SPECTRAL TRANSMISSION AND SHORT-WAVE ABSORBING PIGMENTS IN THE LENSES OF FISH AND OTHER ANIMALS

A Thesis submitted by

ANDREA THORPE

For the degree of Doctor of Philosophy

Department of Optometry & Visual Science
The City University

October 1991

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 8

ACKNOWLEDGEMENTS

A special thank you to Dr. Ron Douglas, my supervisor, who made my research so enjoyable with his unfailing enthusiasm for this subject; I am also indebted to Ron for practical help in all aspects of the study.

Grateful thanks go to numerous people for the supply of materials, including Dr. Gordon Reid of the Horniman Museum; The Plymouth Marine Laboratory; Dr. W. Dunlap, A.I.M.S., Townsville, Australia; Dr. Lindsay Ross of Stirling University; Dr. Jim Bowmaker of The Institute of Ophthalmology (also for MSP analysis); Prof. Gordon Ruskell and Dr. Margaret Travers from City University; Prof. Joachim Wagner of Philips University, Marburg, F.R.G. and also Prof. John Blaxter of The Scottish Marine Biological Association.

I express sincere thanks to Dr. Roger Truscott of The Department of Chemistry, University of Wollongong, Australia for his interest in my work and assistance during the time I spent in his laboratory. I am also grateful to Maribel, Grace and the other research students and Dr John Carver for help with HPLC and NMR while in Australia. A big thank you also to Dr. Jonathan Nugent and the Photosynthesis Group, Department of Biology, U.C.L. for permission to use their facilities and equipment and also their help over the past three years.

Finally, thanks to John, Nancy, Alicja and the other post-graduates in the department for their friendship and Simon for his encouragement and support.

DECLARATION

I grant powers of discretion to the University Librarian to allow this thesis to be copied in whole or in part without further reference to myself. This permission covers only single copies made for study purposes, subject to the normal conditions of acknowledgement.

ABSTRACT

Short-wave absorbing compounds are common in the lenses of fish. Such pigments absorb radiation in the region of 300-500nm, restricting the passage of these wavelengths to the retina. The spectral transmission of lenses from 108 species of freshwater and marine fish from a total of 43 families was investigated here. A large number of fish had lens pigments with maximum absorbance around 320-330nm and/or 360nm, which were identified by HPLC and NMR as mycosporine-like amino acids; a group of low molecular weight, water soluble compounds. However, the short-wave absorbing pigment in the lens of the deep-sea species *Stylephorus chordatus* was isolated and identified as kynurenine, a tryptophan metabolite also found in the primate lens. A number of tropical freshwater species were also identified with high concentrations of another tryptophan derivative, 3-hydroxykynurenine, in the lens.

Variations in both lens pigment type and concentration were observed in several species and were found to be dependent on the age of the animal. A model was proposed to explain the changes occurring in lens transmission with age. An initial increase in pigment concentration produces a steep rise in the wavelength of lens 50% transmission which later levels off as pigment accumulation stops. The final stage is a drop in the 50% transmission wavelength, which occurs when pigment levels in the lens fall sharply as the lens continues to increase in volume.

Raising fish under high light, low light or UV conditions had no effect on either the lens pigmentation or the visual pigments, but did result in changes in both retinal structure and corneal pigmentation. This suggests that lens pigments do not accumulate in response to the environmental illumination.

Lenses of the frog, sheep, guinea pig, squirrel and 3 species of monkey were also found to contain a range of biochemically distinct short-wave absorbing compounds. Age-related changes were also observed in the macaque monkey lens. Three cephalopod species had lens pigments with similar short-wave absorbance to compounds in the fish lens, but HPLC analysis revealed that they are likely to be different.

Lens transmission measurements from a range of species support a role of fish lens pigments in increasing acuity. However there is some evidence that short-wave absorbing compounds may also act to protect the retina from UV damage.

ABBREVIATIONS

3HKG	3-hydroxykynurenine glucoside
HPLC	High performance liquid chromatography
λ_{max}	Wavelength of maximum absorbance
MSP	Microspectrophotometry
NMR	Nuclear magnetic resonance
TLC	Thin layer chromatography
UV	Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 STRUCTURE OF THE LENS

The vertebrate lens is a highly refractive structure and is held in position behind the iris by zonular fibres extending from the ciliary body. Cells in the germinative zone in the anterior lens epithelium migrate to the lens bow in the equatorial region where they lose their nuclei and undergo elongation to form lens fibres. The lens continues to grow throughout life, with new fibres continually added at the equator pushing the older enucleated fibres towards the centre of the lens where, rather than being disposed of, they become compressed to form the lens nucleus. Since protein synthesis takes place only in the outer cortical region of the lens, any post translational changes and damage occurring to lens proteins accumulate in the lens nucleus as the fibres age. The absence of nerves and blood vessels and the highly organised arrangement of the lens fibres help to maintain lens transparency throughout an animal's life.

All vertebrate lenses are composed largely of protein and water. Average protein contents are very high at around 40-50% (w/w), whilst hydration levels are low [Zigman & Yulo, 1979; Lundgren *et al.*, 1986]. The major protein

constituents (around 90% of total lens protein) are the crystallins [de Jong, 1981], water soluble structural proteins which are at high concentrations in the cytoplasm of lens fibre cells. Vertebrate lenses contain three major immunologically distinct families of structural protein: α-, ß- and τ-crystallins. A fourth major crystallin group, the δ-crystallins are found only in lenses of birds and reptiles [de Jong *et al.*, 1989].

1.2 FUNCTION OF THE LENS

The crystalline lens is primarily thought of as a refractive tissue, used for fine focusing to achieve sharp image formation upon the retina. It performs this function to a greater or lesser degree in all vertebrates. The majority of terrestrial vertebrates accommodate by changing the shape of their soft, elliptical lenses [Fisher, 1969]. However, most of the refractive power of the eye in terrestrial animals is provided not by the lens, but by the cornea, by virtue of the difference of its refractive index to that of the air. In contrast, the lens must supply the vast majority of focusing power in the eyes of aquatic animals as the cornea is lost as a refractive surface underwater. Lenses of aquatic animals are therefore often large, spherical and have a high refractive index [Sivak, 1990]. Methods of accommodation vary throughout the animal kingdom; most fish for example, move the lens backwards towards the retina for distant vision using one or two

retractor lentis muscles, although certain species (sharks, rays and also amphibians) move the lens forward towards the cornea with a *protractor lentis* for near vision [Duke-Elder, 1958; Sivak, 1980].

A second function of the lens in many animals, arguably as important as the first, is the absorbance of short-wave radiation. Ultraviolet (UV) radiation below 310nm is almost completely absorbed by the proteins of the cornea [Kinsey, 1948; Kennedy & Milkman, 1956; Lerman, 1984; van Best *et al.*, 1988]. Lens and corneal proteins, in common with most others, contain tryptophan, tyrosine and phenylalanine residues in varying amounts. These residues have an aromatic ring structure which causes them to absorb UV radiation in the region of 260-280nm. Nucleic acids are also present in the lens and absorb around 260nm. The lens, due to its greater axial diameter and high protein concentration is usually the limiting filter within the eye. However, a large number of species have now been found to have pigments in the lens, absorbing UV radiation above 320nm (see Section 1.2), which would otherwise be transmitted by both corneal and lens proteins. The human lens plays an important role in the absorbance of UV radiation, as demonstrated by patients after lens extraction surgery, who are able to read optometry charts illuminated under UV wavelengths where a normal observer can perceive nothing at all [Williams *et al.*, 1983]. Lenses of the squirrel and a number of other animals have such high concentrations of short-wave absorbing compounds that they appear bright yellow and transmit very little UV [Arden &

Tansley, 1955; Cooper & Robson, 1967 & 1969a; Zigman *et al.*, 1985]. In contrast, animals with no lens pigment, such as the pigeon and dogfish, have lenses which transmit significantly in the near UV from 310-400nm [Kennedy & Milkman, 1956; Emmerton *et al.*, 1980].

It is not only the lens which can act as a short-wave filter. The fish cornea often absorbs short wavelengths quite strongly [Moreland & Lythgoe, 1968; Bridges, 1969; Appleby & Muntz, 1979; Kondrashev *et al.*, 1986; Douglas, 1989], while the vitreous humour of one species of fish is reported to be yellow [Lythgoe, 1971]. Short-wave absorbing pigments in the retina are also quite common; many birds, reptiles and turtles have oil droplets within their cones [Walls & Judd, 1933a; Strother, 1963; Goldsmith *et al.*, 1984] and humans have a yellow macular pigment in the fovea [Wald, 1949; Bone *et al.*, 1988; Handelman *et al.*, 1988]. A number of fish have a diffuse yellow pigment throughout the retina [Muntz, 1973; Bowmaker *et al.*, 1988; Denton & Locket, 1989], whilst others have ellipsosomes, modified mitochondria with short-wave absorption within the cone inner segments [MacNichol *et al.*, 1978; Avery & Bowmaker, 1982]. Short-wave absorbing compounds can therefore be present in a combination of ocular tissues, co-existing within the eye, often significantly modifying wavelengths reaching the outer segments of the photoreceptors.

1.3 SHORT-WAVE ABSORBING COMPOUNDS IN THE LENS

The short-wave absorbing compounds found in the lenses of man and other primates are a series of tryptophan catabolites: 3-hydroxykynurenine glucoside, kynurenine and an unknown kynurenine glucoside [van Heyningen, 1971a, 1973a&b; Bando *et al.*, 1981]. The squirrel lens pigment is also a kynurenine derivative, N'-acetyl-3-hydroxykynurenine [van Heyningen, 1971b; Zigman & Paxhia, 1988], slightly different to those found in the primate lens.

It has been suggested that a few shallow water species of fish may also have kynurenine-like compounds in the lens [Zigman, 1987]. However, these assumptions were based only on absorbance profiles which can be misleading (see Chapter 3). Bon *et al.* (1968) and van Heyningen and Linklater (1976) both state that the lens pigments of many fish are quite different to the kynurenine compounds found in other vertebrate lenses. A number of fish lens pigments have recently been identified as mycosporine-like compounds [Dunlap *et al.*, 1989]. Several mesopelagic species of fish have carotenoid-like pigments in the lens which absorb at longer wavelengths (400-500nm) than both the tryptophan and mycosporine derivatives [Somiya, 1976; McFall-Ngai *et al.*, 1986 & 1988; Yu *et al.*, 1991; Douglas & Thorpe, in press].

Many other species have short-wave absorbing compounds in the lens which have not yet been identified, for example the frog lens, although it is not coloured, absorbs significantly in the UV [Kennedy & Milkman, 1956]. Lenses of the lamprey and prairie dog [Walls & Judd, 1933a], pig

and cow [Weale, 1954a] and snake [Walls, 1931] are also reported to either be yellow in colour or have high short-wave absorbance.

There is much literature reporting changes in human lens pigmentation with age, with a rise in the absorption of shorter wavelengths resulting in a reduced spectral range in older humans [Said & Weale, 1959; Boettner & Wolter, 1962; Lerman & Borkman, 1976; Weale, 1988]. Little is known however, about lens ageing processes in other animals, though the data available suggest that other species may go through similar age changes to man. For example, the lens of the cat is shown to absorb UV wavelengths more strongly with age [Dodt & Walther, 1958] as are lenses of the goldfish and brown trout [Douglas, 1989].

1.4 FUNCTIONS OF SHORT-WAVE ABSORBING PIGMENTS

Walls and Judd (1933a&b) made numerous suggestions as to the function of yellow oil droplets in the retina and then postulated that other yellow filters within the eye would have similar effects, which are discussed below. Early work graded the intensity of colouration of the lens: a deep yellow lens was found in the 'sun-worshipping' prairie dog and lenses of squirrels from diminishing light intensities displayed progressively less pigmentation [Walls and Judd, 1933a&b]. The yellow lenses of snakes were found to correlate with a requirement for 'keen vision': secretive and nocturnal species having clear lenses while active

diurnal species had bright yellow lenses [Walls, 1931]. However, not all short-wave pigments in the eye are yellow; a tissue with high absorption in only the 300-400nm region for example would appear colourless. Similarly, a yellow colouration does not necessarily suggest a UV absorbing function; carotenoid pigments are often yellow in colour but transmit significantly in the UV.

All animals possessing lenses with short-wave absorbing pigments inevitably limit their sensitivity and therefore usually have a diurnal lifestyle [Denton & Warren, 1968; Zigman, 1982]. In fish, yellow corneas and lenses are found, with one exception (see below), only in diurnal species [Muntz, 1973; Moreland & Lythgoe, 1968; Bridges, 1969], which often spend the night time buried or inactive [Lowe-McConnell, 1964; Muntz, 1973 & 1976a]. In addition, it is generally observed that nocturnal fish and those exposed to low light levels do not have pigmented lenses [Kennedy & Milkman, 1956; Denton, 1956; Muntz, 1973; Douglas & McGuigan, 1989]. Many fish have corneas which are pigmented only in the dorsal region [Moreland & Lythgoe, 1968]. Such 'eye shades' will preferentially remove bright downwelling short-wave radiation without diminishing the intensity of the relatively dim upwelling illumination [Walls & Judd, 1933a; Lythgoe, 1979; Kondrashev *et al.*, 1986].

However, whilst it appears that animals exposed to higher light levels do in general have short-wave absorbing pigments within the eye, authors concede that many animals do not fit this ideal; most markedly anomalous are the

yellow lenses in a number of deep-sea species [Douglas & Thorpe, in press]. It is doubtful that the yellow lenses of mesopelagic species have evolved to serve the same function as the yellow lenses of terrestrial animals which are exposed to substantially higher levels of illumination. Therefore, a single function for short-wave absorbing pigments within the eye is unlikely. Suggestions for the function of lens and other ocular pigments are numerous and quite varied, they are considered below.

1.4.1 IMPROVEMENT OF IMAGE QUALITY

Image formation is complicated by the attenuation of light rays by absorbance and scatter before they even reach the eye [Heinermann, 1984]. The cornea, lens and retina also scatter light quite considerably within the eye [Lythgoe, 1979]. Light scattered by small particles (Rayleigh scatter) is composed of mainly short wavelengths and can result in glare when the incident radiation is sufficiently bright, creating a dazzling effect within the eye [Walls & Judd, 1933a]. Effects of both glare and scatter are consequently greater at short wavelengths (Fig. 1-1).

Aquatic animals have further problems since the presence of suspended particles greatly increases light scatter underwater [Kennedy & Milkman, 1956]. Short-wave absorbing compounds in the fish lens will therefore remove the veiling light which is composed of short-wave radiation when caused by Rayleigh scattering underwater [Appleby & Muntz, 1979].

The crystalline lenses of all animals, including fish, also have high chromatic dispersion which results in considerable levels of longitudinal chromatic aberration [Wald & Griffin, 1947; Sivak, 1974; Palmer & Sivak, 1981]. Longitudinal chromatic aberration produces superimposed blurred images upon the normal images on the retina [Heinermann, 1984]. Diurnal animals will necessarily be exposed to high levels of short-wave radiation, the very wavelengths which, as for glare and scatter, contribute most strongly to chromatic aberration [Sivak, 1982] (Fig. 1-1).

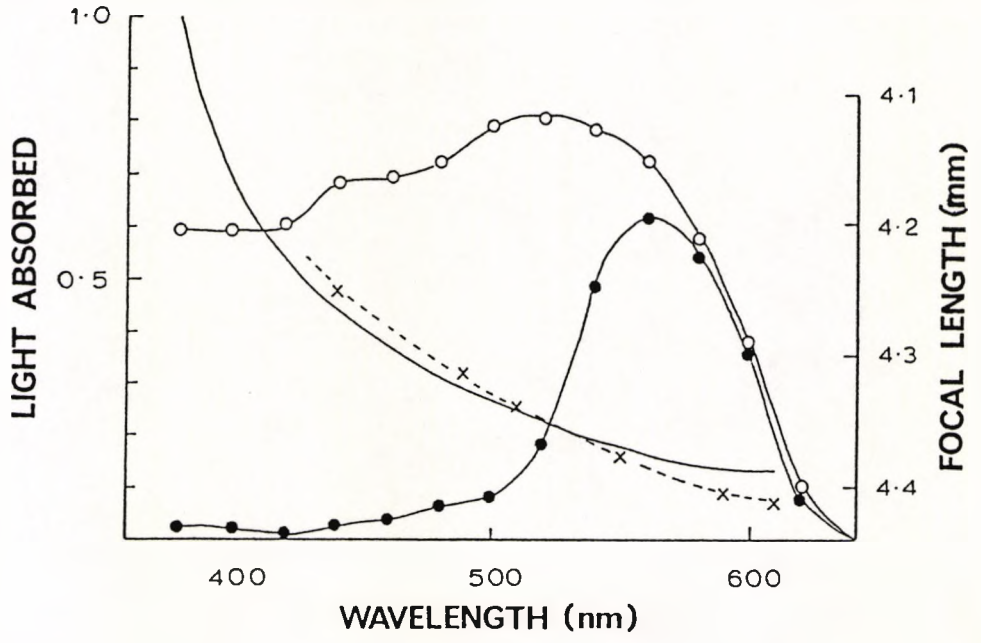


Figure 1-1. Effects of the yellow lens and cornea of the oscar (*Astronotus ocellatus*) on Rayleigh scattering and chromatic aberration. The left hand ordinate gives the proportion of light absorbed by the retina alone (unfilled circles), by the retina when the lens and cornea are present (filled circles) and also the amount of Rayleigh scattering as a proportion of the amount at 380nm (continuous line). The right hand ordinate gives the focal length of a 3.5mm lens (crosses and dashed line). After Muntz, 1972.

Yellow filters have been shown to substantially reduce the chromatic aberration of the fish lens *in vitro* [Sivak & Bobier, 1978] and it is thought that yellow lenses and corneas might similarly limit the effects of longitudinal chromatic aberration *in vivo* [Zigman & Paxhia, 1988]. Therefore, any ocular tissues having pigments specifically absorbing short wavelengths (e.g the oscar, Fig. 1-1) will increase overall image quality and visual acuity at high light intensities by removing the wavelengths responsible for glare, scatter and also chromatic aberration within the eye [Muntz, 1973 & 1976a; Zigman & Gilbert, 1978; Appleby & Muntz, 1979; Stark, 1987].

1.4.2 PROTECTION OF THE RETINA FROM UV

Various authors report detrimental effects of UV on the retina [Zigman & Bagley, 1971; Penn, 1985]. It is thought that one function of the pigmented lenses in the squirrel and primates may be to protect the retinal pigment epithelium and photoreceptors from UV-induced damage [Zigman, 1983a; Collier *et al.*, 1985; Collier & Zigman, 1987]. In support of this, aphakic squirrels exposed to physiologically high UV levels suffered widespread retinal damage [Collier *et al.*, 1989]. But why some animals exposed to high levels of UV, never accumulate short-wave absorbing filters if they do serve such a protective role is unknown. There are for example a large number of fish from a diverse range of habitats, which do not have any lens pigmentation [Douglas & McGuigan, 1989].

It is thought that some lens pigments may also serve a protective role against UV by acting as photosensitisers [Zigler & Goosey, 1984]. The kynurenines found in primate and squirrel lenses for example, sensitise lens proteins upon near-UV exposure, causing them to become aggregated [Dillon *et al.*, 1976], leading to an increase in absorption by the lens.

1.4.3 AID IN PREY DETECTION

Several species of mesopelagic fish and squid have been found to have yellow lenses [Denton, 1956; Somiya & Tamura, 1971; Somiya, 1976, 1979 & 1982; Muntz, 1976b & 1983; McFall-Ngai *et al.*, 1988; Thorpe *et al.*, in press]. It is thought that yellow lenses in predatory deep sea fish could serve to break the bioluminescent camouflage of its prey [Muntz, 1976b]. A yellow lens will affect the sensitivity of the retina, restricting the spectral range of both the blue-green downwelling illumination and the bioluminescence serving as camouflage. But, since the blue/green bioluminescence emits relatively more long-wave radiation than is present in the background illumination [Muntz, 1976b & 1990; Herring, 1983], this will increase the contrast of the supposedly camouflaged fish against the background.

Somiya (1976, 1977, 1979 & 1982) also suggests that yellow lenses may aid in the detection of bioluminescent light, but of longer wavelengths than the blue-green camouflage light. A number of mesopelagic fish have red light organs and have also been shown to be sensitive to such long-wave light [Denton *et al.*, 1985; Bowmaker *et al.*,

1988]. If such fish also have yellow lenses, as is the case in *Malacosteus, Aristostomias* and *Pachystomias* [Somiya, 1982; Douglas & Thorpe, in press], then the blue-green background illumination would be absorbed, yet the longer wavelengths generated by the photophores would be transmitted efficiently. Eliminating the background radiation therefore enhances the contrast of bioluminescent light against the background, which could be extremely useful for both intra-specific communication and prey detection, since such fish will be able to see without being seen by other species [Denton *et al.*, 1985].

1.4.4 SCAVENGING FREE RADICALS

Several species of deep-sea fish have carotenoid pigments in the lens, often resulting in a bright yellow colouration [McFall-Ngai *et al.*, 1986 & 1988; Yu *et al.*, 1991; Douglas & Thorpe, in press]. Carotenoids are commonly found in photosynthetic organisms where they play a protective role against singlet oxygen damage [Gregory, 1989]. Carotenoid pigments in the fish cornea [Moreland & Lythgoe, 1968; Bridges, 1969; Orlov & Gamburtseva, 1976; Appleby & Muntz, 1979] may act in a similar way to quench active oxygen produced within the eye by photooxidation [Kirschfeld, 1982]. Carotenoids are also found in the human macula region, where it is thought they protect the photoreceptors against damaging free radicals [Kirschfeld, 1982].

1.4.5 OTHER FUNCTIONS

It is possible, that certain types of lens and corneal pigmentation, such as the increasing pigmentation of the human lens with age, have no functional significance. Such changes may be due to the accumulation of degradation products which happen to absorb in the UV region [McEwen, 1959; Zigman, 1971]. The yellow lenses of highly diurnal species may therefore result from near UV exposure, with more pigment produced in those lenses exposed to higher levels of UV [Muntz, 1973]. Similarly, since downwelling illumination is more intense than upwelling light, then restriction of pigmentation in the cornea of many teleosts to a dorsal eye-shade may result [Heinermann, 1984].

Alternatively, the presence of lens filters in animals exposed to very high light levels could conceivably represent a method of lowering the intensity of incident radiation, being 'simply the most convenient way of achieving an increase in effective density in a transparent structure' [Muntz, 1982]. Lens pigments have also been suggested to have an effect on lens protein stability. The carotenoid pigment in one deep-sea species was found to be tightly bound to the lens alpha crystallin fraction and it is thought that the pigment may stabilise the lens protein [McFall-Ngai *et al.*, 1986].

Short-wave absorbing compounds may therefore be found in a range of ocular tissues; the cornea, the lens, the vitreous humour and the retina have all been found to have pigments absorbing short wavelengths. It is unlikely that all the functions discussed in this section are applicable

to pigments present in every species. A number of quite different, unrelated pigments have arisen independently in the eyes of vertebrate species, most probably to serve slightly different requirements. That nature should evolve so many methods of filtering short-wave radiation is testament to the functional importance of such compounds within the eye.

1.5 UV RADIATION UNDERWATER

The ultraviolet spectrum may be divided into three regions: UV-A, UV-B and UV-C. UV-C which covers 40-286nm is mainly absorbed by the earth's atmosphere. UV-B extends from 286-320nm and is responsible for skin tanning and the production of vitamin D. UV-B can be mutagenic at high doses but is of little significance for vision since the majority of these wavelengths are absorbed by proteins in the cornea and lens. UV-A covers 320-390nm and is the region in which the UV photoreceptors found in a number of freshwater fish are maximally sensitive [Avery *et al.*, 1983; Whitmore & Bowmaker, 1989]. UV-A, like UV-B can be biologically damaging at high doses [Calkins, 1982].

Both the intensity and spectral content of UV are greatly reduced as a function of water depth (Fig. 1-2). Despite this, intensities of UV sufficient for vision may reach depths of up to 100m in clear oceanic water [McFarland, 1986]. However, natural water is infrequently 'clear'. There are many factors which can reduce light

transmission under water; planktonic blooms and sediment upheavals for example are common and will affect the intensity and/or spectral content of light reaching greater depths.

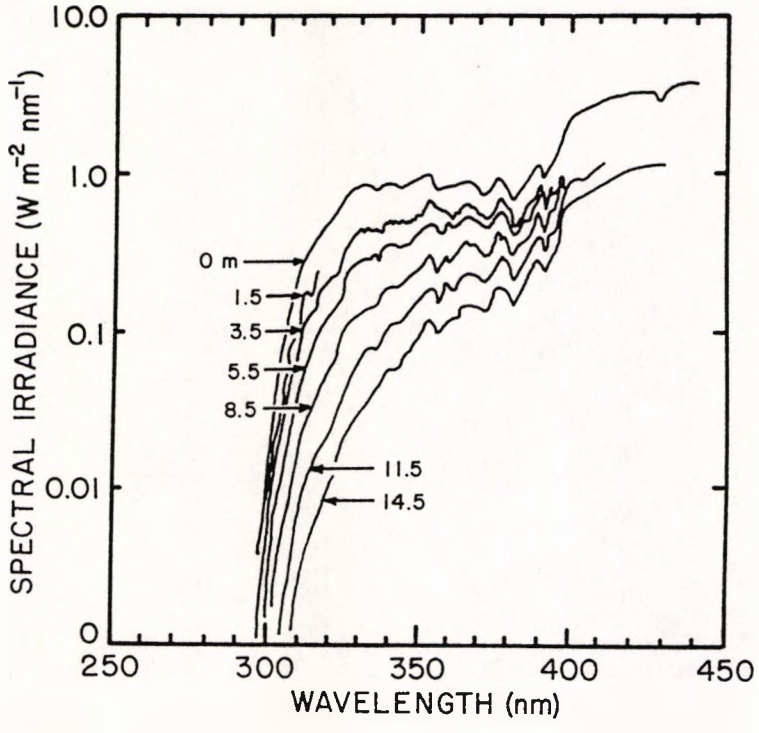


Figure 1-2. Downwelling spectral irradiance over the UV-A and UV-B regions at the water surface (0m) and at various depths. From: Loew & McFarland, 1990.

Marine waters have been classified into several types by Jerlov (1951 & 1976) based on their downwelling spectral irradiance. Different classes of Jerlov water have different compositions of dissolved and particulate matter causing them to transmit light to varying degrees. Coastal waters for example generally have a higher concentration of 'yellow substance' (degraded plant tissues) and transmit less UV and visible light than oceanic waters. The greater

scattering of UV (see Fig. 1-1) relative to longer wavelength radiation means that even low concentrations of phytoplankton or 'yellow substance' can have quite marked effects on UV transmission through water.

1.6 AIMS OF THIS STUDY

It is obvious when surveying the literature that although much work has been carried out on human lenses, relatively little time has been spent on the investigation of lens pigments in other animals. The aim of this project was to study further the types and significance of lens pigmentation, particularly in fish. Numerous species of fish from a wide range of habitats were examined to try and relate the phylogenetic presence of lens filters with possible functions. Biochemical identification of the pigments found in a number of fish lenses was also carried out.

Much is known about age-related changes in human lens pigmentation but correspondingly little is known about lens ageing in other species. Lens transmission was therefore measured for several fish species over reasonable age ranges to determine any changes occurring in lens pigmentation with age and to explore any similarity to human lens ageing.

Effects of the lighting environment on fish lens pigmentation were also investigated to determine whether high light intensities could initiate lens pigment deposition. Fish with and without lens pigments, were

reared under elevated and reduced light levels and also under UV illumination. Lens transmission was recorded from populations at the start and from experimental populations after experimental exposure for a number of months.

For comparison, lens transmission was measured in three invertebrate cephalopods occupying the same habitats as many marine species of fish. The lens transmission of a number of terrestrial vertebrates having quite different habitats to fish was also measured. Pigments were also extracted from a number of cephalopod and vertebrate lenses.

CHAPTER TWO

MATERIAL AND METHODS

2.1 MATERIAL

2.1.1 FISH

Fish from a variety of families inhabiting various environmental niches were studied. Most temperate marine species were obtained during several visits to the Plymouth Marine Laboratory and from a cruise of the R.R.S. Calanus arranged by the Scottish Marine Biological Association, Oban. The Horniman Museum Aquarium, Forest Hill, London provided a number of both freshwater and marine tropical species. Tropical marine species were also obtained from local dealers, as were the majority of the tropical freshwater species. Tilapia were obtained from Dr. L.G. Ross at the University of Stirling. Temperate freshwater fish were purchased from local fish farms and aquaria. The flying fish and *Stylephorus chordatus* were caught by Dr. R.H. Douglas on a North Atlantic cruise of the R.R.S. Discovery (No. 195).

2.1.2 OTHER ANIMALS

Monkey lenses were donated by other studies involving the lens capsule (Dr. M.J. Travers, City University) and retina (Dr. J.K. Bowmaker, Institute of Ophthalmology).

Sheep and chicken eyes were obtained from local abbatoirs. The squirrel and a few reptilian and amphibian species were from the Horniman Museum. The newt and salamander were purchased through Blades Biological. Guinea pig and frog eyes were donated by practical classes at the City University. All cephalopod specimens were provided by the Plymouth Marine Laboratory.

2.2 LENS SPECTRAL TRANSMISSION

After initial identification [Wheeler, 1978; Nelson, 1984; Axelrod & Schultz, 1990], small fish were killed by decapitation and large fish with a sharp blow to the head, followed by pithing. Eyes were enucleated by severing the optic nerve and were then hemisected and the lens removed; on the rare occasions when lenses showed signs of opacity, these lenses were discarded. Fish standard length (from snout to base of tail) and lens diameters were measured.

A number of lens holders with holes drilled in a range of diameters were constructed in aluminium. Each lens could then be placed in a holder of the correct size and held in position in the path of the beam of a spectrophotometer (Fig. 2-1). A Shimadzu UV-240 recording spectrophotometer (1mm x 3.5mm beam size) was used fitted with an integrating sphere (Fig. 2-2) which collected all wavelengths transmitted and scattered by a lens and focused them onto the detector.

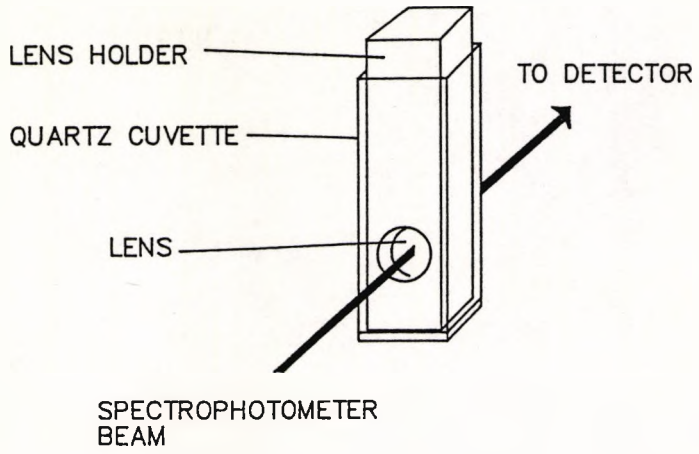


Figure 2-1. Diagram of lens positioned in metal holder within a quartz 1cm pathlength cuvette. The lens is held in position in the path of the spectrophotometer beam.

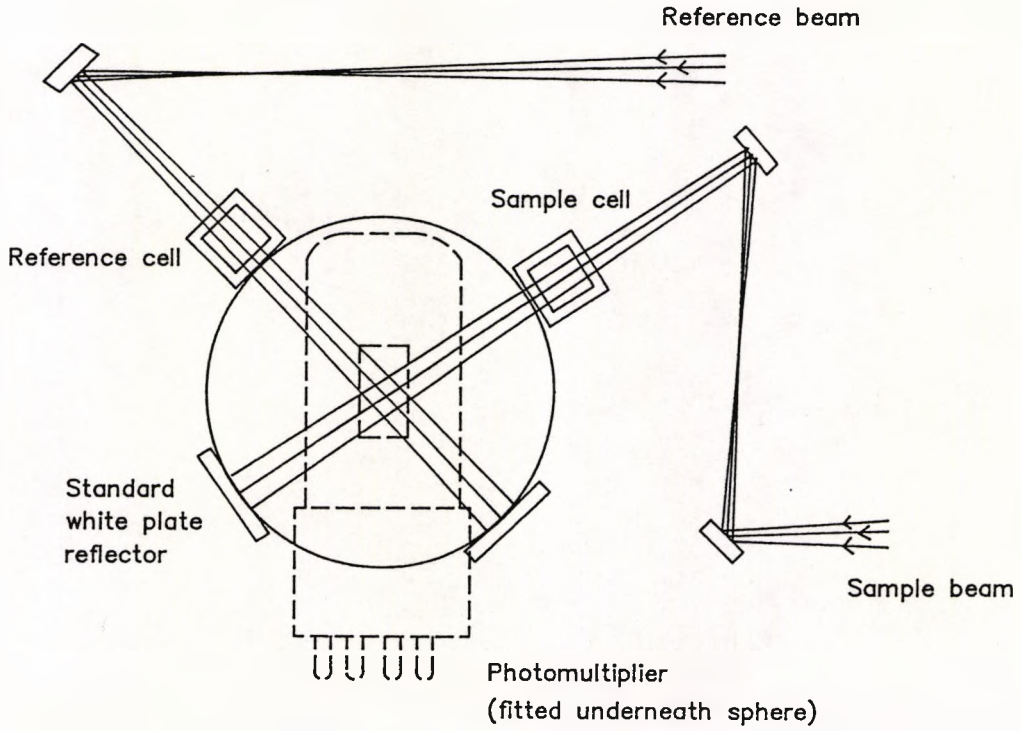


Figure 2-2. Optics of the Shimadzu UV240 integrating sphere.

Without an integrating sphere, only light rays passing through the lens undeflected would be measured, giving inaccurate transmission values, both in intensity and at incorrect wavelengths. Lens transmission and absorbance was recorded over the wavelength range 250nm to 700nm. The lower limit was imposed by the integrating sphere which was not reliable at wavelengths below 250nm. Data were obtained as transmission or absorbance at 5nm intervals and curves fitted on computer using a program written by Dr. D. Thompson (City University). Transmission spectra give a representation of the wavelengths transmitted by the lens which then impinge upon the retina. Ideally, maximum absorbance should be measured as an indicator of lens pigment concentration, but the spectrophotometer was not accurate for optical density values above 3 units. The wavelength at which the transmission fell to 50% of the maximum was therefore used to represent the concentration of pigments in a lens. Comparisons of 50% transmission values with maximum absorbance values (when these could be measured) revealed that 50% transmission was a good representation of pigment concentration.

Both lenses of an animal were examined as quickly as possible, usually within 10 minutes of death. Lenses were not placed in saline before or during scanning since pigments were found to leach readily when a lens was immersed in liquid. The effect of exposing lenses to the atmosphere for 15min. was investigated, but no difference in transmission spectra were noticed over this time (Fig. 2-3).

Corneas were scanned in a similar manner to the lens; cleaned corneas were flattened against the wall of the cuvette and the central portion positioned in the path of the spectrophotometer beam using a metal holder with a hole size of 2mm. For studies on pigmentation in the dorsal region of the cornea, the cornea was positioned so that this area was in the path of the beam.

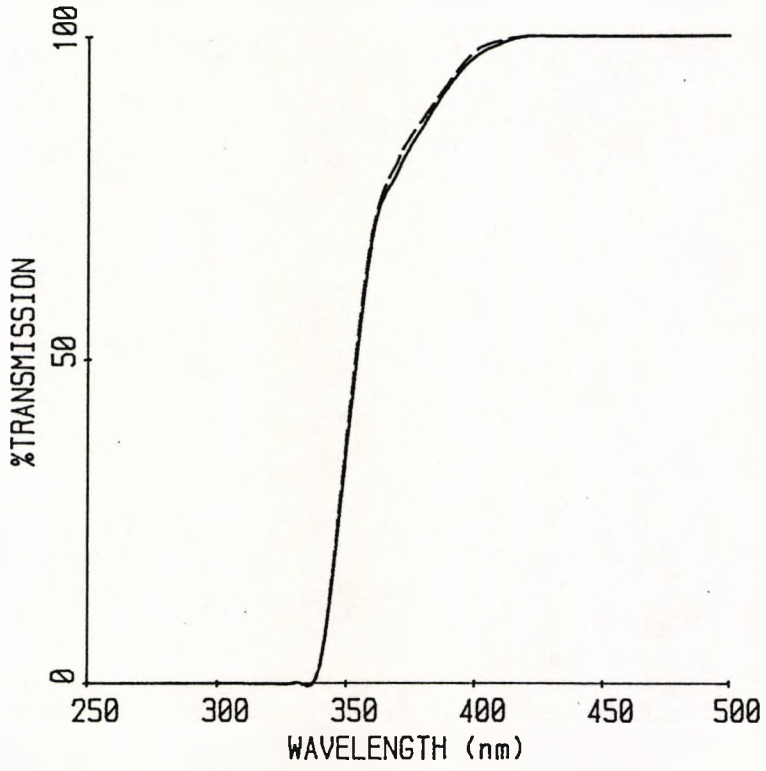


Figure 2-3. Spectral transmission of a *Carassius auratus* lens scanned immediately after death (dashed line) and after 15min. exposure to the atmosphere (solid line).

It was difficult to dissect the nucleus from a fresh lens without retaining cortex adhered to its surface. The easiest method of obtaining the lens nucleus for transmission recordings proved to be to leave the whole lens in saline for 30min., after which the cortex could be easily

slipped off the inner nuclear core. Recordings on nuclei dissected from a fresh lens and one left in saline, show that pigment leaching from the nucleus was minimal over this 30 minute time period (Fig. 2-4). The loss of pigments from the lens nucleus appears to be inhibited by the tightly packed nature of the lens fibres in this region; as the cortical fibres are more loosely arranged they allow the pigment to diffuse more readily. The nucleus was then scanned in the spectrophotometer in the same manner as for the whole lens.

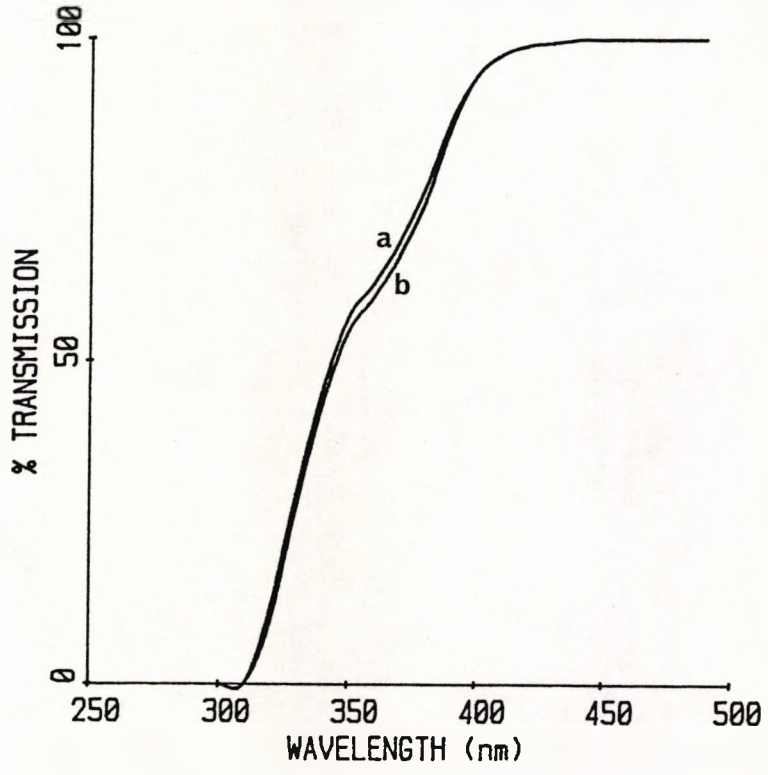


Figure 2-4. Spectral transmission of the lens nuclei of *Carassius auratus*. (a) Right lens nucleus scanned after the whole lens was placed in saline for 30min. and (b) left lens nucleus scanned immediately after death. Both nuclei were 2.01mm in diameter.

Lenses were frozen soon after scanning in a small volume (0.5-1ml) of saline (0.9% w/v.) for later pigment extraction. Corneas when examined, were similarly frozen at -40°C. Whenever possible the spectrophotometer was taken into the field so that lens transmission could be recorded soon after death. However, when this was impractical, lenses (or whole eyes) were frozen immediately after removal and thawed and scanned back at the laboratory. A number of whole frozen animals were also obtained from the Horniman Museum, London. The effect of freezing on lens transmission was therefore examined.

Both eyes were removed from a whiting (*Merlangius merlangus*) and the left eye was frozen intact at -20°C, the lens was removed from the right eye and scanned in the spectrophotometer before freezing. The right lens was thawed at room temperature, scanned and refrozen on 3 consecutive days. A final transmission scan was carried out after 4 weeks. The left eye was also thawed after four weeks and the lens was then enucleated, measured and scanned. The spectral transmission was almost identical for the four scans of the right lens (Fig. 2-5).

The initial scan of the right lens could almost be superimposed upon the scan of the left lens after 4 weeks at -20°C (Fig. 2-6). Therefore, no detrimental effects of the freeze-thaw cycle were observed on the lens transmission, whether the whole eye or just the excised lens was frozen.

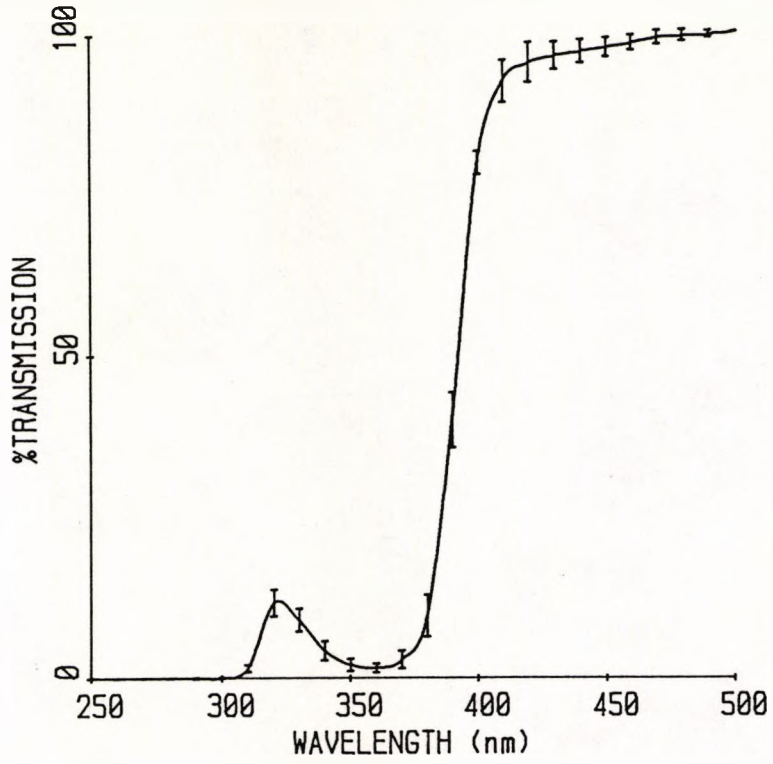


Figure 2-5. Mean spectral transmission of four scans of the right lens of *Merlangius merlangus* carried out for three consecutive days after death and after freezing for four weeks. The error bars indicate standard deviation.

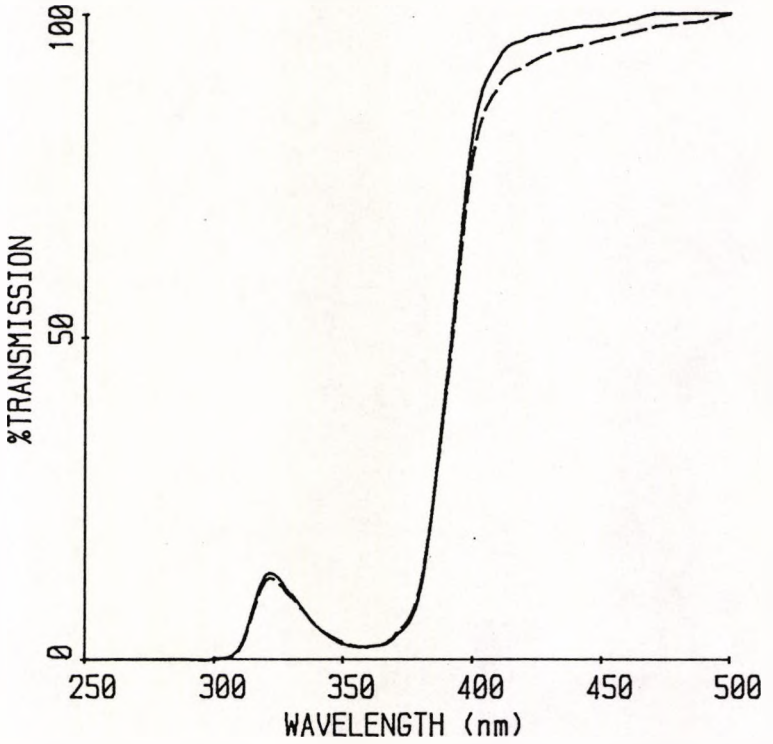


Figure 2-6. Spectral transmission of the right and left lenses of a *Merlangius merlangus*. The right lens was scanned immediately after death (solid line) whereas the left lens was scanned after the whole eye was frozen for four weeks (dashed line).

The spectral transmission of lenses of other animals was measured in practically the same manner as for fish lenses. However, the structure of the lens of most other species was bi-convex rather than spherical. Care was therefore taken to ensure that lenses were positioned in the same orientation as *in vivo*, with the equator of the lens resting against the sides of the holder and the anterior portion of the lens incident to the spectrophotometer beam.

Theoretical lens transmission was calculated for a number of fish lenses in order that changes with age could be predicted. This was based on a constant absorbance per unit pathlength through the lens. If the transmission of a lens with diameter **x**mm at a particular wavelength = T_1, then the transmission of a lens with diameter **y**mm, assuming the validity of Beer's and Lambert's laws, at the same wavelength is represented by: $T_1^{x/y}$. This formula was used to compare changes occurring in the spectral transmission of lenses accumulating pigments with age with theoretical lens transmission changes, based on increase in lens size alone, if no pigments were produced (see Chapter 4).

The transmission of most lenses was high between 500 and 700nm, at around 96-99%. However this absolute value varied quite markedly, especially in smaller lenses, depending on the positioning of the lens within the holder. A small shift of the lens into the holder and away from the detector resulted in quite a large loss in transmission. It was obvious that these values were not real and the absolute transmission at 632.8nm was therefore measured for a number of lenses using another method.

2.3 LENS ABSOLUTE TRANSMISSION

The absolute transmission of a number of lenses was measured using a high performance photodiode with an active area of 100mm^2 (R.S. Components) in a manner similar to that reported by Bassi *et al.* (1984). A He/Ne laser (Spectra-Physics model 13) emitting light at 632.8nm was used as the light source. Transmitted light impinging on the diode was converted to a voltage, amplified and displayed on a voltmeter. High light intensities saturated the diode and low light intensities produced no response. Therefore, a 2.1 neutral density filter was placed in front of the beam which was then focused by a low powered lens on the centre of the experimental lens (Fig. 2-6).

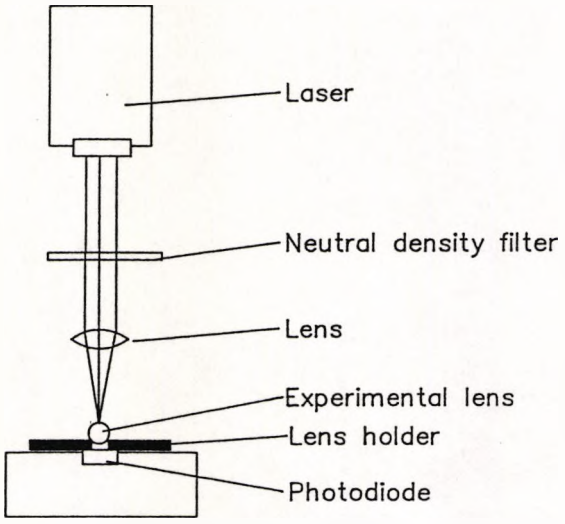


Figure 2-6. Diagram of the apparatus used to measure the absolute transmission of a lens at 632.8nm. The laser beam was focused onto the experimental fish lens and light transmitted passed to a photodiode where it was converted into a voltage reading.

The lens was placed on a metal holder with a 2mm hole, which was positioned on the surface of the diode. As the laser beam was smaller in cross-section than the diameter of the smallest lenses examined, no light could reach the detector without first passing through the lens.

The absolute transmission at 632.8nm was calculated using the voltage recorded with the lens in position (V_{trans}) divided by that recorded without the lens (V_{inc}). The absolute transmission did not vary much between lenses and was generally found to be around 96-99%, which was comparable to readings obtained for larger lenses at 632.8nm on the spectrophotometer. Lens transmission will never reach 100% in reality due to light forward reflected off the anterior and posterior surfaces of a lens. However, whole lens transmission recordings made on the spectrophotometer were adjusted to 100% at 700nm for all lenses before scanning, for ease of comparison of spectra.

2.4 FISH AGE DETERMINATION

Fish age determination by scale annulus counting is time-consuming and can often be inaccurate [Boehlert, 1985]. It was shown for the brown trout that the lens diameter gives a good indication of the relative age [Douglas, 1987]. The assumption was made that older fish would have larger lens diameters than younger fish of the same species. Lens diameter was therefore taken as an indicator of relative age within a species for most fish used in this study.

In a few species of fish, the age was estimated by counting growth rings on the scales. Areas of slow growth are shown as tight concentric rings, whilst rapid growth is indicated by spaced rings. In wild species of fish the drop in temperature in winter is often accompanied by a lack of food and therefore little growth is possible. This results in the production of closely spaced annuli every winter, and therefore estimation of age can be carried out by simply counting these annuli.

A few scales were removed from the fish from above the lateral line just below the dorsal fin. Scales were soaked in 4% Sodium hydroxide for 1 hour and then washed in water to remove pigmented tissue. Scales with imperfect centres were discarded, remaining scales were mounted on a slide with a coverslip and annuli counted under low power on a microscope.

2.5 PIGMENT EXTRACTION

Lens pigment extracts were made from previously frozen lenses. When several lenses had been pooled and frozen, the pigments were extracted from them all together; lenses from individual animals, when large enough, were extracted alone. Lenses were thawed at room temperature and homogenised in a ground glass 10ml tissue homogeniser for 5 minutes. Distilled water was added when necessary to bring the total extract volume to between 1ml and 5ml depending upon the quantity of lens material. Samples were then centrifuged at

100,000g using a Sorvall-RC65 ultracentrifuge fitted with an 8 x 50ml rotor (4^{o}C, 30min.) to pellet the high/medium molecular weight protein.

Low molecular weight soluble proteins and any pigment remained in the supernatant, which was pipetted off the pellet and further purified by ultrafiltration under nitrogen using Amicon YM2 membranes with a pore size of 1000Da fitted to a 50ml Amicon cell. The filtrate was scanned for pigment absorbance on a Phillips PU 8740 spectrophotometer.

Corneal pigment extracts were generally made in the same manner as lens extracts. However the orange pigment from the goldfish cornea was extracted with 100% acetone, since it is not water soluble.

2.6 PROTEIN ESTIMATION

Protein pellets from centrifugation were suspended in 1ml of distilled water and water-insoluble proteins solubilised by the addition of 0.5ml 5% SDS, 2% NaOH. The protein concentration of the lens pellet was determined by diluting to 10ml with water, measuring the absorbance of the sample at 280nm and comparing the absorbance to a series of BSA protein standards. The total absorbance of extracted pigment was expressed as a fraction of both mg of protein in the pellet and the total volume of the lenses used for extraction.

2.7 PIGMENT IDENTIFICATION

Lens pigment samples were prepared and extracted at University College, London. The lyophilised pigments were then taken to the Dept. of Chemistry at the University of Wollongong, Australia, where further purification and identification by HPLC and NMR was carried out.

2.7.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Lyophilised lens pigment extracts were prepared for isocratic reverse-phase high performance liquid chromatography (HPLC) by redissolving approximately 5mg in 1ml 20% methanol (HPLC grade) and filtering through a C-18 Cep Pak cartridge. A Whatman 10 ODS-3 semi-preparative column (250mm x 9.4mm internal diameter) protected by a Bondapak C-18 pre-column was fitted to the system. A mobile phase of 20% methanol and 0.1% Acetic acid (filtered and degassed prior to use) was used as detailed by Dunlap *et al.* (1989), with a flow rate of 2ml/min. Filtrate absorbance was monitored on a Knauer variable wavelength detector at 340nm and recorded on a Shimadzu C-R6A Chromatopac recorder.

Up to 0.5ml of a sample was injected into the system for each run and any peaks were collected, pooled and lyophilised. Dried purified samples were then redissolved in 1ml of 20% methanol and scanned on a Shimadzu UV-250 spectrophotometer. Pigment identification was carried out by injecting an equal volume of a known standard with the sample and monitoring any increase in absorbance at the retention time of the standard.

2.7.2 THIN LAYER CHROMATOGRAPHY

Samples which could not be identified by HPLC methods were analysed further by thin layer chromatography (TLC) using butanol: acetic acid: water (12:3:5) as the solvent. Approximately 40μl of each sample was loaded along with 40μl of a tryptophan solution onto pre-prepared silica plates (Merck), and run for 30min. until the solvent front reached around 3/4 of the way up the plate. After drying, plates were stained with both Ehrlich's reagent (0.1% p-dimethylaminocinnamaldehyde [DMAC] in 1M HCl) and ninhydrin.

2.7.3 NUCLEAR MAGNETIC RESONANCE

The fine structure of molecules can often be elucidated by studying the spectra recorded by nuclear magnetic resonance (NMR). Each proton in a sample produces a characteristic chemical shift according to its environment with respect to the rest of the molecule. The technique is extremely sensitive and requires only around 5mg of pure sample.

Purified dry lens pigments were prepared for proton nuclear magnetic resonance (^{1}H-NMR) by dissolving in 0.7ml of Deuterium oxide (D_2O). 1μl of DSS was added as an internal standard. Samples were run on a Varian Unity 400MHz NMR spectrometer by either Dr. J. Carver or Maribel Nonato at The University of Wollongong, Australia until enough scans were made to overcome the background noise effects. Comparisons with chemical manufacturer's NMR data (BDH) were carried out to investigate possible structures from textbook allocations of signals [Brügel, 1979].

CHAPTER THREE

SPECTRAL TRANSMISSION AND SHORT-WAVE ABSORBING PIGMENTS IN THE LENSES OF FISH

3.1 INTRODUCTION

3.1.1 LENS SPECTRAL TRANSMISSION

Intraocular pigmentation in fish has been a subject of interest for decades. Early work was strictly qualitative, concentrating on the presence of yellow colouration in the ocular tissues [Walls & Judd, 1933a&b]. That a fish lens could absorb much of the incident UV radiation and yet still appear colourless to the human eye was shown by Kennedy & Milkman (1956), who used a spectrophotometer to measure whole lens transmission down to 320nm and also by Denton (1956), who photographed mercury spectra through the lens. These techniques suggested the presence of short-wave absorbing compounds in the lenses of many fish [Denton, 1956]. Most work to date however, has tended to concentrate on absorbance at longer wavelengths and there have, until recently, been relatively few lens transmission and absorbance studies in the UV region [Kennedy & Milkman, 1956; Burkhardt, 1966]. Some recent studies however have measured spectral properties down to around 200nm [reviews: Heinermann, 1984 and Muntz, 1972; Hawrhyshyn *et al.*, 1985; Chou & Hawrhyshyn, 1987; Douglas, 1989; Douglas & McGuigan, 1989].

A number of freshwater species have been shown to have lenses transparent to near UV radiation [Douglas & McGuigan, 1989; Hawrhyshyn *et al.*, 1989; Whitmore & Bowmaker, 1989], with numerous reports of high UV transmission by the ocular tissues of the goldfish [Burkhardt, 1966; van den Berg & Mooij, 1982; Bassi *et al.*, 1984; Hawrhyshyn *et al.*, 1985; Douglas, 1989]. Most proteins have aromatic residues which, due to their physical structure, absorb radiation around 260 to 280nm. Thus, even lenses with no specific short-wave pigments will absorb appreciably below 310nm. In the goldfish, wide intraspecific variations are described, with lens transmission cut off points ranging from 315-370nm [van den Berg & Mooij, 1982; Bassi *et al.*, 1984; Hawrhyshyn *et al.*, 1985; Douglas, 1989]. Such variability may be in part accounted for by the range in lens sizes examined, since a larger lens will correspondingly absorb more UV and have a longer cut off wavelength than a smaller one (see Chapter 4). Another factor to consider is that some goldfish do actually accumulate short-wave absorbing pigments in the lens which shifts the lens cut off, often considerably, to longer wavelengths [Douglas, 1989; see also Section 4.2.2.1].

Lenses of young rainbow trout, roach and rudd, like young goldfish, have also been shown to be free of short-wave absorbing compounds [Douglas, 1986; Hawrhyshyn *et al.*, 1989; Whitmore & Bowmaker, 1989], which is hardly surprising given that all these species are reported to be UV sensitive [Avery *et al.*, 1983; Hawrhyshyn & Beauchamp, 1985; Douglas, 1986; Hawrhyshyn *et al.*, 1989; Whitmore &

Bowmaker, 1989; Bowmaker *et al.*,1991]. A relatively UV-transparent lens is of course essential to ensure that short wavelengths reach the UV sensitive cones in the retina of these species. Interestingly, UV sensitivity is reported to be restricted to younger individuals of brown trout [Bowmaker & Kunz, 1987], when lens short-wave transmission is at its highest; UV sensitivity is absent in older fish, by which time the lens has accumulated short-wave absorbing pigments [Douglas, 1989; Douglas *et al.*, 1989].

Many species have lenses containing specific short-wave absorbing pigments, but the pigment concentration is not always high and frequently results in the transmission of a portion of the near UV (350-400nm) even though higher energy radiation is absorbed [Denton, 1956; Kennedy & Milkman, 1956; Douglas, 1989; Douglas & McGuigan, 1989]. When short-wave absorbing pigments are present in a lens, they compound the effects of protein absorbance, resulting in longer transmission cut off points (from 330 to 400nm) than seen for unpigmented lenses [Muntz, 1973; Douglas & McGuigan, 1989]. Occasionally short-wave absorbing pigments are found at such high concentrations that the lenses appear yellow, since they absorb into the (human) visible region of the spectrum; this is common in wrasses and parrotfish [Muntz, 1973]. Yellow lenses (and often yellow corneas) are also frequently found in cichlids and other highly diurnal species which are exposed to high light intensities [Muntz, 1973; Lythgoe, 1975; Douglas & McGuigan, 1989].

Fish with yellow filters often spend the night time inactive, pressed up against a bank or actually buried in

the sand [Lowe-McConnell, 1964 & 1969]; a natural solution, since yellow filters will undoubtedly adversely affect their sensitivity [Walls & Judd, 1933a; Muntz, 1973 & 1976a]. Daytime populations of fish tend to be replaced at sunset with a variety of nocturnal species [Lowe-McConnell, 1964], which unlike diurnal species, always have clear ocular media [Denton, 1956; Douglas & McGuigan, 1989].

Paradoxically, although yellow lenses in fish tend to be loosely associated with high light environments [Zigman, 1982 & 1987], they have also been reported in a number of mesopelagic species living at depths greater than 100m [Denton, 1956; Somiya & Tamura, 1971; Muntz, 1976b; Somiya, 1976, 1977, 1979 & 1982; McFall-Ngai *et al.*, 1986 & 1988; Thorpe *et al.*, in press; Douglas & Thorpe, in press] where the light intensities are extremely low. The yellow lenses of a number of deep-sea fish, like those of shallow swimming fish, often absorb appreciably in the UV [Douglas & Thorpe, in press]. This is worth emphasising since not all yellow pigments necessarily absorb at such short wavelengths: some carotenoid pigments for example, although yellow in colour, absorb in the 400-500nm region with low absorbance below 400nm [e.g. Moreland & Lythgoe, 1968; see also Fig. 6-1].

3.1.2 BIOCHEMICAL IDENTITY OF PIGMENTS

It has recently been shown that pigments in the lenses of many marine fish are mycosporine-like amino acids [Dunlap *et al.*, 1989]. Mycosporines are a group of closely related, low molecular weight, water soluble compounds absorbing in the mid UV region (300-360nm) [Favre-Bonvin *et al.*, 1976;

Nakamura *et al.*, 1982]. They were first identified associated with light-stimulated sporulation in terrestrial fungi [Leach, 1965]. Since then, mycosporine compounds have been shown to be widespread in the marine environment, especially in invertebrate species such as sea anemones, corals and jellyfish [Takano *et al.*, 1978a&b; Hirata *et al.*, 1979; Dunlap & Chalker, 1986].

It is thought that red algae and the symbiotic algae of reef corals synthesise mycosporines thereby conferring UV protection to the sensitive photosynthetic pigments of the community [Dunlap *et al.*, 1986; Wood, 1989]. Mycosporines are produced in coral in response to UV exposure [Jokiel & York, 1982] and concentrations fall with increasing depth as UV penetration falls off [Maragos, 1972; Dunlap *et al.*, 1986]. Mycosporine-like compounds are also often found at appreciable concentrations in the fish cornea and iris as well as the lens [Dunlap *et al.*, 1989]. The compounds present in fish ocular tissues are palythinol (l_{max} 319nm), palythine (l_{max} 320nm), asterina-330 (l_{max} 330nm) and palythene (l_{max} 360nm) [Dunlap *et al.*, 1989]. One or more of these compounds are generally present at varying concentrations, resulting in a variety of UV transmission and absorbance spectra. The yellow colour of the lenses in some fish [Muntz, 1976a], is due to a high concentration of the longer wavelength absorbing compound (palythene) in the lens [Dunlap *et al.*, 1989]. It is not known if mycosporine pigments are actually metabolised by fish, but it is highly likely, given their widespread distribution, that either mycosporine compounds or precursors form part of the diet.

