The Study of Intertidal Mollusc Polymorphism Using Spectroradiometry and Visual Modelling

Katie Lawson Cruickshanks
Ecology and Evolutionary Biology Group
School of Biological Sciences
University of Southampton

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Abstract

Making associations between certain colours or patterns of an organism and its environments is fundamental in studies of animal coloration. These observations allow researchers to consider the evolutionary forces driving the appearance of the organism. Forces at work include thermoregulation, intraspecific communication and interspecific communication or predation. Where communication is occurring then the human visual sense may not always be appropriate to interpret signals given that visual capabilities may vary between taxa. The evolution and maintenance of genetic polymorphisms in molluscs is well documented although proof of the selective agents is seldom found. Shell polymorphisms in molluscs are proposed to exist by divergent selection in heterogeneous habitats by visually orientated predators although other non selective hypotheses have been suggested. All field correlations and selection tests using polymorphic molluscs have relied upon the human visual sense to judge the colours of the prey and its background. Here I present data concerning the discriminability of different morphs of intertidal littorinid species against natural backgrounds in the visual systems of potential predators. Two distinct habitats were studied, firstly the relatively simple high shore splash zone where *Littorina saxatilis* lives and secondly the complex algal canopy inhabited by *L. obtusata* and *L. fabalis*. By collecting reflectance spectra in the field and modelling it into the visual systems of predators I have shown that the extent of background matching in a particular *Littorina saxatilis* population is dependent on the shore geology and that greater background heterogeneity may provide more opportunities for crypsis. In populations of exposed and sheltered ecotypes of *L. saxatilis* chromatic differences between littorinids and backgrounds were observed which indicated that the populations subjected to predation by birds with high visual acuity are more cryptic than those preyed upon by crabs. On a smaller scale, background heterogeneity differences between lichen covered rock and bare rock were quantified. The extent to which different morphs of *L. saxatilis* matched these two background types was quantified in the visual systems of crabs and birds. I revealed that the more uniform background provided more opportunities for *L. saxatilis* cryptic which contradicts the patterns observed between shores dependent on geology. The algal habitat was studied in terms of visual heterogeneity of two different algal species and the extent to which different morphs of *L. obtusata* and *L. fabalis* matched the backgrounds and certain structural parts of the algae. In agreement with the lichen-bare rock system, the more uniform algal background (*Fucus serratus*) provided more opportunities for crypsis than the more visually heterogeneous algal species (*F. vesiculosus*). The results are discussed in terms of their relevance to visual selection and other non selective agents at different levels of background heterogeneity which may affect intertidal mollusc polymorphisms. Importantly the techniques used are assessed in terms of their appropriateness and suggestions are made for future research incorporating the visual signals available to different predators.

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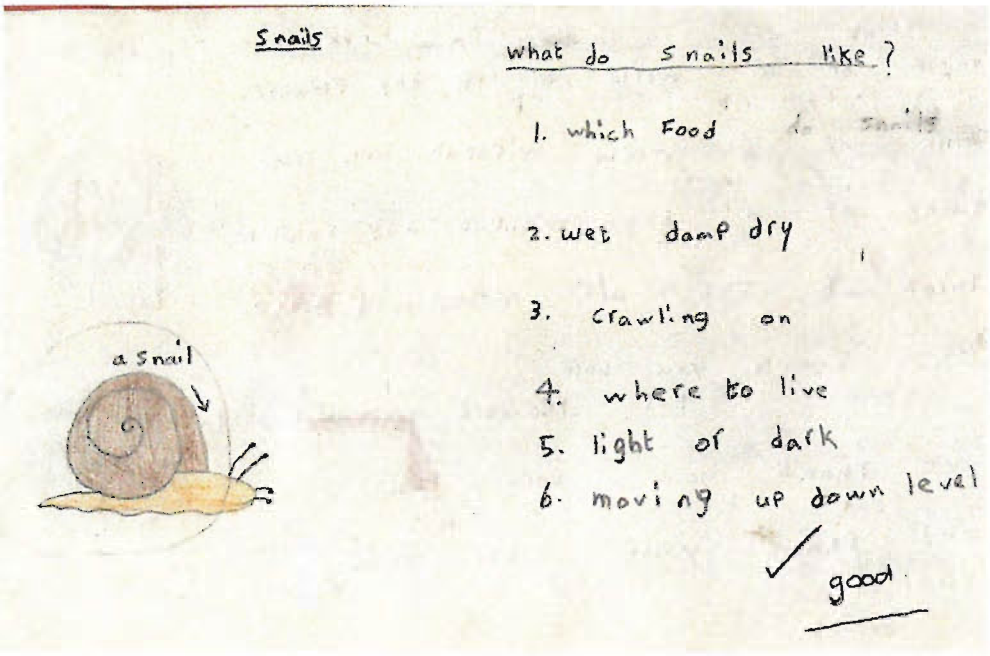
I acquired an interest in malacology at an early age……..



Snails

a snail

what do snails like ?

1. which Food do snails

2. wet damp dry

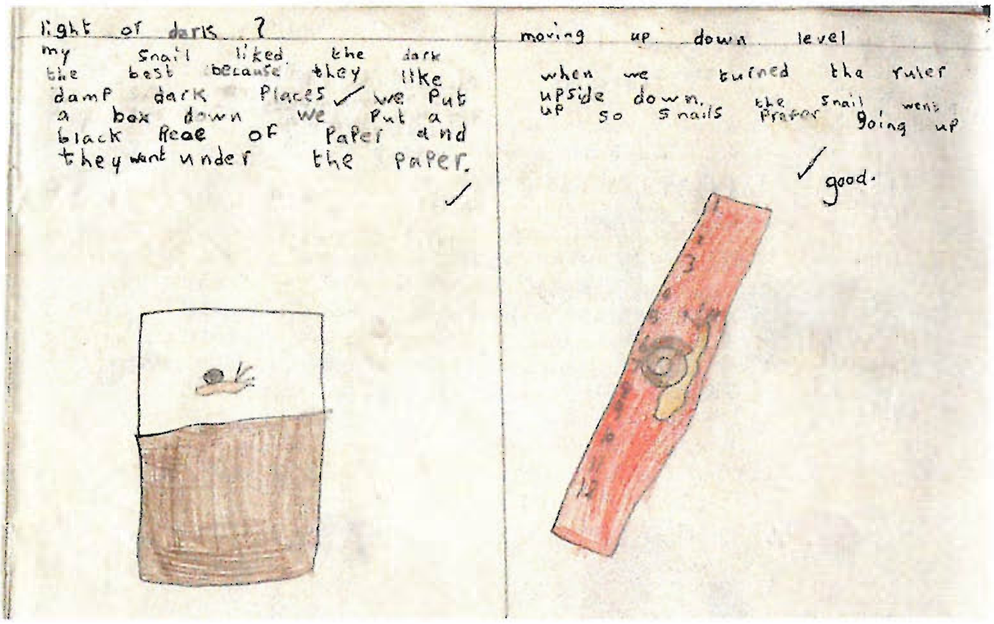
3. crawling on

4. where to live

5. light or dark

6. moving up down level

✓ good.



light or dark ?

my snail liked the dark the best because they like damp dark places ✓ we put a box down we put a black peae of paper and they want under the paper. ✓

moving up down level

when we turned the ruler upside down. the snail went up so snails prafer going up

✓ good.

PART I

INTRODUCTORY CHAPTERS

Chapter 1

Introduction

(1.1) The Visual Environment

Vision is a highly studied sensory mode, shaped by one unifying feature, the behaviour of light (Lythgoe, 1979; Bennett and Cuthill, 1994; Chiao *et al.,* 2000a). By selective absorption, reflection, and scatter, light shapes the photic environment of an organism (Lythgoe, 1979). It is the variety of light environments and the process of evolution that determines the appropriate visual mechanism for each visual task (Endler, 1993; Chiao *et al.,* 2000b; Sumner and Mollon, 2000; Fuller, 2002). Thus, perception of objects and events can differ remarkably not only between species but also between individuals of the same species (Lythgoe and Partridge, 1989).

Colour is a primary characteristic used by human observers in the judgement of appearance. It is a visual attribute of objects that results from the light they emit, transmit or reflect in the visible range of the electromagnetic spectrum (400-700nm). Due to the huge diversity of colours present in the environment, which themselves vary with the illuminating light, the ecology of vision presents many and often complicated avenues for investigation. In general, ecological investigations concentrate on the behaviour resulting from visual stimuli. For example resultant behaviours include courtship displays (Endler and Théry, 1996; Albert and Endler, 2004; Gomez and Théry, 2004), aggressive and territorial displays (Santee and Bakken, 1987; Sankaran, 1996) and defence against predators (Eterovick *et al.,* 1997; Macedonia *et al.,* 2002; Merilaita and Kaitala, 2002). These studies show that the relationship between the reflectance or colour pattern element of an object (Endler, 1978), and the perception by the 'receiver' of elements such as brightness, is often hard to define. It is the quest for this correlation that has led scientists to develop a wide variety of techniques to represent reflectance measurements in a standardised numerical form that is interchangeable between humans and other organisms of interest.

In trying to quantify and label the diversity of animal coloration, previous workers have produced several species-specific classification methods based on colour as perceived by the human observer (Pettitt, 1973a; Crook, 1997; Hanlon *et al.,* 1999). The use of classification systems is somewhat restricted because these methods do not allow the organism's visual appearance to be quantified in the visual system for which its colour signals were designed (*i.e.* that of a predator or conspecific). Moreover, attention must be given to the behaviour and ecology of the animal in question dictating the time of day and locations which determine the visual environment (Lythgoe and Partridge, 1989; Endler, 1990). Therefore, it is important to acknowledge that coloration is not an inherent property of an object, it is a product of the brain of the animal perceiving the object (Bennett *et al.*, 1994).

My thesis investigates shell colour polymorphism in littorinids. Spectroradiometry is used to objectively measure shell and background colour and visual modelling is used to quantify the signals available to predators. The effect of different levels of background heterogeneity on 'background matching' of littorinids is tested.

In the remainder of this introduction I first review the functions of animal coloration and resultant behavioural interactions between individuals using examples from many taxa (1.2). How interspecific interactions are mediated by vision is considered next (1.3), before focussing on selection and polymorphism in molluscs (1.4). The introduction concludes with a brief summary of the intertidal zone (1.5), the focus of my work, before outlining the rationale and specific objectives of the thesis (1.6).

(1.2) Animal Coloration

Coloration varies within a wide variety of animal taxa, including mammals (Kiltie, 1992), birds (Endler and Théry, 1996; Gomez and Théry, 2004), reptiles (Persons *et al.,* 1999; Stuart-Fox *et al.*, 2003), fish (Crook, 1997; Gamble *et al.,* 2003), amphibians (Summers *et al.,* 2003; Siddiqi *et al.,* 2004), crustaceans (Palma and Steneck, 2001) and insects (Cordero, 1992; Andrés *et al.,* 2002). Numerous surveys and experiments have shown that the general colours and tones of animals tend to match their backgrounds (Cain and Sheppard, 1950; Kettlewell, 1955; Endler, 1978, 1984, 1991; Whiteley *et al.,* 1997; Merilaita *et al.,* 1999; Heiling *et al.,* 2003, 2005). Many species within these taxa are genetically polymorphic in the sense that their populations consist of several discrete coexisting colour morphs.

Colour patterns of animals are believed to serve three main functions: thermoregulation, communication, and predator evasion (Endler, 1978). These factors act at intra- and interspecific levels and therefore the ability to separate the causal relationship between any of these three factors and the visible coloration is somewhat confused by their interaction. This interaction may, sometimes, result in a compromise between the separate functions of coloration (Cook, 1986a).

Some researchers have explained correlations between habitat type and body coloration as resulting from climatic selection that favours dark forms in shaded habitats, and lighter forms in open habitats (Lamotte, 1959; Bengston *et al.,* 1976; Etter, 1988; Cowie, 1990; Chang and Emlen, 1993). The role of coloration in thermoregulation is therefore a potentially important factor maintaining polymorphisms in different microclimates where gene flow occurs. Body coloration plays an important role in determining body temperatures of grasshoppers exposed to direct radiation, with black individuals having a mean temperature excess which is 49% greater than white individuals (Forsman, 1997). In particular, the thermoregulatory properties of the dog whelk, *Nucella lapillus* have been extensively investigated; physiological stress resulting from high temperature and desiccation is found to be greater on protected shores where lighter, predominantly white morphs of *N. lapillus* occur (Berry, 1983; Etter, 1988). Considerable variation in shell colour can be found at smaller scales such as within a single shore where sunnier and darker microhabitats are created. Even this small-scale variation can cause genotype-specific microhabitat partitioning, with lighter colour morphs being more common at sunnier sites and spending relatively more time in the sun than darker morphs (Harris and Jones, 1995).

Polymorphisms in the land snail *Theba pisana* have previously been explained as a balance between selection by climatic variables and predation (Heller, 1981; Cowie, 1990; Hazel and Johnson, 1990; Slowtow *et al.,* 1993). More specifically, Heller (1981) described crypsis against the shading of perennial vegetation to explain the distribution of dark morphs of *T. pisana,* however, conspicuous light morphs were maintained in the population due to favouring by climatic selection. The extent of behavioural differences, resulting in this microhabitat selection, was attributed to the relative complexity of the habitat types in the vicinity of the organism (Hazel and Johnson, 1990).

Despite the body of evidence supporting climatic selection, communication between organisms can affect the evolution of coloration signals. Intraspecific communication, in particular during courtship and competition (Allen, 1989), is the second of the three mediums through which selective agents may act. Through the quality of plumage, which can be decreased by parasites, male passerine birds compete for females therefore exemplifying sexual selection based on visual cues (Hamilton and Zuk, 1982). Interestingly, Endler (1991) found evidence of a trade-off between sexual selection and predator avoidance in guppies (*Poecilia reticulate*). Through the evolution of signals that compromise maximum conspicuousness to potential mates and minimum conspicuousness to potential predators, guppy coloration has accounted for the observation that both prospective mates and predators alike utilise the same sensory modes (Endler, 1991). Furthermore, specialised behaviour and colour patterns adapted for use at particular times and places will create a progressive reinforcing feedback; colour patterns and visually guided behaviour should coevolve in predictable directions, (otherwise known as "sensory drive", Endler, 1988) (Endler, 1990). A similar balance of the two selective forces creating and maintaining crypsis for protection and conspicuousness for mating has been observed in species of lekking forest birds (Endler and Théry, 1996).

In sexually dichromatic Australian lizards there is a complex interaction between coloration of body parts visible to conspecifics driven by sexual selection, and coloration of body parts visible to avian predators driven by natural selection when comparing body part coloration to the background (Stuart-Fox *et al.,* 2004). In the case of an environment where there is a steep gradient of change in abiotic conditions, such as intertidal rocky shores, there seems to exist a balance between the selective forces of predation and thermal tolerance, which determines the 'optimum' morph frequency of polymorphic littorinid snails at a particular site (Ekendahl, 1995). The interaction of abiotic and biotic factors acting as selective agents for colour was observed in populations of *Littorina saxatilis* and found to vary in extent both temporally and spatially (Ekendahl and Johannesson, 1997). It is evident that neither climatic selection nor intraspecific communication can act as the adaptive determinant of coloration in isolation. Clearly selective agents such as predation may interact and exert different kinds of selective pressures upon organisms with respect to coloration. Irrespective of any other function of coloration, interspecific communication is an essential factor because predation is an important mortality factor in the life of most animals (Endler, 1986). Furthermore, the evidence supporting the possible adaptive significance of animal coloration is most compelling with respect to defence against predation. Although other explanations are clearly important in driving colour polymorphisms, the following section will concentrate solely on the evidence of predation as a selective agent in creating and maintaining colour polymorphisms.

(1.3) Interspecific Interactions Mediated by Vision

Much of the work on the role of visual perception in ecology and evolution derives from John Endler (Endler, 1978; 1984, 1986, 1988, 1990; Bennett *et al.*, 1994). He focussed on the predator-prey relationship amalgamating the main themes of the earliest research into the adaptive significance of animal coloration by Poulton (1890), Thayer (1909), Mottram (1916), and Cott (1940). When considering animal coloration in terms of predation, a colour pattern can act in three ways (Endler, 1988): preventing detection (crypsis and masquerade), preventing identification as an edible prey (mimicry) or signal to a potential predator that the prey possesses defences which operate during subjugation or consumption (aposematism). The visibility of a colour pattern to a predator in any of these scenarios is described as the result of the interplay of five non-independent factors; inherent properties of the pattern, predator vision, hunting tactics, prey behaviour, and background colour patterns (Endler, 1978). The level of communication that exists between a predator and its prey is fundamental to the role of selection in driving the evolution of the prey's avoidance tactics.

Predation can be divided into five stages: detection, identification, approach, subjugation and consumption (Endler, 1986). Most notably detection is the primary task for the predator to execute; this then initiates the process of natural selection. From the point of view of the prey species, detection may be more likely to occur in the case of species exhibiting warning coloration or aposematism (Grill and Moore, 1998). However, where distastefulness and warning coloration are absent and the species is neither a Bayesian mimic (a palatable mimic which effectively parasitises the model) nor a Mullerian mimic (a mimic with defence which benefits the model- Mallet, 1999; Speed and Turner, 1999), then inconspicuousness to a predator is required (Endler, 1978). In this thesis I have focussed on the role of coloration in attaining crypsis. This will be reviewed here.

Early experiments exploring the adaptive significance of animal coloration reflected the need for animal coloration to vary dependent on the environment inhabited and thus to obtain crypsis (*e.g.* Poulton, 1890). Despite a longstanding body of literature on crypsis, the quantitative implications of background coloration and pattern were first truly expressed by Endler (1978) with the introduction of the term 'background matching'. Of utmost importance are the requirements that must be met for a colour pattern to be regarded as cryptic against the background; "*A colour pattern is cryptic if it resembles a random sample of the background perceived by predators at the time and age, and in the microhabitat where the prey is most vulnerable to visually hunting predators*" (Endler, 1978). For example experiments conducted into the extent of background matching by tree-dwelling caterpillars to the background twigs upon which they rest found reflectance spectra of both the caterpillar and the background to be very similar shapes (Church *et al.,* 1998a). These experiments showed that shape of the twig and caterpillar reflectance spectra provided virtually no chromatic information which an avian predator could utilise to discriminate the caterpillars from the twigs. Thus, it is important to note that crypsis does not occur in isolation; it must involve the interaction of the prey and the background (Endler, 1978, 1984).

Another predator avoidance mechanism is 'masquerade' whereby the predator misinterprets an object of interest (a prey item) as something uninteresting (Endler, 1978; Allen and Cooper, 1985). For example the occurrence of an extensive polymorphism in the bivalve *Donacilla cornea* has been explained by correlations between the density and coloration of stones and pebbles, and the bivalve population morphs, concluding that both crypsis and masquerade were important (Whiteley *et al.,* 1997). Indeed it appears that distinction between the two modes of camouflage (crypsis and masquerade) is less clear in natural systems than it is theoretically (Allen and Cooper, 1985). This thesis, however, is concerned with crypsis and specifically how marine molluscs match their backgrounds in terms of reflectance spectra and predator visual capabilities.

Most animals have inconspicuous or cryptic coloration in their natural habitats (Poulton, 1890; Thayer, 1909; Cott, 1940; Edmunds, 1974; Endler, 1978). Furthermore most natural environments exhibit some level of visual heterogeneity either spatially or temporally (Godfrey *et al.,* 1987; Endler, 1990; 1991; Seehausen *et al.,* 1999; Stuart-Fox, *et al.,* 2004). Therefore opportunities exist for natural selection to maintain multiple morphs in a species rather than a single fittest form as predicted by classical 'Darwinian' theory (Jones, 1980). Therefore some morphs are at an advantage in one part of their habitat but not another; hence the likelihood of this type of selection is increased with the complexity of the habitat. It is commonly thought that environmental heterogeneity reinforces variable selection in both time and space, thus facilitating the maintenance of polymorphisms (Cooper, 1984; Cook, 1986a, 1998; Allen, 1988; Deutsch, 1997; Merilaita, 2001). Therefore the occurrence of different colour morphs in an environment represents an advantage conferred to those morphs that allows their continued existence in the population (Allen, 1989). Direct evidence of the selective force maintaining this advantage is, however, often difficult to deduce from experimentation using the predator and prey of interest (Ekendahl, 1998; Reid, 1987), although Sheppard (1951) and Cain and Sheppard (1954) showed that thrushes exhibited selective predation on *Cepaea nemoralis* by taking a higher proportion of conspicuous individuals in certain habitats. Greater success has, however, been achieved using artificial prey (Allen and Clarke, 1968; Church *et al.,* 1997; Brodie, 1993; Merilaita *et al.,* 2001; Stuart-Fox *et al.,* 2003). Thus animal coloration studies often result in correlations between frequencies of morphs and the environment rather than direct testing of selection as the driving force maintaining morphs in a population. A classic example is the peppered moth, *Biston betularia,* which exhibited extreme industrial melanism to enhance crypsis due to industrialisation, which seems only explicable due to the threat of predators hunting by sight (Kettlewell, 1955).

Association between morphs and certain species of plants which present different coloured backgrounds has been observed in species of stick insects in the genus *Timema* (Sandoval, 1994). In fact, the first phylogenetically based evidence for predation by divergent selection for crypsis in this taxon has been recorded for a small clade in this genus, indicating that speciation can be related to the diversity of backgrounds present in the habitat (Crespi and Sandoval, 2000).

In an environment which changes spatially and temporally such as a forest prone to fires, animals have to evolve accordingly to maintain protection from predators some of the time. Results from studies on polymorphic coastal plain fox squirrels indicate that all-light backed morphs are better static matches to unburned backgrounds than are darker morphs with which they coexist (Kiltie, 1992). Against fire blackened backgrounds, however, intermediate and fully blackened backed individuals are better matches. Hence, not only is the visual crypsis of potential prey important in predator avoidance, but the accompanying behaviour also determines conspicuousness and thus the chance of identification by a predator. The rock crab, *Cancer irroratus* exhibits colour polymorphism in its postsettlement juvenile stages whereas the adults are monomorphic (Palma and Steneck, 2001). The different life stages of the rock crab dictate occupation of a different habitat harbouring predators. Therefore the crab exhibits the appropriate behavioural crypsis to avoid predation.

Achieving crypsis by background matching is a widely studied and well recognised feature of animals. However, crypsis through disruptive coloration is also a recognised mode of crypsis (Endler, 1981, 1984). Marine isopods have been shown to obtain crypsis by partly blending with the background and distracting the attention of the viewer from the contour of the animal to unessential patterns (Merilaita, 1998). Cuttlefish (*Sepia pharaonis*) also exhibit disruptive coloration by 'rapid neurally controlled polyphenism' enabling the animals to switch between background matching and disruptive coloration to appear cryptic when required (Hanlon and Messenger, 1988; Chiao and Hanlon, 2001a, b). Furthermore experiments using artificial prey and wild birds as predators have shown that disruptive coloration can be more effective than general background resemblance as a mechanism of camouflage (Cuthill *et al.,* 2005).

In molluscs, about 30 per cent of species are polymorphic in some taxanomic groups, and there is an association of background homogeneity with monomorphism, and heterogeneity with polymorphism (Clarke *et al.,* 1978; Cain, 1977, 1988). Four hypotheses exist to account for genetic stability of these polymorphisms: a) random processes with selective neutrality; b) balance between disruptive selection and gene flow in a heterogeneous environment; c) frequency-dependent selection; d) heterozygote advantage (Reid, 1987). However colour phenotypes are unlikely to be selectively neutral and selection, rather than random processes, is responsible for maintaining the diversity. Previously, heterozygote advantage had been identified as the cause of such phenotypic polymorphisms (e.g. Cain and Sheppard, 1954), despite there being insufficient conclusive evidence of its operation. Indeed, heterozygote advantage has been refuted through experimentation (Reimchen, 1974; Smith, 1976).

The experiments of Cain and Sheppard (1950) marked the beginning of research into the adaptive significance of the shell colour polymorphism of the terrestrial snail *Cepaea nemoralis*. In some parts of Britain, populations of *C. nemoralis* in 'open' habitats such as grassland tend to have high proportions of banded yellow snails, while in woodlands the commonest morphs are usually unbanded browns and pinks (Cain and Sheppard, 1954; reviewed by Jones *et al.,* 1977; Clarke *et al.,* 1978). Other helicid snails also show associations between morph frequencies and habitat (*e.g. Arianta arbustorum* (Parkin, 1971), *Trichia striolata* (Jones *et al.,* 1974) and *T. hispida* (Shelton, 1984) (Allen, 1989)). Goodhart (1987) observed that *C. nemoralis* is so highly visibly polymorphic compared to other related species that the survival of these related species without visible polymorphism is difficult to envisage. As an explanation, Goodhart suggested that polymorphisms may be non-adaptive, implying that each morph of *C. nemoralis,* albeit pink, yellow, banded or unbanded has no selective advantage over the next (Goodhart, 1987).

Clarke (1962a) reported important evidence that predators of various kinds can act to maintain visible non-mimetic polymorphisms. He called these polymorphisms 'apostatic' due to the selective advantage imparted upon those individuals that stand out from the rest of the population; in other words the selective advantage of apostates. This 'frequency dependent selection' refers to the type of selection whereby the selective value of a certain genotype is a function of its frequency (Clarke, 1962a). The relationship can be either positive or negative such that in the first instance the selective advantage increases as the frequency declines therefore only positive frequency dependent selection can maintain polymorphisms within populations. In short, apostatic selection describes the advantage conferred upon rare prey when a predator consumes disproportionately more common prey (Allen, 1984).

Observing differential predation of morphs in the wild is made inherently difficult by the presence of the experimenter. However, actual divergent selection acting on different morphs or forms of a species in a population has been observed both in the field and the laboratory setting (Kettlewell, 1955; Maskell *et al.,* 1977; Sandoval, 1994; Tullrot, 1994). Due to the difficulty of performing these experiments accurately, the role of predators in creating and maintaining colour polymorphisms in a prey population has been extensively demonstrated by the use of artificial prey, most notably the use of different coloured pastry baits (Allen and Clarke, 1968; Allen, 1972, 1974, 1976). In many experiments using pastry baits the effect of prey density is considered, yet further variables have been investigated including palatability (Greenwood *et al.,* 1981) and the degree of background matching (Bond, 1983; Cooper, 1984). The advantages of artificial prey are that they are stationary, standardised for colour, size and shape, and can be produced in large numbers (Allen, 1989). These advantages of artificial prey have enabled experiments to be conducted in a highly controlled manner using both trained and wild birds (Allen, 1974, 1976; Allen and Anderson, 1984; Allen and Cooper, 1988; Manly, 1973, 1974, 1985; Greenwood, 1985).

Despite the extensive experimentation into apostatic selection using artificial prey, it is clear that predation on live polymorphic prey under natural conditions needs to be studied. Most notably the work of Reid (1987) into the polymorphic mangrove snail *Littoraria filosa* was important in initiating experimentation into the role of selection in polymorphic species in the field, yet direct observations of predation were missing from the experiment.

Experiments on human subjects searching for prey items on computer screens suggest that apostatic selection is an adaptive strategy resulting in faster capture rates (Tucker and Allen, 1991). However, this could be counter-interpreted as increased apostatic selection representing the effect of the increased rate of capture (a search) rather than the cause (Allen, 1989).

In addition to the visual purpose of coloration of animals there is effectively also a non-visual explanation whereby colour is incidental- an epiphenomenon- whereby the colour has no function itself yet it is closely related to some other purpose (Rosenberg, 1997). Whilst it may seem that predation has been a panacea for explaining animal coloration patterns, it must not be forgotten that there exist examples of non-visual causes of animal coloration such as metabolic waste deposition and shell structure reinforcement in molluscs (Giesel, 1970). The white morph of the polymorphic limpet *Lottia digitalis*, has been found to whiten, and hence match its goose barnacle background to a greater extent with age, due to diet or possible infection by a shell eroding fungus (Byers, 1989). In addition Hughes and Mather, (1986) found age related changes in the shell colours of *Littorina* species living on mangroves.

The examples discussed here highlight the importance of colour classification of forms or morphs in ecological study. Apart from any inherent physical properties of a colour pattern and its illuminating light source, the visual acuity of potential predators must also be considered when attempting to interpret the adaptive significance of animal coloration.

How the predator sees a visual scene determines the extent to which fine-grained visual information, that may not be accessible through experimental measurement, is utilised in predation (Church *et al.,* 1998b). It must be noted that differentiation between predators in terms of importance as a selective agent may be dependent on whether only one or all predators possess colour vision (particularly if the population is of high density) (Pettitt, 1975; Endler and Théry, 1996; Endler and Mappes, 2004).

(1.4) Selection and Polymorphism in Intertidal Molluscs

Whilst visible polymorphisms occur in many species, one of the more interesting families is Littorinidae. These marine molluscs inhabit the highly dynamic intertidal rocky shore and thus are presented with a background that is changing on different temporal scales due to both physical and biotic factors.

Intertidal *Littorina* snails and related genera have frequently been examined with regards to the adaptive significance of their shell polymorphisms. These species are distributed within the intertidal zone of most European shores (Reid, 1996) and their shell colour variation is known through breeding experiments to be genetically determined (Reimchen, 1989; Ekendahl and Johannesson, 1997). The reproductive strategies of littorinids vary greatly from production of pelagic egg capsules with high dispersal potential (*Littorina littorea*) to direct development with low dispersal potential such as production of benthic egg masses (*L. arcana, L. obtusata* and *L. fabalis*) and ovoviviparity in *L. saxatilis* which retains embryos in a brood pouch and releases miniature 'crawlaway' juveniles (Raffaelli, 1982; Reid, 1996). The diversity of life history strategies in littorinids offers opportunities to test several evolutionary hypotheses (*e.g.* Wilkins and O'Reagan, 1980; Johannesson, 1988; Kyle and Boulding 2000; Johnson *et al.,* 2001). The study of littorinid polymorphism has, however, more often concentrated on those species with low dispersal.

A large body of research already exists documenting five decades of fieldwork and investigation on the selective and non-selective forces that act on littorinid colour polymorphisms:

- Visual selection by predators with respect to background colour both against the conspicuous morph and apostatically (Heller, 1975; Smith, 1976; Atkinson and Warwick, 1983; Byers, 1990)

- Selection to live on or near algal food species, related to height on the shore (Van Dongen, 1955; Williams, 1990)
- Non-random mating among morphs (Cook, 1992; Rolan-Alvarez *et al.,* 1999; Pickles and Grahame, 1999)
- Selection acting on the pleiotropic effects of shell colour alleles (Raffaelli, 1979; Sergievsky, 1992)
- Morph-specific habitat choice (Reimchen, 1979; Ekendahl, 1995; Rolan-Alvarez and Ekendahl, 1996)
- Genetic drift of alleles controlling morph coloration (Janson, 1985; Ekendahl, 1995)
- Parasitoid insects as selective agents contributing to the maintenance of polymorphism (McKillup and McKillup, 2002)

There is a clear indication that certain (if not all) colour morphs of littorinid species are associated with backgrounds upon which they are cryptic (Pettitt, 1973b; Hughes and Jones, 1985; Ekendahl, 1998; Johannesson and Ekendahl, 2002). In particular, selection for crypsis and apostatic selection appear to be acting on mangrove-dwelling *Littoraria pallescens* maintaining morph ratios such that they are proportional to elements of the background to which they are visually matched (Hughes and Mather, 1986).

Species-specific classification systems for coloration have commonly been applied to littorinids to categorise the polymorphism present in each species. For example Pettitt (1973b) used methods for scoring colour and banding to classify the colour polymorphisms of *Littorina saxatilis* based upon ground colour and superimposed patterns. This 'allele-like' system assumes several "genes" each coding for a separate feature of the shell coloration and despite being more objective, it can result in a large number of different morph classifications to observe meaningful differences in morph frequencies. Variations of this method were later used when correlating the distribution of shell pattern in *L. saxatilis* and its sibling species to background with regards to achieving crypsis (Raffaelli, 1979; Atkinson and Warwick, 1983; Byers, 1990).

Broader, less structured classification schemes have also been adopted (*e.g.* Heller, 1975; Ekendahl and Johannesson, 1997; Ekendahl, 1998). Sacchi (1969) categorised *Littorina* *fabalis* into four principal phenotypes: citrina (yellow), light reticulata (light reticulations on a light brown background), dark reticulata (dark reticulations on a brown ground colour), olivacea (olive-green to olive-brown) and the rare inversicolor morph (striped) (Figure 1.4.1). These morphs are also present in *L. obtusata* (Smith, 1976). For the purposes of this thesis the latin names have been simplified to 'yellow', 'light brown', 'dark brown', 'olive' and 'striped'.

Figure (1.4.1): Principal morphs of *Littorina obtusata* and *L. fabalis* (top row: yellow, olive, light brown, dark brown; bottom row: striped morphs).

These principal morphs of *L. fabalis* may possibly correspond with the colours of several discrete parts of *Fucus serratus*, the major substratum of *L. fabalis* (Reimchen, 1979; Ekendahl, 1995). These subjective correlations using 'human' indices of coloration are an ideal lead into objective colour classification in this family.

Visual selection has often been proposed as an explanation for observed correlations in coloration between littorinid morphs and their backgrounds. However, experiments on littorinid coloration have often yielded indirect rather than direct evidence of visual selection. In an attempt to associate morphs with background characteristics Heller (1975) found that the reddish-brown morphs of two species in the *Littorina saxatilis* complex often reached their highest frequencies on shores in Wales where Old Red Sandstone predominates. Indeed, all ten shores where the frequency of the red morph of *L. saxatilis* exceeded 15% were from Old Red Sandstone locations. In addition, Heller (1975) found that the proportion of white morphs of *L. saxatilis* increased with shore exposure, evidently due to the increasing cover of barnacles, which provided a white background upon which the white morph appears cryptic. Conversely Raffaelli (1979) concluded that red and orange morphs of *L. saxatilis* are no more common on red rocks than elsewhere. Raffaelli (1979) suggested selection on pleiotropic characters to explain the association between shell and substratum colour by instead linking shell colour to the type and structure of the substratum. In this particular study dark patterned shells were found mainly on rocks, while bright unpatterned ones were found more often on boulders (Raffaelli, 1979).

The banded morphs of the algal dweller *Littorina obtusata*, which are always rare, were proposed by Smith (1976) to be maintained in the population by apostatic selection. However, he suggested that the rest of the colour morphs present in higher frequencies had probably evolved under disruptive selection for crypsis with the various morphs favoured temporally and/or spatially in a heterogeneous environment. These conclusions were based upon a comprehensive study of populations from over eighty sites in the British Isles. However, Smith (1976) also postulated the relevance of the genetic diversity apparent at many sites around the UK to be dependent on the nutritional rather than the visual properties of the different seaweeds. Given the littorinids feed on the algae, Smith (1976) proposed that nutrition influenced shell coloration and therefore background matching was caused indirectly rather than directly. It is now accepted that shell colour in littorinids is genetically inherited through a one locus, two allele system (Ekendahl and Johannesson, 1997). Although it seems questionable that such a system could give rise to diverse poilymorphism.

