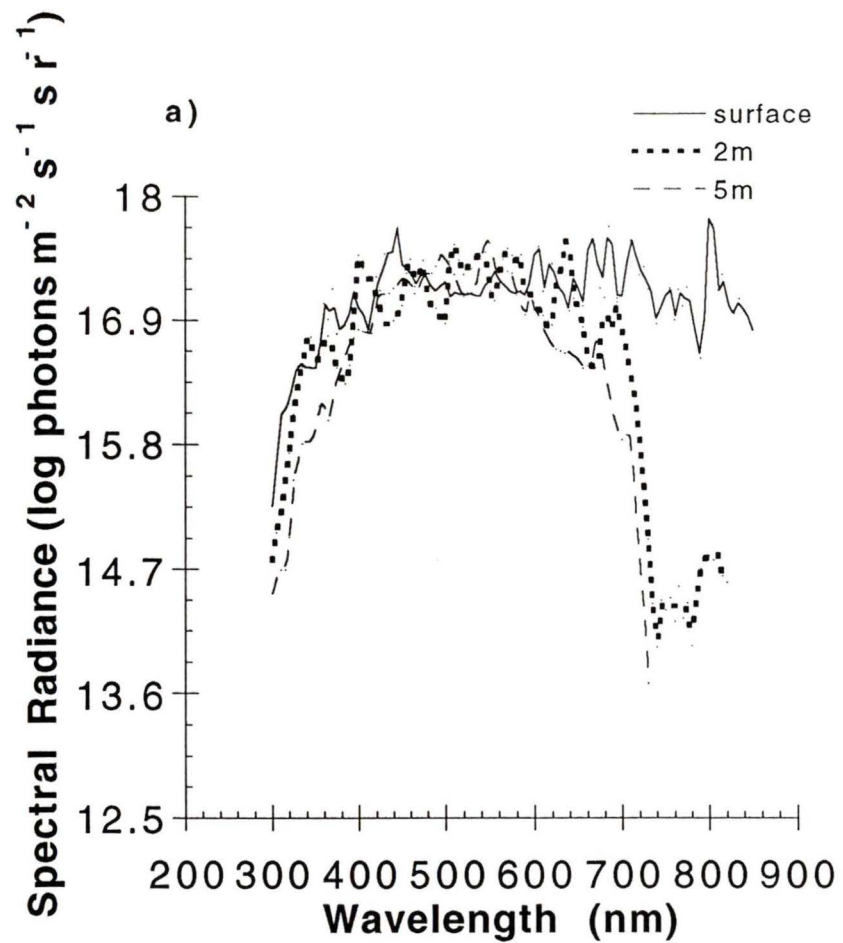
**Figure 2.4.** Spectral distribution of surface reflectance (ratio of downwelling to upwelling irradiance) at 3 study sites. The offshore site had greater surface reflectance and the peak reflectance was shifted to a shorter wavelength than the other two sites.



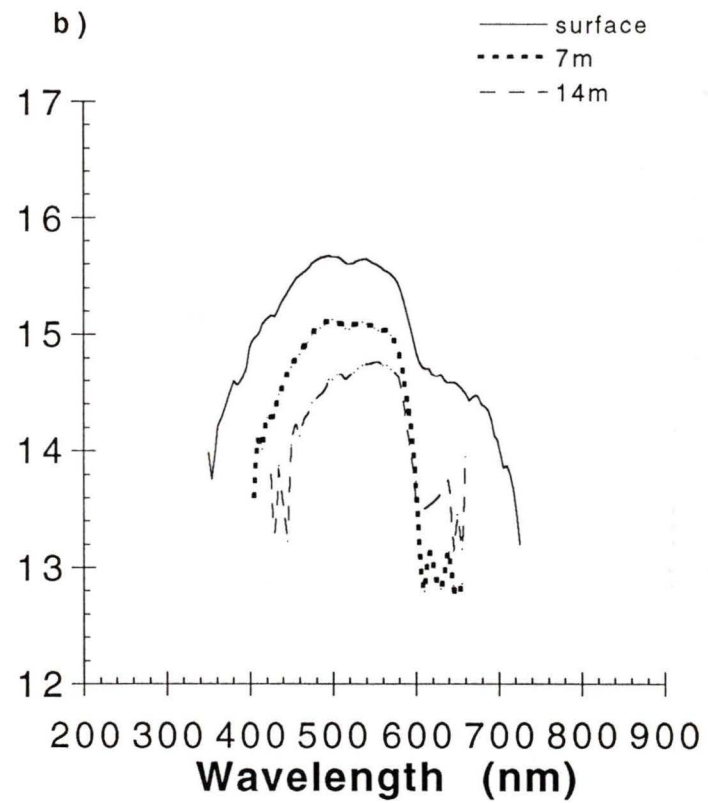
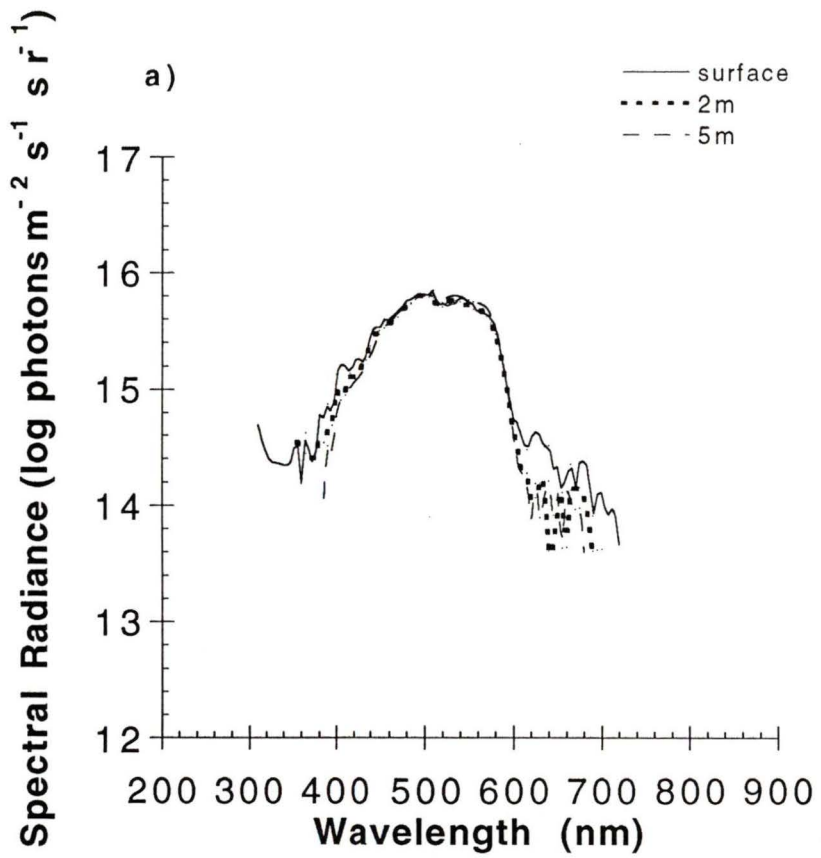
**Figure 2.5.** Spectral distribution of irradiance in 3 directions (DW = downwelling, UP = upwelling, HZ = horizontal) at the surface and maximum depth of each site. Horizontal irradiance data were not obtained from the 25m site. a) surface irradiance at 5m site, b) surface irradiance at 15m site, c) surface irradiance at 25m site, d) 5m irradiance at 5m site, e) 14m irradiance at 15m site and f) 24m irradiance at 25m site. In all circumstances, the amount of upwelling irradiance was least and most narrowly distributed.



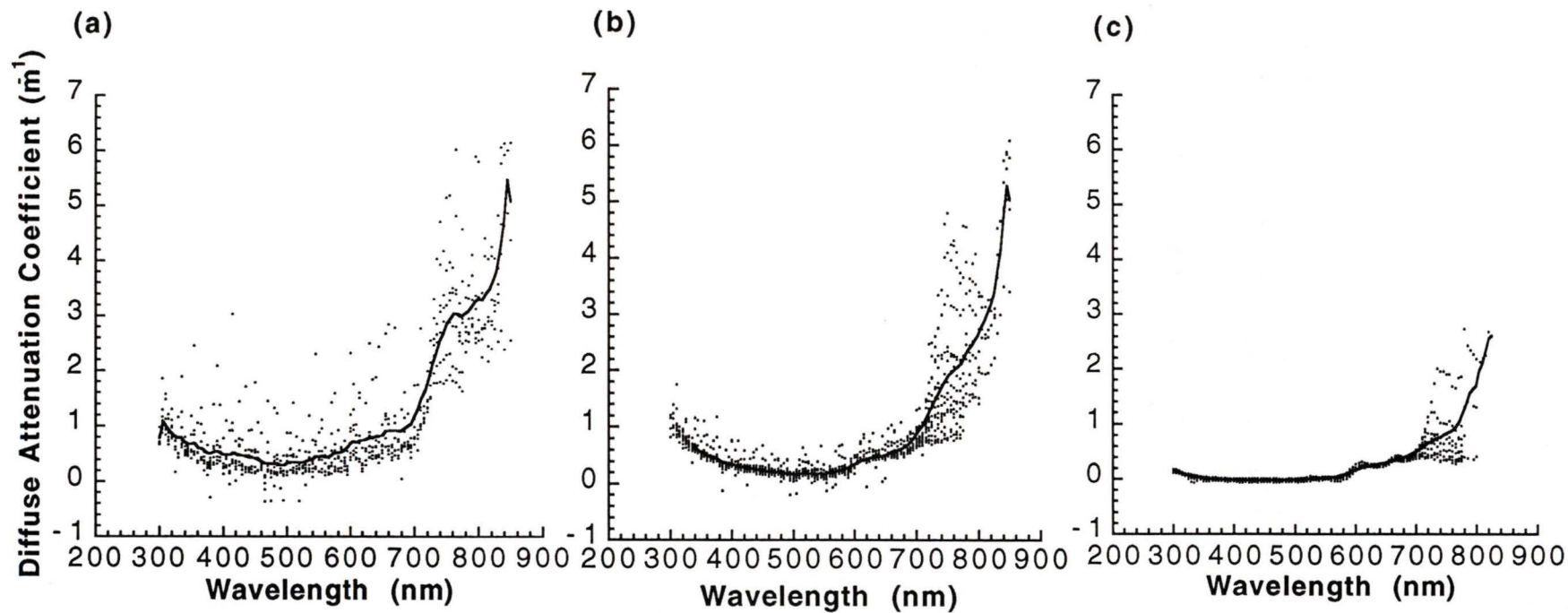
**Figure 2.6.** Depth profile of downwelling radiance for a) 5m site (n=1) and b) 15m site (n=2) under sunny sky conditions. Spectra near the surface were slightly noisy due to the presence of waves. At both sites radiance decreased and the spectral distribution narrowed from 300-850nm to 340-720nm with increasing depth.



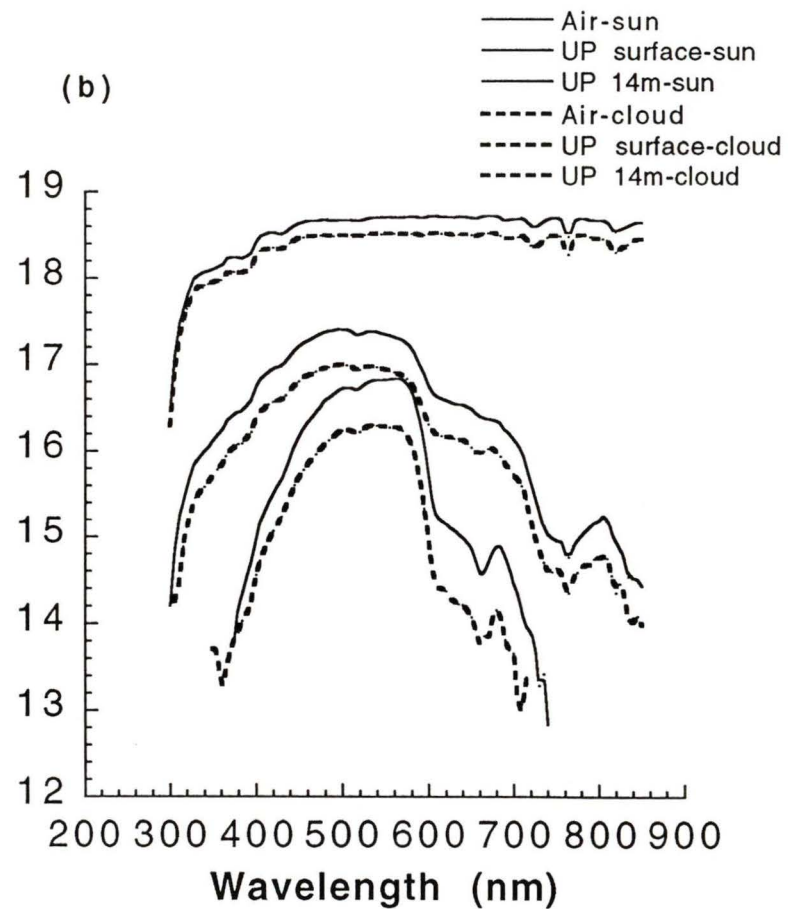
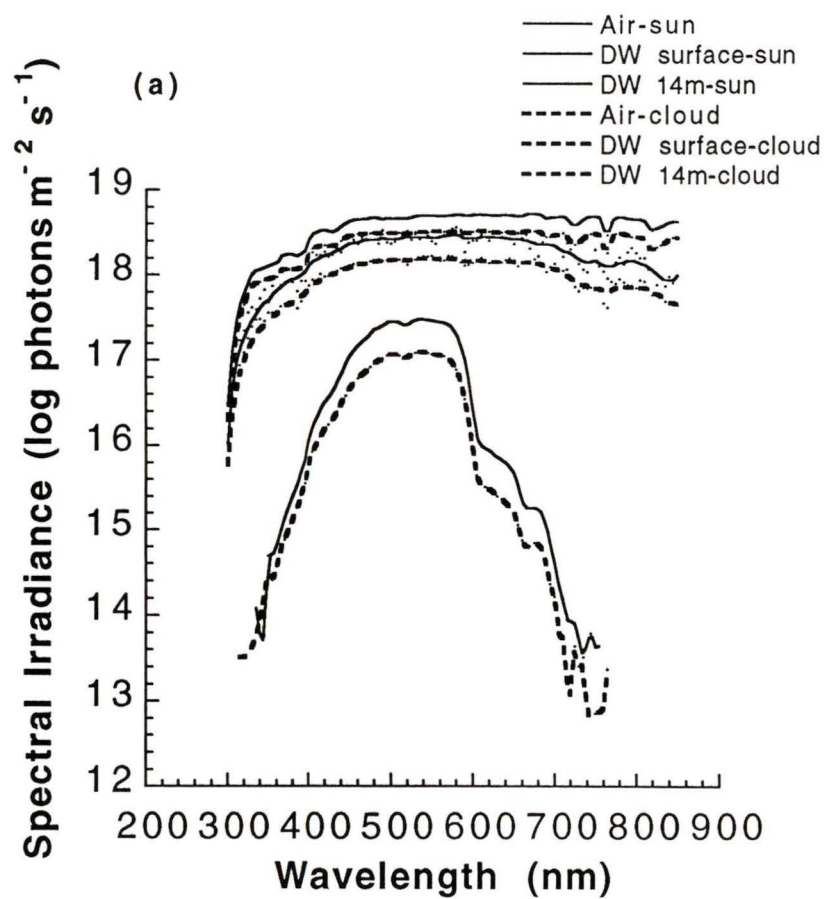
**Figure 2.7.** Depth profile of upwelling radiance at a) 5m site ( $n=1$ ) and b) 15m site ( $n=2$ ) under sunny sky conditions. At the 5m site, radiance was largely unchanged as depth increased. At the 15m site, radiance decreased and the spectral distribution narrowed with depth.



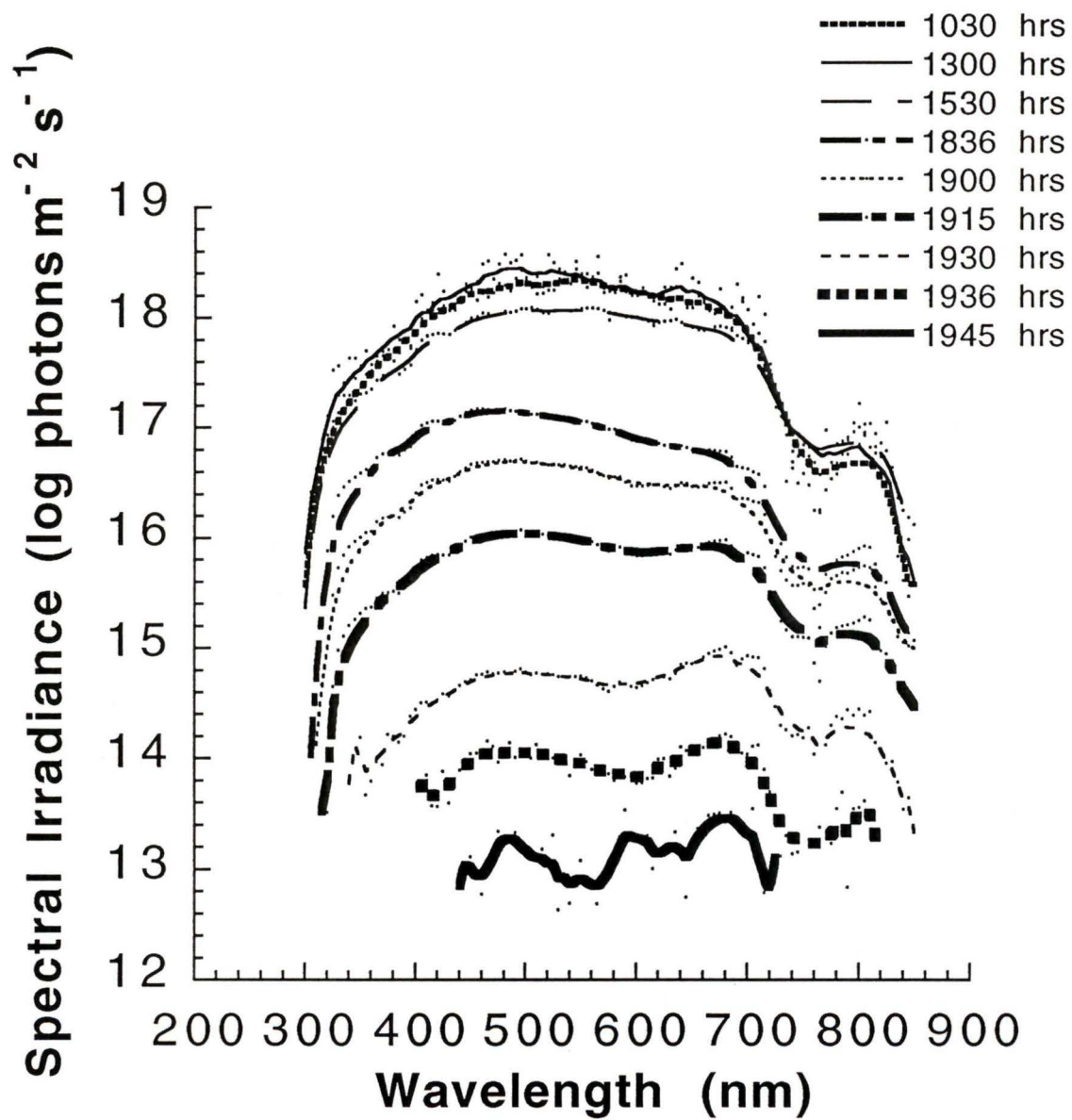
**Figure 2.8.** Mean diffuse attenuation of downwelling irradiance at 3 study sites. a) 5m site ( $K_D = 0.89$ ), b) 15m site ( $K_D = 0.41$ ) and c) 25m site ( $K_D = 0.12$ ). For all sites, light at 700nm and longer was most rapidly attenuated with depth.