Mycosporine compounds are not restricted to the ocular tissues of fish. Gadusol and other mycosporine-related compounds are also found in the eggs of marine fish [Chioccara *et al.*, 1980; Grant *et al.*, 1980; Plack *et al.*, 1981]. The internal organs of adult fish are protected from UV radiation by the external scales, but before its scales are fully developed, an embryo may be at risk from UV damage. Gadusol absorbs short-wave UV (l_{max} 318nm) and could protect the developing embryo from the damaging effects of such short-wave radiation, since it is present at considerable concentrations in the eggs of many marine fish [Plack *et al.*, 1981].

Mycosporine-like compounds, however, are not the only pigments known to occur in the fish lens. Tryptophan metabolites have been found in the lenses of a number of species [van Heyningen, 1973b] and it is possible that they are also present in the fish lens [e.g. Zigman, 1987]. Tryptophan is an essential amino acid, present in the majority of proteins, and therefore forms part of the diet of all fish. Several species of deep-sea fish have carotenoid pigments in the lens, resulting in a bright yellow colouration [McFall-Ngai *et al.*, 1986 & 1988; Douglas & Thorpe, in press].

Carotenoids are commonly found in photosynthetic organisms where they play a protective role against singlet oxygen damage [Gregory, 1989]. They are also found in the fish cornea [Moreland & Lythgoe, 1968; Bridges, 1969; Orlov & Gamburtseva, 1976; Appleby & Muntz, 1979] and may act in an analogous manner to quench active oxygen produced within

the eye by photooxidation [Kirschfeld, 1982]. Although concentrations of zeaxanthin and lutein (carotenoids in the primate macula region) vary widely between individuals [Werner *et al.*, 1987; Bone *et al.*, 1988; Handelman *et al.*, 1988], it is thought that they may protect photoreceptors against damaging free radicals [Kirschfeld, 1982]. Since deep-sea animals are unlikely to be exposed to physiologically damaging amounts of short-wave radiation, it is difficult to imagine that the carotenoids present in the lenses of these species serve such a protective role.

The almost universal nature of carotenoids and oxygenated carotenoids (xanthophylls) in photosynthetic plants, algae and bacteria means that there is always a plentiful source of carotenoids for accumulation into lenses of fish. Carotenoids do not absorb in the UV region and are therefore not equivalent to short-wave absorbing mycosporines: the presence of carotenoids in the lens probably evolved to serve a quite different function.

Other deep-sea species have been found with lens pigments having quite different absorbance spectra to the short-wave absorbing pigments already identified [Somiya, 1982; Muntz, 1976b; McFall-Ngai *et al.*, 1988; Douglas & Thorpe, in press]. A number of these have λ_{max}s around 400nm, which is longer than those of the mycosporine compounds found in surface-dwelling species yet shorter than carotenoid absorbance. A number of deep-sea species do have lens pigments with shorter λ_{max}s, around 340-360nm [Douglas & Thorpe, in press], but the biochemical identities and any similarity to mycosporine compounds have yet to be shown.

Several biochemically distinct lens pigments have therefore been described in fish: mycosporine-like amino acids, carotenoid compounds and a range of biochemically distinct unidentified short-wave absorbing compounds. All these give rise to lenses with a variety of spectral transmission and absorbance characteristics. Although many functions for such pigments have been suggested (Chapter 1), their purpose in most cases remains a matter of debate. This chapter looks at the spectral characteristics of the lenses in a large number of previously unexamined species representing a wide variety of both marine and freshwater families and identifies some of the short-wave absorbing pigments. It is hoped that this will lead to an insight into both the phylogenetic distribution and function of lens pigmentation in fish.

3.2 RESULTS

3.2.1 SPECTRAL TRANSMISSION OF FISH LENSES

Fish lenses fell into three general categories according to their transmission characteristics: those with no lens pigment, those with intermediate pigment concentrations and a final group with high pigment concentrations. The presence or absence of lens pigments was, in the first instance, determined by the appearance of the transmission spectra. Fish with no lens pigmentation had spectra similar to that shown in Fig. 3-1a and the

wavelength at which transmission was at 50% of the maximum was generally around 310-330nm in this group, depending on lens diameter. The drop in transmission at around 300nm is due to absorbance by aromatic residues (mainly tryptophan and tyrosine) within the lens structural proteins. No detectable amounts of specific short-wave absorbing pigments could be extracted from lenses of fish in this group.

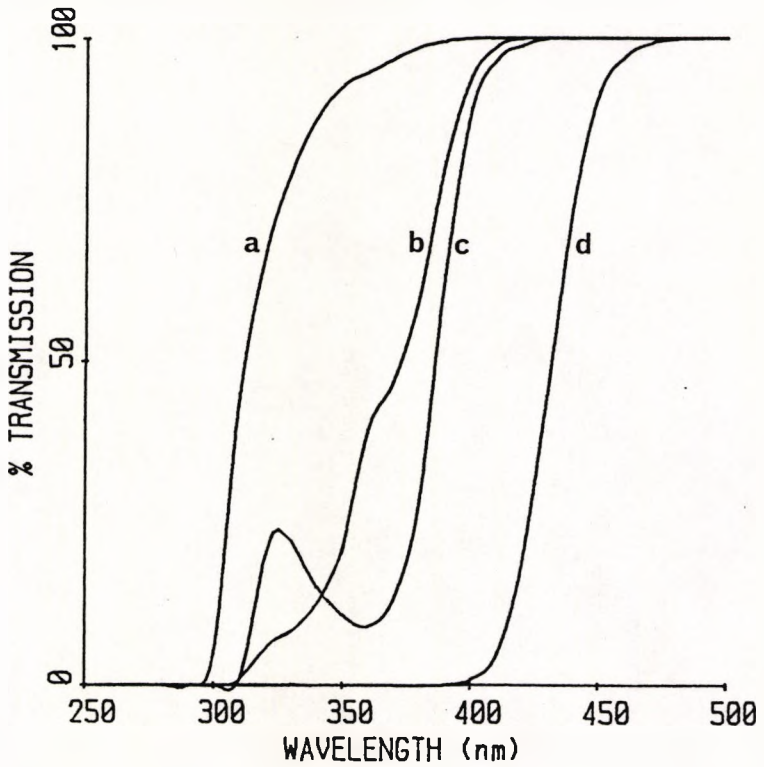


Figure 3-1. Spectral transmission of lenses of (a) *Salmo trutta* with no lens pigment, (b) *Oreochromis niloticus* and (c) *Merlangius merlangus* with intermediate pigment concentrations and (d) *Herotilapia multispinosa* with a high pigment concentration.

When pigments were present at low concentrations, shoulders appeared on the usually smooth transmission spectra (e.g. Fig. 3-1b&c) and the spectra were often shifted to longer wavelengths when compared to spectra of lenses with no pigment. The shape of the transmission trace varied greatly depending on which pigments were present and on their concentrations (see Section 3.2.3), resulting in a large range in 50% transmission wavelengths (from 320nm to around 390nm).

Higher concentrations of pigment shifted the lens transmission quite significantly to longer wavelengths (Fig. 3-1d). Wavelengths transmitted through the lens were severely restricted in these fish so that the 50% transmission wavelength was frequently as high as 450nm. A list of all the species of fish examined with their lens 50% transmission wavelength and the extracted pigment λ_{max} is shown in Table 3-1. The relationship between the whole lens transmission and the extractable pigment is discussed in greater detail in Section 3.2.3.

Table 3-1. List of the 108 species of fish examined, showing the number of fish from each species and the wavelength at which transmission fell to 50% of the maximum for the largest lenses from each species. λ_{max}s of short-wave absorbing compounds extracted from the lenses are also shown.

ORDER/FAMILY	SPECIES	NO.	50%T (nm)	λ_{max} (nm)	GROUP
LAMNIFORMES					
Scyliorhynidae	*Scyliorhinus canicula*	4	340	-	CM
RAJIFORMES					
Rajidae	*Raja naevus*	1	335	-	CM
	Raja batis	1	334	-	CM
	Raja montagui	1	325	-	CM
	Raja microocellata	1	328	-	CM
ANGUILLIFORMES					
Congridae	*Conger conger*	1	341	-	CM
Muraenidae	*Muraena helena*	1	383	+	CM
OSTEOGLOSSIFORMES					
Pantodontidae	*Pantodon buccholzi*	7	355	329,360	TF
Mormyridae	*Gnathonemus petersi*	1	323	-	TF
CLUPEIFORMES					
Clupeidae	*Clupea harengus*	26	425	324,360	CM
	Sprattus sprattus	1	419	+	CM
CYPRINIFORMES					
Cyprinidae	*Barbus callipterus*	1	326	-	TF
	Rhodeus sericeus	1	315	-	CF
	Leuciscus idus	10	330	-	CF
	Puntius tetrazona	1	345	-	TF
	Rutilus rutilus	6	324	-	CF
	Cyprinus carpio	25	378	324,360	CF
	Carassius auratus	198	382	324,360	CF
CHARACIFORMES					
Characidae	*Phygocentrus piraya*	1	370	325,360	TF
	Rooseveltiella nattereri	1	346	320	TF
	Hyphessobrycon callistus	1	325	-	TF
SILURIFORMES					
Siluridae	*Kryptopterus bicirrhis*	1	318	-	TF
Osmeridae	*Osmerus eperlanus*	2	335	-	CM
SALMONIFORMES					
Salmonidae	*Salmo gairdneri*	16	334	-	CF
GADIFORMES					
Gadidae	*Gadus morhua*	7	400	360	CM
	Pollachius virens	6	399	360	CM
	Melanogrammus aeglefinus	4	395	360	CM
	Merlangius merlangus	33	390	360	CM
	Pollachius pollachius	8	397	340,360	CM
	Trisopterus minutus	9	340	-	CM
	Trisopterus luscus	8	347	-	CM
	Ciliata mustela	1	358	-	CM

ORDER/FAMILY	SPECIES	NO.	50%T (nm)	l_{max} (nm)	GROUP
GADIFORMES Cont/d.					
Merlucciidae	*Merluccius merluccius*	5	341	-	CM
LOPHIIFORMES					
Lophiidae	*Lophius piscatorius*	2	400	323,360	CM
Antennariidae	*Antennarius hispidus*	1	391	330,360	TM
CYPRINODONTIFORMES					
Exocoetidae	*Exocoetus obtusirostris*	15	435	395	CM
Poeciliidae	*Poecilia reticulata*	1	315	-	TF
ATHERINIFORMES					
Melanotaenidae	*Melanotaenia parkinsoni*	1	315	-	TF
LAMPRIFORMES					
Stylephoridae	*Stylephorus chordatus*	5	453	360	CM
GASTEROSTEIFORMES					
Gasterosteidae	*Spinacchia spinacchia*	5	321	-	CM
DACTYLOPTERIFORMES					
Syngnathidae	*Syngnathus acus*	1	418	+	CM
	Syngnathus typhle	11	420	360	CM
	Hippocampus ramulosus	1	421	363	CM
SCORPAENIFORMES					
Agonidae	*Agonus cataphractus*	1	395	+	CM
Cottidae	*Cottus gobio*	3	408	+	CM
	Taurulus bubalis	4	400	+	CM
Triglidae	*Trigla lucerna*	7	403	328,360	CM
	Aspitrigla cuculus	8	398	325,360	CM
Scorpaenidae	*Pterois volitans*	4	401	322,360	CM
PERCIFORMES					
Centrarchidae	*Lepomis gibbossus*	3	404	325,360	CF
	Lepomis cyanellus	1	398	+	CF
Chaetodontidae	*Chaetodon sp.*	2	377	331	TM
	Chaetodon frembli	1	374	330	TM
	Chaetodon melanotus	1	404	332	TM
Cichlidae	*Tilapia mariae*	1	388	+	TF
	Oreochromis niloticus	66	398	324,360	TF
	Haplochromis ishmaeli	16	390	320,358	TF
	H. argens	11	328	-	TF
	H. piceatus	2	360	+	TF
	H. sauvagei	5	394	+	TF
	H. pyrrocephalus	2	339	+	TF
	H. xenognathus	4	390	323,360	TF
	Macropleurodus bicolor	3	385	320,360	TF
	Astatorheochromis alluaudi	4	355	+	TF
	Platytaeniodus degeni	3	385	+	TF
	Lamprologus dahlia	1	351	320	TF
	L. tetrocephalus	1	348	321	TF
	Crenicichla lepidota	1	400	+	TF
	Cichlasoma sevrum	2	417	325,360	TF
	Astronotus ocellatus	4	430	324,360	TF
	Herotilapia multispinosa	5	432	327,362	TF
	Apistogramma curviceps	11	436	370	TF

ORDER/FAMILY	SPECIES	NO.	50%T (nm)	l_{max} (nm)	GROUP
PERCIFORMES Cont/d.					
Ciclidae	*Aequidens maronii*	2	440	370	TF
	Aequidens pulcher	100	---	370	TF
Plesiopidae	*Calloplesiops altevelis*	1	407	326	TM
Labridae	*Labrus bergylta*	2	321	-	CM
	Crenilabrus melops	2	319	-	CM
	Ctenolabrus rupestris	1	315	-	CM
	Thalassoma sp.	1	430	361	CM
Anabantidae	*Ctenopoma oxyrhynchus*	1	430	+	TF
	Osphronemus goramy	1	398	326,360	TF
	Helostoma temmincki	20	387	329,360	TF
	Colisa fasciata	2	385	327,360	TF
Belontidae	*Trichogaster microlepis*	13	450	370	TF
	T. trichopterus	10	442	370	TF
	Betta splendens	2	435	370	TF
Acanthuridae	*Acanthurus sp.*	1	404	331	TM
	Zebrasoma flavescens	1	379	+	TM
	Paracanthurus hepatus	3	390	330	TM
Mullidae	*Mullus surmuletus*	2	327	-	CM
Pomacanthidae	*Pygoplites diacanthus*	2	402	331	TM
	Holacanthus ciliaris	1	406	330,360	TM
	Centropyge heraldi	1	395	327	TM
Pomacentridae	*Amphiprion sp.*	2	350	+	TM
Bleniidae	*Parablennius gattorugine*	1	407	+	CM
	Liophrys pholis	2	403	+	CM
Callionymiidae	*Callionymus lyra*	14	385	321,359	CM
Carangidae	*Trachus trachurus*	23	400	327,359	CM
Gobiidae	*Thorogobius ephippicutus*	1	396	+	CM
	Pomatoschistus microps	1	389	+	CM
Percichthyidae	*Dicentrarchus labrax*	4	404	+	CM
Scombridae	*Scomber scombrus*	6	380	325,360	CM
Stichaeidae	*Lumpenus lampretaeformis*	3	374	+	CM
PLEURONECTIFORMES					
Pleuronectidae	*Platichthys flesus*	5	408	323,360	CM
	Pleuronectes platessa	18	405	331,360	CM
	Limanda limanda	6	403	328,360	CM
	Microstamus kitt	1	405	+	CM
	Hippoglossoides platessoides	4	380	+	CM
Soleidae	*Microchirus variegatus*	2	325	-	CM
	Buglosidium luteum	2	322	-	CM
	Solea solea	2	330	-	CM
TETRAODONTIFORMES					
Tetradontidae	*Arothron hipsidus*	1	407	+	TM
Diodontidae	*Diodon lystrix*	1	301	321,360	TF
Balistidae	*Cantherhines pullus*	2	380	326	TM

KEY

TM - Tropical marine
TF - Tropical freshwater
CM - Cold marine
CF - Cold freshwater

\+ Pigment present but not extracted
\- No detectable pigment

3.2.2 LENS PIGMENT ABSORBANCE

Whole lens absorbance was not particularly informative for the majority of lenses since absorbance by pigments was usually too high to achieve an acceptable level of resolution on the spectrophotometer (e.g. Fig. 3-2a). With highly pigmented lenses it was often possible to get an idea of which pigments were present in a lens by scanning just a slice of lens in the spectrophotometer. Since the pathlength was reduced, absorbance was not as high as for the whole lens and higher resolution could be achieved. This technique was used for the oscar (*Astronotus ocellatus*), where a section revealed two main pigments in the lens with estimated l_{max}s at 325nm and 360nm (Fig. 3-2b).

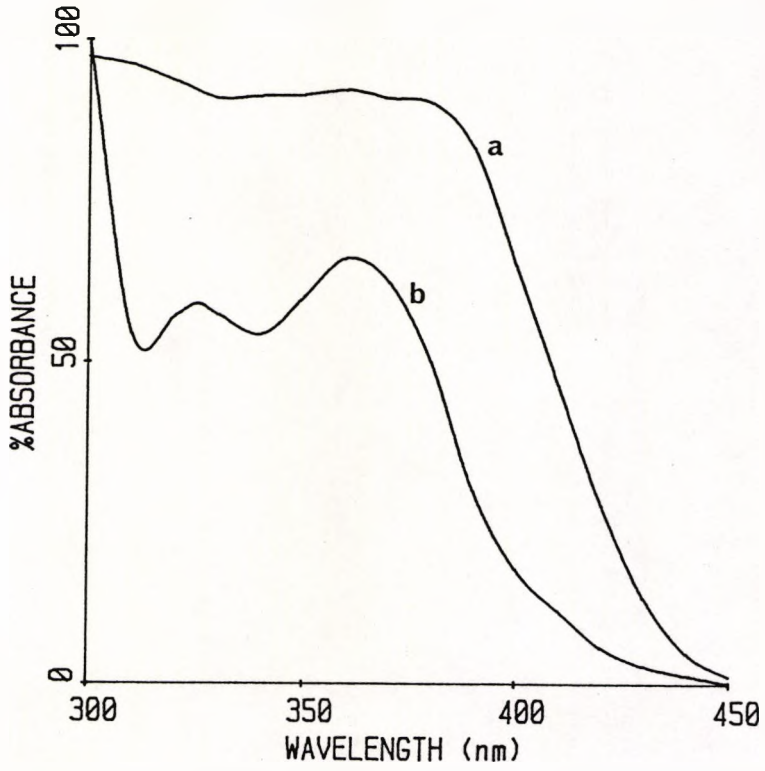


Figure 3-2. Absorbance spectra of (a) the whole lens of the *Astronotus ocellatus* (Lens diameter 2.79mm) and (b) of a section through the same lens (approximately 1mm thick).

Similarly, in the keyhole cichlid (*Aequidens maronii*), the whole lens absorbance gave no clue as to the pigment present, but the absorbance spectrum of a section revealed one pigment with l_{max} 370nm (Fig. 3-3).

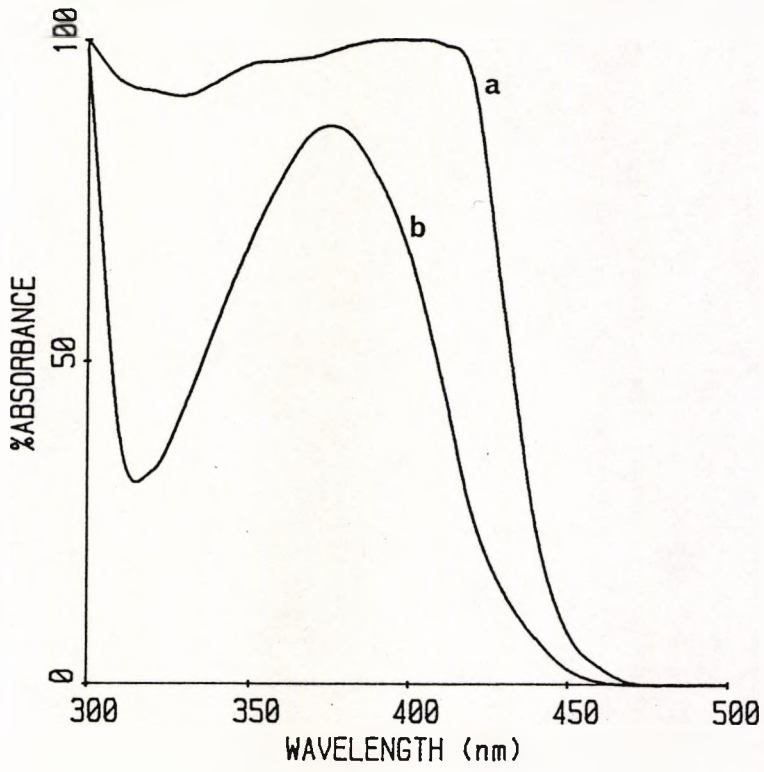


Figure 3-3. Absorbance spectra of (a) the whole lens of *Aequidens maronii* (lens diameter 1.58mm) and (b) of a section cut through the same lens (approximately 1mm thick).

Whole lens transmission spectra of the keyhole cichlid and the oscar looked very similar with only a slight difference in 50% transmission: (keyhole cichlid 440nm, oscar 432nm). It was therefore not possible to even speculate which pigments were present in the lens of these species from whole lens transmission spectra alone. Measuring the absorbance of a lens section therefore permits the study of pigments further than is possible by both

whole lens transmission and absorbance. However, when enough material was available it was preferable to extract the pigments from lenses and to characterise the lens pigments of a species in this manner, especially since l_{max}s of lens extracts were consistently slightly shorter than in the whole lens.

It was noticed that when most lenses were left standing in saline for any length of time they became opaque. The bathing fluid of such lenses when scanned on the spectrophotometer revealed that pigments were leaching out of the lens. This suggested that the pigments were free to move around within the ultrastructure of the lens and were not bound to any large proteins. The lens pigment of the carp (*Cyprinus carpio*) for example, leached into saline which, when scanned, revealed quite low protein absorbance around 270-280nm and a compound with l_{max} 360nm (Fig. 3-4).

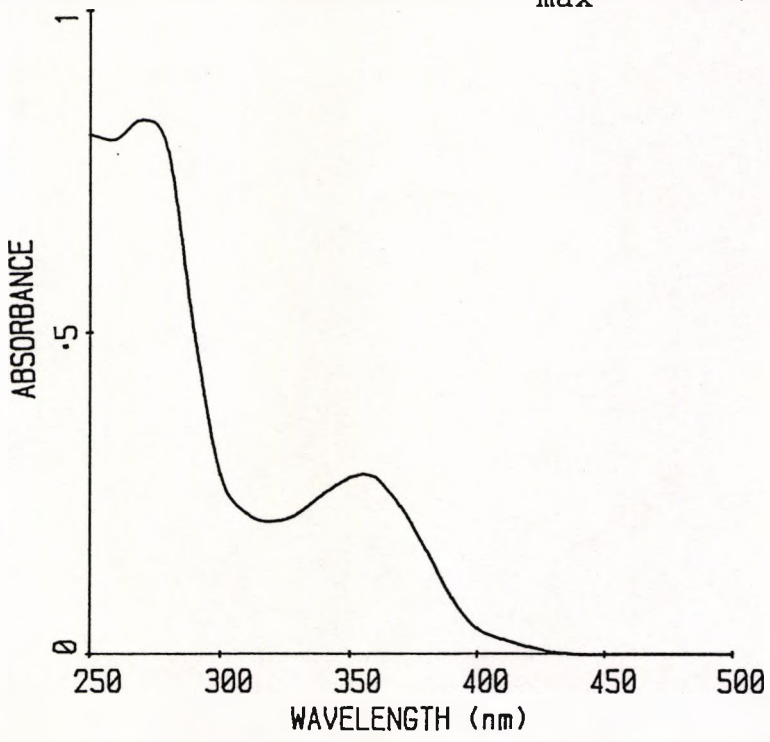


Figure 3-4. Spectral absorbance of the leached component from the lens of *Cyprinus carpio*, obtained by placing the 2 lenses of a fish in 0.9% saline for 6 hours.

As the majority of lens pigments appeared to have low molecular weights and were readily diffusible, pigment extraction was quite simple (see Section 2.5). A high percentage of the pigment could be obtained in the first extraction of lenses (usually around 86%; see Chalker & Dunlap, 1981 for calculation). The histogram of l_{max} values obtained from lens extracts shows three main clusters of pigment, one around 320 to 330nm, one at 360nm and one at 370nm (Fig. 3-5). A pigment with l_{max} 340nm was found in the pollack (*Pollachius pollachius*) and a l_{max} 385nm pigment was found in the flying fish (*Exocoetus obtusirostris*). The l_{max}s of the pigments extracted from each species are listed in Table 3-1.

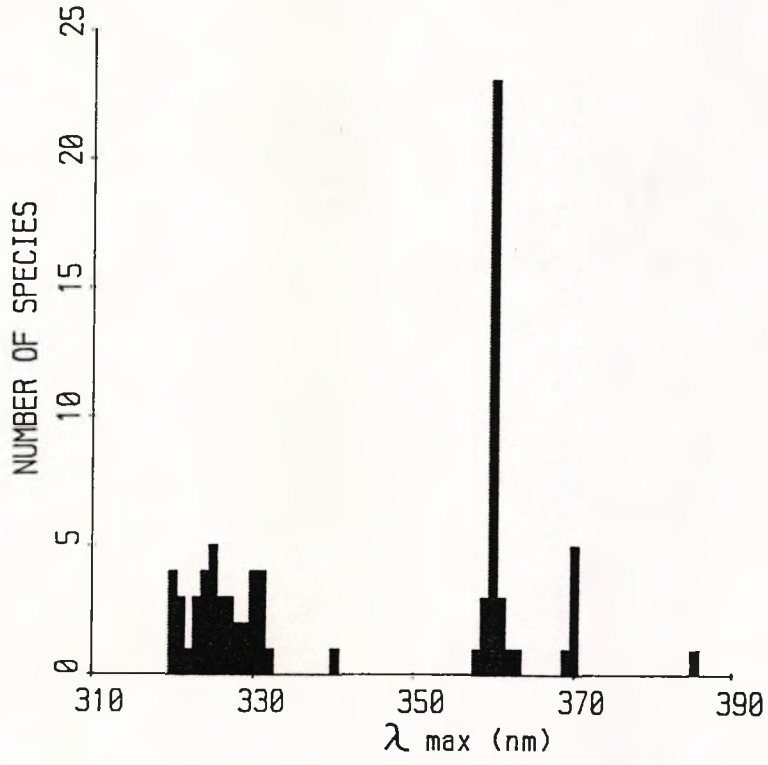


Figure 3-5. Frequency histogram of the l_{max}s of the pigments extracted from the lenses of different species of fish. When more than one lens extraction was carried out for a species, l_{max} values were averaged.

Figure 3-6 shows the absorbance spectra of three pigments extracted from different fish lenses, representing the three major groups seen in Figure 3-5. The pigment bandwidth appears to become wider as the pigment l_{max} increases (a phenomenon also observed in visual pigments [MacNichol, 1986]).

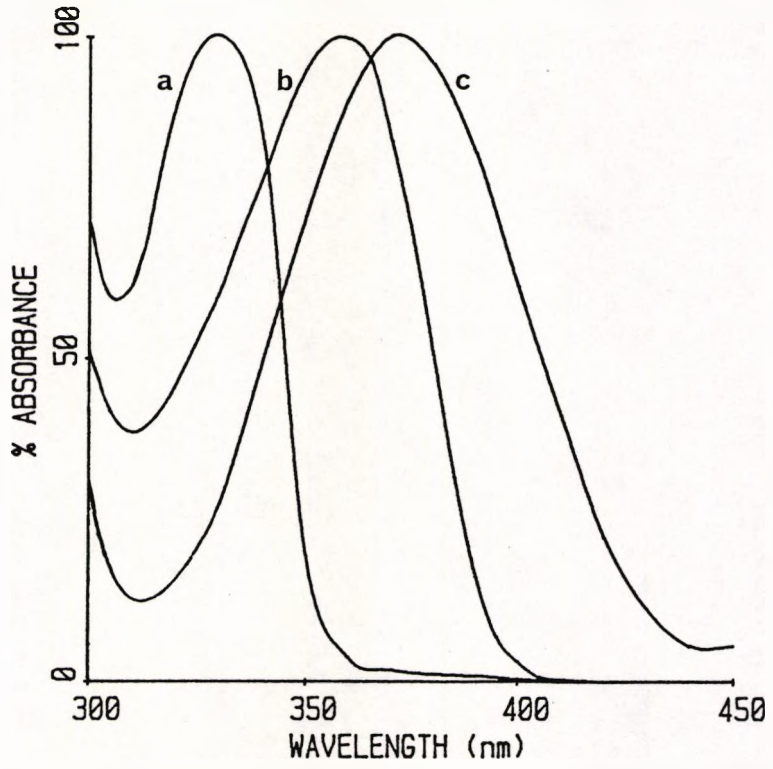


Figure 3-6. UV absorbance spectra of the three pigments commonly extracted from the fish lens. (a) l_{max} 330nm pigment from *Chaetodon sp.* lenses (b) l_{max} 360nm pigment from lens of *Gadus morhua* and (c) l_{max} 370nm pigment from *Trichogaster trichopterus* lenses. The sharp increase in absorbance below 320nm is due to the presence of protein in extracts.

The butterfly fish (*Chaetodon* sp.), consistently showed high concentrations of just a 330nm l_{max} pigment in lens extracts (Fig. 3-6a) whereas the cod (*Gadus morhua*) lens extract revealed a pigment with l_{max} 360nm (Fig. 3-6b). Moonlight gourami (*Trichogaster trichopterus*) lens extracts

similarly had just one pigment, but with a slightly longer l_{max} of 370nm (Fig. 3-6c). Absorbance spectra of other lens extracts however, show that there is frequently more than one class of pigment present in a lens. Relative peak heights often varied between species and several peaks were frequently seen in HPLC profiles (see Section 3.2.5), both suggesting that it is not simply just one pigment with a complex absorbance profile. The lens extract of the tub gurnard (*Trigla lucerna*) for example had roughly equal concentrations of both 328nm and 360nm l_{max} pigments (Fig. 3-7a). But lenses of the flounder (*Platichthys flesus*) and scad (*Trachus trachurus*) had quite different relative amounts of the 320-330nm and 360nm pigments (Fig. 3-7b&c).

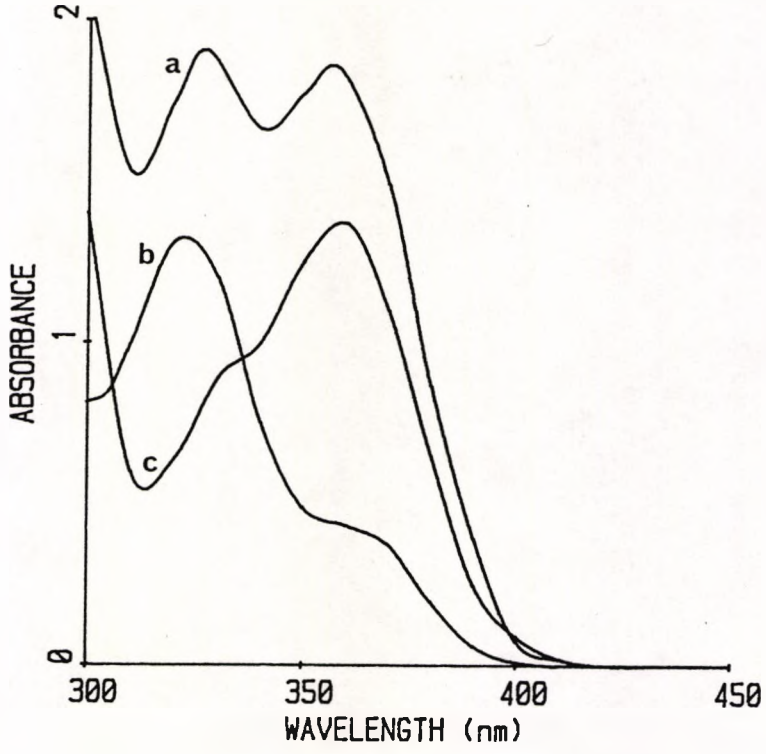


Figure 3-7. UV absorbance spectra of the lens extracts from (a) *Trigla lucerna*, (b) *Platichthys flesus* and (c) *Trachus trachurus*, showing variation in relative 320-330nm and 360nm l_{max} pigment concentrations.

The λ_{max} of the shorter wavelength pigment tended to vary and could be anywhere between 319nm and 333nm (see Fig. 3-5), suggesting that there is a group of compounds with similar absorbance spectra rather than just one compound. These short-wave absorbing pigments were seldom found alone in a lens extract, but more often tended to be accompanied by the 360nm pigment (e.g. Fig. 3-7). However, exceptions to this were found, most notably in the lenses of a number of tropical marine species, for example the chaetodontidae (Fig. 3-6a and Table 3-1).

Attempts were made to correlate lens pigmentation with the phylogenetic grouping of fish (Fig. 3-8). There was no clear pattern, but closely related species did tend to have similar lens transmission characteristics. For example, none of the rays or dogfish examined (Rajiformes and Scyliorhyniformes) were found to have lens pigmentation.

In contrast, many perciforme species and all scorpaeniformes had highly pigmented lenses. When a family displayed lens pigmentation, pigments present were frequently the same, even if concentrations varied. This was demonstrated in lenses of many gadiforme fish which contained mainly λ_{max} 360nm pigment. For example, whiting (*Merlangius merlangus*) had a low concentration, whereas lenses from cod (*Gadus morhua*), a closely related species, had higher lens 50% transmission and contained a higher concentration of the same pigment (Table 3-1).

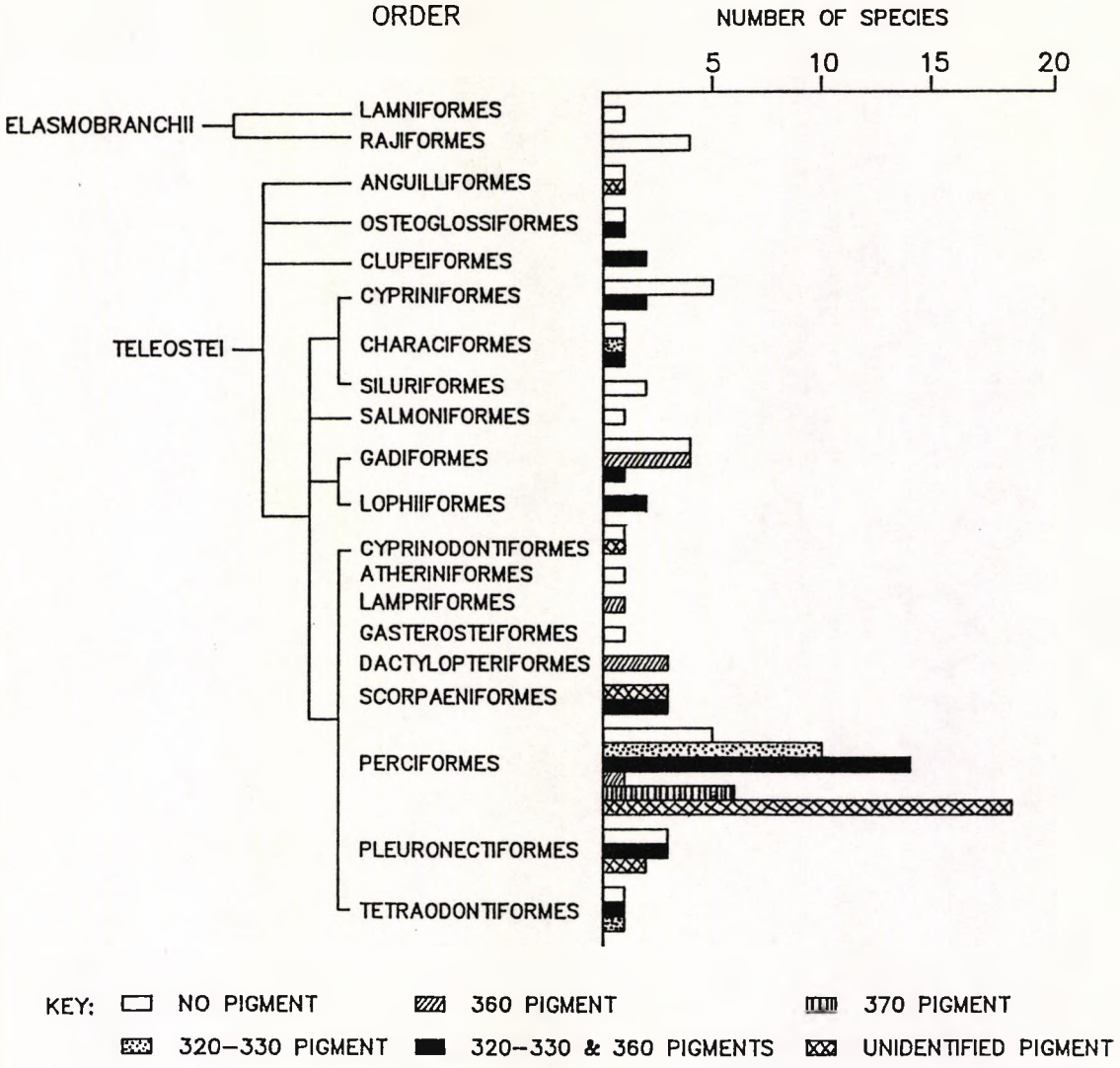


Figure 3-8. Phylogenetic distribution of lens pigmentation in fish. Pigments with identical UV absorbance maxima have been grouped together for simplicity (though this does not necessarily mean that they are identical compounds).

3.2.3 RELATIONSHIP BETWEEN LENS TRANSMISSION AND PIGMENT ABSORBANCE

The whole lens transmission is dependent on both the types of pigment present in the lens and the concentrations of these pigments. Whiting (*Merlangius merlangus*), cod (*Gadus morhua*) and pipefish (*Syngnathus typhle*) had quite different whole lens transmission (Fig. 3-9). However, all

lenses had the same l_{max} 360nm pigment, but at varying concentrations (Fig. 3-10). The high concentration of pigment in the pipefish lens results in a very large shift in the transmission despite its smaller lens diameter, with 50% transmission around 420nm (Fig. 3-9). Lower pigment concentrations in the whiting and cod lead to lower 50% transmission than observed in the pipefish despite their larger lens diameters.

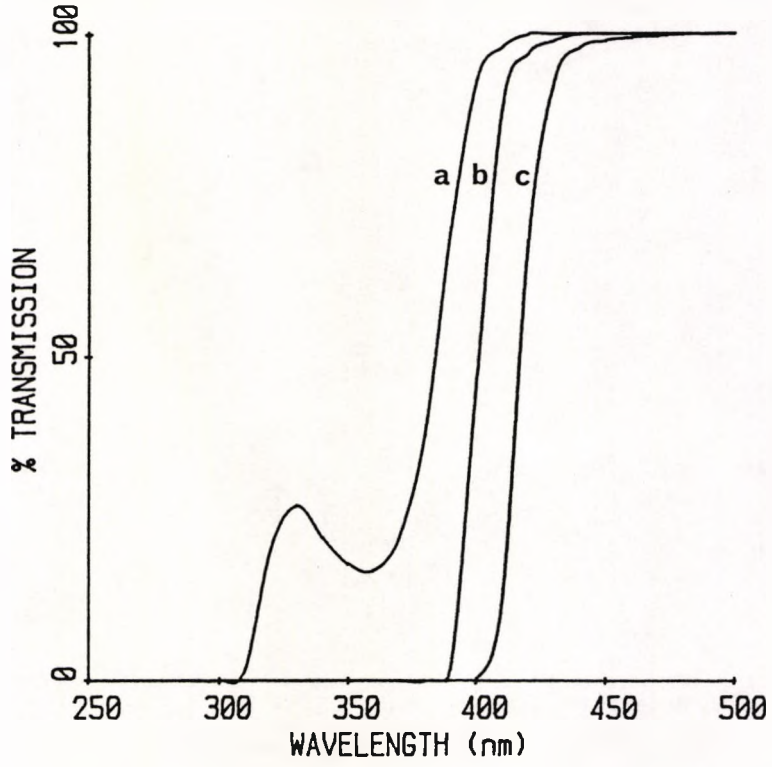


Figure 3-9. Spectral transmission of (a) a 3.33mm diameter *Merlangius merlangus* lens (b) a 5.01mm diameter *Gadus morhua* lens and (c) a 1.56mm *Syngnathus typhle* lens.

It was clear that different classes of lens transmission often represented not only differences in pigment concentrations, but also the presence of different pigments. The position of shoulders on the transmission curve varied and was used as an indication of the l_{max} of pigments present in the lens.

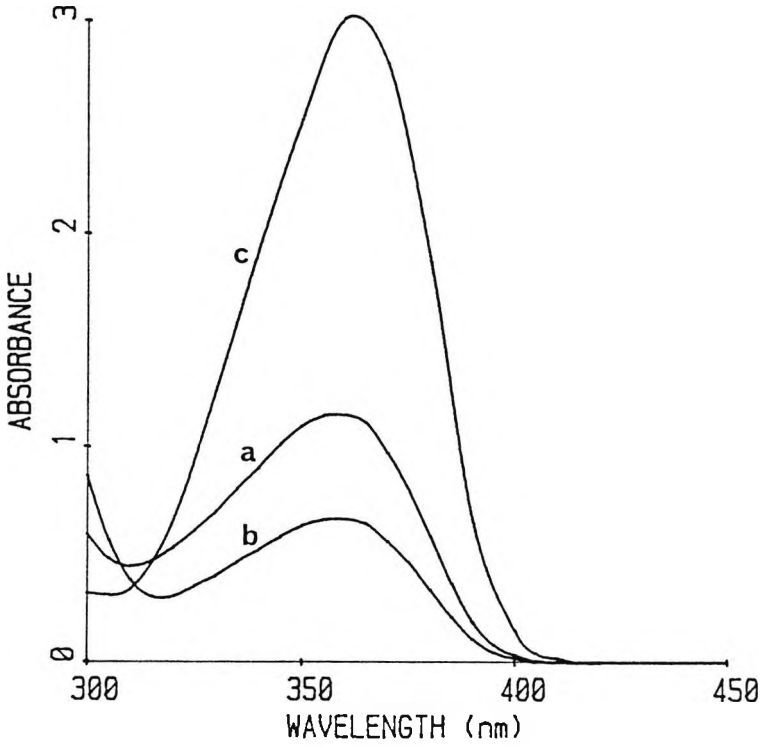


Figure 3-10. Spectral absorbance of lens pigment extracts from (a) *Merlangius merlangus*, (b) *Gadus morhua* and (c) *Syngnathus typhle*. Absorbance values were corrected for differences in both extraction volume and total lens volume.

Lenses with just 360nm l_{max} pigment had a well-defined secondary peak at 330nm and a sharp rise in transmission between 375 and 400nm (e.g. Fig. 3-1 curve c). Lenses with low concentrations of both 320-330 and 360nm pigments had shoulders on the transmission trace in the regions of 330 and 360nm with a rise in transmission between 380 and 400nm (e.g. Fig. 3-1 curve b). Lenses containing l_{max} 370nm pigment were identified by secondary peaks in the transmission around 315-320nm and very low transmission between 350 and 400nm rising sharply between 420 and 450nm (Fig. 3-11, dashed line).

It was impossible to predict the pigment content of a lens from whole lens transmission alone when concentrations were high (e.g. Fig. 3-11, solid line). However, pigments at such high concentrations could usually be extracted from lenses of a single animal. Transmission spectra like Fig. 3-1 (curve d) may be due to either high concentrations of l_{max} 360nm pigment alone (as in Fig. 3-9d) or to high concentrations of 360nm and 320-330nm pigments (as in Fig. 3-11, solid line).

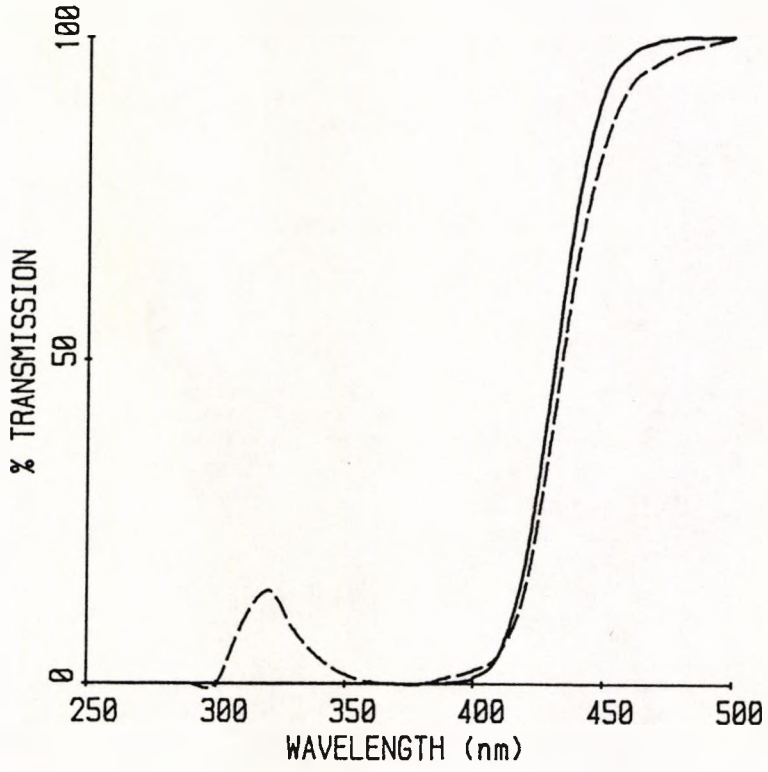


Figure 3-11. Spectral transmission of a 1.09mm diameter *Betta splendens* lens (dashed line) which contains only a 370nm pigment and a 1.89mm diameter *Herotilapia multispinosa* lens (solid line) with both 320 and 360nm l_{max} pigments.

3.2.4 LENS NUCLEUS AND CORTEX

Spectral transmission of the nucleus only from several large goldfish (*Carassius auratus*) was measured; lenses from smaller fish had nuclei with diameters less than 1.0mm which were too small for spectral transmission recordings. The spectral transmission of each nucleus was very similar in shape to that of the whole lens (e.g. Fig. 3-12), but spectra appeared to have shifted slightly to the left, due to the smaller diameter of the nucleus. The similar shape of both curves suggested that the same pigment was present in both the nucleus and cortex in the goldfish. Unfortunately, since the concentration of pigment was low, it was not possible to extract the pigment separately from the nucleus and cortex for confirmation.

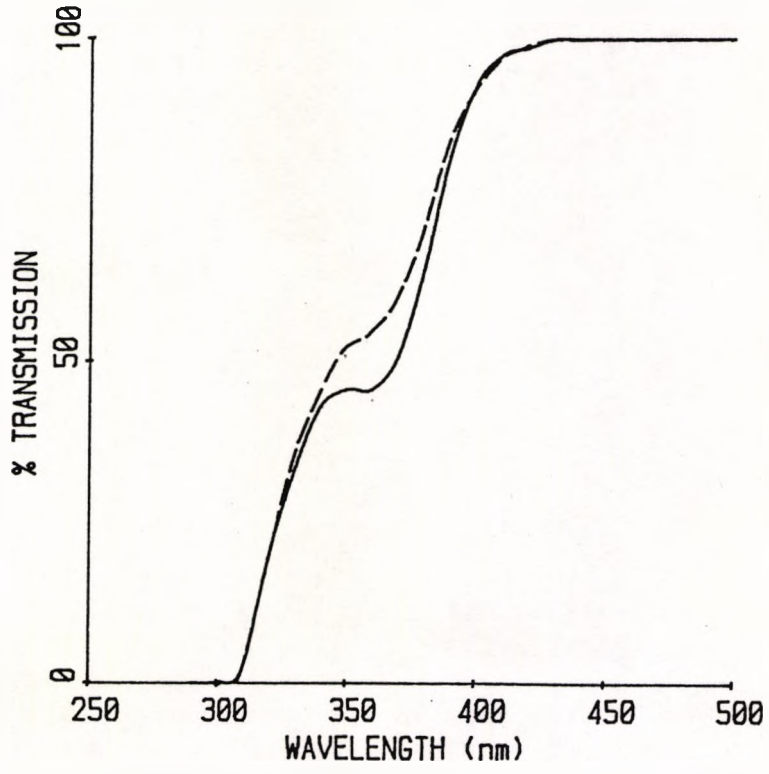


Figure 3-12. Spectral transmission of a 3.45mm diameter lens from *Carassius auratus* (solid line) and the nucleus of this lens, 2.12mm in diameter (dashed line).

Lens nucleus transmission in pollack (*Pollachius pollachius*) was similarly shifted around 5nm from the whole lens spectrum (Fig. 3-13), suggesting that the same compound is present in both the nucleus and cortex of this species.

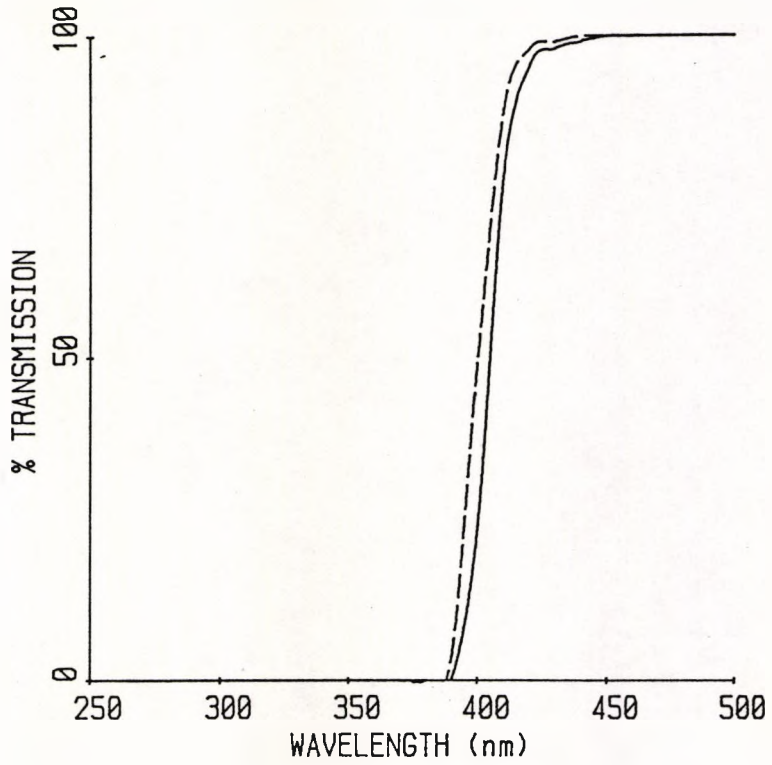


Figure 3-13. Spectral transmission of a 5.36mm diameter lens from *Pollachius pollachius* (solid line) and the lens nucleus, 3.54mm in diameter (dashed line).

The distribution of pigments in the lens was examined further by extracting pigments from the nucleus and cortex of pollack and whiting separately. The lenses of 6 pollack were dissected into nucleus and cortex after 30min. in 0.9% saline (which was extracted along with the cortex fraction to include any pigment which had leached out of the cortex). Absorbance traces of both extracts revealed the presence of the same pigments in the nucleus and cortex, but the relative concentrations of the two pigments differed (Fig.

3-14), the nucleus having slightly more 360nm pigment and less l_{max} 340nm pigment than the cortex.

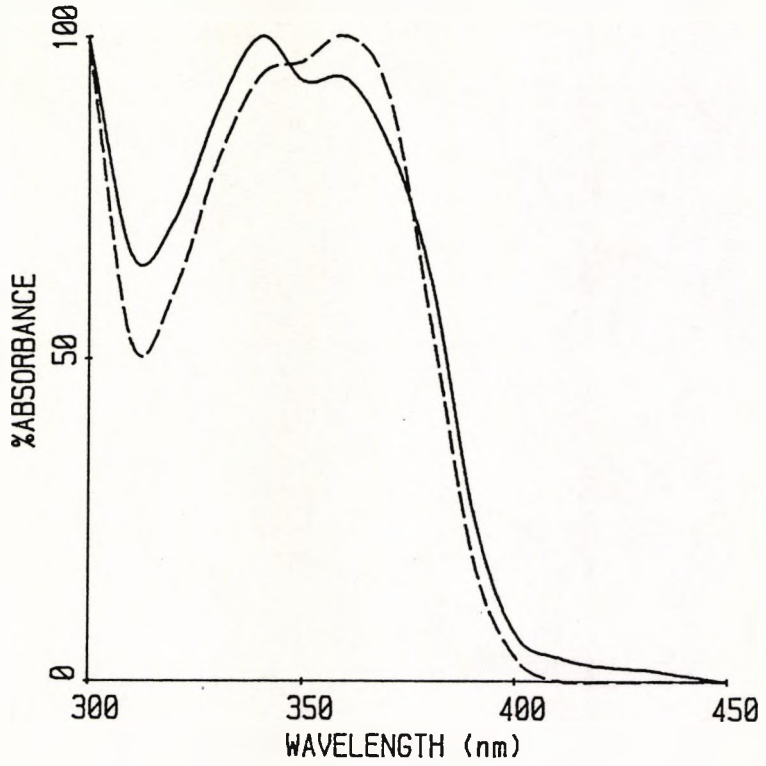


Figure 3-14. Lens pigment extract absorbance from 12 *Pollachius pollachius* lenses. Lens cortex (solid line) and nucleus (dashed line) were extracted separately. Absorbance is expressed as a percentage of the maximum.

Concentrations of each pigment in the nucleus and cortex in absorbance units per cubic centimetre of nucleus or cortex volume were:

	340nm	**360nm**
Nucleus	8.46	8.86
Cortex	7.21	6.79

The pollack lens nucleus therefore had a slightly higher pigment concentration than the cortex. Differences in relative pigment concentration, although small, may indicate that some decomposition of 360nm pigment has occurred in the

cortex of pollack (as shown later for lens extracts, see Chapter 5), or alternatively that more 360nm pigment has been laid down in the nucleus.

Whiting (*Merlangius merlangus*) had only l_{max} 360nm pigment in the lens. The lens nucleus and cortex were dissected from three individuals and the pigment extracted. Absorbance traces of nucleus and cortex extracts were identical in shape, confirming that the same pigment is located throughout the lens, but the total absorbance was slightly higher in the cortex extract. However, when the volume of cortex and nucleus was corrected for, the resulting absorbance per cubic centimetre was 8.325 for the cortex and 13.019 for the nucleus (Fig. 3-15).

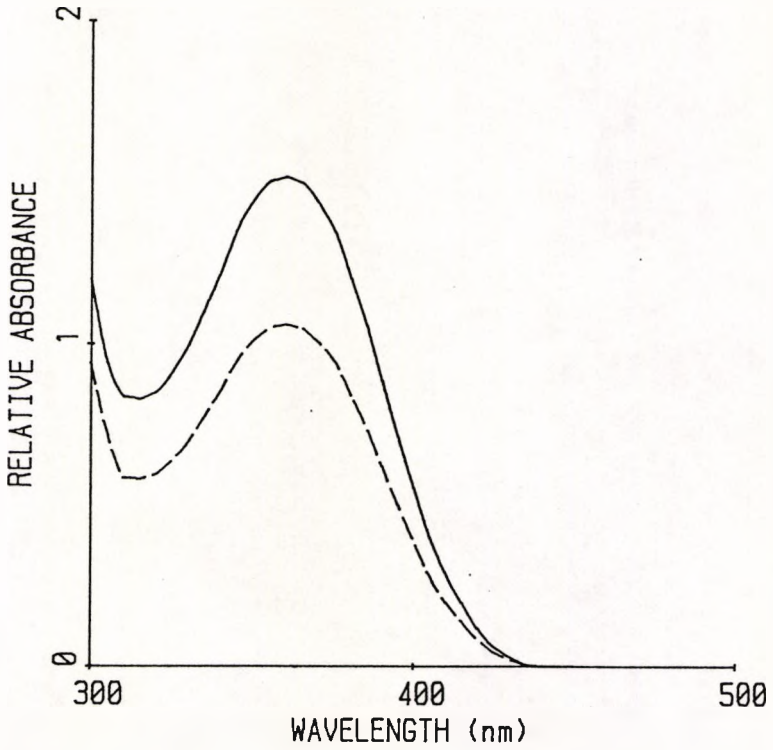


Figure 3-15. Spectral absorbance of the extract from three *Merlangius merlangus* lenses, lens nucleus (solid line) and cortex (dashed line) were extracted separately. Absorbance traces have been corrected for differences in cortex and nucleus volume.

Nucleus diameters were measured for a number of species and the ratio of nucleus diameter to whole lens diameter calculated (Table 3-2 & Fig. 3-16).

SPECIES	NO.	DIAMETER NUC/LENS (%)	S.D.
Gadus morhua	14	65.3	5.3
Merlangius merlangus	26	64.5	2.6
Pollachius pollachius	13	65.7	2.5
Trisopterus luscus	10	68.2	3.1
Pollachius virens	10	62.8	3.3
Clupea harengus	10	63.4	2.1
Aspitrigla cuculus	12	61.8	1.7
Trachus trachurus	6	65.8	1.2
Scomber scombrus	12	63.7	2.6
Carassius auratus	228	62.1	3.2
Oreochromis niloticus	40	63.0	4.0

Table 3-2. List of species for which the ratio of the nucleus diameter over whole lens diameter was measured. The nucleus measurement is expressed as a percentage of the whole lens diameter. The mean value is 64.2%.

The diameter of lens nucleus averaged 64% of that for the whole lens in all species. Furthermore, the percentage was the same whatever the lens diameter, as demonstrated by the highly significant correlation coefficient (Fig. 3-16, r=0.99). Other authors report similar values: the nucleus diameter of the guppy lens is reported to be 72.5% of the whole lens diameter [Eberle, 1968] and a value of 67.4% is reported for *Haplochromis burtoni* [Fernald & Wright, 1983].

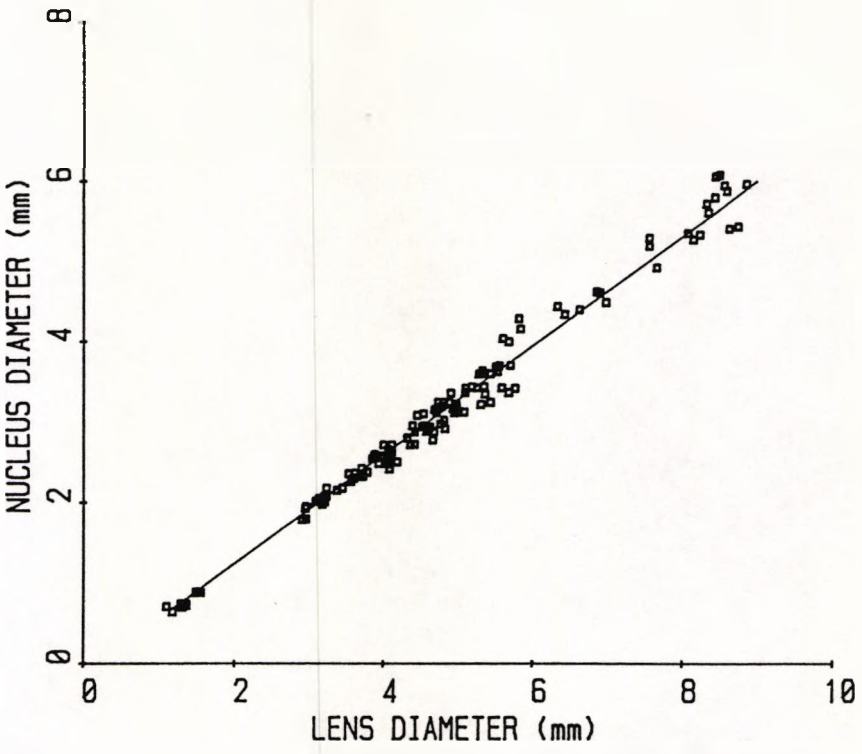


Figure 3-16. Plot of the nucleus diameter as a function of the whole lens diameter for all species in Table 8-2 except *Carassius auratus* (which have been omitted for clarity). The line shows the regression; the correlation coefficient (r) was highly significant at 0.99.

3.2.5 PIGMENT IDENTIFICATION

3.2.5.1 HPLC

High performance liquid chromatography (HPLC) was used to determine the identity of the lens pigments from 14 species of fish. HPLC of unknown pigments was carried out with mycosporine-like amino acids from the herring (*Clupea harengus*) which had been previously identified for us as palythine and palythene by Dr. W. Dunlap of the Australian Institute of Marine Science, Townsville. The herring lens also contains trace amounts of asterina-330 and palythinol.

The chemical structures and l_{max}s of the four mycosporine-like amino acids commonly found in the lenses of fish are shown in Figure 3-17. Dr. Dunlap also kindly supplied standards of lenses containing these four compounds.

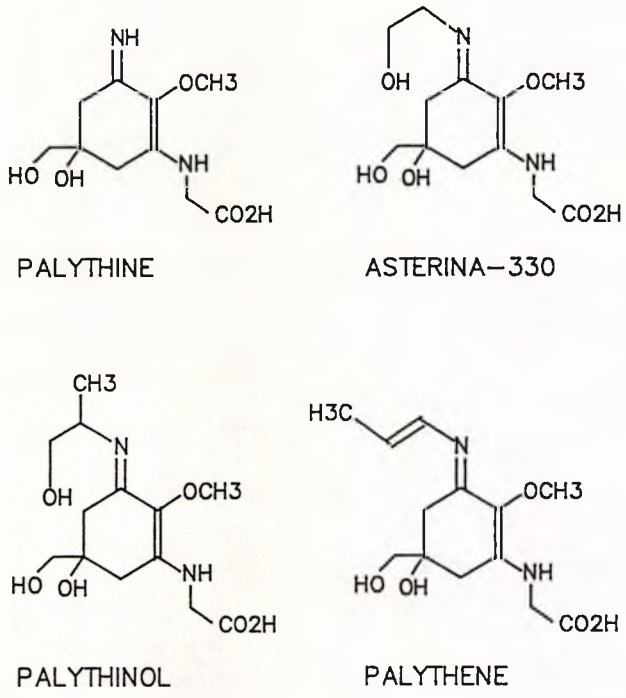


Figure 3-17. Chemical structures of the four mycosporine-like amino acids commonly found in the fish lens. Palythine with l_{max} 320nm, asterina-330 with l_{max} 330nm, palythinol with l_{max} 331nm and palythene with l_{max} 360nm.

HPLC retention times of the various pigment constituents of fish lenses were constant (Fig. 3-18), which enabled the co-injection of standards and unknown samples. An increase in absorbance at the retention time of the standard sample, when the unknown sample was co-injected, indicated the presence of the standard in the sample.