Using the previously discussed subjective classification methods, Pettitt (1973a) extensively described the distribution of colour morphs of *Littorina saxatilis* and surveyed the evidence that visual selection is the factor governing this distribution. Given the observed association between littorinid and background coloration, Pettitt (1975) described the potential predators of *L. saxatilis*. He recognised that whilst littorinids may not constitute the entire diet of any given predator, the impact of the predator on a population (of littorinids) may have a large impact as a selective agent, particularly if the predator possesses colour vision. The shore crab *Carcinus maenas* is the main predator of littorinids lower on the shore (Pettitt, 1975; Heller 1975; Raffaelli, 1978), although some fish (Palmer, 1979; Hughes and Jones, 1985; Boulding *et al.*, 2001), including the shanny (*Lipophrys pholis*), may also be important (Qasim, 1957; Pettitt, 1975; Reimchen, 1979). Birds have also been recorded removing significant numbers of littorinids on rocky shores (Gibb, 1956; Johannesson and Ekendahl, 2002), and also littorinid shell remains have been found in the gizzards of many bird species (see Pettitt, 1975 for a review).

Direct experimental evidence for selection on the polymorphism, as described in the previous section, is more difficult to obtain (Cook and Garbett, 1992). Through laboratory predation experiments, Reimchen (1979) showed that blennies use visual cues in selecting their prey (*L. fabalis*), and when two colour morphs were presented in similar frequencies, they selected the more conspicuous one preferentially. Attempts to observe crabs (possible visually-orientated predators) influencing colour frequencies by selection have failed in the field (Ekendahl, 1998) and the laboratory (Tucker, 1988). In field transplantation experiments involving caged and uncaged plots, Johannesson and Ekendahl (2002) observed fish showing a preference for more conspicuous morphs but surprisingly birds exhibited no selection among the colours despite removing up to 16% of the experimental snails. The colours selected by the birds may, however, have been relevant to the avian visual system rather than the human observers recording the results, given that birds have different visual abilities to humans. It is reasonable, however, to assume that littorinids are subject to predation by visually-orientated predators and the role of these predators may be selective. It is also possible that visually-orientated predators may be non-selective or even selective in a manner which should result in a monomorphic population. Should monomorphism occur, other previously discussed selective agents, such as climatic selection, may be more important in the evolution of coloration for that particular population (Sergievsky, 1992).

Regardless of the lack of direct evidence, the general theme that appears throughout the literature is that visual selection acts directly on colour pattern with respect to background coloration. However, in all cases described here, the classification of morph coloration has relied upon the human visual system, rather than the visual system purported to be responsible for visual selection. A review of the problems associated with the human judgement of coloration will be given in Chapter 2. Therefore the study of littorinid coloration in the context of the visual environment and the observer is of utmost importance. The following section will describe the experimental system which is investigated in this thesis with respect to the adaptive significance of coloration in intertidal littorinids.

(1.5) The Experimental System

Where there is a considerable tidal range, the shore has an intertidal zone subject to cycles of immersion and emersion (Hill *et al.,* 1998). Habitats do not always conform to this description as tides may be more or less absent and generally biological communities occupy the littoral zone that can reach many metres above high tide level (Johannesson, 1989). The combination of vertical tide- and horizontal topography-dependent gradients has significant implications in determining the types of organisms that rocky shores support (Ekendahl and Johannesson, 1997). Thus the visual properties of the background upon which organisms are found, are caused by the local community and, indirectly, determined by physical factors (Lewis, 1964). For example, the dominant mid-shore species in the UK change gradually from fucoids on sheltered shores to barnacles or mussels on more exposed shores (Hawkins and Hartnoll, 1985). Yet biological interactions also create changes in the community and thus the visual environment of the rocky shore. These biological interactions include competition for space (Connell, 1961), grazing (Cubit, 1984) and predation (Hill *et al.,* 1998), which create free space and expose the rocks.

The quality of coastal water can be quite variable depending on the nature of the substratum, weather conditions and proximity to human activities (Lythgoe and Partridge, 1991; White, 2003). The underwater environment is strongly influenced by turbidity, which can be an important factor in determining the visual characteristics of the habitat (Lythgoe, 1979). The exposed rock surface also adds visual complexity to the rocky shore as the rock type may have a specific colour, and in addition there may be two or more different rock types found in the same place, forming a geological transition (Raffaelli, 1979; Byers, 1990). Furthermore, the tidal cycle generates another factor affecting the background through wetting which can create specular reflectance or glare in direct sunlight.

In terms of predators, the rocky shore is accessible to both marine and terrestrial predators. Observations on British shores have even included crows, cats, dogs, and rats foraging upon intertidal communities (Feare, 1967; Pettitt, 1975; Carlton and Hodder, 2003). The existence of a broad range of predators of rocky shore organisms further complicates the understanding of selective pressures creating and maintaining visibly polymorphic species.

Using the analogy of terrestrial environments, we might argue that man's influence on the rocky shore alone may have irreparably altered its 'natural' state to an extent that original selective processes will never be uncovered (Cook, 1986b). However the rocky shore has remained relatively undisturbed in terms of human activity due to the development of a conservation ethos on rocky shores related to their natural beauty. Additionally north European rocky shores provide no, or very few, opportunities for commercial exploitation. The accessibility and two-dimensional structure makes the rocky shore highly suitable for extensive ecological experimentation (Edwards *et al.,* 1982; Underwood, 2000). In addition to such scientifically valuable monitoring attributes, the habitat is home to geographically variable polymorphic species of the family *Littorinidae.*

(1.6) Aims and Rationale

Despite a wide variety of possible explanations for the high occurrence of polymorphisms in molluscs, selection by predators remains an attractive and fruitful line of investigation. My work has focused on the possible role of selection acting to maintain shell colour polymorphism using objective classification of coloration as an investigative tool.

This thesis aims, for the first time to apply modern techniques of spectral reflectance measurement and visual modelling to mollusc polymorphism in the intertidal habitat. It is hoped that through extensive spectral data collection and analysis that patterns relating littorinid coloration and background coloration will test theories of visual selection maintaining genetic polymorphism.

Hence the work described here involves experimentation into background matching by littorinids in visually heterogeneous habitats. More specifically, the aim is to identify the selective role of the predators of polymorphic littorinids by quantifying the appearance of littorinids against their backgrounds. Since colour is not an inherent property of an object but rather a property of the sensory and neural processing of the receiver (Endler, 1978, 1990; Lythgoe 1979; Goldsmith, 1990; Thompson *et al.,* 1992), the appearance of littorinid prey will be modelled into the visual systems of different predator species and background matching will be studied in terms of the predator's visual sensitivity to different wavelengths of reflected light.

The second introductory chapter of Part I (Chapter 2) provides a comprehensive review of spectroradiometry as an objective colour measurement technique and a description of its application to background matching in polymorphic littorinids. Modelling of spectra into the visual systems of potential predators and its application is also reviewed and described in full methodological terms. The specific methodologies of spectroradiometry and visual modelling are not repeated in each chapter. Therefore in each chapter, references are made to Chapter 2 (General Methods: Theory and Practice). In any subsequent publications from my work, the modelling methods described in the following chapter will be provided as appendices.

The *saxatilis* complex inhabits the splash zone which, as a visual environment, is physically determined by the geology of the area and mediated biologically by encrusting algal and lichen growth. Therefore the extent to which littorinid morphs match backgrounds of different geologies and also backgrounds with lichen growth is assessed in Part II. Specifically Chapter 3 investigates the differences between populations of *L. saxatilis* on shores of different geologies. The difference between the polymorphism in *Littorina saxatilis* ecotypes in Sweden is investigated in Chapter 4 to quantify the signals available to different predator visual systems. Visual heterogeneity of the rock surface caused by encrusting lichen is investigated in Chapter 5 with respect to background matching of different *L. saxatilis* morphs.

Littorina obtusata and *L. fabalis* inhabit fucoid algae in the lower to mid- shore zone. This environment is subject to physical variation on a variety of temporal scales, most importantly tides. The tidal cycle enables predation access to fucoid-dwelling prey by fish, crabs and also wading and terrestrial birds. The algal canopy is the focus of exploration of spatial and temporal background matching patterns in littorinids through the techniques of spectroradiometry and visual modelling in Part III. Specifically in Chapter 6 the morphological differences between algal dwelling species will be investigated with a view to determining the extent to which different morphs match the background in the visual systems of different predators. Furthermore consideration will be given to the ability of predators to detect littorinids in turbid water conditions.

Previous studies exploring the visual ecology of interactions have focused on intraspecific communication measuring the often flamboyant spectra in birds, amphibians, flowers and fruits. This work aims to assess selection acting on littorinid polymorphism by analysis of comparably ordinary natural spectra in visual systems of potentially important predators which vary in their acuity. Therefore in Chapter 7 the techniques of spectroradiometry and visual modelling are assessed in terms of their suitability for studying intertidal mollusc coloration using examples from other biological systems.

Chapter 2

General Methods: Theory and Practice

(2.1) Introduction

In this thesis I have investigated the colour polymorphism in littorinid species by the collection of reflectance spectra from their shells and backgrounds in a variety of habitat types on different shores. The techniques of data collection, processing, modelling and analysis are similar throughout. Therefore this chapter describes common techniques and their underlying theoretical basis to avoid repetition in subsequent chapters. The chapter aims to provide the theoretical background to colour quantification (2.2), specific methods relating to spectral data collection (2.3) and objective multivariate analysis of spectral data (2.4). The process of visual modelling is described theoretically (2.5) including the prediction of spectral sensitivities from visual templates (2.5.1). Following on from this, the Vorobyev and Osorio (1998) receptor threshold visual model used throughout this thesis is derived algebraically (2.5.1). Characteristics of the visual systems of predators are described (2.6) including a brief description of foraging ecology, the visual system and the model parameters used. Finally information about the study sites is given in section 2.7.

(2.2) Theoretical and Physical Aspects of Spectroradiometry and Spectra Analysis

The perceived colour of an object depends on (1) the nature of the illuminating light source, (2) its modification by interaction with the object, and (3) the characteristics of the observer's visual system (Lythgoe 1979; Billmeyer and Saltzman, 1981; Endler, 1990). By accounting for all three visual parameters when modelling colour vision in another visual system, the most comprehensive assessment of chromatic discrimination can be achieved. This section describes past efforts in colour measurement and the developments that have led to the current visual modelling techniques using reflectance spectra as applied throughout this thesis.

Human description of colour has been traditionally characterised by creating equally spaced gradations of measure based on human perception of changes in each of the parameters assigned to a particular colour measurement (Zuk and Decruyenaere, 1994). Such descriptions of colour, called colour standards, include the system developed in the 1930s by the Commission Internationale d'Eclairage (CIE), an organisation that specifies methods of measuring colour for the production of dyes and inks. The CIE system used seventeen human subjects to match colour sets whilst specifying the colour in terms of the light source, the object and the observer (Mehta, 1990).

Another system, based on judgement by the human eye is the Munsell colour method (Munsell, 1963). Munsell colour atlases contain 'chips' that are organised into hue, value, and chroma. 'Hue' refers to colour in the colloquial sense of red, orange, green, *etc.* and is arranged in a 100-hue circuit divided into ten colour categories. 'Value' describes the relative darkness in the colour sample and 'chroma' indicates the degree to which the colour is saturated with the hue in question (Zuk and Decruyenaere, 1994). It is the human perception of changes in each of these parameters that forms the colour standards (Zuk and Decruyenaere, 1994). Munsell colour chips were mainly designed for human colour vision, and the relevance of their reflectance spectra to visual systems of other animals is uncertain (Chiao and Cronin, 2002). However, Munsell atlases have been employed by evolutionary ecologists to describe the coloration of organisms in the field with respect to intraspecific communication (Dawkins and Guilford, 1994; De Repentigny *et al.,* 1997), interspecific communication and the role of visually orientated predators (Cain, 1953; Allen and Anderson, 1984; Ellis *et al.,* 1997) and climatic selection in molluscs (Cowie, 1990). Endler (1990) points out that the use of colour standards to subjectively rank objects or individuals does not contribute towards the understanding of predator vision.

Although a great deal of useful information has been gained from classification of colour morphs as judged by humans (*e.g. Cepaea nemoralis*, as summarised by Jones *et al.,* 1977), it must be noted that the categories assumed by researchers may bear no relationship to how other animals perceive differences among the same spectra. It is evident that attempts to study animal coloration have been made inherently difficult by the subjectivity associated with the human visual sense (Crook, 1997). Variation between humans and other animal groups is now widely accepted (Cuthill and Bennett, 1993; Dittrich *et al.,* 1993; Bennett *et al.*, 1994; Church *et al.,* 1998a, b; Vorobyev *et al.,* 1998; Hunt *et al.,* 2001). Therefore, a classification based directly upon the spectra reflected from the object in view rather than human perception will evidently be a better starting point in studies of animal colour patterns (Norris and Lowe, 1964; Endler, 1990).

Spectral data are superior to observational data in several ways. Firstly, spectral data are less susceptible to researcher bias as spectra are objective, graphical, and thus quantitative representations of physical phenomena in the sense that the reflection of light from a sample has not passed through a human 'sensory filter' (Grill and Rush, 2000). Reflectance spectra can be collected by spectroradiometry which is the technique of measuring the spectrum of radiation emitted by a source. In order to do this, radiation must be separated in to its component wavebands and each band measured separately. Thus the spectroradiometer takes an objective, physical measure of the surface in radiometric units at each wavelength, typically from 300-750nm, ultraviolet to near infrared (Fig. 2.1.1).

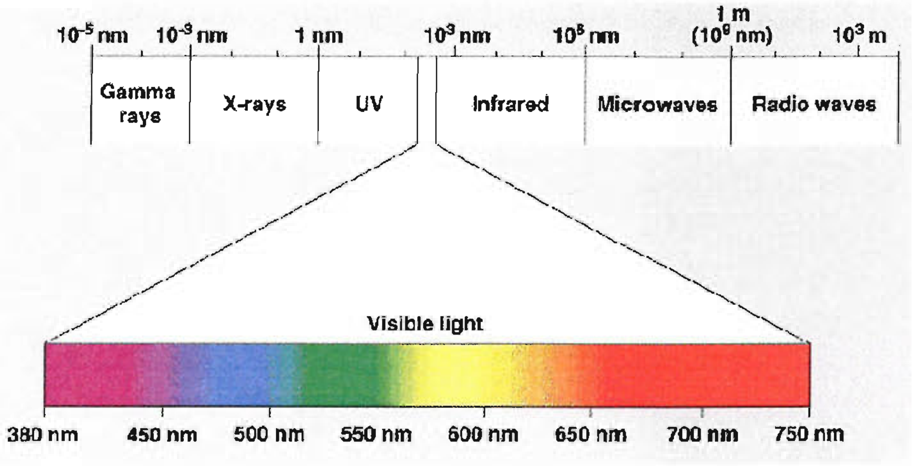


Figure 2.1.1: The electromagnetic spectrum
(from http://fig.cox.miami.edu/~cmallery/255/255phts/255phts03.htm).

Spectral data in this form represent the physical properties of the surface and permit modelling into any characterised visual system of a potential observer. Despite the obvious attraction of visual modelling techniques, it is interesting to firstly assess spectra based purely on their physical properties. This 'objective' analysis enables investigation of spectral reflectance irrespective of visual capabilities of the observers and also human vision. The following section describes the data collection and the techniques used in both 'objective' spectra analysis and 'subjective' visual modelling.

(2.3) General Spectroradiometry Methodology and Spectral Data Management

An SD2000 dual channel spectroradiometer (Ocean Optics, The Netherlands) was used for all spectral data collection. This system comprises a deuterium halogen DH2000 light source which directs light via a bifurcated fibre optic contained within a black plastic (PTFE) probe housing to the sample. The probe housing tip was cut at a 45° angle to allow the collection of light reflected from the sample rather than specular reflectance (glare). The light reflected from the sample travels via the bifurcated fibre optic and is detected by the spectroradiometer. Data were collected and processed in OOIBase32 software (Ocean Optics) on a notebook PC. All files were produced in Excel format.

To calculate reflectance, the probability of reflection at each wavelength (λ) relative to a perfect white reflector is calculated. Therefore the white reference spectrum from a Spectralon^{TM} panel was collected in a dark box prior to spectral collection. After the white reference was recorded, the electrical noise within the spectroradiometer was collected to be subtracted from each spectrum by the OOIBase32 software during processing. The dark current was collected by closing a manual shutter on the lamp and holding the probe tip against a dark, non shiny surface. White reference and dark current were collected in raw digital number or 'scope mode' whereas reflectance spectra, analogous to transmission spectra, were collected in transmission mode. In the field the equipment was set up on the shore (Fig. 2.2.1) and the lamp was warmed up for 20 minutes as recommended by the manufacturer to acquire a stable output. During spectral data collection, the stability of the spectroradiometer was monitored by checking the white reference to see how far the spectroradiometer had strayed from the white standard (100% reflectance). To counteract the white reference straying from 100% reflectance, a new white reference and dark current spectrum was collected approximately every 5 minutes during spectral data collection on the shore.

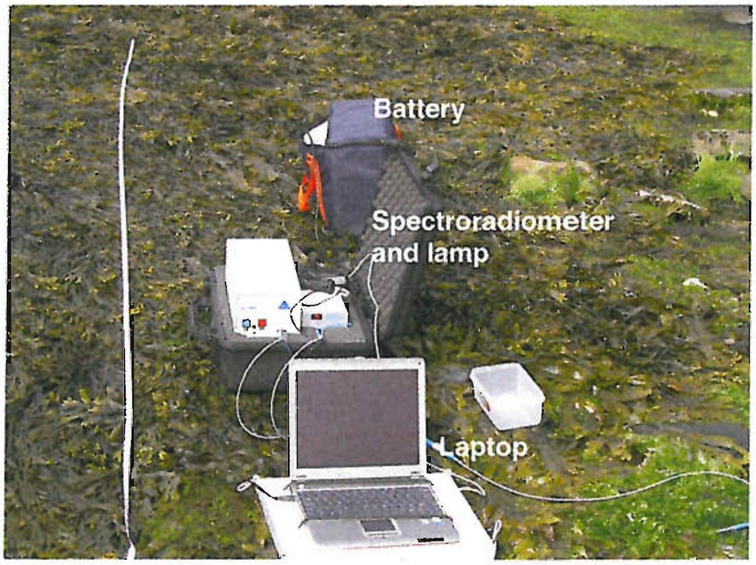


Figure 2.2.1: Field spectroradiometry equipment set up on the shore.

Spectral files created by OOIBase32 were combined to single Excel spreadsheets by first editing in 'R', a system for statistical computation and graphics freely available online (www.R-project.org). In addition to combining files in Excel, each spectrum had an artifactual "spike" removed between 654-660nm which was purely a feature of the light source used (Cuthill *et al.,* 1999). Spectra were arranged in columns in Excel and labelled according to the site, replicate sample within site and spectrum number. For visual modelling, spectra were modelled into the visual systems of selected species using SPEC (Hadfield, 2004); a package which enables colour parameters to be calculated directly from files produced in OOIBase32. SPEC enables rapid processing of spectral data according to the receptor noise model of Vorobyev and Osorio (1998) used throughout the thesis and described in section 2.5.2.

(2.4) Objective Analysis of Reflectance Spectra

Analysing spectra without making assumptions about the physiology of the observer's visual system can be useful (*e.g.* Endler and Théry, 1996; Cuthill *et al.,* 1999; Mays *et al.,* 2004; Thomas *et al.,* 2004) as it does not require data regarding the visual system of the observer. Indeed, assessment of colour by description of spectra alone has yielded ecological insights into background matching (Norris and Lowe, 1964; Götmark, 1996; Cuthill *et al.,* 1999). The non-physiologically related analysis of spectra used in this thesis is based on Principal Components Analysis (PCA). In a PCA of spectral data, each wavelength interval (λ) represents a variable in the analysis and each spectrum, a replicate. PCA can be applied as a preliminary data reduction technique useful for ordination of spectra (Macedonia *et al.,* 2002) and thus allows the identification of wavelengths most important for discrimination of spectra (Grill and Rush, 2000; Mays *et al.,* 2004).

There are four limitations to the use of PCA directly to analyse spectra. Firstly, PCA depends upon independence of the variables and reflectance at one wavelength is strongly correlated to reflectance at the wavelengths either side, particularly if the spectroradiometer is high resolution and divides each wavelength into smaller collection intervals than 1nm. This covariance invalidates the assumptions of PCA. Secondly, all visual systems are more sensitive to certain wavelengths and do not necessarily use all the available wavelengths. Therefore some differences between spectra detected by PCA may be irrelevant to the observer. Thirdly, spectral data are ideally collected in large well replicated sets which are inevitably highly correlated. Thus the assumptions of independence and multivariate normality break down (Endler and Mielke, 2005). Finally, the neurological processing of colour is known to be independent of brightness in both vertebrate and invertebrate visual systems (Chittka, 1996; Vorobyev and Osorio, 1998; Osorio *et al.,* 1999; Kelber *et al.,* 2003). The first principal component (PC) is usually a brightness measure and the remaining PCs are related to colour yet for every analysis which may contain subsets of spectra in prior analyses these PCs vary, making comparisons between separate analyses unviable (Cuthill *et al.,* 1999).

The application of PCA in this work was for the estimation of groupings among spectra based on their physical characteristics. All PCAs were carried out in SIMCA-P, a multivariate statistics package. PCA constitutes a pre-analysis to visual modelling and was used to find general spectral trends in a comparative sense to assess the effectiveness of visual modelling. For each spectral data set subjected to PCA, in most cases both standardised and unstandardised spectra were analysed. Standardised spectra have had the overall spectrum mean subtracted from each wavelength interval reflectance value. This corrects for brightness variation, producing principal components which represent chromatic variation between spectra. For each PCA of unstandardised spectra, the percentage variation explained by PC1 is reported to indicate the level of brightness variation between spectra.

To test whether signals available to a predator's visual system could lead to selection on a prey population, we need to consider the visual system in which the signals are likely to be perceived. To interpret visual signals, PCA of spectra would be inappropriate. The following section justifies the process of visual modelling and explains the model used throughout the thesis.

(2.5) Visual Modelling

Interpretation of the reflectance spectra in terms of another species' vision requires knowledge of the physical and neurobiological properties of photoreceptor and associated cells in the retina and brain of the species in question (Endler, 1990). An objective measurement that is 'psychologically labile' (Bennett *et al.*, 1994), such as reflectance spectra can be quantified independently of the observer, as suggested by Endler (1990), whereas colour cannot. Endler (1978) promoted interest in creating a more uniform and thus rigorous methodology for measuring animal coloration, by addressing how the organism perceives an object rather than how humans perceive the same object. Perception can be understood as a multistage process, with receptor signals transformed by a sequence of neural mechanisms (Vorobyev and Osorio, 1998). Current experimentation is now highly directed firstly to realising the differences between animal groups with regards to perception, and secondly to devising techniques to convert objective measurements of colour into what is available to the photoreceptors of the predator in question.

A model has been developed based on spectral sensitivities and noise associated with receptor types that predicts the discriminability of any two spectra reflected from natural objects (Vorobyev and Osorio, 1998), or in other words whether an object is conspicuous against its background. In simulating retinal responses to real plumage spectra, Vorobyev and Osorio (1998) created a model of the way in which a bird's retina extracts spectral information from its environment. This model is appropriate for analysis of reflectance spectra in this thesis as it accurately predicts behavioural data for a broad range of animal taxa, including birds, while making minimal assumptions provided we know the relative numbers of photoreceptor types and also the wavelengths to which the organism is maximally sensitive (spectral sensitivity, Stuart-Fox *et al.,* 2003).

Visual systems encode contrast signals such that a colour is assessed relative to its background (Vorobyev *et al.,* 1998). In this thesis, littorinid shell colour is contrasted relative to a series of natural background spectra as perceived by potential predators, the specific characteristics of which will be discussed in section 2.6. By using published data on the spectral sensitivities of each cone receptor type and also secondary visual structures such as ocular filters, the loci of spectra relative to the spectral sensitivity peaks of the predator species can be mapped into 'perceptual space' (Vorobyev *et al.*, 1998). Any spectrum will fall on a particular point, as defined by quantum catches of each receptor type, in the perceptual space. The 'distance' (ΔS) between any two points (spectra) in perceptual space is correlated with perceived differences in colours (Endler, 1990). The smaller the ΔS, the more similar are the two spectra. Once the colours are assigned to positions in the perceptual space, the information can then be interpreted in an ecological context.

Birds have been the primary group in which physiological modelling has been developed. Objective measurements of appearance have been applied to the intraspecific relations in groups of birds. By use of spectroradiometry Endler and Théry (1996) investigated the conspicuousness of three species of lekking birds by measuring the reflectance spectra of plumage and the background to deduce the factors affecting female attraction. Furthermore, UV reflectance from bird plumage has been demonstrated by spectroradiometric techniques and suggests the importance of UV in intraspecific communication (Burkhardt and Finger, 1991; Finger and Burkhardt, 1994; Hunt *et al.*, 2001).

Investigation of vertebrate and invertebrate visual systems has been pre-empted by extensive study of the human visual system. The spectral sensitivity of human vision was first measured through psychophysical experiments in the early part of last century (Goodeve, 1936; Wald, 1945), and much later directly measured at the cellular level (Bowmaker and Dartnall, 1980). Interestingly both psychophysical and direct cellular methods gave similar predictions of human cone sensitivities (Kelber *et al.*, 2003). Direct techniques include spectrophotometry using extracted pigments, retinal densitometry, electrophysiological recordings from individual photoreceptors, and microspectrophotometry (Lamb, 1995).

Microspectrophotometry (MSP) is the only method by which the spectral absorption properties of visual pigments can be measured *in situ* from individual retinal photoreceptors (Partridge, 1986; Hart, 2001a), and has been invaluable in the study of animal vision. MSP uses a modified microscope optic which passes a very narrow beam of light through a retinal cell (Hawryshyn *et al.*, 2001). The amount of light transmitted at each wavelength from the retinal cell is detected by a photoelectric device allowing characterisation of the photopigments in the different cone types. The MSP technique has been extensively employed in bird species (*e.g.* the pigeon, *Columba livia,* Bowmaker, 1977; the tawny owl, *Strix aluco,* Bowmaker and Martin, 1978; the budgerigar, *Melopsittacus undulatus* and the zebra finch, *Taeniopygia guttata* Bowmaker *et al.*, 1997; the domestic turkey, *Meleagris gallopavo,* Hart *et al.*, 1999). It has been shown that most diurnal birds have at least four kinds of photopigments in the cone cells of their eyes (Bowmaker *et al.*, 1997), including a spectrally sensitive peak near the ultraviolet (Cuthill *et al.,* 2000). Very short-wavelength sensitivity in birds is possible because, unlike humans, avian ocular media do not absorb UV light before it reaches the retina and also their short-wavelength-sensitive cone is spectrally tuned to be maximally sensitive to shorter wavelengths. Cone cells in the avian retina are sensitive to UV light giving birds the potential to discriminate reflectance spectra that look identical to humans (Smith *et al.,* 2002). When photoreceptors are not characterised for the particular species of interest, the closest related species for which photopigments are known should be chosen (Bennett *et al.,* 1994). Despite relative conservatism in visual acuity in birds, there is one major phylogenetic difference between taxa in that some birds have short-wavelength sensitivity biased towards violet, which is the ancestral state, while others are biased towards ultraviolet, which has evolved independently at least four times (Ödeen and Håstad, 2003). This phylogenetic grouping of avian visual systems, based on short-wavelength sensitivity, has important implications for the study of avian visual ecology.

Given sufficient physiological data pertaining to the spectrophotometric characteristics of a visual system, it is possible to model the spectral sensitivity of an animal. The history of visual pigment modelling to produce spectral sensitivity curves is described by Hart (1998) and therefore the following section will only explain the visual templates used in this thesis.

(2.5.1) Predicting Spectral Sensitivity from Visual Templates

The shape of absorbance spectra of all visual pigments in vertebrates and invertebrates are invariant and can be characterised by a common visual pigment template with a single parameter λ_{max} (Dartnall, 1953; Endler and Mielke, 2005). No relationship between the molecular structure of the receptor pigments and their spectral sensitivity has yet been derived, hence visual templates have been empirically calculated using curve-fitting to recorded data (Govardovskii *et al.*, 2000). Although many have tried to create improved visual templates from the early attempts of Dartnall (1953), Godvardovskii *et al.*, (2000) provides the most refined templates which accurately describe spectral sensitivity. Spectral sensitivity curves depict the level to which a particular species is sensitive to different parts of the spectrum from interrogation of retinal cells by a variety of techniques to be discussed.

Not only is variation in visual systems and hence spectral sensitivities important between species, the illuminating light at the time also determines the appearance of the object to the observer. Hence the visual response to light is a compound of the wavelength of incident light and how the eye reacts. The resultant visual colour of an object is the incident light as modified by the object and again modified by the visual response to the illuminant (Chamberlain and Chamberlain, 1980). This theory is the basis of the Vorobyev and Osorio (1998) model, applied to data throughout this thesis, which will be described in the following section.

(2.5.2) The Model

Reflectance spectra describe the probability of reflection at each wavelength (λ) or wavelength interval (depending on the resolution of the spectroradiometer used) relative to a perfect reflector; a white standard. The light spectrum, or 'stimulus spectrum', reaching the eye of the observer $Q(\lambda,X)$ from a patch on the viewed object at distance X under given environmental conditions is a product, at each λ, of the incident light illuminating the patch, the reflectance spectrum of the patch and the transmission spectrum of the medium between the patch and the viewer (Lythgoe, 1979; Endler, 1990). This can be represented by the following equation:

$$Q(\lambda,X) = I(\lambda)\, R(\lambda)\, T(\lambda,X) + V(\lambda)$$

Where $Q(\lambda,X)$ is the radiance spectrum arriving at the eye at distance , $I(\lambda)$ is the irradiance (ambient light) spectrum illuminating the patch, $R(\lambda)$ is the reflectance spectrum of the patch at the incident light and specified viewing angle, $T(\lambda,X)$ is the transmission spectrum for the medium of distance X between patch and viewer, and $V(\lambda)$ is the veiling light (Lythgoe, 1979; Endler, 1990). To maintain consistency, all spectra were modelled using daylight 65 (D65) irradiance (Fig. 2.5.1) (Wyscecki and Stiles, 1982) which is clear blue sky and commonly used in visual modelling (Vorobyev *et al.,* 2001; Chiao *et al.,* 2000a, b).

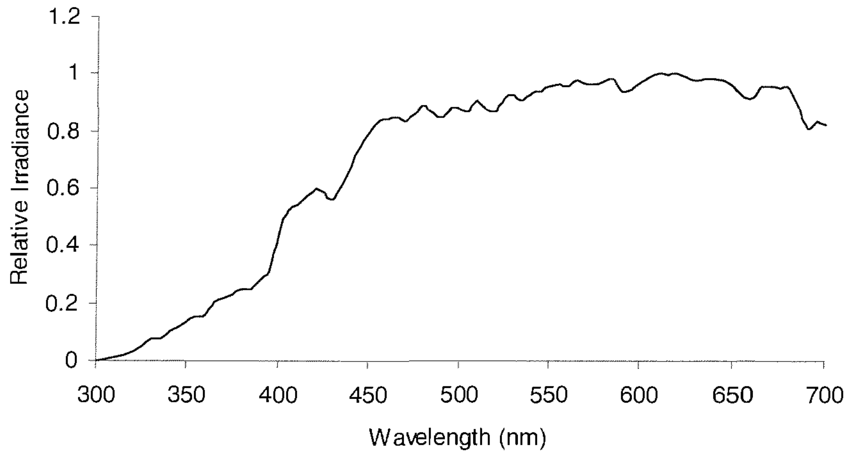


Figure 2.5.1: Daylight 65 irradiance spectrum (from Wyscecki and Stiles, 1982).

Veiling light obscures details of the visual task in question by reducing the level of contrast and in this case is commonly caused by fog or dust particles. When the viewing distance is less than 10m, in the case of all avian predator species to be considered here, in clear air $T(\lambda,X) = 1.0$ and $V(\lambda) = 0.0$. However when considering marine species such as fish and crabs, it is important to consider the behaviour of light in water and in particular the effect of turbidity and attenuation with depth (Lythgoe, 1979, 1991). Based upon this theory, consideration to the visual system of the observer can be given and reflectance spectra can be modelled to interpret the way in which information about a certain visual environment, in particular a patch and its background, will be made available for neural processing. This involves calculating a 'filtered spectrum' which accounts for any absorbance by pre-retinal structures that occurs due to the optical properties of the organism's eye. Mathematically, the stimulus spectrum must be multiplied by the transmission spectra of the ocular media that have significant absorption in the range of wavelengths to which the photoreceptors are sensitive (Sumner and Mollon, 2003). In terms of the visual systems of species to be considered here, ocular media to be considered include the absorption properties of the lens and the macula.

Differences in the angle at which a predator may view an object clearly exist. For example a crab would view objects horizontally more often than birds, which may view small prey items at 45^o or even from directly above. This methodology does not take account for variation in viewing angle and merely calculates quantum cone catches of different objects.

At the cone level knowledge about sensitivity is required and is acquired by methods such as MSP. Hence the total output (receptor quantum catches) of each cone type i present in the observers retina can be calculate for, in this case, all littorinid shell spectra and backgrounds by the following integral:

$$Q_i = \int R_i(\lambda)\, S(\lambda)\, I(\lambda) \mathrm{d}\lambda$$

Where λ represents wavelength, R_i is the spectral sensitivity of cone type i, $S(\lambda)$ is the fraction of incident photons reflected from the colour patch, integrated over the visual spectrum (in this case 300-700nm) and $I(\lambda)$ is the spectrum of light entering the eye (irradiance on the colour patch due to incident light, Vorobyev and Osorio, 1998). Representing quantum cone catches in this way demonstrates the ability for objects to change in appearance according to illumination, yet many organisms are able to discount this variation through colour constancy. To take account of receptor adaptation enabling colour constancy, receptor quantum catches (Q_i) are normalised to give a value $qi = kiQi$. The coefficients ki describe the von Kries transformation, whereby Qi is normalised by the quantum cone catch for the irradiance spectrum. Normalised quantum catch values are calculated for the littorinid (Q_i^a) and the background (Q_i^b) for each cone type i:

$$qi = kiQ_i^a / kiQ_i^b$$

The Vorobyev and Osorio (1998) model depends on psychophysical thresholds set by noise in receptors i. These thresholds are used to investigate a given noise dominated mechanism. The signal of receptor channel f_i is proportional to the natural logarithm of the quantum catch:

$$f_i = \ln q_i$$

Therefore the difference in the receptor signals (Δf_i) for two stimuli, a and b, is defined as:

$$\Delta f_i = f_i, a - f_i, b$$

The following equations model the discriminability (ΔS) of the two stimuli (in this case a littorinid shell and a background) in a) dichromatic visual system, b) trichromatic visual system and c) tetrachromatic visual system:

a)

$$(\Delta S)^2 = \frac{(\Delta f_1 - \Delta f_2)^2}{e_1^2 + e_2^2}$$

b)

$$(\Delta S)^2 = \frac{e_1^2 (\Delta f_3 - \Delta f_2)^2 + e_2^2 (\Delta f_3 - \Delta f_1)^2 + e_3^2 (\Delta f_1 - \Delta f_2)^2}{(e_1 e_2)^2 + (e_1 e_3)^2 + (e_2 e_3)^2}$$

c)

$$(\Delta S)^2 = \frac{((e_1 e_2)^2 (\Delta f_4 - \Delta f_3)^2 + (e_1 e_3)^2 (\Delta f_4 - \Delta f_2)^2 + (e_1 e_4)^2 (\Delta f_3 - \Delta f_2)^2 + (e_2 e_4)^2 (\Delta f_3 - \Delta f_1)^2 + (e_3 e_2)^2 (\Delta f_2 - \Delta f_1)^2 + (e_3 e_2)^2 (\Delta f_1 - \Delta f_4)^2))}{((e_1 e_2\, e_3)^2 + (e_1 e_2\, e_4)^2 + (e_1 e_3\, e_4)^2 + (e_2 e_3\, e_4)^2)}$$

where, noise in receptor type i, (e_i), can be modelled in various ways. However Hadfield (2004) presents a flexible function which recognises three sources of noise:

$$e_i = \frac{\sqrt{(1/(ln(\mathrm{T}(Qia + Qib)/2))^2 + \omega_i^2)}}{n_i}$$

where T is a scaling factor which relates Q_i (expressed as a proportion of the maximal cone catch) to an absolute quantum catch value, ω_i = the Weber fraction for cone type I which represents the signal to noise ratio for the colour channel i:

$$\omega_i = v_i / \sqrt{n_i}$$

where v_i is the signal-to-noise ratio of a single cone and n_i is the number of receptor cells of type i within the receptor field. Weber fraction (ω_i) values are set for either the Pekin Robin, *Leiothrix lutea* (long-wavelength-sensitive cone $\omega_4 = 0.05$) from which the other ω_i values for the remaining receptor classes can be calculated or a human ($\omega_i = 0.02$) (Vorobyev and Osorio, 1998). Estimates of cone ratios (n_i) for the visual systems modelled in this thesis are described in section 2.6.