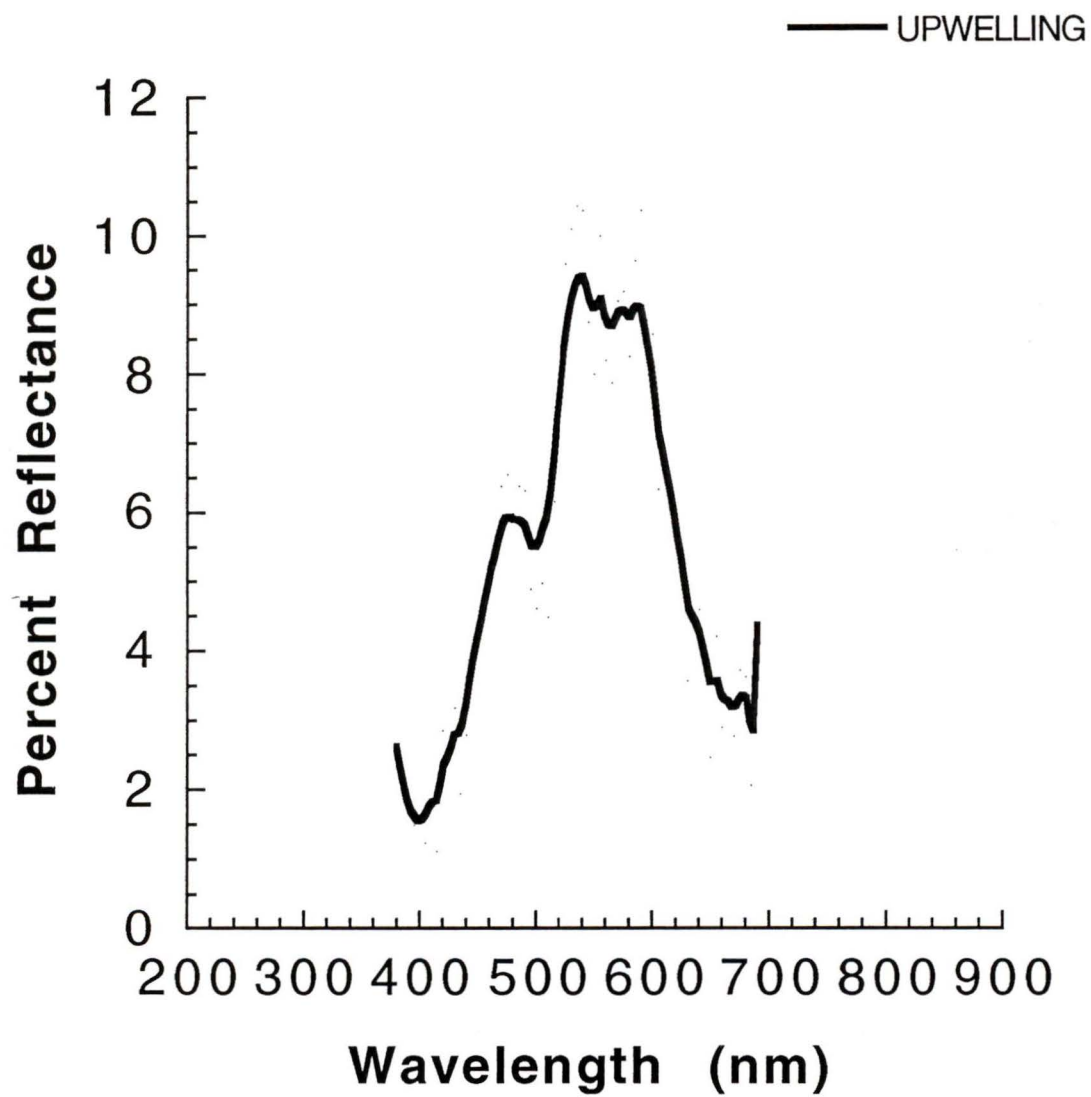
**Figure 2.9.** Downwelling and upwelling irradiance at the 15m site under sunny (n=2) and cloudy sky conditions (n=1) from the surface and 14m in depth a) downwelling irradiance b) upwelling irradiance. There was a decrease in irradiance due to cloud, regardless of direction and depth, however more light tended to be lost between 450nm - 550nm and 620-700nm.



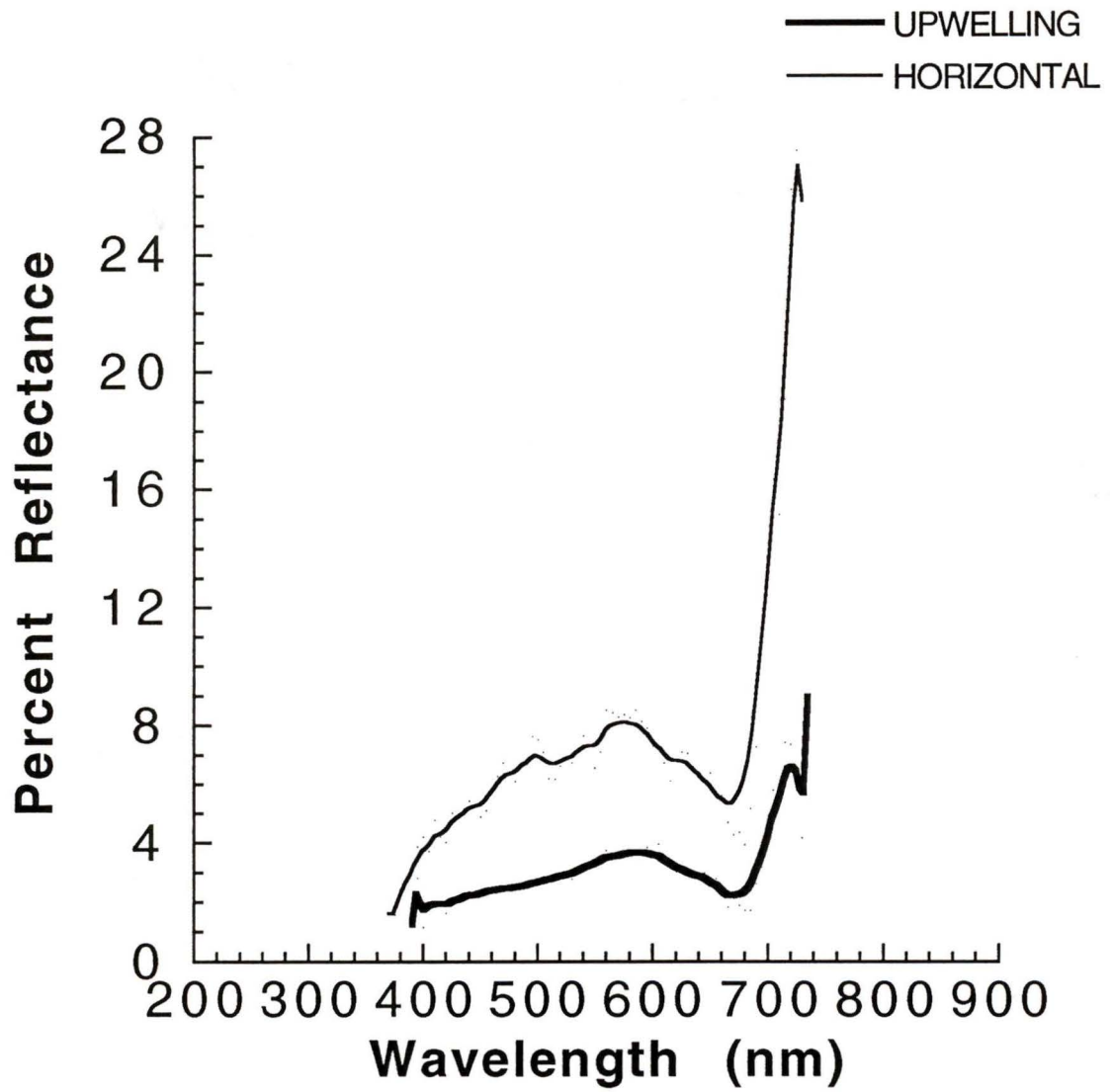
**Figure 2.10.** Time series from mid-morning until sunset for downwelling irradiance at a depth of approximately 1 meter. A small peak in light between 620nm and 700nm occurred at 19:15 hours. Sunset occurred at approximately 20:00 hours.



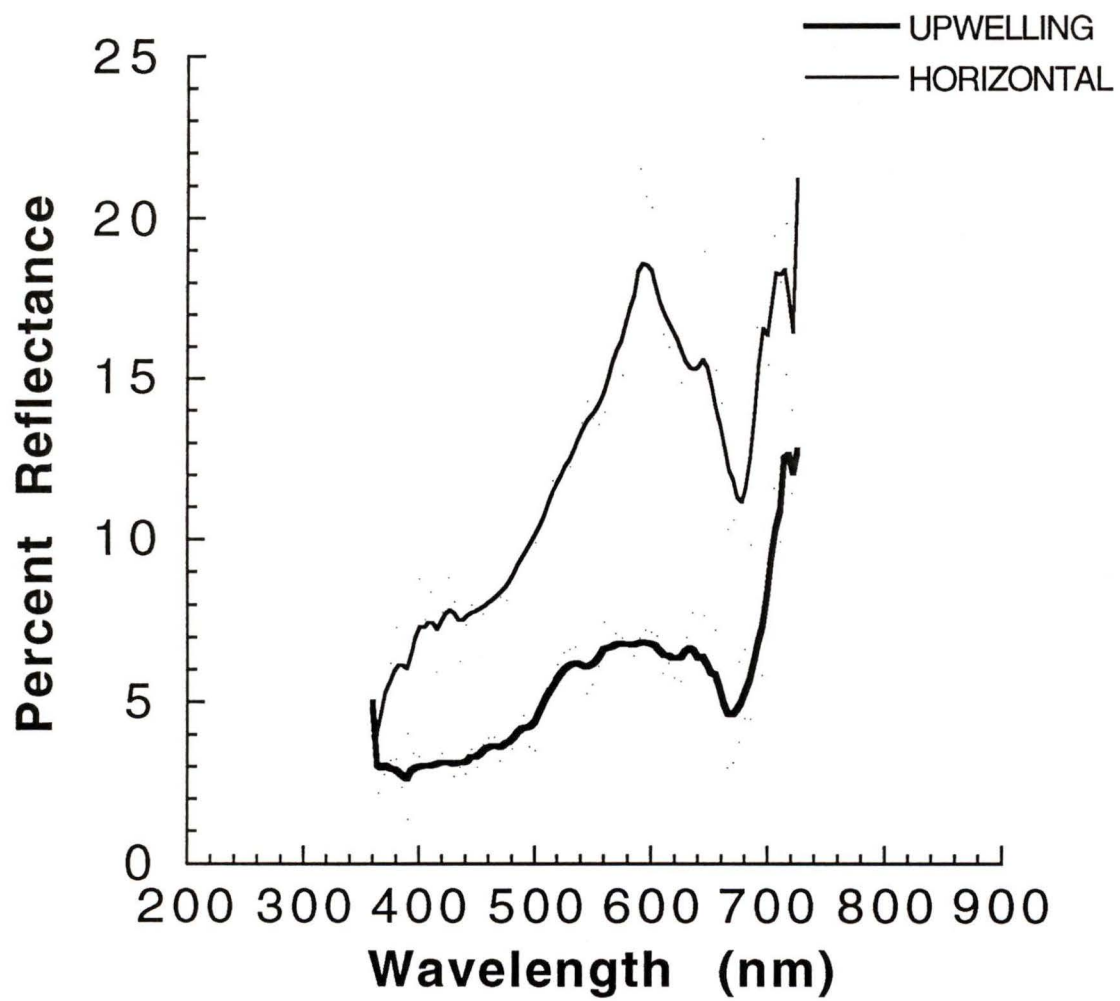
**Figure 2.11.** Percent reflectance of sand in the upwelling direction ( $n = 1$ ) obtained at an inshore patch reef.



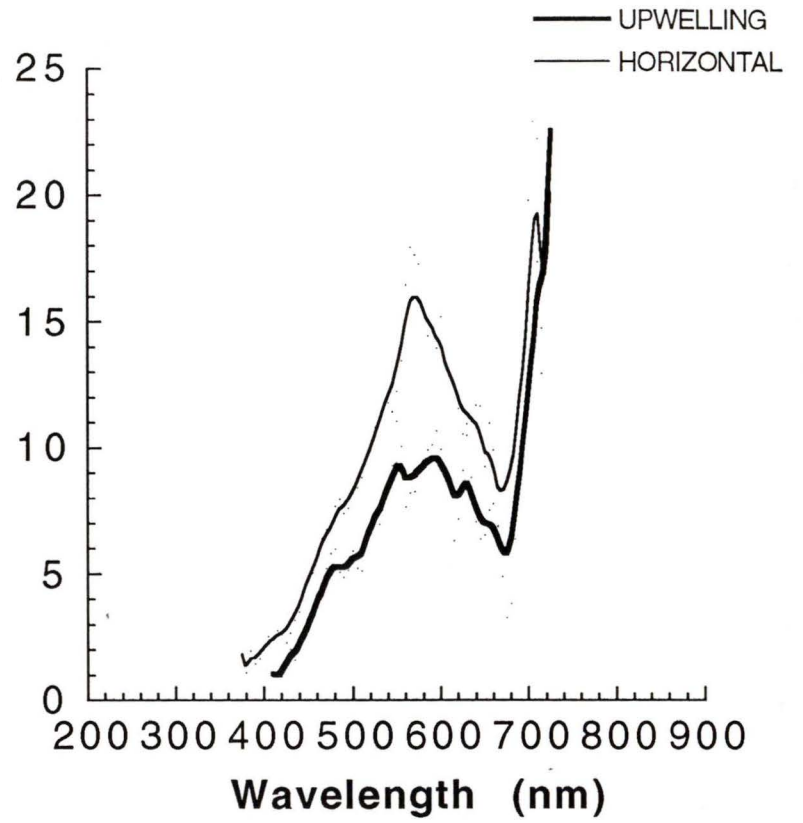
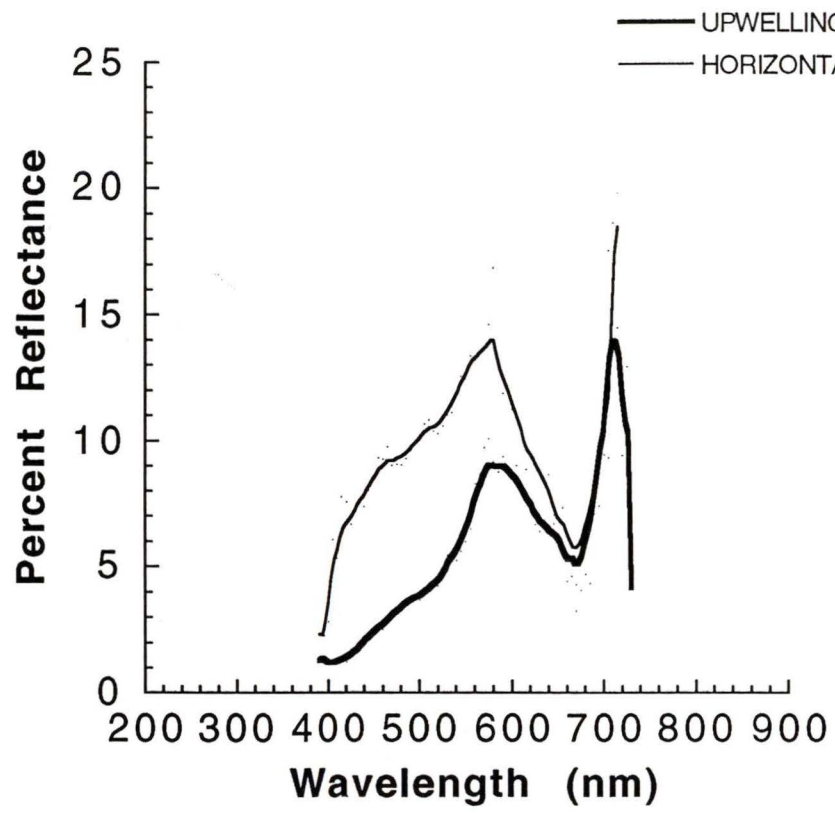
**Figure 2.12.** Percent reflectance of *Montipora verrucosa* in the upward and horizontal direction (n = 10).



**Figure 2.13.** Percent reflectance of coral rubble in the upward and horizontal direction (n = 8).



**Figure 2. 14.** Percent reflectance of *Porites compressa* for a) brown morph (n = 13) and b) green morph (n = 9) in the upward and horizontal direction.



## DISCUSSION

There were differences in photic conditions between inshore and offshore sites in Kaneohe Bay. The optical characteristics of the offshore site are consistent with Jerlov's (1976) ocean water type I, while the inshore areas are more similar to ocean water type II or III. The spectral distribution of light at the offshore site was shifted towards the blue part of the spectrum (460-480 nm) as indicated by spectral depth profiles,  $\lambda_{p50}$  values and surface reflectance spectra. This is consistent with other studies of clear tropical ocean water (Duntley, 1963; McFarland and Munz, 1975; Lythgoe, 1979). The predominant wavelength of light at the inshore sites corresponded to the green region of the spectrum. This is most likely due to a higher amount of suspended particles and organic matter, possibly as a result of the close proximity of the inshore sites to land (Lythgoe, 1979). Therefore, the spectral characteristics of the offshore water were mainly due to the inherent properties of water itself, chiefly Rayleigh scattering of short wavelengths and absorption of long wavelengths. In addition, the attenuation data showed that the offshore water was much clearer than either of the inshore sites, since light penetrated to greater depths at the offshore site.

The spectral characteristics of water were found to vary according to the line of sight. Near the surface, downwelling spectra tended to be rather broad while upwelling and horizontal light were more blue-shifted. The effect of bottom substrate was substantial, since the spectral distribution of upwelling light broadened as proximity to the substrate increased. These trends are similar to earlier findings of McFarland and Munz (1975) although the exact  $\lambda_{p50}$  values differ slightly. This can be attributed to several differences in methodology. Firstly, the previous study did not take into account the ultraviolet region of the spectrum and this affected the calculation of the  $\lambda_{p50}$  index. As well, physical differences in the study sites may reveal themselves in this index.

These results demonstrated that ultraviolet light can penetrate both clear offshore water and inshore water of Kaneohe Bay. Although UV light is subject to a great degree of scattering in the upper depths, there was a considerable amount of UV light present in the bay. In fact, the proportion of UV light found at 25m was greater than an estimate of

10% reported in another study (Fleischmann, 1989). Therefore, UV light can not be ignored as a visually relevant stimulus.

The presence of clouds in the sky did not affect underwater light to a large extent. Previous work in clear tropical water found that clouds only affected total intensity and not spectral distribution. However in this case, there were some effects on spectral quality due to cloud since wavelengths between 450-550nm and 620-700 nm were reduced proportionately more than other wavelengths. Overall, light intensity was found to decrease by about 45% as a result of cloud which is greater than a previously published estimate of 20% (Kirk, 1994).

The effects of time of day on downwelling spectral properties were greatest at crepuscular periods. During the day, light intensity and spectral distribution were relatively constant. Most extensive changes occurred as twilight approached which is similar to the effects that McFarland (1986) observed. Therefore, the photic conditions of this reef system were relatively constant, except during twilight periods. At twilight, the underwater light was shifted towards shorter wavelengths with the least amount of photons at 600 nm. The absolute light intensities at dawn and dusk were mirror images of each other. In the Caribbean, light intensity at sunrise and sunset are around  $2 \times 10^{12}$  photons/cm<sup>2</sup>/sec/nm at 550 nm (McFarland et al., 1979) which is slightly less than the light intensity observed just before sunset in Hawaii. These changes in spectral quality of water at twilight may be used as a diel cue for many reef species.

In general, the various coral substrates did not reflect much light. Very little short wavelength light, i.e. less than 450 nm, was reflected. The dominant wavelength of reflected light from most of these substrates corresponded to the green region of the spectrum (538-551nm), except for *Montipora* which reflected most wavelengths equally, therefore it was highly saturated. The spectral reflectance of the corals is likely to be related to the presence of photosynthetic algae within the coral polyps which are known to absorb blue and red light with light-absorbing pigments.

### **Implications to visual communication**

According to the optical properties of these three study sites, the amount of light should not be a limiting factor for visual communication . Even at the maximum depth of

each site, there was ample light for daytime vision. Thus, downwelling light penetrated deep enough to illuminate targets even under cloudy sky conditions.

When the water column was considered as the background, targets would be seen against a background with dominant wavelengths ranging from 470nm to 536nm in the horizontal direction, depending upon location and depth. Near the surface, the downwelling light in the upward direction created a very bright background composed of medium wavelengths (green) against which targets are viewed. When looking downwards, the background light was composed of short wavelengths, corresponding to blue, when the bottom was far. It has been noted previously that upwelling light tends to have more blue short-wavelength light than downwelling light in clear oceans (Tyler and Smith, 1970). However, when the bottom is close, the spectral properties of the background depend on the spectral characteristics of the substrate.

The coral substrates did not provide very bright backgrounds against which targets would be seen. The spectral properties were dominated by medium wavelengths, typical of green light, except for *Montipora* which reflected all wavelengths equally and therefore it appeared white.

Water clarity has several important implications to visual communication. At the inshore sites, the water was less clear than offshore which means that visual communication would be less efficient over long distance at great depths. Indeed, most cleaning stations occurred within the upper 3 m (pers. obs.) where light is abundant and communicative behaviors can be easily seen. At the offshore site, depth should not limit visual communication since the water was very clear. In fact, cleaning stations can occur up to 15m in depth (pers. obs.).

This work has shown that there are several important factors to consider when characterizing the photic conditions of underwater habitats, such as time of day, weather, total depth of water, reflectivity of the bottom substrate and position in the water column. Furthermore, these factors have different effects and implications to visual communication, depending on the system of interest. Knowledge of the photic environment is critical to the understanding of visual communication systems because it affects the quality of light impinging on the viewer's retina, the light which illuminates

targets and the transmission of visual images. Thus, to effectively study visual communication in the natural environment, the optical properties of the habitat specific to the communication system must be evaluated under all conditions.