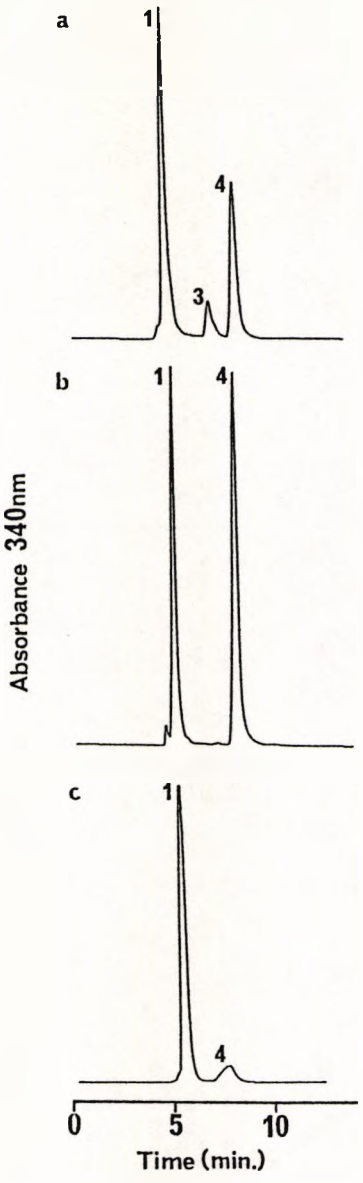


Figure 3-18. HPLC elution profiles from the lens extracts of (a) *Clupea harengus*, (b) *Pollachius pollachius* and (c) *Oreochromis niloticus*. HPLC conditions are detailed in the text. Peak labels refer to mycosporine-like amino acids, 1- palythine, 2- asterina-330, 3- palythinol and 4- palythene (see Fig. 3-17).

Many of the temperate marine and freshwater species have palythene and palythine mycosporines in their lenses (Table 3-3; Fig. 3-18), whereas tropical marine species tended to have a very high concentration of only asterina-330 (Table 3-3; Fig. 3-19).

SPECIES	PIGMENTS PRESENT
Syngnathus typhle	palythene>>>palythine
Clupea harengus	palythene>palythine>>palythinol
Pollachius pollachius	palythene=palythine
Aspitrigla cuculus	palythine>>palythene
Cyprinus carpio	palythine>>palythene
Oreochromis niloticus	palythine>>palythene
Lepomis gibbosus	palythine
Chaetodon sp.	asterina-330
Paracanthurus hepatus	asterina-330
Pygoplites diacanthus	asterina-330
Exocoetus obtusirostris	unknown mycosporine compound
Stylephorus chordatus	kynurenine
Aequidens pulcher	3-hydroxykynurenine
Trichogaster trichopterus	3-hydroxykynurenine

Table 3-3. List of pigments in the fish lens and their relative concentrations identified by HPLC and NMR. > represents more than, >> more than twice as much, >>> more than three times as much, = indicates equivalent concentrations.

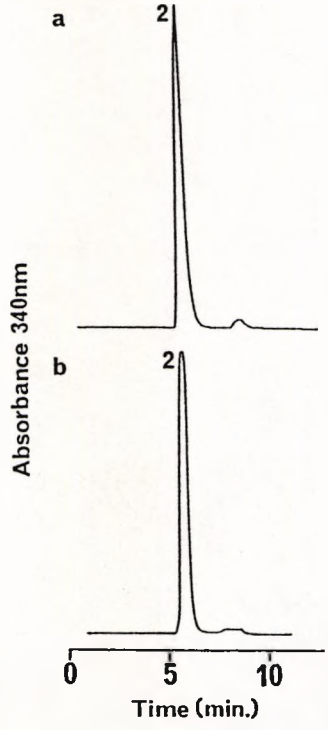


Figure 3-19. HPLC profiles of lens pigment extracts from two tropical marine species, (a) *Pygoplites diacanthus* and (b) *Paracanthurus hepatus*. Both appear to have considerable amounts of only asterina-330 in their lenses. Peak labelling as in Fig. 3-18.

The lens pigment from a species of flying fish (*Exocoetus obtusirostris*) had a different retention time (7.8 min.) (Fig. 3-20a), to that of the most polar mycosporine, palythene at 8.3 min. (Peak 4, Fig. 3-18). The 7.8 min. pigment was found to be unstable on standing and degraded into four separate compounds (Fig. 3-20b), one of which (labelled 1) was identified as palythine by co-injection with standards. The lens pigment of the flying fish is therefore likely to be a rather unstable mycosporine-like compound, slightly less polar and with a longer λ_{max} than palythene.

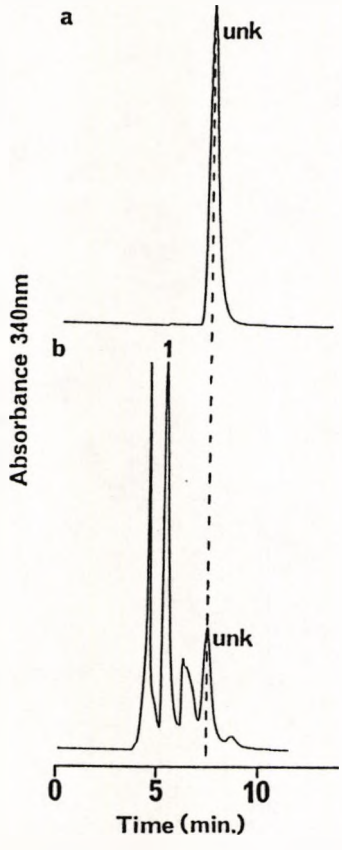


Figure 3-20. HPLC elution profiles of the lens extract from *Exocoetus obtusirostris*. (a) An unknown compound (labelled unk) with a 7.8 min. retention time eluted initially. (b) After several hours in solution, and re-injection, three new peaks were noticed in the profile. One of these, labelled 1, was found to co-elute with palythine from the lens of *Clupea harengus*. Peak labelling as in Fig. 3-18.

Pure mycosporine samples were obtained by HPLC and the spectral absorbance of peaks collected reflected this; much of the absorbance at shorter wavelengths seen with the crude extracts was removed (Fig. 3-21). The unknown flying fish compound (Fig. 3-21, curve d), like the mycosporine compounds (curves a-c), does not absorb in the far UV region, unlike tryptophan derivatives (see Fig. 3-23).

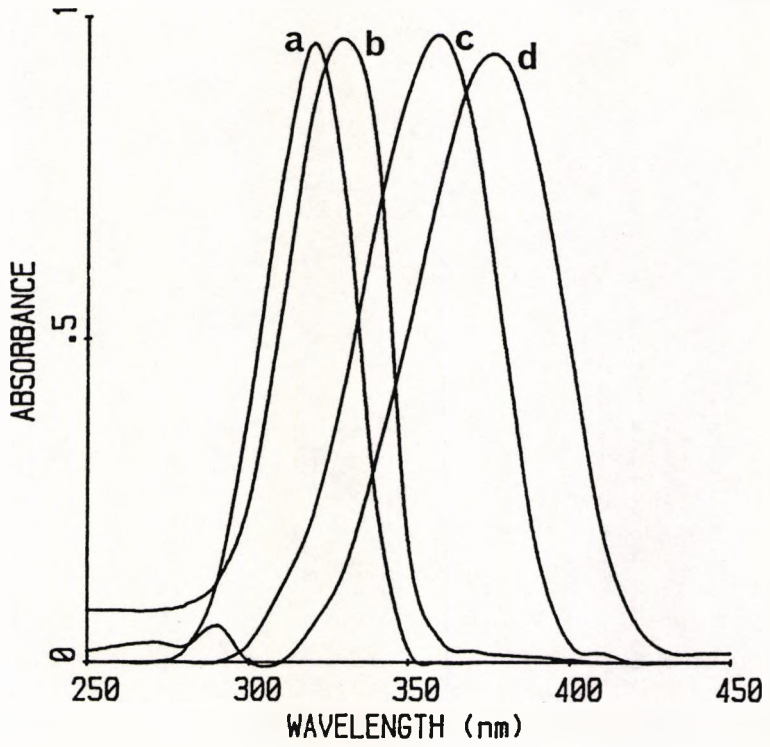


Figure 3-21. Absorbance spectra of HPLC purified samples of a) palythine from the lens of *Oreochromis niloticus*, b) asterina-330 from *Chaetodon sp.*, c) palythene from the lens of *Clupea harengus* and d) the unknown mycosporine-related compound from *Exocoetus obtusirostris*. Lens pigment concentrations are arbitrary.

There were a number of other lens extracts which did not chromatograph well with the mycosporine standards. The extract of *Stylephorus chordatus* for example had a longer retention time (10.4 min.) than palythene (8.3 min.) (Fig.

3-22). The absorbance maxima in the near-UV for palythene and the *Stylephorus chordatus* extract were very similar at 360nm, but an additional peak at 260nm was noticed for *Stylephorus chordatus* (Fig 3-23), suggesting a different structure.

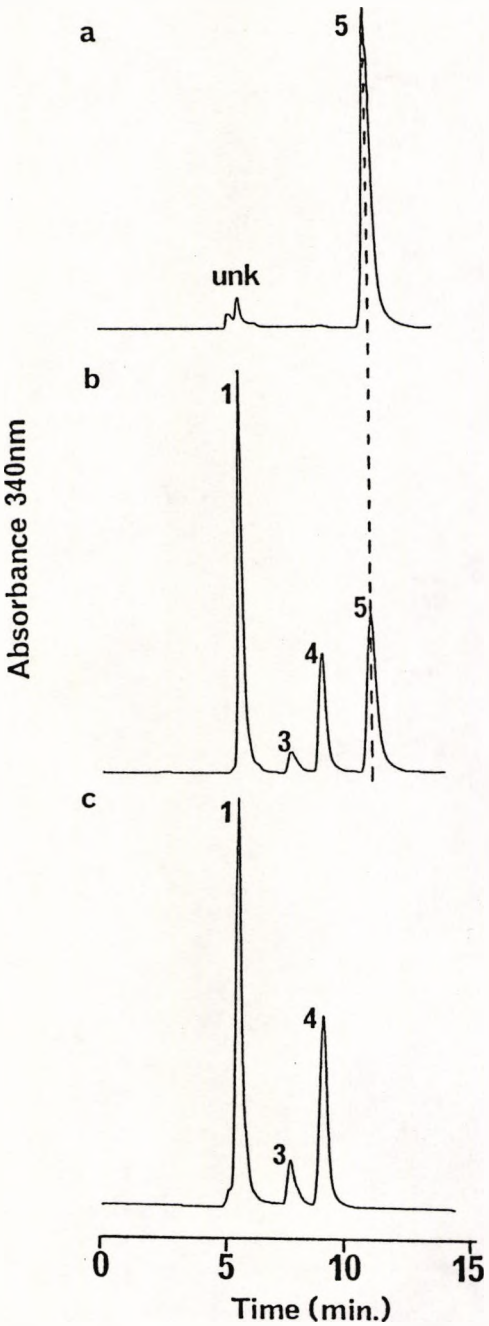


Figure 3-22. HPLC elution profiles for (a) the *Stylephorus chordatus* lens extract, the major peak showed a retention time of 10.6 min., the minor peaks at 6 minutes are unknown, (b) *Stylephorus* lens extract co-injected with *Clupea harengus* lens pigments and (c) *Clupea harengus* lens pigments alone (peak labelling as in Fig. 3-18).

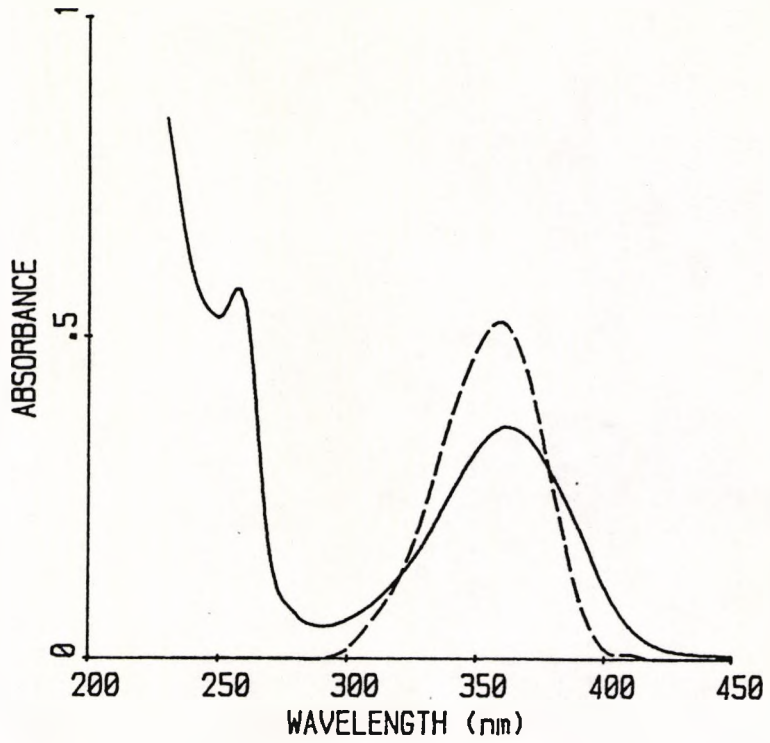


Figure 3-23. Absorbance spectra of purified palythene from the lens of *Clupea harengus* (dashed line) and the unknown pigment (peak 5) from *Stylephorus chordatus* lenses (solid line). Although both have similar l_{max}s in the near UV, far UV absorbance is quite different.

The 370nm l_{max} pigment from lenses of *Trichogaster trichopterus* and *Aequidens pulcher* (Table 3-1), also had a slightly longer retention time than palythene (Fig. 3-24). Both species were shown to have the same pigment, since both their retention times and l_{max}s were identical (Fig. 3-24 & 3-25), but different to those of the mycosporine compounds. The absorbance spectrum of the 370nm pigment (Fig. 3-25), though it was unlike that of the mycosporines in the far UV, resembled that of the lens pigment from *Stylephorus chordatus* (Fig. 3-23 solid line).

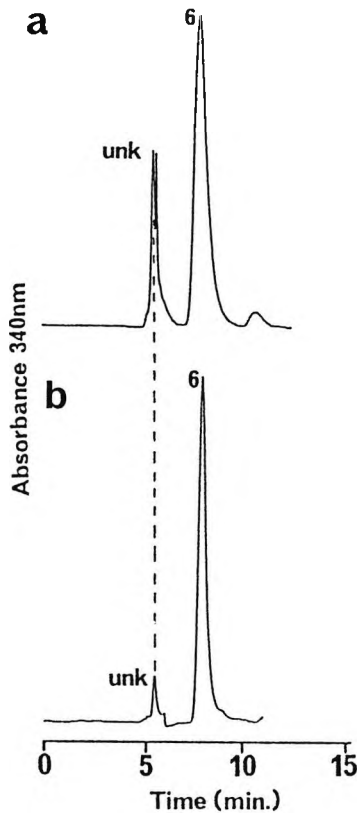


Figure 3-24. HPLC elution profiles of the lens extracts from (a) *Trichogaster trichopterus* and (b) *Aequidens pulcher*. The major peak eluted from both (labelled 6) at 7.7 min.

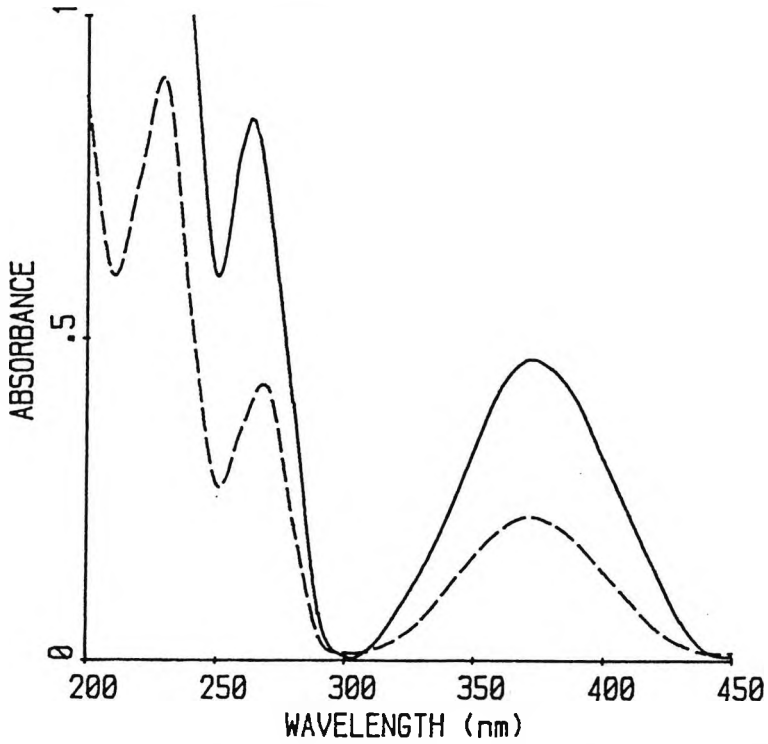


Figure 3-25. Spectral absorbance of the purified lens pigment (Peak 6) from *Trichogaster trichopterus* (dashed line) and *Aequidens pulcher* (solid line). Pigment concentrations are arbitrary.

3.2.5.2 TLC

Stylephorus chordatus and *Trichogaster trichopterus* pigments were investigated further, after HPLC analysis, by thin layer chromatography (TLC) with tryptophan. It has been suggested that tryptophan metabolites may be found in the lenses of fish, as well as in the primate and squirrel lens (Zigman, 1987). Therefore TLC with tryptophan will give an indication as to whether the compounds from these two species have any chemical similarities to tryptophan.

The results show that the *Stylephorus chordatus* and *Trichogaster trichopterus* pigments have very similar R_f values, both of which are slightly lower than that for tryptophan

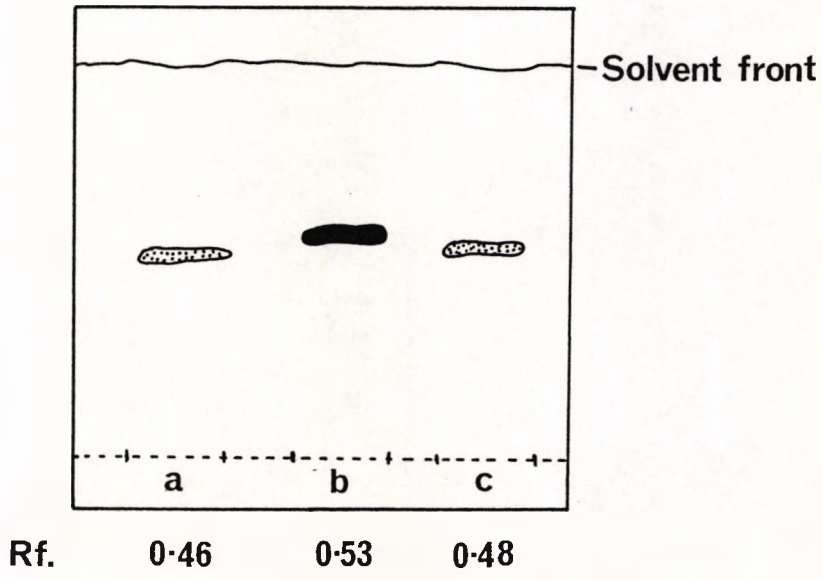


Figure 3-26. Diagram of thin layer chromatograph of (a) *Stylephorus chordatus*, (b) authentic tryptophan and (c) *Trichogaster trichopterus* with the relative R_f values. The tryptophan band stained more strongly with both DMAC and ninhydrin than the other two, simply indicating a higher concentration.

The two lens extracts and tryptophan stained with p-dimethylamino-cinnamaldehyde (DMAC) indicating the presence of an indole or pyrrole ring, and ninhydrin indicating the presence of a primary amino ($-NH_2$) group. Therefore both the two new pigments have a structure and free amino group similar to tryptophan (Fig. 3-27) and unlike any of the mycosporine compounds (Fig. 3-17).

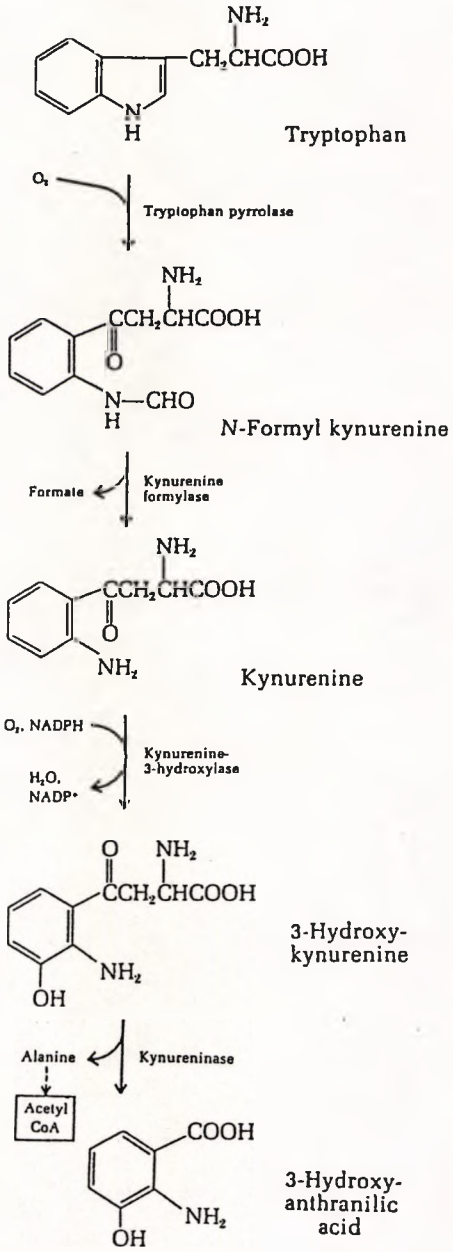


Figure 3-27. Catabolic pathway of tryptophan, showing the relationship between kynurenine found in *Stylephorus chordatus* lenses and 3-hydroxy-kynurenine isolated from *Trichogaster trichopterus* lenses. (From: Lehninger, 1970).

3.2.5.3 Proton NMR

Proton nuclear magnetic resonance (^{1}H-NMR) was carried out on four purified lens pigments. NMR was initially performed on compounds identified by Dr W. Dunlap as palythene (λ_{max} 360nm) and palythine (λ_{max} 325nm) isolated from lenses of herring. Spectra obtained from both samples were quite noisy (Figs. 3-28 & 3-29) due to the low sample concentration (2mg) which meant that the NMR machine was working at the highest limit of its capabilities. The NMR spectrum of the herring palythine sample (Fig. 3-29) corresponded quite well with published information on proton NMR for palythine [Takano, 1978a; Chioccara *et al.*, 1980]. But there were undoubtedly other impurities in our sample.

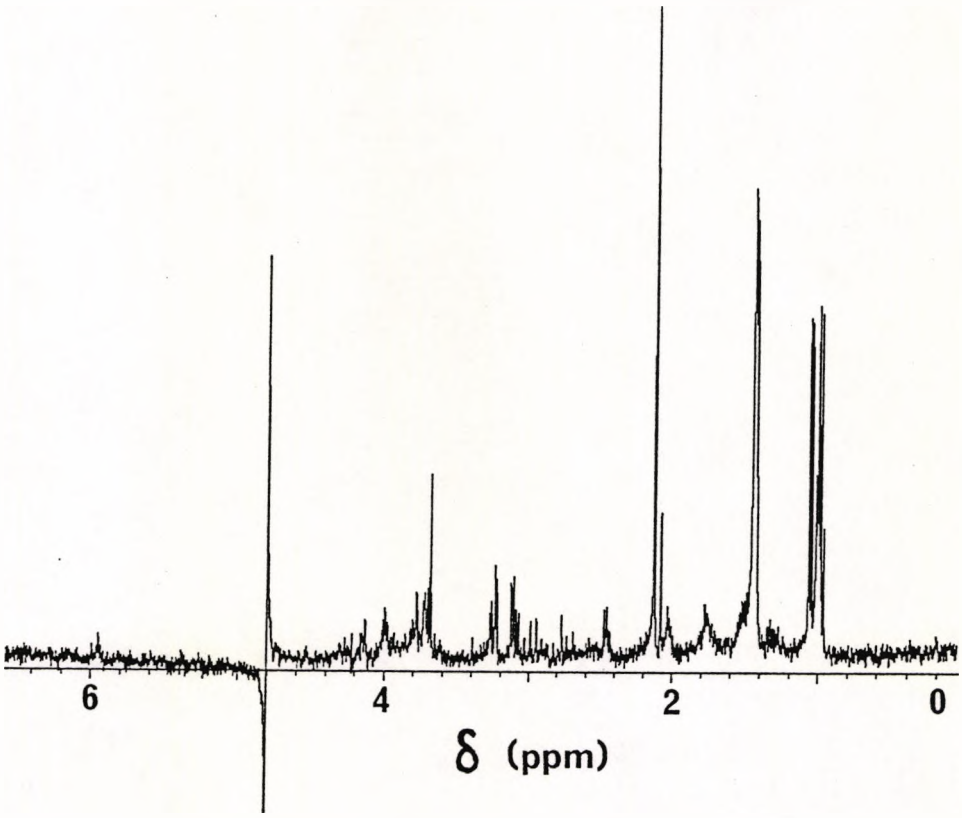


Figure 3-28. Proton NMR spectrum of HPLC purified palythene (λ_{max} 360nm) pigment from the lens of *Clupea harengus*. δ represents the chemical shift in parts per million.

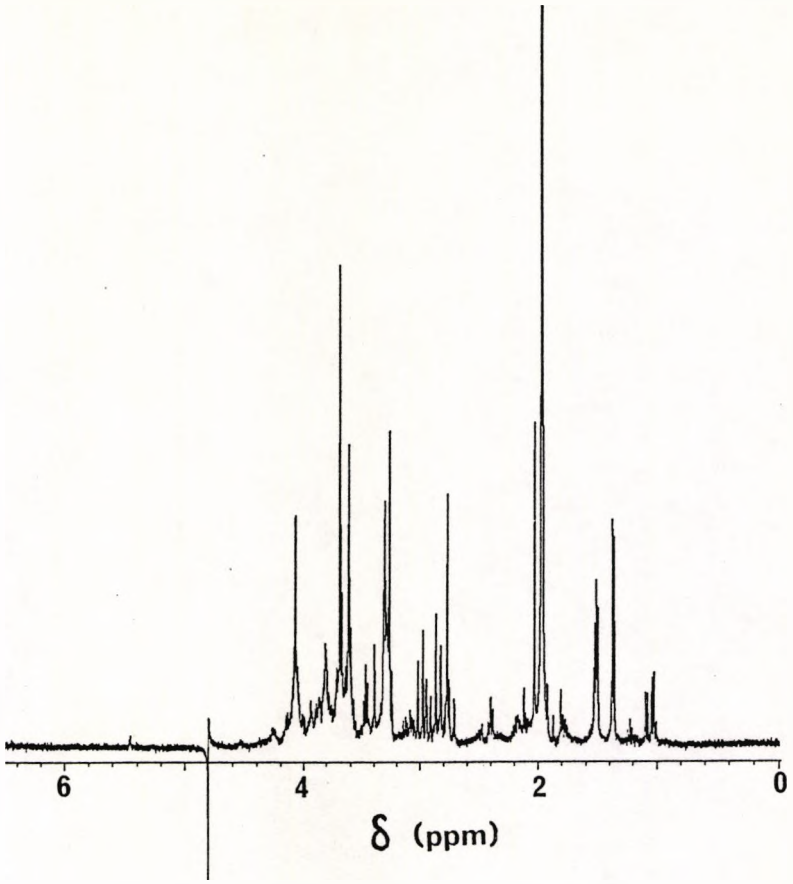


Figure 3-29. Proton NMR spectrum of HPLC purified palythine (l_{max} 360nm) from the lens of *Clupea harengus*.

After NMR, palythene and palythine samples were lyophilised and redissolved in distilled water and absorbance spectra were re-recorded (Fig. 3-30). The traces demonstrate the instability of palythene to the drying process and purification techniques used; the palythene degraded completely, producing palythine and possibly also other compounds. The absorbance of the palythine sample was unchanged.

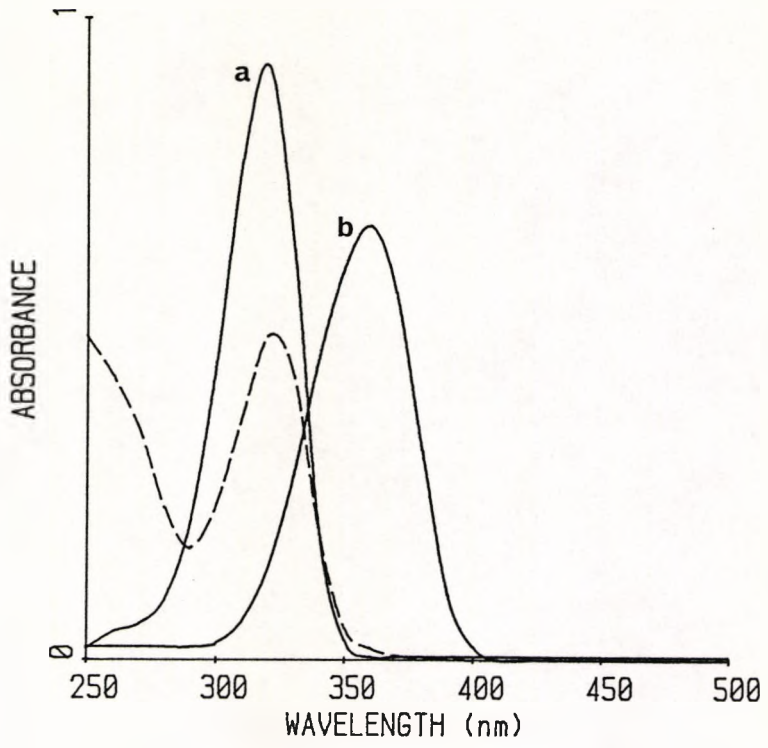


Figure 3-30. Absorbance spectra of HPLC purified a) palythine and b) palythene from the *Clupea harengus* lens before NMR was carried out. The dashed line shows the absorbance of the palythene sample b) after it was dried following nmr.

NMR spectra were also recorded of *Trichogaster trichopterus* and *Stylephorus chordatus* lens pigments after HPLC purification (Fig. 3-31 & 3-32). The spectra were unlike those recorded for mycosporines since they displayed peaks in the aromatic region of the spectrum (6.8 - 8.0 ppm), but spectra were similar to those of kynurenine compounds derived from tryptophan.

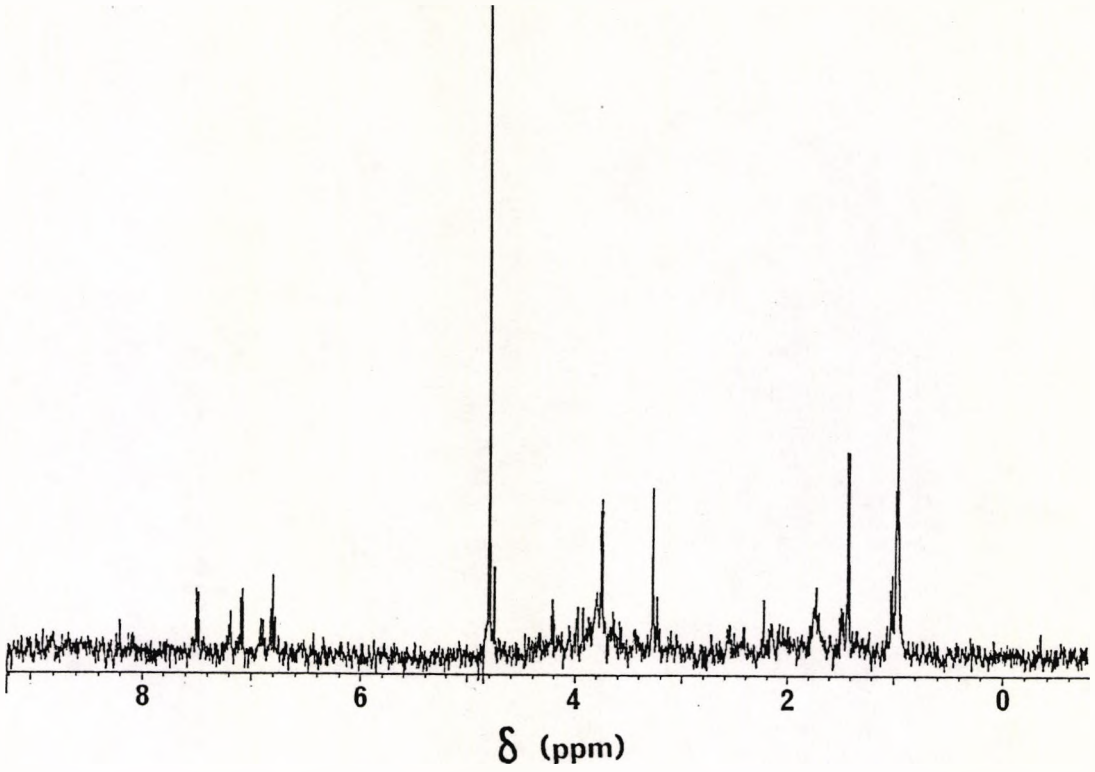


Figure 3-31. Proton NMR spectrum of the HPLC purified lens pigment from *Trichogaster trichopterus*.

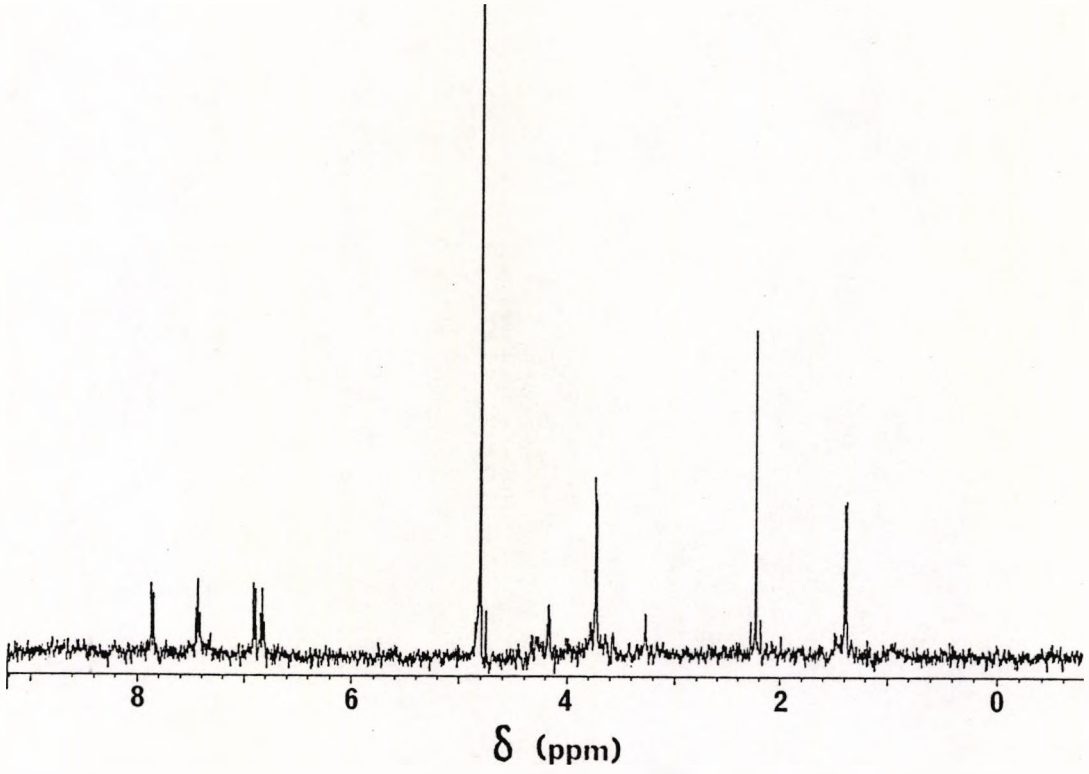


Figure 3-32. Proton NMR spectrum of the purified extract from the lens of *Stylephorus chordatus*.

Comparisons of the *Stylephorus chordatus* spectrum with that of kynurenine itself revealed identical NMR spectra (Fig. 3-33). The gourami spectrum was similar, and was identified later to be 3-hydroxykynurenine [Truscott *et al.*, submitted; see Fig. 3-27].

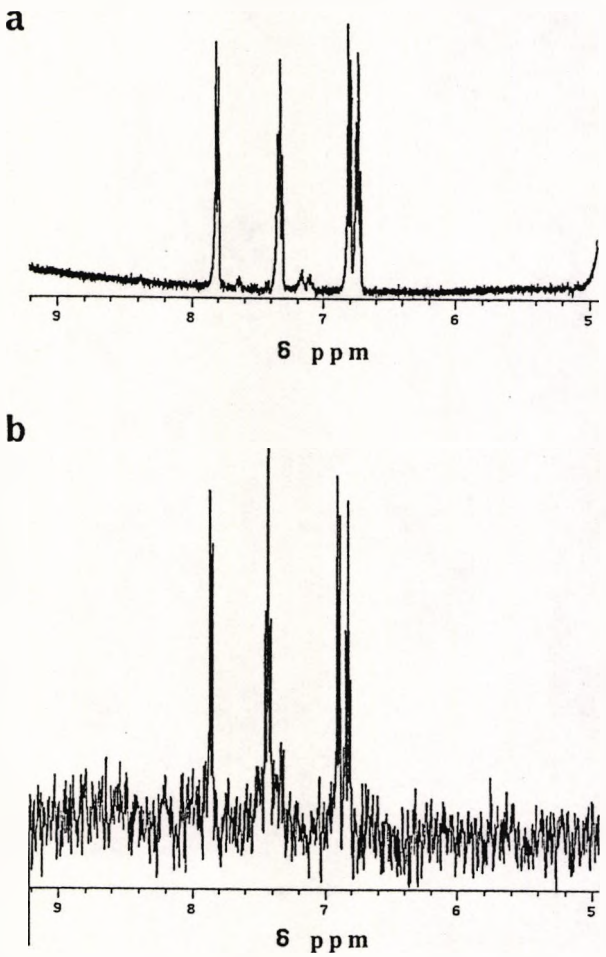


Figure 3-33. Proton NMR spectra of (a) the aromatic region of the authentic leucyl-kynurenine (b) the same region of the *Stylephorus chordatus* lens extract.

Finally, mass spectroscopy was carried out on the *Stylephorus chordatus* pigment to elucidate its molecular weight. The small amount of purified pigment meant that a

highly sensitive mass spectrometer was required. The sample was therefore sent to the Australian National University at Canberra for analysis. The molecular weight was determined to be 208Da, as is that of pure kynurenine, confirming that the compound from *Stylephorus chordatus* lenses was kynurenine (see Fig. 3-27).

3.3 DISCUSSION

These results support previous data predicting that the presence of short-wave absorbing pigments in the fish lens is widespread [Muntz, 1973, 1976a&b; Douglas & McGuigan, 1989; Dunlap *et al.*, 1989]. Over two thirds of the 114 species examined from 49 families have some degree of lens pigmentation (Table 3-1 & Fig. 3-8). In fact, given the limited criteria for the presence/absence of pigmentation used here i.e. evidence from transmission spectra, it may be that most species have at least trace amounts of pigment.

3.3.1 BIOCHEMICAL NATURE OF LENS PIGMENTS

The finding of mycosporine compounds in the lenses of marine fish was not surprising given the almost ubiquitous occurrence of mycosporine-like amino acids throughout the marine invertebrate community [Chioccara *et al.*, 1980; Nakamura *et al.*, 1982]. However, although mycosporine pigments have been previously identified in the lenses of

tropical marine species [Dunlap *et al.*, 1989], they have not until now been identified in lenses of more temperate diurnal marine animals, such as gurnard, herring, pipefish and pollack (Table 3-3). Mycosporine-like compounds are found in a range of tropical invertebrate species and the fact that many temperate species have mycosporine analogues in their eggs [Chioccara *et al.*, 1980; Plack *et al.*, 1981] suggests that there is also a readily available supply of mycosporine precursors in more temperate waters.

More surprising was the identification of mycosporine pigments in the lenses of temperate and tropical freshwater fish such as carp and tilapia (Table 3-3). There is no information on the presence of short-wave absorbing compounds in freshwater algae and invertebrates, but it is highly likely that they will have developed analogous methods of UV protection to their marine equivalents.

The lens pigment of the flying fish (*Exocoetus obtusirostris*) appeared to have a longer l_{max} than other mycosporine pigments at around 385nm (Fig. 3-21). Since one of the degradation products of this pigment was identical to palythine (l_{max} 320nm), the flying fish lens pigment is likely to be a rather unstable mycosporine analogue. The other two degradation products also had l_{max}s around 320nm and may be related to palythine. However, since the pigment was so unstable, it was not possible to establish the identity of the compound by NMR or mass spectroscopy.

Whether the mycosporines in the fish lens play a similar role in photoprotection as in invertebrate species (but in this case to protect the retina) is not known, but

this suggestion has been made previously for the pigments of both fish and higher vertebrate lenses [Collier & Zigman, 1987; Collier *et al.*, 1989]. The fact that mycosporine compounds have been identified in many diurnal species (Table 3-3; Dunlap *et al.*, 1989) supports a role in UV protection (see Chapter 9).

The lens pigments of mesopelagic fish are, in general unidentified, but include carotenoid-like pigments in some animals [McFall-Ngai *et al.*, 1988; Douglas & Thorpe, in press]. The deep-sea species examined here, *Stylephorus chordatus*, was found to have kynurenine as its lens pigment (see Fig. 3-27) - a compound unrelated to either mycosporines or carotenoids. It is possible that there are very low concentrations of mycosporine pigments also present in the *Stylephorus chordatus* lens, since there were small peaks in the HPLC elution profile around the relevant retention times (Fig. 3-22a). The l_{max} 370nm pigment, 3-hydroxykynurenine, isolated from the lenses of several cichlids and belontids (Figs. 3-24 & 3-25) is also a tryptophan catabolite and is related to kynurenine. HPLC profiles of these extracts also demonstrated low concentrations of unidentified absorbing compounds (Fig. 3-24).

Zigman (1987) has suggested that kynurenine-like compounds are present in the lenses of some shallow swimming marine species using only evidence from lens extract absorbance spectra. However, it is unsatisfactory to base conclusions simply on similarities in crude extract absorbance spectra, since a multitude of compounds can often

have identical spectra, at least in certain regions (e.g. see Fig. 3-23). The presence of other absorbing compounds in the lens can complicate the absorbance spectra. For example, nucleic acids absorb maximally at 260nm and, in combination with palythene (l_{max} 360nm), would create a UV absorbance spectrum that resembles that of kynurenine (Fig. 3-23). The identification of kynurenine in the deep-sea *Stylephorus chordatus* and the related compound in the lenses of some cichlid and belontid species, therefore represents the first evidence, based on both HPLC purified samples and NMR, that tryptophan catabolites act as short-wave absorbing compounds in the lenses of some fish.

Kynurenine is produced in the breakdown pathway of tryptophan (Fig. 3-27) and it is probable that tryptophan-related pigments are synthesised within the fish lens from free tryptophan, as is the case in the human lens [van Heyningen, 1973b]. Higher vertebrates such as squirrels and primates also have lens pigments derived from tryptophan catabolites in the same pathway [van Heyningen, 1973a&b; Zigman & Paxhia, 1988]. That a mesopelagic species and a number of freshwater species should have evolved lens pigments closely related to those of higher terrestrial vertebrates is quite surprising. Specific enzymes are required for the reactions in this pathway and it could be that they are active in only a few of the species examined up to now, explaining why kynurenine compounds are not commonly found. Alternatively, it may be that tryptophan itself is at a low concentration in the lenses of most fish, as is reported for a number of other animals [van Heyningen,

1973b]. This would prevent its accumulation and subsequent conversion into active short-wave absorbing pigments, even if the necessary enzymes were present.

Two tryptophan-related compounds and four major mycosporine compounds (including the unidentified mycosporine-related pigment from the flying fish) were isolated from the lenses of fish examined here. There are also a number of carotenoid compounds [McFall-Ngai *et al.*, 1986; Douglas & Thorpe, in press] and a variety of unidentified compounds with absorbance in the region of 340-410nm [Somiya, 1982; Muntz, 1976b; McFall-Ngai *et al.*, 1988; Douglas & Thorpe, in press]. Lens pigmentation in fish therefore represents a good example of convergent evolution: a number of quite different methods of effectively achieving the same result have evolved in the fish lens independently. Although the mycosporine and kynurenine pigments found in fish lenses are quite different structurally, their ultimate effects on the whole lens transmission are identical, suggesting that they both evolved to serve similar functions in absorbing UV wavelengths.

It is not certain how many of the lens pigments listed in Table 3-1 are mycosporine compounds. However, it is likely that all l_{max} 370nm pigments (i.e. from lenses of all belontidae species and several cichlidae species) are 3-hydroxykynurenine, as identified in the gourami *Trichogaster trichopterus*. The 360nm l_{max} pigment extracted from lenses of gadidae and several other species could be either the mycosporine compound palythene or kynurenine, since both have similar near UV absorbance (see Fig. 3-23). As

palythene was identified from extracts of pollack lenses (Fig. 3-18b), it is most likely that lenses of other gadidae species, for example cod, whiting and haddock (Table 3-1) also contain palythene rather than kynurenine. In support of this, van Heyningen and Linklater (1976) also concluded that the lens pigments of several gadidae species were not tryptophan related, as the lenses did not metabolise radio-labelled tryptophan.

Pigments absorbing in the 320-330nm range presented a similar problem; these compounds could be either tryptophan metabolites (N'-formylkynurenine absorbs maximally around 320nm) or the mycosporine compounds: palythine, palythinol and asterina-330 (Fig. 3-17), which absorb in the 320-330 region. The λ_{max}s in this region were extremely variable (Fig. 3-5), suggesting that the majority of lens pigment extracts are more likely to contain a mixture of the mycosporine-related compounds than simply just one compound.

3.3.2 PIGMENT DISTRIBUTION IN THE LENS

Mycosporine pigments are present in both the lens nucleus and cortex of fish. Since the pigments were easily extracted it was assumed that they were able to diffuse within the lens and were not bound to any lens proteins. Lens pigments in the pollack (Fig. 3-14) were at similar concentrations in nucleus and cortex. However, the higher concentration of 360nm pigment in the whiting nucleus compared to the cortex (Fig. 3-15) appears to contradict this and may be explained by the more compressed fibres in the nucleus. Tightly packed lens fibres may make it more

difficult for even very small compounds to travel around freely. Another possibility is that the pigment is attached to the lens crystallins in this species, and since the nucleus has a higher protein concentration, the pigment concentration of the nucleus is correspondingly higher. Earlier work has suggested that lens pigments in cod and a number of other fish are weakly and non-covalently bound to low molecular weight lens protein [Bon *et al.*, 1968]. Other lens pigments, such as the carotenoids of *Argyropelecus affinis*, a deep sea species, are bound to the lens crystallin and therefore can be found at very different concentrations in the nucleus and cortex [McFall-Ngai *et al.*, 1986 & 1988; Yu *et al.*, 1991].

3.3.3 PHYLOGENETIC RELATIONSHIPS

Pigmented lenses have been described for a wide range of freshwater families [Muntz, 1973; Douglas & McGuigan, 1989], with the majority belonging to the perciforme order (Fig. 3-8). In marine species, yellow lenses are described only in perciforme and perciforme-derived species (with the exception of the yellow lenses found in mesopelagic fish) [Muntz, 1976b]. The Syngnathidae (Pipefishes) are neither perciformes nor mesopelagic, yet they too have yellow lenses (Table 3-1; Fig. 3-9c). A number of other non-perciforme species had considerable pigment concentrations although the lenses were not yellow (e.g. cod, whiting etc. and plaice, flounder etc. Table 3-1). Lenses not appearing yellow, but nevertheless having quite efficient short-wave absorbing compounds have now been described in several other orders,

both marine and freshwater [Denton, 1956; Kennedy & Milkman, 1956; van Heyningen & Linklater, 1976; Bon *et al.*, 1968; Douglas & McGuigan, 1989; Douglas, 1989]. Fish containing lens pigments are represented in 14 out of the 20 orders examined here (Fig. 3-8), confirming that lens pigments are phylogenetically widespread.

Closely related species often have the same lens pigments. For example, four members of the gadidae family: cod, saithe, whiting and haddock all have a λ_{max} 360nm pigment, which is likely to be palythene, in the lens (Table 3-1). Other authors confirm the presence of only 360nm pigment in the lenses of several other gadidae species [Bon *et al.*, 1968; van Heyningen and Linklater, 1976]. Although the pigment composition is the same, pigment concentration can vary, with whiting lenses having considerably lower concentrations than lenses of both cod and haddock.

Most cyprinids have lenses with no pigmentation (Fig. 3-8), and even those species sometimes accumulating pigments (goldfish and carp), never have very high concentrations (see also Chapter 4). Similarly, neither the rajidae or scyliorhynidae show any evidence of lens pigmentation (Table 3-1, Fig. 3-8). The rays examined were all from one genus (*Raja*) and demonstrated similar lens transmission spectra. Such correlation within a close family group tends to suggest that these fish retain some common genetic factor governing their lens metabolism.

Perciforme species show the greatest diversity in lens pigmentation (Fig. 3-8), with at least 6 different types of pigment isolated. It may simply be that as more perciforme

species were examined, the variety in lens pigment types observed was higher. However, since the perciforme order contains by far the greatest number of species, it is probable that the genetic diversity is greater than observed in other orders, perhaps leading to the wider variety of lens pigment types.

HPLC profiles (Fig. 3-18) demonstrate quite clearly the interspecific variability often seen in relative pigment concentrations. Even closely related species can have quite different relative pigment concentrations, though the pigment composition of their lenses is often the same (for example note the large range in 50% transmission of the cichlids in Table 3-1, though most appear to have both palythine and palythene pigments). Since the genetic make-up of congeneric species is likely to be quite similar, it may be that other factors, such as the animal's environment, are important for the determination of specific pigment levels within the lens.

3.3.4 ECOLOGICAL RELATIONSHIPS

The presence of both lens and corneal pigments in fish have been linked to a diurnal lifestyle [Muntz, 1973; Zigman, 1982]. Although pleuronectidae and soleidae families are closely related and share the same environment, lenses of soleidae species have no pigment, whereas all pleuronectids have considerable levels of lens pigment (Table 3-1). This fits in with the presence of pigmented lenses in only diurnal animals, since unlike the pleuronectids, soleidae tend to be nocturnal [Bristow,

1987]. Whilst it is true that many of the highly diurnal species, such as those living on coral reefs (e.g. pomacanthidae and pomacentridae families - Table 3-1), often do have highly pigmented lenses, this is not a hard and fast correlation. Animals living in the same body of water sometimes have quite different lens transmission characteristics [Denton, 1956]. Lenses of the dogfish and rays are unpigmented, whereas those of plaice and flounder contain pigment (Table 3-1), although all live close to the sea bed at similar depths. Similarly, although the species of flying fish examined here did have a high lens pigment concentration and therefore also a high lens 50% transmission (Table 3-1, see also Fig. 4-29), other species within the same family and sharing the same environment have very little, if any, lens pigment [Douglas, pers. comm.].

The majority of gadiforme species examined here did contain lens pigments (Fig. 3-9, Table 3-1). However, two gadidae species, of the genus *Trisopterus* and also the hake (*Merluccius merluccius* - a member of the merluccidae family), had no lens pigments (Table 3-1; Fig. 3-8). All three species are found close to the ocean bottom at depths of up to 500m and the hake feeds actively in shallow water only at night [Wheeler, 1978]; lifestyles and habitats which would not appear to benefit from short-wave absorbance.

Animals with pigmented lenses, can be found in a wide range of water colours, clarities and depths [Douglas & McGuigan, 1989]. Cichlids, for example, are found in habitats ranging from murky swamps to clear lakes [Barlow, 1974; Lowe-McConnell, 1964 & 1969]. Haplochromine species

are also found to segregate into different ecological environments within Lake Victoria [Witte, 1981 & 1984], yet adults of all species except *Haplochromis argens*, have the same short-wave absorbing compounds in the lens (Table 3-1; see also Chapter 4). *Haplochromis argens* does not inhabit appreciably greater depths than other species [Goldschmidt, 1988] and in addition is reported to be chiefly zooplanktivorous in common with many other haplochromine cichlids [Goldschmidt, 1988]. Therefore, it could be that some genetic difference is responsible for the absence of lens pigments in this species rather than an environmental or ecological factor.

360nm λ_{max} pigments alone, whether the mycosporine amino acid palythene or tryptophan derived kynurenine, have to date, been found only in marine species of fish (Table 3-1). Whilst 360nm pigments are found in freshwater species, a shorter wavelength 320-330nm pigment is always present too. Similarly, the λ_{max} 370nm pigment, 3-hydroxy-kynurenine, was only isolated from lenses of a number of tropical freshwater species (Tables 3-1 & 3-3). Such observations may indicate the influence of some environmental factor on pigment formation, but could also be a mis-representation due to the relatively small number of species examined.

There are obviously considerable overlaps between trophic groups of fish and no one species of fish is likely to feed exclusively on a food source ignored by all others. Since mycosporine compounds are so widespread in the marine environment, it is likely that most fish include mycosporine

compounds in the diet. Many reef fishes are known to feed on algae [Barlow, 1974], which are thought to synthesise mycosporine pigments [Shibata, 1969; Dunlap & Chalker, 1986; Wood, 1989]. Fish such as *Acanthurus sp.* and related species, grazing on the reef, will have a high mycosporine intake, which may explain the high concentration of mycosporine-like pigments in their lenses (Table 3-1).

Pigmentation of the fish lens is probably a combination of both diet and metabolism, and perhaps also UV-exposure. Sequestration of pigments could conceivably be the result of a number of different pathways. Kynurenine and related pigments in the lenses of *Stylephorus chordatus* and some gouramis and cichlids are derived from tryptophan in the diet (Fig. 3-27). Mycosporine precursors or analogues are also likely to be dietary in origin. Their conversion into the four compounds commonly found in the lens could occur either before transportation into the lens or actually within the lens. Conditions for catabolic processes are likely to vary, for example oxidative conditions may favour production of one pigment, whereas highly acidic conditions may lead to the accumulation of another. The presence of particular enzymes to speed up conversion of mycosporine analogues may also have a bearing on the concentrations of these compounds in the lens. Interspecific differences in metabolic conditions could ultimately lead to the formation of different compounds.

Detailed information on lifestyle, behaviour and feeding preferences is available for relatively few fish species. It is therefore extremely difficult to identify

accurately the functions of short-wave absorbing compounds in the lenses of fish by attempting to correlate their presence/absence to the animal's ecological niche. However, by extrapolation of information available for closely-related species it is possible to generalise about possible lens pigment functions in families or species within the same order or from the same habitats. Results will be discussed with observations from other vertebrates in Chapter 9.

A number of biochemically distinct pigments have now been isolated from fish lenses, many of these, such as the short-wave absorbing compounds in lenses of deep-sea species [McFall-Ngai *et al.*, 1988; Muntz, 1976b; Douglas & Thorpe, in press], have yet to be identified. Many however have been identified including carotenoid-like compounds, tryptophan derivatives and mycosporine-like amino acids.

Examination of a large number of lenses from individuals of a number of species, led to the discovery that considerable intraspecific differences in lens transmission and pigmentation often exist. Older lenses tended to have quite different transmission characteristics compared to younger lenses of the same species. Such age related differences in lens transmission are considered in the following chapter.

CHAPTER FOUR

CHANGES IN FISH LENS PIGMENTATION WITH AGE

4.1 INTRODUCTION

It is widely reported that the human lens is clear at birth but becomes pale yellow in young children with further increases in the intensity of the yellow colouration with normal ageing [Wald, 1952; Said & Weale, 1959; McEwen, 1959; Boettner & Wolter, 1962; van Heyningen, 1972; Weale, 1973 & 1988; Lerman & Borkman, 1976; Lerman, 1980a]. The human lens contains kynurenine and kynurenine glucosides [van Heyningen, 1973a&b] which, in young lenses, absorb 95% of the incident photons in the near UV, the remainder being absorbed by tryptophan residues of the lens protein [Dillon & Atherton, 1990]. The specific content (expressed as μmol/g lens protein) of 3-hydroxykynurenine-glucoside, the major absorbing compound, falls in the human lens with age [Bando *et al.*, 1981], and kynurenine is only detected in lenses of the very young [van Heyningen, 1973a&b]. Insoluble high molecular weight aggregates accumulate in the lens nucleus with age as a result of post-translational changes within the crystallins and cross-linking between the UV-photooxidised residues [Zigman, 1971 & 1983a; Goosey *et al.*, 1980]. This results in a decrease in lens transparency due to an increase in both scattering and lens absorbance in

the near UV and blue regions. The greater absorbance of short wavelengths with age in man is therefore due to a combination of factors, all of which are compounded by an increase in lens axial diameter with age [Mellerio, 1971 & 1987].

In contrast to human lens ageing, reports on ageing in the fish lens are less frequent and often contradictory. A few studies report spectral transmission of the whole eye or just the lens in a number of species. For example, goldfish ocular media transmission was reported not to change with age [McCandless *et al.*, 1969], but later work suggests that it actually does [Bassi *et al.*, 1984; Douglas, 1989]. A reduction in the transmission of short-wave radiation by lenses of goldfish with age was observed by both Bassi *et al.* (1984) and Douglas (1989). Hawryshyn *et al.* (1989), carried out a similar study of the rainbow trout and found a slight age-related difference in ocular media transmission at 320nm (but at no other wavelengths). However, a more definite ageing trend is described for the closely related brown trout [Douglas, 1989]. Douglas (1989) proposed that age-related changes in lens short-wave transmission were not dependent solely on the increasing lens diameter but that short-wave absorbing pigments developed in the lenses of both goldfish and brown trout with age.

Lenses of a mesopelagic fish, *Argyropelecus affinis*, are reported to suddenly commence pigment deposition upon reaching a certain size, with a yellow carotenoid pigment then accumulating steadily as the fish age [McFall-Ngai *et al.*, 1986]. Lenses of young fish are colourless whereas

older animals have deep yellow lenses due to the binding of carotenoid-like pigments to the lens α crystallin, surrounding the colourless inner portion [McFall-Ngai *et al.*, 1986]. More recent work however updates this, with very low concentrations of carotenoid pigment detected even in the 'unpigmented lens core', suggestive of an abrupt increase in the rate of pigment accumulation [Yu *et al.*, 1991; Douglas & Thorpe, in press]. Villermet and Weale (1972) studied lens pigmentation in two age groups of rudd (*Scardinius erythrophthalmus*) and found a quite different age effect. Older fish developed a deep yellow patch of pigment in the anterior portion of the lens nucleus with a transverse area approximately the same size as the pupil.

Even if no pigment accumulates in the lens with age, a slight reduction in the transmission of short-wave radiation will still be seen, due to the increasing size of the lens, which grows steadily throughout a fish's life. Absorbance by aromatic residues in the lens proteins will increase as new proteins are continually added during growth. However, if pigments are laid down in the lens, this reduction in transmission is more pronounced, often with all the UV and even some of the blue wavelengths absorbed by lenses of older individuals.

Methods and timing of pigment accumulation appear to vary between species; the rudd develops its patch of pigmentation quite late in life [Villermet & Weale, 1972], whereas yellow lenses are observed in quite young *Argyropelecus affinis* [McFall-Ngai *et al.*, 1986; Douglas & Thorpe, in press]. Short-wave absorbing compounds also

appear to accumulate in lenses of the brown trout within three years [Douglas, 1989]. It is not yet certain why pigments should develop in the fish lens with age or what the origins of the pigments are, but it is now obvious that quite a number of species go through lens ageing processes.

In this section, age-related changes in the lens transmission of seventeen species of both freshwater and marine species of fish are examined. Wherever possible, fish of a wide age range from a single population were examined, but when this was not practical, fish of different ages were obtained from separate populations.

4.2 RESULTS

There are a number of ways in which lens transmission was observed to change with age. The first and simplest was lens ageing without pigment accumulation, resulting in a steady increase in the wavelength of 50% transmission, which could be accounted for solely by the increasing pathlength through the lens (Section 4.2.1). Other species, although initially unpigmented, showed an increase in pigment concentration with age (Section 4.2.2). A number of species initially accumulating lens pigments then displayed a levelling in the lens 50% transmission wavelength and form the third group (Section 4.2.3), while fish showing a drop in the wavelength of lens 50% transmission after an initial increase, represent the fourth group (Section 4.2.4). A

fifth group displayed only a fall in lens 50% transmission with increasing age (Section 4.2.5). Each of the groups will now be considered in more detail.

4.2.1 LENS AGEING WITHOUT PIGMENT ACCUMULATION

4.2.1.1 Rainbow trout

Lens transmission spectra of 4 year old rainbow trout (*Salmo gairdneri*) were shifted to longer wavelengths when compared to the lens transmission of 2 year old animals (Fig. 4-1). Fish ages were estimated by the dealer and were later confirmed by scale annulus counting.

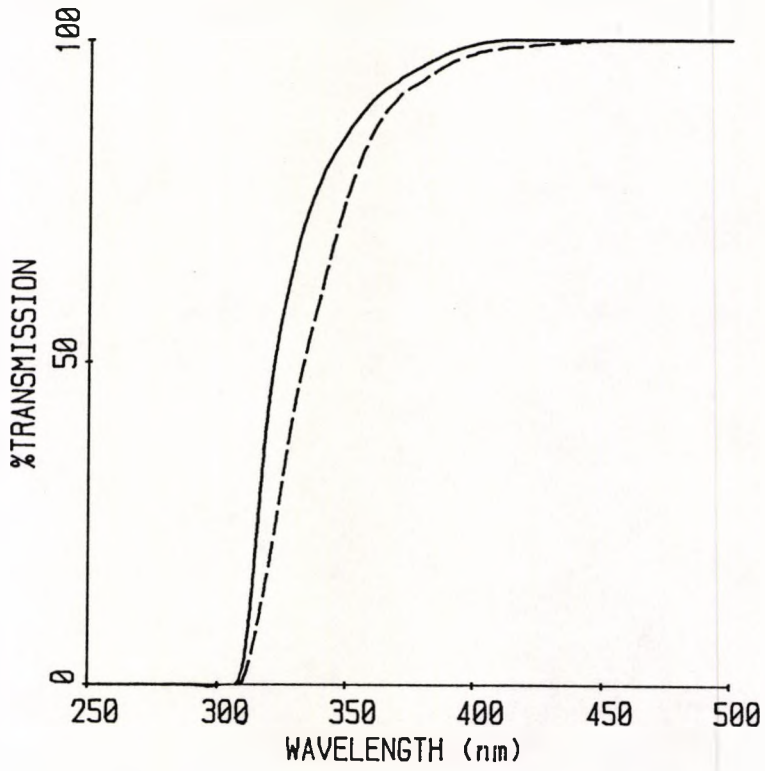


Figure 4-1. Spectral transmission of two lenses from *Salmo gairdneri*, 3.29mm (solid line) and 6.15mm (dashed line) in diameter.