Ideally, ΔS will be in units of "just noticeable differences" (jnds), where less than 1 jnd indicates that the two stimuli are not discriminable. However, difficulty in parameterising the receptor noise model means that there are no units and ΔS values can only be compared relative to each other (J. Hadfield, *pers. comm.*). Therefore conclusions about the discriminability of two stimuli are based upon the relative differences in ΔS between, for example, pairs of littorinids and backgrounds in one comparison (*i.e.* one visual system and irradiance condition).

The Vorobyev and Osorio (1998) model ignores the achromatic signal. However a large amount of variation in natural reflectance spectra is the brightness (achromatic) component (Cuthill *et al.,* 1999). It is thought that the achromatic signal may be important for luminance-based tasks such as motion detection (Osorio *et al.,* 1999; Hart, 2001b). Therefore the achromatic signal received, based on brightness differences alone, when different visual systems observe pairs of stimuli, is calculated using the following equation:

$$\Delta S = \left|\Delta f_i \,/\, \omega_i\right|$$

In birds, the double cone class is assumed to be responsible for achromatic tasks (Maier and Bowmaker, 1993; Osorio *et al.,* 1999; Vorobyev *et al.,* 1998). However, for other species modelled in this thesis the achromatic signal is assumed to be detected from the long-wavelength sensitive receptor type.

Although such models give consideration to the eye physiology of the target species, the methodology makes no assumption about the neurological processing stages involved in perception. Hence, this technique merely accounts for the information available to the observer's brain based on the number of photons absorbed by the different photoreceptor types which are characterised by their spectral sensitivity curves. However, this method can be assumed to be a reasonable estimation of perception as the larger the changes in receptor signals, the larger are the changes in colour appearance (Chiao and Cronin, 2002).

(2.6) Ecology and Visual System Characteristics of Test Organisms

The evolutionary mechanisms determining the visual characteristic of organisms are thought to be determined by the visual environment, and hence visual 'tasks', that the organism experiences. This section will detail the relevant ecological characteristics in prey detection of the known predators of littorinid species concerned in this work. In particular, the visual systems of these predators will be described, including justification for the best estimate of parameters used in visual modelling based on published data and personal communication with other researchers. It must be noted that the information presented on different visual systems varies in detail for the three predator types, therefore the most complete model was applied for each predator type.

(2.6.1) Green Shore Crab (*Carcinus maenas*)

Foraging Ecology

Carcinus maenas is an omnivorous predator, foraging opportunistically on a wide variety of animal and algal material on rocky shores (Crothers, 1968; Shelton and Mackie, 1971; Elner and Hughes, 1978; Behrens Yamada and Boulding, 1996, 1998; Hughes and O'Brien, 2001). Despite their omnivorous foraging behaviour their chelae are well adapted to both peeling (grasping the apex with one chela and rotating the shell with the other until the apex breaks off) and crushing mollusc shells (Pettitt, 1975). Crabs are an important class of predator on marine molluscs (Pettitt, 1975; Smith, 1976; Raffaelli, 1978; Hughes and Elner, 1979; Reid, 1984, 1987; Seeley, 1986; Hughes and Mather, 1986; Hughes and O'Brien, 2001). Experiments have shown that predation by crabs on polymorphic littorinids is difficult to test and visual selection has not been recorded (Tucker, 1988). This species is known to be active throughout a complete 24 hour cycle, yet maximum activity is observed during a high tide at night. In addition, ontogenetic differences in feeding behaviour in *C. maenas* means that its impact on prey populations on the shore is dependent on the size range present, which is known to vary seasonally (Mascaró and Seed, 2001). The sensory mode most commonly employed by *C. maenas* has long been debated in the literature, primarily because it has not been known whether crabs possess colour vision. It has been suggested that crabs do in fact use colour vision to hunt for prey and are better able to detect the object if it is moving, and best of all if it is striped (Waterman, 1961). The use of chemical and tactile stimuli has been commonly evoked as an explanation for foraging behaviour due to confusion surrounding the colour vision capabilities of shore crabs. Presentation of molluscan flesh has been found to illicit a response in sense organs located on the antennae resulting in the attraction of that individual towards the flesh (Shelton and Mackie, 1971; Rebach, 1996; Zhou and Rebach, 1999). Mechanoreceptors in limbs and mouthparts can detect changes in water currents enabling the crab to sense objects creating this disturbance (Crothers, 1967; Tautz *et al.*, 1981; Garm, 2005). Despite support for chemosensory and mechanoreceptor mechanisms of prey detection from afar, upon close range it can be expected that *C. maenas* uses its visual capabilities to distinguish prey from the background.

Visual System

Conflicting results from experimental work has made characterisation of crustacean visual systems notoriously difficult. Horridge (1967) proposed the presence of a two receptor-pigment system in *C. maenas* through motion-detection behavioural experiments. Wald (1968) used selective adaptation of the electroretinogram (ERG) response to physiologically support behavioural evidence for the multiple pigment system. Contrary to both findings, Bruno *et al.*, (1973) concluded the existence of a single pigment system and hence a lack of colour discrimination ability in *C. maenas*. Although no additional visual pigments have been identified, the use of filters in colour screening or reflecting mechanisms has been suggested to explain behavioural evidence of a two pigment visual system (Leggett, 1979). Therefore for the purposes of estimating the appearance of reflectance spectra in the visual system of *C. maenas* a two-pigment visual system has been used. Specifically, the spectral sensitivity based on electroretinogram (ERG) experiments by Martin and Mote (1982) which suggest maximum sensitivity (λmax) of blue (440nm) and green light (508nm) for *C. maenas* has been used. Spectral sensitivity curves (Fig. 2.6.1) were produced by fitting a visual template of normalised data as described in section 2.5.1 (Godvardovskii *et al.*, 2000).

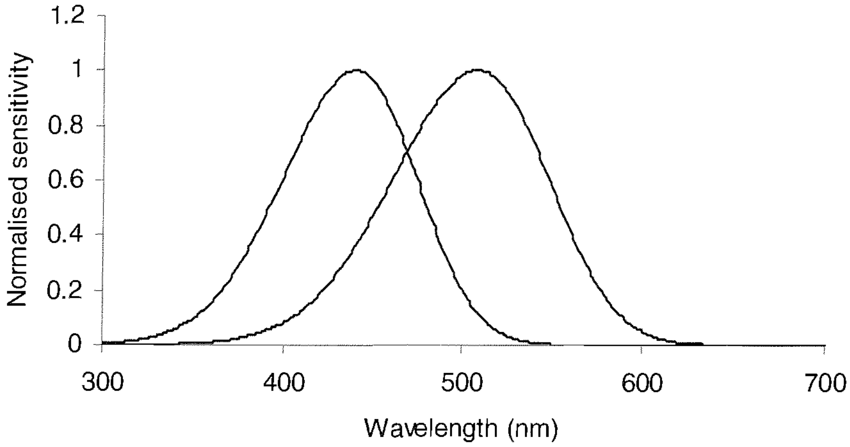


Figure 2.6.1: Spectral sensitivity curves of *Carcinus maenas* (from Martin and Mote, 1982).

Using the SPEC software, quantum catches per receptor type were calculated with Von Kries transformation (Nascimento and Foster, 1997; Dyer, 2001), using D65 irradiance spectra and without any consideration to the optical qualities of the ocular media due to lack of published data.

Model Parameters

For estimation of colour space distances for pairs of spectra (ΔS) high light intensity (T= 1,000,000) was selected to produce the greatest signal to increase values of ΔS and enable greater differentiation between spectral pairs. Weber fractions of 0.02, which is that of a human, were used as it is not yet certain that the threshold contrast for both receptor mechanisms of a crab is less than that of a bird (Vorobyev and Osorio, 1998). Clearly an exact Weber fraction would have been more appropriate, however, determination of threshold values requires at least knowledge of absolute numbers of the different receptor cells. Additionally, equal densities of receptor types were assumed after a trial of different ratios. Furthermore there is a lack of published literature on shore crab receptor cell densities. There is some evidence to suggest that dichromatic brachyuran crabs possess two visual pigments which are expressed in the same receptor cell (Sakamoto *et al.*, 1996) which supports equal densities of receptor cells in *C. maenas*.

(2.6.2) Blenny (*Lipophrys pholis*)

Foraging Ecology

The visual environment encountered by a fish is effectively simpler than that of a terrestrial organism because water has a dimming effect by selective absorption of certain wavelengths of day light (Lythgoe, 1979). Effectively the closer to the surface a species lives, the broader the range of the spectrum that is available and hence the more classes of receptor cells required (Loew and Lythgoe, 1978). Since littorinids occupy the intertidal zone of rocky shores, their fish predators would most likely be coastal species. In addition, there is evidence that coastal species prey upon littorinids readily (Reimchen, 1979; Burrows *et al.,* 1999). Hence, the species chosen for an approximate visual model was the blenny, *Lipophrys pholis.*

As *Lipophrys pholis* is a bottom-dwelling fish of the family Blenniidae (Teleosti), it approaches prey located in the water column from below and prey located on the bottom from above. SCUBA observations have reported a single blenny moving within algal fronds and removing 14 out of 64 *L. fabalis* in one hour (Reimchen, 1979). Laboratory experiments have shown blennies approaching littorinids on algal fronds and this is further discussed in chapter 6 and 7. The nature of the water in which these coastal fish hunt is important as there are striking differences in the spectral distribution of natural water according to chlorophyll content, levels of dissolved products and depth (Lythgoe and Partridge, 1989). Therefore, in Chapters 6 where fish vision modelling was carried out, turbid water conditions were tested.

Visual System

The visual system of the peacock blenny (*Salaria pavo*) has recently been characterised in full allowing an insight into the visual capabilities of a littoral fish species (White *et al.,* 2004). This species is found from Morrocco to Southern France (Zander, 1986) and occupies a visual environment comparable to that of the British blenny (*L. pholis*). *Lipophrys pholis* is known to prey upon littorinids and accordingly possesses both rods and cones with similar wavelength sensitivities to the peacock blenny: a rod pigment with a λmax at 515nm (Loew and Lythgoe, 1978) or 504nm (Partridge, 1986), and medium-wavelength sensitive (MWS) and long-wavelength sensitive (LWS) cones with λmax values of around 538nm (Partridge, 1986) and 570nm (Loew and Lythgoe, 1978). Additionally, *L. pholis* is known to possess a class of short-wavelength sensitive (SWS) cone with λmax 454nm (Partridge, 1986). Therefore *L. pholis* can be classed as a trichromat and the spectral sensitivities used in modelling are according to Partridge (1986) as described above (454nm, 538nm and 570nm) and shown in Figure 2.6.2. Using microspectrophotometry in peacock blennies, White (2003) found that the cornea was unpigmented and transmitted evenly over the range 300-800nm whereas the lens was found to transmit evenly over the range 440-800nm but had a mean short-wavelength cut-off at 420nm (Fig. 2.6.2). This feature of the lens is known to enable short-wavelength cutoff, filtering out UV light (White *et al.*, 2004). Therefore secondary visual structures (lens transmission) in the eye of the blenny will be considered in the visual model.

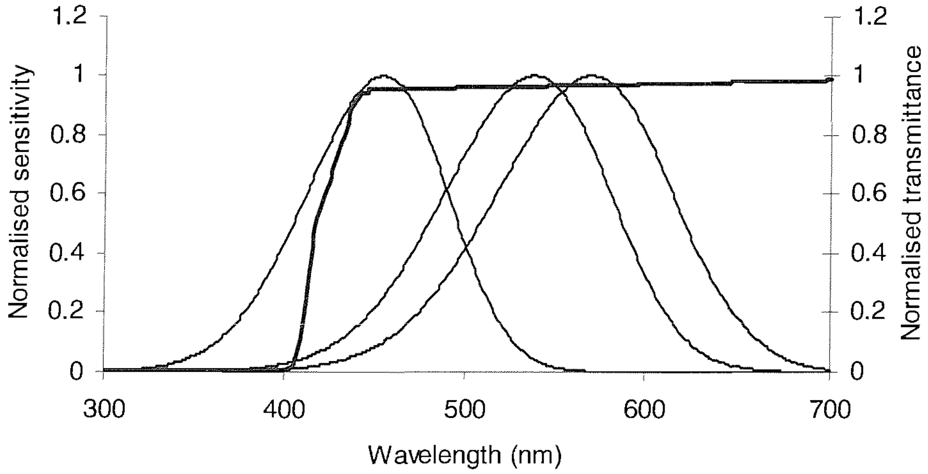


Figure 2.6.2: Spectral sensitivity curves and mean transmission of the peacock blenny lens (adapted from White, 2003). The lens acts as an ultraviolet cut-off filter, with the point of 50% transmission occurring at around 420nm.

Model Parameters

For estimation of colour space distances for pairs of spectra (ΔS) high light intensity (T= 1,000,000) was selected. As with *C. maenas,* Weber fractions of 0.02, which is that of a human (Vorobyev and Osorio, 1998), were used as it is not clear whether a blenny's threshold contrast for both receptor mechanisms is less than that of a bird. As described for the crab model parameterisation, an exact Weber fraction would have been more appropriate. However, determination of threshold values requires at least knowledge of the absolute numbers of the different receptor cells. The ratio of cones used for modelling was 1:1:2 (SWS:MWS:LWS) based on the ratio adopted by White (2003) for MWS and LWS cones with the assumption of a ratio value of 1 for SWS cones as there is no other evidence available. Cornea and lens transmission data were used for the peacock blenny, *Salaria pavo* from White (2003).

(2.6.3) Shorebird

Foraging Ecology

So far, no species of shorebird has had its visual system fully characterised both in terms of spectral sensitivity and pre-retinal structures such as oil droplets. Therefore foraging ecology in this section will be a general review of the foraging characteristics of shorebirds which are known predators of littorinids including species from the families Haematopodidae (Oystercatchers), Laridae (Gulls), Motacillidae (Pipits and Wagtails), Scolopacidae (Sandpipers) and Charadriidae (Plovers).

Depending on the species and the time of year, avian predators impact upon intertidal mollusc communities differently. Migrating knots have been found to remove 1-4 % of littorinids whilst stopping over in Iceland during the spring (Alerstam *et al.*, 1992). Such precise estimates of the impact of bird predation on littorinid populations are often lacking in the literature despite field observations and gut content analysis. This is because it is believed that some individual birds may target, in preference to littorinids, a more profitable food source while the rest of the population concentrate on the latter. The overall impact is therefore unknown (Feare, 1967; Pettitt, 1975). From gut content and gizzard analysis numerous species of shorebird have been found to consume littorinids, and to different extents, often dependent on the individual's preference and availability of the species' usual food source (see Pettitt, 1975 for a review). In terms of the actual consumption of littorinids, an appreciation for the viewing angle and foraging distance of a shorebird must be included. From field observation, shorebirds forage amongst algae when the tide is out, occasionally using their beak/bill to move the algae and uncover prey beneath the surface. The bird will be observing the prey and its background from head height (approximately 30cm) and lower with a viewing angle of approximately 45 degrees (Osorio and Ham, 2002; I. C. Cuthill *pers. comm*). However, the viewing angle could not be incorporated into the model.

Visual System

In contrast to di- and trichromatic visual systems, birds have four classes of daylight-functioning photopigments (cones) that differ in their absorption spectra; hence they are maximally sensitive to different wavelengths of light. The visual systems of birds are highly studied and hence more information is available for parameterising a visual model for a suitable shore bird than either the crab or the fish. No physiologically derived spectral sensitivity data exist for a shore bird; however, molecular methods looking at the SWS1 opsin gene have shown that some species in the family Laridae possess UV sensitive receptors as opposed to violet sensitive receptors with λmax at 371nm (Ödeen and Håstad, 2003). Given that birds are relatively conservative in the maximal sensitivity of cone types (Hart, 2001b), other than for the UV sensitive/violet sensitive cone, it seems appropriate to use well established spectral sensitivities for the SWS, MWS and LWS cones. Therefore, the blue tit (*Parus caeruleus* L.) was used as a model for the remaining three cones with λmax values at 451nm (SWS), 537nm (MWS) and 605nm (LWS) (Hart *et al.,* 2000).

It is known that birds, such as gulls (Laridae), that must look through the surface of the water to locate their prey, tend to have a relatively high proportion of LWS cones (Hart, 2001b). Physiological interrogation of retinal cells of the Silver gull (*Larus novaehollandiae*) has led to estimates of the relative abundance and topographical distribution of retinal cone photoreceptors. The mean percentages of the different cone photoreceptor types in the eye regions of the retina are 9.2% (UVS/VS), 17.2% (SWS), 21.9% (MWS), 21.5% (LWS), 30.3% (LWS double cones) (Hart, 2001a). These values have been calculated as a cone ratio for use in the visual model executed in SPEC; 1:1.9:2.4:2.3 (UVS/VS:SWS:MWS:LWS). Spectral sensitivity curves (Fig. 2.6.3) were produced by fitting a visual template of normalised data as described in section 2.6 (Godvardovskii *et al.,* 2000). Using the SPEC software, quantum catches per receptor type were calculated with Von Kries transformation, using daylight 65 irradiance spectra and with consideration to the optical qualities of the ocular media, including oil droplets, according to Hart *et al.,* (2000) (Figure 2.6.3).

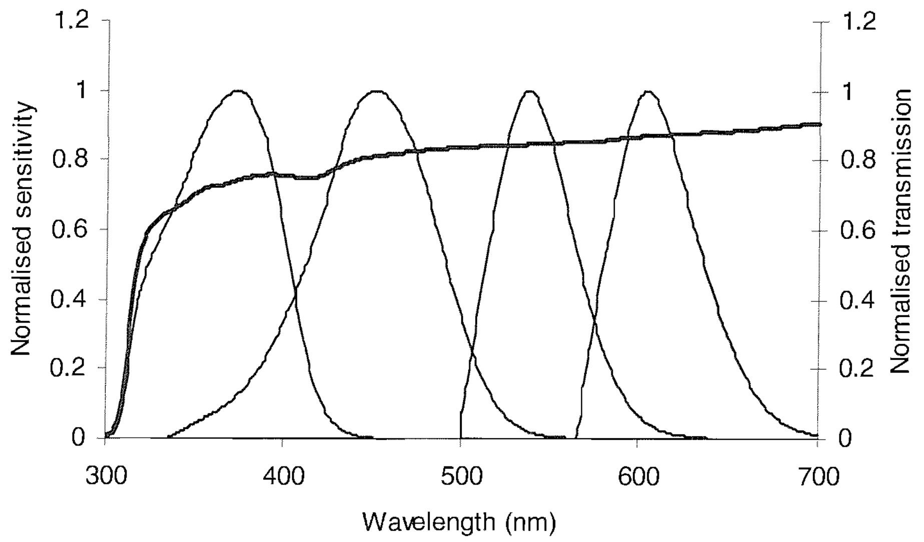


Figure 2.6.3: Spectral sensitivity curves and mean transmission of the ocular media of a shore bird from combining physiological data from the silver gull (*Larus novaehollandiae*, Hart, 2001a) and the blue tit (*Parus aureleus*, Hart *et al.*, 2000).

Model Parameters

For estimation of colour space distances for pairs of spectra (ΔS), the following values were chosen for the model parameters; High light intensity (T= 1,000,000) to maximise the signal, Weber fractions of 0.05 which is an estimation based on values for the Pekin Robin, *Leiothrix lutea* (long-wavelength-sensitive cone ω_4= 0.05) (Vorobyev and Osorio, 1998), and cone ratio based on the densities of cone types of the Silver gull (1:1.9:2.4:2.3) (Hart, 2001a).

(2.7) Study Sites

Spectral data were collected at eight UK sites (Fig. 2.7.1) which were chosen for specific reasons including diversity of colour morphs of the littorinid species, diversity of rock colour, heterogeneity created by lichen coverage and algal species present. Data from only five of the shores are presented in this thesis. All shores had to have good access for carrying heavy equipment and sites which were frequently sampled were chosen to be within one day's driving distance. Data are presented for the algal dwelling species *Littorina fabalis* and *L. obtusata* at Bembridge, Isle of Wight (SZ 659880). The high shore rock dwelling species *L. saxatilis* was studied on four shores: Kimmeridge, Dorset (OS grid reference SY 907784); Swanage, Dorset (SZ 040786); Portland, Dorset (SY 678683); and Heybrook Bay, Devon (SX 495487).

In addition, four shores on the West coast of Sweden were sampled for *L. saxatilis* as part of collaboration with Professor Kerstin Johannesson at Tjarno Marine Biology Laboratory, Göteborg University; details about Swedish sites will be given separately in Chapter 5.

In the following five results chapters the principles and techniques of spectral data collection, objective Principal Components Analysis (PCA) were used to demonstrate spectral differences between littorinids and backgrounds. Additionally, visual models were used to calculate colour space distances (ΔS) between littorinid and background spectra pairs. The differences between spectral pairs and also littorinid morphs are investigated using statistical tests.

PART II

ROCK DWELLING LITTORINIDS

Chapter 3

Does accounting for spectral reflectance and predator vision help explain differences in colour polymorphism in *Littorina saxatilis* on different shores?

Summary

Correlations between the reflectance properties of certain morphs of *Littorina saxatilis* and certain rock colours are known to occur. These correlations suggest that visual selection against conspicuous morphs may be acting to maintain shell polymorphism in a heterogeneous environment. The colour polymorphisms of three populations of *L. saxatilis* were investigated by measurement of reflectance spectra and visual modelling techniques. Principal Components Analysis (PCA) of littorinid and background reflectance spectra revealed that the two shores of the same rock type were similar in spectral diversity, whereas another shore with different rock type was more spectrally diverse. The results of visual modelling show that *L. saxatilis* populations on shores of the same geology show similar background matching, whereas the more spectrally diverse shore provides more opportunities for crypsis. The differences between crabs and birds in their abilities to detect different morphs are discussed. Despite the differences in geology, yellow morphs were conspicuous in the simulated visual systems of both crabs and birds. Possible explanations for the differences between colour morphs and the different geologies are discussed with reference to selective and non-selective processes.

(3.1) Introduction

Selection in heterogeneous backgrounds has often been suggested as an important contributing factor in the maintenance of colour polymorphism (Cain and Sheppard, 1954; Jormalainen and Tuomi, 1989; Cook and Kenyon, 1991; Harris and Jones, 1995; Parsonage and Hughes, 2002; Gonçalves Rodrigues and Silva Absalão, 2005). The extent to which the constituent morphs match their backgrounds has been much discussed, although quantification of this phenomenon is less common (Poulton, 1890; Thayer, 1909; Cott, 1940; Edmunds, 1974; Endler, 1978). Frequently the evolution of crypsis is prevented because conspicuousness is beneficial in intraspecific communication such as mating, intrasexual competition and territorial behaviour (Macedonia *et al.,* 2002; Siddiqi *et al.,* 2004; Håstad *et al.,* 2005). In organisms where vision is not well developed, intraspecific communication occurs via some other channel (*e.g.* tactile response, vibration, olfaction or sound). Therefore the general result is an inconspicuous phenotype largely determined by the behaviour of the predator(s) (Hughes and Jones, 1985; Hughes and Mather, 1986). Other selective forces such as thermoregulation (*e.g.* in *Nucella lapillus,* Etter, 1988; *Littoraria* spp., Parsonage and Hughes, 2002) and selection on pleiotropic effects of coloration (*e.g.* in *Littorina* spp. Raffaelli, 1979) may also be important.

Gastropods are one of the most polymorphic classes in the phylum Mollusca (Clarke *et al.,* 1978; Cain, 1977, 1988) despite their limited vision. Therefore factors influencing the polymorphism in this taxon exclude intraspecific communication, particularly as no evidence of assortative mating based on shell colour has been found (Lamotte, 1959; Cook and Garbett, 1992) and courtship in helicid molluscs does not depend on visual stimuli (Jeppesen, 1976). Accordingly, visual selection by vertebrate predators in a heterogeneous environment has been suggested to help maintain shell polymorphism in a variety of gastropods (Cain and Sheppard, 1954; reviewed by Jones *et al.,* 1977; Clarke *et al.,* 1978; Reimchen, 1979; Johannesson and Ekendahl, 2002). Other processes have also been suggested such as selection pressure via linkage disequilibrium (Sokolova and Berger, 1999) and apostatic selection (Cain and Sheppard, 1954; Smith, 1976; Reid, 1987). Background matching, however, remains the most highly studied process whether it is acting to maintain the polymorphism or reduce the level of variability. In particular the polymorphism in *Littorina saxatilis* complex of species has been extensively documented

(Pettitt, 1973b; Reid, 1996; Atkinson and Warwick, 1983; Ekendahl and Johannesson, 1997; Ekendahl, 1998).

Littorina saxatilis occupies the upper zones of the intertidal rocky shore and is an ovoviviparous species which retains embryos within the brood pouch and releases miniature snails at the crawling stage (Reid, 1996). Dispersal is therefore limited and there is little gene exchange between sites on neighbouring shores (Johannesson and Tatarenkov 1997). Depending on the geology and resultant topography of the area, the upper zone of the intertidal is effectively a two dimensional habitat with crevices subjected to seasonal and tidal variability in the extent of wave splash. Additionally this area of the shore is patchily colonized by epiphytes, encrustations and epifauna resulting in a visually heterogeneous environment with its underlying colour determined by the rock type and weathering.

Littorina saxatilis is polymorphic in many shell traits including, size, shape, ornamentation and colour. To describe accurately shell colour variation in this species, an 'allele-like' system of determining ground colour and patterning separately was described by Pettitt, (1973b) leading to the characterisation of up to 1288 morphs. The morphs at any particular shore can be classified into fewer morphs using ordinary colour names which commonly include white, yellow, fawn, brown, black, red, orange, buff and also banded (Reid, 1996; Ekendahl and Johannesson, 1997). The shell colour polymorphism observed in *L. saxatilis* is also geographically variable in terms of the frequencies of morphs found on different shores and in different countries (Ekendahl and Johannesson, 1997) and has been specifically related to rock colour in the past by the use of morph colour coding based on shell colour and pattern (Heller, 1975; Raffaelli, 1979). Colour coding of morphs, however, does not allow the quantification of colour. Instead, morphs are categorised by the human observer under the lighting conditions at the time.

Littorina saxatilis is potentially preyed upon by avian and crustacean predators (Pettitt, 1975; Edwards *et al.,* 1982; Johannesson and Ekendahl, 2002). Although fish are reported to prey readily upon intertidal gastropods (Reimchen, 1979; Bertness *et al.,* 1981), it is likely that fish predators have little impact on high shore *L. saxatilis* which are seldom fully submerged. Likewise, crab predation will be relatively minimal in the splash zone on macrotidal UK shores. Despite this, crustaceans are likely to affect *L. saxatilis* in other areas with smaller tidal ranges such as Southern Europe and Sweden (Gurriarán and Mendez, 1985; Ekendahl and Johannesson, 1997; Ekendahl, 1998) and for this reason crabs are included as a potential predator. Therefore this work addresses the influence that birds and crabs have on the polymorphism of *L. saxatilis* at multiple sites. The visual capabilities of the predators range from dichromacy with blue and green visual sensitivity in the crab (Leggett, 1979; Martin and Mote, 1982) to tetrachromacy in birds which gives rise to sensitivity across the entire visible spectrum including ultraviolet wavelengths (reviewed by Bennett and Cuthill, 1994).

By taking into consideration the entire visual signal available to a potential predator (the prey item's reflectance spectrum) it is possible to prevent erroneous conclusions based on the visual capabilities of humans. More specifically, measuring differences in reflectance spectra between prey and their backgrounds enables assessment of coloration irrespective of neural processing (and therefore variability) created by a human observer (Cuthill *et al.*, 1999). Spectral reflectance measurement has already given us a better understanding of animal signaling by characterisation of animal coloration in many taxa including arthropods (Heiling *et al.*, 2005), fish (Grill and Rush, 2000; Rush *et al.*, 2003), reptiles (Macedonia *et al.*, 2002), amphibians (Emerson *et al.*, 1990), birds (Hunt *et al.*, 1998; McNaught and Owens, 2002; Perrier *et al.*, 2002), and mammals (Godfrey *et al.*, 1987; Kiltie, 1992). Although there are many definitions of the colour morphs present in mollusc species (*e.g. Littorina obtusata,* Smith, 1976; *Littoraria filosa,* Reid, 1987; *Cepaea nemoralis,* Cain, 1988; *Nucella lapillus,* Etter, 1988; *Theba pisana,* Cowie, 1990), characterisation of shell polymorphism by spectral measurement of shell coloration has never been carried out. *Littorina saxatilis* inhabits a visually heterogeneous habitat and has a low level of dispersal which makes it an ideal species for studying the evolution of colour polymorphism by background matching using spectral measurement techniques such as spectroradiometry.

Spectroradiometry provides a quantitative description of colour by accurate measurement of spectral reflectance. Reflectance spectra can then be used in visual models to estimate the discriminability of objects against their backgrounds in another organism's visual system. The discriminability of two spectra can be quantified by calculation of colour space distances between spectra (ΔS values) using the Vorobyev and Osorio (1998) threshold sensitivity model. Colour space distances represent the abilities of different predators in the detection of prey items against different backgrounds in the sense that the larger the ΔS is between the prey and its background, the more conspicuous the prey item is.

Accordingly, reflectance spectra have been modelled into the visual systems of conspecifics and predators to determine the significance of visual signals in a wide variety of taxa including birds (Maddocks *et al.,* 2001; Vorobyev *et al.,* 2001), reptiles (Stuart-Fox *et al.,* 2003), amphibians (Siddiqi *et al.,* 2004) and arthropods (Théry and Casas, 2002; Dyer and Chittka, 2004). Mollusc shell coloration, however, has never been interpreted in the visual system of potential predators which may be influencing the colour polymorphism. Therefore, my work compared littorinid populations and the background matching of their constituent morphs from three shores on the south coast of England. This was achieved in the present study by collection of spectral data from littorinids at different shores to be assessed objectively and also by the use of visual modelling. Two of the sites were exposed limestone shores and are separated by 40km and the other was a moderately exposed dolomite limestone shore and is approximately equidistant from both of the other two limestone shores. If visually-orientated predators are selecting conspicuous littorinids against a heterogeneous background then the polymorphism in *L. saxatilis* at the two more distant locations with similar geology would have the most similar colour variation.

My overall aim was to investigate whether modelling littorinid and background spectra into the visual systems of potential crab and bird predators contributes to the understanding of the evolution of the polymorphism in *Littorina saxatilis.* The specific questions addressed were whether site specific background matching patterns exist or is the polymorphism driven by the background rock colour and hence geology? Additionally, are morphs spectrally distinct such that they should be categorized as separate colour morphs?

(3.2) Methods

Reflectance spectrum sampling was carried out between June and September 2004 at Portland, Kimmeridge and Swanage in Dorset, England. Sites were chosen on the basis of their geology. Swanage and Portland are exposed oolitic limestone shores and were chosen as they share the same geology yet they are separated by 40km. The geology of

Portland and Peveril Point at Swanage is the result of a Portland limestone outcrop through the Isle of Purbeck (Allison and Kimber, 1998). Kimmeridge is a semi-exposed rocky ledge situated equidistant in between the two limestone shores and is also limestone, known as ferroan dolomite, which has been affected by iron such that it is orange/yellow in colour with brown crevices.

At Kimmeridge, which is a rocky ledge, four 5 x 0.5m horizontal transects were randomly selected and all littorinids encountered in each transect and their backgrounds were measured for spectral reflectance. At Portland and Swanage, which are boulder shores, four densely populated areas were identified and all littorinids were sampled until approximately one hundred littorinids per area and their backgrounds had been included. Reflectance spectra were collected in the centre of the last whorl from littorinid shells larger than 3mm shell height restricted by the reflectance probe aperture. When collecting reflectance spectra from *Littorina saxatilis* and the rock background, sampling strategy varied according to the topography of the shore. At all three shores, background spectra were measured, and on the right hand side, immediately adjacent to each littorinid (as viewed by the experimenter).

All shell reflectance spectra were measured in a dark box. For each snail a description of the rock background and the colour morph approximately according to Pettitt (1973a) was recorded. At each shore, a reference set of morphs was created to ensure consistency of colour characterisation. For all shores, morph frequencies were derived from the spectral data collection. Differences between morph frequencies at the three shores were analysed using a Chi-squared contingency table test.

Each littorinid was assigned to an age category (adult or juvenile) according to the size distribution encountered at each shore. Multivariate data reduction (PCA) and analysis was carried out on raw spectra of all shores together and then separately to determine differences between backgrounds and littorinids at each shore.

(3.2.1) *Spectral Data Analysis*

Mean reflectance spectra of each littorinid morph and sample reflectance spectra of backgrounds are shown for each shore to exemplify the spectra collected. Principal Components Analysis (PCA) was used to assess whether spectral differences alone can identify patterns between littorinid and background spectra within and between shores. All spectra used in PCA were standardised for brightness differences by subtraction of the mean reflectance of each spectrum from the reflectance at each wavelength interval. In PCAs of spectra, littorinids were not assigned to particular morph categories. Instead PCA was used to detect the overall similarities of littorinid spectra and their backgrounds at the different shores. In all principal component analyses, principal component (PC) 1 and PC2 explain 80% or more of the variation between spectra, therefore the subsequent principal components are not described. Firstly all spectral data from the three shores were reduced to the first two principal components and analysed to test for differences between PC1 and PC2 of littorinids and backgrounds dependent on the shore from which they came in a two-way ANOVA. Then within shore differences between PC1 and PC2 of littorinids and backgrounds were detected using individual one-way ANOVAs. PC scores did not always conform to assumptions of normality and homogeneity of variances for ANOVA, however, in each analysis there were more than 30 replicates per factor level therefore the results of the ANOVAs are interpreted with caution. The percentage variation explained by PC1 of unstandardised spectra, and the significance of its correlation with mean reflectance, is described to indicate the extent to which the raw spectra vary dependent on brightness. Furthermore, PCA indicates the diversity of littorinid spectra and whether this correlates with the diversity of background spectra found within each shore. Therefore PCA is a suitable method of data reduction to gain weighted linear sums of the original data which represent variation in the spectra

(3.2.2) *Visual Modelling Data Presentation and Analysis*

Visual models were used to determine the appearance of colour morphs in the visual systems of a bird and a crab as described in section 2.6. The crab and bird visual models described in section 2.6 were used to calculate colour space distances (ΔS) for each littorinid and its immediate background according the receptor threshold noise model of Vorobyev and Osorio (1998). Colour space distances represent the extent to which littorinids are discriminable from their backgrounds according to the assumptions of the crab and bird models and are presented and analysed in the following ways:

1. The numbers of paired comparisons which fall into each colour space distance (ΔS) class for each visual system model were displayed in frequency histograms to demonstrate the discriminability of littorinids at each shore. Each ΔS class was divided into the constituent morphs to indicate the number of individuals of each

morph in that ΔS class. To test whether the three shores differed in their overall extent of background matching, the difference between the distributions of ΔS values per shore were tested using one-way ANOVA on Box-Cox transformed data followed by *post-hoc* Tukey tests.