### **Chapter 3. Conspicuousness of the Hawaiian Cleaner Wrasse (*Labroides phthirophagus*) in the Reef Habitat as an Optical Signal for Cleaning**

#### INTRODUCTION

Fish associated with tropical coral reefs are known to have very distinctive colouration patterns. Past work has attempted to determine the functional significance of these colours to reef fish and several ideas have emerged. Longley (1917) suggested that reef fish possess disruptive colouration, Lorenz (1962) introduced “poster colouration” where colour patterns reduce intraspecific aggression, Zumpe (1965) thought that colouration served in species recognition, and others (Peterman, 1971; Kelley and Hourigan, 1983) proposed that colouration mediates coordination of intraspecific movements during schooling and feeding. All of these ideas stress the importance of colour patterns on visual communication between reef fish. In the case of the Hawaiian cleaner wrasse, the distinctive colouration pattern is likely to play a role in mediating interactions between host fish and the cleaner.

A communicative signal is generally defined as a stimulus which is produced by specialized structures within a single channel, usually within a narrow band of the spectrum of the particular channel (Sebeok, 1968). A colour pattern is a signal since it occupies a narrow portion of the visual channel in terms of shape, colour, brightness, flicker etc. Animals generate optical signals by creating variation in the spectral, temporal and spatial patterning of light through reflection of ambient light (Hailman, 1977; McFarland and Loew, 1983). The generation of these signals depends on the reflective and absorptive properties of the animal’s integument according to the type of pigments and structural components present.

The overall colour pattern of an animal is composed of a mosaic of patches, each with a different size, shape, colour and brightness (Endler, 1978). When the patches of a colour pattern represent a random sample of the background, in terms of size, shape, colour and brightness, the probability of detection is minimized and the colour pattern is said to be cryptic (Endler, 1978). Similarly, a colour pattern which is composed of patches that deviate from each other as well as the background, in size, shape, colour or

brightness, is highly detectable and therefore conspicuous. The degree of crypsis or conspicuousness of a colour pattern clearly depends upon the characteristics of the background against which the animal is seen (Endler, 1978, 1980; Levine et al., 1980).

The importance of background conditions in the evolution of animal colouration patterns has been recognized (eg. Endler, 1984, 1991; Godfrey et al., 1987; Kohda and Watanabe, 1986; Marchetti, 1993), however, very little work has addressed this issue for coral reef fishes. In the coral reef habitat, the background against which fish are viewed is highly complex and spectrally variable in time and space. These background conditions must be considered to evaluate the apparent conspicuousness of fish on coral reefs. The hypothesis of this work was that the colouration pattern of the Hawaiian cleaner wrasse provides a high degree of contrast from within its pattern, i.e. between patches and against the background, thereby rendering it highly conspicuous in the reef environment. In fact, the idea that cleaner wrasses evolved maximum contrast with their surroundings to be highly conspicuous was originally proposed in one of the first accounts of cleaning symbiosis (Limbaugh, 1961) but it has never been tested.

The conspicuousness of a colour pattern is directly related to the amount of contrast provided by the pattern in the natural environment. That is, patterns which have a high degree of contrast are more conspicuous. Contrast is defined as the relative difference between target radiance and background radiance (Lythgoe, 1979). There are two types of contrast which are important in visual communication. Luminance contrast refers to differences in brightness which results from amplitude differences between light spectra. Spectral contrast refers to differences in colour or hue which is described by differences in shapes of light spectra .

The purpose of this component of the project was to evaluate the colour pattern and dance behaviour of the Hawaiian cleaner wrasse as possible optical signals which act to attract host fish. The objectives were first, to assess the conspicuousness of the cleaner wrasse in the natural environment by determining the amount of luminance and spectral contrast provided by the colour pattern of the cleaner wrasse. The apparent conspicuousness was assessed in different microhabitats to determine how conspicuousness was affected by changes in optical properties between locations. The

second objective was to determine if the cleaner wrasse was more conspicuous than other commonly occurring reef fish. Thirdly, the body movements of the dance were analyzed.

## MATERIALS AND METHODS

Four fish species were studied: Threadfin butterflyfish (*Chaetodon auriga*), Hawaiian cleaner wrasse (*Labroides phthiophagus*), Saddle wrasse (*Thalassoma duperrey*) and the Yellow tang (*Zebrasoma flavescens*). Fish were hand caught with nets from Kaneohe Bay at the Hawaiian Institute for Marine Biology. The fish were anaesthetized with 1g/l MS-222. When the fish were completely anaesthetized, they were placed on a flat white surface in about 1-2 cm of water. All measurements took place outside with the sun as the light source, between 12:00-15:00 hours under sunny sky conditions.

Reflectance spectra from individual colour patches (Fig. 3.1) were obtained with a LiCor spectroradiometer (LiCor, Lincoln, Nebraska, model LI-1800UW) fitted with a fiber optic probe (Oriel). The aperture of the fiber optic was placed approximately 1 cm away from the surface of the fish. Each patch was scanned three times and the mean spectrum was used. A reflectance standard made of compressed barium sulfate was scanned and all spectra were expressed relative to this standard reflectance. This standardized the reflectance measurements with respect to time of day and sky conditions. The relative area of each colour patch was determined by digitizing images of each fish species (Bioscan OPTIMAS) and determining the proportional area which each colour patch occupied on the fish's body. The percent reflectances were then corrected for area and these values (%R) were used in all calculations.

### **Contrast Calculations**

Examples of all types of contrast calculations are provided in Appendix 1. All contrast values were calculated over a wavelength range of 300-850nm to correspond to the measurements of the photic conditions in the environment.

Two types of contrast were calculated. Luminance contrast (LC) was calculated with the following equation (Lythgoe, 1988):

$$LC = \left| \frac{R_t - R_b}{R_b} \right|$$

where  $R_t$  is the radiance from the target and  $R_b$  is the radiance from the background.

Luminance contrast was calculated for all pairs of adjacent colour patches. Only adjacent pairs of colour patches were considered because, at a given point in time, the viewer can only focus on one point on the target and contrast results from two patches. The resultant value was negative when the target radiance was less than the background radiance, which means that the target was darker than the background. However, absolute values were used in computations of means, since both darkness or brightness can provide luminance contrast.

Spectral contrast (SC) was determined by calculating Euclidean distances between pairs of reflectance spectra from the colour patches of the fish. Each pair of spectra was corrected for brightness differences (Endler, 1990).

$$SC = \sqrt{\sum_{all \lambda} (S_1 - S_2)^2}$$

where  $S_1$  is the spectrum for patch 1 and  $S_2$  is the spectrum for patch 2.

Contrast values were computed for three conditions. In the first condition, referred to as inherent contrast, the effect of impinging light on the contrast provided by each fish was assessed. That is, the contrast within each pattern, provided between patches, was determined and no background conditions were considered. This contrast was computed by multiplying %R spectra from colour patches by downwelling irradiance (impinging light) from two depths, surface and midwater, at each site. The surface depth that was used was 3m for all sites, while the midwater depth was 5m, 7m and 15m for the 5m, 15m and 25m sites respectively. For the second condition, contrast was determined between the fish and the water as background. In this case, %R from fish patches were multiplied by downwelling irradiance (impinging light) at 3 sites for 2 depths and compared to the mean horizontal radiance at each site (background). For the 25m site, irradiance data was converted to radiance values and since horizontal radiance was not available, radiance in the upwelling direction was used instead. The third condition was an evaluation of the contrast provided by the fish against the coral background. The coral

species which were considered included the substrates for which reflectance spectra were obtained: *Montipora verrucosa*, *Porites compressa* (green and brown morph), coral rubble and sand. For this situation, the product of %R from fish patches and downwelling irradiance incident on each coral substrate (converted from radiance) was compared to horizontal reflectance from the coral.

Mean LC, SC and standard errors for each fish species were calculated from contrast values from all colour patches. Mean overall LC and SC were also calculated for each species.

To test for significant differences between fish in mean overall contrast, the Kruskal-Wallis test for nonparametric data was used. However, mean overall values may underestimate contrast of a fish if many low-contrast patches are present. Therefore, these tests were also run using the contrast value from the patch which provided highest contrast on each fish, i.e. white body for *C. auriga*, yellow head for *L. phthirophagus*, green body for *T. duperrey* and yellow body for *Z. flavescens*. The statistical tests were examined at a probability level of 0.05. Identifying which species were significantly different from each other, in terms of contrast, was accomplished by running the Mann-Whitney test between pairs of data. In this test, the same set of observations was used so the probability of making a Type I error (rejecting the null hypothesis when it is true) is less than 0.05 (Day and Quinn, 1989). Therefore, the Bonferroni method was used to assign significance to the Mann-Whitney test by adjusting the probability to  $0.05/n$ , where  $n$  is the number of tests in the same family. In this case, six unplanned comparisons were conducted for both LC and SC, therefore the Bonferroni adjusted  $p$  value was 0.008.

Luminance and spectral contrast within visual backgrounds was also determined. The contrast within the water column was assumed to be negligible because it is homogeneous at a given point in space. In the case of coral, pairwise comparisons between *Montipora*, sand, rubble and the two colour morphs of *Porites* were used in the contrast equations.

## **Dance**

To study the dance swimming behaviour, underwater video recordings were obtained with an underwater HI-8 (Hitachi model VMH-81A) video camera. Recordings

were taken on patch reefs near the 15m study site. The video tapes were then analyzed to obtain a behavioural sequence representing the swimming pattern of the dance. An outline of the cleaner wrasse's body was traced from the video recordings to determine the postural movements of the dance. An image was obtained about every 5 frames of tape to ensure accuracy. The amplitude of the vertical oscillations was determined by measuring the angle between the horizontal axis along the direction of movement and the mid-line of the cleaner's body.

## RESULTS

### **Spectral Reflectance**

The spectral reflectance curves of fish colour patches show the amount of light reflected by each colour patch, i.e. brightness, as well as the spectral characteristics of the patch. For *C. auriga* (Fig. 3.2), the yellow tail reflected a moderate amount of light (40%) between 540nm-700nm with a sharp cut-off region at 500 nm. The white patch reflected most light (70%) and it had a broad spectrum, indicating it reflected most wavelengths equally. The black eyebar reflected the least amount of light (10%). Unlike the white patch, black did not reflect any particular wavelength very well, therefore the spectrum appears broad and flat. The orange patch reflected about 20% of the incident light, with more light reflected between 600-700nm due to a fairly sharp cut-off from 520 to 580 nm. The yellow stripe on the tail did not reflect much light and it showed a slight cut-off between 500 and 540nm.

The spectral reflectance curve of *L. phthiophagus* (Fig. 3.3) shows that most light was reflected by the yellow head. In fact, beyond 500nm the amount of reflected light increased by about 20%. The black stripe reflected about 10% and the shape of the curve is quite flat. The pink tail reflected about 20% of incident light. This patch showed a peak around 400-420nm and strong absorption between 500-580nm. The percent reflectance of the blue-black patch was also about 20%. This patch had a small, broad peak at 400-460nm and absorbed between 600-650nm.

For *T. duperrey* (Fig. 3.4), all patches reflected a similar amount of light (10-15%). The blue head had a peak around 400nm. The spectrum from the green patch had

a fairly broad shape with a small peak around 520-540nm. The main characteristic of the red patch was an absorption band between 400-580nm.

The spectra from the patches of *Z. flavescens* (Fig. 3.5) were similar in shape. There was a sharp cut-off near 500nm since all three areas are yellow in colour, however they varied in brightness. Most light was reflected by the anal fin, while the main body reflected slightly less. The dorsal part of the body reflected least light, relative to the other patches, and the shape was slightly more broad than the other two patches. This may be due to the presence of melanin granules on this area of the body (pers. obs.).

### **Trends in contrast values**

The light impinging on the fish affected the amount of inherent contrast. The highest LC (Table 3.1) occurred at different sites and depths for each fish. The highest SC values (Table 3.2) for all fish occurred at the 5m site at 3m in depth. Generally, the magnitude of LC was not as variable as SC.

At the inshore sites, SC values were greater for the 3m depth than for the deeper locations. On the other hand, SC values at the 25m site were relatively smaller at the shallow depth (3m) compared to the 15m depth. Thus, as the spectrum of downwelling light underwent changes with depth, this caused the reflectance from fish to differ in spectral quality. Furthermore, the degree to which this occurred depended on the local water properties.

When water was considered as the background, the highest LC (Table 3.3) and SC values (Table 3.4) occurred at 3m in depth at the 25m site. The lowest contrast occurred at the 15m site at 7m in depth for all fish except in the case of the SC for *T. duperrey* and *C. auriga* which occurred at 3m in depth.

With coral as the background, the highest LC (Table 3.5) occurred with the green morph of *Porites compressa* as the background, except for *Z. flavescens* where *Montipora* provided the greatest amount of LC. Lowest LC values resulted when the brown morph of *Porites compressa* was the background for *L. phthiophagus* and *Z. flavescens*. For *T. duperrey*, the lowest LC was provided by *Montipora* as the background and for *C. auriga* it was coral rubble. The highest SC (Table 3.6) occurred when the brown morph of *Porites compressa* was considered. The lowest SC occurred when sand was the

background substrate, with the exception of *L. phthiophagus* which had its lowest SC against coral rubble.

The amount of contrast within the environment due to coral substrates was found to be quite low in terms of LC, but relatively high in SC (Table 3.7). The mean LC provided by all types of coral was 0.556, with the greatest LC (1.511) between rubble and sand and the lowest LC between *Montipora* and green *Porites*. The mean SC of all coral was 28.77. In this case, most SC was provided by *Montipora* and rubble (43.31), while *Montipora* and sand provided least SC (18.7).

For each fish species, there was a specific colour patch which contributed most to the contrast provided by the fish. With respect to inherent luminance contrast, most contrast was provided by the combination of black/white patches on *C. auriga*, the yellow head/blue-black area on *L. phthiophagus*, the red/green patches of *T. duperrey* and the dorsal/body of *Z. flavescens*. The greatest inherent spectral contrast resulted from the combination of orange/white on *C. auriga* for all cases, except when 15m water from 25m site was used as impinging light where the yellow tail/white patch had the highest inherent spectral contrast. For the other fish, most inherent spectral contrast was provided by the yellow head/black stripe on *L. phthiophagus*, the red/green patches of *T. duperrey* and dorsal/body on *Z. flavescens*. When water and coral were considered as the background, the greatest LC and SC were due to the white patch of *C. auriga*, the yellow head on *L. phthiophagus*, the green body of *T. duperrey* and the body of *Z. flavescens*.

### **Differences in contrast between fish species**

When mean overall contrast was considered, the fish did not show significant differences in their contrast values, except for inherent LC and inherent SC. In the case of inherent LC, *L. phthiophagus* provided a significantly greater amount than the other three species (Table 3.8). For inherent SC values, *C. auriga* showed a significantly higher degree of SC than the other fish (Table 3.9). Thus, even though *L. phthiophagus* provided the maximum amount of LC compared to these other fish, it provided a low to moderate amount of SC.

When the analysis was done using the contrast values from the patch which provided highest contrast on each fish, the results were slightly different. For this

situation, significant differences in contrast occurred between fish for inherent SC and LC and when coral was the background for LC. The analysis of inherent contrast values showed the same relationship as was determined using the overall contrast. That is, *L. phthiophagus* had significantly greater LC than the other fish (Table 3.8), while *C. auriga* had significantly greater SC than other species (Table 3.9). When fish were compared to a background of coral substrate, *C. auriga* had significantly higher LC than both *T. duperrey* and *L. phthiophagus* while the LC of *Z. flavescens* was significantly higher than *T. duperrey* (Table 3.8).

### **Dance**

It was expected that the dance would be displayed by almost every individual, however it was observed far less frequently than expected. During field observations, the dance was observed by four individuals and two of these were juvenile cleaner wrasses, which were identifiable by their colouration (Mahon, 1994). Only two sequences were captured on video tape out of a total of 95 minutes of recorded cleaning behaviour of approximately 20 individuals. For those adults who did display the dance, it was performed when hosts were in close proximity as well as when no hosts were apparent. Of the dances which were observed, the behaviour was very consistent. It always involved body oscillations in the vertical direction with minimal horizontal movement. A typical sequence of body postures during the dance is shown in Figure 3.6. This sequence lasted 1.8 seconds and three body oscillations occurred. The amplitude of first and second oscillations was  $5^{\circ}$  from the horizontal and the third was  $25^{\circ}$  degrees from the horizontal. The mean amplitude of the vertical oscillations was  $11.6^{\circ}$  from the horizontal.

**Table 3.1.** Inherent luminance contrast for pairwise comparisons between colour patches of 4 species of reef fish. The pair of colour patches for each contrast determination is shown in parenthesis by abbreviation of the first letter for each patch (as shown in Figure 3.1). The first patch of the pair was arbitrarily chosen as the background and the second was the target. The order of the colour patch comparisons for each fish is consistent across all impinging light conditions. Mean values and standard errors for individual impinging light conditions are shown in bold. Overall mean contrast and standard error from all types of impinging light are shown at the bottom of each column.

Impinging Light Site/Depth	LUMINANCE		CONTRAST	
	<i>C. auriga</i>	<i>L. phthiophagus</i>	<i>T. duperrey</i>	<i>Z. flavescens</i>
5m Site	-0.776 (YS/YT)	1.92 (Y/BS)	-0.25 (R/B)	-0.69 (D/B)
3m	-0.987(B/W) -0.983(O/W) -0.954(YT/W)	-0.467 (BB/BS) -0.583 (PT/BS) 4.54 (YH/BB) 0.559 (BB/P)	-0.822 (R/G)	-0.96 (A/B)
	<b>-0.925±0.05</b>	<b>1.194±0.95</b>	<b>-0.536±0.29</b>	<b>-0.825±0.14</b>
5m	-0.767 -0.988 -0.986 -0.959	1.97 -0.464 -0.571 4.6 0.584	-0.275 -0.826	-0.68 -0.96
	<b>-0.925±0.05</b>	<b>1.224±0.96</b>	<b>-0.551±0.28</b>	<b>-0.82±0.14</b>
15m Site				
3m	-0.778 -0.986 -0.982 -0.953	1.9 -0.476 -0.586 4.53 0.56	-0.246 -0.822	-0.69 -0.96
	<b>-0.925±0.05</b>	<b>1.186±0.95</b>	<b>-0.534±0.29</b>	<b>-0.825±0.14</b>
7m	-0.772 -0.988 -0.985 -0.957	1.94 -0.472 -0.579 4.57 0.568	-0.268 -0.825	-0.686 -0.96
	<b>-0.926±0.05</b>	<b>1.205±0.96</b>	<b>-0.547±0.28</b>	<b>-0.823±0.14</b>
25m Site				
3m	-0.775 -0.987 -0.983 -0.954	1.974 -0.468 -0.575 4.59 0.59	-0.254 -0.823	-0.69 -0.96
	<b>-0.775±0.05</b>	<b>1.222±0.96</b>	<b>-0.539±0.28</b>	<b>-0.825±0.14</b>
15m	-0.767 -0.988 -0.986 -0.959	1.93 -0.472 -0.582 4.55 0.561	-0.275 -0.827	-0.68 -0.96
	<b>-0.925±0.05</b>	<b>1.197±0.95</b>	<b>-0.551±0.28</b>	<b>-0.82±0.14</b>
SPECIES MEAN	-0.996	1.62	0.54	0.82
N	24	30	12	12
SE	0.06	0.28	0.09	0.05

**Table 3.2.** Inherent spectral contrast for pairwise comparisons between colour patches of 4 species of reef fish. The pair of colour patches used in each contrast determination is shown in parenthesis by abbreviation of the first letter for each patch (as shown in Figure 3.1). The order of the colour patch comparisons for each fish is consistent across all impinging light conditions. Mean values and standard errors for individual impinging light conditions are shown in bold. Overall mean contrast and standard error for each fish from all types of impinging light are shown at the bottom of each column.

Impinging Light Site/Depth	SPECTRAL CONTRAST			
	<i>C. auriga</i>	<i>L. phthiophagus</i>	<i>T. duperrey</i>	<i>Z. flavescens</i>
5m Site	0.0554 (YS/YT)	0.923 (Y/BS)	0.11 (R/B)	0.967 (D/B)
3m	0.482 (B/W)	0.197 (BB/BS)	0.264 (R/G)	0.583 (A/B)
	6.699 (O/W)	1.501 (P/BS)		
	4.484 (YT/W)	0.704 (YH/BB)		
		0.317 (BB/PT)		
	<b>2.93±1.6</b>	<b>0.728±0.23</b>	<b>0.187±0.08</b>	<b>0.775±0.19</b>
5m	0.047	0.755	0.084	0.85
	0.249	0.165	0.206	0.5
	5.434	0.124		
	4.198	0.563		
		0.094		
	<b>2.482±1.37</b>	<b>0.34±0.13</b>	<b>0.145±0.06</b>	<b>0.675±0.18</b>
15m Site				
3m	0.028	0.718	0.052	0.488
	0.242	0.095	0.126	0.304
	3.279	0.0857		
	2.284	0.349		
		0.064		
	<b>1.458±0.79</b>	<b>0.262±0.13</b>	<b>0.089±0.04</b>	<b>0.396±0.09</b>
7m	0.019	0.304	0.0359	0.347
	0.13	0.069	0.091	0.214
	1.907	0.0421		
	1.614	0.222		
		0.081		
	<b>0.918±0.49</b>	<b>0.144±0.05</b>	<b>0.0635±0.03</b>	<b>0.281±0.07</b>
25m Site				
3m	0.029	0.434	0.061	0.474
	0.274	0.115	0.139	0.284
	2.899	0.0986		
	2.177	0.369		
		0.0628		
	<b>1.345±0.71</b>	<b>0.216±0.08</b>	<b>0.1±0.04</b>	<b>0.379±0.1</b>
15m	0.035	0.423	0.0788	0.534
	0.233	0.159	0.179	0.305
	2.199	0.117		
	2.382	0.448		
		0.0665		
	<b>1.212±0.62</b>	<b>0.243±0.08</b>	<b>0.129±0.05</b>	<b>0.42±0.11</b>
SPECIES MEAN	1.72	0.322	0.119	0.488
N	24	30	12	12
SE	0.40	0.06	0.02	0.08

**Table 3.3.** Luminance contrast between colour patches of 4 reef fish against water as the background. Colour patches are identified in parenthesis by abbreviation of the first letter for each patch (as shown in Figure 3.1). The order of the colour patches for each species is consistent across all sites and depths of water. Mean values and standard errors for individual water backgrounds are shown in bold. Overall mean contrast values and standard errors for each fish from all sites and depths are at the bottom of each column.