Lenses of rainbow trout did not contain detectable levels of pigment at any age, but 50% transmission increased steadily with increasing lens diameter (Fig. 4-2). The theoretical wavelength of 50% transmission for lenses with diameters 2 and 3 times those of the smallest trout were calculated assuming a constant absorbance per unit diameter (as used by Mellerio, 1971 & 1987 and Douglas, 1989 - see Section 2.2).

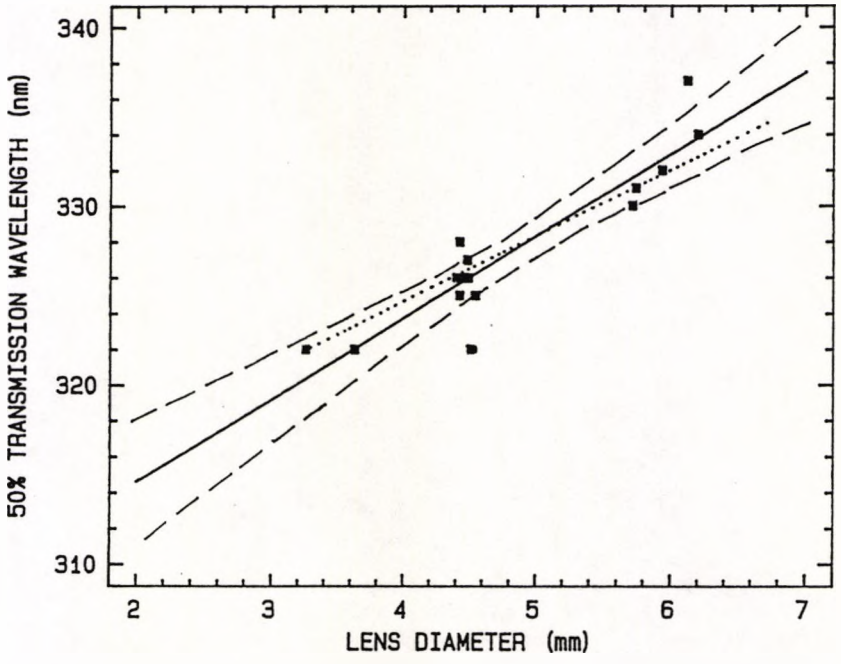


Figure 4-2. Lens 50% transmission wavelength as a function of diameter for all *Salmo gairdneri* lenses examined. The dotted line represents the theoretical increase in transmission if this were dependent on lens growth alone, calculated from the smallest lens. The solid line is the regression with 95% confidence limits (dashed lines).

Such theoretical calculations may have an added degree of accuracy when applied to the fish lens: most fish lens pigments appear to be free to diffuse throughout the lens and the concentrations in nucleus and cortex should therefore be roughly the same (see Chapter 3). The line in Fig. 4-2 shows the theoretical ageing of the trout lens based on an increase in size alone. The observed data conform well to the theoretical model and it can be concluded that no pigments were laid down in the lens of this population of rainbow trout with increasing age.

4.2.1.2 Marine species

Similar lens ageing without pigment production was also observed in several marine species. Lenses of the dogfish (*Scyliorhinus canicula*), hake (*Merluccius merluccius*) and bib (*Trisopterus luscus*) all displayed comparable shifts in their spectral transmission with age (Fig. 4-3).

The 50% transmission wavelength expressed as a function of the lens diameter, as in the trout, conform quite well to the theoretical predictions in all three species (Fig. 4-4), with the theoretical line lying within the 95% confidence limits of regression. This suggests that appreciable amounts of pigment do not accumulate in the dogfish, hake or bib lens over the age ranges examined here.

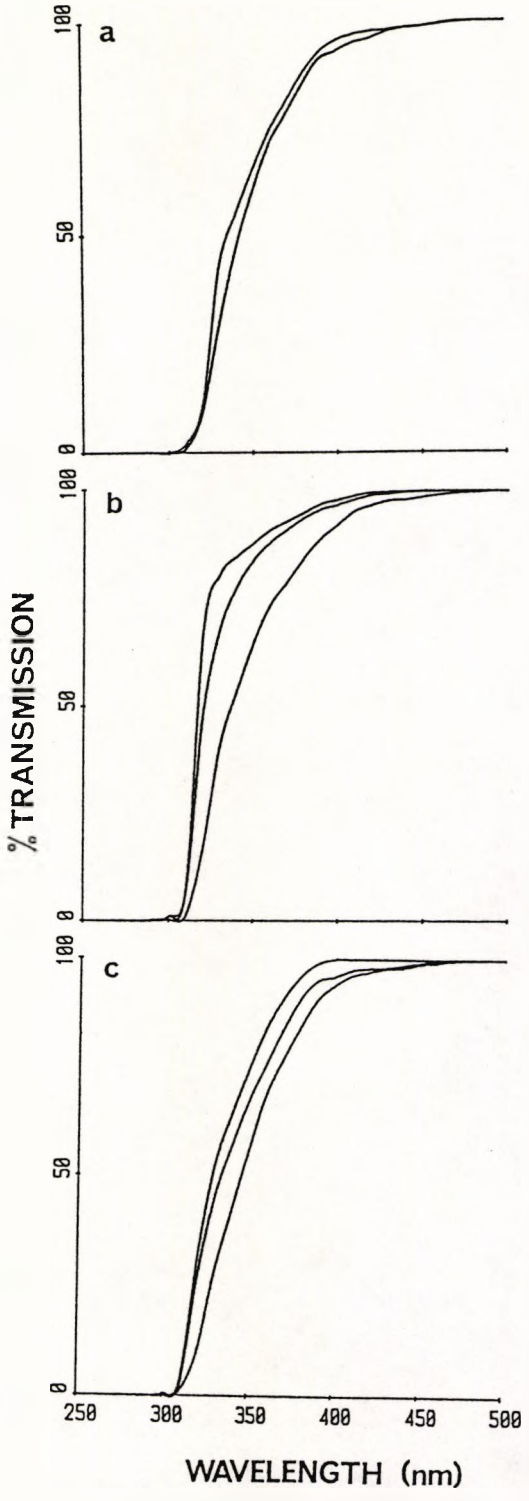


Figure 4-3. Lens spectral transmission in three marine species, with increasing lens diameters left to right in all cases. (a) *Merluccius merluccius* with lens diameters of 2.53mm and 5.8mm (b) *Scyliorhinus canicula*, with lens diameters of 1.34mm, 3.16mm and 7.23mm (c) *Trisopterus luscus* with lens diameters of 3.44mm, 3.94mm and 5.84mm.

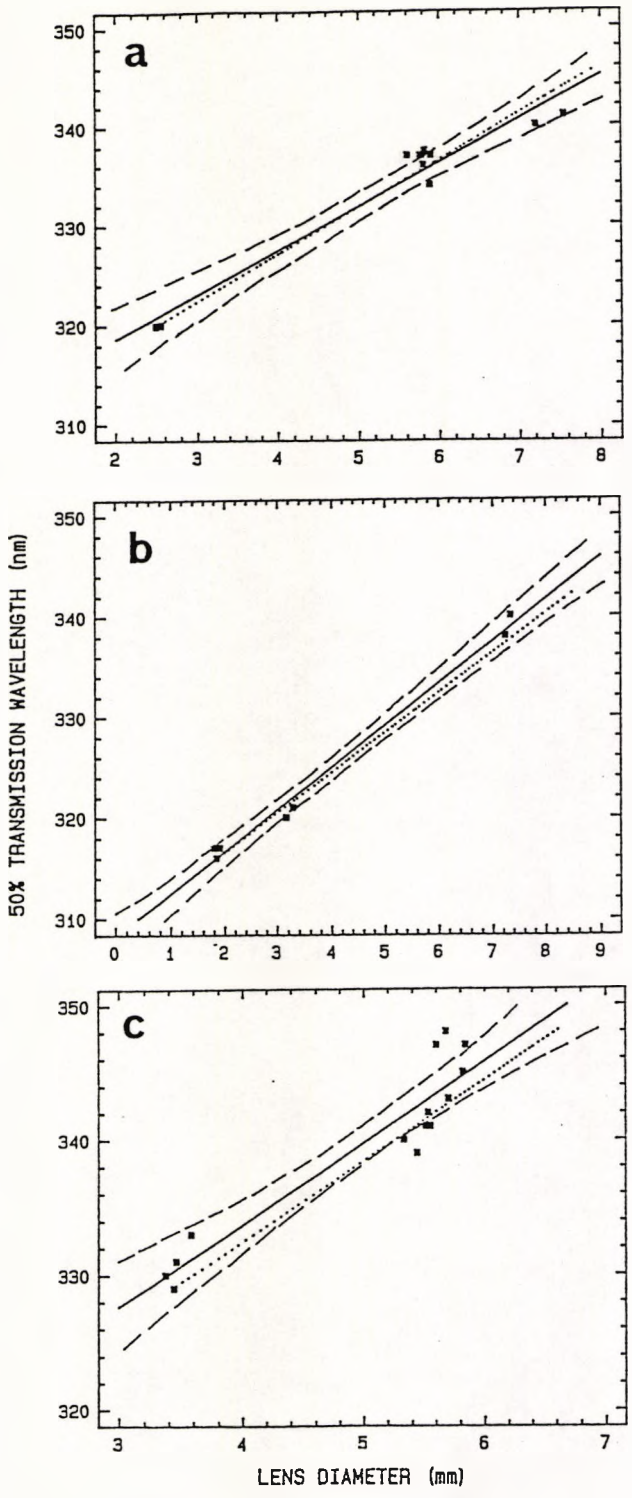


Figure 4-4. Plot of lens 50% transmission wavelength as a function of the lens diameter in (a) *Merluccius merluccius* (b) *Scyliorhinus canicula* and (c) *Trisopterus luscus*. Dotted lines represent theoretical increases in lens 50% transmission, based on increasing lens diameter alone, calculated from the smallest lens in each case. Solid and dashed lines are the regression and 95% confidence limits respectively.

4.2.2 INCREASE IN LENS PIGMENTATION WITH AGE

4.2.2.1 Goldfish

The lenses of many goldfish (*Carassius auratus*) went through ageing changes similar to those involving no pigment accumulation (Fig. 4-5). However, not all goldfish lenses remained free of pigments and there are in fact two ways in which the lens of the goldfish may age, either with (Fig. 4-6) or without (Fig. 4-5) pigment accumulation. Over 200 goldfish, ranging in standard length from 3.9 to 13.7cm were examined, with lens diameters ranging from 0.93mm to 3.93mm.

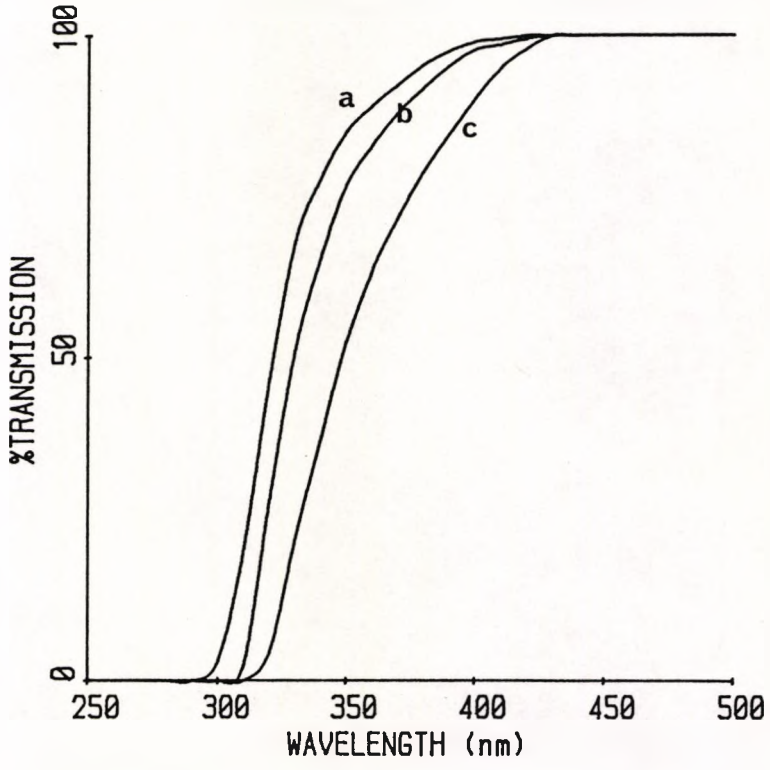


Figure 4-5. Lens spectral transmission of three *Carassius auratus*, with no lens pigment and lens diameters of (a) 1.23mm, (b) 2.53mm and (c) 4.59mm.

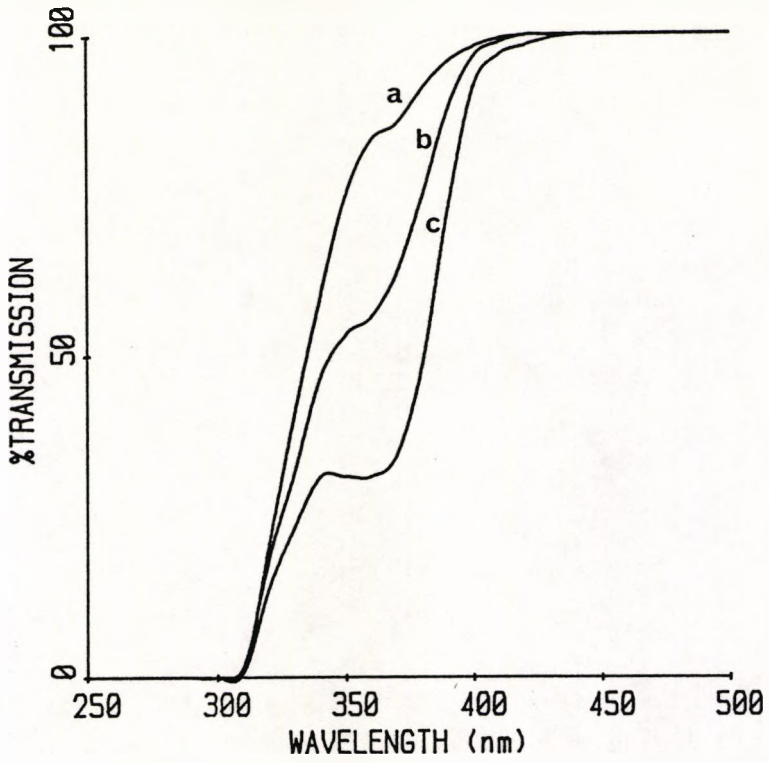


Figure 4-6. Spectral transmission of the lenses of three *Carassius auratus* with lens pigment. The lens diameters are (a) 1.95mm, (b) 2.73mm and (c) 3.38mm.

The 50% transmission wavelength as a function of lens diameter was plotted for all goldfish (Fig. 4-7). Lenses with no pigment correlate quite well with the theoretical line which is based on increase in lens size alone. However, pigmented goldfish lenses generally had 50% transmission points which were much higher than those for unpigmented lenses. A few lenses with low pigment concentrations (e.g. Fig. 4-6, curve a) do not show a large shift in the 50% transmission wavelength from lenses of the same diameter without pigment (Fig. 4-5, curve a).

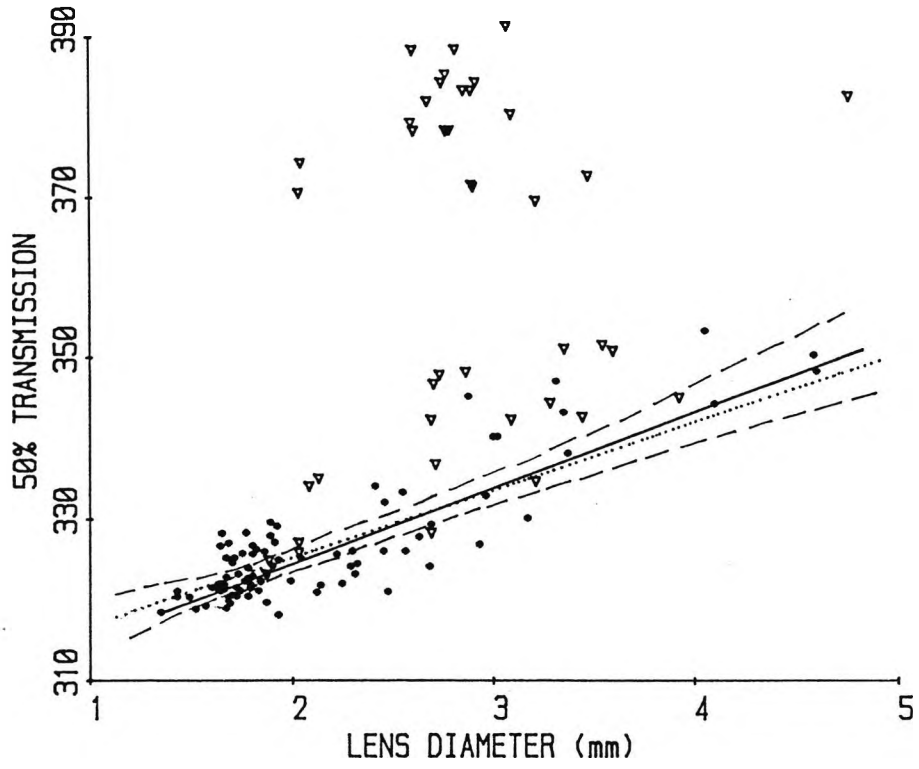


Figure 4-7. 50% transmission wavelength as a function of lens diameter for pigmented (open triangles) and unpigmented (filled circles) lenses from the right eyes of all *Carassius auratus* examined. The solid line is the theoretical shift based on increase in lens diameter alone, which falls within the 95% confidence limits (dashed lines) of the regression (dotted line) for unpigmented lenses.

Goldfish were split into 7 groups according to their standard length. Only 1 of the 9 fish with standard length less than 4cm had any sign of lens pigmentation based on the spectral transmission (see section 3.2.1) compared to 10 out of the 17 examined in the 10.1 - 11.5cm length range (Fig. 4-8). Therefore, in general the smaller goldfish tended to have lenses free of pigmentation, whereas many of the older fish had some degree of lens pigmentation.

Goldfish lens absolute transmission values at 632.8nm were routinely measured and averaged 96-97%, which compared favourably with readings recorded by the spectrophotometer at this wavelength.

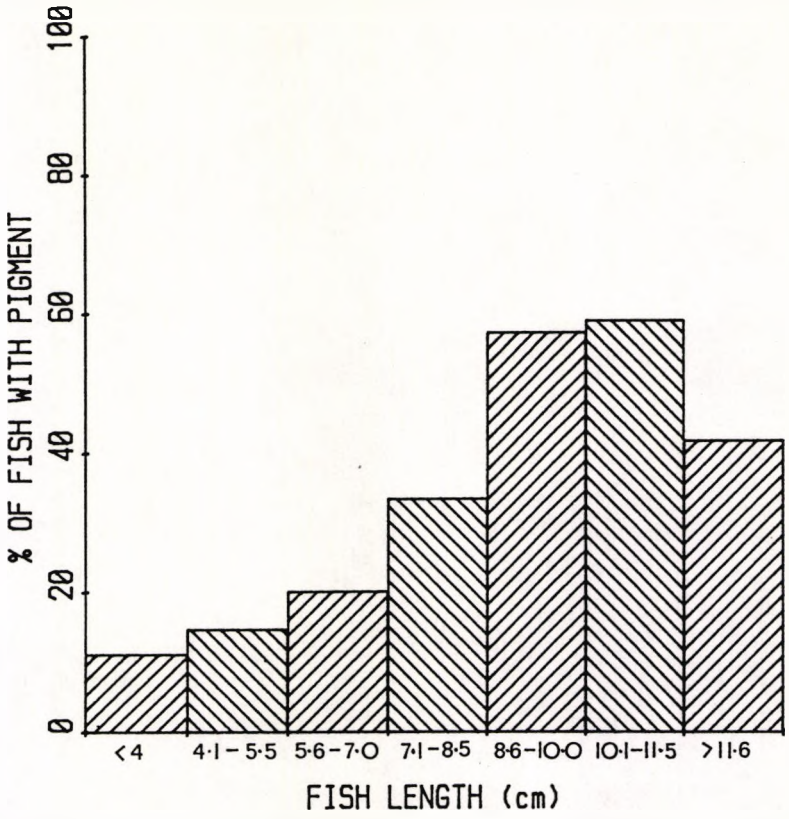


Figure 4-8. Histogram of the number of pigmented lenses in *Carassius auratus* with increasing body length. Data were split into 1.4cm bins on the basis of the fish length.

4.2.2.2 Long rough dab

Only four long rough dab (*Hippoglossoides platessoides*) were available. However, they represented a large size range and showed quite different transmission characteristics (Fig. 4-9). The oldest fish lens transmitted significantly less of the shorter wavelengths than all the younger animals. The plot of 50% transmission wavelength as a function of lens diameter, even though there were so few fish, suggests that a shift in transmission may occur with age (Fig. 4-10).

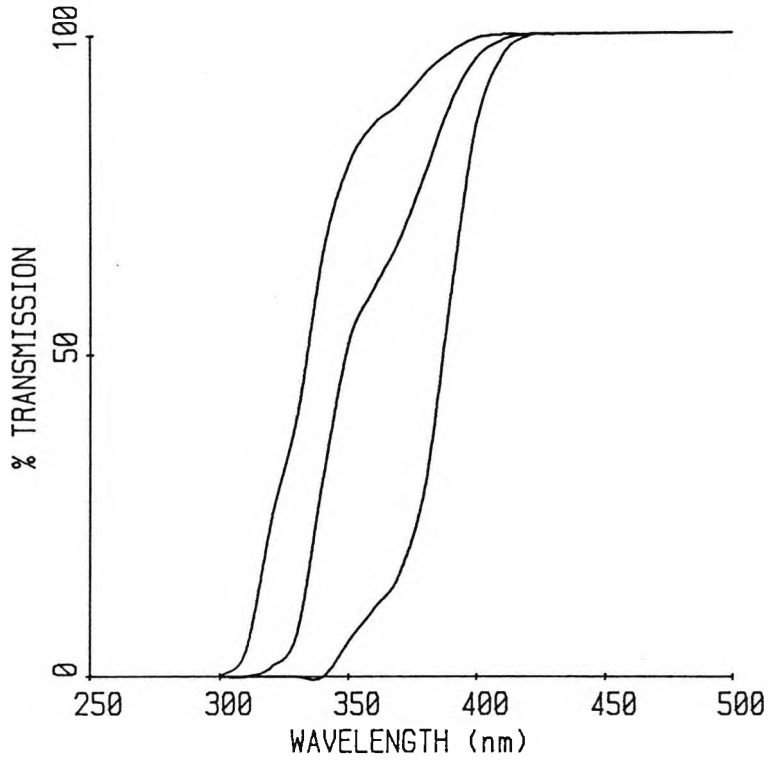


Figure 4-9. Lens spectral transmission variation with age in *Hippoglossoides platessoides*. Lens diameters are 2.27mm, 4.80mm and 5.4mm from left to right.

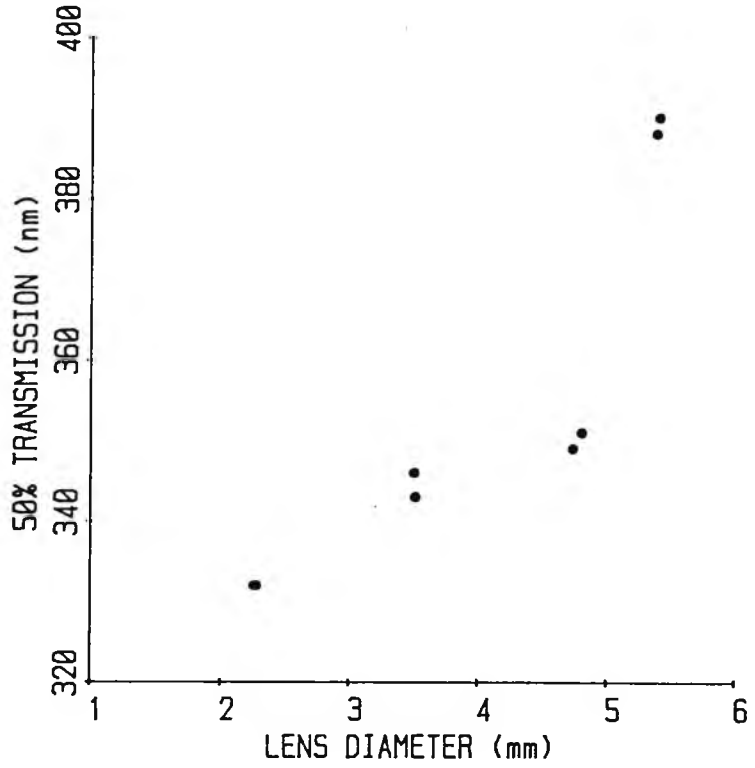


Figure 4-10. Lens 50% transmission wavelength as a function of the lens diameter in the four *Hippoglossoides platessoides* examined.

4.2.2.3 Carp

Carp (*Cyprinus carpio*) of different ages were purchased at various times from numerous local dealers. Most carp had some pigmentation in the lens (e.g. Fig. 4-11). However there were two populations, one of young fish and the other of older fish in which no pigment was detected. Each population appeared to have its own characteristic 50% transmission wavelength (Fig. 4-12), which appeared to vary markedly even between populations with similar lens diameters. This was especially true of younger carp where the range in 50% transmission values was particularly large (320-390nm).

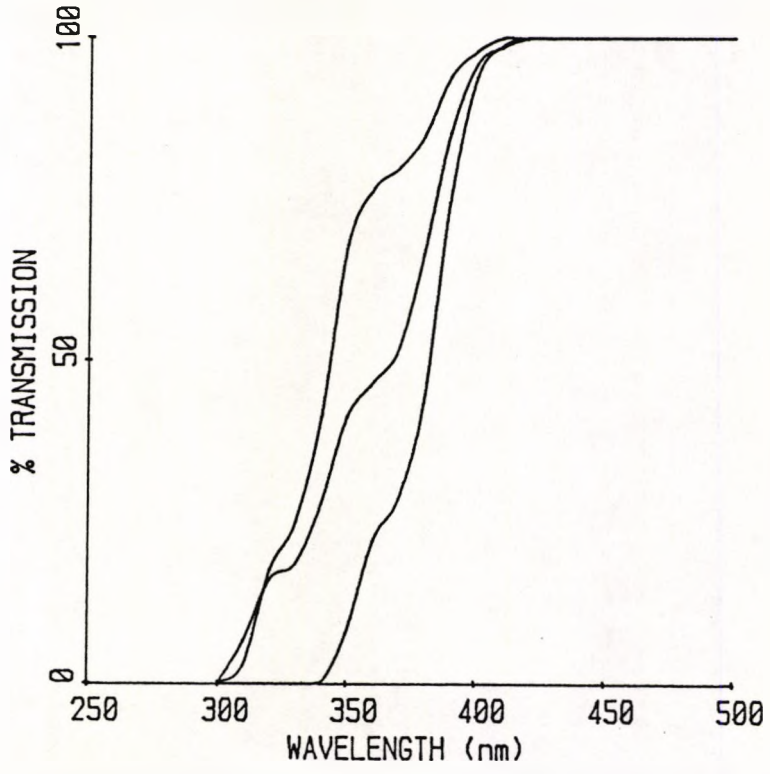


Figure 4-11. Lens spectral transmission of three individual *Cyprinus carpio*, with lens diameters, left to right, of 1.56mm, 1.81mm and 2.3mm.

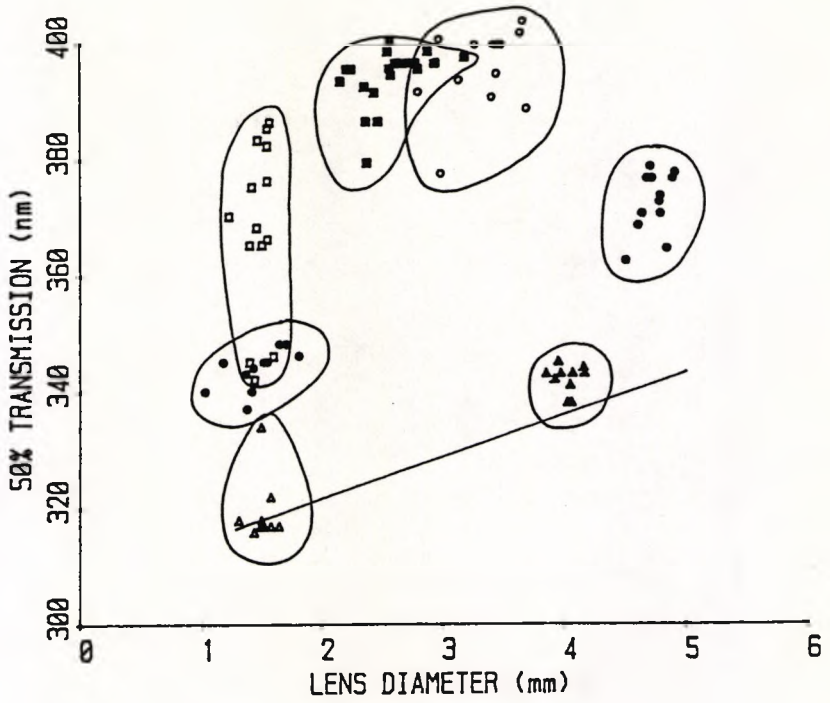


Figure 4-12. 50% transmission wavelength as a function of the lens diameter for seven populations of *Cyprinus carpio*. The line shows the theoretical increase in 50% transmission if this were based on increase in lens diameter alone. The data in each circle represent different populations.

Not only the concentration of lens pigments varied in different populations of carp: there were also differences in the pigment composition. Most mirror carp were found to have both palythine (l_{max} 320nm) and palythene (l_{max} 360nm) in the lens (Fig. 4-13, solid line) while carp of a different (scaly) variety appeared to have only appreciable levels of palythene in the lens (Fig. 4-13, dashed line).

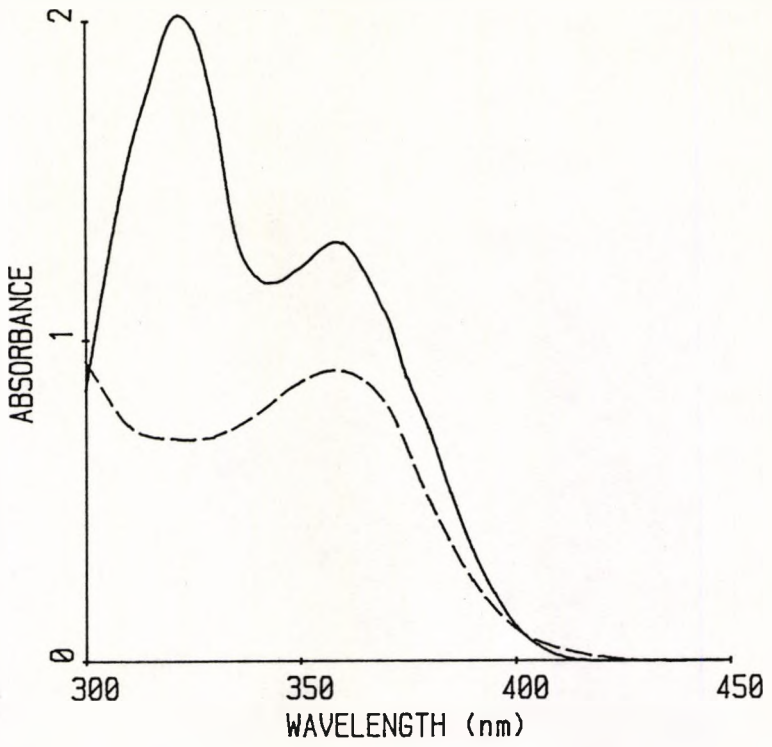


Figure 4-13. Spectral absorbance of lens pigment extracts from *Cyprinus carpio*. One population of fish demonstrated only a l_{max} 360nm pigment (dashed line), whereas the majority had an additional l_{max} 320nm pigment (solid line).

4.2.2.4 Herring

Both young and old herring (*Clupea harengus*) obtained from The Scottish Marine Biological Association had pigmented lenses (Fig. 4-14). Young fish were at the stage of metamorphosis, whereas older fish were estimated to be between two and three years of age. Older animals were split into two groups since a number were caught in the wild and examined immediately, whereas others had been reared in the laboratory for around 1 year. Both groups of older herring were of similar age.

Lenses of the fish from the wild had higher 50% transmission wavelengths, indicating that wild fish had a higher lens pigment concentration than the animals raised indoors, although populations were the same age (Fig. 4-15).

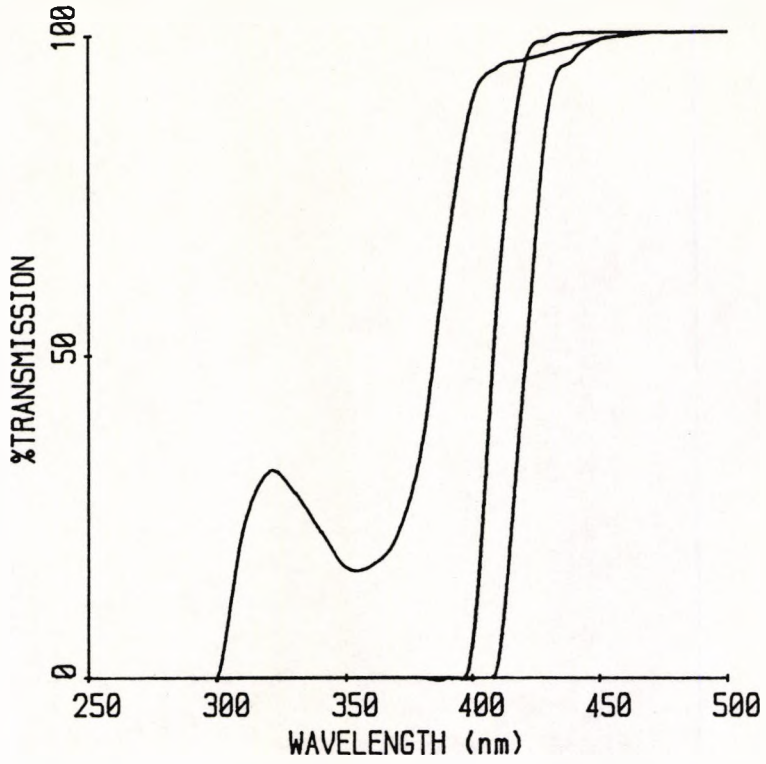


Figure 4-14. Spectral transmission changes in the lens of *Clupea harengus* with age. Lens diameters increase from 0.84mm to 3.02mm to 4.86mm left to right.

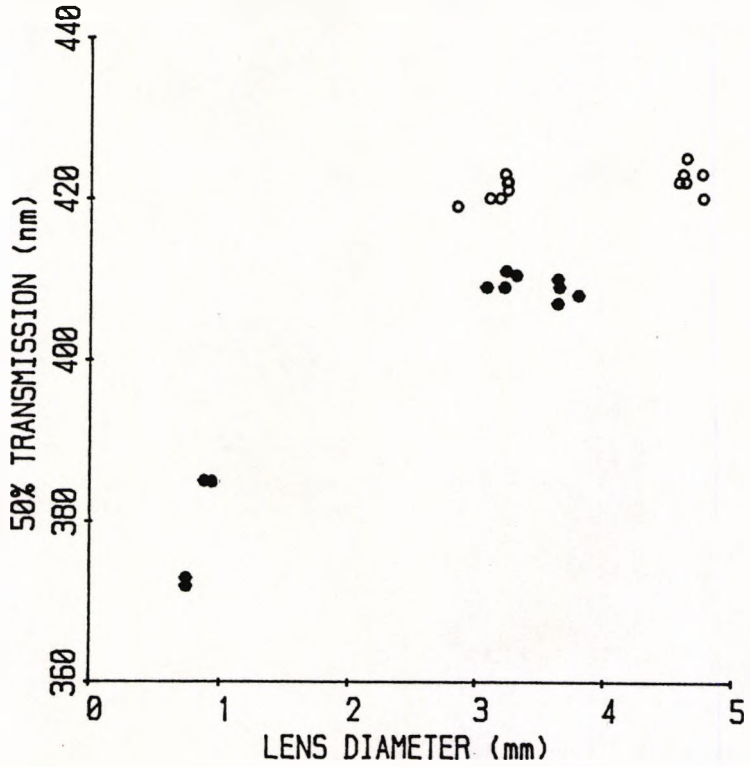


Figure 4-15. Lens 50% transmission as a function of the lens diameter in all *Clupea harengus* lenses examined. Data from fish caught in the wild are represented by open circles, whereas those from fish reared in the laboratory are shown as filled circles.

Older herring lenses were pooled and pigments were extracted from both laboratory-reared and wild fish. Young herring lenses were too small to obtain pigment extracts, but whole lens absorbance suggests the presence of only palythene (l_{max} 360nm) (Fig. 4-16, dashed line). Lens extract absorbance from both classes of older fish showed two absorbance maxima (Fig. 4-16, solid lines), which were identified as palythine (l_{max} 320nm) and palythene (see Section 3.2.5). The concentration of palythene was highest in wild-caught older fish explaining the shift of 50% transmission to longer wavelengths in these animals.

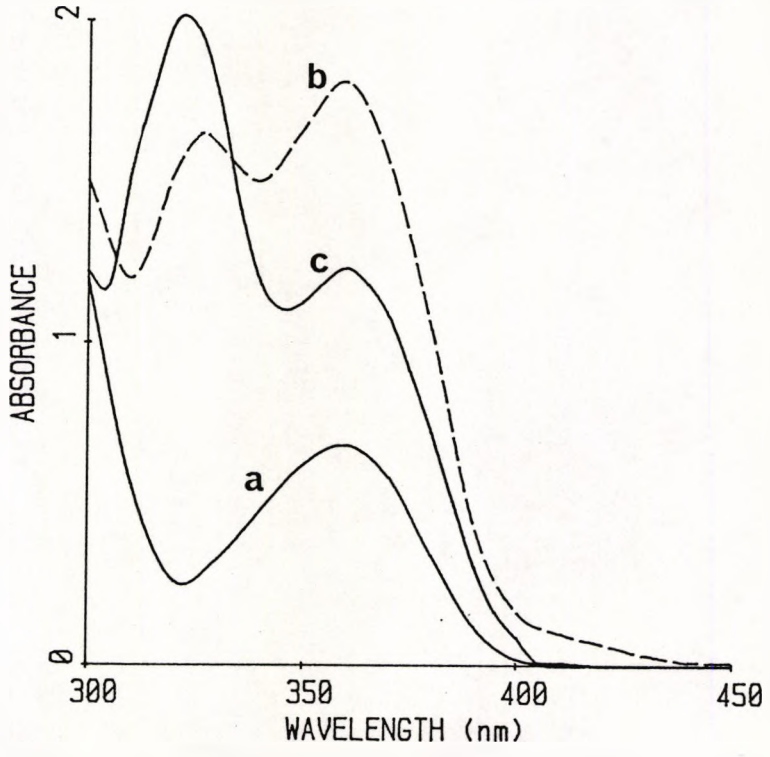


Figure 4-16. Spectral absorbance of *Clupea harengus* lens pigment. (a) young herring whole lens absorbance (b) absorbance of pigment extracted from wild caught and (c) laboratory-reared older fish.

4.2.3 INCREASE FOLLOWED BY LEVELLING IN LENS PIGMENT CONCENTRATION WITH AGE

4.2.3.1 The Haplochromines

A number of closely-related species of the *Haplochromis* family were studied. One species - *Haplochromis argens* did not accumulate lens pigments with age (Fig. 4-17) but showed age-related lens transmission changes similar to those seen in unpigmented goldfish lenses (see Fig. 4-5). A number of other species did however produce pigments in the lens with age. Although young fish had lenses which were quite transparent to UV radiation in the region 310-400nm, all lenses of older *Haplochromis ishmaeli*, *Haplochromis xenognathus* and *Haplochromis sauvagei* showed a marked decrease in short-wave transmission (Fig. 4-18).

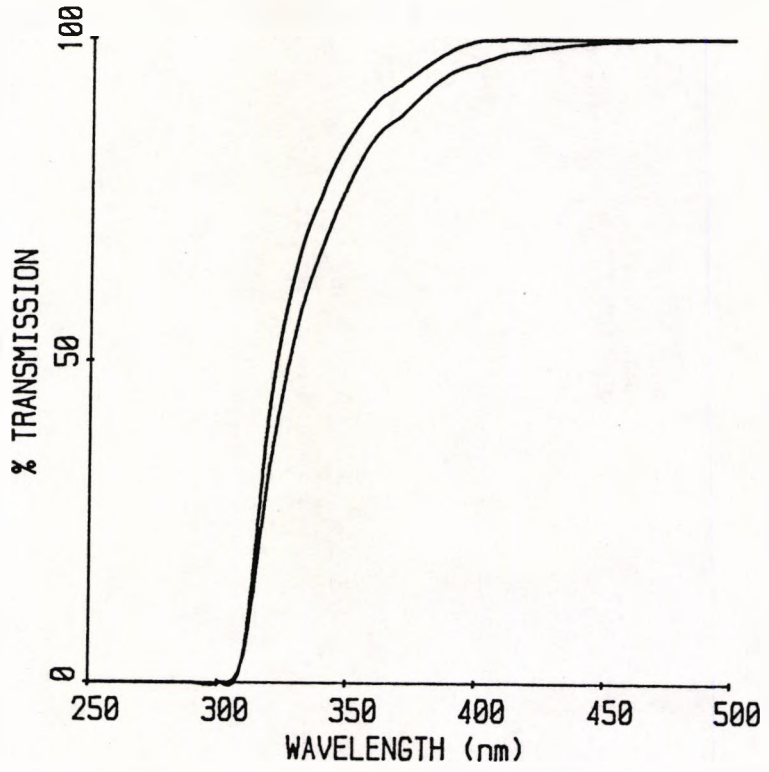


Figure 4-17. Spectral transmission of two *Haplochromis argens* lenses, diameters 1.44mm and 3.34mm left to right.

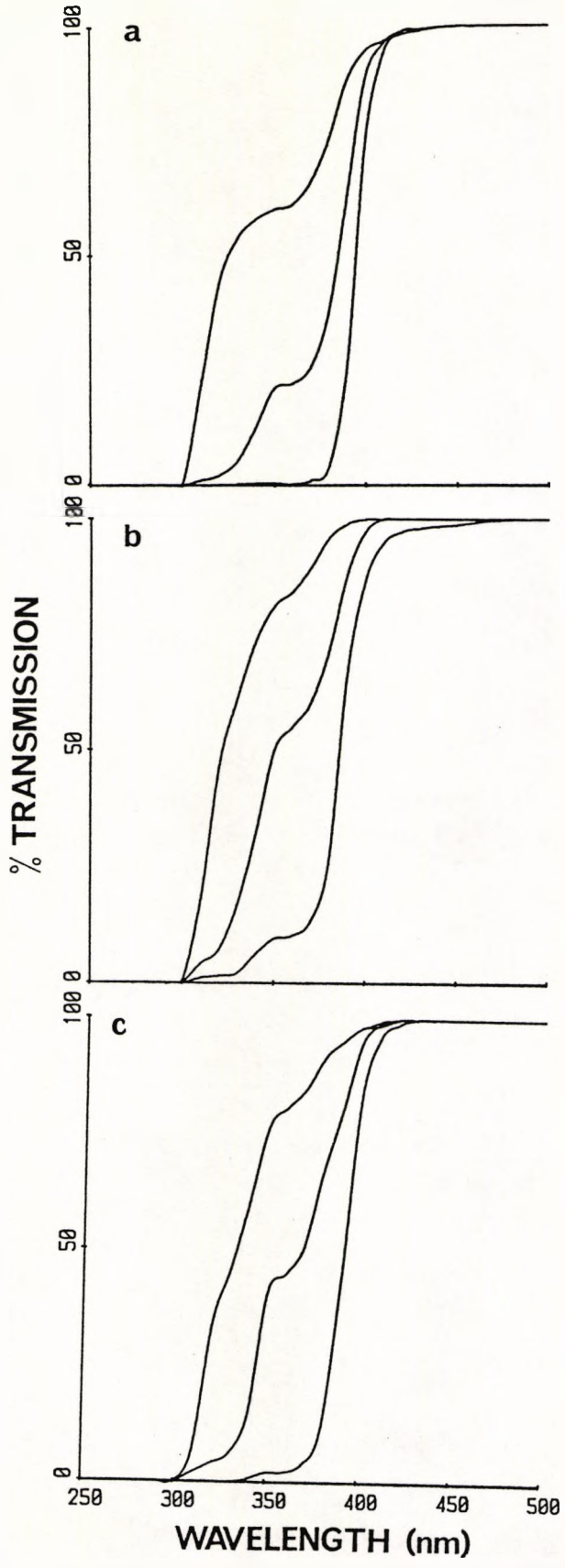


Figure 4-18. Lens spectral transmission of three *Haplochromis* species accumulating lens pigments with age. (a) *Haplochromis ishmaeli*, with lens diameters 0.98mm, 1.69mm and 3.04mm left to right. (b) *Haplochromis xenognathus*, with lens diameters 1.41mm, 1.49mm and 3.41mm. (c) *Haplochromis sauvagei*, with lens diameters 1.5mm, 2.44mm, 3.57mm.

The substantial increase with age in the lens 50% transmission wavelength of three *Haplochromis* species (Fig. 4-19) is due to the increasing concentration of short-wave absorbing pigments. In *Haplochromis argens* the increase in 50% transmission with age fitted the theoretical model based on increasing lens size alone, demonstrated by the closeness of regression and theoretical lines in Fig. 4-19. Older animals from all three *Haplochromis* species with lens pigments displayed a levelling in the wavelength of 50% transmission (Fig. 4-19).

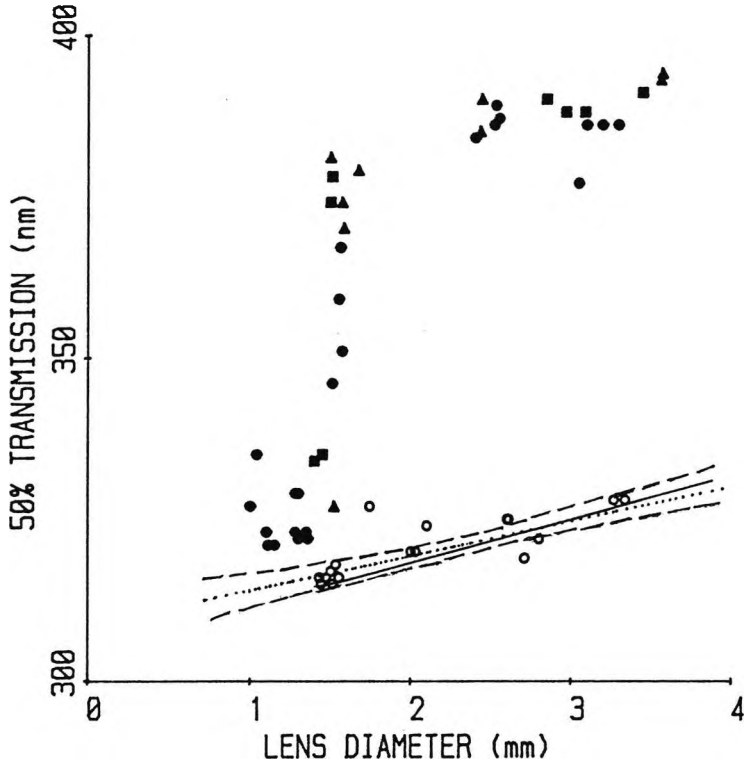


Figure 4-19. Wavelength of lens 50% transmission as a function of the lens diameter in *Haplochromis ishmaeli* (filled circles), *H. xenognathus* (squares), *H. sauvagei* (triangles) and *H. argens* (open circles). The solid line represents the theoretical increase in the lens 50% transmission wavelength with age if this were based solely on increasing lens size. The dotted line is the regression for *H. argens* lenses with 95% confidence limits (dashed lines).

4.2.3.2 Tilapia

A range of different sized tilapia (*Oreochromis niloticus*) supplied by Dr. L.G. Ross from the Institute of Aquaculture, University of Stirling, were split into 5 groups on the basis of lens diameter. There was a marked reduction in the spectral range transmitted by the lenses of older fish (Figs. 4-20 & 4-21). The data suggest that the shift in transmission to longer wavelengths, which is quite marked initially, eventually slows until the 50% transmission stops increasing despite further increases in both lens diameter and fish standard length.

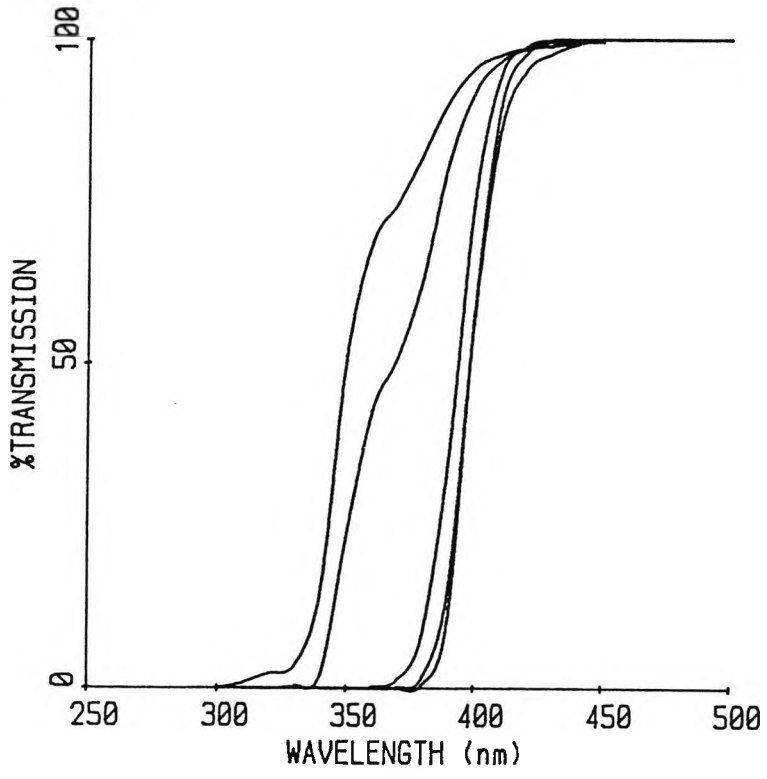


Figure 4-20. Averaged lens spectral transmission from five age groups of *Oreochromis niloticus*. The mean lens diameter for each of the groups increases from left to right, 1.03mm, 1.69mm, 3.63mm, 4.62mm and 6.58mm.

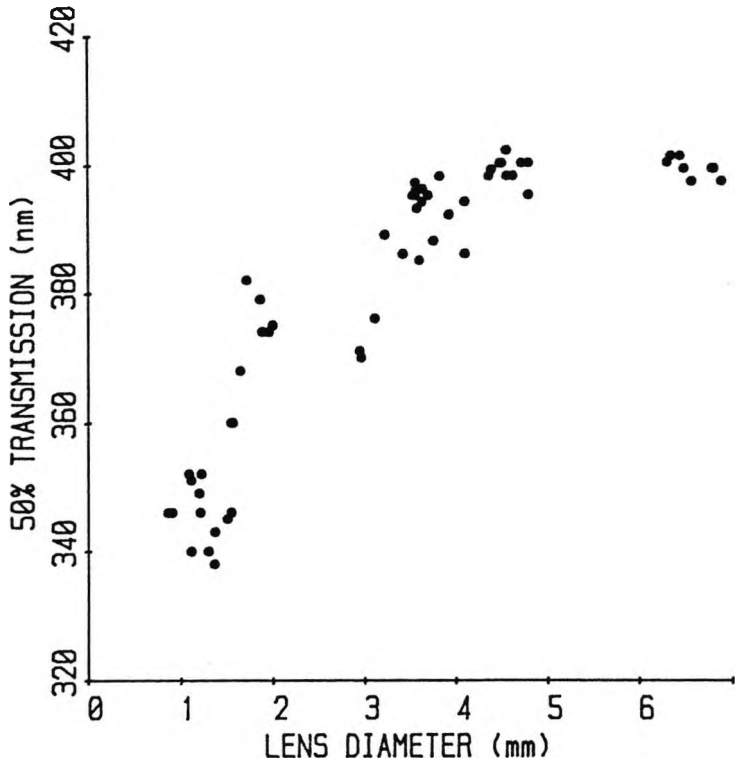


Figure 4-21. Lens 50% transmission values as a function of the lens diameter for all *Oreochromis niloticus* lenses.

The same short-wave absorbing pigments were present in lenses of all five age groups of tilapia studied (Fig. 4-22). HPLC analysis showed that the pigments were the mycosporine-like amino acids, palythine and palythene (Table 3.3). Concentrations of both pigments increased with lens diameter, reaching a maximum in lenses averaging 3.63mm diameter, after which the concentrations of both pigments started to fall (Fig. 4-23).

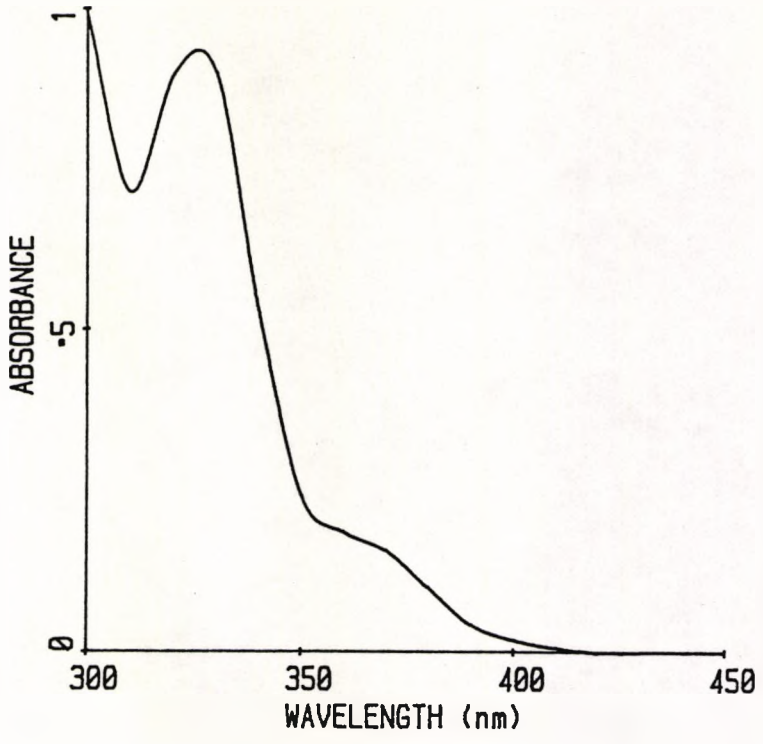


Figure 4-22. Spectral absorbance of the pigment extracted from 8 lenses from large *Oreochromis niloticus* (mean lens diameter 6.58mm). All ages of fish had the same pigments present in the lens.

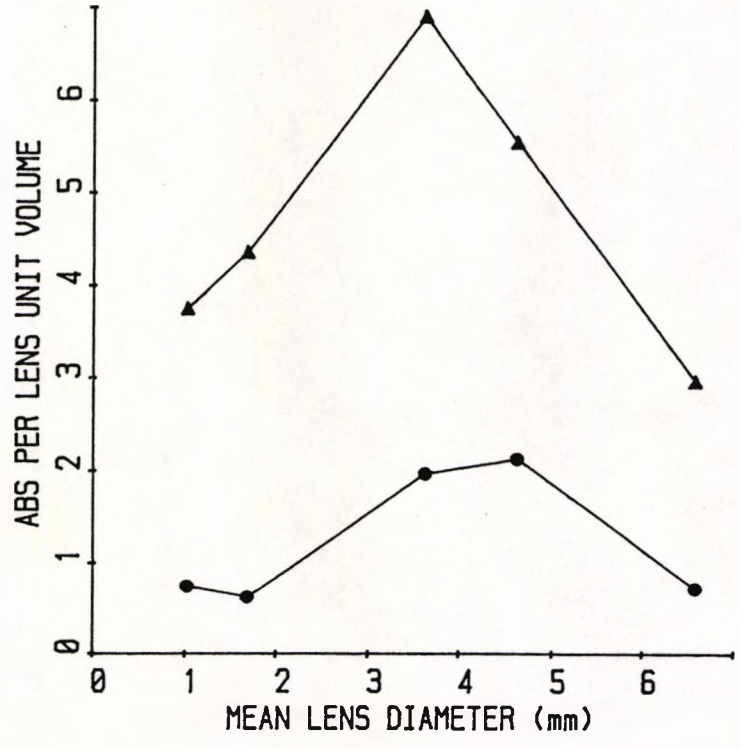


Figure 4-23. Absorbance of both the l_{max} 320nm (triangles) and 360nm (circles) pigments extracted from the five age groups of *Oreochromis niloticus*. Data were corrected for lens volume in each of the groups.

4.2.3.3 *Stylephorus chordatus*

Ageing trends were also observed in lenses containing pigments unrelated to the mycosporine-like compounds. Transmission spectra of *Stylephorus chordatus* lenses, which contain kynurenine (see Chapter 3), were also found to shift to longer wavelengths with age (Fig. 4-24). Only five fish were available, but despite this, it is obvious that the youngest fish lens contained appreciably less kynurenine. 50% transmission in lenses of the oldest fish appear to level off around 410nm, when a slowing down in pigment deposition appears to occur (Fig. 4-25) in a manner analogous to older tilapia lenses.

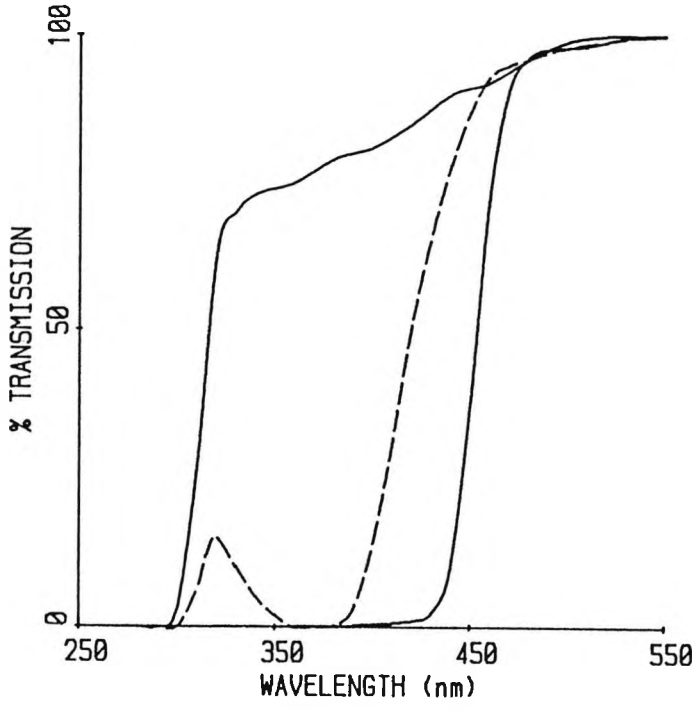


Figure 4-24. Spectral transmission of three *Stylephorus chordatus* lenses with increasing lens diameters, left to right, of 1.10mm, 1.96mm and 5.35mm.

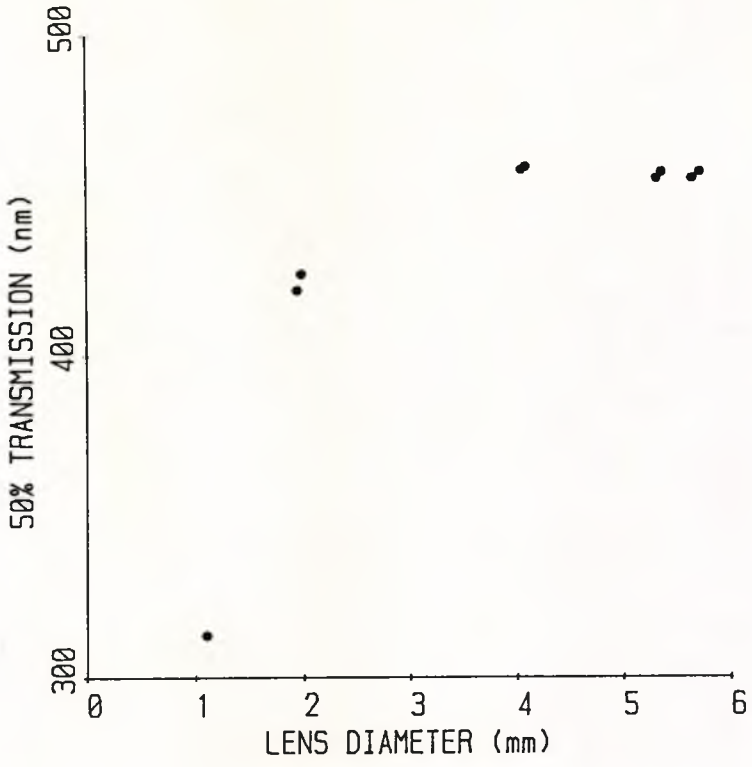


Figure 4-25. Lens 50% transmission wavelength expressed as a function of the lens diameter in all five specimens of *Stylephorus chordatus* examined.

4.2.4 INITIAL INCREASE AND LATER REDUCTION IN LENS PIGMENT CONCENTRATION WITH AGE

4.2.4.1 Pollack

Lens spectral transmission in pollack (*Pollachius pollachius*) initially shifted slightly to longer wavelengths with age (Fig. 4-26). However, lenses from older fish showed a shift in spectral transmission towards shorter wavelengths with the largest lenses displaying the lowest wavelengths of 50% transmission (Figs. 4-26 & 4-27).