2. The median colour space distance of each morph from its own background was displayed as a box plot with the interquartile range to indicate the discriminability of the morph categories assigned on the shore. The discriminability of morphs (ΔS) was tested within each shore using General Linear Models on Box-Cox transformed data followed by *post-hoc* Tukey tests.
3. The difference between the frequencies of morphs in either the 0ΔS class or classes greater than 0ΔS were tested using chi square contingency tables which were partitioned to detect between morph, and between combined groups of morphs differences using the Lancaster (1949) and Irwin (1949) method as described by Everitt (1977).

(3.3) Results

(3.3.1) *Morph Frequencies*

Morph frequencies from all three shores (Table 3.3.1), differed significantly from each other (χ^2= 288.18, *d.f.* 14, P<0.001). From observation of the percentage frequencies in Table 3.3.1, the frequency of the yellow morph at Portland was higher than at either Swanage or Kimmeridge. Similarly the frequency of brown morphs at Swanage was greater than at Portland and Kimmeridge. Additionally the frequency of grey morphs at Kimmeridge was greater than at Portland and Swanage, whereas there were only 3 white littorinids at Kimmeridge compared to 35 and 28 at Portland and Swanage respectively. Furthermore, the black morph was only absent from Swanage and Portland.

Table (3.3.1): Frequencies and percentages (in brackets) of different morphs at Portland, Kimmeridge and Swanage.

	Morphs								
Site	Brown	Buff	Fawn	White	Yellow	Black	Orange	Grey	Sample size
Portland	76 (19.54)	37 (9.51)	82 (21.08)	35 (9)	130 (33.42)	-	15 (3.86)	14 (3.60)	389
Kimmeridge	91 (29.55)	-	58 (18.83)	3 (0.97)	67 (21.75)	38 (12.34)	8 (2.6)	43 (13.96)	308
Swanage	176 (48.22)	31 (8.49)	74 (20.27)	28 (7.67)	37 (10.14)	-	14 (8.83)	5 (1.37)	386

(3.3.2) *Reflectance Spectra*

A random sample of background reflectance spectra and mean reflectance spectra for each littorinid morph found at Portland are shown in Figure 3.3.1A. The background spectra demonstrate the 'yellowness' of the Portland limestone indicated by high reflectance from 500-650nm. The seven morphs identified at Portland vary in their mean spectra, but similarly demonstrate the yellowness of morphs at Portland.

Sample background reflectance spectra (Fig. 3.3.1E) collected at Kimmeridge were more variable in spectral shape than those collected at Portland, this can also be seen from the dispersion of points on the PCA score plot (Fig. 3.3.3). The rock background spectra at Kimmeridge do not show increased reflectance around 500-675nm which can be seen in Portland and Swanage spectra (Fig. 3.3.1D-F). Kimmeridge littorinid spectra (Fig. 3.3.1B) vary to a similar extent as those collected at Portland beyond approximately 500nm. The mean white morph spectrum at Kimmeridge has high reflectance but is similar in spectral shape to other morphs found at Kimmeridge. This difference in brightness could be due to the low number of white littorinids found at Kimmeridge.

Swanage background reflectance spectra varied in brightness like Portland, rather than in terms of spectral shape (Fig. 3.3.1F). From 590nm, mean spectra of littorinid morphs at Swanage were variable in both spectral shape and brightness depending on the 'yellowness' or 'redness' of the shells (Fig. 3.3.1C).

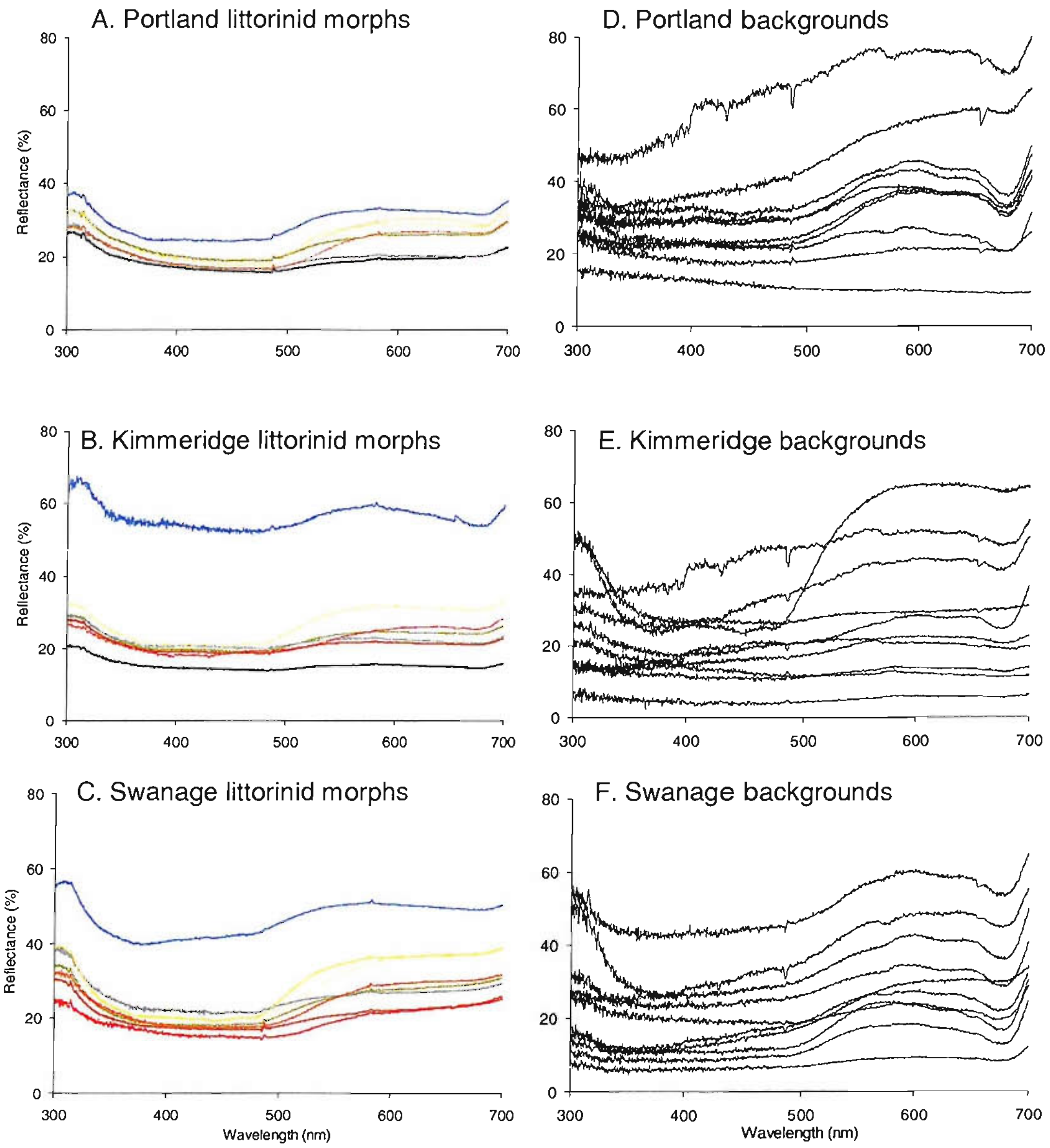


Figure 3.3.1: (A) Mean reflectance spectra of morphs (left hand side) collected at Portland (Brown, brown line, N= 76; Buff, peach line, N=37; Fawn, green line, N= 82; Grey, grey line, N=14; Orange, orange line, N=15; White, blue line, N=35; Yellow, yellow line, N= 130), (B) Kimmeridge (Black, black line, N= 38; Brown, brown line, N= 91; Fawn, green line, N= 58; Grey, grey line, N=43; Orange/terracotta, orange line, N=8; White, blue line, N=3; Yellow, yellow line, N= 67) and (C) Swanage (Brown, brown line, N= 176; Buff, peach line N= 31; Fawn, green line, N= 74; Grey, grey line, N=5; Orange, orange line, N= 8; Terracotta, red line, N=6; White, blue line, N=28; Yellow, yellow line, N=58). Right hand side: randomly selected sample reflectance spectra of backgrounds are variable in brightness at Portland (D), variable in shape at Kimmeridge (E) and variable in terms of brightness and spectral shape variation at Swanage (F).

(3.3.3) *Between-Shore Principal Components Analysis of Spectral Data*

The spectra from Portland, Kimmeridge and Swanage were analysed together using PCA to look for similarity between littorinids and backgrounds dependent on the shore. There was a significant interaction between the type of spectra (background and littorinid); specifically backgrounds are different from littorinids in that the background spectra have more positive PC2 values than littorinids. However, littorinids and backgrounds were not discriminable in terms of PC1 ($F_{(1,2155)}$=0.06, P=0.806). Given that PC1 represents variability between short and long wavelengths, PCA was unable to discriminate the variation between littorinids and their backgrounds when considering all three shores together. Presentation of the score plot and coefficient values of the three shores combined would provide little extra information. Therefore plots of centroids and standard errors are displayed (Fig. 3.3.2) along with tabulated statistics to describe significant differences with reference to the original spectra. There were significant shore-dependent differences between littorinids and backgrounds in PC1 and PC2 scores which represent chromatic variation in the spectra (PC1, $F_{(2,2155)}$=7.01, P=0.001; PC2, $F_{(2,2155)}$=48.15, P<0.001, Table 3.3.2). Specifically backgrounds at Kimmeridge were most variable which can be understood from the range of PC scores at Kimmeridge (PC1=143.81; PC2=100.08) compared to Portland (PC1=134.43; PC2=63.47) and Swanage (PC1=117.57; PC2=81.13). The variance in PC scores for backgrounds at Kimmeridge can also be seen from the scatter in Figure 3.3.2.

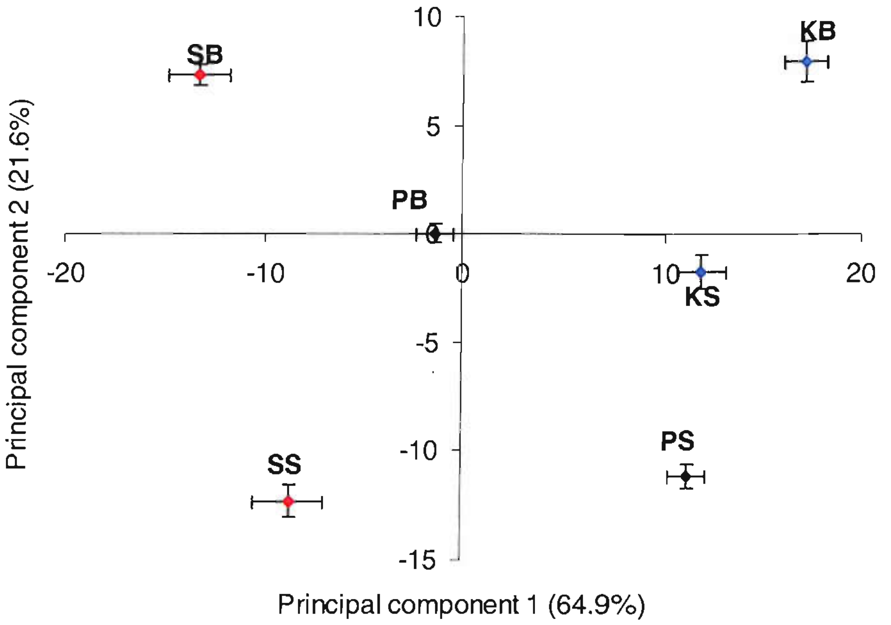


Figure 3.3.2: Principal component score centroids with standard error bars for Portland backgrounds (PB, N=386) and littorinids (PS, N=386), Kimmeridge backgrounds (KB, N=310) and littorinids (KS, N=310) and Swanage backgrounds (SB, N=385) and littorinids (SS, N=385).

Table 3.3.2: Two-way ANOVA comparing principal component scores of backgrounds against littorinids at Portland, Kimmeridge and Swanage.

	d.f.	Sum of squares	*F*	*P*
PC1				
Shore	2	223028	178.45	<0.001
Surface type	1	237	0.06	0.806
Shore x surface type	2	8758	7.01	0.001
Error	2155	1346012		
PC2				
Shore	2	10808	35.06	<0.001
Surface type	1	170431	1039.46	<0.001
Shore x surface type	2	14692	48.15	<0.001
Error	2155	328776		

(3.3.4) *Within-Shore Principal Components Analysis of Spectral Data*

Since analysis of variance of PC scores from all three shores produced statistically significant interactions, shores were investigated separately. Using standardised spectra PC1 and PC2 represent colour differences in the spectra (*i.e.* spectral shape differences), the description of which can be derived from the coefficient of variation plots (Fig. 3.3.3A-C). Apart from PC1 at Portland, all background spectra have significantly greater PC score values than littorinids (ANOVA, Table 3.3.3). More specifically, backgrounds have more positive PC scores than littorinids. PC2 for Portland data shows that backgrounds have high long-wavelength reflectance relative to littorinids, which have high short-wavelength reflectance relative to backgrounds (Fig. 3.3.3D). Therefore, backgrounds at Portland are significantly 'redder' than the littorinids sitting on them (Table 3.3.4).

In the case of PC1 at Kimmeridge, backgrounds reflect more light between 350-450nm relative to littorinids which are redder in colour and reflect more light at longer wavelengths (>450nm) relative to backgrounds (Fig. 3.3.3E). From the Kimmeridge PC2 coefficient of variation (Fig. 3.3.3E), the opposite relationship was apparent: littorinids reflect relatively more short-wavelength light compared to backgrounds which reflect more long-wavelength light relative to littorinids. Therefore PC2 represents the relative amount of short- to long-wavelength reflectance. Although there is a significant difference between littorinid and background spectra at Kimmeridge (Table 3.3.3), the PC score plot (Fig. 3.3.3B) shows that at Kimmeridge there was less distinct grouping of spectra types when compared to either Portland or Kimmeridge.

At Swanage littorinids reflected more short wave-length blue light relative to backgrounds, which reflect more long wavelength red light relative to littorinids. PC2 of spectra collected at Swanage shows that backgrounds had high long-wavelength reflectance relative to littorinids, which had high short-wavelength reflectance relative to backgrounds.

Table 3.3.3: One way ANOVA of principal component 1 and 2 scores for each shore, indicating the difference between backgrounds (B) and littorinids (L) (significance level: ns=not significant, $P<0.05$ *, $P<0.001$ ***).

	Principal component 1			Principal component 2		
Shore	*F*	*d.f.*	Difference	*F*	*d.f.*	Difference
Portland	1.73	1, 770	B=L (ns)	1275.25	1, 770	B>L***
Kimmeridge	6.21	1, 616	B>L*	71.56	1, 616	B>L***
Swanage	574.26	1, 769	B>L***	39.47	1, 769	B>L***

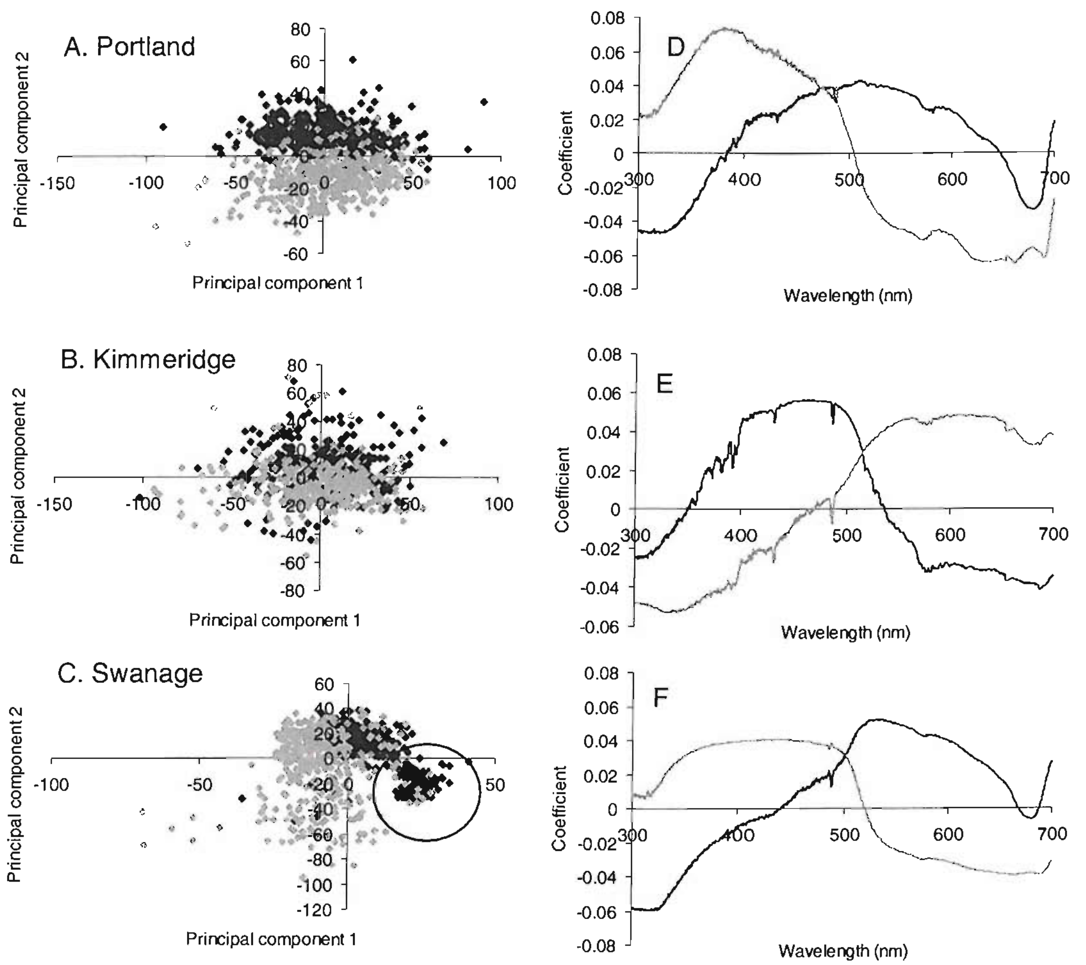


Figure 3.3.3: Principal component score plots of littorinids (grey markers) and backgrounds (black markers) using standardised spectra collected at Portland (A), Kimmeridge (B), and Swanage (C) which demonstrate the differences in PC1 and PC2 of these two types of spectra. The backgrounds collected in transect 4 at Portland are highlighted as these spectra form a discrete group in terms of PC1 and PC2. Corresponding coefficient of variation values of the first two principal components (PC1, black line; PC2, grey line) for Portland (D), Kimmeridge (E), and Swanage (F).

PCA of unstandardised spectra reported that PC1 explained a large percentage of the variation between spectra and also correlated significantly with mean reflectance (brightness) at Portland (90.1% variation explained; Spearman's correlation coefficient with mean reflectance r =0.098, $P<0.01$), Kimmeridge (91.5%; r =0.141, $P<0.001$) and Swanage (83.5%; r = -0.236, $P<0.001$). Additionally PC1 of unstandardised spectra indicated that backgrounds were brighter than littorinids at all three shores (Portland, $F_{(1, 770)}= 89.96$, $P<0.001$; Kimmeridge, $F_{(1, 616)}= 49.89$, $P<0.001$; Swanage, $F_{(1, 771)}= 337.61$, $P<0.001$).

In summary, backgrounds were generally spectrally distinct from littorinids but the way in which the two kinds of spectra were separable was different at each shore. Backgrounds at

Kimmeridge reflected relatively more light between 400-650nm than littorinids. Similarly at Swanage backgrounds reflected relatively more light between 425-675nm. However, the axis of most variation (PC1) did not discriminate between littorinids and backgrounds at Portland. In terms of PC2, Portland and Swanage show the same pattern such that littorinids reflect more long-wavelength red light relative to backgrounds yet this pattern is reversed at Kimmeridge which could be dependent on both Portland and Swanage having the same geology.

(3.3.5) *Visual Modelling of Reflectance Spectra*

Significant differences in overall colour space distances were shown by the bird model (ΔS values) ($F_{(2, 921)}$= 8.26, P<0.001). Specifically littorinids at Portland and Swanage matched their backgrounds to a similar extent but littorinids at Kimmeridge were significantly closer in colour space distance than at both other shores in the bird visual model (Portland, P=0.004; Swanage, P=0.0005). There was no significant difference in the ability of the crab to detect littorinid and background spectra pairs at the three shores.

At all three shores, the difference between the ability of the crab and the bird was apparent by the distribution of ΔS values across the classes. The bird model indicated that between 44.2- 44.9% of littorinids were discriminable versus 18.7- 20.1% indicated by the crab model. Specifically the simulated crab visual system did not enable effective discrimination between littorinid and background colours (Fig. 3.3.4).

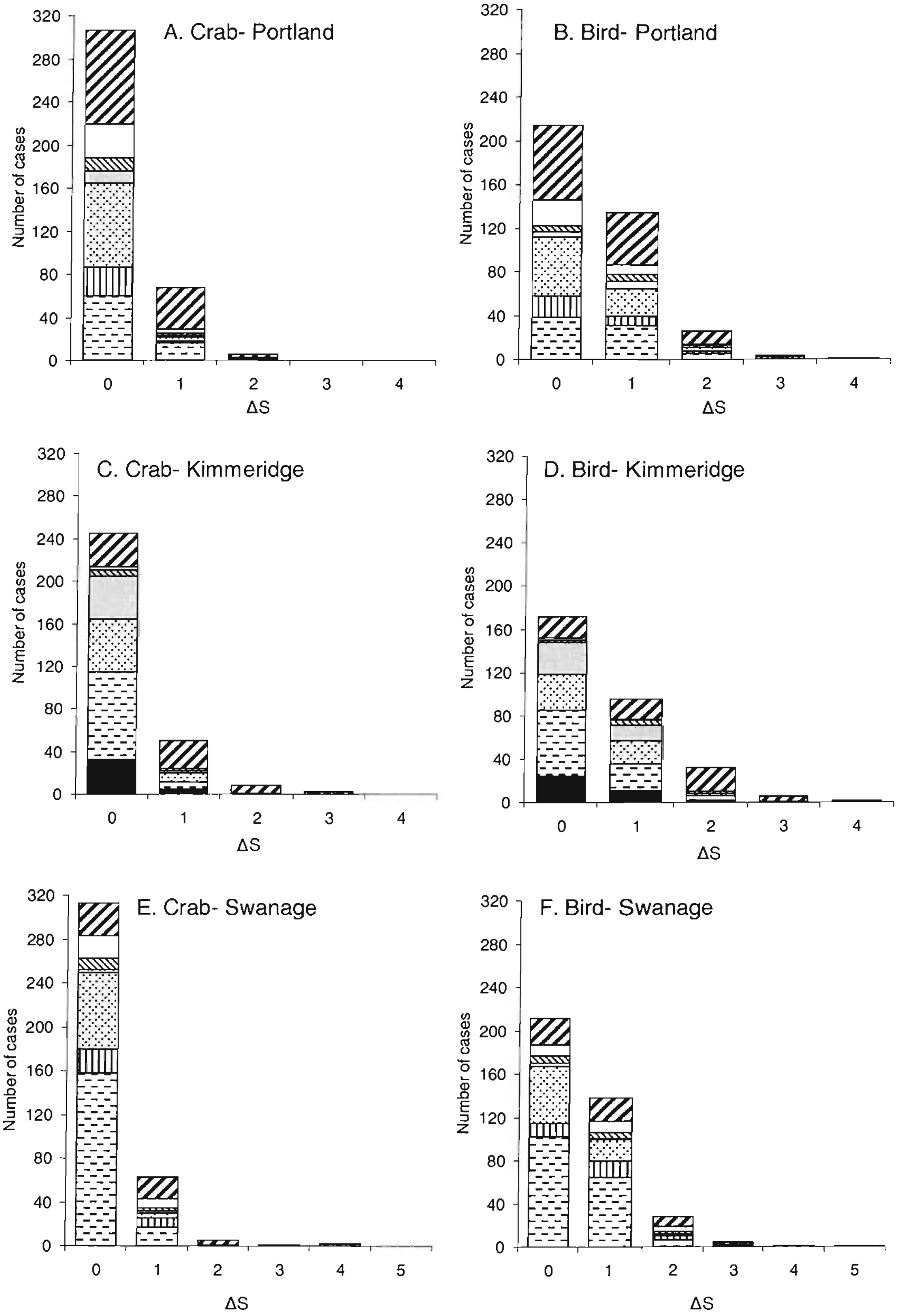


Figure 3.3.4: Number of cases in each ΔS class including division of each ΔS class into number of individuals of each morph: yellow ▨, white ☐ , orange ▧ , grey ▒, fawn ░ , brown ▤, black ■, and buff ▥ , for crab and bird visual models at Portland (A, B), Kimmeridge (C, D) and Swanage (E, F).

For both the crab and bird visual models there were significant differences on all shores between the frequencies of paired contrasts in the 0ΔS class and those ΔS classes greater than zero combined (Chi-squared, $P<0.05$). The Lancaster (1949) and Irwin (1949) method for partitioning contingency tables described by Everitt (1977) was used to test for specific differences between categories responsible for the overall Chi-squared values, the results are shown in Table 3.3.4. To acquire expected values greater than five, morph frequencies were combined in some cases with other morphs which, from inspection of the spectra, were most similar. The results in Table 3.3.4 show that apart from the bird visual model at Portland, all Chi-squared partitioning tests reported significant differences between the yellow morph and all other morph categories. For all three shores and both visual systems, the majority of morphs classified were presented in the 0ΔS class, therefore all littorinids could appear cryptic. At Portland the yellow and fawn morphs were significantly more cryptic to the crab whereas the bird model indicated that buff and fawn individuals were significantly more cryptic. The crab model indicated that brown and grey were more cryptic than other morphs at Swanage whereas the bird model also indicated that fawn was more cryptic.

Table 3.3.4: Chi square partitioning results comparing the ratio of each morph in the $0\Delta S$ against the $>0\ \Delta S$ classes. Morph categories: brown (br), black (bl), buff (bu), fawn (f), grey (gr), orange (o), yellow (y), white (w).

Shore	Visual model	Chi square contingency table partitioning groups					
Portland		(br) **bu**	(br, bu) **f**	(br, bu, f) **gr**	(br, bu, f, gr) **o**	(br, bu, f, gr,o) **w**	(br, bu, f, gr,o, w) **y**
	Crab	NS	$\chi^2 = 5.88$, $P<0.05$	NS	NS	NS	$\chi^2 = 19.738$, $P<0.01$
	Bird	$\chi^2 = 227.08$, $P<0.01$	$\chi^2 = 11.105$, $P<0.01$	NS	NS	NS	NS
Kimmeridge		(bl) **br**	(bl, br) **f/o**	(bl, br, f/o) **gr**	(bl, br, f/o, gr) **y/w**		
	Crab	NS	NS	NS	$\chi^2 = 50.271$, $P<0.01$		
	Bird	NS	NS	NS	$\chi^2 = 21.9$, $P<0.01$		
Swanage		(br/gr) **bu**	(br/gr, bu) **f/o**	(br/gr, bu, f/o) **w**	(br/gr, bu, f/o, w) **y**		
	Crab	$\chi^2 = 5.938$, $P<0.05$	NS	$\chi^2 = 4.574$, $P<0.05$	$\chi^2 = 39.285$, $P<0.01$		
	Bird	$\chi^2 = 4.116$, $P<0.05$	$\chi^2 = 4.068$, $P<0.05$	$\chi^2 = 5.706$, $P<0.05$	$\chi^2 = 7.594$, $P<0.01$		

The crab and bird visual models confirm that there were differences in the discriminability of *Littorina saxatilis* morphs from their backgrounds at Portland, Kimmeridge and Swanage. To illustrate shore-specific differences between morphs as categorised on the shore, the median ΔS values for each morph category were displayed (Fig. 3.3.5A-F). The results in Figure 3.3.5 show that the yellow morphs were more conspicuous than the other morphs against the background. In the crab visual model at Portland, only yellow was significantly more conspicuous than fawn, whereas in the bird model only orange was significantly more conspicuous than fawn (Fig. 3.3.5A, B; Table 3.3.5). At Kimmeridge, however, the yellow morph was significantly more conspicuous than all other morphs on the shore in both the crab and bird visual systems whereas there was no significant difference between the conspicuousness of the other morphs (Fig. 3.3.5C, D; Table 3.3.5). At Swanage, in the crab visual system the yellow morph is significantly more conspicuous than all morphs except orange and white, additionally the white was more conspicuous than fawn (Fig. 3.3.5E, F: Table 3.3.5). The bird model showed, however, that at Swanage both yellow and white were more conspicuous than brown and also that buff was more conspicuous than fawn. Given that white and yellow were not significantly different from each other, they were equally conspicuous to a bird at Swanage.

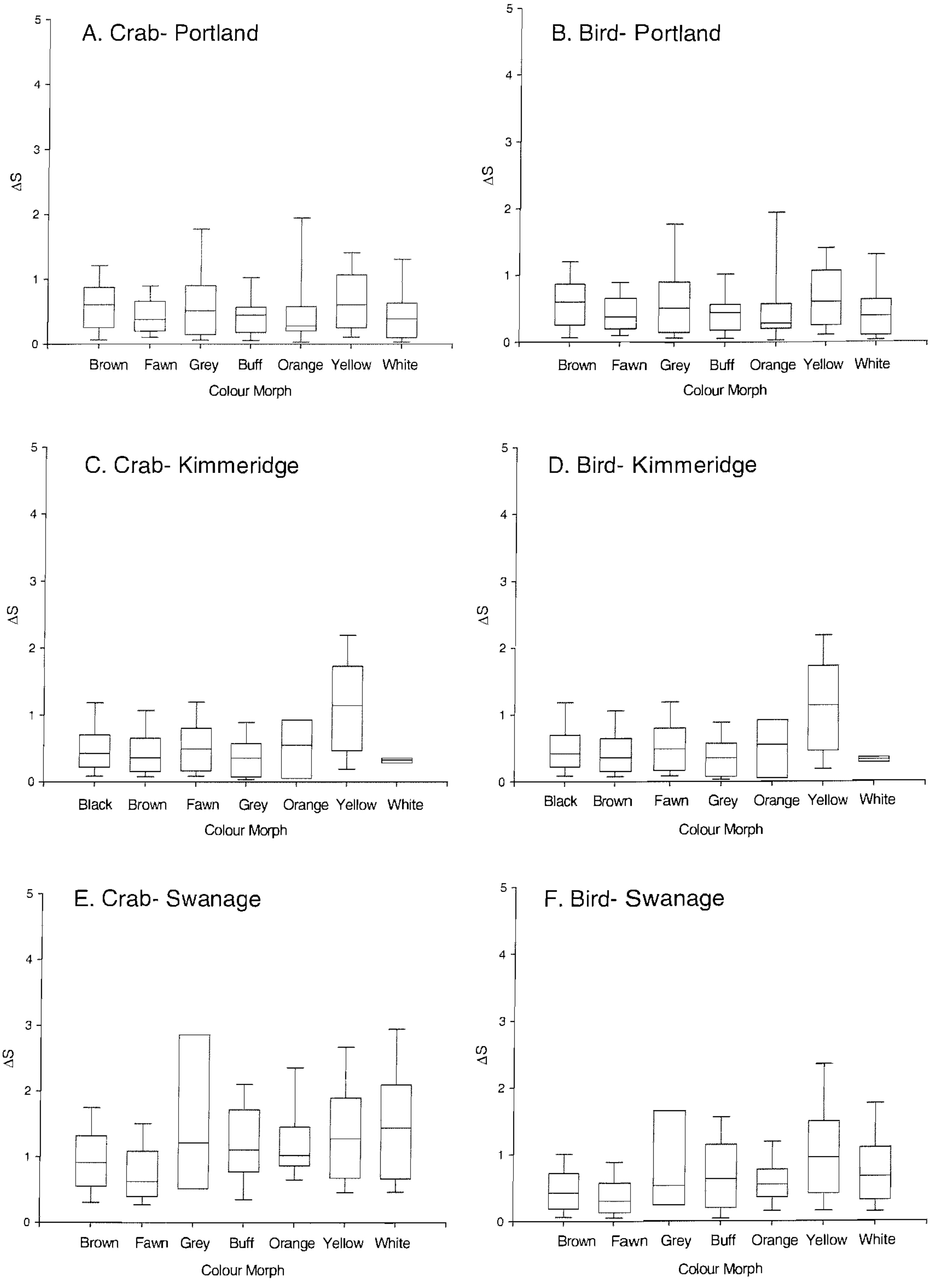


Figure 3.3.5: Median ΔS (± interquartile range) for each colour morph for crab (A) and bird (B) visual models at Portland (Brown, N= 76; Buff, N=37; Fawn, N= 82; Grey, N=14; Orange, N=15; White, N=35; Yellow, N= 130). Kimmeridge median ΔS (± interquartile range) for crab (C) and bird (D) visual models (Black, N= 38; Brown, N= 91; Fawn, N= 58; Grey, N=43; Orange/terracotta, N=8; White, N=3; Yellow, N= 67). Swanage median ΔS (± interquartile range) for crab (E) and blenny (F) visual models (Brown, N= 176; Buff, N= 31; Fawn, N= 74; Grey, N=5; Orange, N= 8; Terracotta, N=6; White, N=28; Yellow, N=58).

Table 3.3.5: General Linear Model comparing Box-Cox transformed ΔS values in each morph category with more than 5 individual littorinid replicates including *posthoc* Tukey tests to identify significant differences between morph pairs (significance level: $P<0.05$ *, $P<0.01$ **, $P<0.001$ ***).

Shore and morphs present	Crab	Bird
Portland	**$F_{(7, 378)} = 3.07$ ***	**$F_{(7, 378)} = 3.26$ ****
(brown, buff, fawn, grey, orange, white, yellow)	Yellow>Fawn *	Orange>Fawn *
Kimmeridge	**$F_{(4, 292)} = 13.3$ *****	**$F_{(4, 292)} = 13.14$ *****
(black, brown, fawn, grey, yellow)	Yellow>Black *** Yellow>Brown *** Yellow>Fawn *** Yellow>Grey ***	Yellow>Black *** Yellow>Brown *** Yellow>Fawn *** Yellow>Grey ***
Swanage	**$F_{(5, 374)} = 12.3$ *****	**$F_{(5, 374)} = 8.3$ *****
(brown, buff, fawn, orange, white, yellow)	White>Fawn * Yellow>Brown ** Yellow>Fawn *** Yellow>Buff *	White>Brown ** White>Fawn *** Yellow>Brown ** Yellow>Fawn *** Buff>Fawn *

To summarise, there were differences between morph ratios at the three shores. In particular there were more yellow littorinids at Portland where the underlying rock surface was more yellow. The colours of littorinids and their backgrounds were different within each shore and the combined PCA of all three shores confirms that these differences were shore dependent. Specifically the background spectra PC scores indicate that, as a shore, Kimmeridge was more heterogeneous than either at Portland or Swanage. Overall yellow littorinids were most conspicuous against all backgrounds. In general, backgrounds were brighter than littorinids at all three shores. Irrespective of morph, match to background was better at Kimmeridge than Portland and Swanage, which were similar.