Site /Depth	LUMINANCE		CONTRAST	
	<i>C. auriga</i>	<i>L. phthiophagus</i>	<i>T. duperrey</i>	<i>Z. flavescens</i>
5m Site	23.2(YT)	130.8 (YH)	29.6 (BH)	0.59 (B)
3m	465 (W)	32.96(BS)	131.96 (G)	495.7 (D)
	3.62(B)	16.97 (PT)	23.9(R)	106.6 (A)
	8.04 (O)	16.78 (BB)		
	1.98 (YS)			
	<b>100.37±91.2</b>	<b>49.38±27.4</b>	<b>61.82±35.1</b>	<b>200.96±150.5</b>
5m	10.2	132.6	13.92	227.6
	221.9	34.47	62.6	49.23
	1.22	17.57	10.8	8.04
	3.14	17.51		
	0.392			
	<b>47.37±43.6</b>	<b>50.54±27.6</b>	<b>29.11±20.5</b>	<b>94.96±82.5</b>
15m Site				
3m	4.57	65.2	5.996	107.4
	104.5	17.31	29.73	23.33
	0.08	8.28	4.75	3.36
	1.04	9.1		
	-0.298			
	<b>21.978±20.6</b>	<b>24.97±13.6</b>	<b>13.49±8.1</b>	<b>44.7±31.9</b>
7m	4.24	62.65	6.59	107.9
	114.3	15.81	30.95	22.57
	0.103	7.59	4.67	3.16
	0.833	7.85		
	-0.334			
	<b>23.84±22.6</b>	<b>23.48±13.2</b>	<b>14.07±8.5</b>	<b>44.54±32.2</b>
25m Site				
3m	292.2	3307.8	300.5	5518.7
	4547.7	779.5	1392.3	1183.7
	48.6	457.5	257.2	225.2
	13.2	429.6		
	34.43			
	<b>1005.2±891.5</b>	<b>1243.6±692.6</b>	<b>650±371.4</b>	<b>2309.2±1628.4</b>
15m	50.31	572.2	75.3	990.9
	1150	152.7	317.3	207.8
	10.46	71.57	54.68	35.75
	15.27	78.23		
	5.34			
	<b>246.3±226.1</b>	<b>218.68±119.3</b>	<b>149.1±84.3</b>	<b>411.5±293.9</b>
SPECIES MEAN	494.6	268.4	152.9	517.6
N	30	24	18	18
SE	131.3	138.2	82.5	327.6

**Table 3.4.** Spectral contrast between colour patches of 4 reef fish against water as the background. Colour patches are identified in parenthesis by abbreviation of the first letter for each patch (as shown in Figure 3.1). The order of the colour patches for each species is consistent across all sites and depths of water. Mean values and standard errors for comparisons against each type of water background are shown in bold. Overall mean contrast values and standard errors for each fish from all sites and depths are at the bottom of each column.

Site/Depth	SPECTRAL CONTRAST			
	C. auriga	L. phthirophagus	T. duperrey	Z. flavescens
5m Site	0.38 (YT)	1.918 (YH)	0.43 (BH)	7.961 (B)
3m	5.906 (W)	0.47 (BS)	1.797 (G)	1.715 (D)
	0.065 (B)	0.277 (PT)	0.386 (R)	0.319 (A)
	0.162 (O)	0.222 (BB)		
	0.047 (YS)			
	<b>1.312±1.15</b>	<b>0.722±0.4</b>	<b>0.841±0.46</b>	<b>3.332±3.1</b>
5m	0.348	4.123	0.392	7.398
	6.065	1.079	1.754	1.64
	0.078	0.62	0.378	0.164
	0.265	0.532		
	0.055			
	<b>1.362±1.18</b>	<b>1.589±0.85</b>	<b>0.871±0.46</b>	<b>3.067±2.21</b>
15m Site				
3m	0.167	1.914	0.185	3.294
	2.527	0.53	0.787	0.756
	0.04	0.3	0.183	0.145
	0.114	0.279		
	0.031			
	<b>0.576±0.49</b>	<b>0.756±0.39</b>	<b>0.385±0.20</b>	<b>1.398±0.96</b>
7m	0.071	0.775	0.081	1.477
	1.083	0.194	0.321	0.322
	0.017	0.122	0.082	0.062
	0.04	0.089		
	0.014			
	<b>0.245±0.24</b>	<b>0.295±0.24</b>	<b>0.161±0.08</b>	<b>0.626±0.43</b>
25m Site				
3m	0.489	5.35	0.425	9.22
	6.489	1.192	2.05	1.94
	0.075	0.722	0.401	0.377
	0.176	0.637		
	0.0566			
	<b>1.457±1.26</b>	<b>1.975±1.13</b>	<b>0.959±0.55</b>	<b>3.846±2.72</b>
	0.12	1.017	0.067	2.253
	1.14	0.0186	0.345	0.41
	0.014	0.059	0.084	0.079
	0.039	0.062		
	0.011			
	<b>0.265±0.22</b>	<b>0.331±0.24</b>	<b>0.165±0.09</b>	<b>0.914±0.68</b>
SPECIES MEAN	0.869	0.938	0.564	2.20
N	30	24	18	18
SE	0.63	0.26	0.15	0.69

**Table 3.5.** Luminance contrast between colour patches of 4 reef fish against coral substrates as the background. Colour patches are identified in parenthesis by abbreviation of the first letter for each patch (as shown in Figure 3.1). The order of the colour patches for each species is consistent across all coral substrates. Mean values and standard errors for individual coral substrates are shown in bold. Overall mean contrast values and standard errors for each fish from all coral substrates are at the bottom of each column.

Coral substrate	LUMINANCE		CONTRAST	
	<i>C. auriga</i>	<i>L. phthirophagus</i>	<i>T. duperrey</i>	<i>Z. flavescens</i>
<i>Montipora verrucosa</i>	49.02 (YT)	576.9 (YH)	20.9 (BH)	1011.7 (B)
	1565.2 (W)	207.5 (BS)	83.4 (G)	235.6 (D)
	12.7 (B)	81.9 (PT)	13.9 (R)	37.9 (A)
	14.8 (O)	104.1 (BB)		
	6.2 (YS)			
	<b>329.58±308.9</b>	<b>242.68±114.7</b>	<b>39.4±22.1</b>	<b>428.4±297.2</b>
<i>Porites compressa</i> (Brown morph)	23.04	320.6	44.1	486.2
	673.9	92.5	183.9	110.1
	5.2	44.8	28.7	17.8
	7.02	53.8		
	2.49			
	<b>142.33±132.9</b>	<b>127.93±65.1</b>	<b>85.57±49.4</b>	<b>204.7±143.2</b>
<i>Porites compressa</i> (Green morph)	42.7	645.5	140.1	901.7
	1886.5	247.9	534.5	233
	15.2	104.1	85.4	35.4
	12.3	137.9		
	6.5			
	<b>392.64±373.5</b>	<b>283.85±134.4</b>	<b>253.3±141.5</b>	<b>390.03±262.1</b>
Coral Rubble	23.4	328.7	56.2	487.4
	816.8	108.7	225.7	113.5
	6.1	44.9	34.4	17.55
	6.4	62.3		
	2.7			
	<b>171.08±161.5</b>	<b>136.15±65.6</b>	<b>105.4±60.5</b>	<b>206.15±143.3</b>
Sand	34.969	511.16	0.092	738.6
	1207.7	161.86	0.335	176.6
	9.84	78.46	0.06	28.1
	10.97	94.39		
	4.73			
	<b>253.6±238.6</b>	<b>211.47±101.5</b>	<b>155.95±0.09</b>	<b>314.5±216.4</b>
SPECIES MEAN	257.9	200.4	96.8	308.7
N	25	20	15	15
SE	139.8	41.1	31.1	87.2

**Table 3.6.** Spectral contrast between colour patches of 4 reef fish against coral substrates as the background. The colour patches are identified in parenthesis by abbreviation of the first letter for each patch (as shown in Figure 3.1). The order of the colour patches for each fish is consistent across all coral backgrounds. Mean values and standard errors for individual coral substrates are shown in bold. Overall mean contrast and standard errors for each fish from all coral substrates are at the bottom of each column.

Substrate	SPECTRAL		CONTAST	
	<i>C. auriga</i>	<i>L. phthirophagus</i>	<i>T. duperrey</i>	<i>Z. flavescens</i>
<i>Montipora verrucosa</i>	0.245 (YT)	1.84 (YH)	0.138 (BH)	4.79 (B)
	3.585 (W)	0.571 (BS)	0.49 (G)	0.892 (D)
	0.033 (B)	0.338 (PT)	0.0995 (R)	0.179 (A)
	0.109 (O)	0.202 (BB)		
	0.019 (YS)			
	<b>0.798±0.62</b>	<b>0.738±0.32</b>	<b>0.243±0.10</b>	<b>1.95±1.17</b>
<i>Porites compressa</i> (Brown morph)	0.851	7.97	1.063	16.04
	13.96	2.23	3.95	3.25
	0.152	2.376	0.759	0.65
	0.48	1.405		
	0.0977			
	<b>3.108±2.71</b>	<b>3.49±1.51</b>	<b>1.924±1.02</b>	<b>6.65±4.76</b>
<i>Porites compressa</i> (Green morph)	0.118	1.615	0.633	2.46
	7.303	0.99	2.175	0.087
	0.063	0.477	0.369	0.116
	0.057	0.564		
	0.024			
	<b>1.51±1.45</b>	<b>0.912±0.26</b>	<b>1.06±0.56</b>	<b>0.888±0.79</b>
Coral Rubble	0.088	0.464	0.169	1.54
	1.967	0.241	0.54	0.202
	0.017	0.138	0.0766	0.048
	0.039	0.188		
	0.0059			
	<b>0.423±0.39</b>	<b>0.258±0.072</b>	<b>0.262±0.14</b>	<b>0.597±0.47</b>
Sand	0.032	5.21	0.092	0.677
	1.163	0.275	0.335	0.18
	0.0113	0.108	0.06	0.031
	0.0155	0.101		
	0.0057			
	<b>0.423±0.23</b>	<b>1.424±1.26</b>	<b>0.162±0.09</b>	<b>0.296±0.19</b>
SPECIES MEAN	1.22	1.37	0.73	2.08
N	25	20	15	15
SE	0.62	0.44	0.27	1.1

**Table 3.7.** Luminance and spectral contrast within the coral background determined by pairwise comparisons between different coral substrates. The first substrate in the pair was arbitrarily chosen as the target and the second the background. Negative luminance contrast values mean that the target coral (first type in pair) was darker than the background coral. Mean values and standard errors are shown in bold.

Coral Substrate	Luminance Contrast	Spectral Contrast
Montipora/Rubble	-0.406	43.31
Montipora/ Porites (brown)	-0.228	20.5
Montipora/ Porites (green)	-0.075	34.11
Montipora/Sand	0.365	18.7
Rubble/Porites (brown)	0.412	29.12
Rubble/ Porites (green)	0.677	40.7
Rubble/Sand	1.511	29.0
Porites (brown)/Porites (green)	0.192	33.06
Porites (brown)/Sand	0.953	19.83
Porites (green)/ Sand	0.738	19.39
<b>MEAN</b>	<b>0.556±0.14</b>	<b>28.77±2.86</b>

**Table 3.8.** Statistical results from Kruskal-Wallis Test (H statistic) for differences in luminance contrast between fish species (Td = *Thalassoma duperrey*, Zf = *Zebrasoma flavescens*, Ca = *Chaetodon auriga*, Lp = *Labroides phthiophagus*). Tests were run using mean overall contrast values and mean values from the patch of highest contrast of each fish. Significance is indicated by asterices. Comparisons show results of pairwise Mann-Whitney tests; fish are ranked in order from least to greatest (left to right). Underlined fish are not significantly different from each other at  $p = 0.008$ .

Luminance Contrast	H statistic	N	p-value	Comparisons
inherent (overall)	20.543	24	<0.001*	Td Zf Ca Lp
inherent (patch)	21.656	24	<0.001*	Td Zf Ca Lp
water background (overall)	1.720	24	0.632	<u>Td Zf Ca Lp</u>
water background (patch)	3.967	24	0.265	<u>Lp Zf Td Ca</u>
coral background (overall)	7.7889	20	0.051	<u>Td Lp Ca Zf</u>
coral background (patch)	12.623	20	0.006*	Td <u>Lp</u> <u>Zf</u> Ca

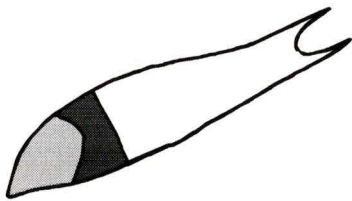
**Table 3.9.** Statistical results from Kruskal-Wallis Test (H statistic) for spectral contrast between fish species (Td = *Thalassoma duperrey*, Zf = *Zebrasoma flavescens*, Ca = *Chaetodon auriga*, Lp = *Labroides phthirophagus*). Tests were run using mean overall contrast values and mean values from the patch of highest contrast of each fish. Significance is indicated by asterices. Comparisons show results of pairwise Mann-Whitney tests; fish are ranked in order from least to greatest (left to right). Underlined fish are not significantly different from eachother at  $p = 0.008$ .







Spectral Contrast	H statistic	N	p-value	Comparisons
inherent (overall)	19.867	24	<0.001*	Td <u>Lp</u> <u>Zf</u> Ca
inherent (patch)	19.463	24	<0.001*	Td <u>Lp</u> <u>Zf</u> Ca
water background (overall)	6.393	24	0.094	<u>Td</u> Ca Lp <u>Zf</u>
water background (patch)	9.220	24	0.027	<u>Td</u> <u>Lp</u> Ca <u>Zf</u>
coral background (overall)	2.063	20	0.559	<u>Td</u> Ca Lp <u>Zf</u>
coral background (patch)	3.309	20	0.346	<u>Td</u> Lp <u>Zf</u> Ca

**Figure 3.1.** Schematic representation of the four study fish species and the location of the colour patches examined in this research. a) *Thalassoma duperrey* (Saddle wrasse) b) *Chaetodon auriga* (Threadfin butterflyfish) c) *Labroides phthirophagus* (Hawaiian cleaner wrasse) d) *Zebrasoma flavescens* (Yellow tang).

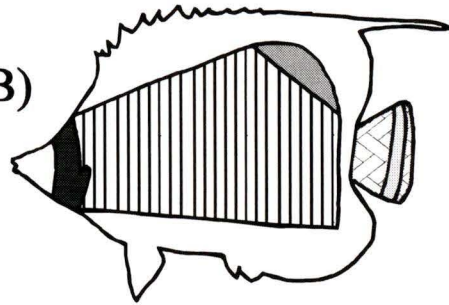
-  Blue Head (BH)
-  Red (R)
-  Green Body (GB)

(A)

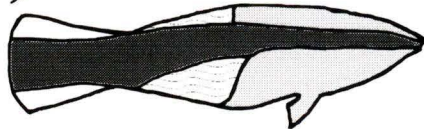


-  Yellow tail (YT)
-  White Body (W)
-  Black Eyebar (B)
-  Orange (O)
-  Yellow stripe (YS)
-  Other

(B)

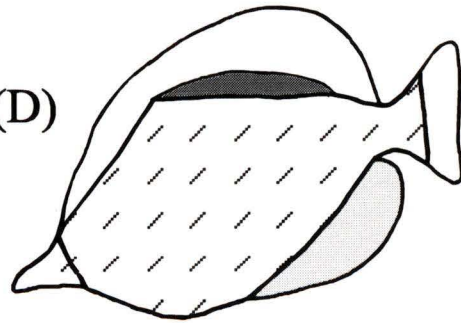




(C)



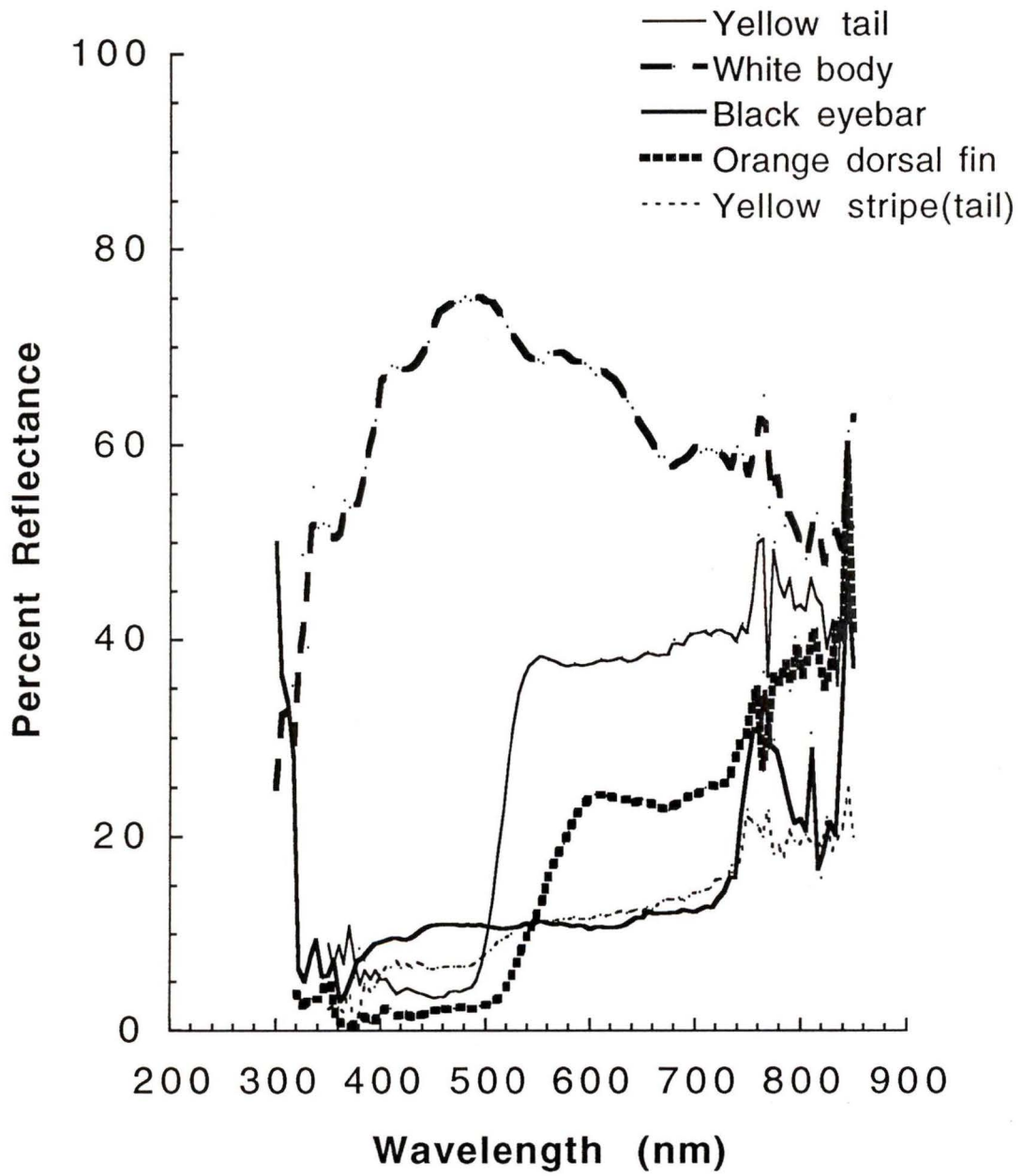
-  Yellow head (YH)
-  Black Stripe (BS)
-  Pink Tail (PT)
-  Blue-Black (BB)

(D)