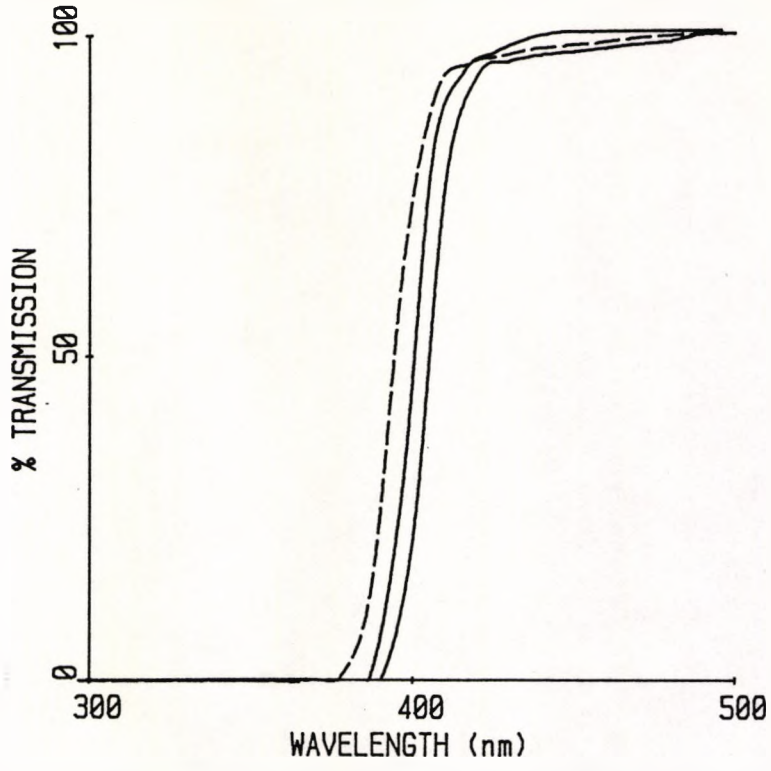


Figure 4-26. Spectral transmission of lenses of *Pollachius pollachius*. The solid lines represent lenses with diameters of 5.69mm and 8.24mm left to right, and the dashed line represents a 10.13mm diameter lens.

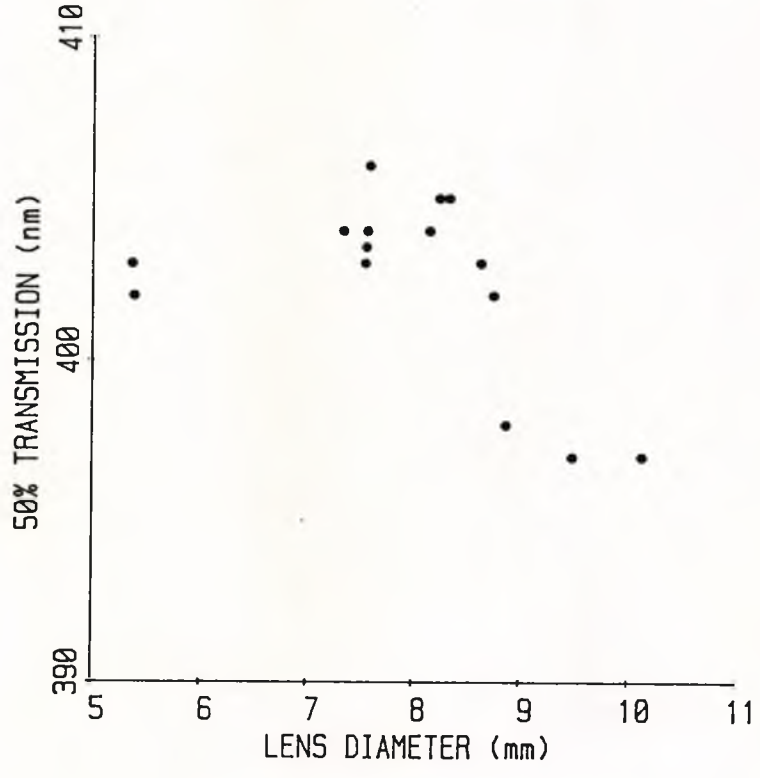


Figure 4-27. Lens 50% transmission wavelength as a function of the lens diameter for all *Pollachius pollachius* lenses examined.

4.2.4.2 Flying fish

A similar effect was observed in the flying fish (*Exocoetus obtusirostris*). Initially, in young lenses, there was a rise in the wavelength of 50% transmission with increasing lens diameter, but lenses from older individuals displayed a slight reduction in the short-wave transmission despite lens diameters of more than double those of the younger animals (Figs. 4-28 & 4-29). Lens pigment was extracted from four age groups of flying fish, and although unidentified (see Chapter 3), was found to decrease in concentration with age (Fig. 4-30). A shift in the pigment l_{max} to longer wavelengths was also observed with age (Fig. 4-30).

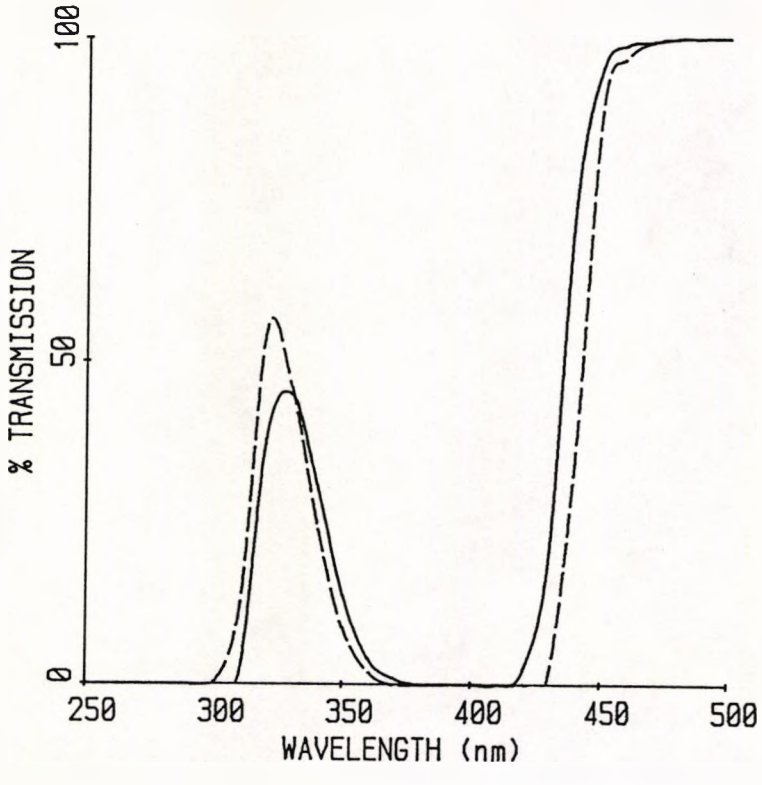


Figure 4-28. Spectral transmission of two *Exocoetus obtusirostris* lenses, 2.32mm (dashed line) and 5.15mm (solid line) in diameter.

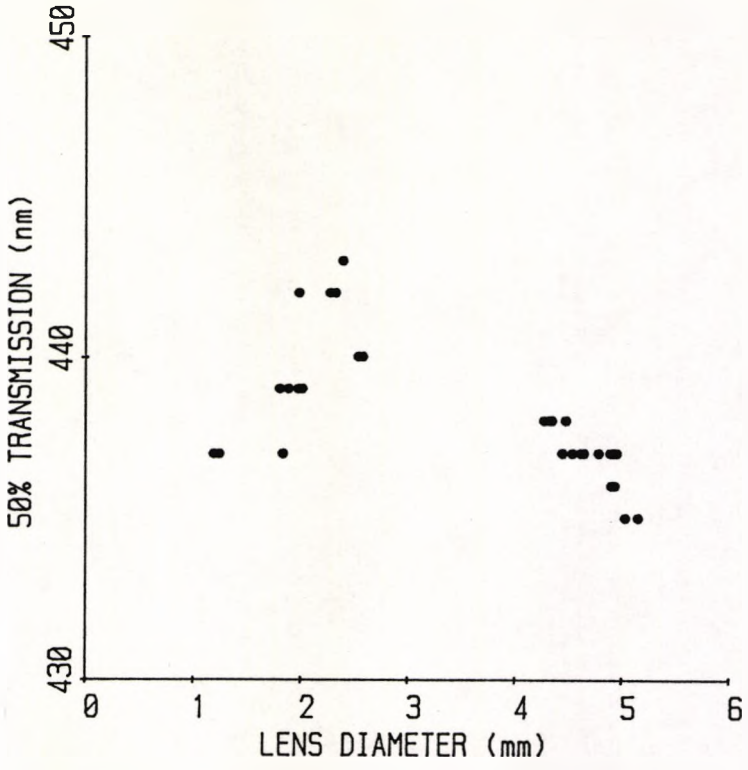


Figure 4-29. Lens 50% transmission wavelength as a function of the lens diameter for all *Exocoetus obtusirostris* lenses examined.

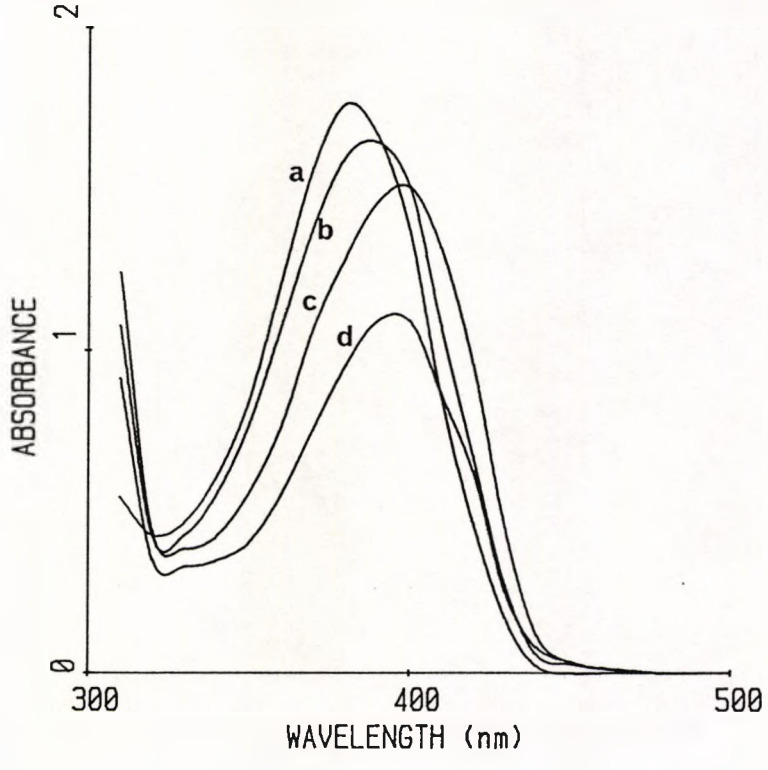


Figure 4-30. Spectral absorbance of the lens extracts from four groups of *Exocoetus obtusirostris* of different sizes. With average lens diameters of (a) 1.72mm, (b) 2.53mm, (c) 4.37mm and (d) 4.74mm. Values were corrected for differences in lens volume.

4.2.5 DECREASE IN LENS PIGMENT CONCENTRATION WITH AGE

4.2.5.1 Kissing gourami

Unlike other species examined, lens transmission in the kissing gourami (*Helostoma temmincki*) appeared to increase, not decrease, at shorter wavelengths with increasing age (Fig. 4-31), resulting in a fall in the wavelength of 50% transmission in the lenses of older fish, despite the increasing lens diameter (Fig. 4-32). Pigment extraction from both groups of lens revealed a higher pigment concentration in lenses of young fish compared to older fish (Fig. 4-33).

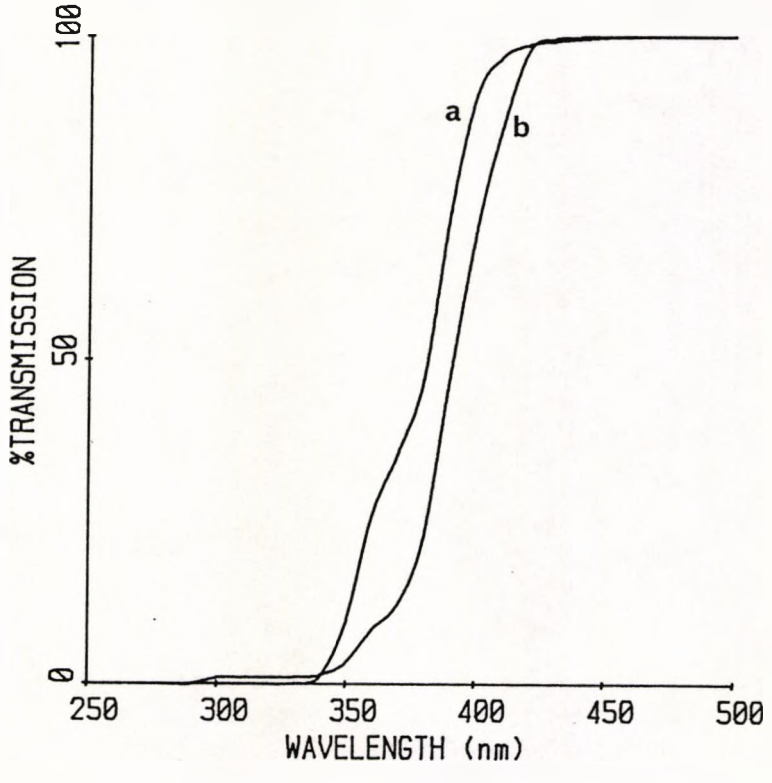


Figure 4-31. Spectral transmission of lenses from two *Helostoma temmincki* with lens diameters of (a) 3.13mm and (b) 1.83mm.

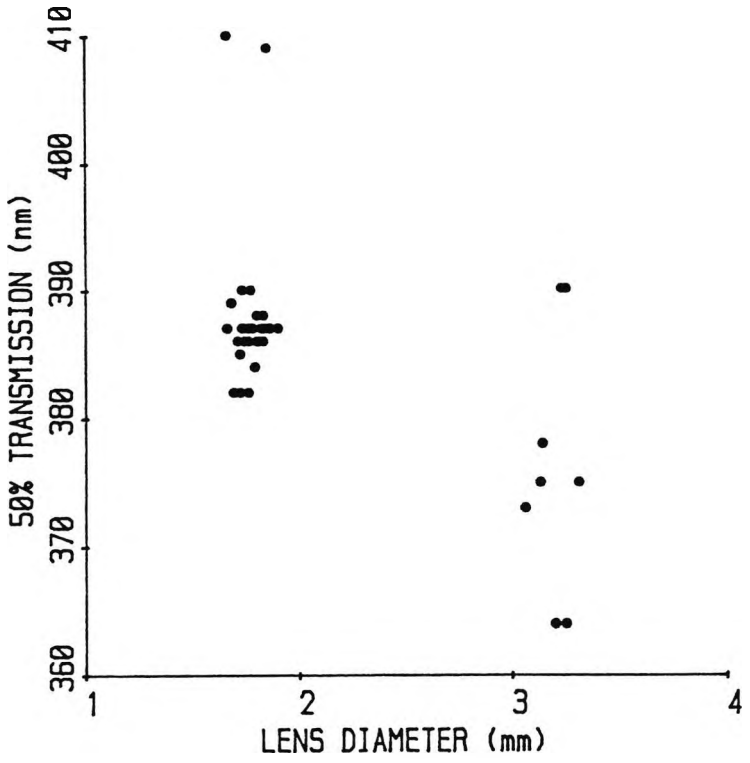


Figure 4-32. 50% transmission wavelength as a function of the lens diameter for all *Helostoma temmincki* lenses examined.

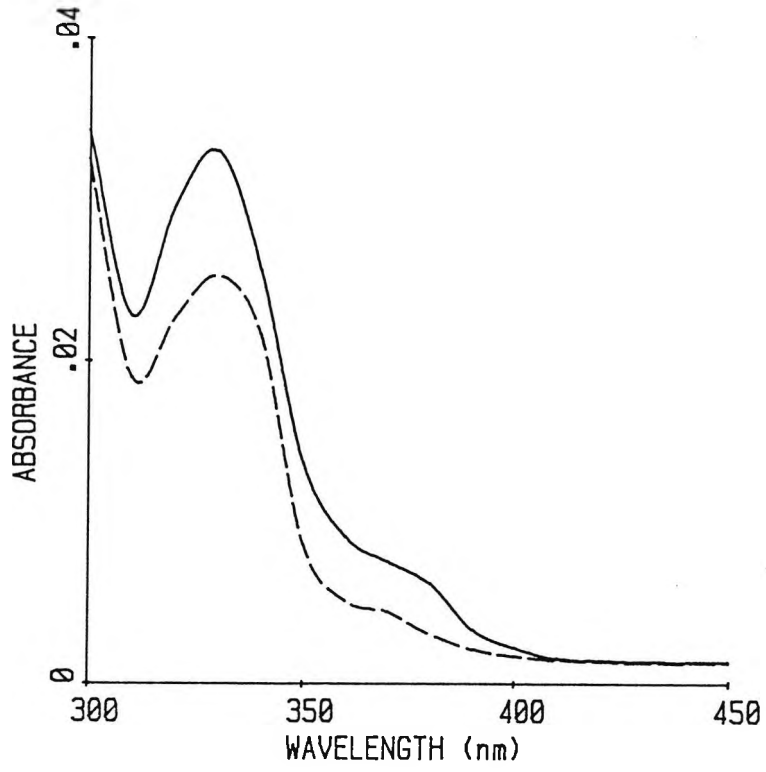


Figure 4-33. Absorbance spectra of lens pigment extracts in $1cm^3$ of water from large (dashed line) and small (solid line) *Helostoma temmincki*, corrected for differences in lens volume.

4.2.5.2 Blue acara

Lenses of the blue acara (*Aequidens pulcher*) were not scanned, but younger fish had a higher concentration of extractable pigment than lenses of older fish which were also a paler yellow in colour (Fig. 4-34).

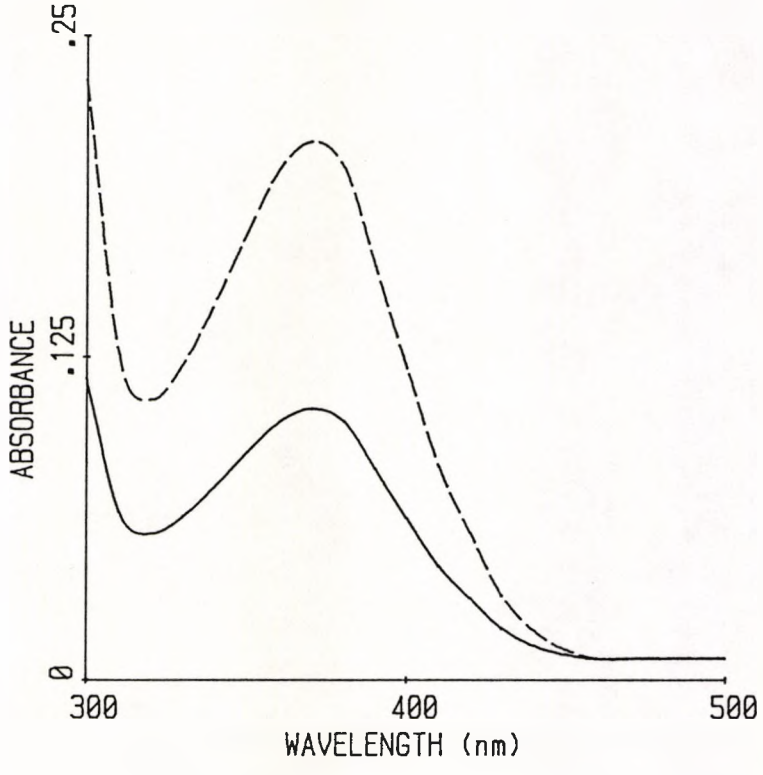


Figure 4-34. Spectral absorbance of lens pigment extracts from small (dashed line) and large (solid line) *Aequidens pulcher* in $1cm^3$ water. Values were corrected for differences in total lens volume.

4.3 DISCUSSION

4.3.1 WHOLE LENS SPECTRAL TRANSMISSION CHANGES WITH AGE

The above transmission data show that a number of things may occur in the lens with age. The lens can either simply increase in size without accumulating lens pigments, or the concentration or type of pigments present in a lens can change with age. When pigments are present in a lens, ageing can be highly variable. Species such as the long rough dab (Fig. 4-10), increase the lens pigment concentration with age, while others, for example the kissing gourami (Fig. 4-32), display a drop in concentration of pigment with age and several other species such as tilapia (Fig. 4-23), show an initial rise and then a drop in pigment concentration. Additionally, new pigments may be produced in a lens with age and these too may then increase in concentration, as occurs in the herring (Fig. 4-16).

Age-related changes in the spectral transmission of lenses without pigment are easily understood. All species with no pigment in the lens show small shifts in 50% transmission with increasing age (Figs. 4-1 to 4-4). The increasing diameters result in longer pathlengths through the lens and therefore increased absorbance by lens proteins. The increasing absorbance is proportional to increases in the lens diameter and the 50% transmission data therefore fit the theoretical lines based on lens growth alone (e.g. Fig. 4-2).

Pigment changes, whilst appearing initially rather confusing, can all be fitted into a common framework (Fig. 4-35). This assumes that the pigment composition of the lens does not change, that the pigments are freely diffusible throughout the lens, and that lenses continually increase in size with age.

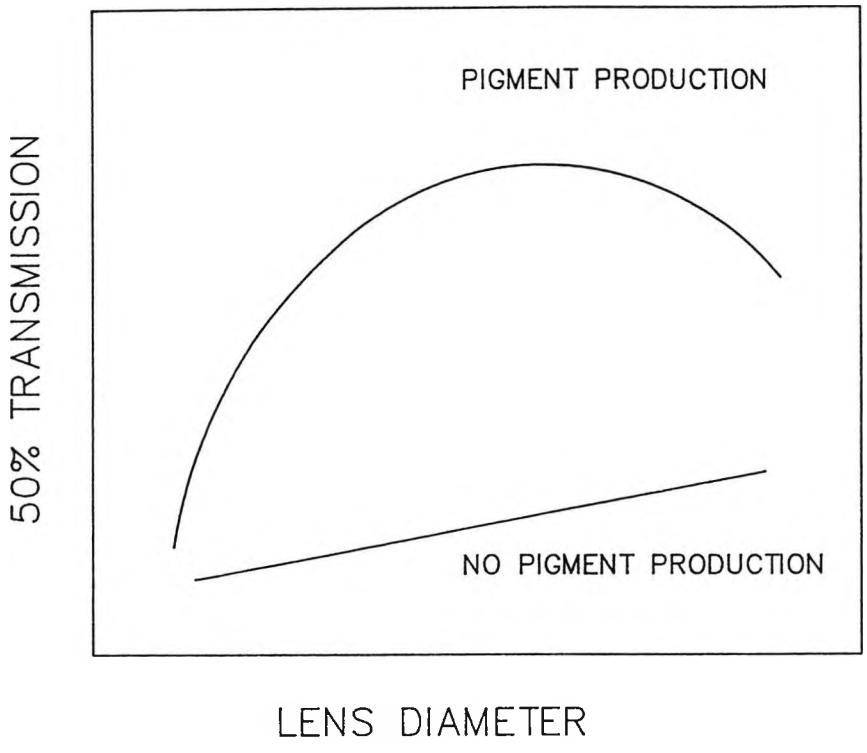


Figure 4-35. Plan of the age-related changes in the lens 50% transmission wavelength in fish. Lenses of some species do not become pigmented with age and fit the lower line, whereas many fish lenses do accumulate short-wave absorbing pigments and can be explained by the curve above (for explanations of a,b & c see text)

Ageing in pigmented lenses can be split into three phases according to the proposed model. The first phase represents relatively rapid pigment accumulation which exceeds the increase in lens volume and results in a sharp increase in the wavelength of 50% transmission (Fig. 4-35a).

In the second phase, pigment accumulation in the lens slows down. However, the lens volume continues to increase with age and both the pathlength through the lens and therefore also protein absorbance increases. This means that lens 50% transmission remains at a constant level for a time, despite the fall in the rate of pigment accumulation (Fig. 4-35b). The third and final phase occurs after lens pigment accumulation has ceased. Further increases in lens volume will effectively 'dilute' the pigment and the concentration will consequently fall, resulting in a drop in 50% transmission wavelength (Fig. 4-35c). It is possible that different species go through each of these three phases at different rates and at different points in their life cycles.

Lens ageing in the long rough dab (Fig. 4-10) can be explained on the basis of phase a alone. Lenses of *Stylephorus chordatus*, tilapia and pigmented *Haplochromis* species show both phases a and b (Figs. 4-25, 4-21 & 4-19). The levelling of the 50% transmission wavelength in larger lenses of these species represents the slowing down and eventual stop in pigment accumulation in the lens. It may be that if older individuals had been examined, they would have shown the phase c - that of decreasing lens 50% transmission (Fig. 4-35c). All three phases may be represented in carp. Although carp were from separate populations, the 50% transmission of pigmented lenses display the initial sharp increase, the levelling as pigment accumulation stops, and the later fall in the wavelength of 50% transmission in the oldest individuals (Fig. 4-36).

Growth rates in different populations can be quite different, but Douglas (1987) has shown that lens diameter is a better indicator of age than body length. Carp with large lens diameters are therefore likely to be older than those with smaller lenses.

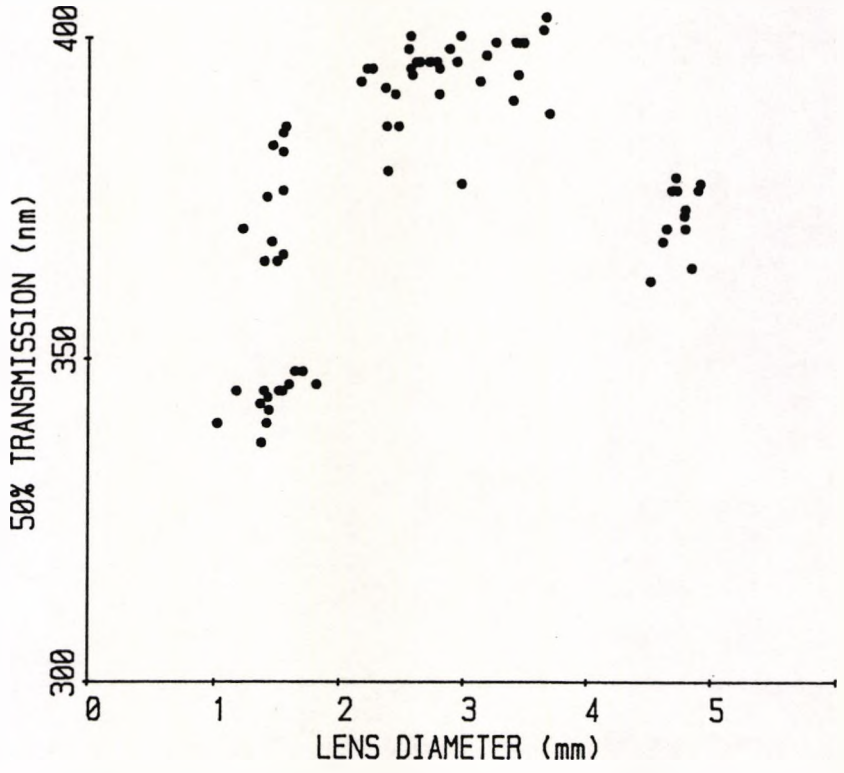


Figure 4-36. Lens 50% transmission as a function of the lens diameter for all pigmented lenses of *Cyprinus carpio*.

Unlike the majority of species, lenses of very young blue acara and kissing gourami had high pigment concentrations (Figs. 4-34 & 4-33) and these species are therefore probably born with pigmented lenses. Accumulation of pigments, phases a and b, must commence during embryonic development. After hatching, lenses become less pigmented and show the phase c of the general ageing plan (Fig. 4-35), with a reduction in the wavelength of lens 50% transmission, even though the lens diameter continues to increase.

It is not clear why so many species of fish display lens ageing processes, often at quite different stages in the life cycle, or even why some species produce no lens pigments at all. Such a wide variation may be attributable to a number of factors which will now be considered separately.

4.3.2 FACTORS AFFECTING LENS AGEING

Many factors could cause the observed changes in fish lens transmission seen with age. UV radiation exposure and diet in particular, have been implicated as causative factors [Zigman, 1971]. Alternatively, it may be that the predisposition of some species for high levels of short-wave absorbing compounds in the lens is purely a genetic factor.

Although lenses containing short-wave absorbing compounds are generally to be found in animals inhabiting high intensity light environments (see Chapter 3), it is clear that UV is not required for pigment formation in many cases. Deep-sea fish for example, are almost certainly never exposed to significant amounts of UV, yet a large number have now been identified as possessing lenses with high levels of short-wave absorbing compounds [Denton, 1956; Somiya & Tamura, 1971; Muntz, 1976b; Somiya, 1976, 1977, 1979, 1982; McFall-Ngai *et al.*, 1986 & 1988; Douglas & Thorpe, in press]. Direct effects of UV on the mycosporine pigments found in fish lenses have not been widely investigated, but are examined in the next chapter.

4.3.2.1 Genetic factors

Occasionally, there are two quite distinct ways in which lenses of individuals of the same species may age. Such intraspecific variation in lens pigment constitution and concentration seems to be a feature of both carp and goldfish. Some carp and goldfish lenses do not accumulate lens pigments with age whilst other lenses become increasingly pigmented as they increase in size (Figs. 4-7 & 4-12). Both these species have been highly inbred for centuries to produce the wide range of varieties available today, which could conceivably have had an effect on the genetic processes of pigment accumulation in certain strains.

The fact that lenses of individuals within other species age in very similar ways may also suggest some genetic control. In tilapia, for example, all the older fish lenses had 50% transmission which appeared to plateau, none of the lenses displayed any further increase in pigment concentration (Fig. 4-21). The passage of pigments or pigment precursors into the lens may be regulated by a certain hormone or other compound. If tilapia were to alter this regulatory process at a certain point in their life cycle, then you might expect all lenses to simultaneously cease pigment accumulation upon reaching this stage. Since all fish were reared indoors on the same food, it is unlikely that differences in diet play any part in this case.

4.3.2.2 Diet

Effects of diet on the lens pigment composition should be considered since lens pigments are probably dietary in origin, as are the carotenoids found in some deep-sea fish lenses [McFall-Ngai *et al.*, 1986; Douglas & Thorpe, in press]. The introduction of a new pigment with increasing age could be due to a change in diet enforced by a change in habitat. The migration of fry to deeper water is a common phenomenon in the life-cycle of many marine fish [Hawrhyshyn & Beauchamp, 1985]. The young feed on planktonic organisms at the water surface, whereas older fish are commonly found at greater depths where the selection of prey may be quite different. A change in diet could plausibly result either in the termination or initiation of pigment accumulation depending on the composition of the new food source. The wide variation in the degree of lens pigment concentration in the different populations of carp could possibly be explained by this: fish from separate sources are likely to have had quite different diets.

4.3.2.3 Light

The lens ageing described in this section is unlikely to be related to the patches of yellow pigmentation developing in the lenses of rudd [Villermet & Weale, 1972] and sharks [Zigman & Gilbert, 1978] with age. There is some evidence that such deep yellow-brown pigmentation may be caused by modification to protein residues upon exposure to sunlight [Zigman, 1971; Zigman & Gilbert, 1978]. Lens pigments, such as the mycosporines and kynurenine

derivatives, could not form a patch of colouration unless they bind to larger proteins, since they appear to be both freely diffusible and water soluble. There could therefore be another way in which fish lens spectral transmission may change with age, involving photo-oxidation of lens proteins (as is thought to occur in the older human lens [Zigman, 1983a]). None of the animals examined in this study had any sign of anterior patches of pigmentation within the lens, but both the earlier studies examined animals kept in captivity, perhaps in shallow tanks where the sunlight reaching the animals would be greater than normally experienced in the wild (see Fig. 1-2).

It is likely that UV-initiated colouration of the lens does not occur when short-wave absorbing compounds are present. Mycosporine-like amino acids, when present in a lens at high concentrations, absorb UV more efficiently than the lens proteins (the extinction coefficient of tryptophan at 280nm is $6310M^{-1}cm^{-1}$ [Weast, 1975], falling sharply towards longer wavelengths, whilst that for palythine in the fish lens is $36,200M^{-1}cm^{-1}$ at 320nm [Takano *et al.*, 1978a]). This would serve to protect those protein residues susceptible to photo-oxidative attack and prevent lens yellowing, in a manner equivalent to the role played by 3-hydroxykynurenine glucoside in the human lens for the first few years of life [Dillon & Atherton, 1990]. In support of this, lenses of the rudd and dogfish, species in which deep-yellow lens colouration was observed [Villermet & Weale, 1972; Zigman & Gilbert, 1978] possibly due to exposure to sunlight, do not contain specific short-wave absorbing

compounds [rudd: Whitmore & Bowmaker, 1989; dogfish: Table 3.1].

Although effects of UV on mycosporine-like pigments have not been widely investigated, coral polyps exposed to high levels of UV have accumulated higher concentrations of protective mycosporine pigments than members of the same species living at greater depths and hence lower UV levels [Dunlap *et al.*, 1986]. Lifelong cumulative UV exposure would perhaps explain increased pigment accumulation in the lenses of some species with age. Directly opposed to this idea however is the fact that numerous animals show phase 3 of the model discussed earlier (Fig. 4-35), where pigment levels in the lens fall with age.

If UV radiation was a major causative effect in lens ageing one might expect animals raised indoors, where the UV component is low, to show little change in lens transmission with age. Tilapia were reared indoors, yet older animals clearly had higher lens pigment concentrations than younger ones, only in the oldest fish did the pigment concentration start to fall (Fig. 4-23). However, lenses of the blue acara, also reared indoors where the UV component of light is low, did show a drop in pigment concentration with age (Fig. 4-34). Furthermore, lenses of herring reared in the laboratory had different <u>relative</u> pigment concentrations (although absolute levels were similar) compared to herring of the same age caught in the wild (Fig. 4-16). Evidence up to now therefore appears contradictory and is investigated further in the next chapter.

4.3.3 CHANGES IN LENS PIGMENT COMPOSITION WITH AGE

Most species examined here had lenses which contain the same pigments throughout the animal's life. Age-related changes in lens transmission in such species relate only to variations in pigment concentration (e.g. tilapia, Fig. 4-23 and blue acara, Fig. 4-34). Some species however, such as the herring and flying fish, accumulate new pigments in the lens with age. Young herring have a high concentration of palythene in the lens as early as metamorphosis and are therefore probably born with pigmented lenses (Fig. 4-16). Older herring were found to have a higher concentration of palythene and in addition, a high concentration of a new pigment, palythine.

Little is known about the chemistry of mycosporine compounds and even their origins are uncertain [Takano *et al.*, 1978a & 1978b]. But it is possible that oxidation/reduction processes could lead to conversions of one pigment to another. Kennedy and Milkman (1956) and also van Heyningen and Linklater (1976) reported the unstable nature of the 360nm pigment from the fish lens and its conversion into a λ_{max} 320nm pigment. The 360nm pigment they describe is likely to be palythene which was shown earlier to be unstable, forming palythine upon lyophilisation (Fig. 3-30, see also Section 5.3.3). It is possible that something similar could occur to lens pigments *in vivo*, perhaps as a result of photooxidation. It may therefore be that fish exposed to lower levels of UV do not show these pigment inter-conversions.

The lens pigment in flying fish, in addition to falling in concentration with age, also appears to increase its λ_{max} to slightly longer wavelengths (Fig. 4-30). There appears to be some spectral evidence for the appearance of a second pigment, absorbing at slightly longer wavelengths in the oldest lenses (Fig. 4-30d), with a slight bump on the downward slope of the absorbance peak. However, the purified lens pigment from HPLC initially revealed the presence of only one compound in the filtered extract (Fig. 3-20), with a λ_{max} of 385nm (Fig. 3-21, curve 4). The additional lens pigment produced with age may therefore be bound to a low molecular weight lens protein, such as the δ-crystallins. Accumulation of a second pigment with a longer λ_{max} than the pigment already present would cause the observed shift in the overall λ_{max} of the crude lens extract (Fig. 4-30).

Therefore, while it is obvious that age has a marked effect on lens transmission, it may be that other environmental facts are important too. Changes in lens pigmentation with age may be due to a number of environmental and genetic factors. To investigate the effects of different diets in such a large number of fish species would be very time consuming and difficult, therefore investigations into other possible influences on lens pigmentation were pursued. The effects of different lighting conditions on fish lens pigments have not been fully investigated and the next chapter deals with an attempt to 'age' fish lenses *in vivo* by raising animals under various levels of illumination.

CHAPTER FIVE

EFFECTS OF DIFFERENT LIGHTING CONDITIONS ON THE TISSUES OF THE FISH EYE

5.1 INTRODUCTION

The age dependent variation of lens transmission described in the last section may be, at least in part, due to some effect of the environment on the pigments in the lens. The wide range in lens transmission observed in carp (Fig. 4-12) and also the differences in pigment concentration between herring reared in the laboratory or caught in the wild (Fig. 4-16), could be explained in a number of ways. Different populations of both herring and carp will have had quite different exposure to light, food sources, and maybe other physiological factors such as pH and temperature. Of these, effects of the lighting environment were investigated further whilst controlling other environmental factors.

It is clear that fish living in high light environments often have more highly pigmented lenses than nocturnal species or those living in lower light intensities [Chapter 3; Denton, 1956; Douglas & McGuigan, 1989]. It might therefore be that the high pigment concentrations in lenses of these species are a direct result of exposure to high levels of illumination.

Lifelong exposure to UV radiation in sunlight has been implicated as a cause of the yellowing of human lenses with age [Grover & Zigman, 1972; Lerman, 1980a] and more especially age-related cataract [Zigman, 1983b for review] via different pathways [Lerman, 1980a]. In support of this, *in vivo* exposure to high doses of near UV (300-400nm) has been shown to cause reduced transmission and cataract in a number of animals [Zigman *et al.*, 1974, 1988 & 1991; Zigman & Vaughan, 1974; Yu *et al.*, 1990] including man [Zigman, 1983b for review; Lerman, 1980b].

Many researchers have examined UV effects further *in vitro*, finding that high intensity UV irradiation leads to the production of free radicals and photoexcited states within the lens [Yamanashi *et al.*, 1979; Zigler & Goosey, 1981; Andley & Clark, 1989; Lerman *et al.*, 1991]; these may eventually lead to the protein cross-links, increased coloration and reduced transmission described for a number of species [van Heyningen, 1973c; Dillon *et al.*, 1976 & 1989; Zigman *et al.*, 1991]. In marked contrast to the vast literature on UV effects on the rodent, squirrel and human lens, little is known about analogous mechanisms in the lenses of fish. However, Zigman (1971) found that dogfish lenses incubated under UV illumination in a tryptophan rich medium became yellow or brown as reported for the human lens [Grover & Zigman, 1972; van Heyningen, 1973c].

Earlier work revealed that the l_{max} 360nm pigment from the fish lens degrades in solution to form a l_{max} 320nm pigment [Kennedy & Milkman, 1956; Bon *et al.*, 1968; van Heyningen & Linklater, 1976]. These compounds are likely to

be palythene (l_{max} 360nm) and palythine (l_{max} 320nm) since these lens pigments were identified in the lenses of the same species in Chapter 4. The rather unstable nature of HPLC purified palythene was also observed earlier during NMR investigations (Fig. 3-30).

This section investigates the role of the lighting environment in lens pigment formation *in vivo* and also lens pigment conversions *in vitro*. Goldfish and carp were chosen for the lighting experiments since young goldfish in general do not have lens pigments (Fig. 4-8), whereas young carp usually do have significant lens pigment concentrations (Fig. 4-12). To determine if the different lighting levels were physiologically significant, corneal pigmentation, retinal structure and visual pigments were also investigated.

5.2 MATERIALS AND METHODS

Two separate experiments were carried out: in Experiment 1 goldfish were kept in high or low light levels for 11 months, Experiment 2 involved raising carp under high, low or UV illumination for 6 months.

5.2.1 EXPERIMENTAL TANKS

Three tanks were used for the two experiments, a 'high' light tank, a 'low' light tank and a UV tank. The basic tank designs were identical (Fig. 5-1) with dimensions of

35cm x 90cm x 35cm high. They were painted externally, first with white paint to reflect light back within the tank and then with black paint to prevent the entry of stray external light. Experimental tanks were located in a dark room and care was taken not to expose low light fish to any bright illumination during feeding and cleaning.

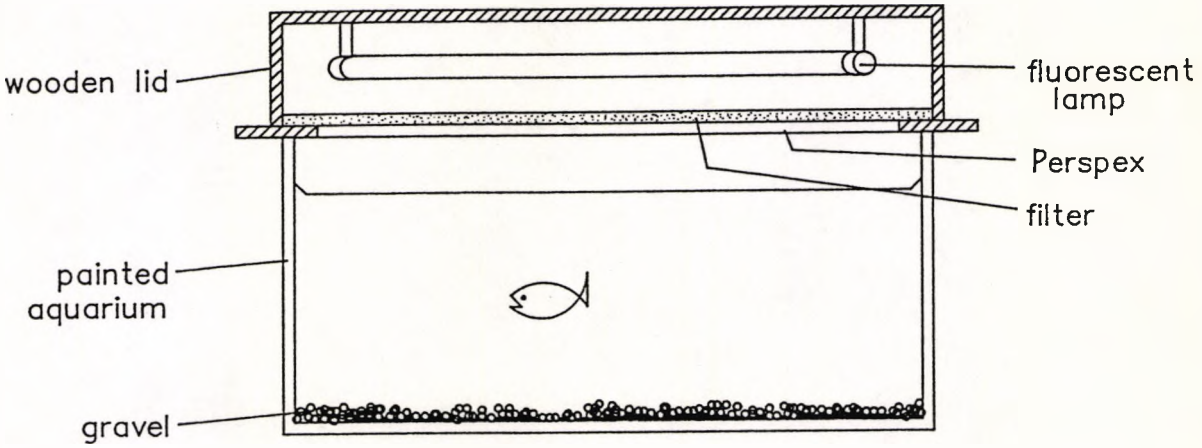


Figure 5-1. Diagram of the experimental tanks used in the lighting experiments. The lid of the high light tank contained two fluorescent lamps with no filter. The low light tank had just one fluorescent lamp and a total of 3.0 log unit neutral density filters. The UV tank lid had two UV tubes with no filter and 2mm thick glass in place of the Perspex.

The low light tank was illuminated with a single fluorescent lamp (Philips TLD 18W/35 White) shielded with a total of 3.0 log unit Lee neutral density filters laid on 5mm thick Perspex in the lid. The high light tank had two fluorescent lamps (with the same specifications as the low light) fitted into the lid with Perspex as in the low light

tank, but without neutral density filters. The UV tank lid was fitted with 2 UV fluorescent strips (Philips Tl 20W/09; maximum spectral output at 360nm). The Perspex in the lid was replaced with 2mm glass which absorbs UV radiation less efficiently. All tank lids were fitted with fans, operative only when the lights came on, to prevent heating effects.

Measurements of minimum and maximum water temperatures in the tanks monitored over a 2 month period established that there were no differences between tanks, with temperatures ranging from 14 to 17^{o}C.

5.2.2 EXPERIMENTAL ANIMALS

Small goldfish, approximately one year old, were purchased from a single population at a local dealer. Twenty five fish were sampled at the start of the experiment and 25 animals were placed in each of the high and low light tanks. Similarly, for the second experiment, a population of one year old carp were purchased and 25 sampled initially. 25 more were placed in each of the high, low and UV tanks used in the second experiment.

5.2.3 LIGHTING MEASUREMENTS

Measurements were made of the wavelengths and intensities of radiation at the water surface in the experimental tanks. Photometric measurements were made at the level of the water surface using a UDT (10AP) detector in the high and low light tanks. A HA3 heat glass was placed over the detector to cut out infra-red radiation. As a rough indication of lighting intensity, values in lux were

measured. Final readings were around 9000lux in the high light tank and 0.9lux in the low light tank, levels roughly equivalent to a noon daylight reading on a cloudless day in the high light tank and a dusk reading in the low light tank.

Radiometric measurements were similarly recorded using a UDT (10DF) detector and a series of interference filters. After corrections for heat glass absorbance and sensor efficiency the readings represented absolute intensities at various wavelengths and gave an indication of the spectral composition of light in the two tanks (Fig. 5-2).

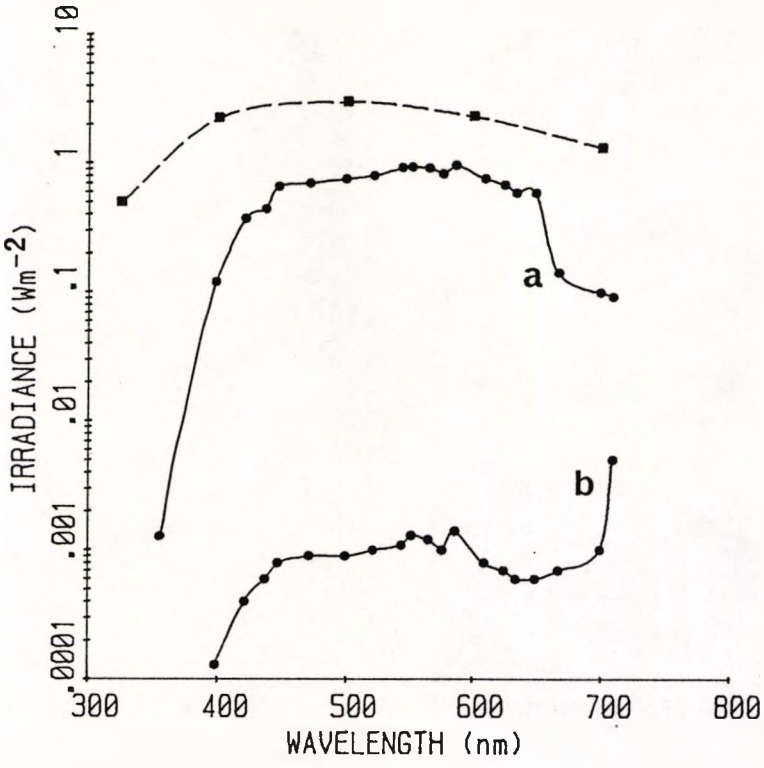


Figure 5-2. Downwelling irradiance at various wavelengths measured at the water surface in the high (a) and low (b) light aquaria. Crosses and dashed line represent natural sunlight levels at noon measured at the sea surface, units: $Wm^{-2}nm^{-1}$ (From: Baker *et al.*, 1982).

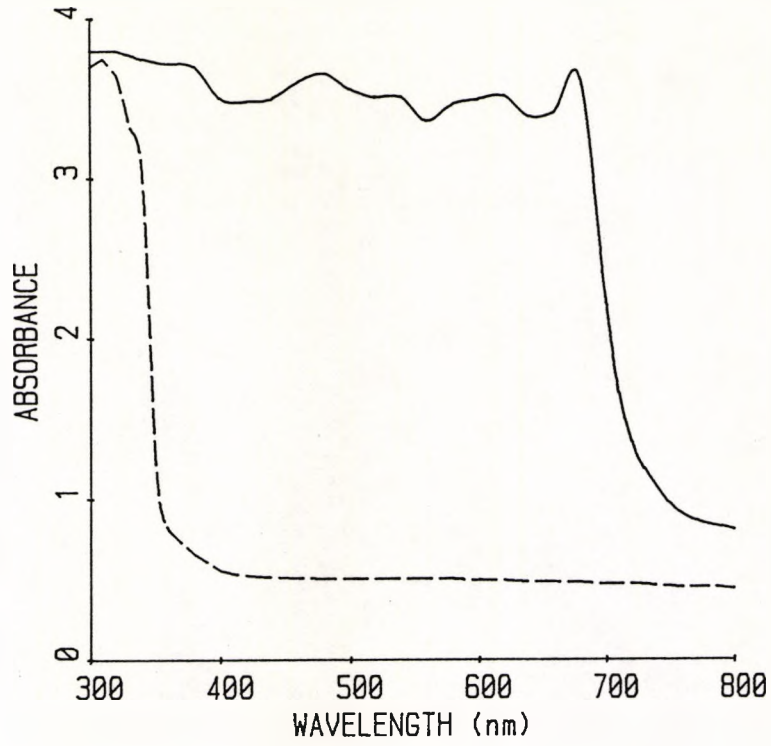


Figure 5-3. Spectral absorbance of the 5mm Perspex fitted into the lid of the high light tank (dashed line), and 5mm Perspex plus 3.0 log unit neutral density filters fitted into the lid of the low light tank (solid line).

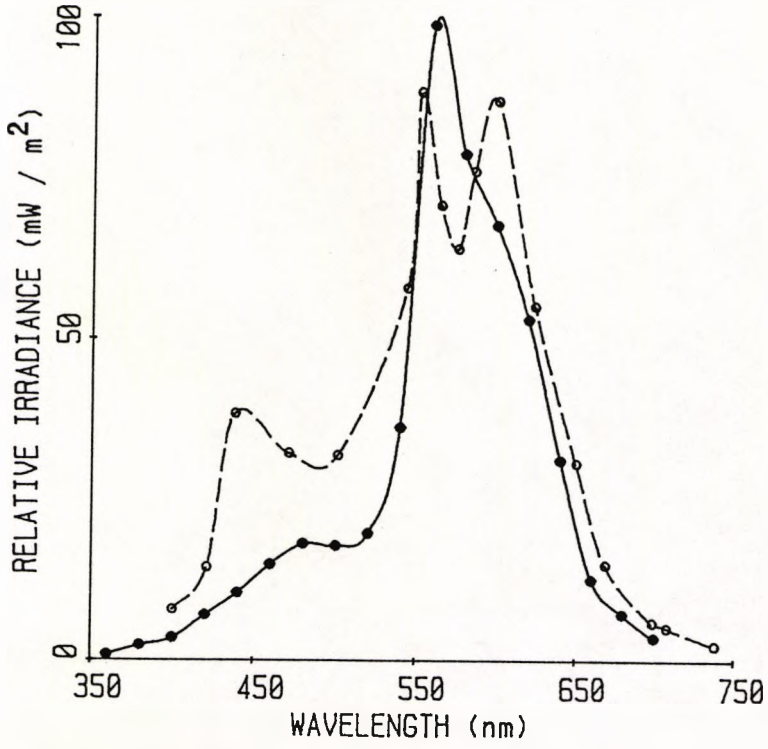


Figure 5-4. Relative spectral content of radiation reaching the water surface in the high light tank. Measured data (dashed line) compare reasonably well to the estimated output from the lamp manufacturer's data after correction for Perspex absorbance (solid line).

Absorbance of the Perspex used in the lids and the neutral density filters in the low light lid was measured over 300-800nm (Fig. 5-3). These enabled verification of the radiometric results when used in conjunction with the manufacturer's data (Philips Lighting Handbook 1986) of the spectral output from the fluorescent lamps (Fig. 5-4).

Unfortunately, a detector capable of measuring in the UV region was not available, so absolute values of the radiation in the UV tank could not be measured. However, the spectral absorbance of the glass used in the UV tank (Fig. 5-5) together with the manufacturer's data (Philips Lighting Handbook, 1986) was used to estimate the spectral content of UV radiation reaching the water surface in the UV tank (Fig. 5-6).

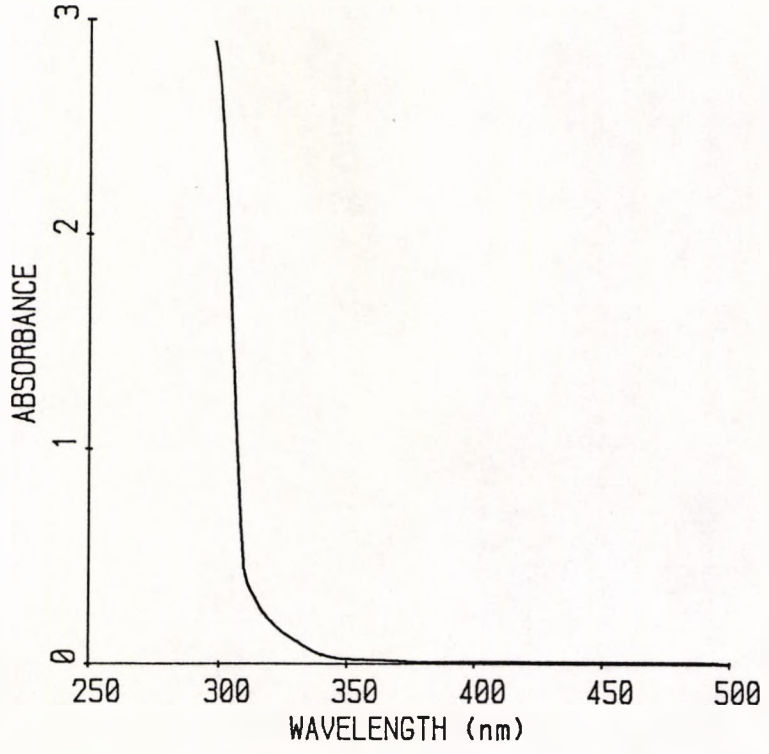


Figure 5-5. Spectral absorbance of the 2mm glass fitted to the lid of the UV tank.

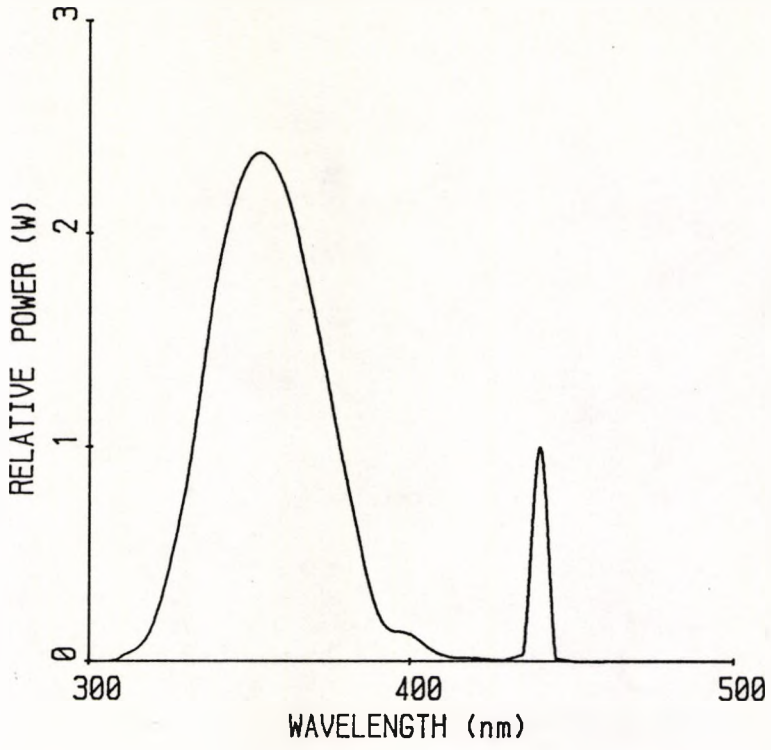


Figure 5-6. Estimate of the spectral content of the output from the UV lamps corrected for the transmission of the 2mm glass fitted in the lid of the UV tanks.

The total output from the UV lamps is highly variable with both age and temperature, but when new, the lamps have an estimated total output of 6.5W (Philips technical data, pers. comm.), making the total from the two lamps around 13W.

5.2.4 LIGHTING AND FEEDING REGIMES

In Experiment 1, high light goldfish were subjected to their elevated light levels for 22 hours per day (22L:2D) and low light fish their subdued levels for only 2 hours per day (2L:22D). Experiment 2 used the same high and low light cycles but also had the UV tank which was on an 18L:6D cycle. All fish were fed identical goldfish pellet food 5 days a

week during the two hours for which the low tank was illuminated.

5.2.5 LENS AND CORNEAL PIGMENT EXTRACTION

All lenses were scanned in the spectrophotometer as detailed earlier (Section 2.2). Lenses of 25 goldfish and carp were measured at the start of the experiment as controls. Pigments from carp lenses were extracted into water as detailed earlier (Section 2.5). Goldfish corneal pigments were extracted initially in water, but since the carotenoid-like pigment they contained was insoluble in water, acetone extracts were also made on the pellet after centrifugation.

5.2.6 MICROSPECTROPHOTOMETRY

Microspectophotometry (MSP) was carried out on photoreceptors from four experimental goldfish, two of which had been reared in the high light environment and two from the low light cycle. Fish were dark-adapted overnight and then killed by cervical transection and pithing. The eyes were enucleated and hemisected under a red safelight. The retinas were removed and one from each fish was placed in ice-cold saline while the other was lightly fixed for 15s in 2% glutaraldehyde. Small pieces of retina, about $1mm^2$ were cut and used for MSP analysis by Dr. J.K. Bowmaker at the Institute of Ophthalmology [Mollon *et al.*, 1984; Bowmaker *et al.*, 1991]. Absorbance spectra were recorded from 750nm to 370nm.

5.2.7 RETINAL HISTOLOGY

Retinas from each of the experimental populations of both goldfish and carp were immersion-fixed in phosphate buffered 2.5% Glutaraldehyde/1% Paraformaldehyde for 2-24 hours. Fixed tissue was then washed three times in phosphate buffer. Retinal pieces were post-fixed in 2% Osmium tetroxide for 1 hour and then rinsed with water. Dehydration through a graded series of ethanol was carried out and tissues were cleaned with xylene and then infiltrated with 1:1 xylene/araldite mix. Tissues were transferred to araldite, agitated overnight at room temperature and finally embedded in fresh araldite which was then baked at 60°C for 48 hours. 1μm retinal sections were cut with glass knives on a Reichert-Jung Ultracut E microtome in radial section. Araldite resin was removed from the sections before staining using sodium methoxide, methoxide diluent and finally acetone. Sections were stained with 1% toluidine blue in 2.5% sodium carbonate.

In order to investigate the presence of any regional differences in receptor density, interrupted serial sections (every 500μm) were made for two complete retinas (from one high and one low light goldfish). The number of rod nuclei and cone ellipsoids were counted from each section at 500μm intervals along the nasal to temporal plane, resulting in a map of rod:cone ratios over the whole retina (see Fig. 5-12). Counts were corrected for the larger 3-dimensional size of the cones using the method of Abercrombie (1946).

1μm radial sections were also cut from the retinas of right eyes of a further 10 high light and 11 low light

goldfish. Sections were taken from the central retina and three counts of rod nuclei and cone ellipsoids made for each retina. Retinas from the right eyes of 11 UV carp, 11 high light carp and 12 low light carp from Experiment 2 were similarly processed and examined. The thickness of the photoreceptor layer, outer nuclear layer and the remainder of the retina was also measured in all the goldfish and carp retinas examined (Fig. 5-7).

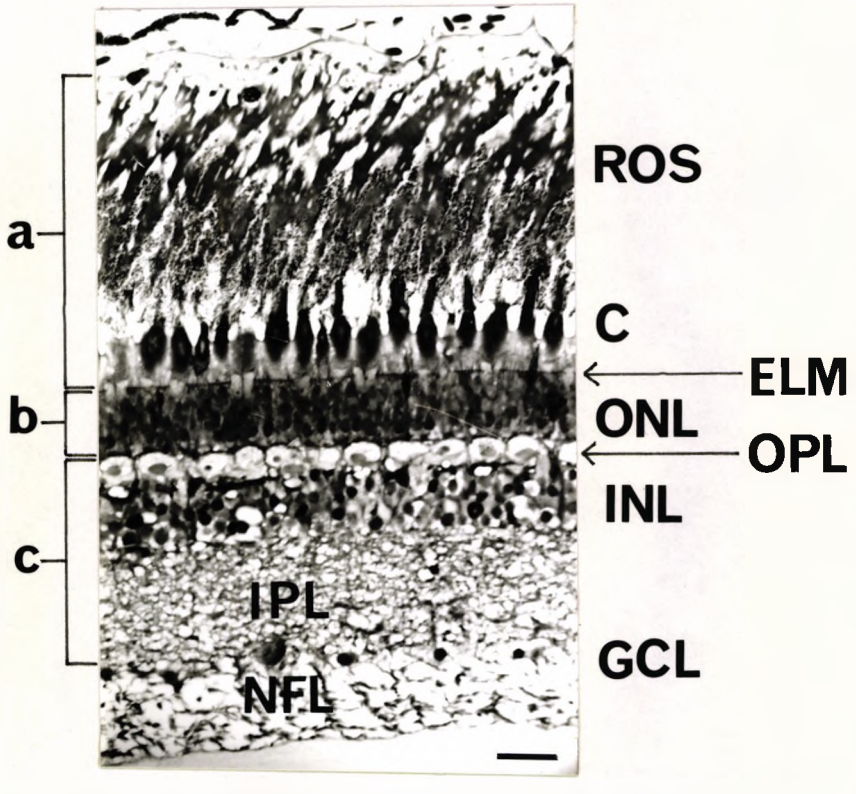


Figure 5-7. Diagram of the regions of the retina measured (a) The photoreceptor layer (b) outer nuclear layer (ONL) and (c) the rest of the retina. ROS = rod outer segments; C = cone ellipsoid; ELM = external limiting membrane; OPL = outer plexiform layer; INL = inner nuclear layer; IPL = inner plexiform layer; GCL = ganglion cell layer; The nerve fibre layer (NFL) was excluded from measurements due to its widely varying thickness. Bar indicates 20μm.

The nerve fibre layer was excluded from measurements due to its variable thickness in different retinal regions.

5.2.8 LENS PIGMENT CONVERSION *IN VITRO*

Lenses from three pumpkinseed (*Lepomis gibbosus*) were pooled and the pigments extracted. Lenses contained both l_{max} 325nm and 360nm pigments (identified by HPLC as palythine and palythene, see Table 3-3). The filtered lens extract was diluted with distilled water to obtain an optical density at 320nm of 0.5 for a 1cm pathlength cuvette. The pH of the pigment solution was adjusted to pH 3.5 with concentrated hydrochloric acid. 1ml aliquots of the diluted pigment extract were placed in two quartz cuvettes and sealed with parafilm. One sample was left on the bench exposed to normal room fluorescent lighting, while the other was illuminated using a 650W Wotan Halogen supershot light (64 540; 400-700nm radiation only). The second sample was irradiated for a total 20 mins.; after 5, 14 and 20 minutes the light was switched off and the absorbance spectrum of the sample re-recorded.