(3.4) Discussion

Studies of animal coloration have recently included information and techniques which account for the visual capabilities of the organisms for whom colour signals are important. Here, I have investigated the spectral characteristics of polymorphic littorinids and the way in which the shore geology could influence the colour morphs present. The results indicate the extent to which different colour morphs of *Littorina saxatilis* match the background in the visual systems of two predator types on shores of different geologies. This investigation addresses two central questions relating to littorinid and background coloration and their discriminability using objective spectral analysis and also visual models approximated to a shorebird and the green shore crab (*Carcinus maenas*). Firstly, are littorinid and background spectra different and is this dependent upon the shore from which they came? Secondly, are any spectral differences better explained once the spectra have been modelled into the visual systems of two potential predators? These questions are subject to assumptions relating to the techniques used and also the interactions of predators and their prey on the intertidal rocky shore. Therefore any caveats regarding the conclusions will be addressed first.

The shore crab was included as a potential predator of *Littorina saxatilis* although on macrotidal UK shores such as Portland, Kimmeridge and Swanage, the opportunities for a crab to forage in the splash zone would be limited as crabs prefer to forage at high tide at night (Crothers, 1968). However, in microtidal areas such as Sweden crabs are known to feed on littorinids readily as indicated by evidence of shell breakage (Ekendahl, 1998). Despite this, the crab model was included to present the range of potential visual systems selecting *Littorina saxatilis*.

Although Swanage and Portland are both limestone shores formed by Portland limestone, they are both exposed boulder shores whereas Kimmeridge is a semi-exposed rocky ledge. This difference in topography should mean that the two shores of the same geology are more similar granted that topography and exposure should affect the type of predators acting at a particular shore. For example topography and exposure were found to be significant predictors of colour morph variation in Swedish *L. saxatilis* (Ekendahl and Johannesson, 1997). Therefore, between-site variation shown in this chapter takes into account the differences in topography.

Littorinids matched their backgrounds better at Kimmeridge than at either Swanage or Portland. Interestingly, Kimmeridge backgrounds were more spectrally diverse than Swanage and Portland. Greater heterogeneity can result in more opportunities for crypsis (Endler, 1978, 1984). Therefore the background heterogeneity at Kimmeridge could be responsible for the increased matching of all morphs. Indeed in *Cepaea nemoralis,* woodland populations are dominated by pink and brown unbanded morphs whereas grassland habitats are dominated by yellow and various banded morphs (Cain and Sheppard, 1950, 1952). The woodland habitat is considered to be more uniform than the grassland habitat (Jones *et al.,* 1977) and therefore supports fewer morphs of *C. nemoralis* (Goodhart, 1987).

In accordance with this evidence, if visual selection is controlling the polymorphism of *L. saxatilis*, the extent of background matching should be the same at all three shores. However, littorinids are mobile and can travel between between 10-15m in 2-3 days which has implications for the effect of different scales of background heterogeneity (Rochette and Dill, 2000). Relative to the spatial frequency of different background elements on the rocky shore, littorinid movement is significant as backgrounds can change over ranges of a few centimetres. Therefore it is important to recognise that the spectral data collection was merely a snapshot of background matching at one point in time. Given the better background matching at Kimmeridge where the background heterogeneity is greater, littorinids of any morph whilst stationary or feeding may have a higher chance of appearing cryptic.

Interestingly there was no evidence to suggest that *L. saxatilis* at Kimmeridge were more polymorphic than littorinids at either Portland or Swanage based on the number of morphs identified, although black individuals were present at Kimmeridge only. This finding is contrary to other littorinids such as *Littoraria pallescens* which is polymorphic in mangrove foliage yet monomorphic in uniform mangrove bark areas (Reid, 1997; Cook 1990a). Therefore the polymorphisms at Portland, Kimmeridge and Swanage may represent the proportions of the background elements to which each morph is a good match (Endler, 1978). Using this justification it can be understood why yellow is most common at Portland, brown and yellow most common at Kimmeridge and brown most common at Swanage. In reality there were differences between Portland and Swanage in rock colour despite similarities in geology. Specifically the rock at Swanage was not as light as the rock at Portland, the reasons for which could not be ascertained.

Given that yellow is the most conspicuous morph at all shores, selection against conspicuous morphs would result in low fitness of the yellow morph. At Portland, however, yellow is the most common morph. A generally cryptic appearance should be favoured by natural selection (Endler, 1978). Indeed in *C. nemoralis* there is evidence to suggest that the most common morph is the most conspicuous and not the most cryptic as predicted by natural selection (Goodhart, 1987).

It appears that spectroradiometry and visual modelling have served to quantify the differences in background matching between the three shores. However, the extent to which individual littorinids match their backgrounds is not only dependent on the background heterogeneity, but also incident lighting and the visual capabilities of the observer. Therefore for studies of morph frequencies within a polymorphism, the human classification of morphs is sufficient (*e.g. C. nemoralis*, Cook *et al.*, 1999; chitons, *Ischnochiton striolatus*, Gonçalves Rodrigues and Silva Absalão, 2005; *Theba pisana*, Cowie, 1990). However, without spectral techniques, shore-wide background matching could not be quantified especially if the habitat could not be easily divided into certain background elements

Chapter 4

Ecotypic variation in Swedish populations of *Littorina saxatilis*: a predator's eye view

Summary

The colour polymorphism of two ecotypes of *Littorina saxatilis* on the Swedish west coast were investigated using spectral reflectance techniques. Sheltered shore populations are mainly at risk from predation by the green shore crab (*Carcinus maenas*) whilst exposed shore populations are potentially preyed upon by birds. Shell and background reflectance spectra were collected from the two ecotypes and modelled into the visual systems of crabs and birds. Chromatic distances between littorinid and background spectra showed that the exposed ecotype were more cryptic to both predators. Furthermore, the visual models revealed that achromatic brightness signals were larger to the crab simulated model rather than the bird simulated model. Previous observations have concluded that certain rare exposed morphs are very conspicuous to the human eye. These rare and conspicuous morphs (red, white and banded) which only appear at frequencies of less than 10% on exposed shores were actually relatively cryptic to birds on exposed shores but more conspicuous against rock on the sheltered shores. Overall, irrespective of morph, littorinids matched backgrounds from their own exposure type better than the alternative. The ecological significance of these findings is discussed with reference to the impact of predators at each exposure.

(4.1) Introduction

On the Swedish west coast, two distinct morphological phenotypes ('ecotypes') of *Littorina saxatilis* exist, the 'E morph' from exposed rocky shores, and the 'S morph' from nearby sheltered shores (Janson, 1982). The 'E morph' is characterized by a small, thin shell with a large aperture which reduces dislodgement, whereas the 'S morph' has a larger, thicker shell and relatively smaller aperture against crab predation (Janson 1982; Johannesson *et al.,* 1993; Johannesson and Johannesson, 1996; Ekendahl and Johannesson, 1997) (Fig. 4.1.1). Sheltered shores, although less affected by wave action, are affected by the movements of stones and predation by dense populations of *Carcinus maenas.* Crabs are rare, if not absent, from exposed shores (Janson, 1983; Ekendahl, 1998). Birds, however, may be more important predators of *L. saxatilis* on exposed shores (Janson, 1983). *Littorina saxatilis* generally appears cryptic against exposed shore backgrounds, yet 2-20% of morphs appear conspicuous which may be due to selection.

Figure 4.1.1: *Littorina saxatilis* exposed ecotype (top row) and sheltered ecotype (bottom row).

It has been suggested that some, and probably most parts of the phenotypic shell differences reflect genotypic differences between the 'E' and 'S' ecotypes (Janson, 1982;

Johannesson and Johannesson 1996), and thus there are polymorphic gene loci within the species controlling shell form that are strongly influenced by divergent selection (Janson, 1983). These quantitative shell characters such as shell size and shell thickness have been found to be fixed in different habitats (Janson, 1982; Johannesson *et al.,* 1993). In addition to morphological characteristics, 'E' and 'S morphs' differ in their shell colour polymorphisms. On sheltered shores the polymorphism is less extensive and littorinids are mostly brown, fawn and yellow (Ekendahl and Johannesson, 1997). In contrast, on exposed shores *L. saxatilis* occur in white, banded, tessellated and red forms (Ekendahl, 1998). Specific colour morphs absent at sheltered sites are: white, banded and red (these occur in frequencies of 1-5% at exposed sites, or less than 1% for red). At exposed shores brownish and tessellated forms are present in frequencies of around 80%. The different characteristics of the 'E' and 'S' morphs are summarised in Table 4.1.1.

Table 4.1.1: *Littorina saxatilis*: physical and morphological characteristics of the two ecotypes and their habitats.

	Sheltered	Exposed
Physical shore characteristics	Boulder shores with fucoid algae	Rocky ledges with mussels and barnacles
Shell size	Large (>6.5mm shell height)	Small (<6.5mm shell height)
Shell Thickness	Thick	Thin
Aperture	Small relative to shell size	Large relative to shell size
Dominant morphs	Brown and Fawn	Brown and Tessellated
Rare morphs	None	Red, White, Striped,
Morphs absent	Red, White, Striped, Tessellated	None
Predators	Crabs (*Carcinus maenas*)	Birds (various species), crabs absent

The genetic basis of shell colour in *L. saxatilis* populations is unclear, although it is certainly an inherited trait which is polymorphic at all hierarchical levels from within species to within demes (Atkinson and Warwick, 1983; Johannesson *et al.,* 1993; Ekendahl and Johannesson, 1997).

At the scale of geographic area (countries) and habitat (exposed versus sheltered shores), colour morphs of *L. saxatilis* are non-randomly distributed (Ekendahl and Johannesson, 1997). More specifically, the morphs found at exposed shores differ from those found at sheltered shores. Purported explanations of mechanisms affecting colour variation include visual selection by predators in a heterogeneous environment, indirect effects of non-random mating, selection for thermoregulation, habitat choice and genetic drift (Heller, 1975; Byers, 1990; Cook 1992; Sergievsky, 1992; Rolán-Alverez and Ekendahl, 1996; Ekendahl, 1995; Ekendahl and Johannesson, 1997; Pickles and Grahame 1999;

Johannesson and Ekendahl, 2002). Evidence of predation on *L. saxatilis* has been supported by field and laboratory investigations and is thought to be due to birds, fish and crabs (Reimchen, 1979, 1989; Hughes and Mather, 1986; Reid, 1987; Tucker, 1988; Alerstam *et al.,* 1992; Ekendahl, 1998). However, perhaps due to the limited visual acuity and limited reliance upon vision for hunting, no selection on the basis of shell colour by the green shore crab (*Carcinus maenas*) has been observed (Tucker, 1988; Ekendahl, 1998).

The difference in polymorphism between the sheltered and exposed habitats of Swedish shores coincides with, among other things, crab abundance, which is high on sheltered shores whereas they are virtually absent on exposed shores (Johannesson, 1986). Crabs readily eat littorinids both in the field and aquaria (Crothers 1968; Tucker 1988; Johannesson 1988; Ekendahl, 1998) and probably represent the most important predators on sheltered shores. Given the absence of crabs on exposed rocky shores it has been suggested that birds and to a lesser extent, fish could act as selective agents at these sites (K. Johannesson, *pers. comm.*). Bird species foraging on Swedish shores include oystercathers (*Haematopus ostralegus*), redshanks (*Tringa totanus*) and pied wagtails (*Motacilla alba*) (K. Johannesson, *pers. comm.*). Furthermore birds and fish are also probable predators at sheltered sites (Ekendahl, 1998) although *L. saxatilis* may live above the zone accessible to fish predators (Johannesson and Ekendahl, 2002). Birds less frequently forage among boulders than on exposed rocks and might therefore be less important predators on the S morph (K. Johannesson, *pers. comm.*). Here I use reflectance spectradiometry techniques to estimate the discrimination of colour morphs of the two ecotypes against the background by the two predator species most likely to attack *L. saxatilis* on the shore; crabs and birds. While I predict birds to have a high ability to discriminate different *L. saxatilis* colour morphs, crabs are assumed to discriminate poorly, if at all.

Since birds forage on Swedish rocky shores it is reasonable to investigate the differential effect of predator visual acuity on the evolution of the shell polymorphism where multiple predator types are foraging. Previously, similar studies involving mollusc colour polymorphisms have generally used colour names and categories based solely on human judgement (*e.g. Lottia digitalis*, Giesel, 1970; Smith, 1976, *Littorina obtusata*; Byers, 1989, *L. saxatilis*; *Cepaea* spp., Jones *et al.*, 1977; *Nucella lapillus*, Berry, 1983; *Littoraria* *pallescens*, Cook, 1983; *Littoraria filosa*, Reid, 1987; *Bullia digitalis*, Heller, 1992; *Euhadra peliomphala*, Hayashi and Chiba, 2004). The coloration of these phenotypically discrete Swedish ecotypes of *L. saxatilis* cannot be understood without an appreciation of the visual ability of predators with which this species interacts and the difference in heterogeneity of the backgrounds in the environments they inhabit.

Reflectance spectroscopy and visual modelling were used to investigate the influence of potential differential selection pressures in terms of visual acuity of predators on the shell colour polymorphism in two ecotypes of *L. saxatilis* (sheltered and exposed) to answer the following questions:

1. Does the colour polymorphism differ between sheltered and exposed habitats in the visual systems of the potential crab and bird predators?
2. Do *L. saxatilis* from one exposure match their own backgrounds better than backgrounds from the alternative exposure (sheltered versus exposed)?
3. Are rare white, banded and red exposed morphs more conspicuous against sheltered shore backgrounds, perhaps explaining why these morphs are not found on the sheltered shore?

(4.2) Methods

(4.2.1) Study sites

Littorina saxatilis were sampled during August 2004 from two sheltered shores and two exposed shores in the northern part of the west coast Swedish archipelago near to Tjärnö Marine Biological Laboratory (58°52'5''N; 1°19'0''E). Despite the use of only two shores per exposure, the shores were chosen to be representative of the ecotypes on the Swedish west coast. Although the area is atidal it experiences small fluctuations in sea level of 0.3m during spring tides predominately due to local air pressure differentials (Johannesson, 1989). Thus the habitats are littoral rather than intertidal. The two types of habitats studied are a consequence of the primary bedrock (granite) and the action of the inland ice and sea during glacial and post glacial time (Janson, 1986).

Exposed shores

Exposed sites are characterised by low cliffs exposed to wave exposure where large algal masses are absent. *L. saxatilis* is found on both vertical and horizontal rock faces predominantly in and around crevices. In general, the granite is a single ground colour with irregular speckling. The bare rock surface is punctuated by patches of mussels (*Mytilus edulis*), barnacles (*Semibalanus balanoides*) and the non calcified encrusting red alga (*Hildenbrandia rubra*). The sites studied were on the small islands of Lilleskär (exposed shore 1) and Inre Vattenholmen (exposed shore 2) (Fig. 4.2.2).

Sheltered shores

Sheltered shores were characterised by various sized boulders of different colours of granite. The background against which the littorinids were found was heterogeneous at two scales; between boulders (different coloured granite), and within boulders (different coloured striations and a mosaic of *H. rubra* and the lichen *Verrucaria maura*). The sites studied were the island of Långholmen (sheltered shore 1) and a southwest facing shore on the island of Saltö (sheltered shore 2) (Fig. 4.2.2).

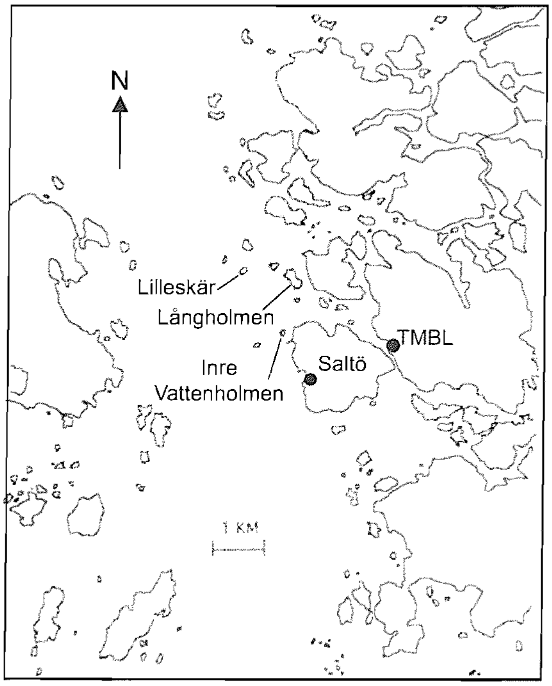


Figure 4.2.2: Study sites on the Swedish west coast near Tjärnö Marine Biological Laboratory (TMBL).

(4.2.2) Spectroradiometry

At each of the four shores, four sampling areas were selected on the basis of density of littorinids and accessibility for the equipment. On sheltered shores, sampling areas (plots) were individual boulders at the same height on the shore. Boulders were selected to cover the range of granite colours present on each shore. On exposed shores, plots were randomly selected. Irrespective of shore, within each plot 20 littorinids were chosen at random in an area less than $1.5m^2$ which contained 20 or more littorinids. When each littorinid had been spectrally measured it was removed from the rock face to avoid repeating measurements on the same individual. Both the littorinid shell reflectance and the background surface reflectance immediately adjacent and to the right of the shell were measured when wet and dry. The procedure for measuring spectral reflectance is described in section 2.7.

Each littorinid was described in the field by morph category (Pettitt, 1975), therefore spectral data collection also provided a random morph survey of each exposure type. The difference in morph frequencies between exposures was tested using a Chi-squared contingency table and Chi-squared partitioning (Lancaster, 1949; Irwin, 1949). The spectroradiometric technique does not take account of patterning and tessellated morphs were recorded under their 'ground' or base colour.

The random nature of sampling at the exposed shores meant that not all rare littorinids were encountered. Therefore a sub-sample of rare littorinids (red, banded and white) was collected from other populations to ensure a complete collection of spectra from all exposed morphs known to be present in the population. Shell reflectance spectra from this sub sample were collected outdoors when specimens were both wet and dry following the same procedure as carried out in the field.

(4.2.3) Analysis

Reflectance spectra were edited using 'R' (statistical software) and all visual modelling was completed using SPEC (see section 2.7 for detailed methodology). All colour space distances (ΔS) and achromatic distances (also ΔS) between spectra pairs were calculated in bird and crab visual models, the characteristics of which are described in section 2.7.

Colour space distances were calculated for all pair-wise comparisons of littorinid and background spectra specific to sampling areas within shores. Since littorinid and background spectra were collected when wet and dry, repeated measures general linear models were used to determine differences in background matching between ecotypes. Given that each littorinid was assigned to a morph category, the extent to which each morph matched the background was tested using repeated measures general linear models.

To test the expectation that ecotypes should match their own backgrounds better than those of the other exposure, littorinid spectra from exposed shores were compared to background spectra from sheltered shores and *visa versa.* Paired t-tests were used to test Box-Cox transformed ΔS values of 'paired' spectra (littorinids against their own backgrounds) with 'swapped' spectra (littorinids against one randomly selected background per snail from the alternative exposure).

(4.3) Results

(4.3.1) *Morph Frequencies*

Morph ratios, as derived from spectral data collection, differed significantly between the two ecotypes ($\chi^2 = 43.9$, 6 *d.f.*, $P<0.001$; Table 4.3.1). Using Chi-squared partitioning, the significant difference in the morph ratios of the two ecotypes was due to black individuals being absent from the sheltered shores ($\chi^2 = 5.99$, 1 *d.f.*, $P<0.05$). Very few white individuals were found at both exposures, with the black morph being absent from the two sheltered shores.

Table 4.3.1: Morph frequencies at sheltered and exposed shores (Chi-squared expected values in brackets).

Exposure	Black	Brown	Grey	Fawn	Orange	Yellow	White
Sheltered	0 (15.5)	63 (51.5)	5 (6.5)	27 (22.5)	10 (13.5)	52 (45.5)	3 (5)
Exposed	31 (15.5)	40 (51.5)	8 (6.5)	18 (22.5)	17 (13.5)	39 (45.5)	7 (5)

(4.3.2) *Visual Modelling of Reflectance Spectra*

The shore-specific results are displayed as histograms which show the number of cases that fall into each colour space distance 0-5ΔS (Fig. 4.3.1). The bird simulation discriminated the most contrasts as shown by a less skewed distribution than the crab simulation (Fig.

4.3.1). Between 90-97% of all simulated comparisons of crab vision were in the 0ΔS class (littorinids were not discriminable from the background), compared to 27-35% of sheltered shore comparisons and 49% of exposed shore comparisons when viewed by the bird (Fig. 4.3.1). In both the crab and bird visual models, all morphs were represented in the 0ΔS class except for the grey morph in the bird visual model on sheltered shores (Figs. 4.3.1 E and F). The distribution of cases of spectral pairs across the ΔS classes differed between sheltered and exposed shores in the bird visual system both when surfaces were wet and dry (Figs. 4.3.1 E-H). Specifically the frequencies of spectral pairs (number of cases) in 0, 1, 2 and >3 ΔS classes differed significantly between sheltered and exposed shores in the bird visual system (Table 4.3.2). These results indicated that *L. saxatilis* was more cryptic on exposed rather than sheltered shores. However, there was no significant difference between the frequencies of cases in 0ΔS and 1ΔS classes between sheltered and exposed shores in the crab visual system (Table 4.3.2).

Table 4.3.2: Number of cases of paired comparisons in ΔS classes at sheltered and exposed shores when wet and dry in the crab and bird visual models. Differences between the frequencies cases in each ΔS class at the two exposures determined by Chi-squared statistical tests (significant results in *italics*).

Visual system	Surface condition	Exposure	Colour space distance (ΔS) class				χ^2	*d.f.*	*P*
			0	1	2	>3			
Bird	Dry	Sheltered	56	56	36	12	11.17	3	*0.011*
		Exposed	79	55	17	9			
	Wet	Sheltered	43	63	32	22	20.41	3	*<0.001*
		Exposed	79	54	17	10			
Crab	Dry	Sheltered	145	15	0	0	1.09	1	0.298
		Exposed	150	10	0	0			
	Wet	Sheltered	154	6	0	0	0.63	1	0.428
		Exposed	151	9	0	0			

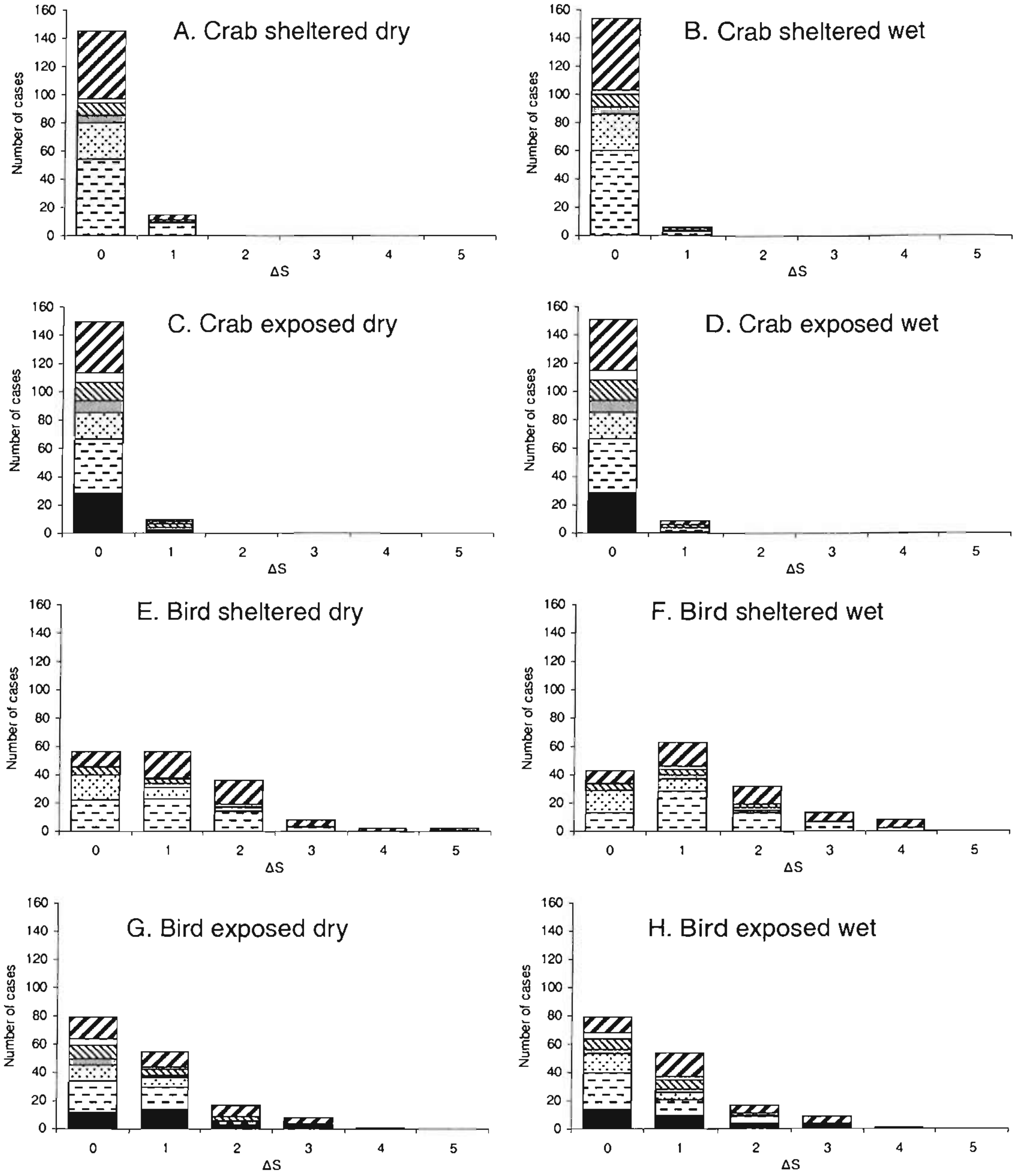


Figure 4.3.1: Number of cases of littorinid and background comparisons which fall into each ΔS category, divided into number of individuals of each morph (black ■ ; brown ▤; grey ▒; fawn ░ ; orange ▧; yellow ▨; white □). Crab visual model (A-D; top row, sheltered; bottom row, exposed; left hand side dry; right hand side, wet) and bird visual model (E-H; top row, sheltered; bottom row, exposed; left hand side dry; right hand side, wet).

There were significant differences in ΔS values of wet and dry spectra pairs in the visual system of the crab for both sheltered shores and the bird for sheltered shore 1 (Table 4.3.3). Therefore wet and dry ΔS values could not be pooled and given that exposure was a factor, the analysis of variance model was a repeated measures design. ANOVA was used to test whether colour distance (ΔS) varies differently in terms of exposure (sheltered versus exposed), replicate shores, plots within shores and wetness (wet versus dry, repeated measurements of the same surfaces) when modelling crab and the bird visual systems (Table 4.3.4). There was no significant interaction of wetness and exposure in the bird visual system but this interaction was significant in the crab visual system ($F_{1,304}$ = 9.69, *P*=0.002) $F_{1,304}$ = 2.56, *P*=0.256) (Table 4.3.4). Importantly there was a significant effect of exposure for the crab ($F_{1,304}$ = 43.14, *P*<0.001) and the bird ($F_{1,304}$ = 9.69, *P*=0.002). Specifically, exposed littorinids were more cryptic than sheltered littorinids to both simulated predators (crab and bird). However, the replicate shores nested within exposure type were significantly different in both visual systems (crab, $F_{2,304}$ = 23013, *P*<0.001; bird, $F_{2,304}$ = 63, *P*<0.001). Plots were different when nested within shore and exposure in both visual systems (crab, $F_{12,304}$ = 14.5, *P*<0.001; bird, $F_{12,304}$ = 18.64, *P*<0.001).

Table 4.3.3: Differences between ΔS values of wet and dry spectra at each shore in both visual models (crab and bird) as tested by t tests on Box-Cox transformed data (significant probabilities in *italics*).

	Crab visual model		Bird visual model	
	t	*P*	*t*	*P*
Sheltered shore 1	3.04	*0.003*	-3.59	*0.001*
Sheltered shore 2	2.23	*0.029*	-1.45	0.150
Exposed shore 1	0.65	0.52	-1.23	0.223
Exposed shore2	-1.57	0.12	0.81	0.418

Table 4.3.4: Four-way repeated measures ANOVA investigating the effects of exposure (E, sheltered versus exposed), wetness (W), interaction between exposure and wetness, shore (S) nested within exposure, interaction between wetness and shore nested within exposure, plot (P) nested within shore and exposure, in terms of Box-Cox transformed ΔS values for the crab and bird visual models. Significant results and interactions in *italics*.

Visual Model	Source	*df*	SS	MS	F	*P*
Crab	Exposure	1	1.06	1.06	43.14	*<0.001*
	Wetness	1	0.03	0.03	1.3	0.256
	E X W	1	0.24	0.24	9.69	*0.002*
	Shore(E)	2	1.14	0.57	23013	*<0.001*
	W X S(E)	2	0.14	0.07	2.83	0.061
	Plot(E S)	12	4.29	0.36	14.5	*<0.001*
	W X P(E S)	12	0.22	0.02	0.74	0.71
	Error	304	7.49	0.03		
	Total	639	50.2			
Bird	Exposure	1	1.61	1.61	140.38	*<0.001*
	Wetness	1	0.05	0.05	4.63	*0.032*
	E X W	1	0.04	0.04	3.54	0.061
	Shore(E)	2	1.45	0.72	63	*<0.001*
	W X S(E)	2	0.05	0.02	2.12	0.122
	Plot(E S)	12	2.57	0.21	18.64	*<0.001*
	W X P(E S)	12	0.11	0.01	0.81	0.644
	Error	304	3.49	0.01		
	Total	639	30.38			

In summary there was a difference in the extent to which both types of simulated predators could discriminate littorinids from their backgrounds at the exposed shore and the sheltered shore irrespective of the morphs measured. Furthermore there were significant differences between shores within exposures and also plots nested within shores and exposures. Despite this, those spectra collected from each exposure were dealt with in a combined way in morph specific analyses as these results represent the natural variability in the system due to background heterogeneity and the movement of the littorinids.

(4.3.3) *Morph-Specific Background Matching*

The extent to which the different morphs match their backgrounds at the two exposures is demonstrated in Figure 4.3.2. Due to a low incidence of white *L. saxatilis*, white could not be included in the general linear models. There were significant differences between the background matching of different colour morphs on both sheltered and exposed shores in the bird visual system (Table 4.3.5). There were only significant differences between background matching of the different morphs in the crab visual model on sheltered shores (Table 4.3.5). Wetness of littorinids and backgrounds significantly interacted with colour morph in the bird visual model on exposed shores ($F_{5,\,146} = 3.06$, $P=0.012$). Wetness also had a significant effect on discriminability in the bird visual model on the sheltered shore.

In the visual system of a crab at the sheltered shore, brown littorinids were significantly more conspicuous than fawn littorinids (*posthoc* Tukey test, $P=0.012$) and also yellow littorinids ($P=0.018$) (Table 4.3.5; Fig. 4.3.2A and B). There was no significant difference in the discriminability of different morphs using the crab visual model at the exposed shores (Table 4.3.5; Fig. 4.3.2.C and D). Using the bird visual model at the sheltered shore, brown littorinids were more discriminable from the background than fawn littorinids ($P=0.002$), grey were more discriminable than fawn ($P=0.04$) and yellow were more discriminable than fawn ($P<0.001$) (Table 4.3.5; Fig. 4.3.2 E and F). In the bird visual model at the exposed shore orange littorinids were more discriminable from the background than grey littorinids ($P=0.002$) and yellow littorinids were more discriminable than fawn littorinids ($P=0.024$) (Table 4.3.5; Fig. 4.3.2G and H).

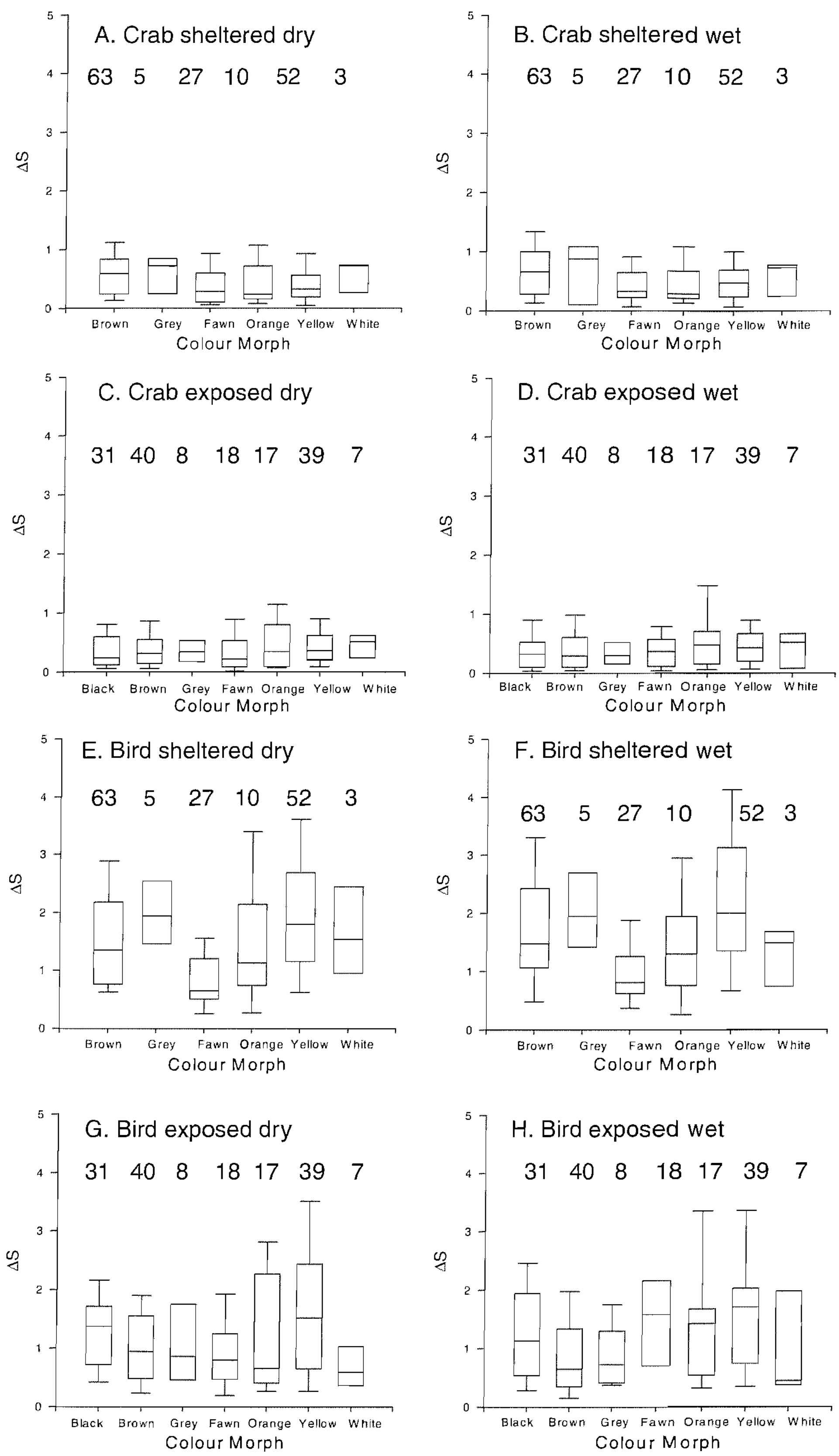


Figure 4.3.2: Median ΔS (± interquartile range) for the colour morphs on sheltered shores in the crab visual system when dry (A) and wet (B), on exposed shores in the crab visual system when dry (C) and wet (D), on sheltered shores in the bird visual system when dry (E) and wet (F) on exposed shores in crab vision when dry (G) and wet (H). Sample sizes are shown above each morph category. Absence of error bars indicates fewer than 10 replicates.