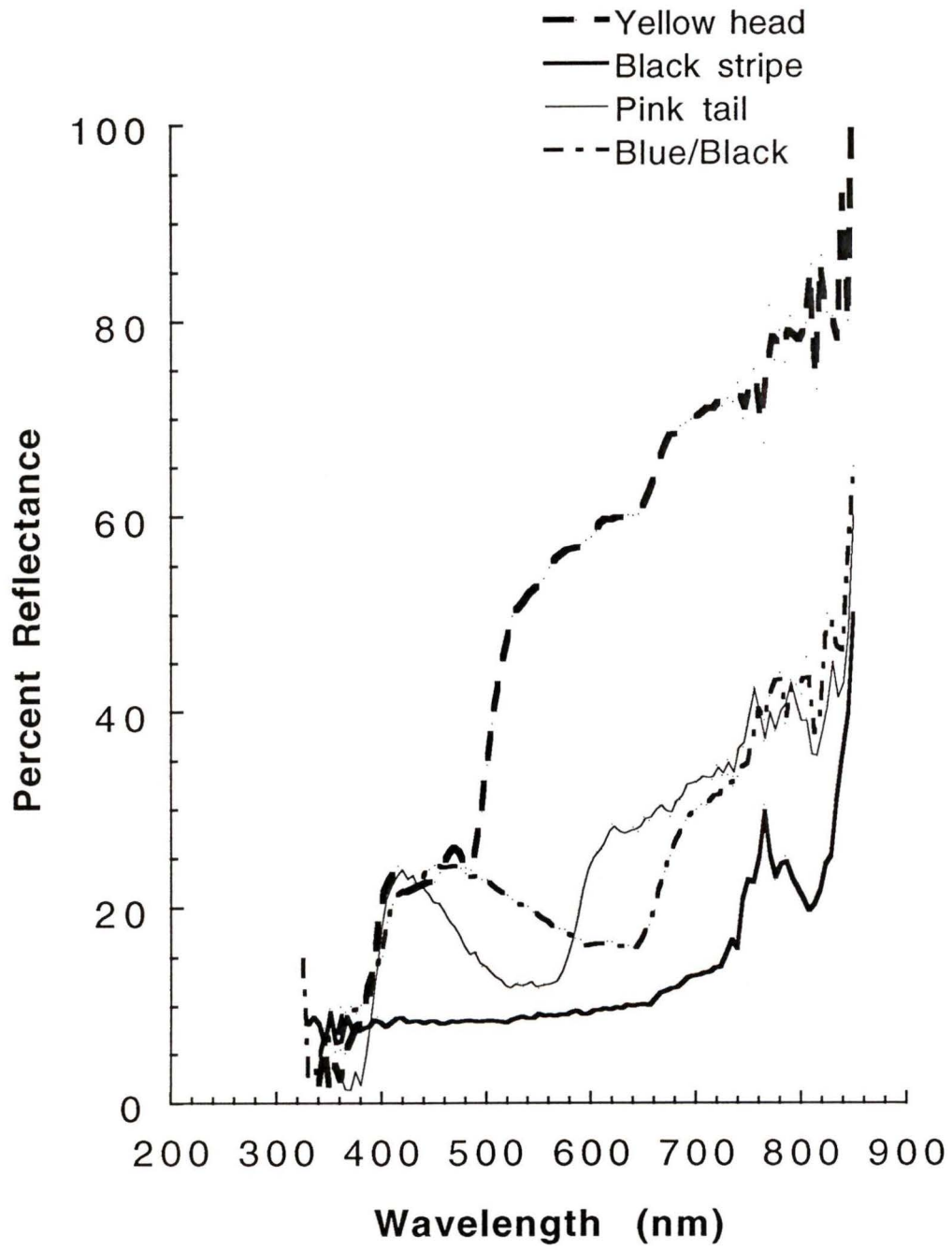


-  Main Body (B)
-  Dorsal (D)
-  Anal Fin (A)
-  Other

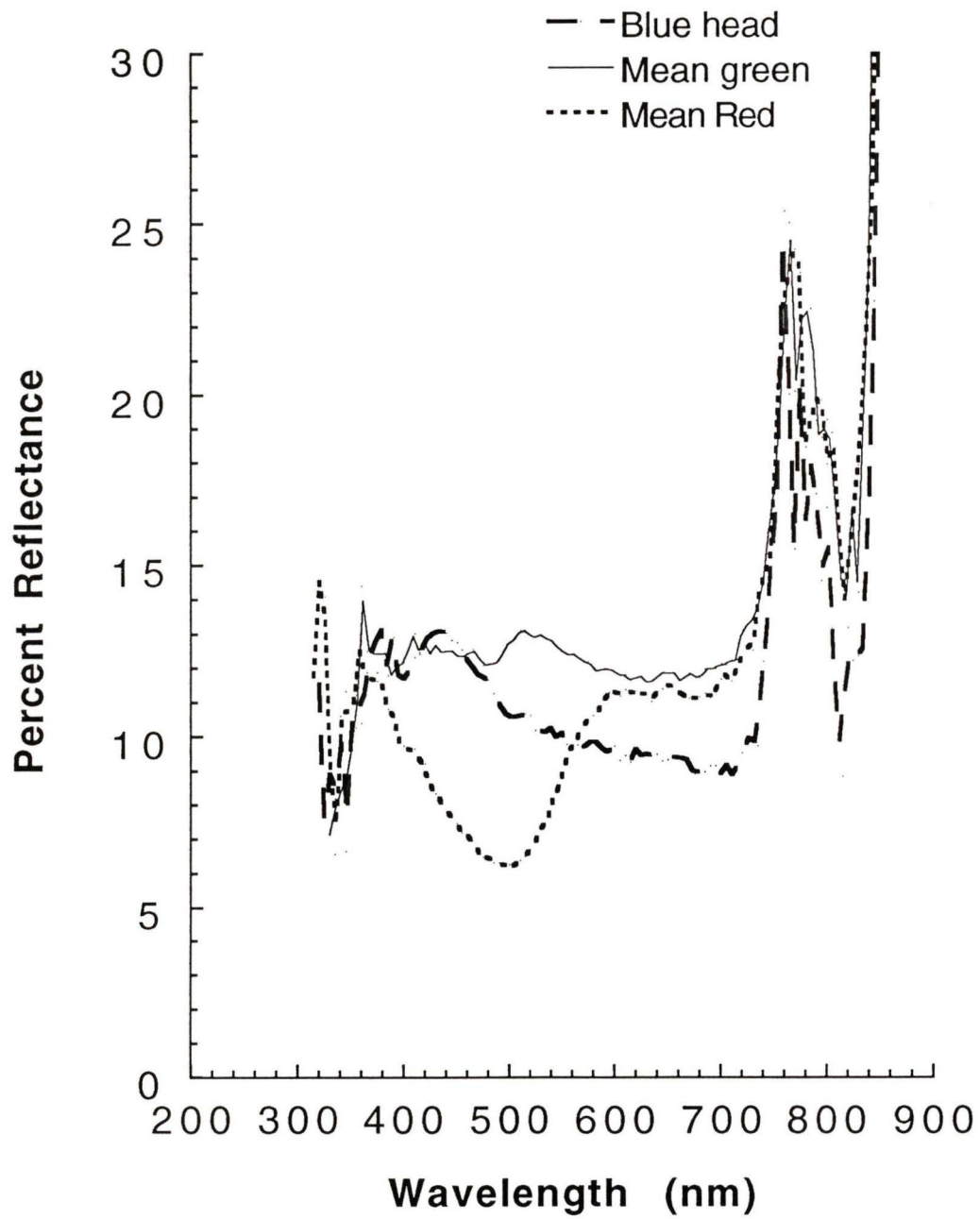
**Figure 3.2.** Percent spectral reflectance from the colour patches of *Chaetodon auriga*



**Figure 3.3.** Percent spectral reflectance from the colour patches of *Labroides phthirophagus*.



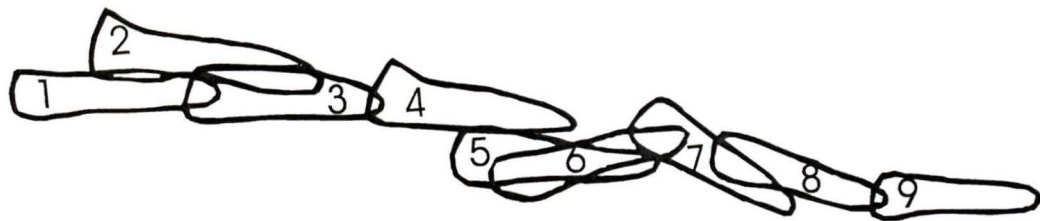
**Figure 3.4.** Percent spectral reflectance from the colour patches of *Thalassoma duperrey*.



**Figure 3.5.** Percent spectral reflectance from the colour patches of *Zebrasoma flavescens*.



**Figure 3.6.** A typical series of body positions in the dance behaviour of *Labroides phthirophagus* obtained from video tape. The numbers indicate the order in which each posture occurred. This sequence was 1.8 seconds in duration. It includes three vertical body oscillations (2, 4, 7) with a mean amplitude of 10 degrees.



Direction of Movement 

3.5 cm

## DISCUSSION

These results suggest that the *L. phthiophagus* should be conspicuous in the reef environment and thus it is possible that the colour pattern may act as an optical signal. The inherent luminance contrast of the cleaner wrasse was greater than the other fish examined in this study. In addition, the inherent spectral contrast on *L. phthiophagus* was greater than at least one common reef species, *Thalassoma duperrey*. Although the backgrounds affected the amount of contrast provided by the fish, not all of the differences in contrast between the four species were significant. This means that conspicuousness within the pattern itself was greater than the conspicuousness due to the overall contrast of each patch against the background.

Conspicuousness can also be assessed by comparing the total contrast within the pattern to the total contrast within the background surrounding the fish at a given point in space. There are two types of backgrounds which are recognized in visual communication, those which are homogenous with respect to luminance and spectral properties and those which are heterogeneous. The open water depths of aquatic systems are known to be fairly homogeneous (Hailman, 1977). Therefore, the amount of luminance and spectral contrast within the fish colour patterns was greater than that within the water column. In other words, all of these fish, including *L. phthiophagus*, were conspicuous against the water column. In fact, the cleaner wrasse was most conspicuous in terms of luminance contrast, since it had the greatest amount of luminance contrast within its pattern. *C. auriga* was most spectrally conspicuous against the water due to the high spectral contrast within its colour pattern.

The reef areas tend to be more heterogeneous in their optical characteristics than water. For the coral substrates studied in this work, the amount of luminance contrast within the coral was quite low. All fish, with the exception of *T. duperrey*, had greater luminance contrast within their colour patterns than within the coral habitat. Although, *C. auriga*, *Z. flavescens* and *L. phthiophagus* were all conspicuous against coral in terms of luminance contrast, *L. phthiophagus* was the most conspicuous since it had the greatest amount of luminance contrast within its pattern. On the other hand, the amount of spectral contrast within the coral substrates was higher than the amount within the

pattern of any of these four reef fish. Thus, these fish were spectrally inconspicuous against coral. Therefore, luminance contrast contributed more to conspicuousness of fish on the reef than spectral contrast.

In general, conspicuousness within a colour pattern is maximal when the properties of adjacent patches differ greatly. This may occur through one or more of the following ways (Endler, 1992). Firstly, if the colour pattern has light patches adjacent to dark patches, the pattern will be conspicuous in almost all light conditions, even to those animals without colour vision, since this type of conspicuousness is related to differences in brightness. This situation occurs for *L. phthiophagus* where the bright yellow head is next to the dark black stripe and for *C. auriga* where a black eyebar is adjacent to the white patch. This may explain why the luminance contrast of *L. phthiophagus* was greatest of all the fish.

The second way in which a pattern's conspicuousness is maximized occurs when the pattern matches the brightness of ambient light and water transmission spectra. When colored patches which reflect high intensity ambient light are positioned next to patches which reflect low intensity wavelengths, the pattern is highly conspicuous. This occurs in the case of the white and black patches of *C. auriga*, the yellow head and black stripe of *L. phthiophagus* and the red and green patches of *T. duperrey*.

A third mechanism which enhances the conspicuousness of a colour pattern takes place when adjacent patches are composed of complimentary colours having spectra with few or no wavelengths in common, such as red and green or yellow and blue. This occurs in *L. phthiophagus* with the yellow head next to a blue-black patch and in *T. duperrey* with a red patch next to the green body. However, the contrast provided from these patches on *T. duperrey* is relatively lower than that of *L. phthiophagus*. This may be because the colours of *T. duperrey* are unsaturated, that is they are not pure. Indeed, the green body of this fish has small flecks of other colours on its body, including white, red and black (pers. obs.).

Finally, there is a fourth way in which conspicuousness of a colour pattern is optimized. This occurs for adjacent patches composed of complimentary colours whose cutoffs occur at the wavelength of greatest ambient light intensity. To be seen as

coloured, the spectral reflectance curve of the patch must change in a region of the spectrum which coincides with available light. This occurs for *L. phthiophagus* with the yellow head and blue-black patches and for *T. duperrey* for the red and green patches. It is for this reason that yellow has been identified as the most readily visible colour in seawater (Lythgoe, 1968). In this study, yellow patches were important in providing contrast for *L. phthiophagus*, *C. auriga* and *Z. flavescens*.

Although the purpose of the dance remains unclear after this study, it is undoubtedly a stereotyped swimming behaviour which has evolved into a ritualized display. The dance motions observed in this study are consistent with those described from previous work on *L. phthiophagus* (Youngbluth, 1967; Losey, 1971). Indeed, ritualization of communicative displays is quite common in social behaviours and is thought to increase the signal value of the behaviour by minimizing ambiguity (Losey, 1971).

The reason that the dance was observed more frequently in juvenile cleaner wrasses as opposed to adults may be due to two factors. Firstly, juveniles are subject to a great deal of aggression from conspecific adults (Mahon, 1994). The dance may help to appease the adults in some manner thereby reducing the possibility of aggressive encounters. In the second place, since the juveniles tend to occupy sheltered areas on the reef, the dance may attract hosts for the juvenile to clean.

In summary, luminance contrast appears to be an important optical signal for detection of the Hawaiian cleaner wrasse on the reef. The role of the dance may not be evident as yet, however it has some characteristics typical of optical signals. It is consistent in form, it is obvious because it takes place during the day in open areas above the reef (except for juveniles) and it seems to be unique among swimming motions of reef fish. Furthermore, it is possible that the dance behaviour, as an optical signal, has more than one purpose. Finally, this work shows that the optically complex coral reef environment includes many factors which can affect conspicuousness of reef fish, including depth, local water properties and background conditions.

## **Chapter 4. Spectral Sensitivity of the Saddle Wrasse, *Thalassoma duperrey* (Family Labridae): a model for the detection of the Hawaiian Cleaner Wrasse by Host Fish.**

### INTRODUCTION

In general, there has been very little research focused on colour vision in coral reef fishes. Most of the existing information stems from quantifying the absorbance maxima for cone pigments (Munz and McFarland, 1975; Loew and Lythgoe, 1978; Levine and MacNichol, 1979; Lythgoe et al., 1994; McFarland and Loew, 1994). Several reef species have been studied in this type of research and many cone pigments have been identified. Some reef fish may possess up to 4 different cone pigments. The general types of pigments which have been found include a pigment sensitive in the ultraviolet region of the spectrum ( $\lambda_{\max}$  360nm), a violet-sensitive pigment ( $\lambda_{\max}$  408nm), a blue-sensitive pigment ( $\lambda_{\max}$  440-502nm) and a green-sensitive pigment ( $\lambda_{\max}$  513-560nm).

Although this information is valuable, absorbance maxima of visual pigments can only indicate the number of cone types, not how the cone classes interact to produce spectral sensitivity. Therefore, to assess the characteristics of a colour vision system, wavelength-discrimination tests must be performed under stringently controlled conditions to prevent other visual cues, such as brightness, from interfering. This type of information is crucial to the understanding of how fish view targets in the natural environment.

Cleaning symbiosis between coral reef fish is a diurnal phenomenon which takes place in a spectrally complex environment, therefore it is likely to be mediated by vision, specifically colour vision. Thus the visual abilities of host fish influence how hosts identify and respond to cleaner fish. *Thalassoma duperrey* is a common host fish of Hawaiian cleaner wrasses, in fact it is one of the preferred host species (Gorlick, 1980). However, little information is available for the visual biology of this species. The purpose of this study was to evaluate the spectral sensitivity of *Thalassoma duperrey* through electrophysiological recording of compound action potentials (CAP) from retinal ganglion cells (RGC).

Retinal ganglion cells collect and integrate visual information from the retina and convey it to the brain. This information leaves the retina via two pathways, involving either ON or OFF ganglion cells (Wheeler, 1979; DeMarco and Powers, 1991). The ON cells carry information about increasing light intensity, while the OFF cells carry information about decreasing light intensity. The receptive fields of RGC are comprised of concentrically arranged antagonistic center and surround areas. The cell's response depends on the degree to which the center and surround areas are stimulated and whether the cell is an ON or OFF type (Schiller, 1992).

The ON and OFF pathways have been shown to exhibit different spectral sensitivities which may encode colour opponency. Colour opponency exists when cells respond with one type of response to certain wavelengths and an opposite response to other wavelengths. This is referred to as reciprocal antagonism for specific pairs of colours since the cell depolarizes for some wavelengths and hyperpolarizes for other wavelengths (Daw, 1973).

Differences in spectral sensitivities for ON and OFF pathways can be attributed to differential input from various cone classes, each with different absorbance maxima. The spectral sensitivity of both ON and OFF pathways is thought to be related to the spectral characteristics of the photic habitat and the types of visual tasks involved (Beaudet et al., 1993). In this study, the spectral sensitivity of the ON and OFF channels of the reef fish *Thalassoma duperrey* will be evaluated. The visual abilities will then be related to detection of the optical signal provided by the colour pattern of the Hawaiian cleaner wrasse.

It is predicted that this fish should possess a visual system sensitive to UV light, blue light i.e. short wavelengths (S), and green light i.e. middle wavelengths (M) since these wavelengths are abundant in the reef environment. It is expected that this fish would not be highly sensitive to red light, i.e. long wavelengths (L), because long wavelengths attenuate rapidly in this type of water.

## MATERIALS AND METHODS

### Study Animals

Adult *Thalassoma duperrey*, 9-11 cm standard length, were obtained from Kona, Hawaii and housed in saltwater aquaria with a water temperature of 22°C and a 12hr light:dark photoperiod. Illumination was provided by 40W fluorescent aquarium lamps (Hagen) with a spectral distribution ranging from 350 to 650nm with peaks in the blue region (425-475nm), green (530nm) and red (600nm). Fish were fed TetraMin flakes for tropical fish and frozen plankton every second day. The fish were held for a minimum of 2 days before experimentation and experiments were completed within one month of each shipment. Although *T. duperrey* appears to have no retinomotor movements (McFarland, pers. comm.), experiments were conducted between 10:00 and 20:00 hrs local time to minimize diel variability.

### Preparation of the experimental fish

Each fish was anaesthetized by immersion in 50 mg/L Metomidate (Syndel). Following general anesthesia, the fish was given an intramuscular injection of 0.01 mg/g body weight of Pavulon (pancuronium bromide, Organon) to ensure immobilization during the experiment. The fish was then placed in a neoprene-lined stereotaxic holder and respired with 22°C aerated saltwater with 7 mg/L Metomidate to maintain general anaesthesia. Throughout the procedure, the fish was covered with a wet sterile gauze to ensure that the skin was moist. The right optic tectum was exposed by removing the overlying tissue and cranial bone. A local anaesthetic salve (Pontocaine) was applied to the surgical site. After completion of experiments, fish were euthanized by double pithing. All housing and experimental protocols were in accordance with the current guidelines of the Canadian Council for Animal Care.

### Experimental apparatus

After surgery, the fish was transferred to the experimental apparatus and respired with 22°C saltwater. The experimental set up consisted of a 3 channel optical system which included one stimulus channel and two background channels surrounded by a

copper wire Faraday cage. The 3 channels were positioned 1 cm away from the left eye of the fish.

250 W Tungsten-Halogen lamps (EJH Spectro) were used as light sources for the background channels. A condenser lens, field lens, diaphragm and projection lens were placed in front of each background source to collect and focus the light into oil-filled fiber optic probes (Oriel). Both intensity and spectral characteristics of the background light could be altered by adding Inconel-coated neutral density filters (Corion) or short and longwave pass interference filters (Corion).

Two types of background adapting conditions were used to evaluate the cone mechanisms in *T. duperrey*: 1) white light with no bandpass filters, and 2) yellow/orange light achieved with 550nm longpass filters. A mechanism is defined as a neural circuit which is comprised of the photoreceptors, retinal neurons and central nervous system targets of retinal neurons (Hawryshyn, 1991). The second type of adapting field was used to isolate any short wavelength mechanisms which might not have been detected under the white light background. An isolated mechanism refers to a mechanism which dominates the spectral sensitivity function as a result of selective bleaching of visual pigments due to the spectral characteristics of the background (Hawryshyn, 1991). With the second type of background, the sensitivity of the middle (M) and long (L) mechanisms was suppressed, hence this is referred to as the M+L adapting field.

The stimulus light was provided by a 300W Xenon lamp (Oriel) with condenser lens, filter lens and projection lens leading to a fiber optic probe. In addition, light stimuli were controlled by an electronic shutter (Uniblitz) so that each stimulus was 750 ms in duration. An Inconel coated neutral density wedge (nominal 4.0 ND) was used to control stimulus intensity and the spectral characteristics were controlled by a monochromator (ISA Instruments).

### **Recording from the retinal ganglion cells**

Electrodes were made from teflon-coated silver wire (0.075mm diameter) with a 1 mm sharpened tip. The reference electrode was placed in the right nares and inserted into the olfactory epithelium. The recording electrode was inserted through the optic tectum

and into the optic nerve. Prior to experimental recording, the fish was allowed to adapt to the background light conditions for a minimum of 30 minutes.