5.3 RESULTS

5.3.1 EXPERIMENT 1 - EFFECT OF THE LIGHTING ENVIRONMENT ON GOLDFISH OCULAR TISSUES *IN VIVO*

5.3.1.1 Behavioural Observations

It was noticed that low light fish did not in general consume as much food as high light fish, possibly due to problems locating it in the dim light. Low light animals were typically more dormant and lethargic than high light fish which were very skittish and alert. High light animals were found to cluster under the water filter, which provided the only relief from the high intensity lighting within the tank.

5.3.1.2 Lens Transmission and Corneal Pigmentation

After 11 months fish were sacrificed and lens transmission spectra compared to those of a group of control fish sampled at the start of the experiment (Fig. 5-8). No significant differences in the wavelength of lens 50% transmission were apparent ($p>0.05$); Fig. 5-9) and neither of the experimental groups of fish produced pigments within the lens.

However, both groups of goldfish had a λ_{max} 325nm pigment in the cornea, which was at approximately the same concentration in both groups (Fig. 5-10). In addition, a carotenoid-like pigment was present in the dorsal cornea which was at twice the concentration in high light fish compared to low light animals (Fig. 5-11).

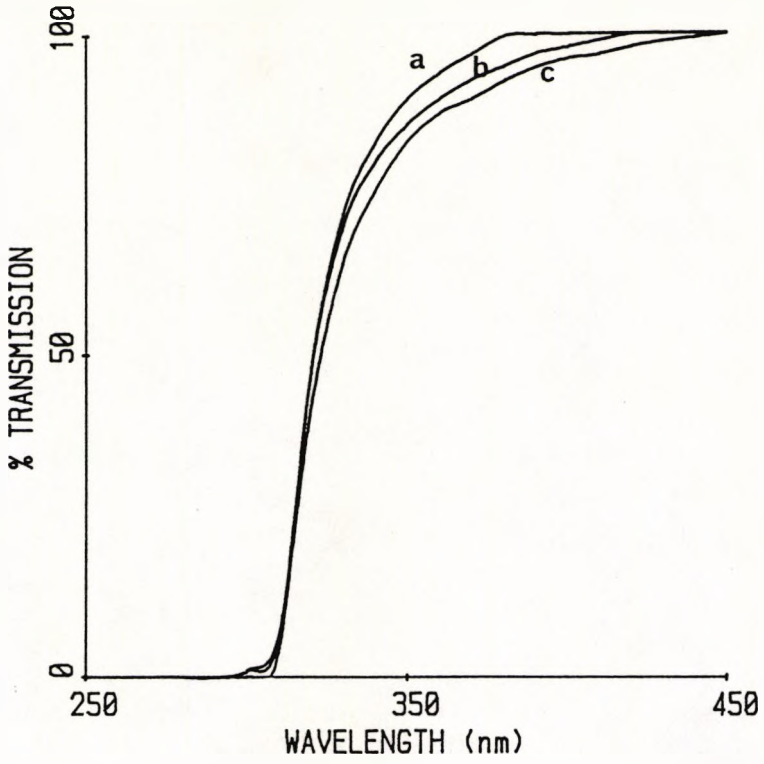


Figure 5-8. Spectral transmission of experimental *Carassius auratus* lenses. (a) the lens from a fish sampled at the start of the experiment with a diameter of 1.89mm (b) the lens from a fish raised under high light conditions with diameter 2.03mm and (c) the lens from a fish raised under low light conditions with a diameter of 1.92mm.

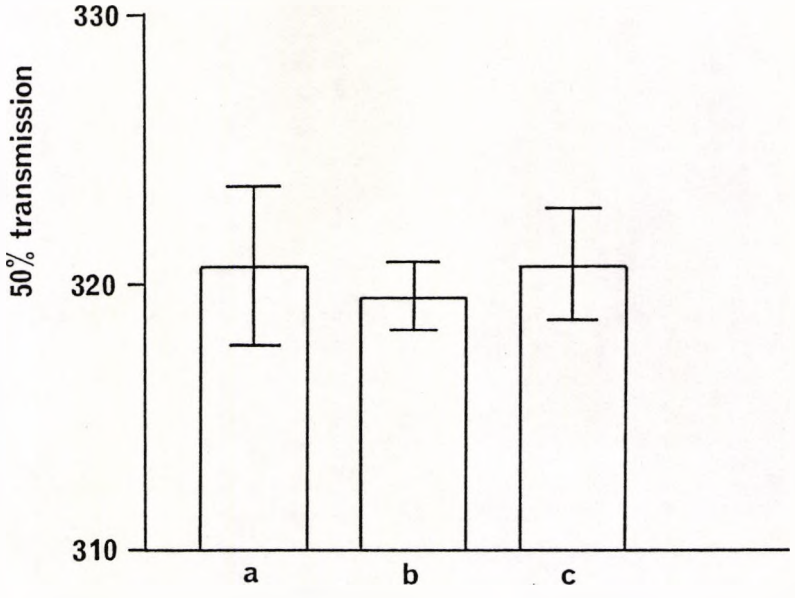


Figure 5-9. Histogram of mean lens 50% transmission wavelengths from three groups of *Carassius auratus*. (a) fish at the start of the experiment, (b) high light fish and (c) low light animals. Error bars indicate the standard deviation.

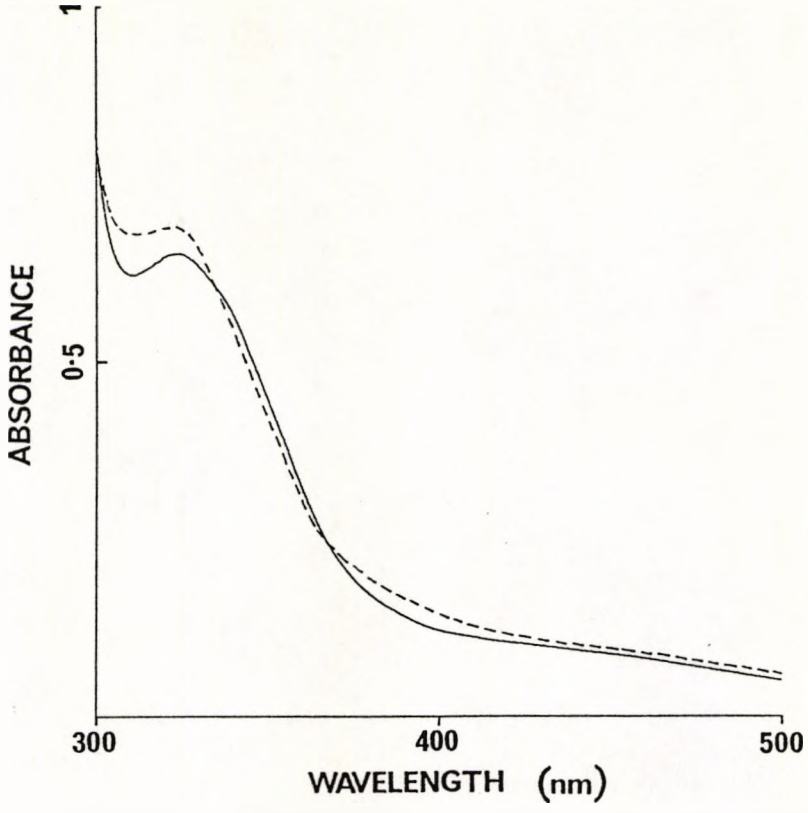


Figure 5-10. Spectral absorbance of water extracts from the corneas of high (dashed line) and low (solid line) light *Carassius auratus*, corrected for differences in extract volume and number of corneas in each extract.

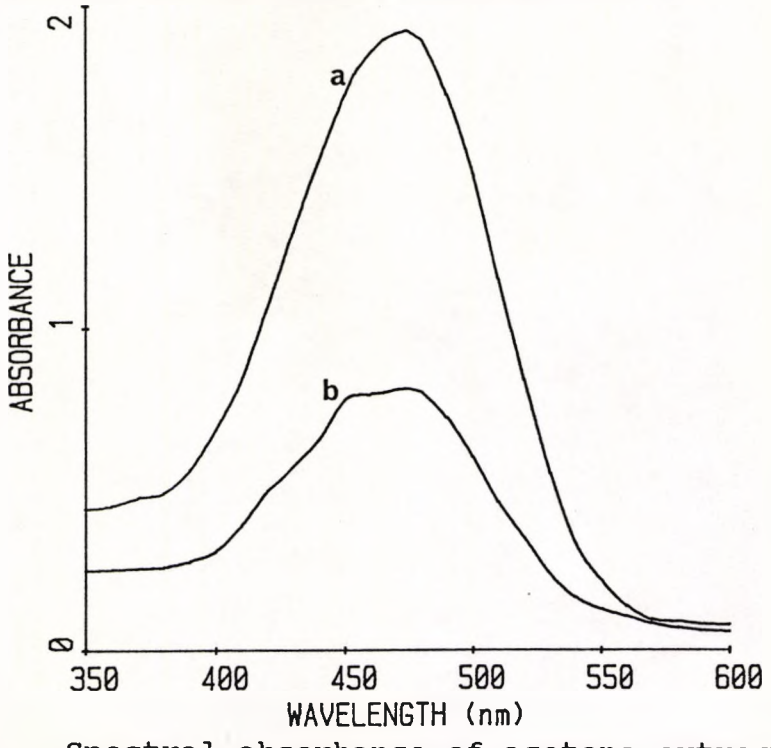


Figure 5-11. Spectral absorbance of acetone extracts of the corneal dorsal eye-shade carotenoid-like pigment from *Carassius auratus* raised under (a) high and (b) low (solid line) lighting conditions. Traces were corrected for differences in extract volume and number of corneas in each sample.

5.3.1.3 Retinal Morphology

The rod:cone ratio was relatively constant throughout the retina in both high and low light goldfish, except for an increase in the number of rods towards the periphery (Fig. 5-12). Consequently, regional variation in retinal structure need not be considered and rod:cone ratios calculated for the central region of the retina from different individuals may be directly compared and averaged.

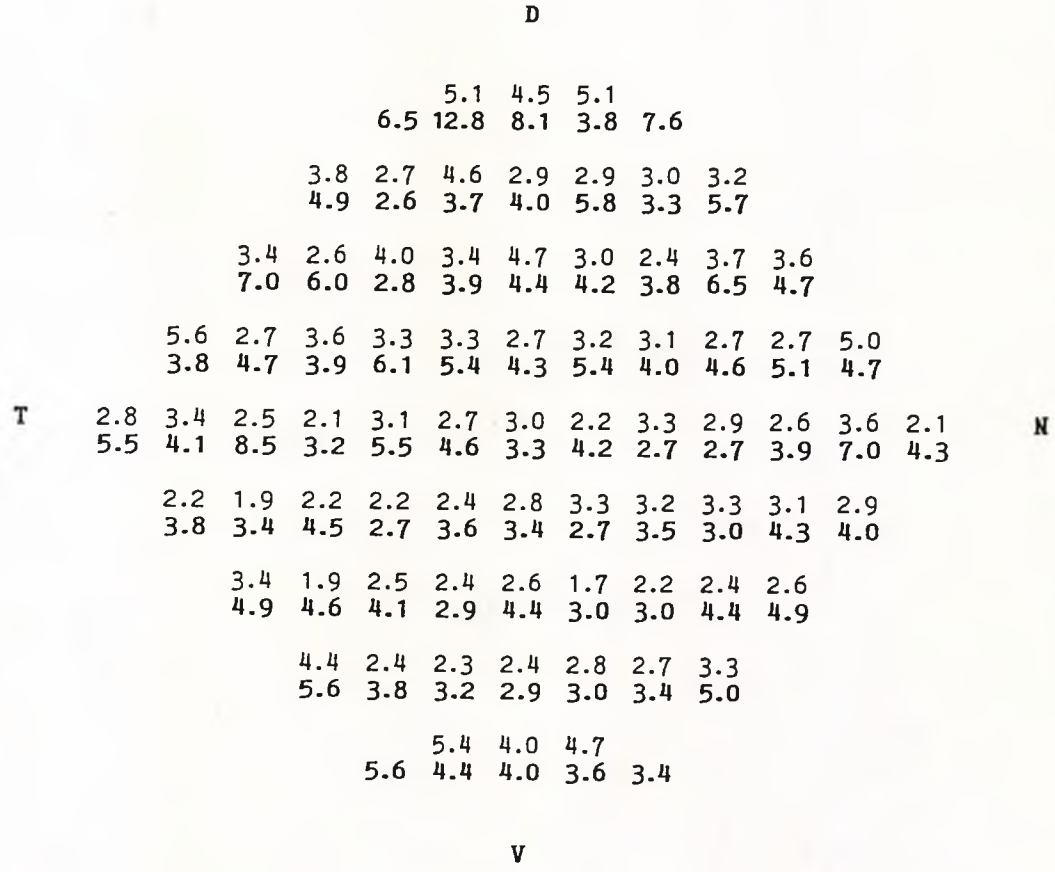


Figure 5-12. Map of the retinas from an individual *Carassius auratus* raised under high levels of illumination (standard type) and a fish raised under much lower levels of illumination (bold type). Measurements represent counts of the number of rods per cone made every 500μm. D=dorsal, N=nasal, V=ventral and T=temporal regions.

Low light fish had a mean rod:cone ratio of 9.01:1 (S.D. 3, n=11) whereas the high light fish had a significantly lower ($p<0.05$) ratio of 5.13:1 (S.D. 1, n=10). The number of cones was similar in all retinas; however, low light fish had a greater number of rods resulting in their higher rod:cone ratio.

Differences were also found in the thickness of retinal layers between high and low light goldfish. The photoreceptor layer was significantly ($p<0.01$) thicker in low light retinas (Table 5-1, Fig. 5-13), suggesting that as well as a higher number of rods, the outer segments of these rods were longer than those found in high light fish.

	HIGH	LOW
PHOTORECEPTORS	98 (26)	125 (53)
OUTER NUCLEAR	16 (4)	21 (7)
OTHER LAYERS	58 (6)	49 (10)
TOTAL	172μm	195μm

Table 5-1. Thickness of the photoreceptor layer, the outer nuclear layer and the rest of the retina (except the nerve fibre layer) in *Carassius auratus* raised in high and low light levels. 3 values were obtained for each retina and retinas from a total of 10 high and 11 low light fish were examined. The numbers in brackets are the standard deviation.

The outer nuclear layer was also significantly thicker ($p<0.05$) in low light fish, as would be expected, given that the number of rods is higher in these retinas. The thickness of the remaining layers of the retina (i.e. all except the photoreceptors, outer nuclear layer and also the

nerve fibre layer) was found to be significantly higher ($p<0.05$) in the high light goldfish.

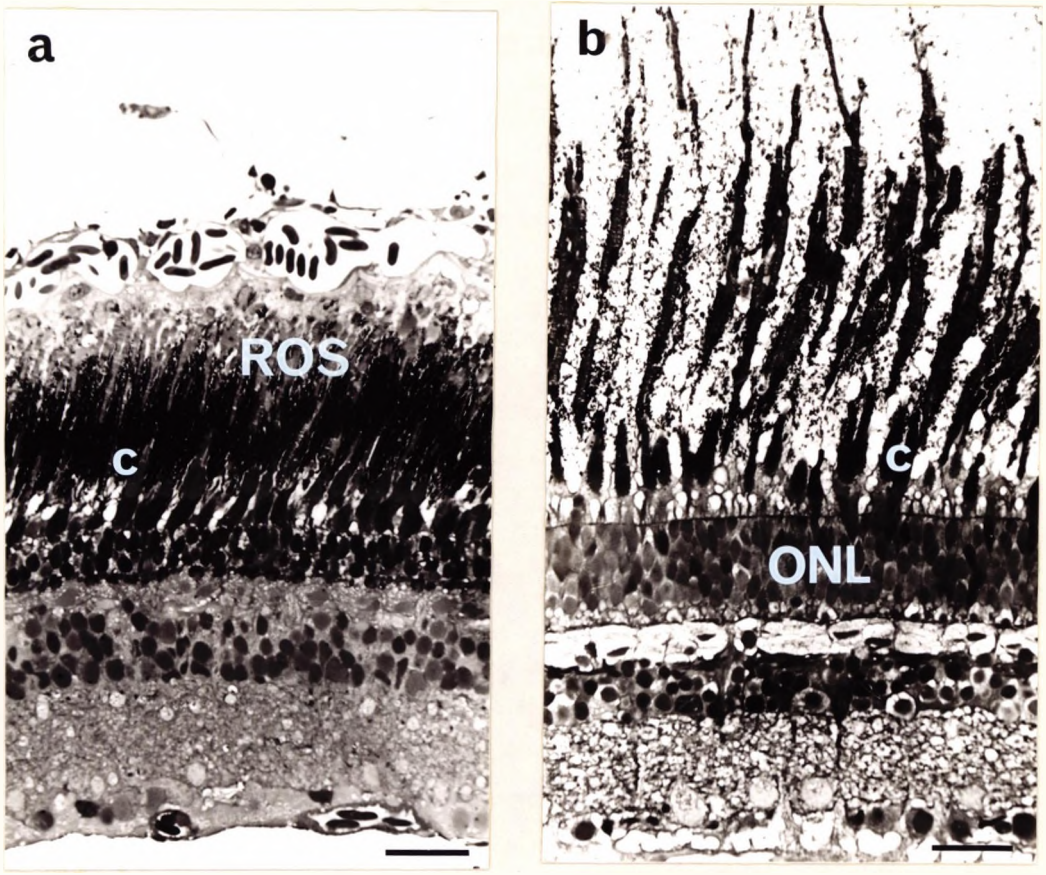


Figure 5-13. Light micrographs of 1μm radial sections from the retina of *Carassius auratus*. (a) a fish reared in a high light environment and (b) a fish reared in a low light environment. ROS=rod outer segments, ONL=outer nuclear layer, C=cone. Bars represent 20μm.

5.3.1.4 Visual Pigments

Nearest fit porphyropsin template curves were fitted to the raw MSP data (for details see Bowmaker *et al.*, 1991), revealing the presence of four cone pigment types (Fig. 5-14). A population of large single cones and the larger member of double cones were found to contain a λ_{max} 614nm

pigment. The smaller member of double cones and another population of large single cones had a pigment with l_{max} around 532nm. Small single cones contained a l_{max} 452nm pigment and a second population of slightly smaller single cones, had a UV pigment with l_{max} around 355-360nm. The UV pigment l_{max} is an estimate, based on curve fitting, since the MSP machine was not equipped to measure below 370nm.

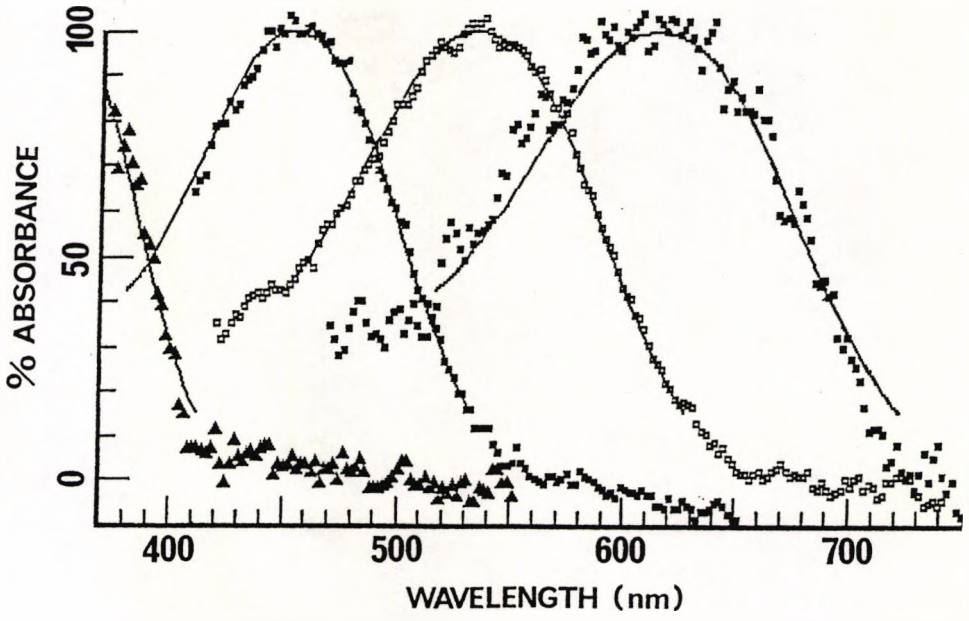


Figure 5-14. Spectral absorbance of the four cone photoreceptor pigments measured by microspectrophotometry in the *Carassius auratus* retina. Pigment l_{max}s were the same for fish raised in either low or high light environments.

UV-sensitive cones were relatively uncommon, but they were found in all four fish. It was not possible to measure any red-sensitive cells in the retinas of the high light fish, but no differences between high and low light goldfish

were found in the l_{max}s of the UV, green or blue sensitive cones, or the rod visual pigments (Table 5-2).

	LOW LIGHT FISH	HIGH LIGHT FISH
RED	614.1 (4.9)	-
n	10	
GREEN	533.6 (1.2)	529.1 (3.2)
n	16	9
BLUE	450.8 (1.4)	453.2 (1.4)
n	10	13
UV	355-360	355-360
n	3	3
RODS	521.1 (3.2)	521.1 (3.9)
n	3	4

Table 5-2. Averaged visual pigment l_{max}s in nanometres with the standard deviation (in brackets), of cells measured from four high and four low light *Carassius auratus* retinas.

5.3.2 EXPERIMENT 2 - EFFECT OF THE LIGHTING ENVIRONMENT ON CARP OCULAR TISSUES *IN VIVO*

5.3.2.1 Lens Transmission and Pigment Content

Young carp were selected for the second lighting experiment since they tended to have higher lens pigment concentrations than most small goldfish (see Chapter 4). Carp have highly variable lens transmission depending on the population examined (see Fig. 4-12), but as the fish used here were from a single population, it was assumed that the spectral transmission of all lenses at the start of the experiment was the same. This was confirmed by the similar transmission spectra of lenses from fish measured at the start of the experiment (Fig. 5-15a). Lens transmission

from fish in each of the experimental groups was measured and averaged after 6 months (Fig. 5-15b,c&d).

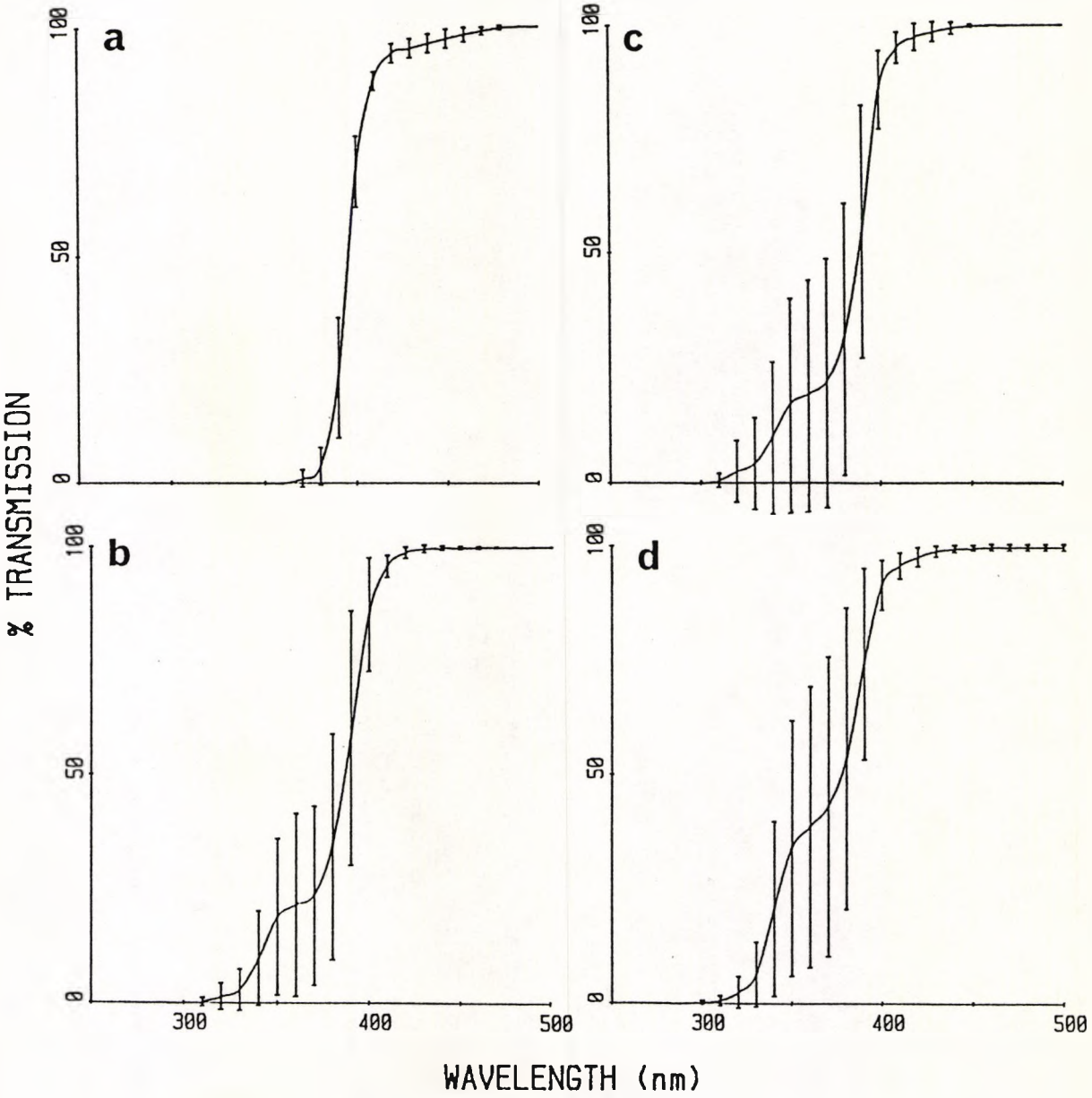


Figure 5-15. Average spectral transmission of experimental *Cyprinus carpio*, (a) fish at the start of the experiment, (b) UV, (c) high and (d) low light carp after 6 months under their experimental lighting conditions. Error bars represent the standard deviation.

All lenses, except those from a single low light fish were pigmented. However, the degree of pigmentation varied greatly between experimental fish (demonstrated by the large standard deviations). Mean 50% transmission wavelengths for all experimental groups were lower than that for the carp sampled at the start ($p<0.05$; Fig. 5-16), suggesting that pigment concentrations in the lens had fallen during the course of the experimental light exposure.

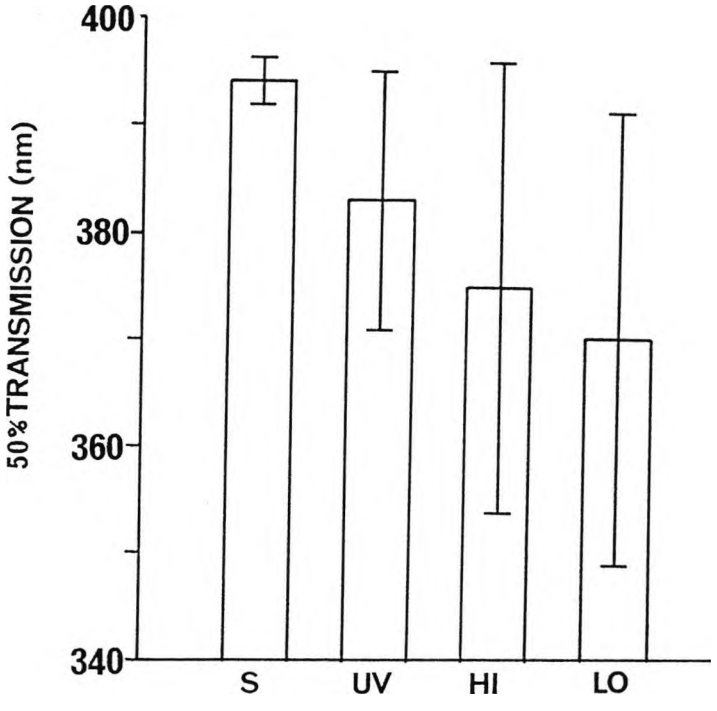


Figure 5-16. Mean lens 50% transmission for four groups of *Cyprinus carpio*. S - fish at the start of the experiment, UV - fish raised in a tank with high levels of UV radiation, HI - fish raised in a high light environment and LO - fish reared in a low light environment.

The same lens pigments were present in all classes of carp, with λ_{max}s of 325nm and 360nm, identified earlier as palythine and palythene respectively (Table 3-3). However, pigment concentrations were markedly lower in all experimental fish than in carp sampled at the start of the experiment (Fig. 5-17). The fish sampled at the start also had higher lens concentrations of palythene with respect to palythine than all other groups (Fig. 5-17).

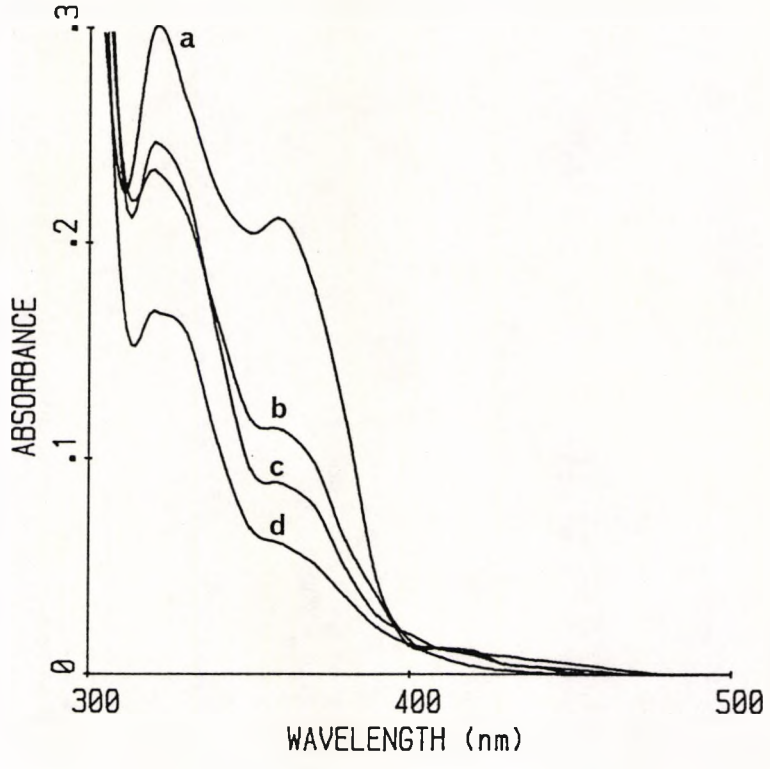


Figure 5-17. Spectral absorbance of the pigments extracted from the lenses of each of the four experimental populations of *Cyprinus carpio* (a) fish at start, (b) UV fish, (c) high light and (d) low light fish.

5.3.2.2 Corneal Pigmentation

Short-wave absorbing pigments were also extracted from the corneas of all four groups of carp. Pigments appeared to be similar to those in the lens, with λ_{max}s around 325nm and 360nm, but there was also a small peak around 410nm in each extract (Fig. 5-18). However, the very low pigment concentrations made accurate comparisons between groups impossible.

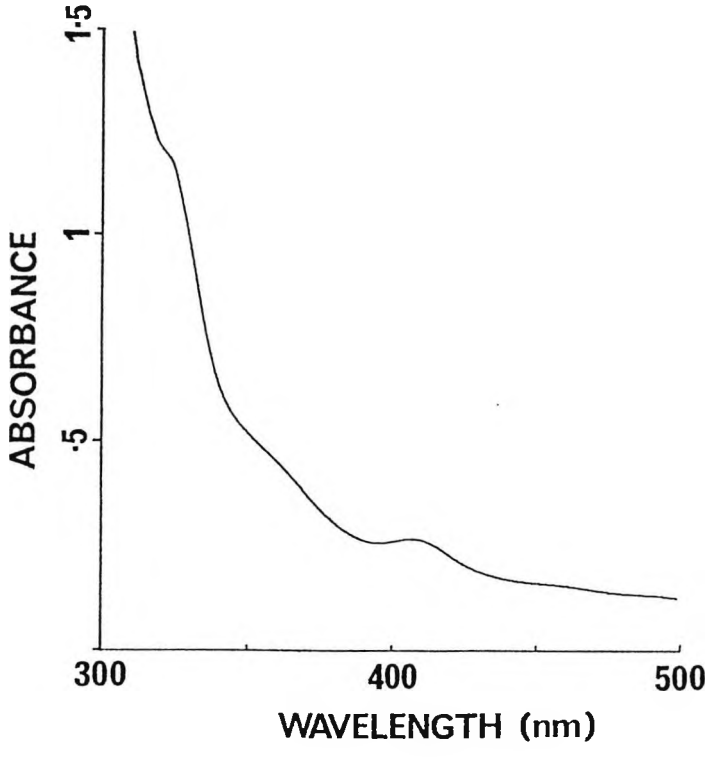


Figure 5-18. Spectral absorbance of the extracted pigment from the corneas of the high light *Cyprinus carpio*. Corneal extracts from the remaining three groups of carp were very similar.

5.3.2.3 Retinal Morphology

The photoreceptor layer, as for goldfish, was found to be significantly thicker in the retinas of low light carp; high light and UV fish had very similar photoreceptor layer thicknesses (Table 5-3, Fig. 5-19; ANOVA $p<0.01$).

	HIGH	LOW	UV
PHOTORECEPTORS	98 (22)	127 (21)	102 (25)
OUTER NUCLEAR	23 (3)	27 (3)	24 (4)
OTHER LAYERS	82 (12)	83 (11)	85 (19)
TOTAL	203μm	237μm	211μm

Table 5-3. Thickness of the photoreceptor layer, the outer nuclear layer and the rest of the retina (excluding the nerve fibre layer) in the three experimental groups of *Cyprinus carpio*. Values are averages of 55 retinal sections from 11 UV and high light carp and 60 sections from 12 low light carp retinas. The numbers in brackets are the standard deviations.

Thicknesses of the outer nuclear layer and the rest of the retina, excluding the nerve fibre layer, were similar for all groups of carp ($p>0.05$; Table 5-3). A significant difference was also found between rod:cone ratios of high and UV fish (considered together) and low light carp (ANOVA, $p<0.01$; Low 7.98 rods per cone; High 6.82 rods per cone; UV 6.54 rods per cone). The number of rods was greater in low light fish, but the number of cones was constant in all three groups.

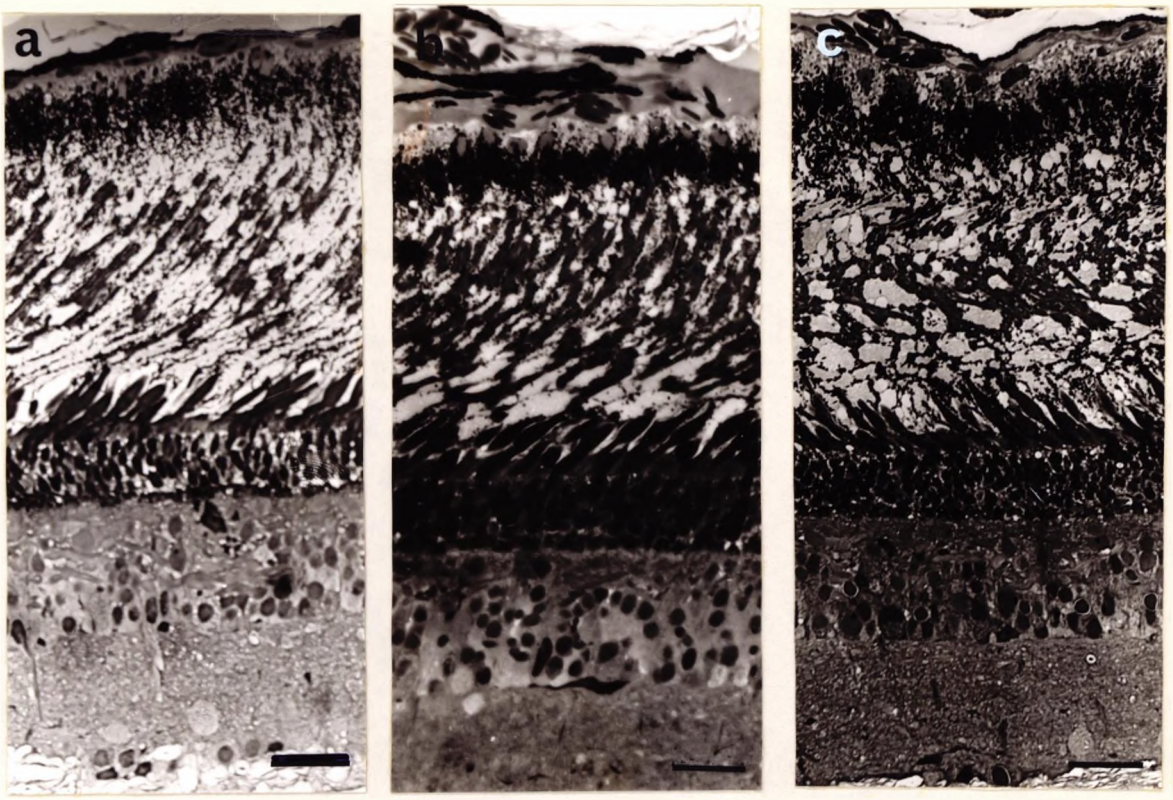


Figure 5-19. Light micrographs of 1μm radial sections from retinas of experimental *Cyprinus carpio* (a) a fish raised under low light (b) a fish raised under high light and (c) a fish raised under UV illumination for 6 months. Bars indicate 20μm.

5.3.3 EFFECTS OF THE LIGHTING ENVIRONMENT ON LENS PIGMENTS *IN VITRO*

The pumpkinseed lens extract had altered significantly after exposure to normal room lighting for 8 hours (Fig. 5-20). The concentration of palythine (l_{max} 320nm) had increased whereas that of palythene (l_{max} 360nm) had fallen, suggesting that palythene was decomposing to form palythine. Exposure to high intensity white light had an accelerating effect on this process (Fig. 5-20). Palythene concentrations in the lens extract gradually fell whereas

palythine levels increased markedly. After 20min. exposure to the high intensity light the concentration of palythene was lower and the concentration of palythine was higher than in the sample left on the bench for 8 hours.

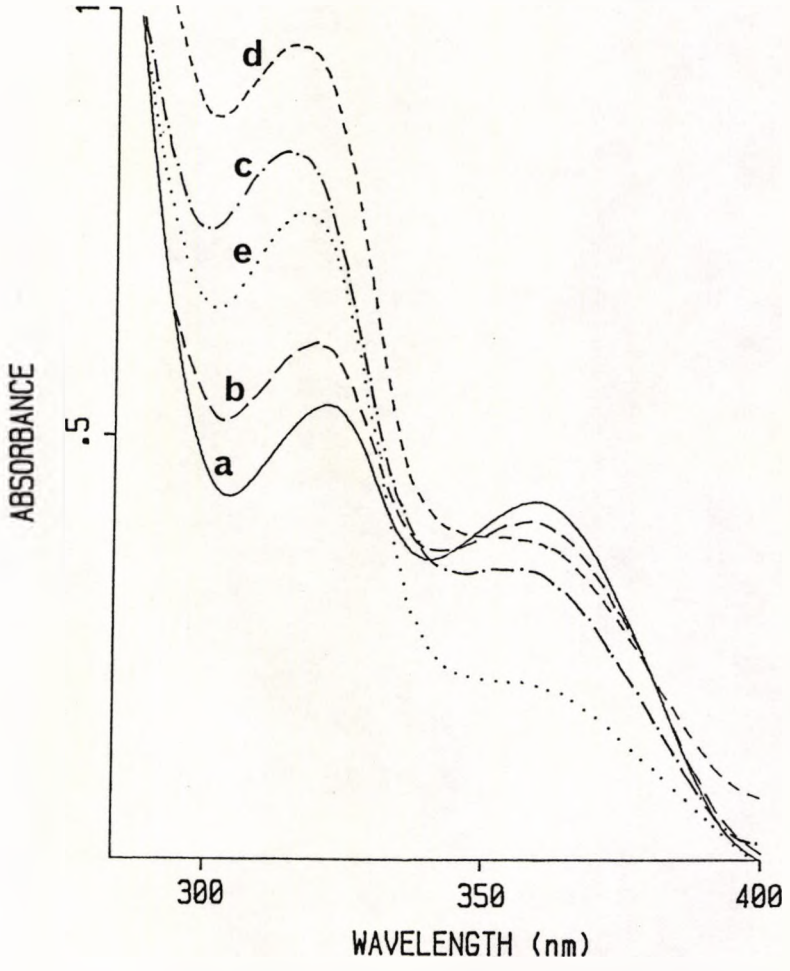


Figure 5-20. Spectral absorbance of the pigments extracted from the lens of *Lepomis gibbosus*. Absorbance of sample at pH 3.5 (a) at the start, (b) after 5min. (c) after 14min. and (d) after 20 min. exposure to a 650W white studio light. Curve (e) is from a sample exposed to normal fluorescent room lighting for 8 hours.

5.4 DISCUSSION

5.4.1 LENS PIGMENTATION

No differences in lens pigmentation were noticed in goldfish or carp reared under high, low or UV illumination. The lighting environments used here therefore had no effect on lens pigmentation. The high light intensities used did not initiate pigment production in goldfish (Fig. 5-8) nor did it cause an increase in concentration of pigments already present in lenses of carp (Fig. 5-17).

However, an effect of the lighting environment on lens pigmentation in fish cannot be ruled out. It is conceivable that exposure to intense light for prolonged periods is required, perhaps in addition to other factors. In the human lens, continued exposure to bright sunlight is implicated as a causative factor in lens yellowing with age [Zigman, 1983b]. It appears that the situation in fish is quite different and that lens pigments are not produced as a direct result of exposure to high levels of illumination.

Goldfish lens pigments, when present, are probably the same as those found in the closely related carp - palythine and palythene mycosporine compounds (Table 3-3). The source of mycosporine pigments, as discussed earlier (Chapter 3), is likely to be the diet. If the fish food pellets used did not contain mycosporine constituents then none of the goldfish or carp would accumulate mycosporine pigments in the lens. As no new mycosporine compounds accumulated in the lens, any pigments present initially will be 'diluted'

as the lens volume increases with age, resulting in a drop in 50% transmission (See Chapter 4). In fact, all three experimental groups of carp had, on average, lower 50% transmission values than the carp sampled initially (Figs. 5-15 & 5-16). This supports the fact that no mycosporine compounds were available from the food source.

It may also be that the lighting conditions used here were not sufficiently bright or were of the incorrect wavelengths to initiate pigment production. However, this is unlikely since changes were observed in both corneal pigmentation (Section 5.4.3) and retinal structure (Section 5.4.5), suggesting that the lighting conditions were biologically significant.

5.4.2 *IN VITRO* PIGMENT CONVERSIONS

It is clear from earlier observations (Fig. 3-30), where a HPLC-purified sample of pure palythene (l_{max} 360nm) decomposed to form palythine (l_{max} 320nm) after lyophilisation, and also from the results of the pumpkinseed lens extract in this chapter (Fig. 5-20), that changes in relative pigment concentrations can occur *in vitro*. Evidence of similar conversions occurring *in vivo*, is provided by the experimental carp. Concentrations of palythene (l_{max} 360nm) relative to palythine (l_{max} 320nm) were higher in fish at the start of the lighting experiment than in all other groups after six months (Fig. 5-17). It is therefore clear that pigment conversions may occur in the lens *in vivo* as well as in pigment extracts *in vitro*.

It is not clear what initiates the conversion of

palythene to palythine *in vivo*. In the herring, higher concentrations of palythene relative to palythine were found in the lenses of wild-caught animals compared to lenses of animals reared in the laboratory (see Fig. 4-16). The two populations of fish will have been exposed to quite different environmental conditions such as pH, temperature and food supply as well as differences in lighting. Any one, or a combination of these may have caused the conversion of palythene to palythine in the lens.

All experimental carp lens extracts revealed very similar relative pigment concentrations irrespective of the lighting condition (Fig. 5-17), which is inconsistent of an effect due to the light intensity: this would lead to lower palythene concentrations in lenses of fish from high light tanks. Since even low light fish showed a loss of palythene, it appears that the environmental radiation has little part to play in the process *in vivo*. It may be that the presence of enzymes or oxidising compounds in the lens has a greater effect than the environmental illumination. High concentrations of acid (as used for the pumpkinseed lens extract) also act as oxidising agents.

The evidence reported here therefore does not support a <u>direct</u> role of light in the conversion of one mycosporine compound to another within the fish lens *in vivo*. A high light intensity can however, accelerate the conversion of palythene to palythine under acidic conditions *in vitro* (Fig. 5-20).

5.4.3 CORNEAL PIGMENTATION

Spectral absorbance of the carotenoid-like pigment from the goldfish cornea was identical to that published for pigment extracted from goldfish integument [Hata & Hata, 1971]. The goldfish corneal eyeshade is therefore probably simply an extension of the body colouration as suggested by Douglas (1989), being composed mainly of carotene and astaxanthin esters. The body colouration of the goldfish which were raised in the dark was noticeably paler than in fish reared under high intensity illumination. As the carotenoid-like corneal pigment appears to be an extension of the body colouration, it is not surprising that it too was lost in goldfish raised under low light conditions (Fig. 5-11).

5.4.4 VISUAL PIGMENTS

Seasonal variations are known to occur in visual pigments of several species of fish [Loew & Dartnall, 1976; Whitmore & Bowmaker, 1989] and are thought to be controlled by daylength. However, the elevated and depressed artificial daylengths used in this experiment resulted in no changes in rod or cone visual pigment λ_{max} in either high or low light goldfish compared to data available for fish raised under normal daylength cycles [Harosi, 1985]. Although UV sensitivity has been observed in the goldfish previously [Hawryhshyn & Beauchamp, 1985; Neumeyer, 1986], this represents the first measurement of UV cones in this species [Bowmaker *et al.*, 1991].

5.4.5 RETINAL MORPHOLOGY

Although minimal effects were observed on lens pigmentation in both goldfish and carp, the lighting environments were physiologically significant, as shown by the results from the corneas of the goldfish and also the retinas of both species. Different lighting environments had quite marked effects on a number of retinal parameters. Both populations of low light animals had more rods which were also longer than those found in fish from other lighting conditions (Figs. 5-13 & 5-19; Tables 5-1 & 5-3), while the number of cones was constant in all goldfish and carp. Differences between high and low light fish retinas were more noticeable in goldfish than carp, which could be due to the longer period for which goldfish were exposed to the various light levels.

The fact that low light animals had a greater number of rods may be interpreted in two ways: either the low light fish have adaptively increased the number of rods or high light fish have lost rods. The phenomenon of rod loss in fish exposed to constant light is widely documented [Marotte *et al.*, 1979; Penn, 1985; Raymond *et al.*, 1988]. Here, a similar situation may result from high light exposure for 23 hours of the day. However, the greater number of rods in low light animals (Fig. 5-13, Section 5.3.2.3) suggests an adaptive response to the low light environment since more rods and longer outer segments will improve sensitivity.

Goldfish reared under high levels of illumination had thicker plexiform layers (Table 5-1) than low light fish, which suggests an increase in the degree of post receptor

visual processing in high light animals. Animals reared under high levels of illumination will place more importance on cone based (colour) vision than on rod-mediated sensitivity, which would require a greater complexity of retinal wiring and maybe increased plexiform layer thickness compared to vision in low light levels.

There is also some evidence to support a reduction in rod density when fish are raised in constant darkness [Raymond *et al.*, 1988], however, this is in disagreement with the high rod densities measured for goldfish raised under low levels of illumination here (Section 5.3.1.3). Allowing fish even very dim light (as used in low light tanks) for a short period will give them a chance to feed and establish a diurnal cycle. Onset of illumination will also trigger disc-shedding. Complete darkness will not allow this and is therefore not a true physiological situation. Such differences could possibly account for the discrepancies in rod numbers observed between the present study and that of Raymond *et al.* (1988).

It is interesting that although the number of rods was found to differ depending on the light intensity, the number of cones was constant. Raymond *et al.* (1988) similarly found no change in the number of cones in animals exposed to either constant light or constant dark. Cone development and maintenance therefore appears to be independent of environmental conditions in both goldfish and carp aged between one and two years.

CHAPTER SIX

SPECTRAL ABSORBANCE OF OTHER OCULAR TISSUES

6.1 INTRODUCTION

Short-wave absorbing compounds are not restricted to the lens of the eye. Corneas, humours and retinas from several species have all been found to have pigments absorbing short-wave radiation [Lythgoe, 1971; Muntz, 1973; Heinermann, 1984 for review]. Wherever the pigment, the ultimate effect on vision will be the same: short-wave radiation will be prevented from reaching the photoreceptors. Corneal pigments are commonly found in fish, with many species having yellow and orange pigments in the dorsal region of the cornea [Walls & Judd, 1933a&b; Moreland & Lythgoe, 1968; Muntz, 1973; Douglas, 1989]. The importance of corneal eye-shades, as discussed in Chapter 1, appears to be their preferential absorbance of downwelling short-wave illumination [Walls & Judd, 1933a]. Such corneal pigmentation is fixed in many species, but an interesting adaptation has evolved in others, such as members of the hexagrammidae and tetraodontidae which have occlusable corneal pigments [Orlov & Gamburtseva, 1976; Appleby & Muntz, 1979]. The cornea of fish in these species is deeply coloured in bright sunlight and colourless at lower light intensities. Other species of fish restrict the spectral

composition of light reaching the retina in a different manner - by corneal iridescence [Lythgoe, 1971, 1975] which, like corneal pigments, may also be light-induced [Shand & Lythgoe, 1987].

Mycosporine-like amino acids, identical to those found in the lenses of many fish, were isolated from the cornea, iris and aqueous humour of a number of marine species by Dunlap *et al.* (1989). However, concentrations in the cornea and aqueous were considerably lower than levels found in the lens. Since the lens obtains many of its metabolites from the aqueous humour [e.g. van Heyningen, 1973b], it is possible that the aqueous is the source of the mycosporine-like compounds found in many fish lenses.

Diffuse photostable yellow and orange pigments have also been observed in the retinas of a freshwater cichlid [Muntz, 1983] and also a number of deep-sea fish [Bowmaker *et al.*, 1988; Denton & Locket, 1989; Douglas, pers. comm.]. The function of such retinal pigments may be the same as short-wave absorbing compounds closer to the front of the eye, but Bowmaker *et al.* (1988) suggest that they may act as photosensitisers. Carotenoid pigments are also found in oil droplets within cones in the retina of other vertebrate species [Walls & Judd, 1933a&b; Goldsmith *et al.*, 1984], these pigments quite clearly act as filters and usually absorb maximally at longer wavelengths than the short-wave absorbing compounds found closer to the front of the eye. However, colourless oil droplets absorbing at shorter wavelengths than coloured droplets have also been described in a number of species of birds and turtles [Partridge,

1989; Ohtsuka, 1985]. A number of fish have also been shown to have rose-pink retinae due to the presence of ellipsosomes [Avery & Bowmaker, 1982], modified mitochondria with short-wave absorption, in cone ellipsoids [MacNichol *et al.*, 1978].

Although uncommon, pigmented vitreous humours have been described. The vitreous of one species of fish *Siganus rostratus*, is reported to be yellow [Lythgoe, 1971] and therefore will filter out some proportion of the incident short-wave radiation.

In this section, the spectral characteristics of a number of fish corneas and vitreous humours, which were measured incidentally, are examined. The relationship of these pigments with any short-wave absorbing compounds present in the lens is also considered.

6.2 METHODS

Different areas of the cornea were placed over a 2mm hole in a metal holder, as also used for lens transmission measurements (Fig. 2-1). The holder was then placed in a quartz cuvette and the spectral absorbance measured in a Shimadzu UV-240 spectrophotometer (see Chapter 2). Vitreous humour from several individuals of a few species was collected with a 5ml syringe and due to its rather viscous consistency, could be placed into the metal holders and scanned as for the lens.

6.3 RESULTS

Most species of fish have no short-wave absorbing compounds in the cornea. However, around 10% of fish examined did show some degree of corneal short-wave absorbance, which was due to the presence of either mycosporine-like compounds as commonly found in the lens, or carotenoid-like compounds, or a combination of both (Table 6-1).

SPECIES	LENS PIGMENT λ_{max} (nm)	CORNEAL PIGMENT λ_{max} (nm)	
		cornea	carotenoid dorsal shade
Astronotus ocellatus(F)	324,360	320	430,450,475
Herotilapia multispinosa(F)	327,362	325,360	-
Pterois volitans(M)	322,360	324,360	-
Helostoma temmincki(F)	329,360	330	-
Phygocentrus piraya(M)	325,360	-	415,455,490
Osphronemus goramy(F)	326,360	-	425,450,480
Haplochromis ishmaeli(F)	320,358	-	-
Liophrys pholis(M)	unknown	360	-
Cantherhines pullus(M)	326	325	-
Chaetodon sp.(M)	331	331	-
Pygoplites diacanthus(M)	331	328	-
Centropyge heraldi(M)	327	330	-
Hippocampus ramulosus(M)	363	325,360	-
Apistogramma curviceps(F)	370	363	408,453,487
Trichogaster trichopterus(F)	370	-	413,457,493
Ctenopoma oxyrhynchus(F)	370	-	425,450,470
Trichogaster microlepis(F)	370	-	425,450,485
Rutilus rutilus(F)	-	360	-

Table 6-1. Corneal pigment λ_{max}s from freshwater (F) and marine (M) fish. Values have been split into carotenoid and short-wave absorbing pigments. Pigments underlined are probably mycosporine compounds, as identified in many of the lenses. The λ_{max} of pigments extracted from the lens are also shown for comparison.

Where no pigments were present in the cornea, the short-wave absorbance increased rapidly at wavelengths below 300nm (Fig. 6-1a). Corneas which were otherwise clear of pigment, having absorbance spectra as in Fig. 6-1a in the ventral region, often had carotenoid-like pigments located in the dorsal part of the cornea. Absorbance spectra of such areas of the cornea were characteristic of carotenoid pigments with l_{max}s in the region of 400-500nm (Table 6-1, Fig. 6-1b). However, corneas with short-wave absorbing mycosporine compounds in the lens had quite different absorbance spectra (Fig. 6-2).

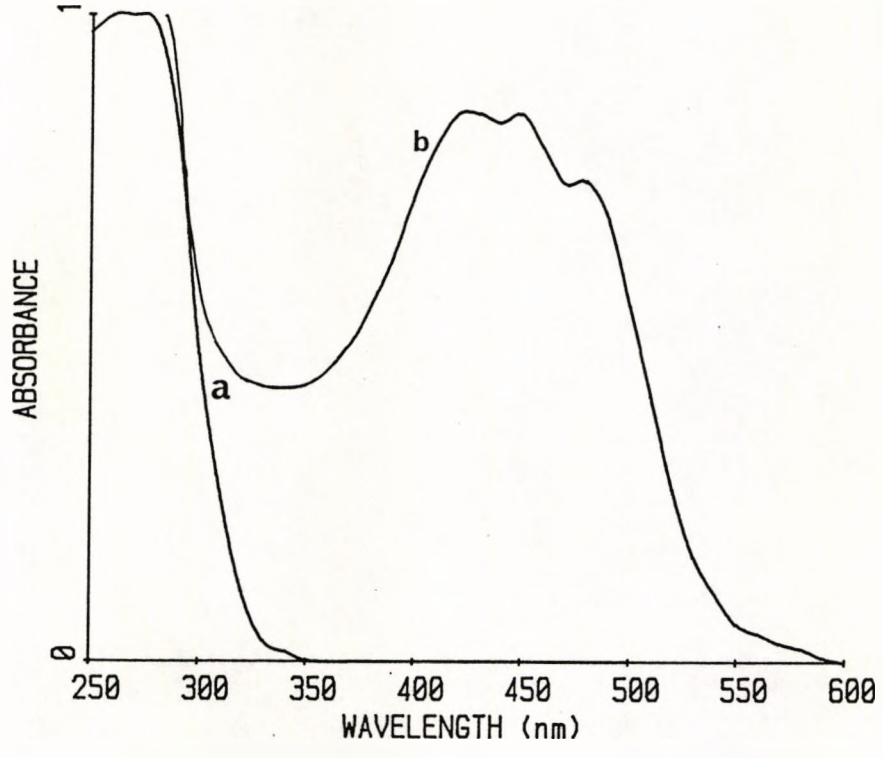


Figure 6-1. Spectral absorbance of the corneas of (a) *Haplochromis argens* with no short-wave absorbing compounds present and (b) *Ctenopoma oxyrhynchus* which has a carotenoid-like dorsal eye-shade.

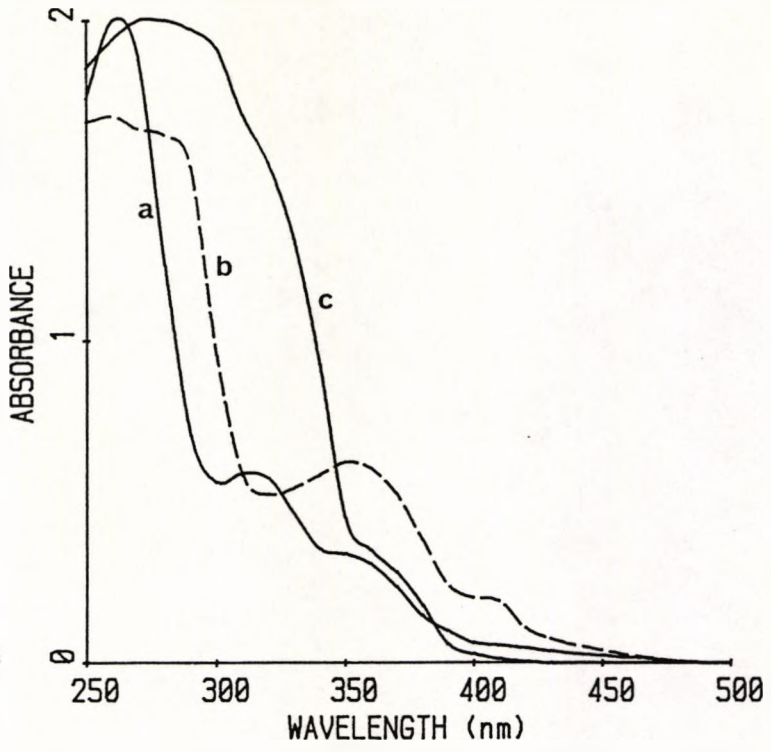


Figure 6-2. Spectral absorbance of the corneas of (a) *Herotilapia multispinosa* (b) *Liophyris pholis* and (c) *Hippocampus ramulosus*. All three species have pigments in the cornea absorbing between 300 and 400nm.

Mycosporine-like compounds, unlike carotenoids, appeared to be located throughout the cornea. The cornea of the oscar (*Astronotus ocellatus*), for example, had a pigment with a l_{max} around 320nm, which was present in both the dorsal and ventral cornea, but in addition, contained a carotenoid-like pigment in the dorsal cornea (Fig. 6-3).

Pigments in the cornea often had similar l_{max}s to those extracted from the lens (Table 6-1). This was particularly true of tropical marine species, where both the corneas and lenses contained a pigment with l_{max} 330nm (see Fig. 6-4).

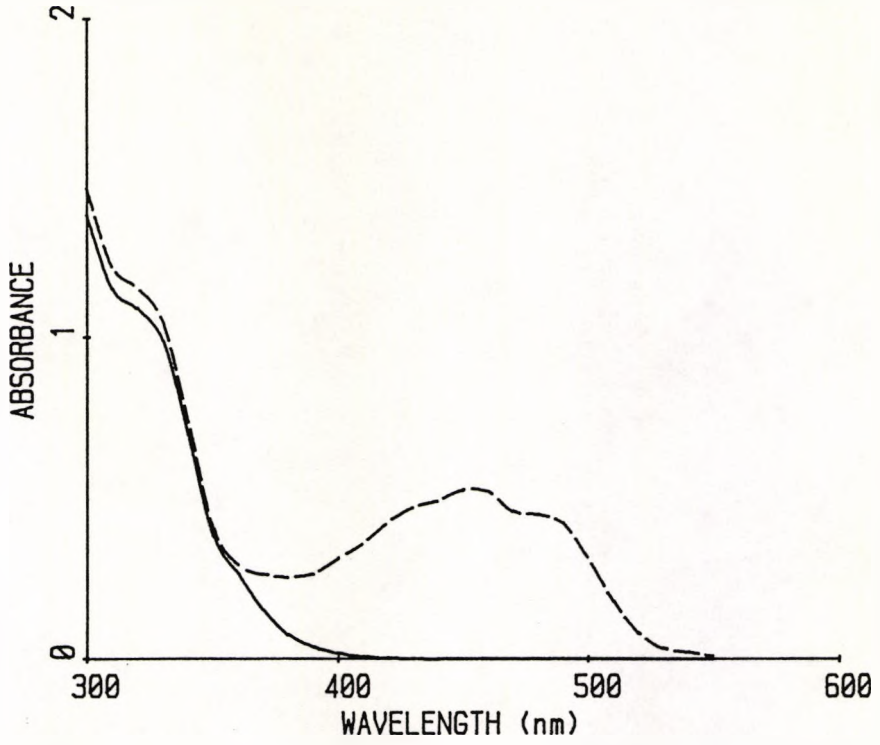


Figure 6-3. Spectral absorbance of the cornea of *Astronotus ocellatus*. The dashed line represents the absorbance of an area 2mm from the dorsal edge of the cornea whereas the solid line is the absorbance 7mm from the dorsal edge. Carotenoid-like pigment is present only in the upper region of the cornea whereas the l_{max} 320nm pigment is located throughout the cornea.