Table 4.3.5: Repeated measures general linear model of colour space distances (ΔS) for each colour morph, with wetness (2 levels) carried out separately at the two different exposures. Significant effects and interactions are in *italics*.

Visual Model	Exposure	Source		*df*	Mean square	F	*P*
Crab	Sheltered	Between-subjects	Morph	4	0.4	3.69	*0.007*
			Error	152	0.12		
		Within-subjects	Wetness	1	$5.12\text{x}10^{-2}$	1.65	0.201
			W x M	4	$1.15\text{x}10^{-2}$	0.09	0.985
			Error	152	4.71		
	Exposed	Between-subjects	Morph	5	$5.35\text{x}10^{-2}$	0.58	0.714
			Error	147	$9.19\text{x}10^{-2}$		
		Within-subjects	Wetness	1	$3.64\text{x}10^{-3}$	0.82	0.368
			W x M	5	$6.92\text{x}10^{-3}$	1.55	0.178
			Error	147	$4.47\text{x}10^{-3}$		
Bird	Sheltered	Between-subjects	Morph	4	0.56	8.12	*<0.001*
			Error	151	$6.94\text{x}10^{-2}$		
		Within-subjects	Wetness	1	0.19	15.66	*<0.001*
			W x M	4	$2.25\text{x}10^{-2}$	1.88	0.118
			Error	151	$1.2\text{x}10^{-2}$		
	Exposed	Between-subjects	Morph	5	0.3	3.82	*0.003*
			Error	146	$7.73\text{x}10^{-2}$		
		Within-subjects	Wetness	1	$4.55\text{x}10^{-2}$	2.41	0.122
			W x M	5	$5.77\text{x}10^{-2}$	3.06	0.012
			Error	146	$1.89\text{x}10^{-2}$		

(4.3.4) *Between-Shore Visual Modelling Comparisons*

In both the crab and bird visual systems, littorinids consistently matched their own backgrounds (paired) to a greater extent than those using swapped spectra (littorinids compared to a background of the alternative exposure) when surfaces were both wet and dry (*i.e.* paired ΔS values were smaller than swapped ΔS values) (Table 4.3.6). To summarise, *L. saxatilis* ecotypes generally matched their own backgrounds better than backgrounds of the alternative exposure.

Table 4.3.6: Paired t-tests comparing Box-Cox transformed ΔS values of paired spectra at each shore when wet and dry against swapped ΔS values for each shore in the crab and bird visual systems. The colour space distance (ΔS) has been calculated between littorinids from one exposure and backgrounds of the other exposure and compared against ΔS values of paired spectra from the littorinid's shore of origin. Each comparison is labelled by the origin of the littorinid spectra, therefore 'sheltered shore1' represents a comparison of sheltered shore 1 littorinids against sheltered shore 1 backgrounds with sheltered shore 1 littorinids against exposed shore 1 backgrounds.

Visual		Dry spectra			Wet spectra		
Model	Shore	Effect	t	*P*	Effect	t	*P*
Crab	Sheltered shore 1	Pair<Swap	-11.63	*<0.001*	Pair<Swap	-13.22	*<0.001*
	Sheltered shore 2	Pair<Swap	-11.51	*<0.001*	Pair<Swap	-10.09	*<0.001*
	Exposed shore 1	Pair<Swap	-6.93	*<0.001*	Pair<Swap	-6.43	*<0.001*
	Exposed shore 2	Pair<Swap	-6.09	*<0.001*	Pair<Swap	-9.65	*<0.001*
Bird	Sheltered shore 1	Pair<Swap	-2.09	*0.038*	Pair<Swap	-4.90	*<0.001*
	Sheltered shore 2	Pair<Swap	-3.81	*<0.001*	Pair<Swap	-3.18	*0.002*
	Exposed shore 1	Pair<Swap	-2.91	*0.03*	Pair<Swap	-2.40	*0.017*
	Exposed shore 2	Pair<Swap	-3.56	*<0.001*	Pair<Swap	-4.68	*<0.001*

(4.3.5) *Achromatic signals*

The mean achromatic ΔS values of both wet and dry spectra pairs were displayed for each morph (Fig. 4.3.3). In all cases, Figure 4.3.3 indicates that the crab was better at discriminating littorinids based on their achromatic brightness signal than the bird (paired t-test of Box-Cox transformed ΔS values, sheltered shore, t =7.54, *P*<0.001; exposed shore, t =7.92, *P*<0.001).

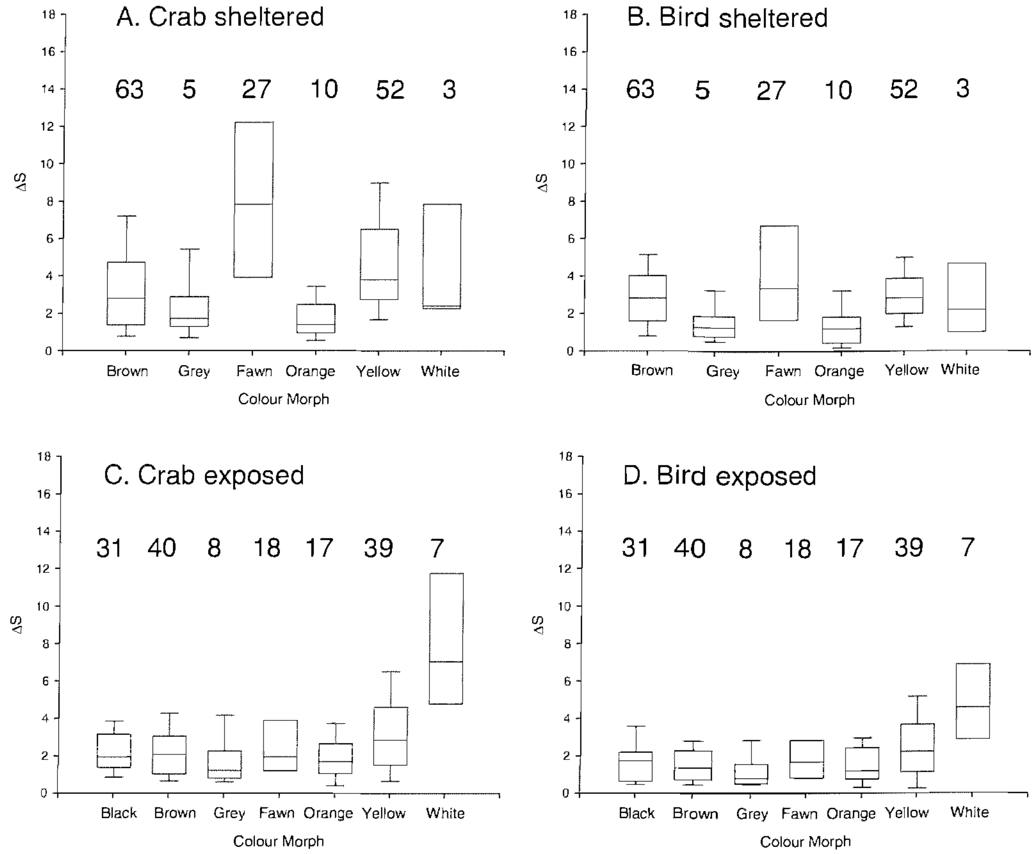


Figure 4.3.3: Median ΔS (± interquartile range) for achromatic signals (mean values of wet and dry colour space distances) of the morphs found on sheltered shores in the crab visual system (A), on sheltered shores in the bird visual system (B), on exposed shores in the bird visual system (C) and on exposed shores in the bird visual system (E). Sample sizes are shown above each morph category. Those morphs with fewer than ten individuals are shown without error bars.

Given that the achromatic signal may be more important to crabs on the sheltered shore, the differences in achromatic signals between morphs in the visual system of a crab were investigated for wet spectra only, since crabs mainly forage more under water or in wet conditions. There were significant differences between the morphs (excluding white due to low numbers) when surfaces were wet (F=5.61, 4 *d.f.*, P=0.001) see Table 4.3.7.

Table 4.3.7: Differences in achromatic signal between morphs on the sheltered shore in the crab visual system when surface were wet, as tested by *post hoc* Tukey test results (* indicates significant difference between morphs $P<0.05$, ns indicates no significant difference between morphs).

Morph comparisons	Tukey test result
Brown-Fawn	ns
Brown-Grey	Grey>Brown*
Brown-Orange	Brown>Orange*
Brown-Yellow	Yellow>Brown*
Fawn-Grey	Grey>Fawn*
Fawn-Orange	ns
Fawn-Yellow	Yellow>Fawn*
Grey-Orange	Grey>Orange*
Grey-Yellow	ns
Orange-Yellow	Yellow>Orange*

(4.3.6) *Rare Morphs*

The highly conspicuous (to the human eye) banded, red and white morphs of *Littorina saxatilis* which are present on exposed shores alone are only present at frequencies of less than 10% (Ekendahl and Johannesson, 1997). Red and banded individuals were not included in the spectral data collection on the exposed shores. Furthermore white individuals were found at such a low frequency (1.8% on sheltered shores), that they could not be included in the analysis of ΔS values of different morphs at each exposure (section 4.3.2; Table. 4.3.5). Therefore a sample of red, banded and white littorinids was collected and their spectral reflectance modelled against a different randomly assigned sheltered shore 1 and exposed shore 1 background per snail.

There was no significant difference in ΔS values of wet and dry rare morph spectra in the visual system of the crab (paired t test sheltered, t= 0.54, P=0.952; exposed, t=1.69, P=0.096) or the bird (t= 0.09, P=0.928; exposed, t=0.59, P=0.558). Since the signals to both visual systems were of importance, the differences in ΔS values between the different morphs were tested using the mean ΔS values of wet and dry spectra. Rare morphs were significantly more cryptic on exposed shore backgrounds than on sheltered shore backgrounds in the bird visual system (Fig. 4.3.4; Table 4.3.8). However, there was no interaction between exposure and morph type when spectra were modelled into the crab visual system (Table 4.3.8). In the simulated bird visual model the red morph was the most cryptic on sheltered shores, whereas on the sheltered shore the white morph was the most cryptic (Fig. 4.3.4).

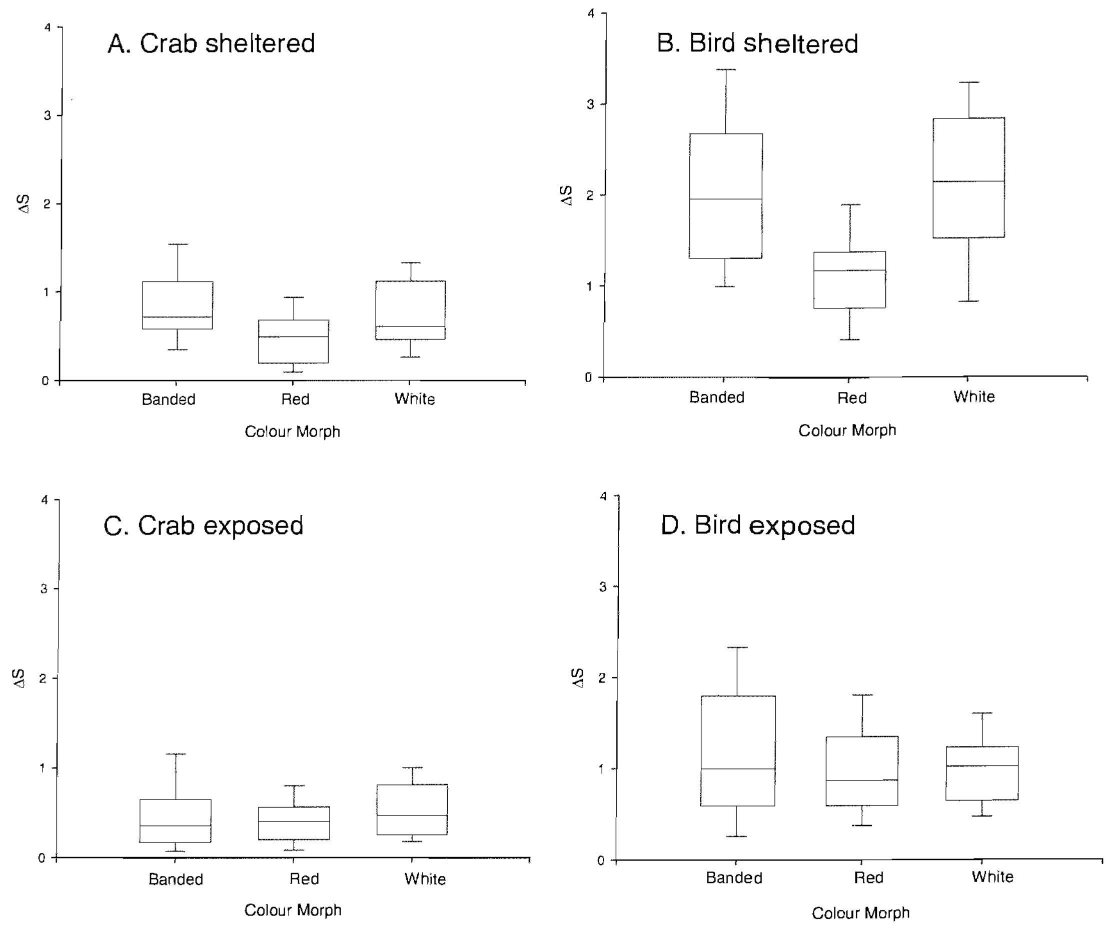


Figure 4.3.4: Median ΔS (± interquartile range) for the rare morphs (banded, N=20; red, N=23; white, N=24) compared against sheltered shore backgrounds in the crab visual system (A), and the bird visual model (B) and against exposed shore backgrounds in the crab visual model (C) and the bird visual model (D).

Table 4.3.8: Two-way ANOVA of Box-Cox transformed colour space distances (ΔS values, mean of wet and dry) for each morph (M), exposure (E), carried out separately in the two visual system models. Significant effects and interactions are in *italics*.

Visual Model	Source		*df*	Mean square	F	*P*
Crab	Between-subjects	Exposure	1	1.46	12.49	*0.001*
		Morph	2	0.61	5.21	*0.007*
		E x M	2	0.21	1.83	0.164
		Error	128	0.12		
Bird	Between-subjects	Exposure	1	15.94	37.38	*<0.001*
		Morph	2	4.68	10.97	*<0.001*
		E x M	2	2.98	7	*0.001*
		Error	128	0.43		

In summary, the rare conspicuous morphs (red, white and banded), found only on the exposed shores, matched exposed shore backgrounds better than sheltered shore backgrounds. Furthermore the three different rare morphs differed in the extent to which they matched the backgrounds dependent on the type of background against which they were viewed in both the crab and bird visual systems.

(4.4) Discussion

Across the two shores the bird visual system was better at discriminating littorinids from the background. Differences between the two *L. saxatilis* ecotypes were also detected. The following sections specifically address the questions set out in the introduction in the context of the two ecotypes of *L. saxatilis*, their polymorphisms and the potential predators.

Does the colour polymorphism differ between sheltered and exposed habitats when modelled in the visual systems of the potential crab and bird predators?

It is generally accepted that crabs are the most important predators of *Littorina saxatilis* on sheltered shores in the study region (Ekendahl, 1998). To the human eye, the morphs from the sheltered shore are plain in coloration, not only because they lack black, banded, red and tessellated morphs but also because white individuals are rare. Therefore it has been hypothesised that selection by crabs results in a less diverse polymorphism on the sheltered shore in terms of fewer discrete morphs and also a lower level of patterning (Ekendahl and Johannesson, 1997). Since crabs have limited vision, or perhaps do not use vision at all when foraging it is unlikely that highly conspicuous morphs would evolve as a means of escaping predation through apostatic selection which has been proposed for other littorinids (Smith, 1976; Hughes and Mather, 1986; Reid, 1987). On the exposed shore, however, selection is probably controlled predominantly by bird predation as crabs are rare if not absent (Ekendahl, 1998).

Essentially all *L. saxatilis* morphs are potentially indistinguishable from the background by colour in the visual system of a crab. Therefore either the background is suitably heterogeneous such that all morphs can, at some point, appear cryptic to crab predators under daylight conditions or, alternatively, the morphs are actually poorly discriminated by crabs. In reality crabs may more often use tactile or chemosensory cues to detect *L.*

saxatilis. However, on the exposed shores there was no difference in the extent to which the different morphs matched the backgrounds in the crab visual system, whereas on the sheltered shores brown morphs were more conspicuous than fawn and yellow morphs.

In the crab's visual system there was no overall difference in background matching, irrespective of morph, at the two exposures. All morphs at both exposures were generally cryptic in the crab visual system (less than 2ΔS) and yet there were differences between morphs at the sheltered shore. How then does the sheltered shore *L. saxatilis* polymorphism exist? It is certainly possible that birds could occasionally forage in a selective manner on sheltered shores. Intensive crab predation in molluscs, however, results in defensive shell characteristics which also interact with adaptation against dislodgement and desiccation (Atkinson and Newbury, 1984; Crothers, 1985; Etter, 1988; Harris and Jones, 1995; Behrens Yamada and Boulding, 1996, 1998). The resultant morph is large thick shells and small aperture size of sheltered shore *L. saxatilis* which may not be attractive to birds given that they either consume small thin shelled littorinids whole (Gibb, 1956; Pettitt, 1975; Summers and Smith, 1983) or alternatively remove body parts from the aperture of larger littorinids (Robertson, 1992). If birds of the same species prey upon both the sheltered ecotype and the small, thin shelled exposed shore *L. saxatilis* then they may not be able to handle and successfully consume adults on the sheltered shore (Gibb, 1956; Summers and Smith, 1983). Therefore birds on sheltered shores may select juveniles (<4mm shell height) which are generally not identifiable as a particular morph and are brown in coloration (Ekendahl and Johannesson, 1997).

High predation in Iceland and the absence of crabs appears to have led to a very diverse polymorphism with conspicuous coloration and also a high frequency of banding and tessellation (Ekendahl and Johannesson, 1997). Therefore the plainness of the sheltered shore *L. saxatilis* could suggest that birds are not an important visual selection agent. The polymorphism at the sheltered shore could merely reflect underlying heterogeneity in pleiotropic loci (Goodhart, 1987). Such effects could include differential thermal properties of shell colour in *L. saxatilis*. There is evidence from White Sea populations that light and dark morphs show different thermal properties under natural conditions (Sergievsky, 1992). Therefore those lighter littorinids could have a higher fitness in positions more exposed to sunlight, whereas darker morphs may persist in shaded areas. If this were the case in the Swedish *L. saxatilis* populations then the morph with optimal thermal properties would reach 100% in a certain location if climatic selection was important. Early work suggested correlations between morphs of the dogwhelk, *Nucella lapillus*, and certain rock colours (Cooke, 1895; Blaney, 1906). Although *N. lapillus* is polymorphic in most localities, it is purported not to be under visual selection since the highest diversity of morphs occurs at wave exposed sites (Etter, 1988) and yet birds are more likely to be important on shores of mid-exposure (Berry, 1983).. Thermal tolerance and dessication stress have more commonly been used to explain the existence of multiple morphs in the dogwhelk (Etter, 1988; Harris and Jones, 1995). Like *L. saxatilis*, no clear relationship between morphs of *N. lapillus* and certain background elements exists. The differences between *L. saxatilis* colour morphs found on Swedish sheltered shores in my work, however, were not as extreme as those found in *N. lapillus* or indeed White Sea *L. saxatilis* populations.

Alternatively, in the absence of visual selection for colour on sheltered shores, achromatic signals could be used for detection of littorinids. Indeed there is evidence that colour blind rabbits (*Oryctolagus caniculus*) and rodents preferentially consume pink individuals of *Cepaea nemoralis* on the basis of achromatic signals (Cain, 1953; Murray, 1962). Interestingly, the crab is better at discriminating *L. saxatilis* based on their achromatic signal relative to the bird, potentially because birds possess superb chromatic discrimination ability (Siddiqi *et al.*, 2004). Furthermore in the crab visual system the common brown morph is more cryptic in terms of achromatic signal than both the fawn and yellow morphs and yet the brown is achromatically more conspicuous than the orange morph. This may imply that crabs using achromatic signals select other morphs more than the brown morph. However, this does not explain the relative commonness of yellow individuals. In a laboratory experiment, Tucker (1989) found no evidence of selection by crabs preying upon *Littorina obtusata* under artificial light conditions. Potentially crabs could select against the most conspicuous morphs using achromatic signals under low light levels when they are more active instead of bright artificial illumination (Tucker, 1988).

The situation on exposed shores appears to be somewhat less complicated: on the exposed shores, where birds are important predators, avian discrimination of all morphs is worse than on the sheltered shores where crabs are the main predators, suggesting that selection by birds on exposed shores has promoted the evolution of crypsis (Pettitt, 1973b). Given that birds are better than crabs at discriminating hues, it is unsurprising that avian predation on exposed shores coincides with more cryptic and also more diverse morphs than those found on sheltered shores (Hayashi and Chiba, 2005). On the exposed shores brightly coloured orange and yellow individuals are the most conspicuous morphs, which is in agreement with the results found on English shores (see Chapters 3 and 5).

It has been suggested that birds will only resort to consuming littorinids when their usual food source is depleted, for example during a particularly harsh winter (Pettitt, 1975). It can be assumed that if birds are predators of *L. saxatilis* on exposed shores, for example whilst on migration, they may select prey in a manner which promotes a stable polymorphism. This process of selection may, however, be slow as birds will remove only a small proportion of the prey population each year (Gibb, 1956; Feare, 1966, 1967; Alerstam *et al.,* 1992).

Although differences related to 'exposure' have been discussed it must be noted that there were significant 'plot' related and 'shore' related differences in background matching within exposures. High colour variation among study sites has previously been observed in Swedish populations of *L. saxatilis* and has been attributed to 'area effects' caused by colonisation by a few founder individuals (Ekendahl and Johannesson, 1997). Although only two shores per exposure were studied, it is clear that the discrimination of colour morphs is different between the two ecotypes. This situation could be compared with that observed in the terrestrial pulmonate snail *Cepaea nemoralis* whereby high levels of between-site variation have been observed when considering apparently identical habitats (Cain and Currey, 1963).

Do littorinids from one exposure match their own backgrounds better than backgrounds from the alternative exposure (sheltered versus exposed)?

Swapping littorinids and backgrounds revealed that in the visual system of crabs, *L. saxatilis* matches the backgrounds of the shores from which they came better than the exposure at which they are not found. This suggests that sheltered shore littorinids are cryptic against their natural background in the eyes of the most common predator, the shore crab. This finding agrees with the possibility of differential polymorphisms existing which relate to the background heterogeneity dependent on the exposure, the predators and potentially shore level (Ekendahl and Johannesson, 1997).

Are rare white, banded and red exposed morphs more conspicuous against sheltered shore backgrounds?

In the bird visual system the rare and supposedly conspicuous morphs are more cryptic against exposed shore backgrounds rather than sheltered backgrounds. Given that these morphs are so conspicuous to humans, it has been hypothesised that they persist in the population through apostatic selection (Ekendahl and Johannesson, 1997). However my results suggest that the rare, 'conspicuous' morphs may actually be quite cryptic when considering shell colour alone as opposed to pattern or brightness. Therefore exposed backgrounds may be more heterogeneous than sheltered shore backgrounds at a scale that is relevant to the movement of *L. saxatilis* (Rochette and Dill, 2000). When foraging in an environment such as the rocky shore, the ability to detect prey items is significantly affected by the visual heterogeneity of the background (Gonçalves Rodrigues and Silva Absalão, 2005). In a more heterogeneous environment there may be more opportunities for crypsis and therefore there is the possibility for a more diverse polymorphism to persist under visual selection (Endler, 1978, 1984).

Although it has been recorded that white, banded and tessellated morphs are not found on sheltered shores (Ekendahl, 1998) in this work the black morph was absent from the two sheltered shores surveyed and very few white individuals were recorded at both exposures. Furthermore the spectroradiometric technique does not take account of patterning. Therefore tessellated morphs were recorded under their 'ground' or base colour and no banded or red morphs were encountered on exposed shores, most likely due to their low frequency of less than 10% (Ekendahl and Johannesson, 1997).

Preliminary results from a 10 year manipulation study suggest that artificial *L. saxatilis* populations on exposed skerries are changing very slowly over time (K. Johannesson in prep.). These populations were created with greatly increased frequencies of conspicuous morphs and so far there is evidence that in particular the frequencies of the white morph are decreasing. The rare morphs that were collected from this manipulation project are more cryptic to birds when modelled against exposed shore backgrounds rather than sheltered shore backgrounds. These supposedly conspicuous morphs are thus more protected in the exposed shore habitat than expected. If the rare morphs were genuinely conspicuous then we might expect to see no difference in conspicuousness between the two exposures. When these rare morphs were compared to the other morphs on the exposed shores in both visual systems, there was no difference between any morphs in the crab visual system. In the bird visual system, however, the rare red morph was more conspicuous than the yellow morph. From the data it seems, that in terms of relative conspicuousness, only the red morph stands out against the background of the exposed shore to a bird while the other rare morphs (banded, white) are relatively cryptic.

The two habitats, sheltered and exposed shores, exhibit differences in the relative importance of different factors which has previously been observed to result in complex spatial patterns of colour polymorphism (Cain and Currey, 1963; Cook and Pettitt, 1998; Cowie and Jones, 1998). A number of evolutionary forces have been identified which cooperate in the evolution and maintenance of visual appearance in *C. nemoralis* and my results suggest a similarly complex interaction of forces. However, this work has detailed the first interpretation of visual signals available to potential predators foraging for *L. saxatilis* on Swedish shores and this work contributes further to the understanding of predator-prey interactions in the intertidal environment.

Chapter 5

Can background heterogeneity caused by lichen encrustation affect the extent to which polymorphic littorinids are cryptic?

Summary

Background heterogeneity in natural habitats can occur through both physical and biological means. The effect of encrusting lichen (*Verrucaria maura*) on the extent to which *Littorina saxatilis* morphs match their backgrounds was investigated. Reflectance spectra were collected from populations of *L. saxatilis* on a red sandstone shore. Principal components analysis of reflectance spectra showed that bare rock backgrounds were more spectrally diverse than lichen backgrounds. Interestingly, visual modelling of reflectance spectra in the visual systems of crabs and birds indicated that *Littorina saxatilis* was more cryptic on the relatively uniform lichen background rather than the more heterogeneous bare rock background. Specifically the crab visual model was found to discriminate *L. saxatilis* morphs against lichen better than bare rock, whereas the bird visual model was good at discriminating spectra overall. The ecological relevance of this finding is discussed in relation to predator visual systems, background heterogeneity, habitat type and colour polymorphism.

(5.1) Introduction

Divergent selection in heterogeneous environments has long been proposed as a mode for maintaining genetic variation in organisms (Cain and Sheppard, 1950; Kettlewell, 1955; Maynard Smith, 1966; Hedrick, 1976; Endler, 1978; Maynard Smith and Hoekstra, 1980; Cook, 1986a). The association of monomorphic coloration in visually homogeneous habitats and polymorphic coloration in visually heterogeneous habitats provides evidence for maintenance of colour polymorphisms by visual selection against varied backgrounds (Cook, 1986a). As described in the preceding chapter, *Littorina saxatilis*, which lives in a visually heterogeneous habitat, is an ideal species for studying the relationship between colour polymorphism and background matching. Of particular interest is the effect of spatially and temporally variable biological background elements on the conspicuousness of different morphs in populations of *L. saxatilis*.

Geographical variation in the polymorphism of *L. saxatilis* and the extent of background matching within and between shores has been explained by heterogeneity of the background geology and its coloration (Heller, 1975; Byers, 1990) and exposure (Johannesson and Ekendahl, 2002). Heterogeneity of background mangrove vegetation has also been implicated in maintenance of shell colour polymorphism in some tropical littorinids (Hughes and Mather, 1986; Cook, 1986b; Reid, 1987). Birds, fish and crabs are all predators that potentially prey upon littorinids (Pettitt, 1975; Alerstam *et al.,* 1992; Robertson, 1992). In an attempt to associate morphs with background characteristics, Heller (1975) found that the reddish-brown morphs of two species in the *Littorina saxatilis* complex often reached their highest frequencies on shores in Wales where Old Red Sandstone predominates. Indeed, all ten shores where the frequency of the red morph of *L. saxatilis* exceeded 15% were from Old Red Sandstone locations.

For rocky shore littorinids, the background against which they live is not only geologically variable but also temporally and spatially variable because of biological background elements such as epiphytes and epifauna. Heller (1975) found that the proportion of white morphs of *L. saxatilis* increased with shore exposure, evidently due to the increasing cover of barnacles, which provide a white background against which the white morph appears cryptic. In contradiction to the evidence for background matching of intertidal snails on different rock types, Raffaelli (1979) concluded that red and orange morphs of *L. saxatilis* were no more common on red rocks than elsewhere. Raffaelli (1979) suggested selection on pleiotropic characters such as salinity tolerance and adhesion ability rather than on shell colour itself, explained the association between shell and substratum colour. Specifically, Raffaelli (1979) showed that dark patterned shells were found mainly on rocky ledges, while bright unpatterned ones occurred more often on boulders.

The discrepancy between human classification of coloration and the actual signals available to predators has been discussed in the preceding chapter. Spectroradiometry and visual modelling allow the interpretation of prey coloration signals in the visual systems of predators. I investigated littorinids inhabiting a spectrally heterogeneous habitat to determine whether lichen growth differentially affects the extent to which different morphs match the background compared to bare rock background. The advantages of objective classification of colour by spectral data collection have been discussed in Chapter 3. In this chapter the same techniques are used to compare littorinid and background coloration (and hence their visual signals to predators) to investigate whether background heterogeneity caused by the presence or absence of lichen epiphytes affects crypsis in polymorphic littorinids.

Overall, this chapter investigates whether different coloured littorinids tend to be found on different background types (bare rock and lichen covered rock) and whether biologically generated background heterogeneity can explain any patterns of morph distribution observed. The following three questions were addressed:

1. Is there a difference between the extent of background matching between morphs of *L. saxatilis* on bare rock and lichen-covered backgrounds?
2. Do crab and bird predators have the same level of discriminability when observing littorinids on bare and lichen covered backgrounds?
3. Is there a relationship between the frequency of a morph and the extent to which it matches the background when the backgrounds are bare rock or lichen covered?

(5.2) Methods

To investigate the role of biologically-mediated background heterogeneity on the extent of background matching in *L. saxatilis,* 50 reflectance spectra were collected from littorinids and backgrounds on bare red sandstone and also 50 from lichen-covered (*Verrucaria maura*) red sandstone at Heybrook Bay, Devon (Ordnance Survey grid reference SX 495487). Shell reflectance spectra were measured in a dark box as described in Chapter 2 (section 2.3). A reference set of littorinids from each morph category was collected to define the morphs when measuring their reflectance. Colour morphs were recorded according to the scheme described by Pettitt (1973a). Each littorinid was assigned to an age category: adult, greater than 6mm shell height, and juvenile, 3-6mm shell height. Those littorinids less than 3mm shell height were not measured as they were too small for collection of reflectance spectra. Since the sample size for reflectance spectra was smaller than at other shores described in chapter 3, an additional survey of littorinids was carried out to determine the morph frequencies of *L. saxatilis* at Heybrook Bay. Therefore the frequencies of different morphs found in ten randomly selected 25cm^2 quadrats in each background type were recorded in a 25m^2 area where spectral data collection also occurred. Differences between morph ratios recorded on the two background types (displayed in Table 5.3.1), were made using chi squared contingency tables tests. The resultant χ^2 value was partitioned to detect differences between morphs using the Lancaster (1949) and Irwin (1949) method (see Everitt, 1979). Since the littorinids encountered in spectral reflectance sampling were not a complete balanced sample of all morphs present, a sub-sample was collected for spectral analysis in the laboratory.

(5.2.1) *Multivariate Analysis of Reflectance Spectra*

Principal components analysis (PCA) was carried out on 'standardised' spectra collected from littorinids and backgrounds. Standardisation implies that spectra have had the mean reflectance subtracted at each wavelength interval to account for chromatic variation between spectra rather than brightness variation. General linear models were used to analyse PC1 and PC2 scores to determine whether there was an association between the two backgrounds (bare rock and lichen) and the colours of littorinids found on them. Therefore PCA was used to illustrate differences between *Littorina saxatilis* and the background against bare and lichen covered rocks on the same shore in terms of spectral shape. Additionally the principal component loadings were used to determine the wavelengths at which the spectra varied and also where they were similar. In PCA of unstandardised spectra the first principal component, which explains the majority of variability in natural spectra, is commonly a correlate of mean reflectance or brightness (Cuthill *et al.,* 1999). Therefore PCA was also carried out on unstandardised spectra to derive the importance of brightness variability between spectra and the correlation between PC1 and mean reflectance tested by Spearman's rank correlation. Brightness differences between background types and also between different littorinid colour morphs were determined statistically using general linear models on Box-Cox transformed mean reflectance values per spectrum.

(5.2.2) *Visual Modelling of Reflectance Spectra*

Using the crab and bird visual models described in section 2.6, colour space distances (ΔS) were calculated for each individual littorinid and its immediate background, whether bare rock and lichen covered rock. These colour space distances were presented and analysed in the following ways:

1. To demonstrate the discriminability of individual littorinids in each background type, the numbers of paired comparisons which fall into each ΔS class for each visual system model are displayed in frequency histograms. Each ΔS class is divided into the constituent morphs to indicate the number of individuals of each morph in that ΔS class.
2. To indicate the discriminability of the morph categories assigned on the shore the median colour space distance of each morph from its own background is displayed as a box plot with interquartile range.
3. To compare the extent of background matching between littorinids on bare rock and lichen-covered rock backgrounds, irrespective of morph category, a t-test was carried out on Box-Cox transformed ΔS values.
4. To determine whether littorinids were matching the background against which they were sitting better than the average background on the shore, a paired t-test on Box-Cox transformed was carried out. The ΔS values of littorinid and background pairs were compared to the mean ΔS values of each littorinid to all backgrounds (either bare or lichen covered) on the shore.