Sensitivity to the test wavelengths was determined by presenting the fish with a stimulus whose intensity was incrementally increased. For most experiments, 13 stimulus wavelengths were presented to the fish, ranging from 380 to 620 nm, in 20 nm intervals. The test wavelengths were presented in a randomized order to prevent selective adaptation of any one cone mechanism. The resultant signal was differentially amplified with a Grass preamplifier (P50 series), with low and high frequency filters set at 0.3Hz and 0.1kHz, respectively. For each intensity trial, the response was averaged 2 or 3 times. In some cases, the energy of the resultant signal was not sufficient to allow testing of wavelengths below 420nm. Throughout the experiment, neural activity was monitored on an oscilloscope (Hitachi VC-6025) and the amplified signal (Fig. 4.1) was relayed to a computer via an A/D port for analysis with ASYST programs.

### **Analysis**

For each test wavelength, the amplitude ( $\mu\text{V}$ ) of the ON and OFF responses was plotted against log photon radiance to generate an intensity-response curve (Fig. 4.2). The threshold intensity values were determined by applying a criterion response level to the intensity-response curve. Sensitivity was then determined as the reciprocal threshold intensity for a given wavelength. The log sensitivity values were standardized to generate relative sensitivity versus wavelength curves. An eighth order polynomial template for cone visual pigments was used to generate theoretical absorption curves (Bernard, 1987; pers. comm.)

### **Detection of the Hawaiian cleaner wrasse**

To assess detection of the potential signal, i.e. the contrast provided by the colour pattern of *Labroides phthirophagus*, the contrast values were recalculated in relation to the spectral sensitivity of *T. duperrey*. That is, contrasts were determined in the same way as previously described but over a narrower region of the spectrum to match the wavelength range for spectral sensitivity of *T. duperrey*. This served to eliminate any misleading values resulting from high contrast in wavelength regions where host fish may not be sensitive.

## RESULTS

### **White Light Background Conditions**

The ON response was dominated by a broad sensitivity peak in the mid (M) and long-wavelength (L) part of the spectrum, i.e. 500-600nm (Fig. 4.3). There was also a small shoulder in the short wavelength (S) region between 480 and 500 nm. The sensitivity at longer wavelengths was approximately 0.8 log unit greater than the sensitivity at shorter wavelengths (380-420nm). Under white light background conditions, a mid-wavelength nomogram (550nm) was found to fit best to the ON data.

The OFF response showed a similar trend of greater sensitivity to longer wavelengths (Fig. 4.4). In this case, the long wavelength sensitivity was about 0.5 log unit greater than the sensitivity at 400-480nm. The best fit for the OFF curve was a mid-wavelength nomogram with maximum absorbance at 545nm.

Difference spectra allow identification of peaks which are specific to one pathway. A difference spectrum between ON and OFF curves revealed essentially no differences in the shapes of the sensitivity curves (Fig. 4.5). Therefore, the ON and OFF pathways have similar spectral sensitivity functions.

### **Adaptation Experiments with 550 Long-Pass Filters**

In these experiments, the background was composed of wavelengths greater than 550nm which acted to selectively lower the sensitivity of medium (M) and longwave (L)-sensitive cone mechanisms through bleaching of the visual pigments.

There was a marked difference in the ON response obtained in these experiments compared to the white light experiments. In this case, the ON response showed a large peak sensitivity at short wavelengths, between 460 and 480 nm, which was approximately 0.5 log unit greater than the sensitivity in the 500-620 nm range (Fig. 4.6). A 460nm nomogram provided the best fit to the ON curve. Furthermore, a difference spectrum between ON curves obtained under each type of background adapting field also revealed a large sensitivity peak in the blue region, 460-480 nm (Fig 4.7). For this difference spectrum a 460 nm nomogram was the best fit.

The OFF response resulting from this adaptation did not show any large increase in relative sensitivity at short wavelengths (Fig. 4.8). In fact, the overall curve was fairly

flat with the exception of one wavelength in the blue region (460 nm) and a small peak between 560 and 600 nm. The best fit to the OFF data was a 570 nm nomogram since no shortwave peak was present.

A difference curve between the adaptation of ON and OFF responses showed that the ON response had a sensitivity peak at shorter wavelengths, while the OFF had greater sensitivity at longer wavelengths, relative to the ON (Fig. 4.9). A 460 nm nomogram provided the best fit to the ON and OFF difference spectrum from the short-wave isolating experiments. Therefore, the ON and OFF functions have different spectral sensitivity shapes under these background adapting conditions.

### **Visual contrast of the Hawaiian cleaner wrasse**

The original contrast calculations were done over a wavelength range of 300-850nm to represent the total amount of contrast in the environment. However, host fish are not sensitive to all wavelengths in this range. Therefore, the contrast provided by the colour pattern of *L. phthiophagus* was recalculated over a wavelength range of 380 to 620nm to correspond to the spectral sensitivity of *T. duperrey*.

For inherent contrast, the overall LC and SC did not differ substantially when the spectrum was narrowed to match the sensitivity of *T. duperrey* i.e. 380-620nm (Table 4.1). The greatest differences in contrast between the original wavelength range (300-850nm) and the range used here occurred for LC (0.039) with light from the 15m site at 3m in depth. The greatest difference in SC (0.013) occurred at the 25m site at 3m in depth. When the patch of highest contrast was considered (i.e. the yellow head), LC and SC from both wavelength ranges were very similar. In this case, the greatest difference between contrast values occurred at the 5m site at 3m for both LC and SC, with the greatest difference being 0.226 and 0.076 respectively.

When water was considered as the background against which *L. phthiophagus* is viewed, the overall LC from 380-620nm was about half of that determined over 300-850nm (Table 4.2). The exception was at the 25m site at 15m deep where LC was slightly higher for the narrowed spectrum. The mean SC values were essentially the same for both wavelength ranges. When calculations were done with the patch of highest contrast, LC was once again reduced by half under the spectral range to which a host fish

is sensitive (380-620nm), except for 25m site at 15m. The SC were the same under both spectral ranges, except for the 25m site at 3m, where SC increased for the narrower spectrum.

With coral substrate as the background, the overall LC became slightly less when the spectrum was narrowed to 380-620nm (Table 4.3). The greatest difference (45.28) occurred with sand as the background. The overall SC values were basically the same, with greatest difference of 0.219. Using the patch of highest contrast, LC was found to decrease from its original value under the narrowed wavelength range with greatest difference for sand (139). The SC values decreased slightly (greatest difference 0.80) when range was reduced from 300-850nm to 380-620nm.

**Table 4.1.** Inherent luminance and spectral contrast provided by the colour patches (shown in parenthesis for each column) of *Labroides phthirophagus* determined over 2 wavelength ranges. The range 300nm-850nm represents the maximum amount of contrast within the environment. The range 380nm - 620nm corresponds to the visual sensitivity range of a host fish (*Thalassoma duperrey*). Negative luminance contrast values mean that the target patch was darker than the background patch. Mean values and standard errors are shown in bold for each impinging light conditions.

Impinging Light Site/Depth	Luminance Contrast 300nm - 850nm	Luminance Contrast 380nm - 620nm	Spectral Contrast 300nm - 850 nm	Spectral Contrast 380nm - 620nm
<b>5m Site</b>				
3m	1.92 (Y/BS)	2.053(Y/BS)	0.923 (Y/BS)	0.85(Y/BS)
	-0.467 (BB/BS)	-0.395(BB/BS)	0.197 (BB/BS)	0.195(BB/BS)
	-0.583 (PT/BS)	-0.59(PT/BS)	1.501 (P/BS)	1.425(PT/BS)
	4.54 (YH/BB)	4.374(YH/BB)	0.704 (YH/BB)	0.658(YH/BB)
	0.559 (BB/P)	0.68(BB/P)	0.317 (BB/PT)	0.307(BB/P)
	<b>1.194±0.95</b>	<b>1.225±0.92</b>	<b>0.728±0.23</b>	<b>0.687±0.22</b>
5m	1.97	2.053	0.755	0.732
	-0.464	-0.395	0.165	0.164
	-0.571	-0.59	0.124	0.112
	4.6	4.374	0.563	0.547
	0.584	0.68	0.094	0.086
	<b>1.224±0.96</b>	<b>1.225±0.92</b>	<b>0.34±0.13</b>	<b>0.329±0.13</b>
<b>15m Site</b>				
3m	1.9	2.053	0.718	0.687
	-0.476	-0.395	0.095	0.094
	-0.586	-0.59	0.0857	0.0712
	4.53	4.374	0.349	0.33
	0.56	0.68	0.064	0.055
	<b>1.186±0.95</b>	<b>1.225±0.92</b>	<b>0.262±0.13</b>	<b>0.247±0.12</b>
7m	1.94	2.053	0.304	0.303
	-0.472	-0.395	0.069	0.069
	-0.579	-0.59	0.0421	0.0411
	4.57	4.374	0.222	0.221
	0.568	0.68	0.081	0.08
	<b>1.205±0.96</b>	<b>1.225±0.92</b>	<b>0.144±0.05</b>	<b>0.143±0.05</b>
<b>25m Site</b>				
3m	1.974	2.053	0.434	0.406
	-0.468	-0.395	0.115	0.112
	-0.575	-0.59	0.0986	0.087
	4.59	4.374	0.369	0.355
	0.59	0.68	0.0628	0.0562
	<b>1.222±0.96</b>	<b>1.225±0.92</b>	<b>0.216±0.08</b>	<b>0.203±0.07</b>
15m	1.93	2.053	0.423	0.416
	-0.472	-0.395	0.159	0.158
	-0.582	-0.59	0.117	0.114
	4.55	4.374	0.448	0.446
	0.561	0.68	0.0665	0.066
	<b>1.197±0.95</b>	<b>1.225±0.92</b>	<b>0.243±0.08</b>	<b>0.24±0.08</b>

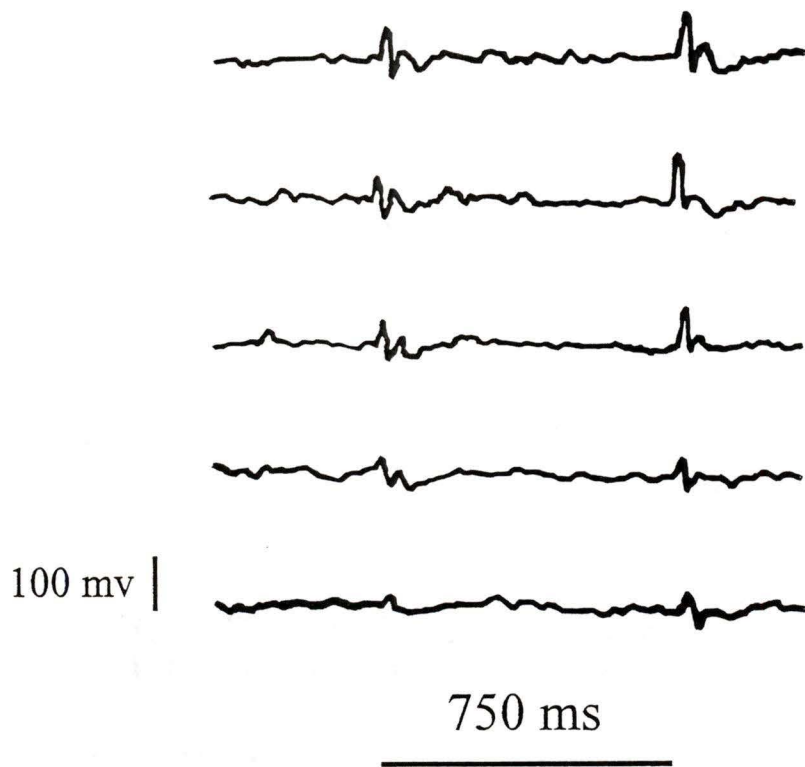
**Table 4.2.** Luminance and spectral contrast for the colour patches (shown in parenthesis) of *Labroides phthirophagus* against water as the background determined over 2 wavelength ranges. The range 300nm-850nm represents the maximum amount of contrast within the environment. The range 380nm - 620nm corresponds to the visual sensitivity range of a host fish (*Thalassoma duperrey*). Mean values and standard errors are shown in bold.

Site/Depth	Luminance Contrast 300nm - 850nm	Luminance Contrast 380nm - 620nm	Spectral Contrast 300nm - 850nm	Spectral Contrast 380nm - 620nm
5m Site				
3m	130.8 (YH)	56.19	1.918 (YH)	1.911
	32.96 (BS)	14.91	0.47 (BS)	0.469
	16.97 (PT)	6.4	0.277 (PT)	0.276
	16.78 (BB)	7.55	0.222 (BB)	0.221
	<b>49.38±27.4</b>	<b>21.26±11.8</b>	<b>0.722±0.4</b>	<b>0.719±0.4</b>
5m	132.6	45.224	4.123	4.12
	34.47	13.22	1.079	1.08
	17.57	5.49	0.62	0.62
	17.51	6.744	0.532	0.531
	<b>50.54±27.6</b>	<b>17.67±9.3</b>	<b>1.589±0.85</b>	<b>1.59±0.85</b>
15m Site				
3m	65.2	19.923	1.914	1.911
	17.31	5.365	0.53	0.530
	8.28	1.7885	0.3	0.296
	9.1	2.554	0.279	0.278
	<b>24.97±13.6</b>	<b>7.408±4.2</b>	<b>0.756±0.39</b>	<b>0.754±0.39</b>
7m	62.65	31.643	0.775	0.774
	15.81	9.06	0.194	0.194
	7.59	3.45	0.122	0.123
	7.85	4.644	0.089	0.089
	<b>23.48±13.2</b>	<b>12.19±6.6</b>	<b>0.295±0.24</b>	<b>0.295±0.16</b>
25m Site				
3m	3307.8	1273	5.35	5.224
	779.5	314.92	1.192	1.165
	457.5	144.88	0.722	0.71
	429.6	162.71	0.637	0.621
	<b>1243.6±692.6</b>	<b>473.88±269</b>	<b>1.975±1.13</b>	<b>1.93±1.1</b>
15m	572.2	607.8	1.017	1.051
	152.7	163.28	0.0186	0.185
	71.57	66.51	0.059	0.091
	78.23	90.57	0.062	0.06
	<b>218.68±119.3</b>	<b>233.29±110</b>	<b>0.331±0.24</b>	<b>0.347±0.2</b>

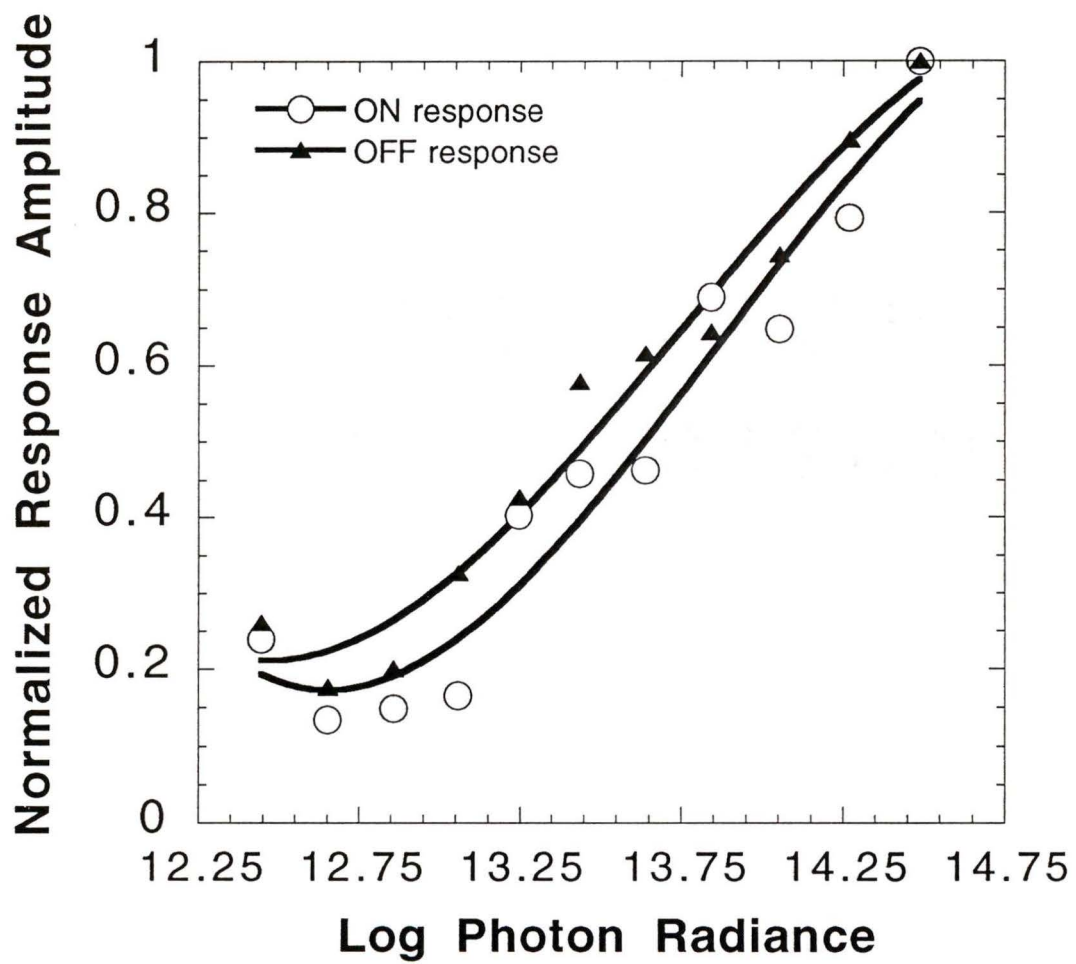
**Table 4.3.** Luminance and spectral contrast for the colour patches (shown in parenthesis) of *Labroides phthiropagus* against coral backgrounds determined over 2 wavelength ranges. The range 300nm-850nm represents the maximum amount of contrast within the environment. The range 380nm - 620nm corresponds to the visual sensitivity range of a host fish (*Thalassoma duperrey*). Mean values and standard errors are shown in bold.

Substrate	Luminance Contrast 300nm - 850nm	Luminance Contrast 380nm - 620nm	Spectral Contrast 300nm - 850nm	Spectral Contrast 380nm - 620nm
<i>Montipora verrucosa</i>	576.9 (YH)	516.04	1.84 (YH)	1.488
	207.5 (BS)	175.26	0.571 (BS)	0.531
	81.9 (PT)	72.32	0.338 (PT)	0.307
	104.1 (BB)	103.08	0.202 (BB)	0.185
	<b>242.68±114.7</b>	<b>216.68±102.1</b>	<b>0.738±0.32</b>	<b>0.628±0.3</b>
<i>Porites compressa</i> (Brown morph)	320.6	236.9	7.97	7.302
	92.5	78.545	2.23	2.136
	44.8	34.7	2.376	2.289
	53.8	48.606	1.405	1.357
	<b>127.93±65.1</b>	<b>99.69±46.6</b>	<b>3.49±1.51</b>	<b>3.271±1.36</b>
<i>Porites compressa</i> (Green morph)	645.5	569	1.615	1.518
	247.9	253.57	0.99	0.969
	104.1	100.91	0.477	0.473
	137.9	146.14	0.564	0.546
	<b>283.85±124.4</b>	<b>267.41±105.5</b>	<b>0.912±0.26</b>	<b>0.877±0.24</b>
Coral Rubble	328.7	305.4	0.464	0.32
	108.7	104	0.241	0.22
	44.9	43.3	0.138	0.132
	62.3	64.75	0.188	0.174
	<b>136.15±65.6</b>	<b>129.36±60.0</b>	<b>0.258±0.07</b>	<b>0.212±0.04</b>
Sand	511.16	372.26	5.21	4.407
	161.86	141.47	0.275	0.274
	78.46	63.34	0.108	0.108
	94.39	87.686	0.101	0.101
	<b>211.47±101.5</b>	<b>166.19±70.6</b>	<b>1.424±1.26</b>	<b>1.223±1.06</b>

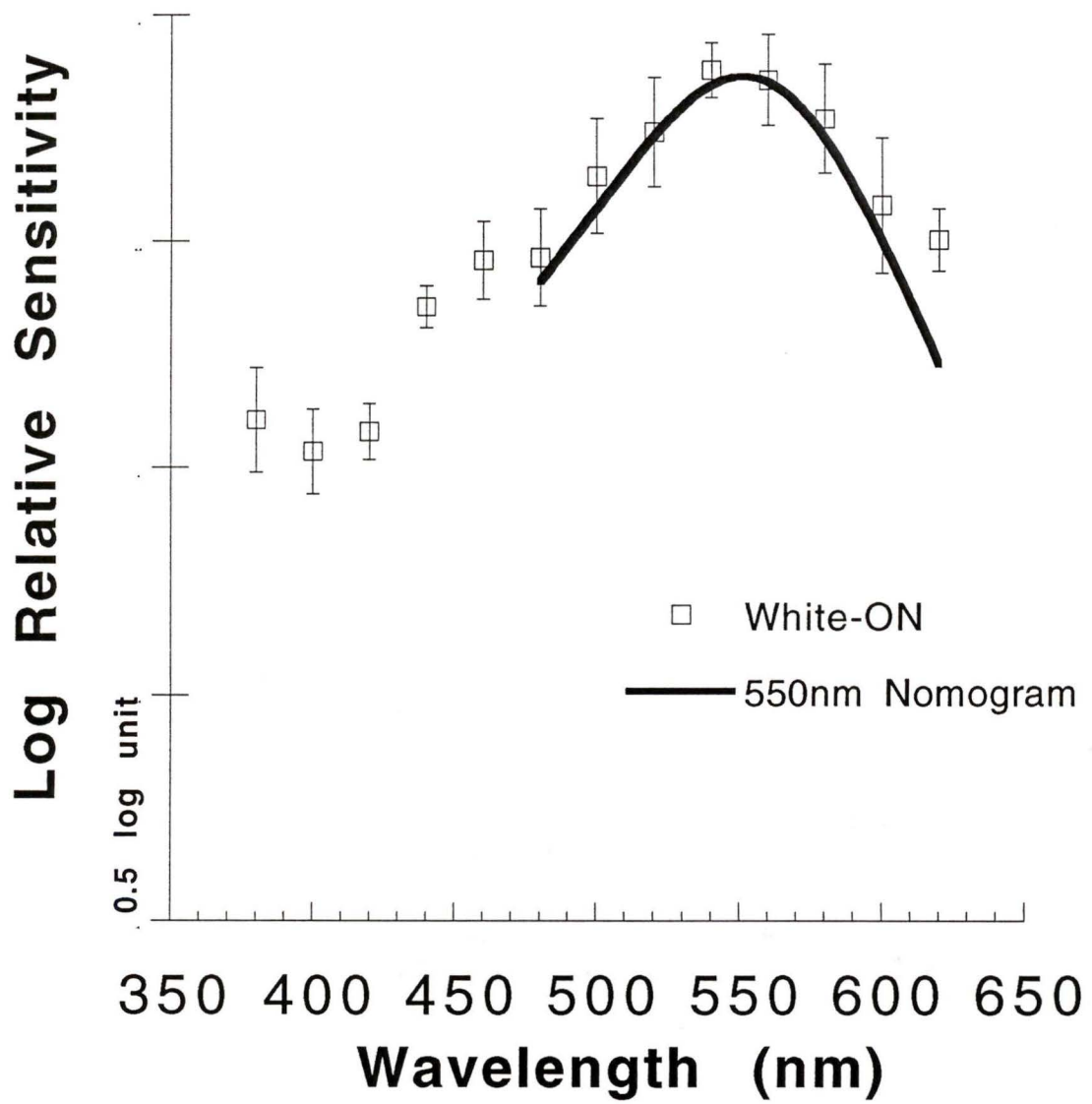
**Figure 4.1.** Compound action potential recordings from the optic nerve of *Thalassoma duperrey* presented with a 600nm stimulus. At each trace from bottom to top, the photon irradiance of the stimulus increased by 0.2 log units. The amplitude of the ON and OFF responses was measured from the first deflection. Deflections in the upward direction correspond to hyperpolarizations.