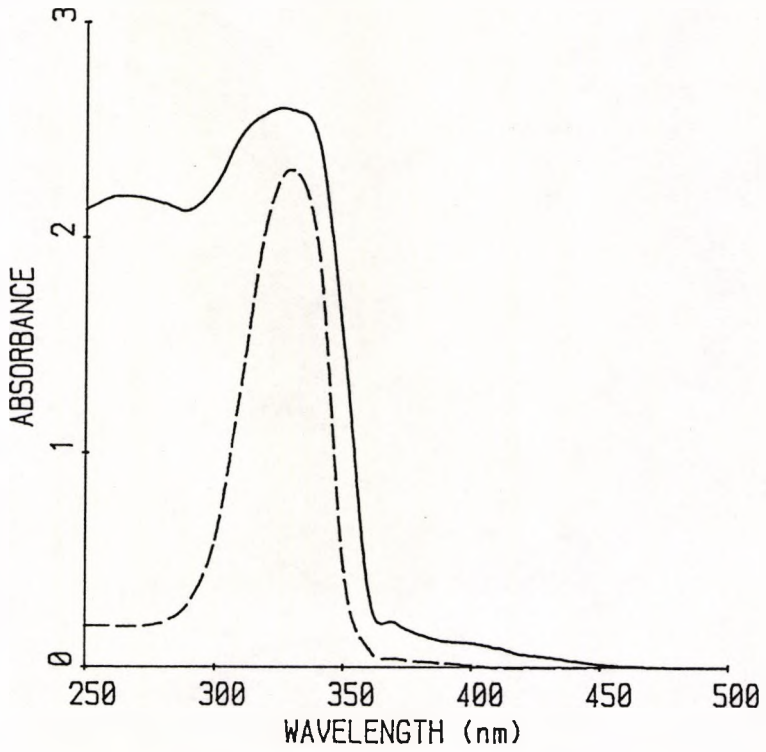


Figure 6-4. Spectral absorbance of the cornea of *Chaetodon sp.* (solid line) and the pigment extracted from the lens of this species (dashed line).

Vitreous humour was collected from eyes of several goldfish (*Carassius auratus*) and roach (*Rutilus rutilus*); the spectral absorbance was very low between 300nm and 400nm (Fig. 6-5), suggesting the absence of appreciable concentrations of short-wave absorbing compounds.

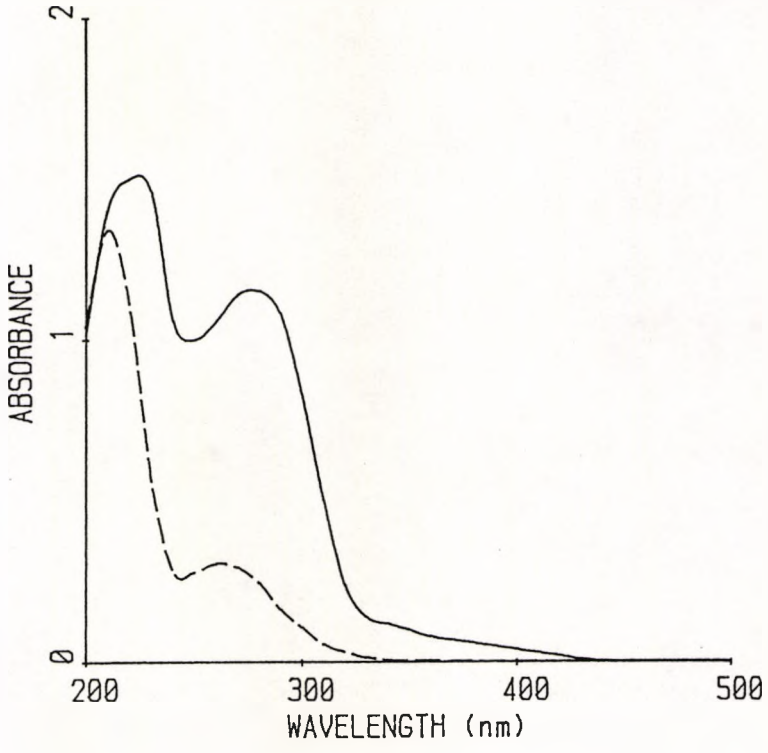


Figure 6-5. Spectral absorbance of the vitreous humour of *Carassius auratus* (solid line) and *Rutilus rutilus* (dashed line). The pathlength through the humours was around 5mm when scanned, which is similar to the distance between the lens and retina for fish of this size *in vivo*.

6.4 DISCUSSION

The cornea, like the lens, can often act as an effective short-wave absorbing tissue within the fish eye. However, absorbance is rarely as high as observed in the lens due to the cornea's thinner structure.

Carotenoid-like compounds were quite common in the fish corneas examined here, absorbing radiation in the 400-500nm region (Table 6-1). The presence of carotenoid pigments in the fish cornea is widely documented [Moreland & Lythgoe, 1968; Bridges, 1969; Orlov & Gamburtseva, 1976; Appleby & Muntz, 1979; Douglas, 1989]. Appleby & Muntz (1979) found that the occlusable corneal carotenoid pigment in a number of tetraodontids was the same as that responsible for coloration of the skin, an observation also true for goldfish corneal pigment [Douglas, 1989; also see Chapter 5]. The predominant carotenoids in the goldfish integument are astaxanthin-ester and 4-keto-lutein ester [Hata & Hata, 1971] and these compounds are therefore also likely to be found in the cornea of this species. Other fish have comparable corneal absorbance to that of the goldfish (Fig. 6-1 & 6-3; Appleby & Muntz, 1979) but the pigments responsible have yet to be positively identified.

The lenses of deep-sea fish have also been found to contain carotenoid compounds [McFall-Ngai *et al.*, 1986; Douglas & Thorpe, in press], but these compounds appear to have quite different absorbance spectra to those found in the cornea of surface dwelling species. Fish corneal

pigments do however appear to be quite similar to the carotenoid pigments found in the macula region of mammalian retinas, which have been identified as lutein and zeaxanthin [Bone *et al.*, 1985 & 1988].

Wavelengths shorter than 400nm are known to penetrate surface waters quite significantly [Jerlov, 1951; see also Section 1.5], and radiation in the region 300-400nm will be transmitted by corneas having only carotenoid pigmentation (Fig. 6-1). However, the oscar and also *Apistogramma curviceps* had corneas with both a carotenoid pigment in the dorsal region and a pigment with a shorter λ_{max} distributed throughout the cornea (see Fig. 6-3, Table 6-1), as was observed earlier for the cornea of the goldfish (Figs. 5-10 & 5-11). The two classes of pigment acting together will effectively reduce the transmission of all wavelengths below 500nm.

Several species such as the lion fish (*Pterois volitans*) and seahorse (*Hippocampus ramulosus*) had no carotenoid-like pigment in the cornea and spectral transmission was reduced only by pigments absorbing radiation below 400nm (Table 6-1, Fig. 6-2). Although the precise chemical nature of the short-wave absorbing non-carotenoid compounds in the fish cornea has not been elucidated, by virtue of the similar λ_{max}s of pigments in both the cornea and the lens, it is likely that the majority of the pigments are mycosporine-like amino acids (Fig. 3-17). It may be that the pigments in the lens and cornea have a common origin such as the aqueous humour. Mycosporine compounds have previously been identified in

corneas and aqueous humours of fish with similar compounds in the lens [Dunlap *et al.*, 1989].

The tropical marine species examined are therefore likely to have asterina-330 (l_{max} 330nm) in both the lens (Chapter 3) and the cornea (Table 6-1). Similarly, the 360nm l_{max} pigment in the seahorse cornea (Fig. 6-2) is likely to be palythene as found in the lens of this species (Chapter 3). The l_{max} 325nm pigment also present in the seahorse cornea is likely to be palythine, although this pigment is not found at appreciable concentrations in the lens. One explanation for this may be that since palythene is known to be unstable, degradation into palythine may occur more rapidly in the cornea. The cornea will be exposed to higher intensity radiation than the lens which could possibly be a factor in the conversion of palythene to palythine (but see also Chapter 5).

In *Apistogramma curviceps*, the corneal pigment also has a different l_{max} to that found in the lens (Table 6-1). The lens pigment is likely to be 3-hydroxykynurenine with l_{max} 370nm (see Chapter 3), and is therefore a tryptophan catabolite rather than a mycosporine compound. However, whether the corneal pigment is palythene or kynurenine, both of which have near UV l_{max}s of 360nm, cannot be determined from the absorbance spectrum alone.

Although pigmented vitreous humours have been observed in fish before (for example the yellow vitreous observed in *Siganus rostratus* by Lythgoe, 1971), the humours examined here did not appear to have spectrally significant amounts of short-wave absorbing pigments (Fig. 6-5), the increasing

absorbance at wavelengths below 300nm is due to the vitreous proteins.

That two or more structures within a single eye may contain different pigments often absorbing in slightly different regions of the spectrum points to an important role in the absorbance of short-wave radiation.

CHAPTER SEVEN

LENS TRANSMISSION AND SHORT-WAVE ABSORBING COMPOUNDS IN OTHER VERTEBRATE LENSES

7.1 INTRODUCTION

Although considerable interest has been shown in the lens transmission of fish, little work has been carried out on the spectral characteristics and pigments of lenses in other vertebrate species with the notable exception of primates and squirrels [Cooper & Robson, 1959a&b; van Heyningen, 1971a&b; Zigman & Paxhia, 1988]. Early work reported the presence of yellow lenses in highly diurnal squirrel, snake and prairie dog species [Walls, 1931; Walls & Judd, 1933a&b], whilst lenses of many other species were colourless. Lens transmission spectra of a few species have also been available for a number of decades: cat [Weale, 1954a; Dodt & Walther, 1958], cattle [Pitts, 1959], rabbit [Kinsey, 1948; Wiesinger *et al.*, 1956] and pigeon [Emmerton *et al.*, 1980], but none of these appear from the spectra to have appreciable levels of pigment in the lens.

Kennedy & Milkman (1956) were the first to recognise that the high absorbance of the frog lens in the 300-400nm region was, as for a number of fish species, due to an extractable pigment, which remains unidentified. Cooper & Robson (1969b) later found that lens pigments in a number of primates could also be extracted and that all appeared to

have the same l_{max}. The major pigment in human and baboon lenses was identified as 3-hydroxykynurenine glucoside, by van Heyningen (1971a&b, 1973b), who also identified the squirrel lens pigment as the related N'acetyl-3-hydroxykynurenine, another tryptophan derivative [van Heyningen, 1971b].

Lens transmission was measured here in a number of bird, amphibian, reptilian and mammalian species and age-related changes in the lens of the macaque were investigated.

7.2 RESULTS

7.2.1 BIRDS

Lens transmission was examined in a number of 1 year old chickens (*Gallus gallus*). The chicken lens transmitted most of the incident radiation above 300nm (Fig. 7-1), with a mean 50% transmission of 334nm (S.D. 2.71, n=12). Although no obvious peaks were noticed, the transmission spectra did appear to have a slight shoulder around 320nm. The chicken lenses were therefore pooled for pigment extraction, but no absorbance was recorded above 300nm in the lens extract, indicating the absence of a detectable amount of short-wave absorbing compounds. The spectral absorbance of the cornea revealed that if any short-wave absorbing compounds were present, they were at very low concentrations (Fig. 7-2).

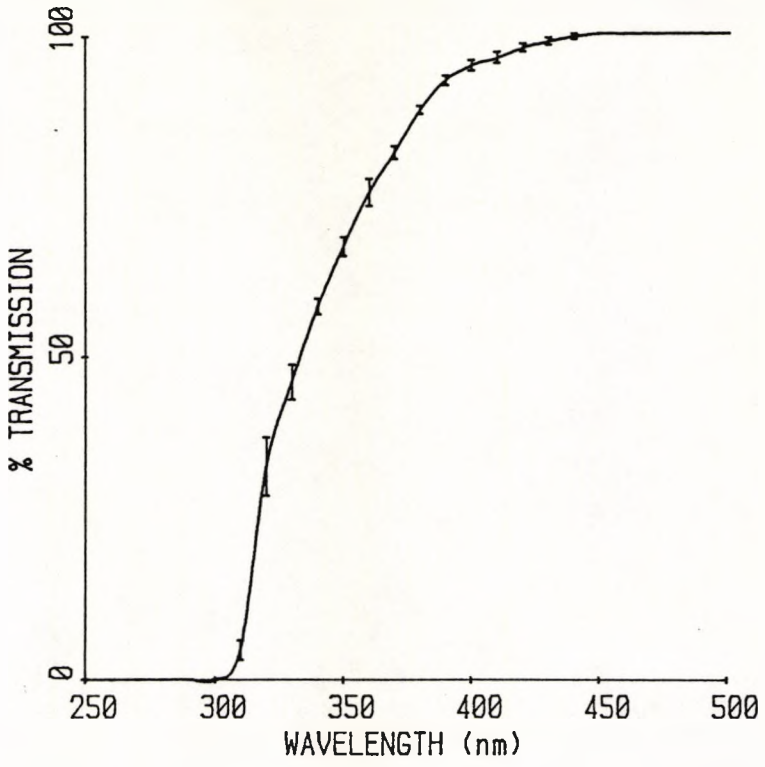


Figure 7-1. Averaged spectral transmission of the lenses of ten chickens with mean lens diameter 4.79mm measured in the anterior-posterior axis. The error bars represent the standard deviation and demonstrate that all lenses had very similar transmission.

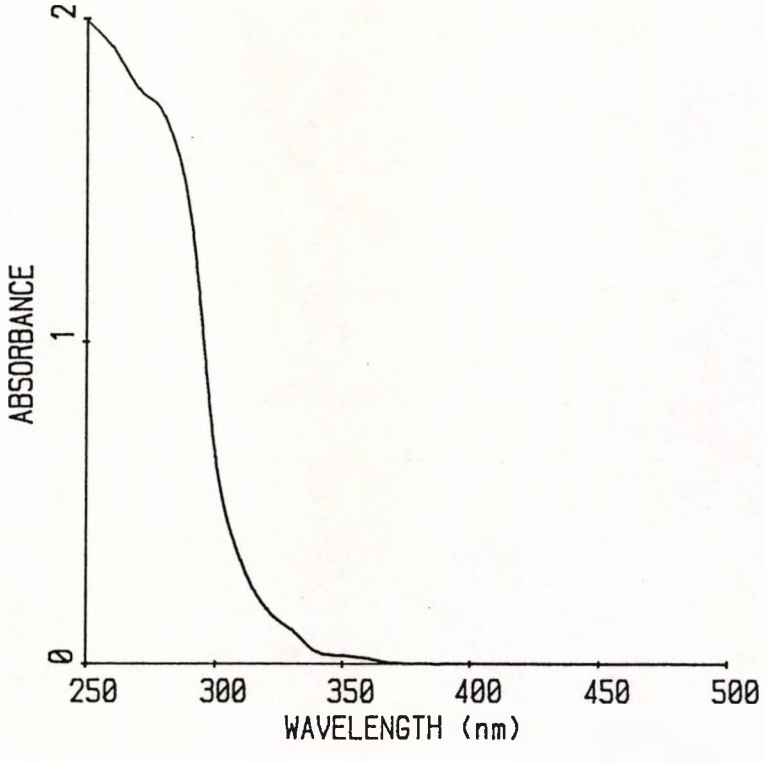


Figure 7-2. Spectral absorbance of the chicken cornea.

Spectral transmission measurements of the quail lens also indicated that no short-wave absorbing compounds were present in this species (data not shown).

7.2.2 AMPHIBIA

The transmission of the lenses from 4 amphibian species were measured. Lenses of the salamander (*Salamandra salamandra salamandra*), fire-bellied newt (*Cynops pyrrhogaster*) and axolotl (*Ambystoma mexicanum*) were found to have no pigmentation (Fig. 7-3, curves a, b & c), with lens transmission resembling that of many unpigmented fish lenses (Chapter 3). Corneas of the newt and salamander did not contain short-wave absorbing pigments (Fig. 7-4).

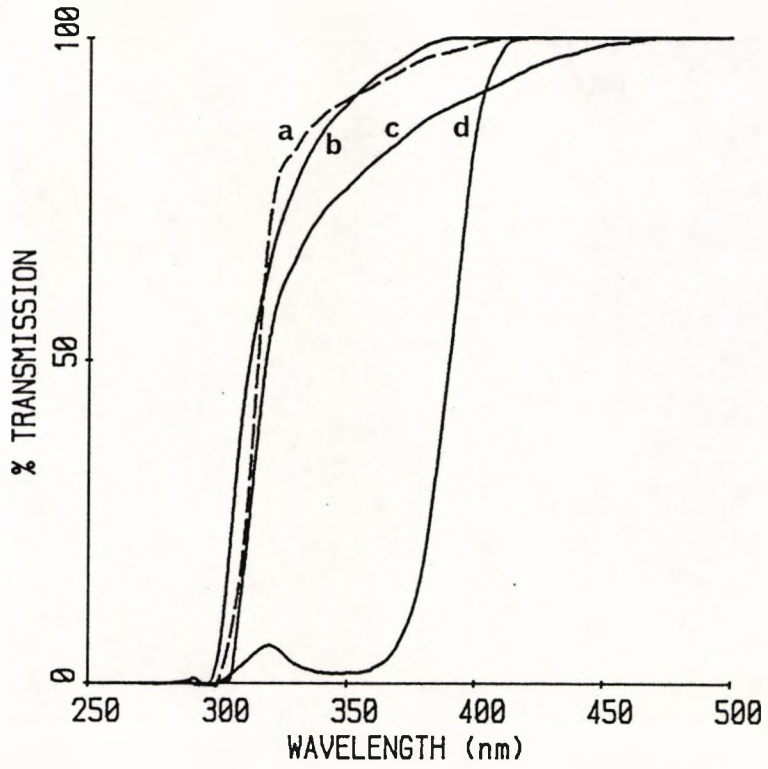


Figure 7-3. Spectral transmission of the lenses of four amphibians. (a) salamander with a lens diameter of 2.01mm, (b) newt with lens diameter 0.88mm, (c) a 1.63mm axolotl lens and (d) a 3.22mm frog lens.

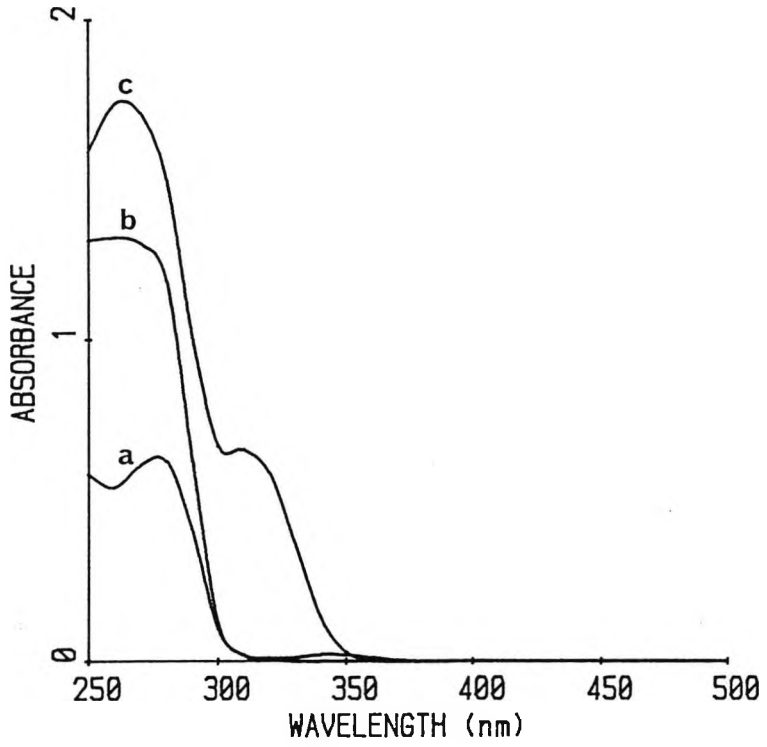


Figure 7-4. Spectral absorbance of the corneas of (a) newt, (b) salamander and (c) frog.

Lens spectral transmission in the common frog (*Rana pipiens*) was quite different to that of the other amphibian lenses examined (Fig. 7-3d), with a mean 50% transmission at 392nm (S.D. 1.80, n=16), indicating the presence of one or more short-wave absorbing compounds. Subsequently, a single pigment was extracted from the lens with a l_{max} of 347nm (Fig. 7-5), quite unlike the l_{max}s of pigments found in fish or other animals to date.

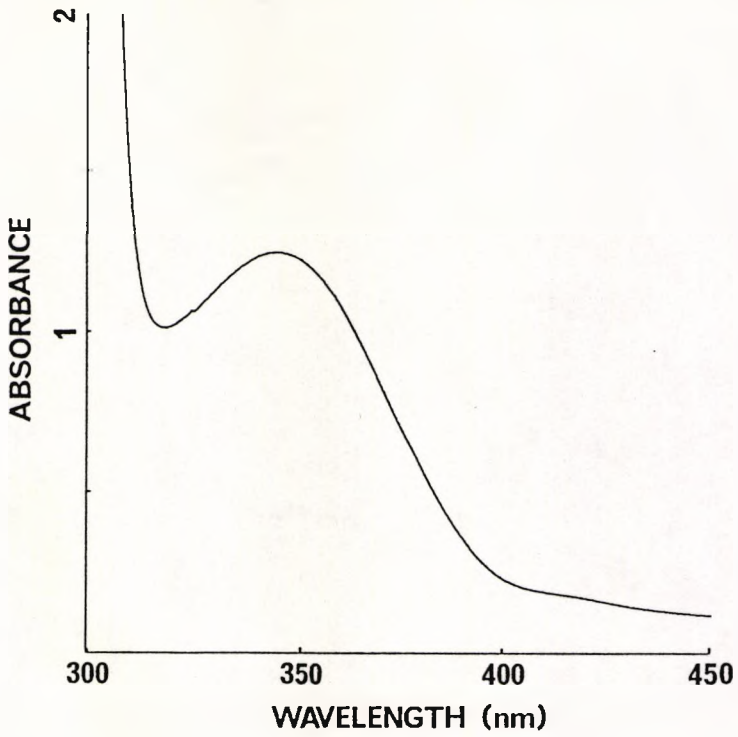


Figure 7-5. Spectral absorbance of the lens pigment extracted from 10 frog lenses.

Further identification of the frog lens pigment were impossible due to the instability of the isolated compound upon lyophilisation. The frog cornea also appears to contain a short-wave absorbing compound, with a l_{max} around 310nm (Fig. 7-4, curve c), the nictitating membrane however, did not contain detectable amounts of pigment.

7.2.3 REPTILES

Lens transmission was measured in two reptilian species, the yellow rat snake (*Elaphe guttata*) and the waterdragon (*Physignathus leseueri*). The waterdragon had no lens pigmentation, but the rat snake did appear to have low concentrations of short-wave absorbing compounds which caused shoulders on the transmission spectrum (Fig. 7-6).

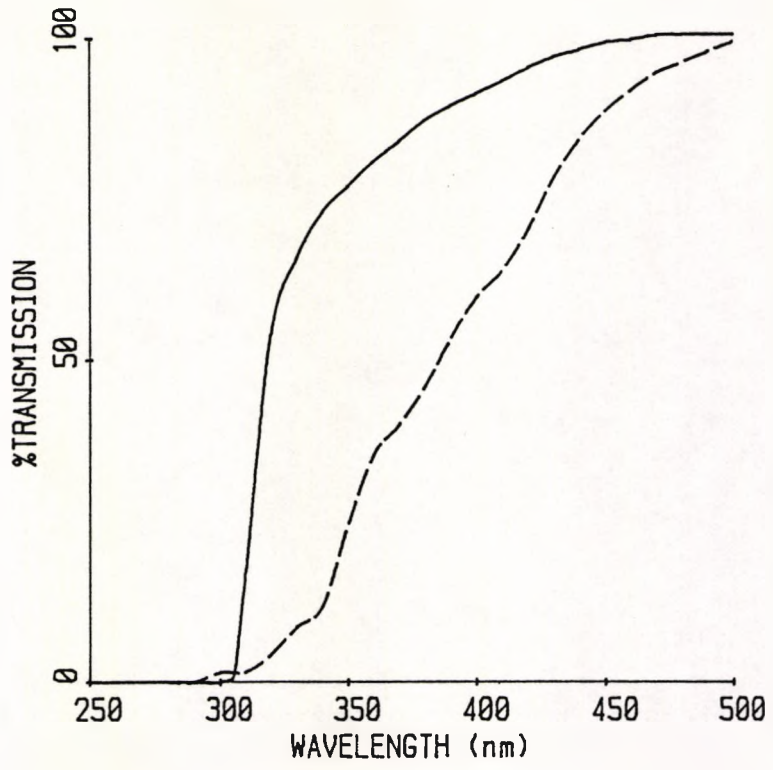


Figure 7-6. Spectral transmission of a 2.52mm diameter waterdragon lens (solid line) and a 2.89mm lens from the rat snake (dashed line).

Such shoulders (Fig. 7-6) may indicate the presence of more than one pigment in the lens. Unfortunately only one lens was available and no identification could therefore be attempted since the pigment concentration was low.

7.2.4 MAMMALS

7.2.4.1 Guinea Pig

The lenses of eight adult albino guinea pigs (*Cavia porcellus*) were found to have very similar transmission (Fig. 7-7b) with mean 50% transmission at 377nm (S.D. 2.02, n=11). Absorbance spectra of the crude lens extract (before filtration) revealed the presence of a short-wave absorbing pigment in the lens with a λ_{max} around 325nm (Fig. 7-8).

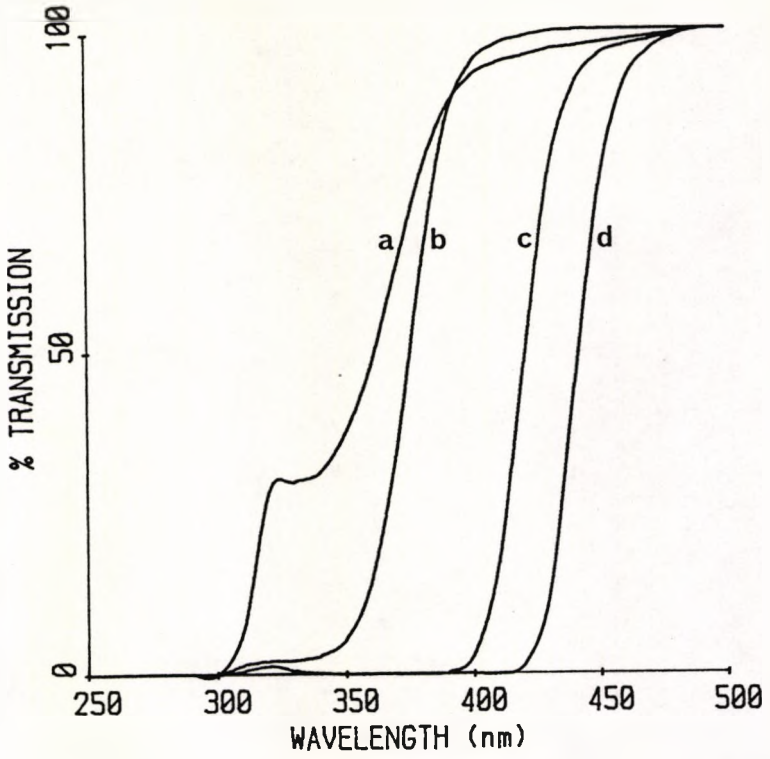


Figure 7-7. Spectral transmission of the lenses of four mammals. (a) a 2mm slice of the sheep lens, (b) a whole guinea pig lens with diameter 3.70mm (c) a 2.79mm macaque monkey lens and (d) a 3.60mm grey squirrel lens.

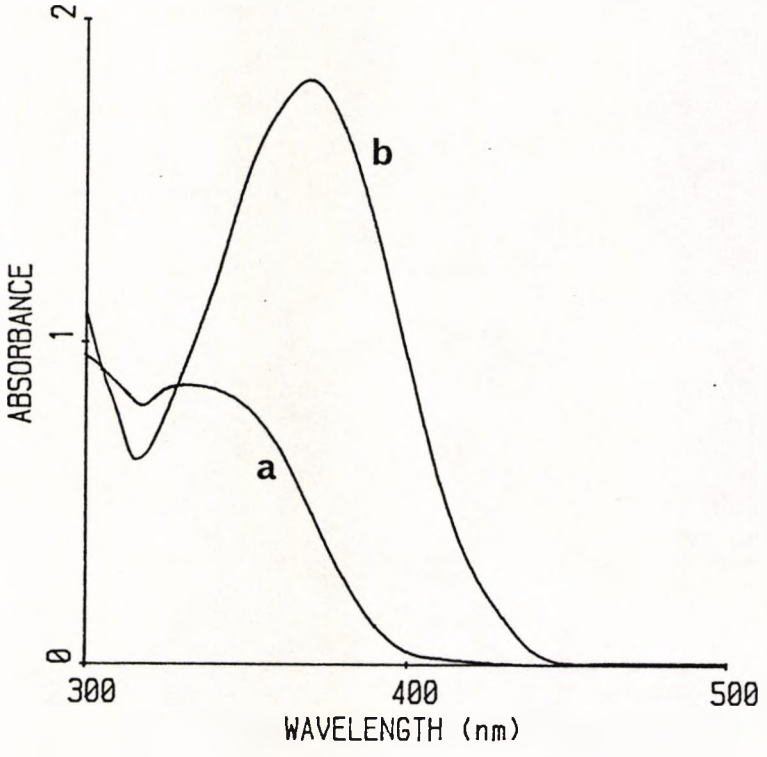


Figure 7-8. Spectral absorbance of the lens pigment extracts from (a) guinea pig and (b) squirrel.

The compound extracted from the lenses did not pass through the ultrafiltration cell used to purify pigments (see Chapter 2), suggesting either that it has a molecular weight greater than 1000Da or, more probably, that it is bound to the lens proteins. No short-wave absorbing compounds were present in the guinea pig cornea (Fig. 7-9).

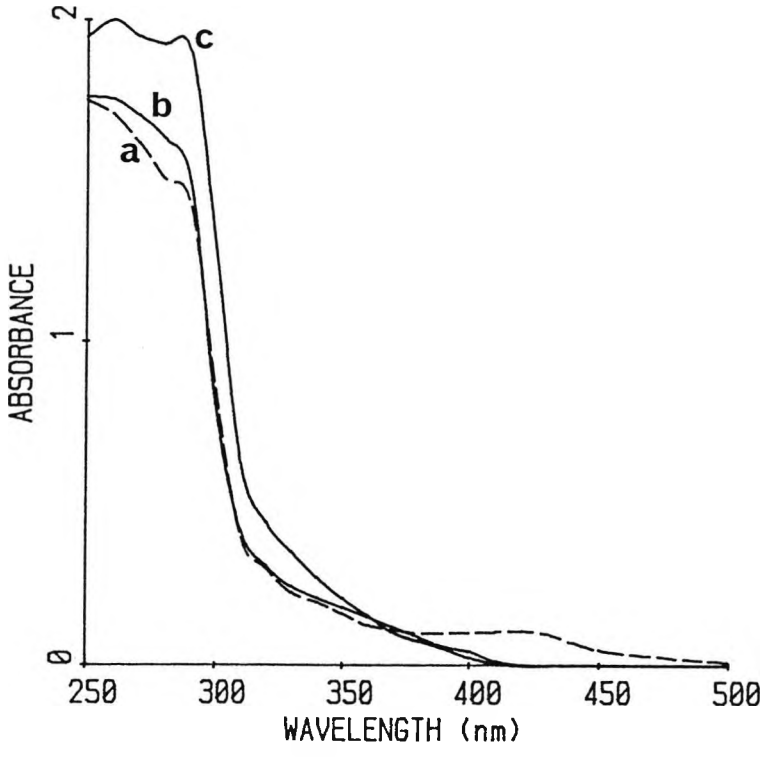


Figure 7-9. Spectral absorbance of the cornea of (a) guinea pig (b) squirrel and (c) sheep.

7.2.4.2 Sheep

Lenses were obtained from two freshly killed sheep (*Ovis ammon aries*). The lenses were above 15mm in their largest dimension and were therefore too large to scan whole in the spectrophotometer. The transmission of a 2mm section of a lens however, showed that it contained a compound

absorbing in the UV region (Fig. 7-7a), with a l_{max} around 330nm. The 50% transmission of the whole lens was estimated from that of the lens section to between 380-390nm.

There was no evidence from the spectral absorbance for a short-wave absorbing compound in the cornea of the sheep (Fig. 7-9). The lens pigment in the sheep appeared to be bound to the lens protein since it could not be extracted.

7.2.4.3 Squirrel

Both eyes of a single grey squirrel (*Sciurus carolinensis*) were obtained frozen. After allowing the eyes to thaw the lenses were removed and measured as usual. Both lenses were yellow in colour and the whole lenses transmitted little UV radiation with a 50% transmission wavelength of 441nm (Fig. 7-7d). The absorbance of the lens pigment extract confirmed the high concentration of a single pigment in the lens of the grey squirrel with a l_{max} of 368nm (Fig. 7-8). There was no indication, from the absorbance spectrum, of any short-wave absorbing pigments in the cornea (Fig. 7-9).

7.2.4.4 Primates

Lenses of three species of monkey were examined, the macaque (*Macaca fascicularis*), the marmoset (*Callithrix jacchus*) and the capuchin (*Cebus appelus*). Only one lens was available from each of 5 macaques. Two animals, one aged 10.5 years and one aged 12 years were considerably older than the other three whose ages ranged from 3 months to 1 year. The lenses were decapsulated as they had been donated

from another study involving lens capsules. Therefore the transmission spectra do not include the absorbance of the lens capsule.

In addition, as the capsules have been removed, the lens dimensions (Table 7-1) are slightly different to those for the intact lens. The dorsal to ventral lens measurements are a little higher and the anterior to posterior measurements correspondingly a little lower than is measured in the intact capsulated lens, due to the supportive role played by the capsule in determining lens shape.

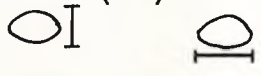
AGE (Yrs)	LENS MEASUREMENTS (mm) 		PIGMENT CONC. (Abs/mg protein)
0.27	3.70	6.40	0.534
0.92	3.10	7.10	0.552
1.07	3.60	7.40	0.498
10.5	3.10	8.10	0.429
12.0	3.00	8.20	0.213

Table 7-1. Measurements of both axial and equatorial diameters of the lenses of five macaque monkeys (*Macaca fascicularis*) of different ages. The concentration of the extracted λ_{max} 365nm pigment per mg of lens protein is shown for each lens (only one lens was available from each animal).

Spectral transmission was measured for four of the lenses and despite the large age differences, was remarkably similar for all lenses (Fig 7-10).

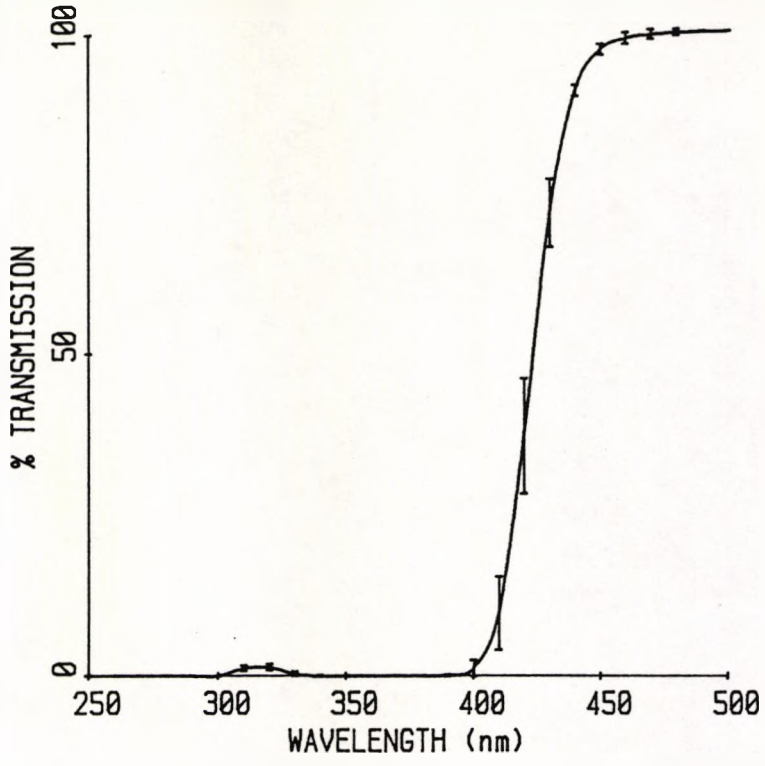


Figure 7-10. Average spectral transmission of five macaque monkey lenses, with average lens diameter 3.30mm. The error bars show the standard deviation.

The lenses examined were pale yellow and therefore had a high 50% transmission wavelength averaging 424nm (S.D. 1.9). Lens spectral transmission in the other two species of monkey was almost identical to that of the macaque (Fig. 7-11). Lenses of the capuchin and marmoset were generally scanned within 3 hours of death, however, a few lenses were frozen for up to 48 hours before measurements were made. No short-wave absorbing compounds were present in the cornea of the macaque (Fig. 7-12).

Absorbance at 370nm was measured for each of the primate lenses (e.g. Fig. 7-12). Values were very similar: macaque 2.57 (n=4, SD. 0.07), marmoset 2.52 (n=11, SD. 0.15), capuchin 2.46 (n=2). All lenses therefore contained equivalent concentrations of pigment.

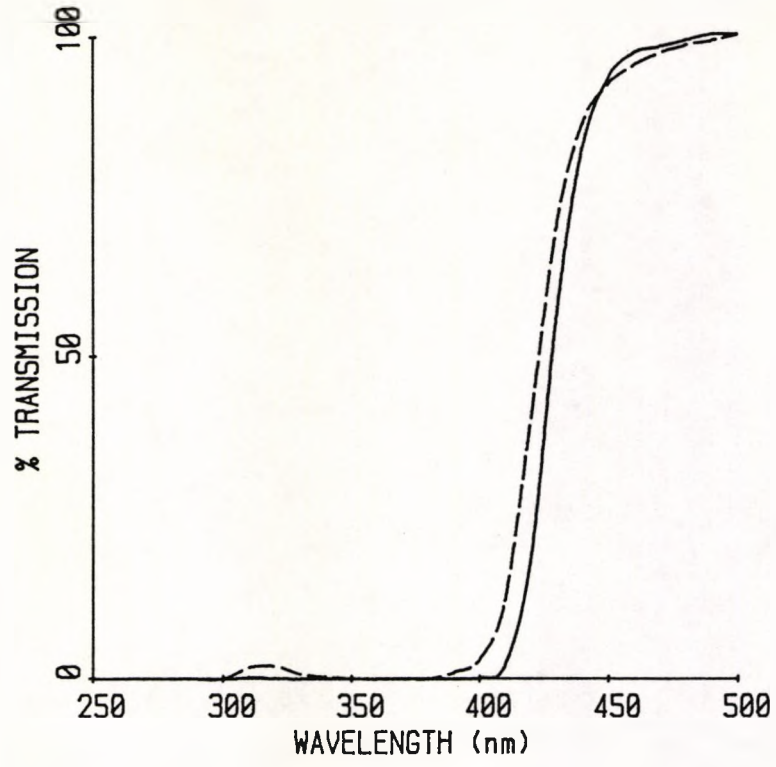


Figure 7-11. Spectral transmission of the lenses of a capuchin monkey with lens diameter 3.92mm (solid line) and a marmoset monkey with lens diameter 2.97mm (dashed line).

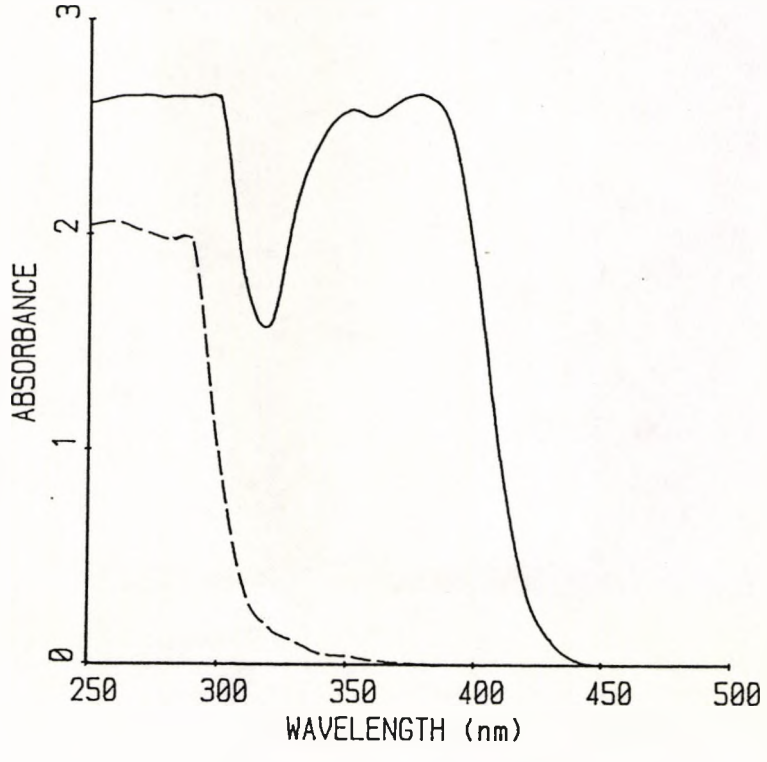


Figure 7-12. Spectral absorbance of the lens (solid line) and the cornea (dashed lens) of a macaque monkey.

When available both lenses of an animal were pooled and extracted together. Absorbance spectra of lens extracts revealed a single pigment with a l_{max} around 365nm in lenses of all three species (Fig. 7-13).

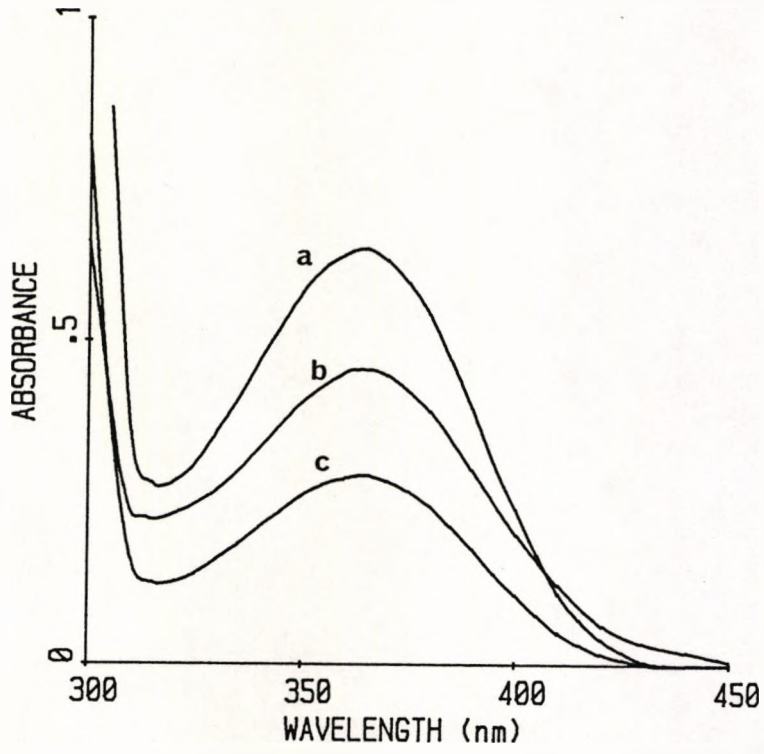


Figure 7-13. Absorbance of the crude lens pigment extracts from the three species of monkey examined. (a) macaque, (b) marmoset and (c) capuchin. Pigment concentrations are arbitrary, in reality all lenses contained approximately equal concentrations of pigment.

Mean pigment concentrations expressed in absorbance per mg of lens protein were also similar for all three species (Table 7-2). However, the concentration was considerably lower in the lens of the oldest macaque (Table 7-1).

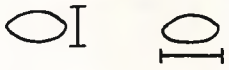
SPECIES	AGE (yrs)	LENS MEASUREMENTS (mm) 		LENS 50% T. (nm)	PIGMENT l_{max} (nm)	PIGMENT CONC. (abs/mg)
Macaque	0-12	3.30	7.44	424	364.1	0.445
Marmoset	2-3	2.58	4.46	427	365.2	0.390
Capuchin	30	3.56	8.24	426	364.5	0.480

Table 7-2. Summary of mean whole lens measurements and 50% transmission wavelengths for three species of monkey. The age ranges of each species are given as are the extracted lens pigment l_{max} and concentration.

The pigment extracted from marmoset and macaque monkey lenses was purified further by HPLC. Co-injection of the macaque lens pigment with 3-hydroxykynurenine from human lenses revealed it contain the same compound as the major pigment in young human lenses (Fig. 7-14). The marmoset lens pigment had a HPLC retention time of 5.9min., identical to that of the macaque and human lens (Fig. 7-14) and the marmoset lens too probably contains 3-hydroxykynurenine glucoside.

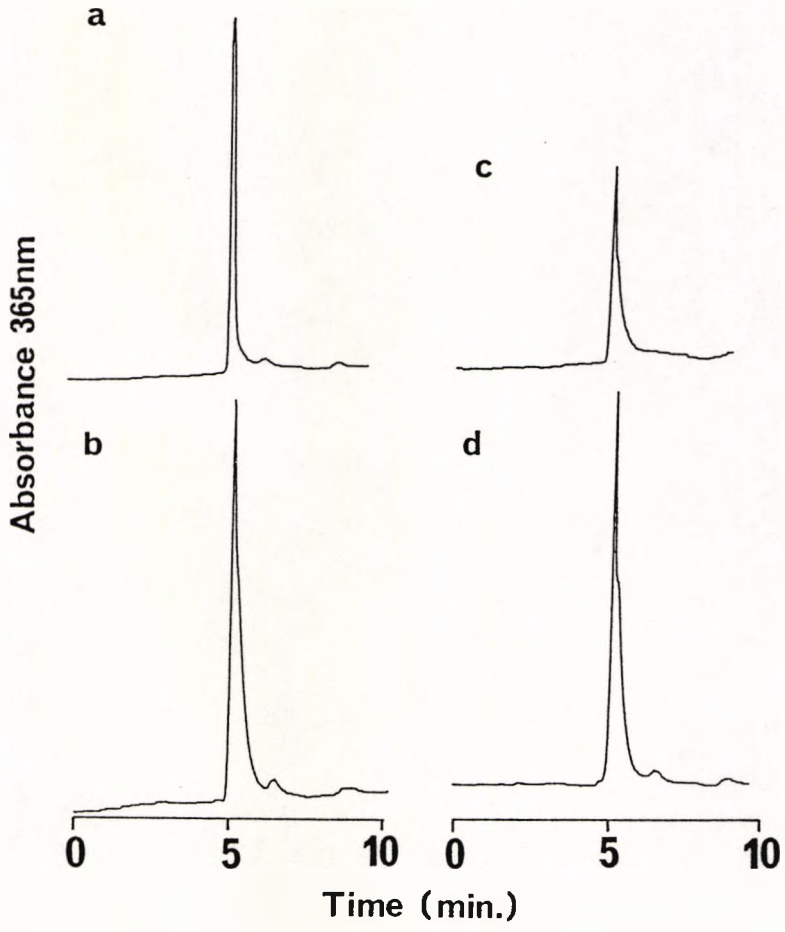


Figure 7-14. HPLC elution profiles of (a) the human lens pigment 3-hydroxykynurenine glucoside, (b) co-injected samples from macaque and human lenses, (c) macaque lens pigment alone and (d) marmoset lens pigment alone. All lenses contain a major compound eluting at 5.9min.

The short-wave absorbance confirmed the presence of a compound with l_{max} at 365nm and also a shorter wavelength l_{max} at 263nm (Fig. 7-15).

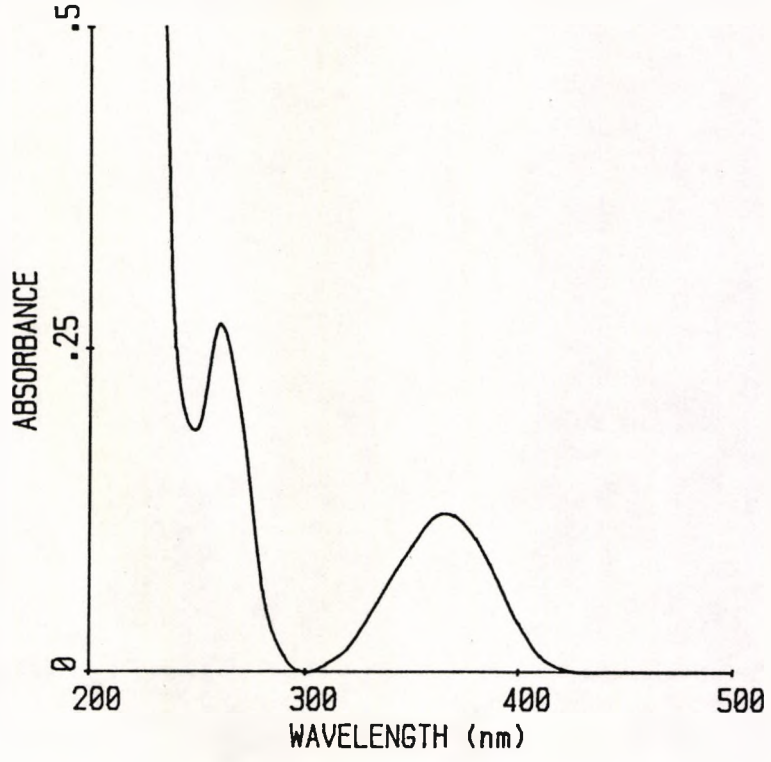


Figure 7-15. Spectral absorbance of the HPLC purified pigment from the macaque lens.

7.3 DISCUSSION

7.3.1 BIOCHEMICAL NATURE OF LENS PIGMENTS

Short-wave absorbing compounds were found in lenses of animals in three of the four remaining vertebrate groups including fish, and are therefore represented in four of the five classes of vertebrate. Lenses of the birds examined here (chicken - Fig. 7-1 and also quail) did not appear to have significant levels of short-wave absorbing compounds.

Both marmoset and macaque monkeys have been shown here to co-chromatograph with 3-hydroxykynurenine glucoside (3HKG), the major pigment in human and also baboon lenses [van Heyningen, 1971b], lending support to the prediction of Cooper and Robson (1969b) that the majority of diurnal primate species have the same lens pigments. It is likely therefore that the presence of 3HKG in the primate lens is a primeval characteristic which evolved in a common ancestor.

Lens extracts from the monkey and squirrel appeared to be reasonably stable upon lyophilisation and subsequent freezing. However, the relative instability of the extracted frog lens pigment and also its quite different λ_{max} of 345nm (Fig. 7-5), suggest that it is unlike either tryptophan-related pigments in primate and squirrel lenses or the mycosporine compounds found in fish lenses. Since frogs lead a predominantly aquatic lifestyle, it is possible that mycosporine-like compounds form a proportion of their diet as for fish and are present in the frog lens. Although only five mycosporine compounds have been isolated from fish

lenses (Chapter 3), many other different mycosporine analogues exist in other marine (and possibly also freshwater) invertebrates [Nakamura *et al.*, 1982]. However, without further analysis, no firm conclusions can be reached as to the identity of the frog lens pigment.

Also interesting is the presence of a short-wave absorbing compound in the frog cornea (Fig. 7-4), previously corneal pigments have been found only in fish (see Chapter 6). The pigment appears to have a l_{max} around 320nm and could be a tryptophan derivative (such as N'formyl-kynurenine, which has a l_{max} at 320nm) or one of the shorter wavelength absorbing mycosporines (see Fig. 3-17). Although the nature of the compounds is uncertain, pigments in the cornea and lens of the frog undoubtedly act as effective filters and together will restrict quite considerably the passage of wavelengths below 370nm.

Short-wave absorbing compounds are at low concentrations in the lenses of the rat snake, sheep and guinea pig (Figs. 7-6 & 7-7) and therefore do not produce as great a shift in transmission as seen in monkey and squirrel lenses (Fig. 7-7). Since pigments from neither sheep nor guinea pig lenses could be extracted, it is likely that they are bound to lens proteins and have a different biochemical nature to the free kynurenine derivatives in the lenses of the monkey and squirrel (as also indicated by different pigment l_{max}s).

7.3.2 AGE-RELATED CHANGES IN LENS PIGMENT CONCENTRATION IN MACAQUES

The concentration of 3HKG in the macaque lens, expressed per mg of lens protein, appears to fall with age (Table 7-1), whereas the 50% transmission wavelength (Fig. 7-10) and absorbance at 370nm were very similar. In addition, the axial diameters were more or less the same (Table 7-1), indicating similar concentrations of pigment within all lenses. A possible explanation for this discrepancy is that the short-wave absorbing pigments may bind to the lens proteins of older animals, as is thought to occur in the human lens [Grover & Zigman, 1972]. It would therefore be impossible to extract these pigments, but they would still contribute to whole lens absorbance.

Functions of the short-wave absorbing compounds in the lenses of vertebrates are discussed later in Chapter 9.

CHAPTER EIGHT

LENS TRANSMISSION AND SHORT-WAVE ABSORBING COMPOUNDS IN CEPHALOPOD LENSES

8.1 INTRODUCTION

Animals sharing the marine environment with fish include the invertebrate cephalopod molluscs: cuttlefish, octopods and squids. They have attained the largest size of any invertebrates, with one species of giant squid reported to have a total length of 16m [Barnes, 1980]. Cephalopod eyes are often very large, comprising up to 50% of the body weight in young animals [Saibil, 1990], making them attractive candidates for lens studies.

It has long been known that the development of the invertebrate eye occurs by a quite distinct pathway to that of the vertebrate [Lankester, 1875]. The cephalopod lens develops from lentigenic areas in the optic vesicle rather than from the ectoderm in contact with the optic vesicle as occurs in vertebrate lens development [Brahma, 1978]. The cephalopod eye does however, show remarkable functional similarities to the eyes of vertebrates, with the same optics and comparable sensitivity and resolution to the vertebrate eye [Saibil, 1990].

Lens pigment studies up to now, have been restricted to just one mesopelagic squid family - the Histioteuthidae.

These animals are unusual in that they have one large and one small eye. As the large lens points upwards its suggested function is to detect down-welling surface light, while the downward facing small lens may detect bioluminescence [Young, 1975]. Denton and Warren (1968) found that the large lenses of *Histioteuthis* were yellow whereas the much smaller lenses transmitted all wavelengths down to 310nm. Spectroscopic studies of *Histioteuthis meleagroteuthis* lens slices by Muntz (1976b) suggested the presence of two pigments in the large lens with l_{max}s of 385nm and 400nm. The smaller lens, although less pigmented than the larger one did contain some pigment [Muntz, 1976b], unlike the species examined earlier [Denton & Warren, 1968]. However, pigments in Histioteuthidae lenses have yet to be identified and their relationship, if any, to pigments in the fish lens ascertained.

Lenses from two species of squid and also the cuttlefish were examined here. The cuttlefish was studied over a wide size range to determine any ageing effects on the lens pigmentation.

8.2 METHODS

Lens diameter was measured in both the dorsoventral and anterior posterior plane. Whole lenses were scanned in the spectrophotometer (if size permitted) as for fish. The lens was then dissected into its three constituent parts (Fig. 8-1), and the thickness of each of these was

determined before they were scanned individually. Pigments were extracted as for fish (see chapter 2) from each separate part of the cephalopod lens, right and left eye lens parts of each animal being pooled.

8.3 RESULTS

8.3.1 CEPHALOPOD LENS STRUCTURE

The cephalopod lens is composed of three easily dissectible parts: a hard nucleus, an anterior bowl-like cortex and a more gelatinous posterior cortex (Fig. 8-1A). The suspensory ligaments attached to the lens at the junction between posterior and anterior lens parts. The nucleus was mushroom shaped with definite lips close to the front (Fig. 8-1B).

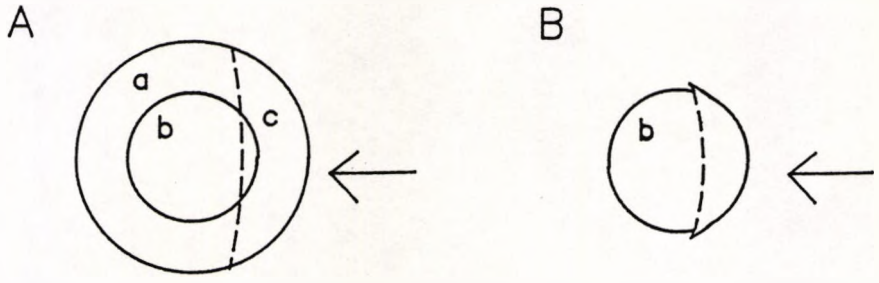


Figure 8-1. Diagrammatical representation of the structure of the cephalopod lens. A. Whole lens with three constituent parts: (a) posterior cortex (b) nucleus and (c) bowl-shaped anterior cortex. The dashed line represents the position of attachment of the suspensory ligaments. B. Mushroom shaped lens nucleus with a visible lip. The arrows in both diagrams represent the direction of incident radiation.

8.3.2 *Sepia officinalis*

Both whole lens and nucleus diameters (measured along the visual axis) in *Sepia officinalis* were directly related to mantle length (Fig. 8-2). Lenses were almost spherical, but the diameter in the anterior-posterior axis was consistently 93% (S.D. 3, n=36) of the dorsoventral diameter irrespective of lens size.

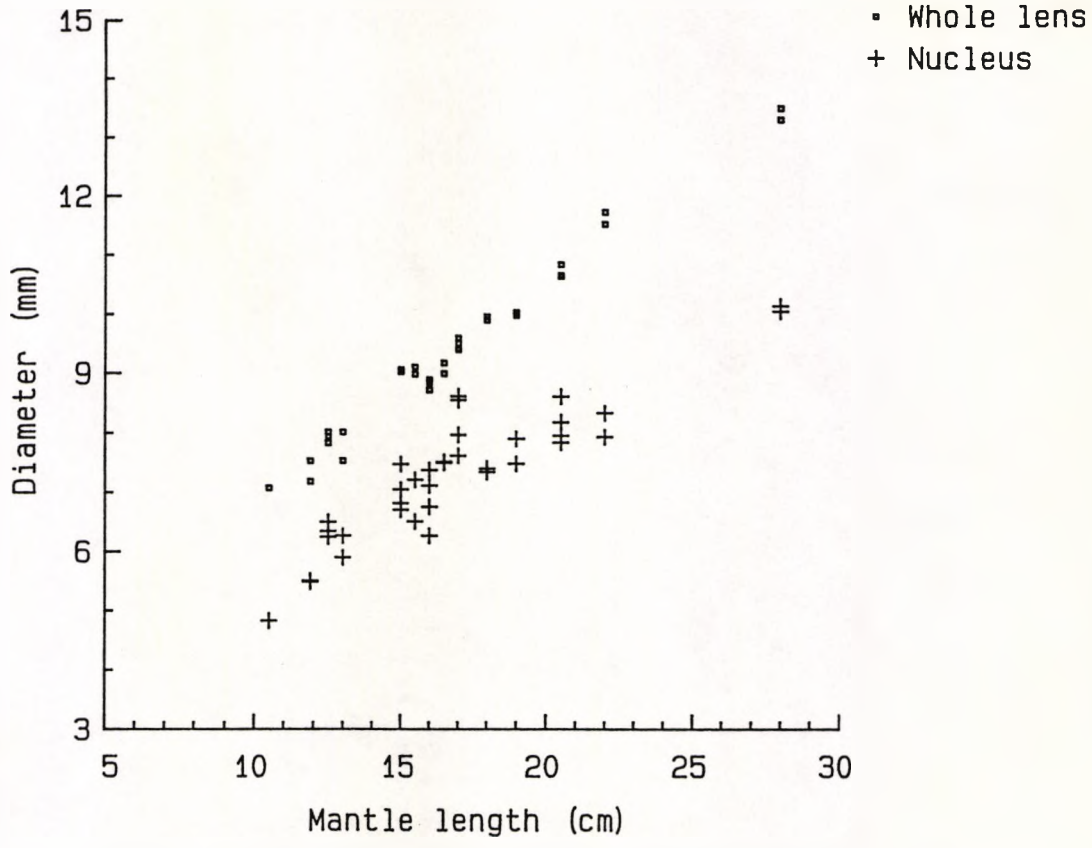


Figure 8-2. Nucleus and whole lens diameters, measured along the visual axis, as a function of the mantle length of all specimens of *Sepia officinalis* examined.

Although a large size (age) range of *Sepia officinalis* was examined (see Fig. 8-2), lens transmission was similar in all animals (e.g. Fig. 8-3).

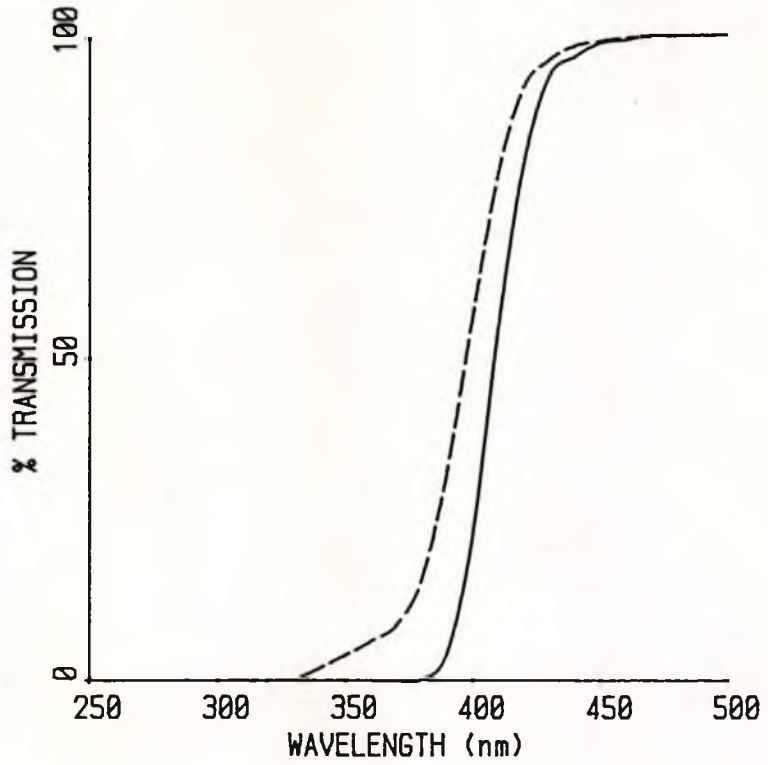


Figure 8-3. Spectral transmission of two whole *Sepia officinalis* lenses with lens diameters of 7.06mm (dashed line) and 9.05mm (solid line).