(5.3) Results

(5.3.1) *Morph Frequencies*

The morph frequencies on the two backgrounds differ statistically significantly from each other (χ^2=33.88, 5 *d.f.*, P<0.01) with more banded, black and fawn individuals on the bare rock than on lichen covered rock. Chi-squared partitioning (Lancaster, 1949; Irwin, 1949) showed specifically that this significant result is dependent on the frequencies of the banded morph and the black morph differing from the brown morph frequencies (χ^2=15.78, 1 *d.f.*, P<0.01) and also the banded, black and brown morph frequencies differing from the fawn morph frequencies on the two backgrounds (χ^2= 6.1, 1 *d.f.*, P<0.05) (Table 5.3.1).

Table 5.3.1: Frequencies and percentages (in brackets) of littorinids of each morph on each background type.

Background type	Banded	Black	Brown	Fawn	Red	White	Total (n)
Bare rock	83 (29.2)	55 (19.4)	44 (15.5)	87 (30.6)	8 (2.8)	7 (2.5)	284
Lichen covered rock	43 (29.4)	15 (10.3)	50 (34.3)	28 (19.1)	8 (5.5)	2 (1.4)	146

(5.3.2) *Reflectance Spectra*

Reflectance spectra demonstrated that although littorinids could be separated into morph categories on the shore, the variability in mean spectra of each morph was dominated by brightness differences (Fig. 5.3.1A). A general linear model analysis of Box-Cox transformed mean reflectance for each morph combined with spectral data collected in the laboratory showed that morphs differ in mean brightness ($F_{(5,\,110)}$=14.099, P<0.001). *Post hoc* Tukey tests showed that the banded morph is significantly brighter than the black morph (P=0.042) and also that the white morph is much brighter than all morphs except banded (P<0.001). The red morph, however, had a markedly different spectral shape from the other morphs, particularly between 600-700nm (Fig. 5.3.1A).

Sample background spectra for bare rock (Fig. 5.3.1B) were more variable in brightness than *Verrucaria maura* backgrounds because the lichen is very dark in colour and had more uniform spectral reflectance than the bare rock (Fig. 5.3.1C). Using Box-Cox transformed mean reflectance for each background spectrum, a general linear model analysis showed that lichen covered backgrounds had significantly brighter spectra than bare backgrounds ($F_{(1,\,96)}$=229.9, P<0.001). Furthermore, bare backgrounds and littorinids do not differ in brightness ($F_{(1,\,98)}$=1.61, P=0.208) whereas lichen backgrounds were significantly brighter than the littorinids ($F_{(1, 94)}$=17.93, P<0.001). Heybrook Bay is a red sandstone shore and thus on bare rock an increase in reflectance was noticeable at 575nm (red), whereas *V. maura* produced a flatter spectrum (Fig. 5.3.1B, C).

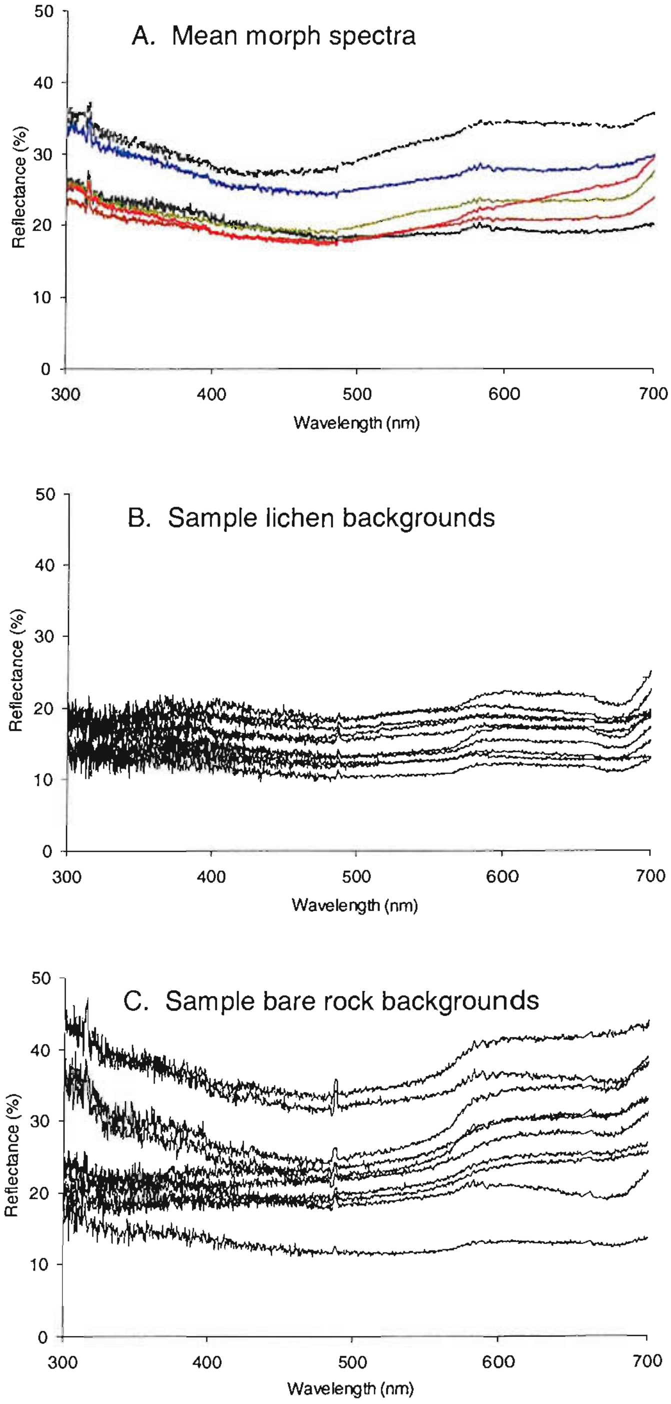


Figure 5.3.1: (A) Mean reflectance spectra of morphs at Heybrook Bay (Banded, blue line, N= 6; Black, black line, N=3; Brown, brown line, N= 54; Fawn, green line, N= 12; Red, red line, N= 19; White, dashed line, N=4), (B) sample spectra from *Verrucaria maura* covered backgrounds and (C) samples spectra of bare backgrounds including the brightest and darkest spectra collected.

(5.3.3) *Multivariate Analysis of Reflectance Spectra*

The uniformity of the lichen background is demonstrated in Figure 5.3.2A, whereby the lichen background spectra were grouped with mostly negative values of PC1 and PC2. The two backgrounds were separated well by PC1 which describes variation in the relative amounts of reflectance below and above 550nm (Fig. 5.3.2B). PC2 describes variation between spectra in terms of relative reflectance below and above 410nm (Fig. 5.3.2B). In terms of PC1 there was a significant interaction of background type and surface type indicating that the spectra of snails vary in accordance to the background type albeit bare rock or lichen ($F_{(1, 192)}$=81.24, P<0.001). No significant interaction of background type and spectrum type was found for PC2 ($F_{(1, 192)}$=1.49, P=0.223) although a significant effect of background was observed irrespective of whether the spectra were backgrounds or littorinids ($F_{(1, 192)}$=45, P<0.001). Using unstandardised spectra, the most variability between spectra was in terms of brightness as PC1 explained 94.5% of the variation between spectra and correlated significantly with mean reflectance (r = -0.739, P<0.001).

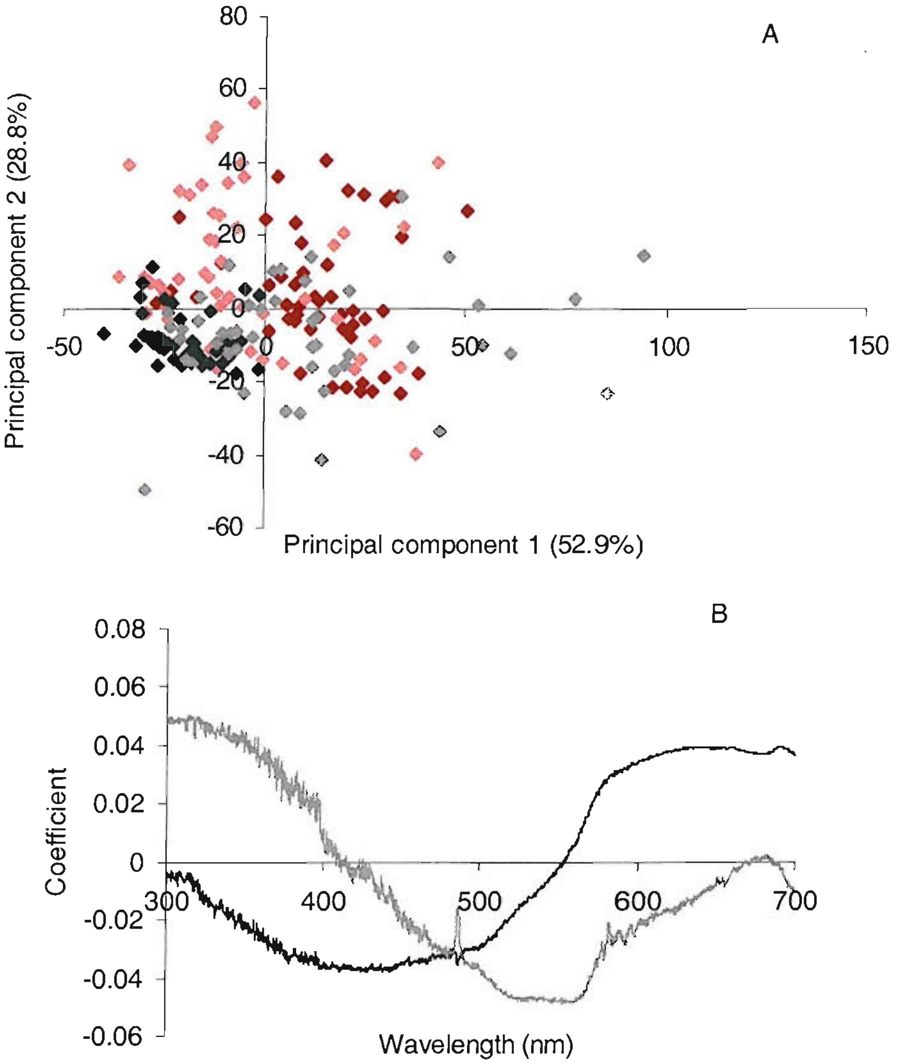


Figure 5.3.2: (A) PCA score plot of spectra collected from bare rock backgrounds (red diamonds) and littorinids (pink diamonds) and *Verrucaria maura* covered rock backgrounds (black diamonds) and corresponding littorinids (grey diamonds). (B) coefficient values of PC1 (black lines) and PC2 (grey line) from a PCA using standardised spectra shown in A, collected at Heybrook Bay.

(5.3.4) *Visual Modelling of Reflectance Spectra*

Overall, there was a low level of discrimination of littorinids against both backgrounds and for both simulated visual systems at Heybrook Bay. The bird model was consistently better at discriminating spectra pairs than the crab. The crab visual model, however, was better at discriminating littorinids on lichen than on bare rock (there were more comparisons in ΔS classes greater than zero for lichen when compared to bare rock) (Fig. 5.3.3A-D).

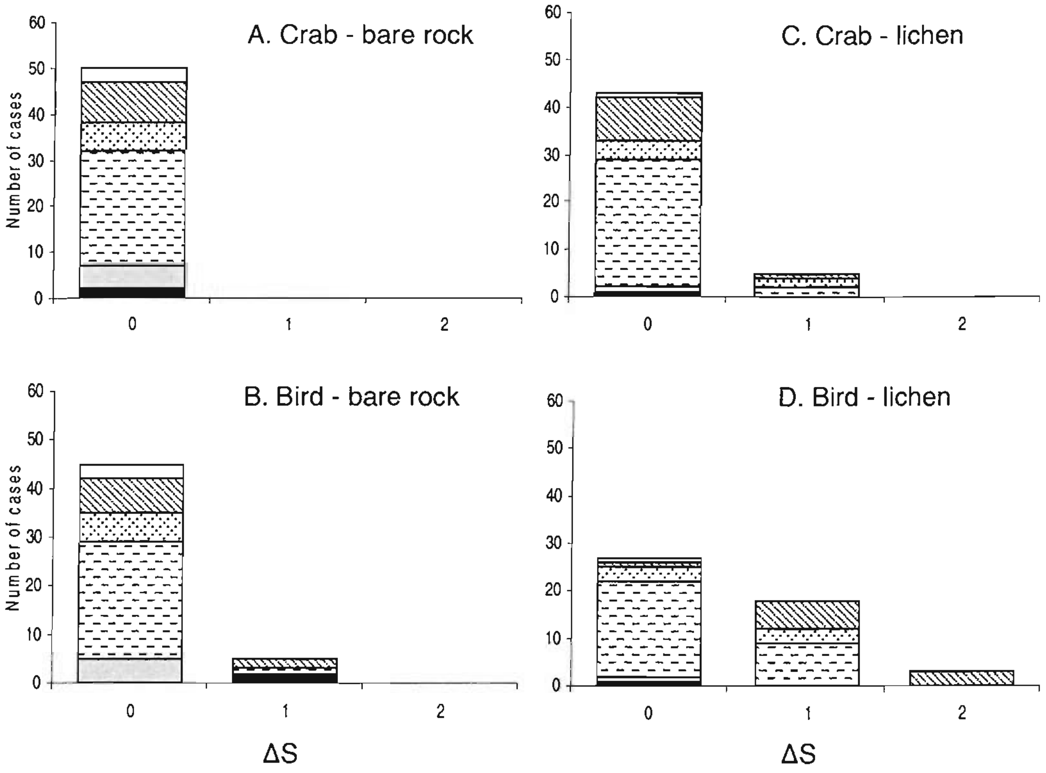


Figure 5.3.3: Number of cases of littorinid and background comparisons which fall into each ΔS category, divided into number of individuals of each morph (White ☐; red ▨ ; fawn ░; brown ▤; black ■; banded ▩)

Littorinia saxatilis on bare rock backgrounds were significantly more cryptic than on lichen covered backgrounds as indicated by significant difference between the distributions of ΔS on the two different backgrounds in crab (t-test on Box-Cox transformed ΔS values, t= -6.88, 75 *d.f.* P<0.001) and bird visual systems (t= -3.25, 75 *d.f.* P=0.002) and from examination of median ΔS values (Fig. 5.3.4A-D). In the crab visual system littorinids matched their own backgrounds better than the average background (paired t-test on Box-Cox transformed ΔS values; N=100, t= -3.53, P=0.001) whereas there was no significant difference between the two in the bird visual model (N=100, t= -1.01, P=0.317).

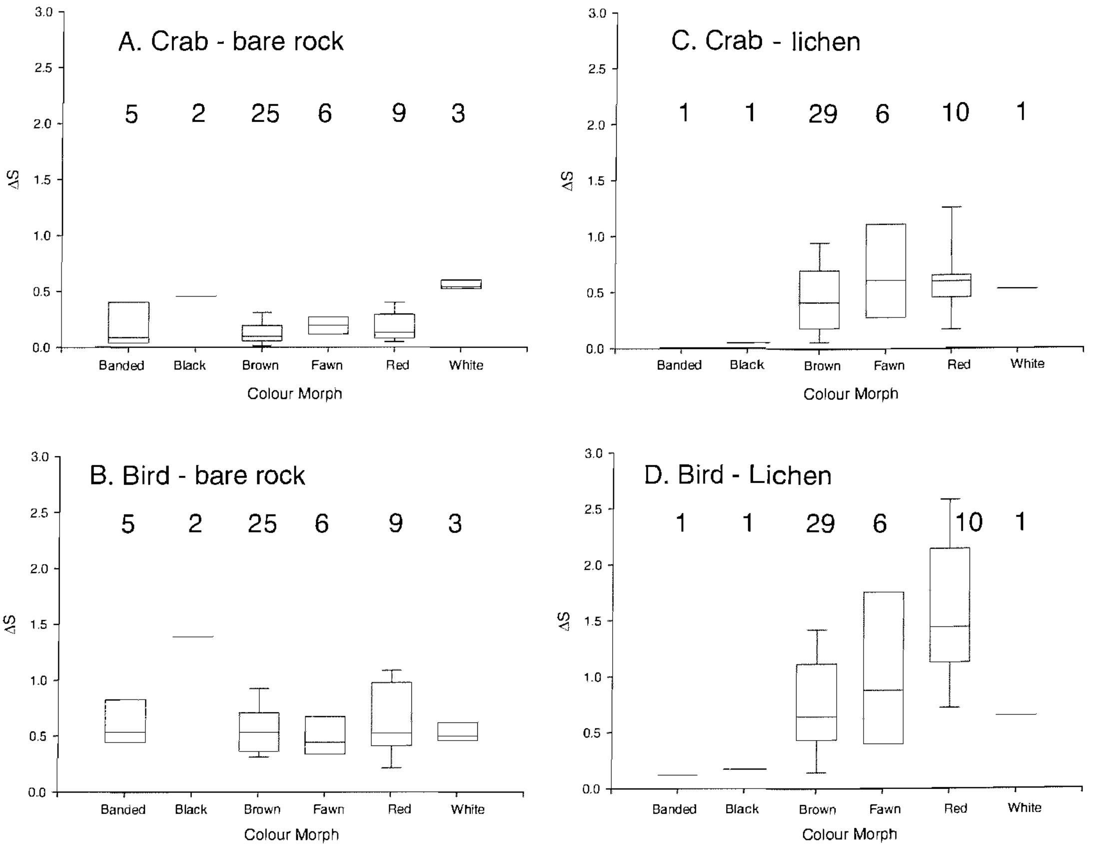


Figure 5.3.4: Median ΔS (± interquartile range) for the morphs found on bare rock in crab (A) and bird (B) visual systems and on lichen-covered rock in crab (C) and bird (D) visual systems. Numbers of individual littorinids per morph are shown above each morph category, where numbers are less than 10 error bars are omitted.

The model simulations indicated that crabs were likely to be better at discriminating *L. saxatilis* on lichen covered rock than they were at discriminating littorinids on bare rock, although birds would most likely be consistently better at discriminating *L. saxatilis* from the backgrounds overall (Fig. 5.3.5).

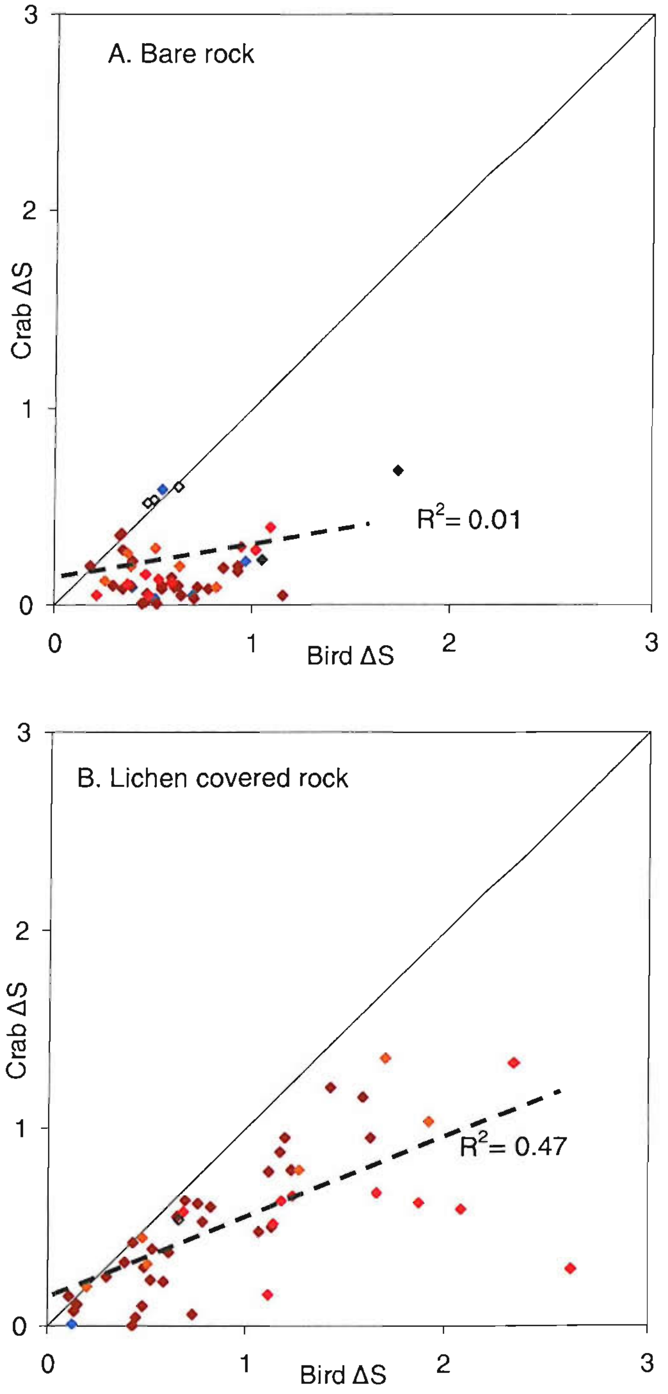


Figure 5.3.5: Differences in discriminability of *L. saxatilis* morphs (banded, blue; black, black; brown, brown; fawn, orange; red, red; white, white) and background spectra on bare rock (A) and lichen covered rock (B) in the visual systems of crabs and birds with regression slopes and R^2 values.

There was no statistically significant relationship between the frequency of a morph found on a certain background type and the percentage of the backgrounds upon which each morph was more cryptic than the mean contrast for the all littorinids on each background type (R^2 values displayed on Fig. 5.3.6). Despite the absence of strong correlation between background matching and frequency of the morph, there was a positive relationship for both backgrounds in both visual systems, hinting that the more common morphs are the most cryptic.

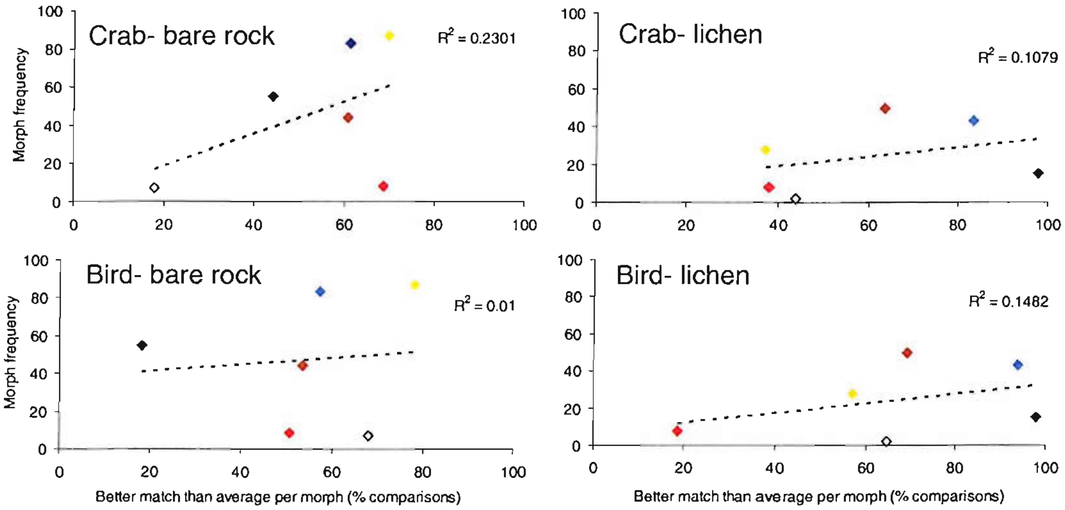


Figure 5.3.6: The percentage of backgrounds against which each morph is more conspicuous than the general match of any morph against any background in relation to the frequency of that particular morph. Results from both the crab and bird models are displayed (Black, black diamonds; banded, blue; brown, brown; fawn, yellow; white, open; red, red) with regression slopes and R^2 values.

In summary, bare rock backgrounds were more spectrally variable than lichen backgrounds. *Littorina saxatilis* matched the lichen backgrounds better overall than the bare backgrounds. The simulated crab model was better at discriminating *L. saxatilis* from lichen backgrounds rather than from bare backgrounds whereas the bird model was better at discriminating *L. saxatilis* overall. When considering all morphs and backgrounds together, littorinids match their own background better than the average background in the crab visual system but not the bird visual system. Therefore, the crab visual model must discriminate *L. saxatilis* against lichens better than bare rock to a greater extent than the bird model.

(5.4) Discussion

The data presented here indicate that simulated crab and bird predators differ in the extent to which they may detect *Littorina saxatilis* against the background. This difference is dependent on whether the background is bare rock or lichen covered rock. However, the parameters of the bird and crab visual models are subject to assumptions and the visual models represent the best approximation of the chromatic information available to the crab and bird visual systems for neural processing. Most importantly the receptor noise-limited model predicts chromatic contrast by assuming bright illumination for relatively large static targets resulting in low achromatic signals (Vorobyev and Osorio, 1998). Although littorinids are small in size, they would be viewed at close distances by both crabs and shorebirds therefore subtending a large visual angle. Thus the chromatic contrasts produced by the models should be interpreted with caution.

Ecological assumptions were made about the predators. For example, they were modelled to forage under clear blue daylight skies (D65 irradiance). D65 is a standard illumination condition and although it is unnatural, the relative difference in discrimination of spectral pairs is still informative. The crab visual model, based on the green shore crab (*Carcinus maenas*), was used despite uncertainties surrounding its use of vision in foraging and also its impact on British rocky shores. On Mediterranean and southern European shores with microtidal ranges the shore crab, *Pachygrapsus marmoratus,* is an important predator of littorinids (Gurriarán and Mendez, 1985). Likewise, the green shore crab *Carcinus maenas* is an important predator of littorinids in atidal areas of Sweden (Johannesson, 1986) and also on the North-East Atlantic coast (Rangeley and Thomas, 1987). Therefore a crab model was included in this work to account for the potential for tidal conditions to occur where predation could impact upon the littorinid population.

The effect of background heterogeneity resulting from the presence or absence of the lichen *Verrucaria maura* was investigated. However, lichen patches were often less than $1m^2$ in area with no more than 1m between two patches; therefore movement of *L. saxatilis* between the two backgrounds could occur. Even though littorinids only travel between 1-4m in 3 months (Janson, 1983), it is possible that an individual littorinid could occur in both lichen covered and bare rock areas during one grazing excursion. Therefore this work was limited to a snapshot of the locations of littorinids at that particular time and does not account for their movement over a 24hour cycle.

Seasonal changes in morph frequencies of littorinids have been shown by Hughes and Mather (1986) and by Reid (1987). Not only is the local movement of littorinids over short timescales an important factor in determining the visual stimuli available to predators, but also seasonal changes in background may also potentially have an effect. Furthermore, the background is subject to temporal variation in *V. maura* growth (Johannesson, 1989) which could, in certain localities, be a cause of changes in morph frequency.

Despite these limitations the study has shown that *Verrucaria maura* provides a background against which *L. saxatilis* morphs in general are more conspicuous when compared to the underlying red sandstone irrespective of morph type. Also on the bare rock areas, individual littorinids match their own backgrounds better than average whereas there is no difference on the lichen backgrounds. This could indicate background matching in the bare rock areas but not in the lichen areas. The lichen background tends to be more uniform than the bare rock background and therefore the bare rock background should provide more opportunities for crypsis (Endler, 1978; Hedrick, 1986; Gonçalves Rodrigues and Silva Absalão, 2005). The survey of morphs found in each microhabitat type, however, does not demonstrate increased diversity of morphs on the bare rock background, although the morph ratios in the two areas do differ significantly. In the morph frequency surveys almost twice as many snails were recorded in bare rock areas than in lichen areas. Despite this, black and fawn littorinids were more frequent on bare rock, yet brown littorinids were more frequent on lichen. This observation could be due to the movement of littorinids between bare rock and lichen-covered rock areas.

Heller (1975) compared morph ratios of species in the *saxatilis* complex at two areas separated by 140km and found associations between background colours and morphs. In my study, background types varied across shorter distances and unlike the work of Heller (1975), no association between bare red sandstone areas and a high frequency of red littorinids was observed. Investigations into background matching in *Cepaea nemoralis* have looked at habitat types which extend over tens of metres or more which represent discrete habitat patches of different heterogeneity levels (Cain and Currey, 1963). Cain and Sheppard (1950) found a strong correlation between the amount of green vegetation in the habitat background and the frequency of banded yellow shells. This result was confirmed by the classic work of Sheppard (1951, 1952) on the numbers of *C. nemoralis* eaten by Thrushes, *Turdus philomelos*, in which he showed that conspicuous forms tend to be removed.

Over small distances, associations have been observed between light morphs of the limpet *Acmaea digitalis* and barnacle covered rocks and dark morphs with dark rocks (Giesel, 1970). However, it is likely that limpets would move further distances on a single foraging excursion than a littorinid and yet limpets are still found to be associated with certain backgrounds. Additionally, Cooper (1984) found that birds still strongly selected artificial prey in a frequency dependent manner when background patches were very small coloured stones. Polymorphic chitons, *Ischnochiton striolatus* are more diverse and more frequent on the undersides of medium sized rocks as opposed to small or large rocks (Gonçalves Rodrigues and Absalão, 2005). This is potentially because medium rocks experience an intermediate level of disturbance and therefore provide an intermediate habitat which supports the most diverse epibiota which in turn provides background heterogeneity and crypsis for more chiton morphs (Gonçalves Rodrigues and Absalão, 2005). Therefore, although *Verrucaria maura* does not represent a discrete habitat which is separate from bare rock, it is still possible that over small distances between different microhabitats, selection against certain *Littorina saxatilis* morphs could occur.

Although no evidence of selection against particular morphs living on a certain background has been proven or indeed tested, habitat choice is a possible explanation for littorinid colour morphs sometimes matching their backgrounds (Hughes and Jones, 1985). This mechanism would require there to be a link between genes controlling behaviour and shell colour or a pleiotropic effect of a single gene controlling behaviour and shell colour. Such behavioural differences have been observed in the land snail, *Arianta arbustorum,* which resulted in yellow individuals occupying exposed areas and brown individuals occupying shaded areas (Abdel-Rehim, 1983). Visual cues are not used by littorinids in food detection and are therefore unlikely to be important in microhabitat selection (Newell, 1965; Land, 1968). Despite this, other mechanisms exist by which morphs could choose different microhabitats related the conditions and resources provided by the microhabitat (*e.g.* mucus trail following, Davies and Beckwith, 1999). Norton *et al.,* (1990) conclude that foraging in littorinids is influenced by algal exudates to minimize unnecessary movement and this could apply to lichen patches. Temperature is known to influence the morph distribution in the intertidal snail *Nucella lapillus* (Etter, 1988) and Cook (1986b) concluded that the thermal properties of different structural parts of mangroves dictate the colour morph positioning.

Verrucaria maura is most common in wave exposed (Lewis, 1964) and shaded sites (Johannesson, 1989). Areas of rock covered by *V. maura* may retain moisture creating an environment which may, on occasions, limit desiccation in *L. saxatilis. Verrucaria maura* is grazed by littorinids but is known to provide low food value (Fletcher, 1980). However, there is no evidence that littorinids actively choose lichen patches. Therefore despite the increased conspicuousness of littorinids when viewed against *V. maura,* the lichen may be a beneficial microhabitat providing moisture and food. Therefore other physiological factors and requirements may determine the positioning of individual littorinids.

Although the receptor threshold model deals with chromatic differences alone it is important to note that the lichen is brighter than the littorinids whereas the bare rock is less bright than the littorinids. This could be an important factor in the survival of littorinids against different backgrounds when viewed by predators as brightness contrast is known to be important for birds (Maier and Bowmaker, 1993; Von Campenhausen and Kirschfeld, 1998) and crabs (Crothers, 1968). Indeed, the achromatic signal (brightness contrast) provides the greatest stimulus power in natural images (Vorobyev and Osorio, 1998). Crabs in particular may be more likely to use brightness as a method for discriminating objects as they commonly forage in low light (Crothers, 1968). Thus it is fitting that crabs were better at discriminating littorinids against the black lichen than against the bare red sandstone.

Although littorinids and their backgrounds do not reflect wavelengths that are undetectable to the human eye (*i.e.* ultraviolet light), biologically important signals, or lack of signals, may be difficult to detect using human observation if human peak sensitivities differ greatly from the predators observing the littorinids (Bennett *et al.*, 1994).
Spectroradiometry has allowed a more precise assessment of the appearance of these snails in their natural environment and in the eyes of their predators. Previous conclusions on the adaptive significance of colour polymorphism in *L. saxatilis* have rested heavily on the

potential for predation to act in a selective manner in a heterogeneous environment. Here, I have taken account of the visual systems of potential predators and found that, although there is no association between particular colour morphs and certain background types, lichens in the splash zone do make littorinids appear more conspicuous to predators, especially crabs. Furthermore, although there is some evidence that those individuals that are more common are also more cryptic, there is no direct relationship between morph type and background. Therefore it seems that visual predation, thermal properties, background heterogeneity caused by geology and epibiota and also other selective factors may all contribute to explaining the maintenance of the polymorphism in *Littorina saxatilis.*

PART III

ALGAL DWELLING LITTORINIDS

Chapter 6

Can algal morphology and background heterogeneity affect crypsis in polymorphic littorinid pr

Summary

Rocky shore algal habitats provide a spatially and temporally variable visual habitat for intertidal snails. *Littorina obtusata* and *L. fabalis* are both polymorphic for shell colour. Genetic polymorphism is thought to be maintained in heterogeneous environments enabling different spatial or temporal fitness of the different morphs. On a moderately exposed shore with no clear algal zonation *Fucus serratus* and *F. vesiculosus* provide backgrounds of different heterogeneity: *F. vesiculosus* with gas vesicles and *F. serratus* without gas vesicles. The extent to which different morphs of *L. obtusata* and *L. fabalis* matched these two algal backgrounds was investigated. Treating the two sibling species as one set of polymorphic prey, reflectance spectra of algal dwelling littorinid shells were collected in the field along with the reflectance spectra of the background immediately adjacent to each littorinid as it was found. Principal Components Analysis (PCA) was used to determine the spectral diversity of the two algal backgrounds and the differences between morph spectra. PCA indeed confirmed the difference in spectral diversity between the two algal species. Visual modelling of reflectance spectra into the visual systems of potential crab, fish and bird predators showed that *F. serratus* provides more opportunities for dark morphs to appear cryptic. However all morphs were more conspicuous on *F. vesiculosus*. Simulated turbid water conditions affected the conspicuousness of some morphs to underwater predators. Differences between predator abilities to detect littorinids on the two algal species is discussed with reference to background heterogeneity.

(6.1) Introduction

The polymorphic sibling species, *Littorina obtusata* and *L. fabalis* dwell amongst algae on rocky shores and provide an alternative model system to study background matching and crypsis using objective colour classification techniques and visual modelling. *Littorina obtusata* and *L. fabalis* often occupy the same algal species at sites which are moderately exposed (Smith, 1976; Reimchen, 1982; Williams, 1994). Both species are polymorphic for shell colour and the specific morphs are shown in Figure 1.4.1 (Chapter 1). The different colour morphs of these two species appear to match different parts of the algae when viewed from certain positions (Reimchen, 1974). More specifically, the algal laminae are yellow when sunlight is transmitted through the surface. Backlit or 'transmitted' backgrounds could provide a transiently occurring background against which the normally highly conspicuous yellow morph is actually cryptic. Conversely, dark morphs of the two species appear more cryptic when light is reflected from the algal lamina surface (Reimchen, 1979). This observation suggests that yellow morphs may be more common on the underside of algal fronds, whereas darker morphs may be more common on the frond surface and dark brown holdfasts of fucoids. Evidence of this pattern has indeed been observed in the field (Reimchen, 1979; Ekendahl, 1994) and in habitat selection experiments in the laboratory (Ekendahl, 1995). Furthermore digital colour pattern analysis has revealed that some morphs of *L. obtusata* resemble structural parts of *A. nodosum* (Wilbur and Steneck, 1999).