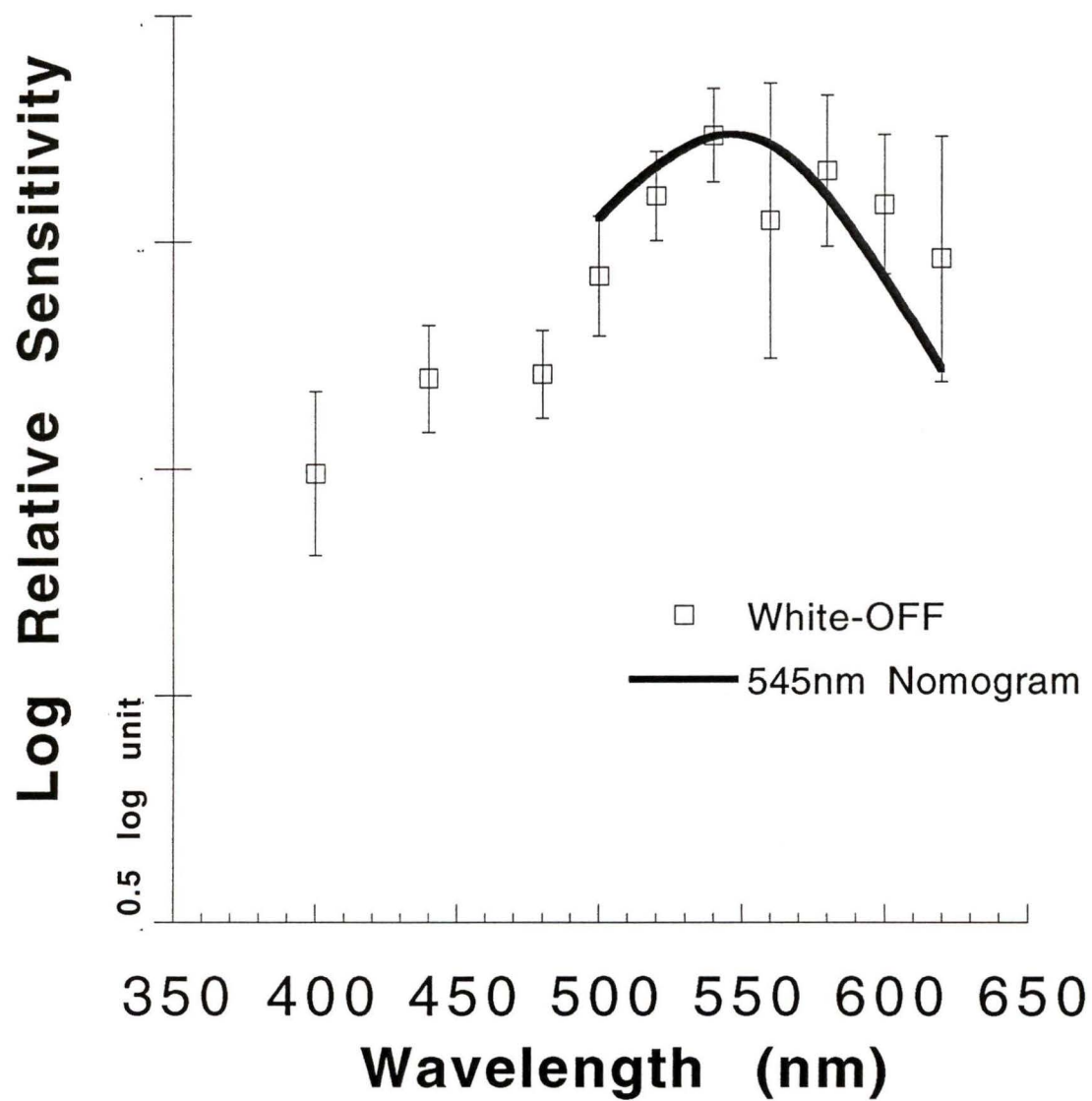
**Figure 4.2.** Normalized response amplitude plotted against intensity to determine threshold sensitivity of *T. duperrey* presented with a 600nm light stimulus. A third order polynomial function was fit to the data.



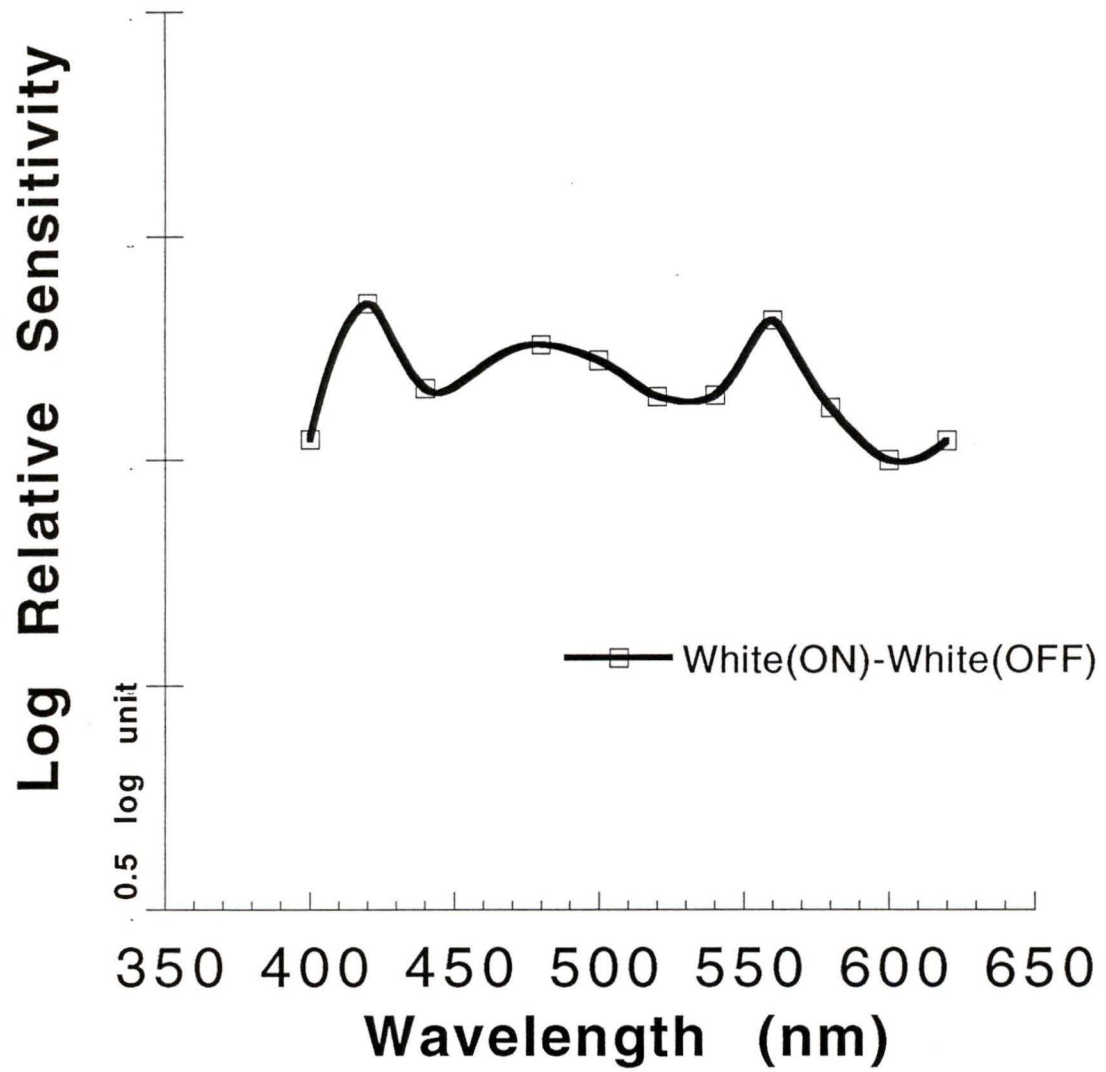
**Figure 4.3.** Mean spectral sensitivity ( $n = 5$ ) of the optic nerve ON response under white light background conditions. Bars represent 1 standard error of the mean. A 550nm visual pigment absorption curve is shown as a solid line. Goodness of fit (least mean difference squared between the theoretical absorption curve and data) was 0.496 log unit.



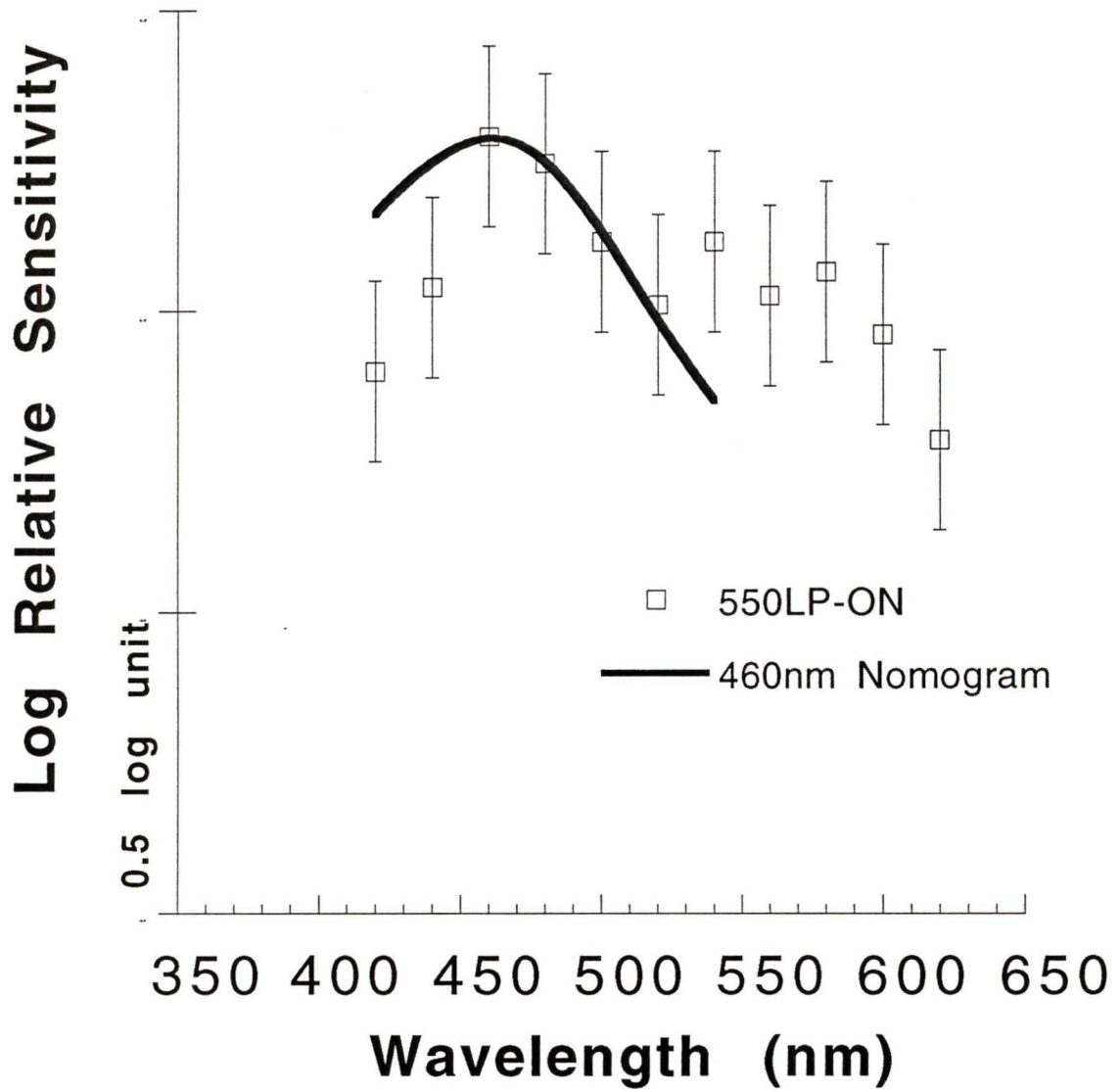
**Figure 4.4.** Mean spectral sensitivity ( $n = 4$ ) of the optic nerve OFF response under white light background conditions. Bars represent 1 standard error of the mean. A 545nm visual pigment absorption curve is shown as a solid line. Goodness of fit between theoretical absorption curve and data is 0.282 log unit.



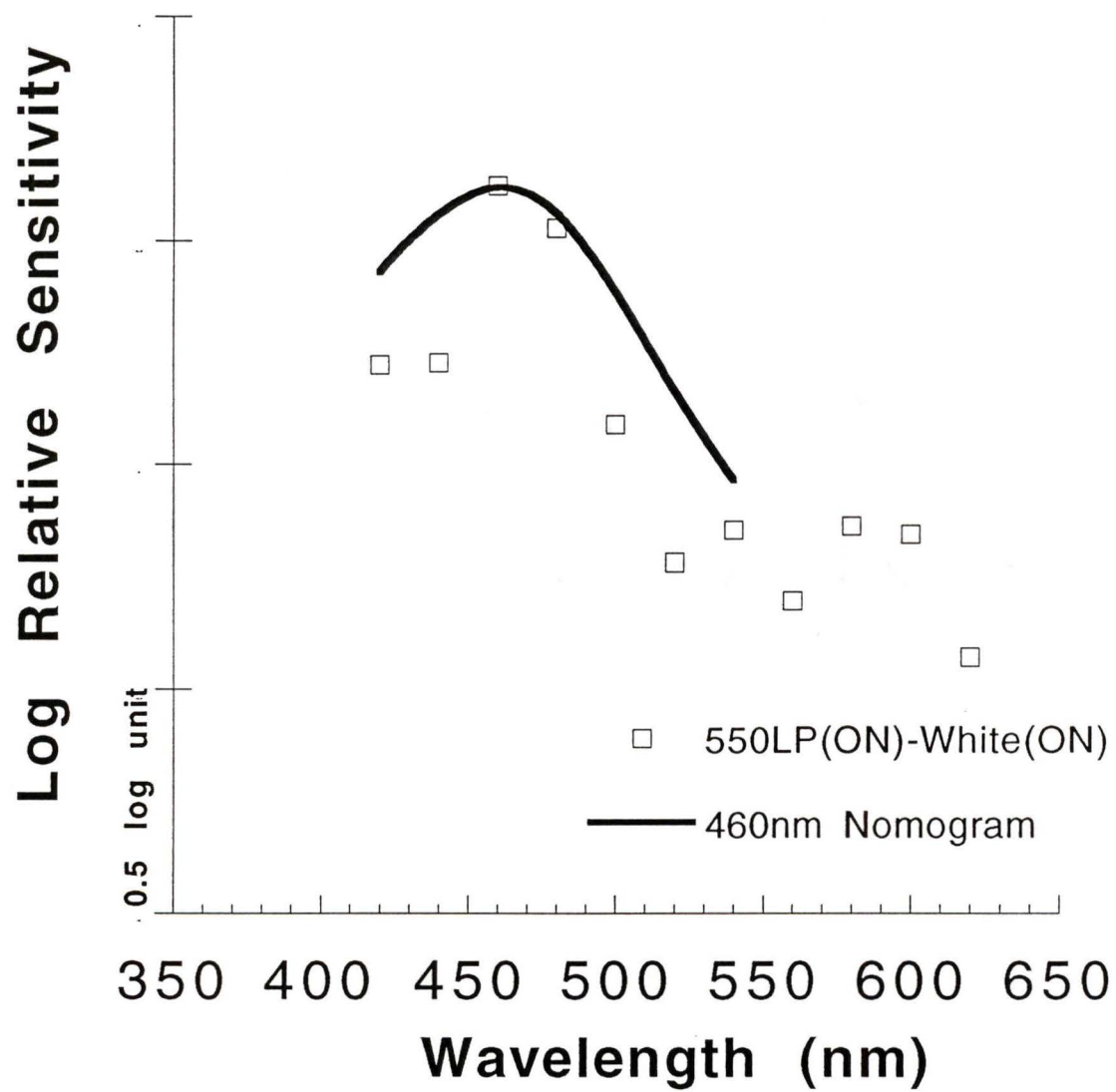
**Figure 4.5.** Difference spectrum from ON and OFF spectral sensitivity functions obtained under white light conditions. The black line is an interpolation of the differences between the two curves. The sensitivity curves differ by less than 0.5 log units.



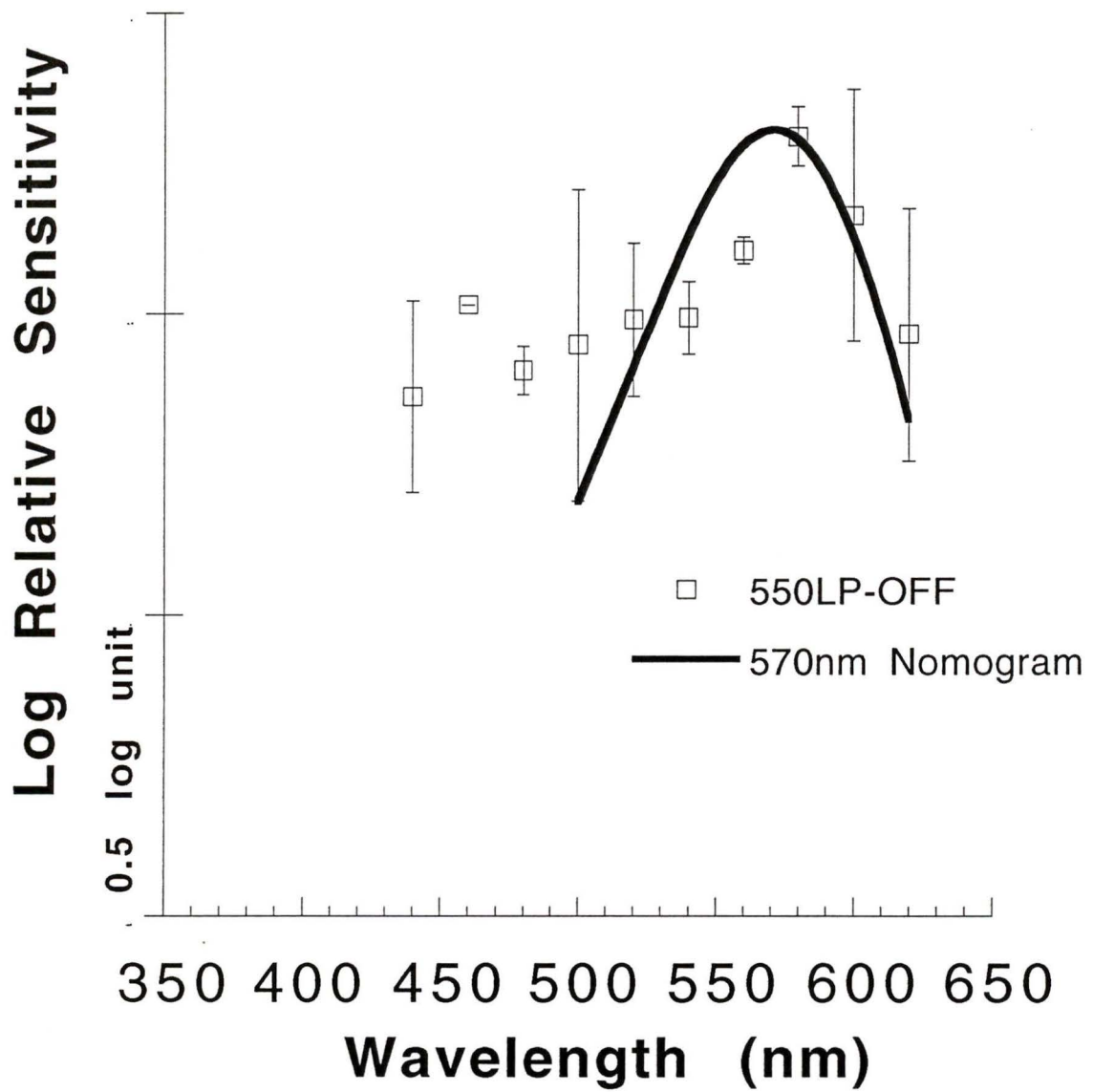
**Figure 4.6.** Mean spectral sensitivity of ON ganglion cell responses ( $n = 3$ ) under an M + L adapting background. Bars represent 1 standard error of the mean. A 460 nm visual pigment absorption curve is shown as a solid line. Goodness of fit between theoretical absorption curve and data is 0.0664 log unit.