However, many *Sepia* lenses were too large to scan whole in the spectrophotometer. These lenses were split into the three constituent parts which were then scanned separately. All three parts of each lens contained pigments, although concentrations were generally highest in the anterior portion (part c, Table 8-1; Fig. 8-4c). This was also observed visually: the front bowl-like cortex occasionally appeared slightly yellow, whereas the other portions had no distinguishable colouration.

LENS PART	ABS/mm	S.D.
a	0.58	0.28
b	0.16	0.06
c	0.90	0.32

Table 8-1. Mean absorbance at 360nm per mm, for lens parts (a), (b) and (c) [refer to Fig. 8-1] of all 28 *Sepia officinalis* examined. Mean lens diameter 9.39mm (S.D. 1.61; range 7.06mm - 13.46mm).

Pigment concentrations in the nucleus were very much lower than measured in the two cortex lens parts (Table 8-1). Despite the large size range of animals examined (mantle lengths ranged from 8.9cm to 28.0cm), no changes in lens pigment concentration were observed for any of the lens parts (Table 8-1).

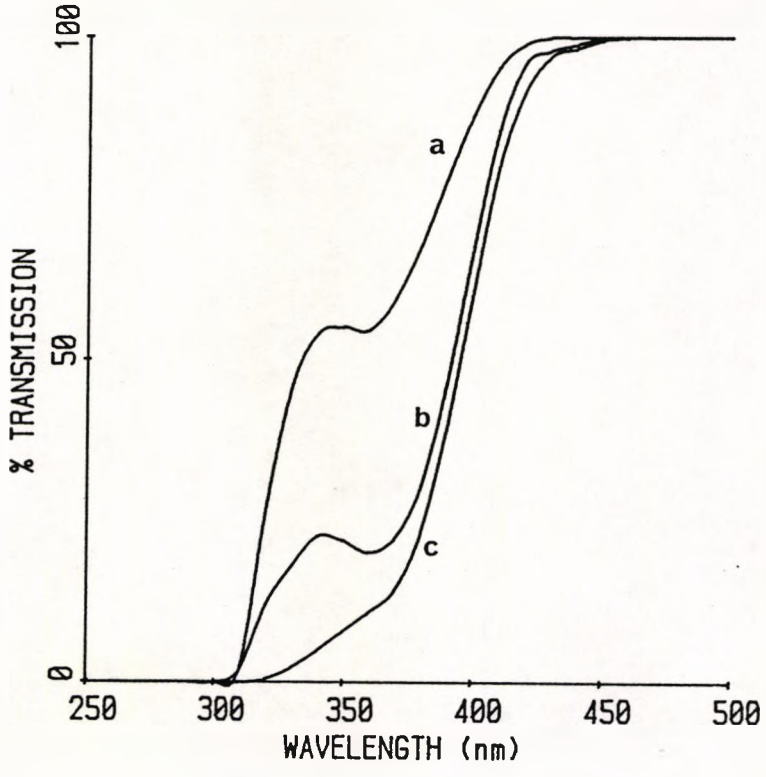


Figure 8-4. Spectral transmission of the lens parts of a *Sepia officinalis* lens. (a) the posterior cortex, 1.43mm thick, (b) the lens nucleus, 6.50mm in diameter and (c) the anterior cortex, 1.16mm thick.

Pigment extraction revealed that there were often differences, not only in the pigment concentration, but also in the pigment composition of the three lens parts. Most lenses contained only a l_{max} 360nm pigment but the anterior cortex from a number of individuals also had pigments with l_{max}s in the region of 320-340nm (Fig. 8-5).

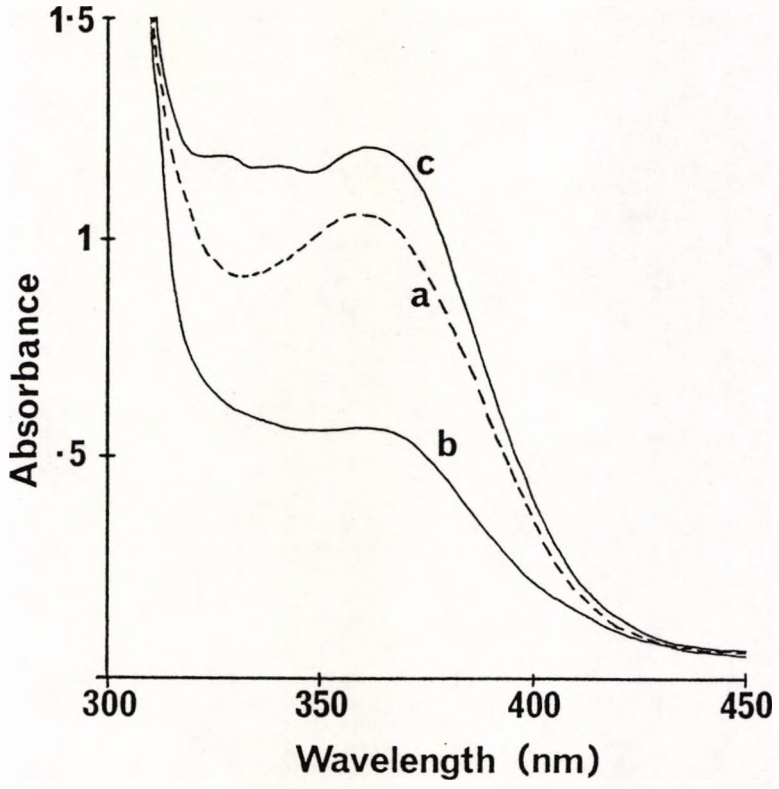


Figure 8-5. Spectral absorbance of the extracts from the 3 lens parts of 5 individual *Sepia officinalis* of similar mantle length (mean 18cm). (a) posterior cortex, (b) lens nucleus and (c) anterior cortex. Spectra were corrected for differences in extract volume.

8.3.3 *Loligo forbesi*

5 *Loligo forbesi* squid with mantle lengths between 16cm and 22cm were examined. Lenses were too large to scan whole in the spectrophotometer, with diameters averaging 9.29mm along the anterior posterior axis. The constituent parts were therefore scanned separately (Fig. 8-6). All three parts of the lenses of *Loligo forbesi*, contained short-wave absorbing compounds with a l_{max} at 320nm and a shoulder at 360nm (Fig. 8-7).

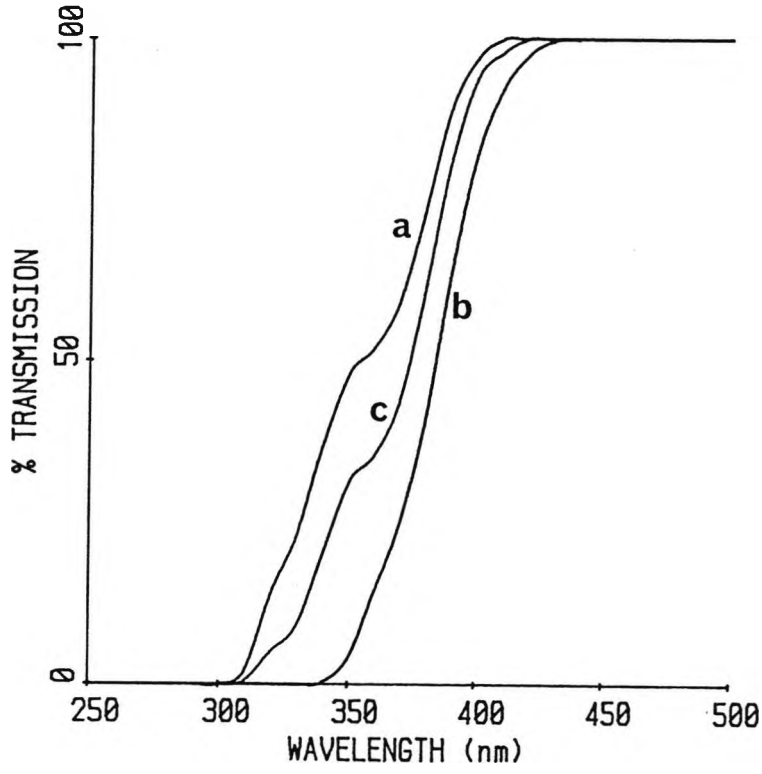


Figure 8-6. Spectral transmission of the three constituent lens parts of *Loligo forbesi*. (a) posterior cortex, 1.06mm thick (b) lens nucleus 6.86mm in diameter and (c) lens anterior cortex, 0.96mm thick.

In general, the anterior cortex (c) had the highest pigment concentration, whereas the nucleus (b) displayed the lowest concentration (Fig. 8-7). However, transmission was lowest in the nucleus (see Fig. 8-6) due to its longer pathlength and increased protein absorbance.

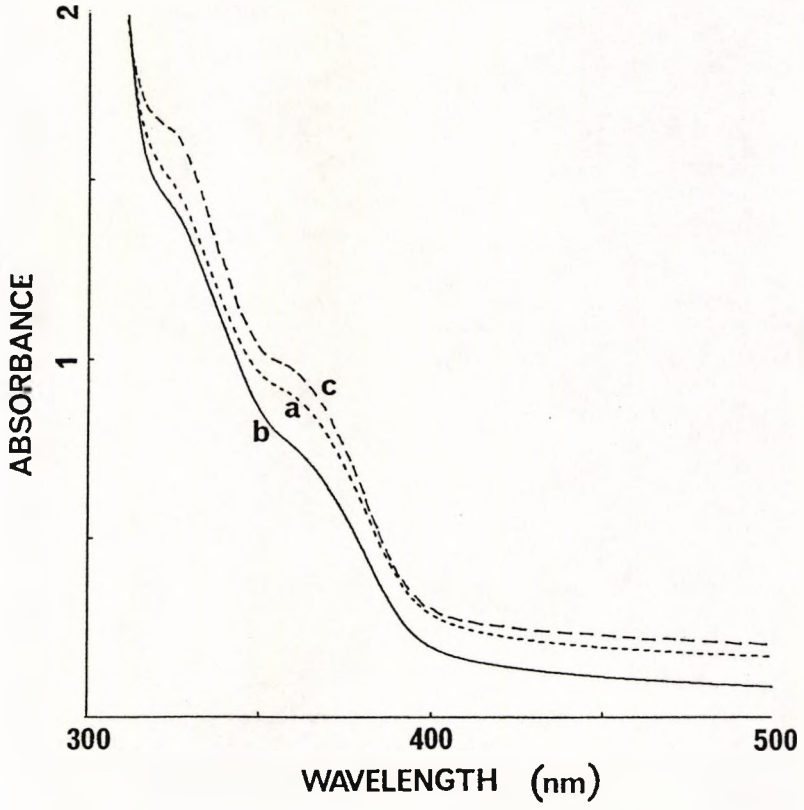


Figure 8-7. Spectral absorbance of extracts of the three constituent parts of the lenses from 5 *Loligo forbesi*. (a) posterior cortex, (b) lens nucleus and (c) anterior cortex. Spectra were corrected for differences in extract volume.

Lens extracts were purified further by HPLC (see Chapter 3 for methods). The pigment had a retention time of 5.13min. (Fig. 8-8) and collected peaks showed a complex absorbance spectrum with maxima at 320 and around 360nm (Fig. 8-9).

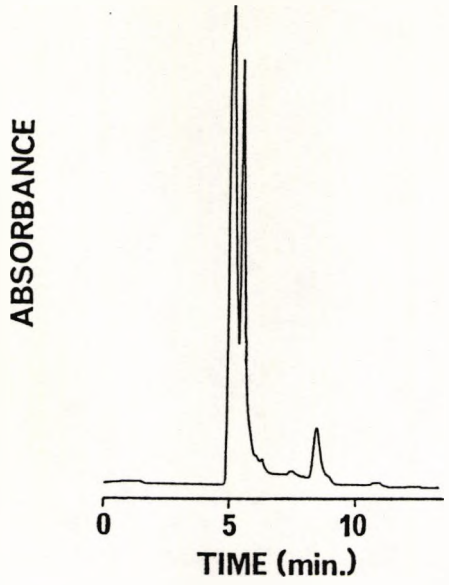


Figure 8-8. HPLC elution profile of the *Loligo forbesi* lens pigment. The major constituent elutes at 5.13min.

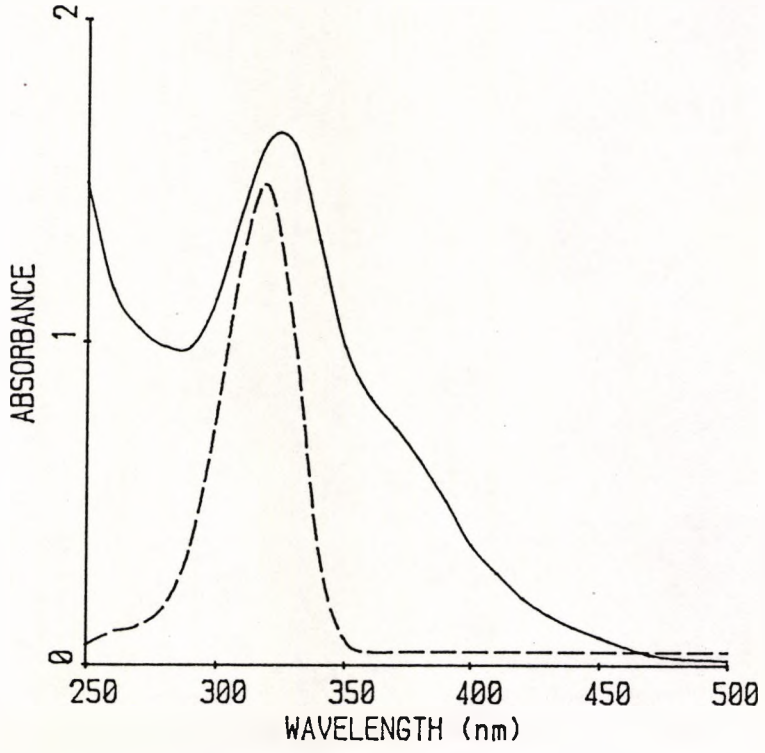


Figure 8-9. Spectral absorbance of the HPLC purified lens pigment from *Loligo forbesi*. The absorbance of palythine (l_{max} 320nm) from *Clupea harengus* is also shown (dashed line).

8.3.4 *Alloteuthis subulata*

Four *Alloteuthis subulata* ranging from 6.2cm to 15cm in mantle length were examined. Lenses were too small to split into their constituent parts, so were scanned whole in the spectrophotometer. Short-wave absorbing compounds were present, resulting in 50% values around 370-380nm (Fig. 8-10). No significant differences were observed in the lens transmission of the smallest and largest animals. Since the pigment concentration was low, and the lenses were quite small, no pigment extraction was possible.

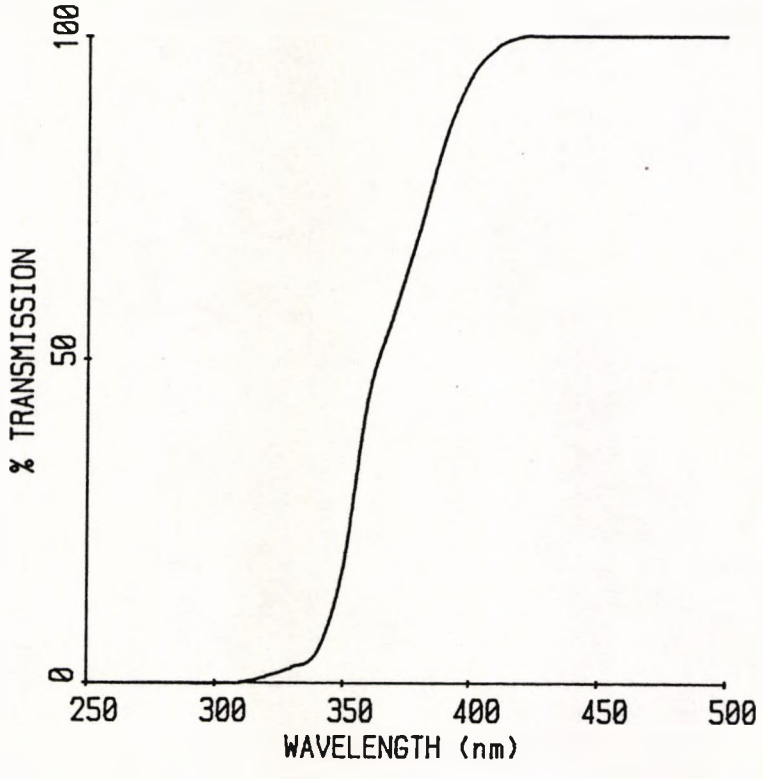


Figure 8-10. Spectral transmission of the whole lens of *Alloteuthis subulata*, with an axial lens diameter of 3.38mm.

8.4 DISCUSSION

8.4.1 CEPHALOPOD LENS STRUCTURE

The largest portion of the cephalopod lens, the nucleus, was rather like the nucleus of the fish lens (see Section 1.1), consisting of more tightly packed material and being much harder than the two cortex parts. The anterior cortex of the cephalopod lens and also the front portion of the nucleus (in front of the lips in Fig. 8-1B) develop from quite different lentigenic areas than the posterior portion of the nucleus and posterior cortex [Arnold, 1967a&b; Brahma, 1978].

8.4.2 LENS PIGMENT IDENTIFICATION AND DISTRIBUTION

The nucleus of both the *Sepia officinalis* and *Loligo forbesi* species examined here contained pigment, albeit at a consistently lower concentration than in the outer layers of the lens. The fact that the different lens portions have quite different pigment concentrations suggests that there are no junctions between the processes of the constituent parts through which the pigment could diffuse.

Although all three *Sepia officinalis* lens parts generally contained the same l_{max} 360nm pigment, the front portion of the lens in a few animals had additional l_{max}s at shorter wavelengths, around 320 and 340nm (Fig. 8-6). Muntz (1976b) also found that the pigment in the posterior lens portion of *Histioteuthis meleagroteuthis* had a different l_{max} from those found in the other two lens portions.

However, Muntz (1976b) found that pigment concentrations were highest in the posterior portion whereas for the animals examined here, pigment concentrations were usually much higher in the front portion of the lens (e.g. Fig. 8-5). It may be, since the anterior cortex will be exposed to the incident radiation, that photooxidative damage is occurring as is thought to cause yellowing of the lenses in some vertebrate species [Zigman, 1971] (but see also Chapter 5).

It is quite obvious that pigments in lenses of the epipelagic species of squid examined here are quite different to those in lenses of their relatives living at mesopelagic depths. Deep-sea squid have yellow lenses with pigment λ_{max}s of around 385 and 400nm [Muntz, 1976b]. The epipelagic species examined here were not yellow in colour but contained short-wave absorbing compounds with λ_{max}s of 320nm and 360nm (Figs. 8-6 & 8-8). A similar situation is observed in fish: a number of deep-sea fish lenses also have pigments with λ_{max}s around 400nm [Muntz, 1976b; Douglas & Thorpe, in press], whereas surface dwelling fish generally have shorter λ_{max}s in the region of 320-370nm (see Chapter 3).

As mycosporine compounds are widespread in the marine environment [Nakamura *et al.*, 1982], it would not be too surprising if they were also present in the cephalopod lens. The λ_{max}s of the pigments found in the lenses of *Sepia officinalis* and *Loligo forbesi* were similar to those of both the mycosporine-like and tryptophan compounds found in many fish lenses (Table 3-1). However, the two λ_{max}s of *Loligo*

lens extracts (Fig. 8-10) appear to belong to just one compound, unlike the 320nm and 360nm pigments in fish.

Lens proteins in cephalopods are functionally similar to vertebrate crystallins, but are chemically distinct since no immunological cross-reactions occur [Bon *et al.*, 1968]. HPLC analysis revealed that the compound in *Loligo* lenses appears quite different to both tryptophan and also to the mycosporine compounds isolated previously from fish lenses since the pigment had a different retention time (Fig. 8-9). The absorbance of the HPLC purified compound below 300nm (Fig. 8-10) is quite unlike that of pigments extracted earlier (Fig. 3-23) and initial NMR studies also point to a quite different structure.

CHAPTER NINE

FUNCTION OF SHORT-WAVE ABSORBING COMPOUNDS IN THE LENS

The fact that many vertebrate species from a range of habitats have short-wave absorbing pigments in the lens supports an important functional role within the eye. Suggested functions of lens pigments are diverse, but include increasing visual acuity, protection of the retina, stabilising lens protein, scavenging free radicals and as an aid in breaking bioluminescent camouflage in deep-sea animals (see Chapter 1).

Lens spectral transmission data, from both fish and other vertebrates, were consistent with the theory that nocturnal species and those living in low light environments generally do not have lens pigments, thereby maintaining optimum sensitivity, whereas animals which are diurnal tend to have quite highly pigmented lenses. The grey squirrel, for example, is highly diurnal, and has a cone dominated retina and a highly pigmented lens (Fig. 7-7). It is therefore ill-equipped for night vision. Many snakes are also reported to be diurnal and have highly pigmented yellow lenses [Walls, 1931]. The species examined in Chapter 7 (rat snake), although usually diurnal, is often nocturnal in summer months [Whitfield, 1983], which may explain the fact that the lens of this species is not as deeply pigmented as many highly diurnal species (Fig. 7-6).

Results from Chapters 3 and 7 suggest that short-wave absorbing compounds in the lens may serve to increase acuity since they are commonly found in animals with a requirement for high visual performance. Cichlid species for example often have highly pigmented lenses (Table 3.1; Douglas & McGuigan, 1989). All cichlids are predatory on other fish [Knoppel, 1970; Barlow, 1974] and display some level of parental care [Lowe-McConnell, 1969], both of which would appear to benefit from the potential increase in visual acuity offered by the presence of a short-wave absorbing compound in the lens. Similarly, gadidae, pleuronectidae and scorpaenidae species are all predatory [Wheeler, 1978; Bristow, 1987] and the majority also have high lens pigment concentrations (Table 3-1). In contrast, cyprinid fish, feed chiefly on zooplankton and detritus [Bristow, 1987] and usually have very little if any, lens pigmentation (Fig. 3-8).

The frog also has a high lens pigment concentration (Fig. 7-3), although it inhabits the same environment as salamanders and newts (which have no lens pigments, Fig. 7-3). Frogs however tend to be more diurnal and have lifestyles which result in exposure to much higher light levels than many other amphibians. They also have a feeding repertoire which involves catching insects in flight and are therefore animals relying heavily on vision. It could be that removal of the UV wavelengths from radiation incident on the retina has a role to play in the improvement of image quality in this species.

Alternative functions of lens pigments such as protection of the retina from effects of UV radiation, may also be of importance to animals exposed to high levels of illumination. It has not been definitely proved that short-wave absorbing compounds in the fish lens play a protective role against UV radiation damage, but there is evidence suggesting that mycosporine compounds similar to those found in the fish lens, do perform such a role in marine invertebrates [Jokiel & York, 1982; Dunlap *et al.*, 1986]. UV irradiation is reported to cause widespread, rapid photoreceptor loss in vertebrates [Lanum, 1978; Collier *et al.*, 1989]. However, all layers of the retina in the carp reared under high levels of UV (Chapter 5) appeared intact and were comparable to those of carp reared under both high and low levels of visible radiation (Fig. 5-19). It is therefore likely that UV fish had suffered minimal, if any, damage to retinal cells as a result of their intense and prolonged UV exposure. These animals had high concentrations of short-wave absorbing pigments in the lens (Figs. 5-15b & 5-17b) and the majority of the incident UV radiation will be absorbed before it has a chance to reach the retina. This suggests that lens pigments in fish may provide effective protection against UV radiation damage to the retina. Investigations into the effect of UV exposure on the retinas of aphakic squirrels and monkeys also support the theory that the lens acts to protect the retina from UV initiated damage [Collier *et al.*, 1989; Ham *et al.*, 1979 & 1982].

Evidence for a UV protective role played by short-wave absorbing lens pigments therefore appears quite strong. It is worth remembering however that the very animals requiring UV protection, that is those exposed to high light levels, will also be prone to the effects of glare and scatter of short-wave radiation. Therefore, even though lens pigments may be playing a primarily protective role, a secondary consequence of short-wave absorbing lens pigments will be the enhancement of visual acuity. Of course, as suggested in Chapter 1, not all animals with high concentrations of short-wave absorbing compounds are exposed to high light levels. The function of lens pigments in deep-sea animals for example, is definitely not one of UV protection since UV does not penetrate to the depths which they inhabit [Jerlov, 1951]. It could be that exposure to high light levels during embryonic stages of the life cycle is responsible for the pigmentation in some deep-sea species [Douglas & Thorpe, in press].

It is possible that the short-wave absorbing compounds in the lenses of some of the species examined here, serve another, quite different, function. For example, it has been suggested that the lens pigment in a deep-sea fish serves to stabilise the lens proteins to which it is bound [McFall-Ngai *et al.*, 1986]. Both the sheep and guinea pig lens pigments also appear to be bound to lens proteins and could possibly play a similar role. What is unlikely however, is that vertebrate lens pigments are completely functionless by-products of lens metabolism. Around 70% of the species examined here had some degree of lens

pigmentation. The pigments extracted from these lenses were identified as a variety of biochemically distinct compounds: mycosporine-like pigments, tryptophan derivatives and a number of unidentified pigments, all absorbing maximally in the region of 300-400nm (Chapters 3 & 7). Moreover, distinct short-wave absorbing compounds are often present in different tissues within the same eye (see Chapter 6). Such biochemical diversity confirms that short-wave absorbing lens pigments have evolved separately a number of times to serve an important function.

REFERENCES

Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239-247.

Andley, U.P. & B.A. Clark (1989). Generation of oxidants in the near-UV photooxidation of human lens α-crystallin. *Invest. Ophthalmol. Vis. Sci.* **30**, 706-713.

Appleby, S.J. & W.R.A. Muntz (1979). Occlusable yellow corneas in Tetraodontidae. *J. exp. Biol.* **83**, 249-259.

Arden, G.B. & K. Tansley (1955). The spectral sensitivity of the pure-cone retina of the grey squirrel (*Sciurus carolinensis leucotis*). *J. Physiol.* **334**, 592-602.

Arnold, J.M. (1967a). On the occurrence of microtubules in the developing lens of the squid *Loligo pealii*. *J. Ultrastruct. Res.* **14**, 534-539.

Arnold, J.M. (1967b). Fine structure of the development of the cephalopod lens. *J. Ultrastruct. Res.* **17**, 527-543.

Avery, J.A. & J.K. Bowmaker (1982). Visual pigments in the four-eyed fish *Anableps anableps*. *Nature* **298**, 62-63.

Avery, J.A., Bowmaker, J.K., Djamgoz, M.B.A. & J.E.G. Downing (1983). Ultra-violet sensitive receptors in a freshwater fish. *J.Physiol.* **334**, 23-24P.

Axelrod, H.R. & L.P.Schultz (1990). *Handbook of Tropical Aquarium Fishes*. Revised edition. T.F.H. Publications, Neptune City, New Jersey.

Baker, K.S., Smith, R.C. & A.E.S. Green (1982). Middle ultraviolet irradiance at the ocean surface: Measurements and models. In: *The Role of Solar UV Radiation in Marine Ecosystems*. J. Calkins (Ed.). Plenum Press, New York. Pp. 79-91.

Bando, M., Nakajima, A. & K. Satoh (1981). Spectrophotometric estimation of 3-OH L-kynurenine-O-ß-glucoside in the human lens. *J. Biochem.* **89**, 103-109.

Barlow, G.W. (1974). Contrasts in social behaviour between Central American cichlid fishes and coral-reef surgeon fishes. *American Zool.* **14**, 9-34.

Barnes, R.D. (1980). *Invertebrate Zoology*. 4th Edition. Holt-Saunders, Tokyo.

Bassi, C.J., Williams, R.C. & M.K. Powers (1984). Light transmittance by goldfish eyes of different sizes. *Vision Res.* **24**, 1415-1419.

Boehlert, G.W. (1985). Using objective criteria and multiple regression models for age determination in fishes. *Biol. Bull.* **83**, 103-117.

Boettner, E.A. & J.R. Wolter (1962). Transmission of the ocular media. *Invest. Ophthalmol.* **1**, 776-782.

Bon, W. F., Ruttenberg, G., Dohrn, A. & H. Batink (1968). Comparative physicochemical investigations on the lens proteins of fishes. *Exp. Eye Res.* **7**, 603-610.

Bone, R.A., Landrum, J.T. & S.L. Tarsis (1985). Preliminary identification of human macular pigment. *Vision Res.* **25**, 1531-1535.

Bone, R.A., Landrum, J.T., Fernandez, L. & S.L. Tarsis (1988). Analysis of the macular pigment by HPLC: retinal pigment distribution and age study. *Invest. Ophthalmol. Vis. Sci.* **29**, 843-849.

Bowmaker, J.K. & Y.W. Kunz (1987). Ultraviolet receptors, tetrachromatic colour vision and retinal mosaics in the brown trout (*Salmo trutta*): Age-dependent changes. *Vision Res.* **27**, 2101-2108.

Bowmaker, J.K., Dartnall, H.J.A. & P.J. Herring (1988). Long-wave sensitive visual pigments in some deep-sea fishes: segregation of 'paired' rhodopsins and porphyropsins. *J. Comp. Physiol. A* **163**, 685-698.

Bowmaker, J.K., Thorpe, A. & R.H. Douglas (1991). Ultraviolet-sensitive cones in the goldfish. *Vision Res.* **31**, 349-352.

Brahma, S.K. (1978). Ontogeny of lens crystallins in marine cephalopods. *J. Embryol. Exp. Morph.* **46**, 111-118.

Bridges, C.D.B. (1969). Yellow corneas in fishes. *Vision Res.* **9**, 435-436.

Bristow, P. (1987). (Ed.) *The Illustrated Book of Fishes.* Octopus Books, Prague.

Brügel, W. (1979). *Handbook of NMR Spectral Parameters.* Volume 1. Heydon & Sons, London.

Burkhardt, D.A. (1966). The goldfish electroretinogram: relation between photopic spectral sensitivity functions and cone absorption spectra. *Vision Res.* **6**, 517-532.

Calkins, J. (1982). (Ed.). *Light and Life in the Sea.* Plenum Press, New York.

Chalker, B.E. & W.C. Dunlap (1981). Extraction and quantitation of endosymbiotic algal pigments from reef-building corals. *Proc. 4th Int. Coral Reef Symp.* **2**, 45-50.

Chioccara, F., Della Gala A., De Rosa, M., Novellino, E. & G. Prota (1980). Mycosporine amino acids and related compounds from the eggs of fishes. *Bull. Soc. Chim. Belg.* **89**, 1101-1107.

Chou, B.R. & C.W. Hawrhyshyn (1987). Spectral transmittance of the ocular media of the bluegill (*Lepomis macrochirus*). *Can. J. Zool.* **65**, 1214-1217.

Collier, R. & S. Zigman (1987). The gray squirrel lens protects the retina from near-UV radiation damage. In: *Degenerative Retinal Disorders. Clinical and Laboratory Investigations.* J. Hollyfield (Ed.). Alan Liss, New York. Pp. 571-585.

Collier, R.J., Merrill, D., Waldron, W. & S. Zigman (1985). Increased susceptibility of rods to near-UV photic insult. *Photochem. Photobiol.* **41**, 123S.

Collier, R.J., Waldron, W.R. & S. Zigman (1989). Temporal sequence of changes to the gray squirrel retina after near-UV exposure.*Invest. Ophthalmol. Vis. Sci.* **30**, 631-637.

Cooper, G.F. & J.G. Robson (1967). Yellow lens pigment of the grey squirrel (*Sciurus carolinensis leucotis*). *J. Physiol.* **188**, 33-34P.

Cooper, G.F. & J.G. Robson (1969a). The yellow colour of the lens of the grey squirrel (*Sciurus carolinensis leucotis*). *J. Physiol.* **203**, 403-410.

Cooper, G.F. & J.G. Robson (1969b). The yellow colour of the lens of man and other primates. *J. Physiol.* **203**, 411-417.

de Jong, W.W. (1981). Evolution of lens and crystallins. In: *Molecular and Cellular Biology of the Eye Lens.* H. Bloemendal (Ed.). John Wiley & Sons, New York. Pp. 221-278.

de Jong, W.W., Hendriks, W., Mulders, J.W.M. & H. Bloemendal (1989). Evolution of eye lens crystallins: the stress connection. *T.I.B.S.* **14**, 365-368.

Denton, E.J. (1956). Recherches sur l'absorption de la lumière par le cristallin des poissons. *Bulletin of the Institute of Oceanography* **1071**, 1-10.

Denton, E.J. & F.J. Warren (1968). Eyes of the Histioteuthidae. *Nature* **219**, 400-401.

Denton, E.J. & N.A. Locket (1989). Possible wavelength discrimination by multibank retinae in deep-sea fishes. *J. Mar. Biol. Ass. U.K.* **69**, 409-435.

Denton, E.J., Herring, P.J., Widder, E.A., Latz, M.F. & J.F. Case (1985). The roles of filters in the photophores of oceanic animals and their relation to vision in the oceanic environment. *Proc. R. Soc. Lond. B* **225**, 63-97.

Dillon, J. & S.J. Atherton (1990). Time resolved spectroscopic studies on the intact human lens. *Photochem. Photobiol.* **51**, 465-468.

Dillon, J., Spector, A. & K. Nakanishi (1976). Identification of ß-carbolines isolated from fluorescent human lens proteins. *Nature* **259**, 422-423.

Dillon, J., Roy, D., Spector, A., Walker, M.L., Hibbard, L.B. & R.F. Borkman (1989). UV laser photodamage to whole lenses. *Exp. Eye Res.* **49**, 959-966.

Dodt, E. & J.B. Walther (1958). Netzhautsensitivitat, linsenabsorption und physikalische lichtstreuung. *Pflugers Archiv.* **266**, 167-174.

Douglas, R.H. (1986). Photopic spectral sensitivity of a teleost fish, the roach (*Rutilus rutilus*), with special reference to its ultraviolet sensitivity. *J. Comp. Physiol. A* **159**, 415-421.

Douglas, R.H. (1987). Ocular lens diameter as an indicator of age in brown trout, *Salmo trutta*. *J. Fish Biol.* **31**, 835-836.

Douglas, R.H. (1989). The spectral transmission of the lens and cornea of the brown trout (*Salmo trutta*) and goldfish (*Carassius auratus*) - Effect of age and implications for ultraviolet vision. *Vision Res.* **29**, 861-869.

Douglas, R.H. & McGuigan, C.M. (1989). The spectral transmission of freshwater teleost ocular media - an interspecific comparison and guide to potential ultraviolet sensitivity. *Vision Res.* **29**, 871-879.

Douglas, R.H. & A. Thorpe (in press). Short-wave absorbing pigments in the ocular lenses of deep-sea teleosts. *J. Mar. Biol. Soc. U.K.*

Douglas, R.H., Bowmaker, J.K. & Y.W. Kunz (1989). Ultraviolet vision in fish. In: *Seeing Contour and Colour.* (Proc. 3rd Int. Symp. of the Northern Eye Inst.). Kulikowski, J.J., Dickinson, C.M. & I.J. Murray (Eds.). Pergamon Press, Oxford. Pp. 601-616.

Duke-Elder, S. (1958). *The Eye in Evolution.* Volume I. System of Ophthalmology. Henry Kimpton, London.

Dunlap, W.C. & B.E. Chalker (1986). Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. *Coral Reefs* **5**, 155-159.

Dunlap, W.C., Chalker, B.E. & J.K. Oliver (1986). Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. *J. Exp. Mar. Biol. Ecol.* **104**, 239-248.

Dunlap, W.C., Williams, D.McB., Chalker, B.E. & A.T. Banaszak (1989). Biochemical photoadaptation in vision: UV-absorbing pigments in fish eye tissues. *Comp. Biochem. Physiol.* **93B**, 601-607.

Eberle, H. (1968). Zapfenbau, Zapfenlänge und Chromatische Aberration im Auge von *Lebistes reticulatus* Peters (Guppy). *Zool. Jb. Physiol.* **74**, 121-154.

Emmerton, J., Schwemer, J., Muth, I., & P. Schlecht (1980). Spectral transmission of the ocular media of the pigeon (*Columba livia*). *Invest. Ophthalmol.* **19**, 1382-1387.

Favre-Bonvin, J., Arpin, N. & C. Brevard (1976). Structure de la mycosporine (P-310). *Can. J. Chem.* **54**, 1105-1113.

Fernald, R.D. & S.E. Wright (1983). Maintenance of optical quality during crystalline lens growth. *Nature* **301**, 618-620.

Fisher, R.F. (1969). The significance of the shape of the lens and capsular energy changes in accommodation. *J. Physiol.* **201**, 21-47.

Goldschmidt, T. (1989). *Ecological Segregation in Zooplankivorous Haplochromine species (Pisces, Cichlidae) from Lake Victoria.* Ph.D. Thesis. University of Leiden.

Goldsmith, T.H., Collins, J.S. & S Licht (1984). The cone oil droplets of avian retinas. *Vision Res.* **24**, 1661-1671.

Goosey, J.D., Zigler, J.S. & K.H. Kinoshita (1980). Cross-linking of lens crystallins in a photodynamic system: a process mediated by singlet oxygen. *Science* **208**, 1278-1280.

Grant, P.T., Plack, P.A. & R.H. Thomson (1980). Gadusol, a metabolite from fish eggs. *Tetrahedron Lett.* **21**, 4043-4044.

Gregory, R.P.F. (1989). *Biochemistry of Photosynthesis* 3rd Edition. John Wiley & Sons, Chichester.

Grover, D. & S. Zigman (1972). Coloration of human lenses by near ultraviolet photo-oxidised tryptophan. *Exp. Eye Res.* **13**, 70-76.

Ham, W.T., Mueller, H.A., Ruffolo, J.J. & A.M. Clarke (1979). Sensitivity of the retina to radiation damage as a function of wavelength. *Photochem. Photobiol.* **29**, 735-739.

Ham, W.T., Mueller, H.A., Ruffolo, J.J., Guerry, D. & R.K. Guerry (1982). Action spectrum for retinal injury from near-ultraviolet radiation in the aphakic monkey. *Am. J. Ophthalmol.* **93**, 299-304.

Handelman, G.J., Dratz, E.A., Reay, C.C. & F.J.G.M. van Kuijk (1988). Carotenoids in the human macula and whole retina. *Invest. Ophthalmol. Vis. Sci.* **29**, 850-855.

Harosi, F.I. (1985). Ultraviolet- and violet-absorbing vertebrate visual pigments : dichroic and bleaching properties. In: *The Visual System.* MBL Lectures in Biology 5. Fein, A. & J.S. Levine (Eds.). Alan R. Liss, New York. Pp.41-55.

Hata, M. & M. Hata (1971). Carotenoid pigments in goldfish (*Carassius auratus*) I. Composition and distribution of carotenoids. *Int. J. Biochem.* **2**, 11-19.

Hawrhyshyn, C.W. & R.D. Beauchamp (1985). Ultraviolet photosensitivity in goldfish: an independent UV retinal mechanism. *Vision Res.* **25**, 11-20.

Hawrhyshyn, C.W., Chou, B.R. & R.D. Beauchamp (1985). Ultraviolet transmission by the ocular media of goldfish: implications for ultraviolet photosensitivity in fishes. *Can. J. Zool.* **63**, 1244-1251.

Hawrhyshyn, C.W., Arnold, M.G., Chaisson, D.J. & P.C. Martin (1989). The ontogeny of ultraviolet photosensitivity in rainbow trout (*Salmo gairdneri*). *Visual Neuroscience* **2**, 247-254.

Heinermann, P.H. (1984). Yellow intraocular filters in fishes. *Exp. Biol.* **43**, 127-147.

Herring, P.J. (1983). The spectral characteristics of luminous marine organisms. *Proc. R. Soc. Lond. B* **220**, 183-217.

Hirata, Y., Uemura, D., Ueda, K. & S. Takano (1979). Several compounds from *Palythoa tuberculosa* (Coelenterata). *Pure & Appl. Chem.* **51**, 1875-1883.

Jerlov, N.G. (1951). Optical studies of ocean waters. *Reports of the Swedish Deep-Sea Expedition* **3**, 1-59.

Jerlov, N.G. (1976). *Marine Optics*. Elsevier, Amsterdam.

Jokiel, P.L. & R.H. York (1982). Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. *Bull. Marine Sci.* **32**, 301-315.

Kennedy, D. & R.D. Milkman (1956). Selective light absorption by the lenses of lower vertebrates, and its influence on spectral sensitivity. *Biol. Bull.* **111**, 375-386.

Kinsey, V.E. (1948). Spectral transmission of the eye to ultraviolet radiations. *Arch. Ophthalmol.* **39**, 508-513.

Kirschfeld, K. (1982). Carotenoid pigments: their role in protecting against photooxidation in eyes and photoreceptor cells. *Proc. R. Soc. Lond. B* **216**, 71-85.

Knoppel, H.-A. (1970). Food of central Amazonian fishes - contribution to the nutrient-ecology of the Amazonian rainforest streams. *Amazoniania II* **3**, 257-352.

Kondrashev, S.L., Gamburtzeva, A.G., Gnjubkina, V.P., Orlov, O.Ju. & P.T. My (1986). Coloration of corneas in fish. A list of species. *Vision Res.* **26**, 287-290.

Lankester, E.R. (1875). Observations on the development of the Cephalopoda. *Quart. J. Microscop. Sci.* **15**, 37-47.

Lanum, J. (1978). The damaging effects of light on the retina. Empirical findings, theoretical and practical implications. *Surv. Ophthalmol.* **22**, 221-249.

Leach, C.M. (1965). Ultraviolet-absorbing substances associated with light-induced sporulation in fungi. *Can. J. Bot.* **43**, 185-200.

Lehninger, A.L. (1970). *Biochemistry*. Worth Publishers Inc. New York.

Lerman, S. (1980a). Lens transparency and aging. In: *Ageing of the lens*. Regnault, F., Hockwin, O. & Y. Courtois (Eds.). Elsevier, Amsterdam. Pp. 263-279.

Lerman, S. (1980b). Human ultraviolet radiation cataracts. *Ophthal. Res.* **12**, 303-314.

Lerman, S. (1984). Biophysical aspects of corneal and lenticular transparency. *Current Eye Res.* **3**, 3-14.

Lerman, S. & R. Borkman (1976). Spectroscopic evaluation and classification of the normal, aging, and cataractous lens. *Ophthal. Res.* **8**, 335-353.

Lerman, S., Mandal, K., Misra, B., Schechter, A. & J. Schenck (1991). Phototoxicity involving the ocular lens: *in vivo* and *in vitro* studies. *Photochem. Photobiol.* **53**, 243-248.

Loew, E.R. & H.J.A. Dartnall (1976). Vitamin A_1/A_2-based visual pigment mixtures in the cones of the rudd. *Vision Res.* **16**, 891-896.

Loew, E.R. & W.N. McFarland (1990). The underwater visual environment. In: *The Visual System of Fish*. Douglas, R.H. & M.B.A. Djamgoz (Eds.). Chapman & Hall, London. Pp. 1-43.

Lowe-McConnell, R.H. (1964). The fishes of the Rupununi savanna district of British Guiana, South America. Part I. Ecological groupings of fish species and effects of the seasonal cycle on the fish. *J. Linn. Soc. (Zoology)* **45**, 103-144.

Lowe-McConnell, R.H. (1969). The cichlid fishes of Guyana, South America, with notes on their ecology and breeding behaviour. *J. Linn. Soc. (Zoology)* **48**, 255-302.

Lundgren, C.H., Williams, T.R. & J.M. Nunnari (1986). Determination of the state and content of water in normal avian, fish, porcine, bovine and human lenses as studied by differential scanning calorimetry. *Ophthalmic Res.* **18**, 90-97.

Lythgoe, J.N. (1971). Iridescent corneas in fishes. *Nature* **233**, 205-207.

Lythgoe, J.N. (1975). The ecology, function and phylogeny of iridescent multilayers in fish corneas. In: *Light as an Ecological Factor II*. Evans, G.C., Bainbridge, R. & O. Rackham (Eds.). Halstead Press, New York. Pp.211-247.

Lythgoe, J.N. (1979). *The Ecology of Vision*. Clarendon Press, Oxford.

McCandless, R.L., Hoffert, J.R. & P.O. Fromm (1969). Light transmission by corneas, aqueous humor and crystalline lenses of fishes. *Vision Res.* **9**, 223-232.

McEwen, W.K. (1959). The yellow pigment of human lenses. *Am. J. Ophthalmol.* **47**, 144-146.

McFall-Ngai, M., Crescitelli, F., Childress, J. & J. Horwitz (1986). Patterns of pigmentation in the eye lens of the deep-sea hatchetfish *Argyropelecus affinis*, Garman. *J. Comp. Physiol. A* **159**, 791-800.

McFall-Ngai, M., Ding, L., Childress, J. & J. Horwitz (1988). Biochemical characteristics of the pigmentation of mesopelagic fish lenses. *Biol. Bull.* **175**, 397-402.

McFarland, W.N. (1986). Light in the sea - correlations with behaviors of fishes and invertebrates. *Am. Zool.* **26**, 389-401.

MacNichol, E.F., Kunz, Y.W., Levine, J.S., Harosi, F.I. & B.A. Collins (1978). Ellipsosomes: organelles containing a cytochrome-like pigment in the retinal cones of certain fishes. *Science* **200**, 549-551.

Maragos, J.F. (1972). A study of Hawaiian reef corals. Ph.D. Thesis, University of Hawaii. Cited by: Jokiel & York, 1982.

Marotte, L.R., Wye-Dvorak, J. & R.F. Mark (1979). Retinotectal reorganisation in goldfish: II. Effects of partial tectal ablation and constant light on the retina. *Neuroscience* **4**, 803-809.

Mellerio, J. (1971). Yellowing of the human lens: Nuclear and cortical contributions. *Vision Res.* **27**, 1581-1587.

Mellerio, J. (1987). Light absorption and scatter in the human lens. *Vision Res.* **11**, 129-141.

Mollon, J.D., Bowmaker, J.K. & G.H. Jacobs (1984). Variations of colour vision in a New World primate can be explained by a polymorphism of retinal photopigments. *Proc. R. Soc. Lond. B.* **222**, 373-399.

Moreland, J.D. & J.N. Lythgoe (1968). Yellow corneas in fishes. *Vision Res.* **8**, 1377-1380.

Muntz, W.R.A. (1972). Inert absorbing and reflecting pigments. In: *Handbook of Sensory Physiology*. Vol VII/1: *Photochemistry of Vision*. H.J.A. Dartnall (Ed.). Springer-Verlag, Berlin. Pp. 529-565.

Muntz W.R.A. (1973). Yellow filters and the absorption of light by the visual pigments of some Amazonian fishes. *Vision Res.* **13**, 2235-2254.

Muntz, W.R.A. (1976a). The visual consequences of yellow filtering pigments in the eyes of fishes occupying different habitats. In: *Light as an Ecological Factor II*. Evans, G.C., Bainbridge, R. & O. Rackham (Eds.). Halstead Press, New York. Pp. 271-285.

Muntz, W.R.A. (1976b). On yellow lenses in mesopelagic animals. *J. Mar. Biol. Ass. U.K.* **56**, 963-976.

Muntz, W.R.A. (1982). Visual adaptations to different light environments in Amazonian fishes. *Rev. Can. Biol. Exp.* **41**, 35-46.

Muntz, W.R.A. (1983). Bioluminescence and vision. In: *Experimental Biology at Sea*. Macdonald, A.G. & I.G. Priede (Eds.). Academic Press, London.

Muntz, W.R.A. (1990). Stimulus, environment and vision in fishes. In: *The Visual System of Fish*. Douglas, R.H. & M.B.A. Djamgoz (Eds.). Chapman and Hall, London. Pp. 491-511.

Nakamura, H. Kobayashi, J. & Y. Hirata (1982). Separation of mycosporine-like amino acids in marine organisms using reversed-phase high-performance liquid chromatography. *J. Chromatog.* **250**, 113-118.

Nelson, J.S. (1984). *Fishes of the World*. 2nd Edition. John Wiley & Sons, New York.

Neumeyer, C. (1986). Wavelength discrimination in goldfish. *J. Comp. Physiol. A.* **158**, 203-213.

Ohtsuka, T. (1985). Relation of spectral types to oil droplets in cones of turtle retina. *Science* **229**, 874-877.

Orlov, O.Y. & A.G. Gamburtzeva (1976). Changeable coloration of cornea in the fish *Hexagrammos octogrammus*. *Nature* **263**, 405-407.

Palmer, D.A. & J. Sivak (1981). Crystalline lens dispersion. *J. Opt. Soc. Am.* **71**, 780-782.

Partridge, J.C. (1989). The visual ecology of avian cone oil droplets. *J. Comp. Physiol. A* **165**, 415-426.

Penn, J.S. (1985). Effects of continuous light on the retina of a fish, *Notemigonus crysoleucas*. *J. Comp. Neurol.* **238**, 121-127.

Pitts, D.G. (1959). Transmission of the visible spectrum through the ocular media of the bovine eye. *Am. J. Optom. Physiol. Opt.* **36**, 289-298.

Plack, P.A., Fraser, N.W., Grant, P.T., Middleton, C., Mitchell, A.I. & R.H. Thomson (1981). Gadusol, an enolic derivative of cyclohexane-1,3-dione present in the roes of cod and other marine fish. *Biochem J.* **199**, 741-747.

Raymond, P.A. (1985). Cytodifferentiation of photoreceptors in larval goldfish - delayed maturation of rods. *J. Comp. Neurol.* **236**, 90-105.

Raymond, P.A., Bassi, C.J. & M.K. Powers (1988). Lighting conditions and retinal development in goldfish: photoreceptor number and structure. *Invest. Ophthalmol. Vis. Sci.* **29**, 27-36.

Saibil, H.R. (1990). Structure and function of the squid eye. In: *Squid as Experimental Animals*. Gilbert, D.L., Adelman, W.J. & J.M. Arnold (Eds.). Plenum Publishing, New York. Pp. 371-397.

Said, F.S. & R.A. Weale (1959). The variation with age of the spectral transmissivity of the living human crystalline lens. *Gerontologia* **3**, 213-231.

Shand, J. & J.N. Lythgoe (1987). Light-induced changes in corneal iridescence in fish. *Vision Res.* **27**, 303-305.

Shibata, K. (1969). Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant & Cell Physiol.* **10**, 325-335.

Sivak, J.G. (1974). The refractive error of the fish eye. *Vision Res.* **14**, 209-213.

Sivak, J.G. (1980). Accommodation in vertebrates: A contemporary survey. In: *Current Topics in Eye Research 3*. Zadunaissky, J.A. & H. Davson (Eds.). Academic Press, New York. Pp. 281-330.

Sivak, J.G. (1982). The contribution of the crystalline lens to chromatic and spherical aberrations of the eye. *Can. J. Optom.* **44**, 89-91.

Sivak, J.G. & W.R. Bobier (1978). Chromatic aberration of the fish eye and its effect on refractive state. *Vision Res.* **18**, 453-455.

Somiya, H. (1976). Functional significance of the yellow lens in the eyes of *Argyropelecus affinis*. *Marine Biology* **34**, 93-99.

Somiya, H. (1977). Bacterial bioluminescence in chlorophthalmid deep-sea fish: A possible inter-relationship between the light organ and the eyes. *Experientia* **33**, 906-909.

Somiya, H. (1979). 'Yellow lens' eyes and luminous organs of *Echiostoma barbatum* (Stomiatoidei, Melanostomiatidae). *Jap. J. Ichthyol.* **25**, 269-272.

Somiya, H. (1982). 'Yellow lens' eyes of a stomiatoid deep-sea fish *Malacosteus niger*. *Proc. R. Soc. Lond. B* **215**, 481-489.

Somiya, H. & T. Tamura (1971). On the eye of the 'yellow lens' fish *Chlorophthalmus albatrossis*. *Bull. Japan. Soc. Sci. Fish.* **37**, 840-845.

Stark, W.S. (1987). Photopic sensitivities to ultraviolet and visible wavelengths and the effects of the macular pigments in human aphakic observers. *Current Eye Res.* **6**, 631-638.

Strother, G.K. (1963). Absorption spectra of retinal oil globules in turkey, turtle and pigeon. *Exp. Cell Res.* **29**, 349-355.

Takano, S., Uemura, D. & Y. Hirata (1978a). Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **26**, 2299-2300.

Takano, S., Uemura, D. & Y. Hirata (1978b). Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **49**, 4909-4912.

Tamura, T. (1957). A study of visual perception in fish, especially on resolving power and accommodation. *Bull. Japan. Soc. Sci. Fish.* **22**, 536-557.

Thorpe, A., Truscott, R.J.W. & R.H. Douglas (1991). Kynurenine identified as the short-wave absorbing lens pigment in the deep-sea fish *Stylephorus chordatus*. *Exp. Eye Res.*, in press.

Truscott R.J.W., Carver, J.A., Thorpe, A. & R.H. Douglas (submitted). The identification of 3-hydroxykynurenine as the lens pigment in the gourami *Trichogaster trichopterus*. *Exp. Eye Res.*

van Best, J.A., Bollemeijer, J.G. & C.C. Sterk (1988). Corneal transmission in whole human eyes. *Exp. Eye Res.* **46**, 765-768.

van den Berg, T.J.T.P. & J.E.M. Mooij (1982). Eye media absorption in goldfish. *Vision Res.* **22**, 1229-1231.

van Heyningen, R. (1971a). Fluorescent glucoside in the human lens. *Nature* **230**, 393-294.

van Heyningen, R. (1971b). Fluorescent derivatives of 3-hydroxy-L-kynurenine in the lens of man, the baboon and the grey squirrel. *Biochem. J.* **123**, 30-31P.

van Heyningen, R. (1972). The human lens III. Some observations on the post-mortem lens. *Exp. Eye Res.* **13**, 155-160.

van Heyningen, R. (1973a). Assay of fluorescent glucosides in the human lens. *Exp. Eye Res.* **15**, 121-126.

van Heyningen, R. (1973b). The glucoside of 3-hydroxy-kynurenine and other fluorescent compounds in the human lens. In: *The Human Lens in Relation to Cataract.* Ciba Foundation Symposium 19. Elsevier, Amsterdam. Pp. 151-171.

van Heyningen, R. (1973c). Photo-oxidation of lens proteins by sunlight in the presence of fluorescent derivatives of kynurenine, isolated from the human lens. *Exp. Eye Res.* **17**, 137-147

van Heyningen, R. & J. Linklater (1976). Serine and threonine ethanolamine phosphate diesters, and some other unusual compounds in the lens of the cod fish (*Gadus morhua*) and haddock(*Gadus aeglefinus*). *Exp. Eye Res.* **23**, 29-34.

Villermet, G.M. & R.A. Weale (1972). Age, the crystalline lens of the rudd and visual pigments. *Nature* **238**, 345-346.

Wald, G. (1949). The photochemistry of vision. *Doc. Ophthalmol.* **3**, 94-137.

Wald, G. (1952). Alleged effects of near ultraviolet on human vision. *J. Opt. Soc. Am.* **42**, 171-177.

Wald, G.L. & D.R. Griffin (1947). The change in refractive power of the human eye in dim and bright light. *J. Opt. Soc. Am.* **37**, 321-336.

Walls, G.L. (1931). The occurrence of colored lenses in the eyes of snakes and squirrels, and their probable significance. *Copeia* **3**, 125-127.

Walls, G.L. & H.D. Judd (1933a). The intra-ocular colour-filters of vertebrates. *Brit. J. Ophthalmol.* **17**, 641-675.

Walls, G.L. & H.D. Judd (1933b). The intra-ocular colour-filters of vertebrates. *Brit. J. Ophthalmol.* **17**, 705-725.

Weale, R.A. (1954a). Light absorption in the crystalline lens of the cat. *Nature* **173**, 1049-1050.

Weale, R.A. (1954b). Light absorption by the lens of the human eye. *Optica Acta* **1**, 107-110.

Weale, R.A. (1973). The effects of the ageing lens on vision. In: *The Human Lens in Relation to Cataract.* Ciba Foundation Symposium 19. Elsevier, Amsterdam. Pp. 5-24.

Weale, R.A. (1988). Age and the transmittance of the human crystalline lens. *J. Physiol.* **395**, 577-587.

Weast, R.C. (1975). Ed. *Handbook of Chemistry and Physics*. 56th Edition, 1975-1976. CRC Press, Ohio.

Werner, J.S., Donnelly, S.K. & R. Kliegl (1986). Aging and human macular pigment density. *Vision Res.* **27**, 257-268.

Wheeler, A. (1978). *Key to the Fishes of Northern Europe*. Frederick Warne Ltd., London.

Whitfield, P. (1983). *Reptiles and Amphibians*. Longman Wildlife Library. Longman Group Ltd., Essex.

Whitmore, A.V. & J.K. Bowmaker (1989). Seasonal variation in cone sensitivity and short-wave absorbing visual pigments in the rudd *Scardinius erythrophthalmus*. *J. Comp. Physiol. A* **166**, 103-115.

Wiesinger, H., Schmidt, F.H., Williams, R.C., Tiller, C.O., Ruffin, R.S., Guerry, D. & W.T. Ham (1956). The transmission of light through the ocular media of the rabbit eye. *Am. J. Ophthalmol.* **42**, 907-910.

Williams, T.T., White, C.W. & W.S. Stark (1983). Ultraviolet visibility after cataract extraction. *Invest. Ophthalmol. Vis. Sci.* **24** (suppl.), 185.

Witte, F. (1981). Initial results of the ecological survey of the haplochromine cichlid fishes from the Mwanza Gulf of Lake Victoria (Tanzania): Breeding patterns, trophic and species distribution. *Neth. J. Zool.* **31**, 175-202.

Witte, F. (1984). Ecological differentiation in Lake Victoria haplochromines: Comparison of cichlid species flocks in African Lakes. In: *Evolution of Fish Species Flocks*. Echelle, A.A. & I. Kornfield (Eds.). University of Maine at Orono Press, Orono, Maine. Pp. 155-167.

Wood, W.F. (1989). Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet radiation. *Aquatic Botany* **33**, 41-51.

Yamanashi, B.S., Hacker, H. & G.K. Klintworth (1979). Wavelength dependence and kinetics of UV-induced free radical formation in the human cornea and lens. *Photochem. Photobiol.* **30**, 391-395.

Young, R.E. (1975). Function of the dimorphic eyes in the mid-water squid *Histioteuthis dofleini*. *Pacific Science* **29**, 211-218.

Yu, N.-T., Bando, M. & J.F.R. Kuck (1990). Localization of UV-induced changes in mouse lens. *Exp. Eye Res.* **50**, 327-329.

Yu, N.-T., Cai, M.-Z., Lee, B.-S., Kuck, J.F.R., McFall-Ngai, M. & J. Horwitz (1991). Resonance Raman detection of a carotenoid in the lens of the deep-sea hatchetfish. *Exp. Eye Res.* **52**, 475-479.

Zigler, J.S. & J.D. Goosey (1981). Photosensitized oxidation in the ocular lens: Evidence for photosensitizers endogenous to the human lens. *Photochem. Photobiol.* **33**, 869-875.

Zigler, J.S. & J.D. Goosey (1984). Singlet oxygen as a possible factor in human senile nuclear cataract development. *Current Eye Res.* **3**, 59-65.

Zigman, S. (1971). Eye lens color: Formation and function. *Science* **171**, 807-809.

Zigman, S. (1982). Yellow lens pigment: An adaptation for activity in bright sunlight. In: *The Role of Solar UV Radiation in Marine Ecosystems*. J. Calkins (Ed.). Plenum Press, New York. Pp. 399-405.

Zigman, S. (1983a). Effects of near ultraviolet radiation on the lens and retina. *Doc. Ophthalmol.* **55**, 375-391.

Zigman, S. (1983b). The role of sunlight in human cataract formation. *Surv. Ophthalmol.* **27**, 317-326.

Zigman, S. (1987). Tryptophan and ocular lens pigments. In: *Progress in Tryptophan and Serotonin Research 1986*. Bender, D.A., Joseph, M.H. & H. Steinhart (Eds.). Gruyter & Co., Berlin. Pp. 399-403.

Zigman, S. & S.J. Bagley (1971). Near ultraviolet light effects on dogfish retinal rods. *Exp. Eye Res.* **12**, 155-157.

Zigman, S. & P.W. Gilbert (1978). Lens colour in sharks. *Exp. Eye Res.* **12**, 155-157.

Zigman, S. & T. Paxhia (1988). The nature and properties of squirrel lens yellow pigment. *Exp. Eye Res.* **47**, 819-824.

Zigman, S. & T. Vaughan (1974). Near-ultraviolet light effects on the lenses and retinas of mice. *Invest. Ophthalmol.* **13**, 462-465.

Zigman, S., Yulo, T. & J. Schultz (1974). Cataract induction in mice exposed to near-UV light. *Ophthal. Res.* **6**, 259-270.

Zigman, S., Paxhia, T. & W. Waldron (1985). Biochemical features of the grey squirrel lens. *Invest. Ophthalmol. Vis. Sci.* **26**, 1075-1082.

Zigman, S., Paxhia, T. & W. Waldron (1988). Effects of near-UV radiation on the protein of the grey squirrel lens. *Current Eye Res.* **7**, 531-537.

Zigman, S., Paxhia, T., McDaniel, T., Lou, M.F. & N.-T. Yu (1991). Effect of chronic near-ultraviolet radiation on the gray squirrel lens *in vivo*. *Invest. Ophthalmol. Vis. Sci.* **32**, 1723-1732.

BIBLIOGRAPHY

Animal Behaviour.
McFarland, D. (1985). Pitman Press, Bath.

Molecular and Cellular Biology of the Eye Lens.
Bloemendal, H. (Ed.) (1981). John Wiley & Sons, New York.

Nuclear Magnetic Resonance Spectroscopy.
Harris, R.K. (1986). Longman Group Ltd., UK.

The Vertebrate Eye and its Adaptive Radiation.
Walls, G.L. (1963). Hafner, New York.

The Visual System of Fish.
Douglas, R.H. & M.B.A. Djamgoz (Eds.) (1990). Chapman & Hall, London.