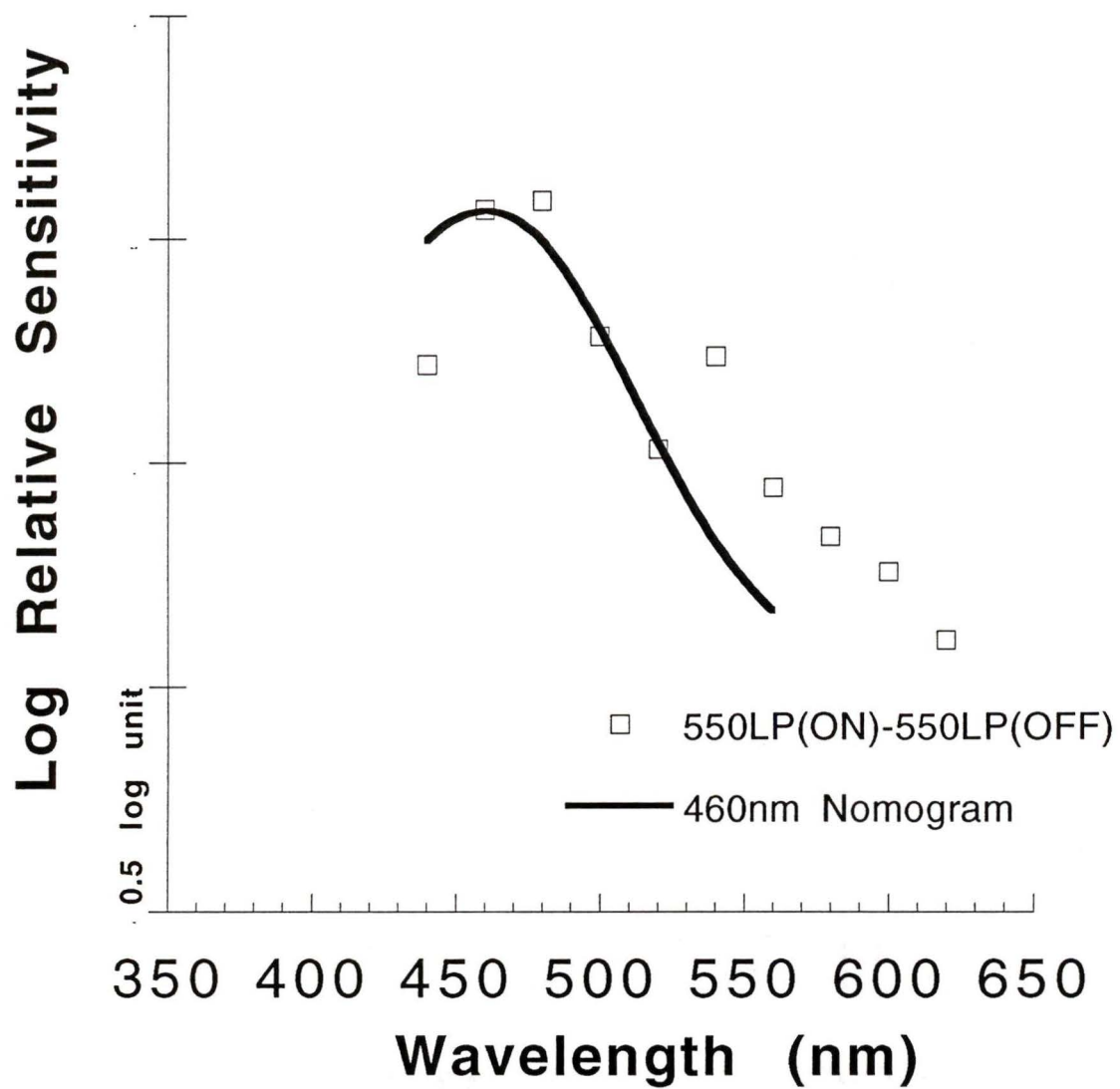
**Figure 4.7.** Difference spectrum for ON responses obtained with white light and M + L adapting background. A 460nm visual pigment absorption curve is shown as solid line. Goodness of fit between theoretical absorption curve and difference data was 0.495 log unit.



**Figure 4.8.** Mean spectral sensitivity ( $n = 4$ ) of the optic nerve OFF response under an M + L adapting background conditions. Bars represent 1 standard error of the mean. A 570nm visual pigment absorption curve is shown as a solid line. Goodness of fit between theoretical absorption curve and data is 1.140 log unit.



**Figure 4.9.** Difference spectrum between ON and OFF sensitivity functions obtained under the M + L adapting background. A 460nm visual pigment absorption curve is shown as a solid line. Goodness of fit between theoretical curve and data 0.290 log unit.



## DISCUSSION

Spectral sensitivity is closely related to the absorbance maxima of the photopigments residing in the photoreceptors, however peak sensitivities often differ from such factors as spectrally opponent interactions and ocular media absorption (Hawryshyn, 1991). Although CAP recordings can reveal several characteristics of the animal's spectral sensitivity, there are assumptions associated with this methodology. Firstly, it is assumed that the sensitivity of the RGC center is higher than that of the periphery. The second assumption is that at low intensity, the sensitivity peaks represent the activity of discrete cone mechanisms (Beaudet et al., 1993; DeMarco and Powers, 1991).

This work shows that *Thalassoma duperrey* is capable of detecting both short (460nm) and medium (550nm) wavelength light. The presence of these sensitivity peaks provide evidence for at least two cone mechanisms in this species.

Under white light conditions, the spectral sensitivity for both the ON and OFF responses were dominated by a medium wavelength sensitivity peak (545-550 nm). This reflects the large contribution of the medium wavelength mechanism which is made up of M-cones with ON-center and OFF-center RGC.

Under the M + L adapting conditions, the peak sensitivity at 460 nm in the ON response indicates that the short wavelength cone mechanism contributes most to the ON response. The lack of a short wavelength peak in the OFF response suggests an absent or reduced contribution of the shortwave mechanism to the OFF channel. This is probably due to a small number of shortwave sensitive OFF-center RGC. A third, long wavelength mechanism may also be present in this species since the OFF response shows a peak around 570 nm, however this requires further investigation. The possibility that wrasses may be highly sensitive to long wavelength light has been noted previously (Munz and McFarland, 1977). However, at this point it remains speculation until further information is gained.

The S-cone mechanism had a sensitivity peak of about 460nm which is slightly shorter than that of other reef fish, which tend to possess blue sensitive pigment with  $\lambda_{max}$  around 480nm (McFarland and Loew, 1994). Nevertheless, these findings are in

good agreement; some variation is expected since  $\lambda_{\max}$  were obtained from different fish species with two different techniques.

The  $\lambda_{\max}$  for the M-cone mechanism reported here is slightly longer than that found for other wrasses. While *Thalassoma duperrey* had a medium-wavelength sensitivity peak of 545-550 nm, other wrasses typically range from 521 to 539 nm (Munz and McFarland, 1977). In general, fish from the family Labridae tend to possess an M-cone mechanism with  $\lambda_{\max}$  values which are shifted about 10nm longer than M-cone  $\lambda_{\max}$  values from reef fish of different families (Munz and McFarland, 1977). For example, tropical marine fishes belonging to the Family Pomacentridae possess a medium sensitive visual pigment with  $\lambda_{\max}$  of 532 - 537nm. Furthermore, wrasses represent an unusual group since many species have their visual pigments based on vitamin A2 which is rare for marine fishes (Lythgoe, 1972). As is usually the case, visual pigments based on vitamin A2 have  $\lambda_{\max}$  values shifted to longer wavelengths relative to visual pigments based on vitamin A1. Therefore, it is possible that *T. duperrey* has visual pigments based on vitamin A2 which may account for the longer wavelength sensitivity of their M-cones compared to other reef fish.

No evidence of UV sensitivity was found for *T. duperrey*. This is somewhat surprising since UV light is abundant in the reef environment and UV sensitivity has been confirmed in some marine reef fish (McFarland and Loew, 1994). If lack of UV sensitivity is indeed the case, this could be due to several factors. Firstly, it is possible that the cornea and the lens of this species are not transparent to UV light. *T. duperrey*, as in several wrasses, possesses corneal iridescence which may act to block UV light as an adaptation to prevent damage from the intense UV radiation typical of tropical marine waters. The other possibility is that this species may simply lack UV receptors in the retina. This may be an adaptation related to the blue spacielight of reef water. A reduction in sensitivity to short wavelength light has been proposed as a mechanism to enhance visual contrast by decreasing sensitivity to those wavelengths which comprise scattered light (Lythgoe, 1979). However in this work, it was not possible to test the response of *T. duperrey* to wavelengths shorter than 380nm because of small response sizes.

### **Vision and the photic environment**

In order to elucidate the ecological importance of an animal's visual ability, the photic conditions of the habitat must be considered. For *T. duperrey*, the S-cone mechanism with a  $\lambda_{\max}$  of 460nm provides sensitivity to blue light which is abundant in the reef offshore environment. This may function as a matched cone pigment with respect to the colour of the water. That is, the wavelength of maximum absorbance of this cone type is similar to the dominant wavelength of the water (Lythgoe, 1988). On the other hand, the M-cone mechanism has a peak sensitivity at 545-550nm. This sensitivity maximum is not matched to the predominant wavelength of the offshore underwater light. This cone type is sensitive to medium wavelengths corresponding to green light which is less abundant, although present, in the water column. Therefore this cone may function as an offset visual mechanism.

At the inshore sites, the M-cone mechanisms would be spectrally matched to the ambient light which is composed primarily of medium (green) wavelengths. The S-cone mechanism would therefore act as an offset cone type in inshore areas.

With respect to coral substrates, the cones may still function as matched and offset mechanisms. The M-cone mechanism has a maximum absorbance which matches the colour of the coral while the S-cone mechanism has an offset peak sensitivity.

Thus, possession of two cone mechanisms allows for a visual system with sensitivities which are matched and offset from the spectral properties of backgrounds and natural targets. This way, vision is optimal in different types of photic environments for a variety of coloured targets. In fact, colour vision is thought to evolve from matched and offset visual pigment systems which first appeared as a means to enhance detection of photocontrast (McFarland and Munz, 1975; Munz and McFarland, 1977; Levine and MacNichol, 1982).

### **Detection of the Hawaiian cleaner wrasse**

When the visual range of *T. duperrey* was considered, LC changed most substantially. This means when the original wavelength range (300-850nm) was used, there may have been contrast peaks in the shortwave or very longwave regions of the spectrum which affected mean contrast values. Whether these peaks were real or an

artifact due to noise from light measurements remains uncertain. Unlike LC, the SC values did not undergo major changes when the spectral sensitivity of *T. duperrey* was taken into account. In other words, the main SC peaks occurred in the range where this host fish was sensitive. Overall, the trends in contrast values were consistent under both wavelength ranges, except for LC and SC against water from the 25m site at 15m deep where the contrast increased under the narrower wavelength range. This may be because the water was nearly monochromatic blue and the highest contrast values were concentrated within the visual range of *T. duperrey*.

Conspicuousness is related to the perception of both luminance and spectral contrast. In a model colour vision system, fine spatial resolution is enhanced when both types of contrast are involved. However, gradual changes in colour are more detectable than gradual changes in luminosity (Jacobs, 1981). Different animals show varying levels of dependence on luminance and spectral contrast for target detection. For honeybees, both luminance and spectral contrast are important, but only one of these types of contrast is sufficient for target detection as long as it is at a detectable level (Lehrer and Bischof, 1995). In the case of anoline lizards, luminance contrast is the main cue used in target detection and spectral contrast has minimal influence except as it affects apparent brightness contrast (Fleishman, 1995).

The visual system of *T. duperrey* is adapted to the optically complex photic conditions on the reef. Furthermore, there is abundant luminance and spectral contrast provided by the colour pattern of *L. phthirophagus* within the sensitivity range of this host species. Therefore to ensure detection, the colour pattern of the cleaner wrasse may be adapted to the spectral sensitivity of host fish which is, in turn, shaped by the photic conditions of the reef. To conclude, both luminance and spectral contrast probably play a role in mediating host response to the cleaner wrasse, however the relative importance of each may differ for different hosts, depending on visual physiology and ecological habits.

## Chapter 5. General Discussion and Conclusions

Visual communication systems are shaped by natural selection in response to many inter-related factors, including the photic environment and visual tasks of the animal. This study attempted to understand the role of visual communication in mediating cleaning symbiosis between Hawaiian reef fish. It is likely that host recognition and response to cleaners is visually mediated to a certain extent. However, the initial recognition of the Hawaiian cleaner wrasse by its host is not well understood. There is probably a genetic component as well as learning involved (Herald, 1964; Losey et al., 1994), yet the relative importance of each is unknown. In this research, the photic conditions on the reef, the optical signal provided by the cleaner and visual biology of host fish were studied and this information was integrated to elucidate how visual communication works in the natural reef environment.

The photic conditions of this study site included green and blue water which ranged in turbidity from moderately clear to very clear. Also, the coral substrates provided backgrounds which were white, green and brown in colour. Thus within Kaneohe Bay, there was a range of local photic conditions under which the cleaner's signal must be effective. Luminosity contrast provided by the cleaner's colour pattern indicated that it was more conspicuous in the natural environment than the other reef fish studied. Therefore, luminance contrast from the colour pattern of the cleaner wrasse may be a visible optical signal to which host fish respond. In addition, the amount of luminance contrast from the colour pattern did not degrade to the same extent as spectral contrast when the photic conditions underwent changes due to location and depth. Thus, this optical signal was optimal under a variety of environmental light regimes.

According to the visual sensitivity of one host fish, *Thalassoma duperrey*, the optical signal of the cleaner wrasse was detectable. That is, *T. duperrey* should be able to detect a sufficient amount of photons reflected from the cleaner wrasse's body. However, the amount of luminance contrast which host fish can detect was less than that predicted from contrast values which was calculated over a large wavelength range (300-850nm). This means that there was more luminance contrast present in the physical

environment than this host fish can actually see. However, there was ample luminance contrast within the sensitivity range of *T. duperrey* so that it was an important visual stimulus.

Conspicuousness of aquatic animals in the natural environment can result from several types of visual stimuli. First of all, the size of the target affects conspicuousness (Hailman, 1977). Larger targets tend to be more conspicuous than smaller ones because they have a greater chance of being detected. In this study, the colour patches of the fish were weighted for size and larger patches were, in fact, more conspicuous.

Movement of the target contributes to conspicuousness in several ways, including speed, direction, repetition, sudden onset and rhythmicity (Hailman, 1977). Although the dance of the cleaner was not very fast in the horizontal direction, the vertical oscillations were quite rapid. The example provided in Chapter 4 showed that the rate of oscillation was 1.67 oscillation per second. In addition, the dance movements were repetitive and rhythmic which may add to its conspicuousness. This is evidence that the dance is a ritualized behaviour which is typical of many optical signals.

Another component of conspicuousness is visual contrast which is dependent upon brightness, hue and colour saturation (Hailman, 1977). In this work, conspicuousness due to brightness and hue was examined, however the amount of saturation of the colour patches was not treated as a separate factor. This can, nevertheless, be important since two patches of the same brightness and hue may differ in the level of conspicuousness, depending on saturation level. For instance, the green body of *T. duperrey* was probably not highly saturated because small flecks of other colours (white, red) were present (pers. obs.).

Reversed counter shading of animals can be highly conspicuous in aquatic systems (Hailman, 1977). Unlike normal counter-shading, reversed counter shading occurs when a fish is lighter on its dorsal surface than the ventral surface. However this did not occur in the fish studied here, although it may be present in other species of reef fish.

Finally, enhancement of body shape can increase conspicuousness (Hailman, 1977). Uniform colouration, as seen in *Z. flavescens*, results in a conspicuous target

because it presents a uniform, continuous image. Also, outlines on the body can affect conspicuousness of body shape. For instance, the black eyebar of *C. auriga* is believed to act as a disruptive pattern reducing the conspicuousness of the fish by making it appear as a different shape. That is, it breaks up the outline of the fish which is thought to serve as an anti-predator detection mechanism (Neudecker, 1989). Alternatively, the cleaner wrasse had a continuous dark stripe along the length of its body which acted to increase the conspicuousness of this fish by emphasizing its shape. A body shape which is unusual may also create conspicuousness by virtue of its novelty within the environment (Hailman, 1977). The fish in this research had body shapes typical of reef fish, namely terete forms as in *L. phthirophagus* and *T. duperrey* and laterally-compressed forms as in *C. auriga* and *Z. flavescens*. Thus, novel body shape was probably not an important factor which contributed to the conspicuousness of these species.

In conclusion, visual communication is important in mediating cleaning symbiosis between Hawaiian reef fish. The conspicuousness of *L. phthirophagus* to other reef fish may be enhanced with specific optical signals, such as the highly contrasting colour pattern and dance which were studied here. These types of visual cues may serve to identify the cleaner wrasse to host fish and ultimately lead to the behavioural response of the host, i.e. posing. Since past research has shown that cleaners and hosts generate and respond to signals which are specific to the relationship, these signals must have evolved for the purpose of mediating cleaning activity.

Both the cleaner and host fish display communicative behaviours which act to maintain the relationship (dancing, posing), therefore cleaning symbiosis between Hawaiian reef fish is most likely based on mutualism. This means that both parties accrue some type of advantage from the relationship. Finally, this relationship is likely to remain stable as long as both participants gain essentially equivalent benefits.

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## APPENDIX 1

$S_1$ = percent reflectance spectrum from colour patch 1

$S_2$ = percent reflectance spectrum from colour patch 2

$I_{s,d}$ = downwelling irradiance spectrum at a specific site and depth

$x$ = brightness correction coefficient for spectrum 1

$y$ = brightness correction coefficient for spectrum 2

$S_{corr1}$ = Corrected spectrum 1

$S_{corr2}$ = Corrected spectrum 2

$I_b$ =Irradiance from water or coral background

### Calculation of inherent contrast

Luminance Contrast:

$$LC = \left| \frac{(S_1 * I_{s,d}) - (S_2 * I_{s,d})}{(S_2 * I_{s,d})} \right| \quad \text{Eq.1}$$

Spectral Contrast:

-brightness correction of reflectance spectra

$$\bar{S} = \frac{S_1 + S_2}{2} \quad \text{Eq.2}$$

$$x = \frac{\bar{S}}{S_1} \quad y = \frac{\bar{S}}{S_2} \quad \text{Eq.3}$$

$$S_{1corr} = (S_1 * x) \quad S_{2corr} = (S_2 * y) \quad \text{Eq.4}$$

-Calculation of Euclidean distance between spectra corrected for brightness differences

$$SC = \sqrt{\sum_{all\lambda} [(S_{1corr} * I_{s,d}) - (S_{2corr} * I_{s,d})]^2} \quad \text{Eq.5}$$

Contrast against water or coral backgrounds

Luminance Contrast:

$$LC = \frac{(S_1 - I_b)}{I_b} \quad \text{Eq.6}$$

Spectral Contrast:

-Spectra are corrected for brightness differences in the same way as above (Eq. 2, 3, 4)

-Euclidean distance calculated between spectra corrected for brightness differences

$$SC = \sqrt{\sum_{all\lambda} (S_{corr} - I_b)^2} \quad \text{Eq.7}$$

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