There is no direct evidence of selective predation on the two littorinid species in the field. Laboratory predation experiments using blennies (*Lipophrys pholis*) showed that the fitness of brown and yellow *L. fabalis* morphs was a function of the light regime and structural part of the algae (Reimchen, 1979). The blennies tended to remove the conspicuous morphs (Reimchen, 1979).

It is commonly thought that environmental heterogeneity reinforces variable selection in both time and space, thus facilitating the maintenance of polymorphisms (Cain and Sheppard, 1950; Endler, 1978; Cook, 1986a; Hedrick, 1986). Therefore, if these molluscs are under strong selection by predators or indeed exhibiting habitat selection (or both) then these conditions may lead to a stable polymorphism (Goodhart, 1987).

The brown algal species (Phaeophyta) upon which *L. obtusata* and *L. fabalis* forage are likely to be visually heterogeneous. *Fucus serratus* and *F. vesiculosus* are particularly common on British shores, differing in their visual heterogeneity mainly because *F. vesiculosus* possesses gas vesicles and prominent reproductive structures, whereas *Fucus serratus* does not.

This chapter explores whether or not there is a difference in the extent to which morphs of *L. obtusata* and *L. fabalis* gain crypsis on these two algal species and their different structural parts. In addition, the ability of potential predators to discriminate littorinid morphs from algal backgrounds of differing heterogeneity will be investigated using spectroradiometry to accurately measure colour and visual modelling to determine the signals available to potential predators (see Chapter 2).

Predators capable of selection of littorinids on the basis of shell colour include birds, fish and potentially crabs (Pettitt, 1975). Crabs are most notably recognised as predators of *L. obtusata* and *L. fabalis* by evidence of shell damage by crushing or peeling (Pettitt, 1975; Smith, 1976; Raffaelli, 1978; Reimchen, 1982; Reid, 1984, 1987; Seeley, 1986; Ekendahl, 1998; Rolan-Alvarez *et al.,* 1997; Trussell, 2000). The sensory mode most commonly employed by the common shore crab, *Carcinus maenas*, has long been debated in the literature primarily because it has not been known whether crabs possess colour vision. Previously it had been suggested that crabs do use vision to hunt for prey and are better able to detect the object if it is moving, and best of all if it is striped (Waterman, 1961). The use of chemical and tactile stimuli have been commonly evoked as explanations for foraging behaviours as the colour vision capabilities of shore crabs had never been physiologically proven (Shelton and Mackie, 1971; Crothers, 1967). However, *C. maenas* is known to possess two classes of photoreceptor (Martin and Mote, 1982). Despite support for chemosensory and mechanoreceptor mechanisms of prey detection from afar, upon close range it can be expected that *C. maenas* uses its visual capabilities to distinguish prey from the background.

There is evidence that coastal fish species, in particular the blenny *Lipophrys pholis*, prey upon littorinids readily (Reimchen, 1979; Burrows *et al.,* 1999). SCUBA observations have reported a single blenny moving within algal fronds and removing 14 out of 64 *L. fabalis* in 1 hour (Reimchen, 1979). Laboratory experiments have shown blennies removing littorinids on algal fronds (Reimchen, 1979). The optical characteristics of the water in which these coastal fish hunt is dependent on chlorophyll content, levels of dissolved products and suspended material causing striking differences in attenuation characteristics and hence ambient spectra of light (Lythgoe, 1979; Lythgoe and Partridge, 1989, 1991; Hovárth and Varjú, 1995; Cummings, 2004).

The intensity of avian predation on intertidal mollusc communities varies greatly, both with the time of year and among bird species. Thus it is possible that the intensity of selection by birds is similarly variable. Migrating Knots (*Calidris canutus*) have been found to remove 1-4 % of *Littorina* species whilst stopping over in Iceland during the spring (Alerstam *et al.,* 1992) and also whilst feeding in Scotland (Summers and Smith, 1983). Thus if selection on littorinids by migrating birds occurs, it could be relatively strong (Janson, 1983). Furthermore, some individual birds may target littorinids whilst the rest of the birds concentrate on a more profitable prey type, therefore the overall impact of bird predation may be unknown (Feare, 1967; Pettitt, 1975). From gut content and gizzard analysis numerous bird species are known to consume littorinids, the extent of which is often dependent on the individual's preference and availability of the species' usual food source (see Pettitt, 1975 for a review). From personal observation in the field, shorebirds often forage amongst algae when the tide is out, occasionally using their beak/bill to move the algae and uncover prey beneath the surface.

The main purpose of this study was to interpret *Littorina obtusata* and *L. fabalis* coloration as perceived in the visual systems of their predators by measurement of chromatic distances between littorinid and background spectra. I predicted that there would be different levels of discriminability of littorinid colour morphs due to the differences in the visual systems of the potential predators against a visually heterogeneous background. Using spectroradiometry and visual modelling I investigated the conspicuousness of polymorphic littorinids against two algal species which exhibit different levels of visual heterogeneity. The ability to detect littorinids against algal backgrounds was assessed by determining colour space distances in each potential predator's visual system. My study, for the first time, takes account of the visual systems which potentially have a role in the evolution of colour signals in *L. obtusata* and *L. fabalis*.

(6.2) Methods

(6.2.1) *Spectroradiometry*

Spectroradiometric reflectance measurements of *Littorina obtusata* and *L. fabalis* and their backgrounds were obtained in the field. The two sibling species were not separated for the purposes of this work given that Bembridge is a mixed shore in terms of algal coverage and there is no clear zonation of algal species, therefore the two littorinid species co-occur and their morph frequencies are known to converge in such situations (Reimchen, 1974; Reid, 1996). Furthermore, this work was concerned with littorinids as prey items and how they matched the two fucoid backgrounds given that both species are at risk from predation by crabs, fish and birds (Pettitt, 1975; Williams, 1990). The reflectance spectra were collected in September 2004 at Bembridge Ledges, Isle of Wight, UK (Ordnance Survey grid reference. SZ 659880) using equipment and methods described in Chapter 2. An area $100m^2$ was selected at mid-shore level where both *Fucus serratus* and *F. vesiculosus* occurred. Four $0.5m^2$ quadrats were randomly positioned by blindly throwing the quadrat within the $100m^2$area. Individual quadrats were separated by at least 3m. From previous population surveys at Bembridge it is known that *L. obtusata* and *L. fabalis* occur in densities between 80-160 individuals m^{-2}. Therefore within each quadrat, the first 30 littorinids encountered were measured, together with the reflectance of the immediate background adjacent and to the right of the littorinid relative to my position. The part of the algal frond measured (stem, lamina, lamina tip and also vesicle and reproductive bodies on *F. vesiculosus* only) was recorded. The colour morph of each littorinid encountered was classified using the system defined by Sacchi and Rastelli (1966) as light brown, dark brown, yellow, olive and striped.

(6.2.2) *Morph ratio surveys*

The ratios of the morphs on each algal species were calculated from the littorinids used for spectral data as this was assumed to be a random sample of the population. These ratios were then compared statistically using Chi-squared tests.

(6.2.3) *Multivariate Analysis of Reflectance Spectra*

To test for spectral differences between backgrounds and littorinids, Principal Components Analysis (PCA) was carried out on 'standardised' spectra collected from littorinids and backgrounds for each algal species separately. Standardisation implies that spectra have had the mean reflectance subtracted at each wavelength interval to account for chromatic variation between spectra rather than brightness variation. A further PCA was carried out on background spectra alone for the two algal species to determine spectral differences between the *F. serratus* and *F. vesiculosus*. Two-sample Kolmogorov-Smirnov tests were used to test the differences between the two algal species in terms of principal component (PC) 1 and 2 scores. Additionally the principal component coefficient loadings were used to determine the wavelengths at which the spectra varied and also where they were similar.

(6.2.4) *Visual Modelling of Reflectance Spectra*

Firstly spectra were edited in the software 'R' to remove artifactual spikes caused by the light source. Colour space distances (ΔS) were then calculated for each individual littorinid and its immediate background by modelling spectra into the visual systems of a crab, fish and bird under daylight 65 (D65) irradiance (see Chapter 2, section 2.5 for details regarding visual modelling). The discriminability of individual littorinids on each background type and the numbers of paired comparisons which fall into each ΔS class for each visual system model were displayed in frequency histograms. Each ΔS class was divided into the constituent morphs to indicate the number of individuals of each morph in that ΔS class. Chi-squared tests were used to determine the differences in the frequencies of principal morphs in the $0\Delta S$ class (low relative discriminability) and those classes greater than $1\Delta S$ (higher relative discriminability) between *F. serratus* and *F. vesiculosus*.

To indicate the discriminability of the morph categories assigned on the shore the median colour space distance of each morph from its own background was displayed as a box plot with interquartile range. The difference in conspicuousness between each morph on the two algal species was tested by a General Linear Model on Box-Cox transformed ΔS values.

For each algal species, the median ΔS of yellow and dark brown littorinids to structural parts were calculated. Given that the bird has the highest discrimination ability, only the bird visual model was used, thus giving the most information about the extent to which different morphs match the different structural parts of the algae. The spectra used were collected in the laboratory from littorinids and algae collected at Bembridge. For comparisons of structural parts all littorinid and algal spectra were paired randomly using a random number generator.

(6.2.5) *Effects of Water Turbidity on Conspicuousness*

Attenuation coefficient data for moderately turbid coastal water between 0-10m was used to model the affect of turbidity on D65 irradiance (Jerlov, 1976 in Lythgoe, 1979) (Fig. 6.2.1). ΔS values were calculated for littorinid and background spectra pairs to model the affect of reduced light due to turbidity on the discriminability of littorinids in the visual systems of their predators. Repeated measures General Linear Model of Box-Cox transformed ΔS values was used to determine the effect of turbidity on the conspicuousness of different morphs on the two different algal species.

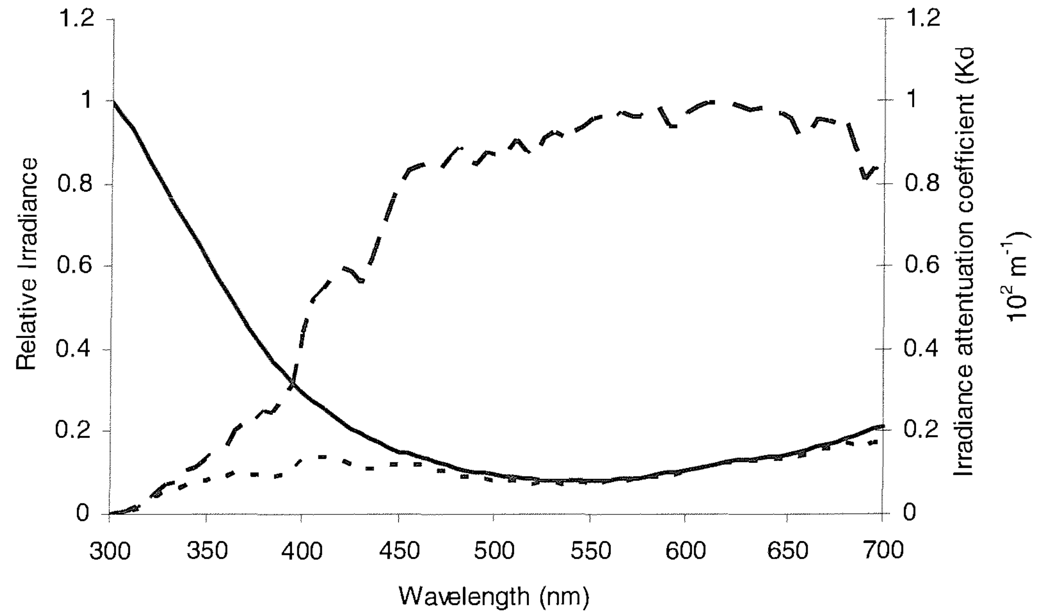
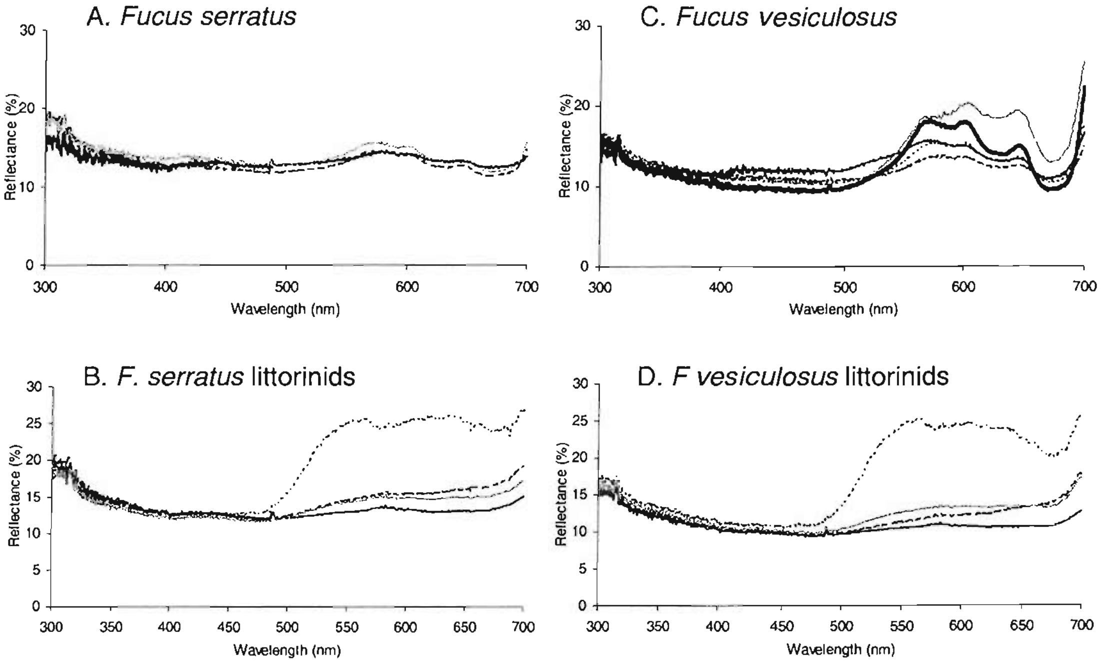


Figure 6.2.1: Spectra used for modelling turbid water conditions; D65 (large dash), attenuation coefficient in turbid coastal waters (black line), resultant irradiance in turbid water (small dash).

(6.3) Results

(6.3.1) *Reflectance Spectra*

After removal of unusable spectra, a total of 226 viable spectra (113 from littorinids and 113 from backgrounds) were collected on *Fucus serratus* and 242 on *F. vesiculosus* (121 from littorinids and 121 from backgrounds). Figure 6.3.1 shows the mean reflectance spectra of littorinid morphs and the algae structural parts collected on the two algal species.



A. *Fucus serratus*

C. *Fucus vesiculosus*

B. *F. serratus* littorinids

D. *F vesiculosus* littorinids

Figure 6.3.1: Mean reflectance spectra of algae and littorinids. (A) *Fucus serratus* structural parts (frond tip, grey; frond, large dash; stem, black). (B) Littorinid spectra of main morphs (yellow, small dash; olive, large dash; light brown, black; dark brown, grey). (C) *F. vesiculosus* structural parts (frond, small dash; frond tip, thin black; stem, large dash; gas vesicle, thick black; reproductive body, grey). (D) Littorinid spectra of main morphs (key as per B).

(6.3.2) *Morph Frequencies*

There was no significant difference between the morph ratios on the two algal species (χ^2=7.1, 5 *d.f.*, *P*=0.231; Table 6.3.1).

Table 6.3.1: Numbers of *L. obtusata* and *L. fabalis* morphs on the two algal species. Expected values in brackets.

Algal species	Dark Brown	Light Brown	Black	Striped	Olive	Yellow
F. serratus	27 (31.9)	27 (20.3)	5 (5.3)	6 (6.3)	17 (20.3)	31 (29)
F. vesiculosus	39 (34.1)	15 (21.7)	6 (5.7)	7 (6.7)	25 (21.7)	29 (31)

(6.3.3) *Multivariate Analysis of Spectra*

(6.3.3.1) *Fucus serratus*

Looking at the PCA coefficients (Fig. 6.3.2B), PC1 indicated variation along a yellow-blue dimension (positive values between 300-510nm and negative values between 510-700nm). In general, *Fucus serratus* backgrounds had positive PC1 scores which indicated low reflectance at low wavelengths (Fig. 6.3.2A). Yellow littorinids had mainly negative PC1 components (high reflectance between 510-700nm) and were also the most variable, whereas it was difficult to make any statements about the distribution patterns of the other morphs (Fig. 6.3.2A).

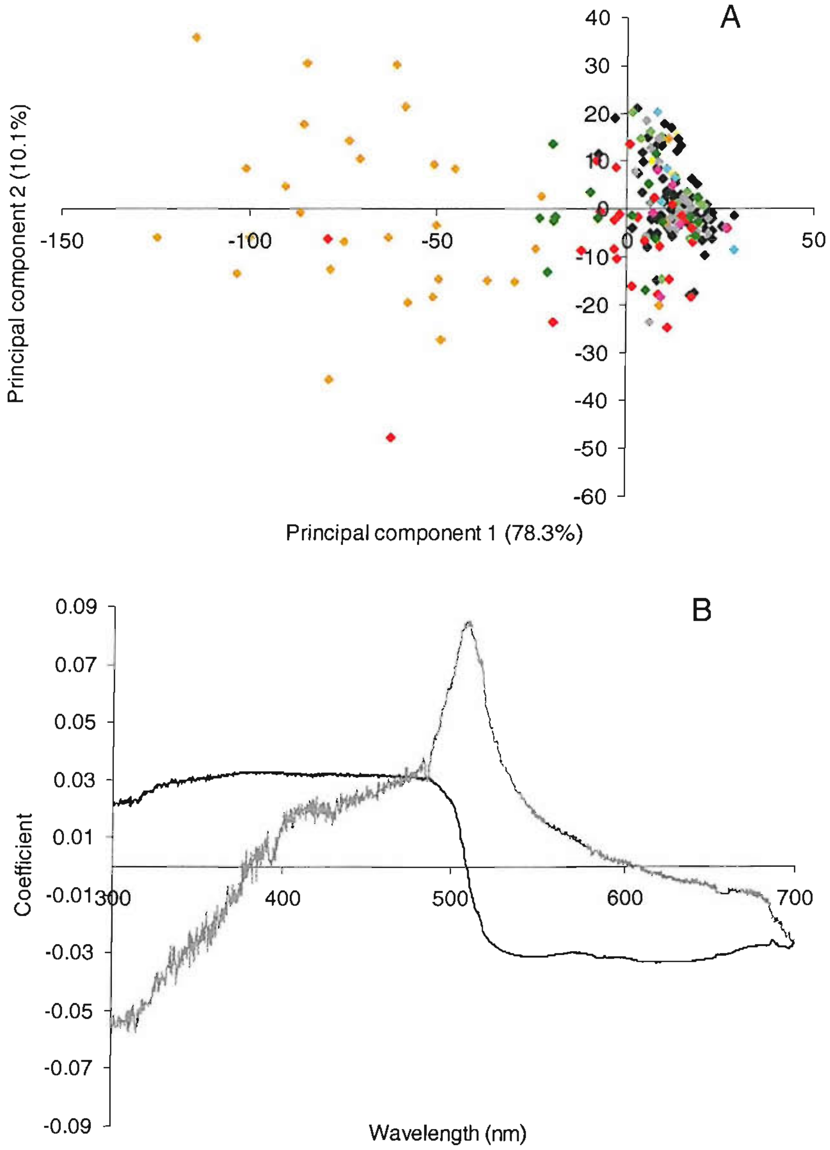


Figure 6.3.2: A, PCA score plot of littorinids (yellow, orange; dark brown, grey; light brown, red; black, turquoise; striped, pink; olive, dark green) and *Fucus serratus* backgrounds (lamina, black; stem, yellow; lamina tip, light green) using standardised spectra and B, coefficients of the first two principal variables (PC1, thick black line; PC2, grey line) from a PCA of all spectra shown in A.

(6.3.3.2) *Fucus vesiculosus*

Like *F. serratus, F. vesiculosus* backgrounds and littorinids were separable on the basis of PC2 (Fig. 6.3.3A). More specifically, the majority of the background spectra are spectrally different from each other due to variability from 490nm onwards. This can be seen from Figure 6.3.3B in which the PC2 coefficient loadings, which explained 8.55% of the variation, clearly showed the algal photo pigments were between 550-650nm. Given that nearly all littorinid spectra have negative PC2 components, they had high reflectance between 490-550nm and also 650-700nm. PC1 explains 75.6% of the variation between spectra and describes short to longer wavelength differences. Despite explaining a large part of the variation between spectra, PC1 components did not provide very much information about the spectra, apart from the fact that yellow was the most variable morph, potentially because yellow individuals are more likely to change colour with ageing and in particular darken with the growth of epiphytes (Smith, 1976) (Fig. 6.3.3A).

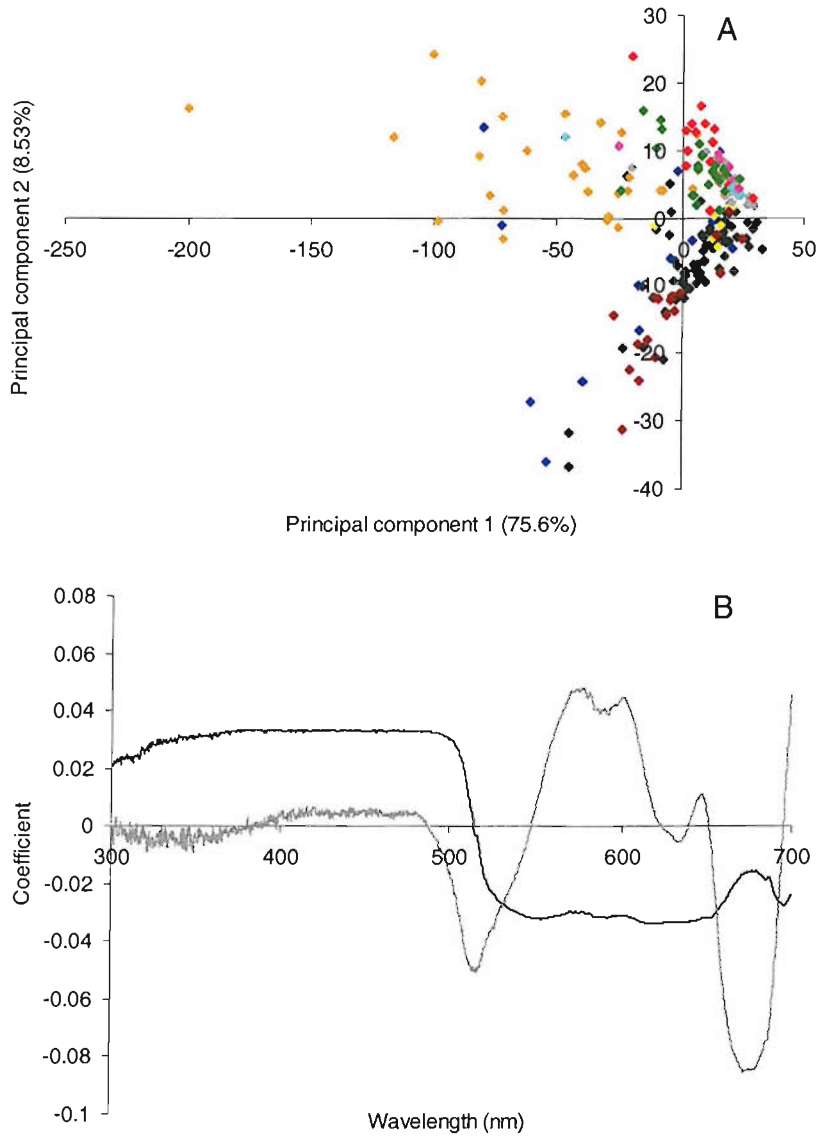


Figure 6.3.3: A, Principal variable score plot of littorinids (yellow, orange; dark brown, grey; light brown, red; black, turquoise; striped, pink; olive, dark green) and *Fucus vesiculosus* backgrounds (lamina, black; reproductive body, blue; stem, yellow; gas vesicle, brown) using standardised spectra and B, coefficients of the first two principal variables (PC1, thick black line; PC2, grey line) from a PCA of all spectra shown in A.

(6.3.3.3) *Fucus serratus* and *Fucus vesiculosus* backgrounds

Using algal spectra alone, the difference in the heterogeneity of spectra from *F. serratus* and *F. vesiculosus* was demonstrated using Principal Components Analysis (PCA) (Fig. 6.3.4A). Although this method of data presentation ignores the visual capabilities of the observer, it did demonstrate that *F. vesiculosus* is more heterogeneous in terms of PC1 which explains 65.1% of variation between spectra (two-sample Kolmogorov-Smirnov test; significant difference in distributions, $Z=3.94$, $P<0.001$). PC1 described variation from short to longer wavelengths and the variability beyond 650nm. *Fucus serratus* and *F. vesiculosus* were more similar in variability in terms of PC2 which explained 14.4% of the variation between spectra. Specifically there was no significant difference between the PC2 distributions of *F. serratus* and *F. vesiculosus* (*Z*=0.89, *P*=0.412). PC2 described low variation between spectra between 300-500nm and described very distinctive differences between spectra between 500-700nm (Fig. 6.3.4B).

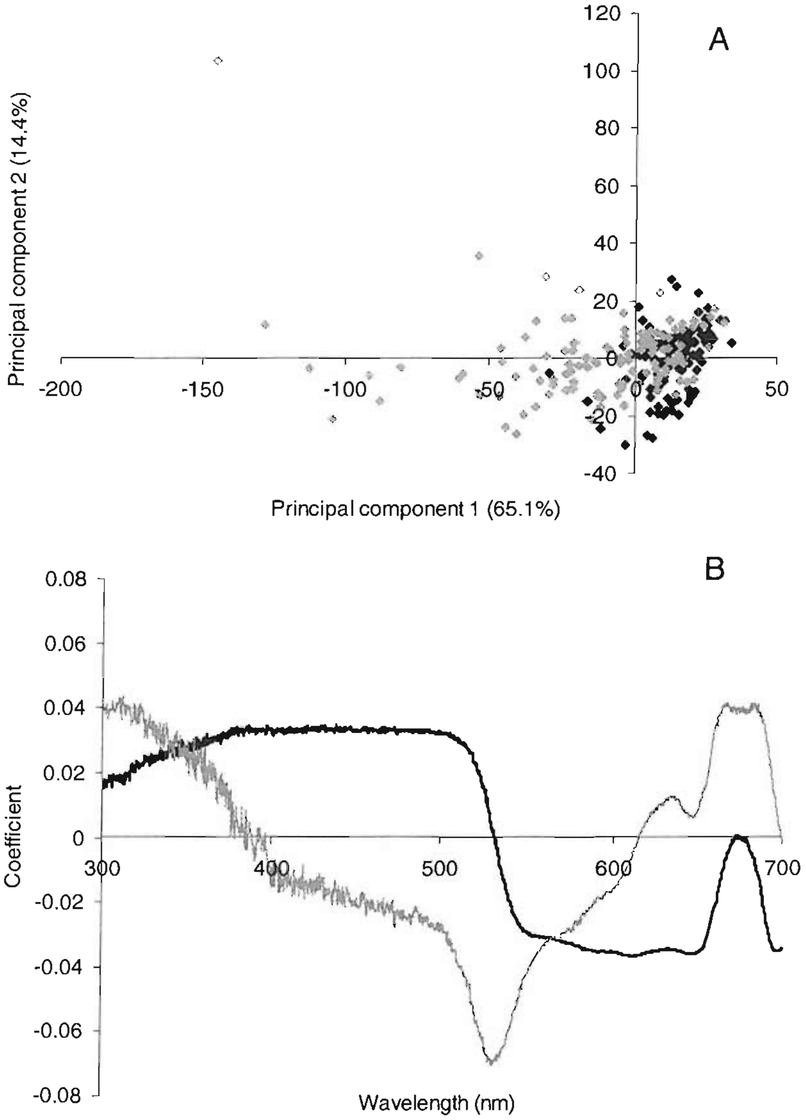


Figure 6.3.4: A, Principal variable score plot of *Fucus serratus* (black markers) and *F. vesiculosus* (grey markers) using standardised spectra and B, coefficients of the first two principal variables (PC1, thick black line; PC2, grey line) from a PCA of all spectra shown in A.

To summarise, on both *F. serratus* and *F. vesiculosus*, the yellow morphs were the most variable in spectral shape. Furthermore *F. vesiculosus* backgrounds were more spectrally heterogeneous than *F. serratus* backgrounds.

(6.3.4) *Visual Modelling of Reflectance Spectra*

There was no significant difference in the frequencies of yellow morphs in the 0ΔS class and ΔS classes greater than 1 between the two algal species for the bird (χ^2=1.618, 1 *d.f.* *P*=0.203) or the fish (χ^2=0.388, 1 *d.f.*, *P*=0.56). In the visual system of the crab the yellow morph was more conspicuous against the *F. serratus* background (χ^2=10.96, 1 *d.f.*, *P*=0.001). However, from Figure 6.3.5, it can be seen that there was a marked difference between the numbers of dark morphs in the 0ΔS class and ΔS classes greater than 1 between the two algal species (crab, χ^2=10.95, 1 *d.f.* *P*=0.001; fish, χ^2=7.41, 1 *d.f.* *P*=0.006; bird, χ^2=23.11, 1 *d.f.* *P*=0.001). Specifically, in all three visual systems there were more dark brown morphs which are cryptic (0ΔS) when viewed against *F. serratus* than against *F. vesiculosus.*

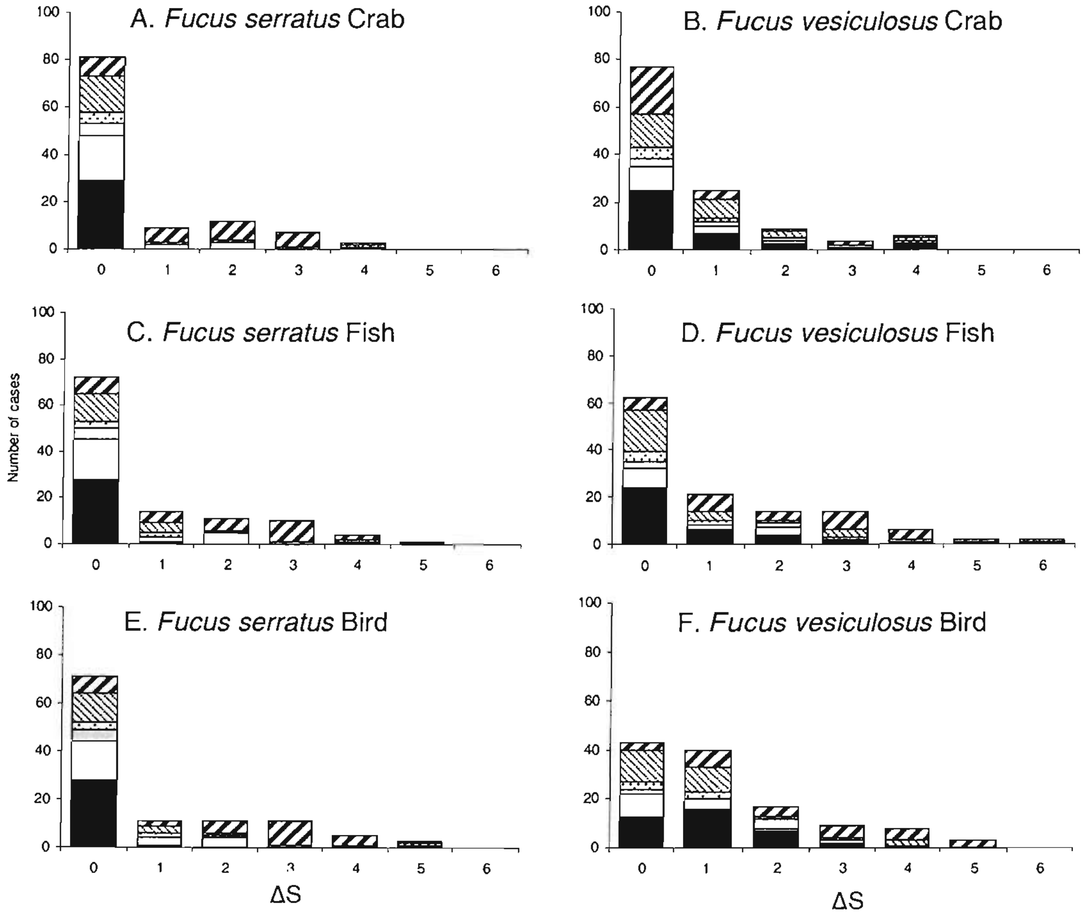


Figure 6.3.5: Number of cases of littorinids against their backgrounds in each ΔS class including division of each ΔS class into number of individuals of each morph: yellow,▨ ; olive,▧; striped,░ ; black,▩ ; light brown,□ ; dark brown, ■ . Left, *Fucus serratus*, right, *F. vesiculosus*; crab (A, B), fish (C, D), bird (E, F).

When looking at morphs on *F. vesiculosus* (Fig. 6.3.6) the median ΔS of each morph from its background showed that yellow was the most conspicuous morph except for the crab. There was more variability in median ΔS values between morphs on *F. vesiculosus* than on *F. serratus*, although the yellow morph was highly different from all other morphs on *F. serratus* (Fig. 6.3.6A, C, D). Also it is important to note that the dark brown morph matched the *F. serratus* background far better than on *F. vesiculosus* (Fig. 6.3.6). Indeed there were significant interactions between morph and algal species in the crab ($F_{(3, 200)}$ =7.6, P<0.001) and bird visual systems ($F_{(3, 200)}$ =4.17, P=0.007) but not in the fish visual system ($F_{(3, 200)}$ =0.68, P=0.564) (Table 6.3.2). Overall the littorinids were more different from the *F. vesiculosus* background than they were from *F. serratus*, irrespective of colour morph (crab, $F_{(1, 200)}$ =7.95, P=0.005; fish, $F_{(1, 200)}$ =8.86, P=0.003; bird, $F_{(1, 200)}$ =13.51, P<0.001) (Table 6.3.2).

Table 6.3.2: General Linear Model comparing Box-Cox transformed colour space distances of morphs on *Fucus serratus* (dark brown, N=30; light brown, N=25; olive, N=17; yellow, N=29) and *F. vesiculosus* (dark brown, N=39; light brown, N=15; olive, N=26; yellow, N=27). Significant results and interactions in *italics*.

Visual system	Source	*d.f.*	Sum of squares	*F*	*P*
Crab	Morph	1	0.15	7.95	*0.005*
	Algae	3	0.37	7.13	*<0.001*
	Morph x Algae	3	0.42	7.6	*<0.001*
	Error	200	3.67		
Fish	Morph	1	0.49	8.86	*0.003*
	Algae	3	3.62	19.27	*<0.001*
	Morph x Algae	3	0.13	0.68	0.564
	Error	200	12.6		
Bird	Morph	1	0.16	13.51	*<0.001*
	Algae	3	0.69	22.63	*<0.001*
	Morph x algae	3	0.13	4.17	*0.007*
	Error	200	2.08		

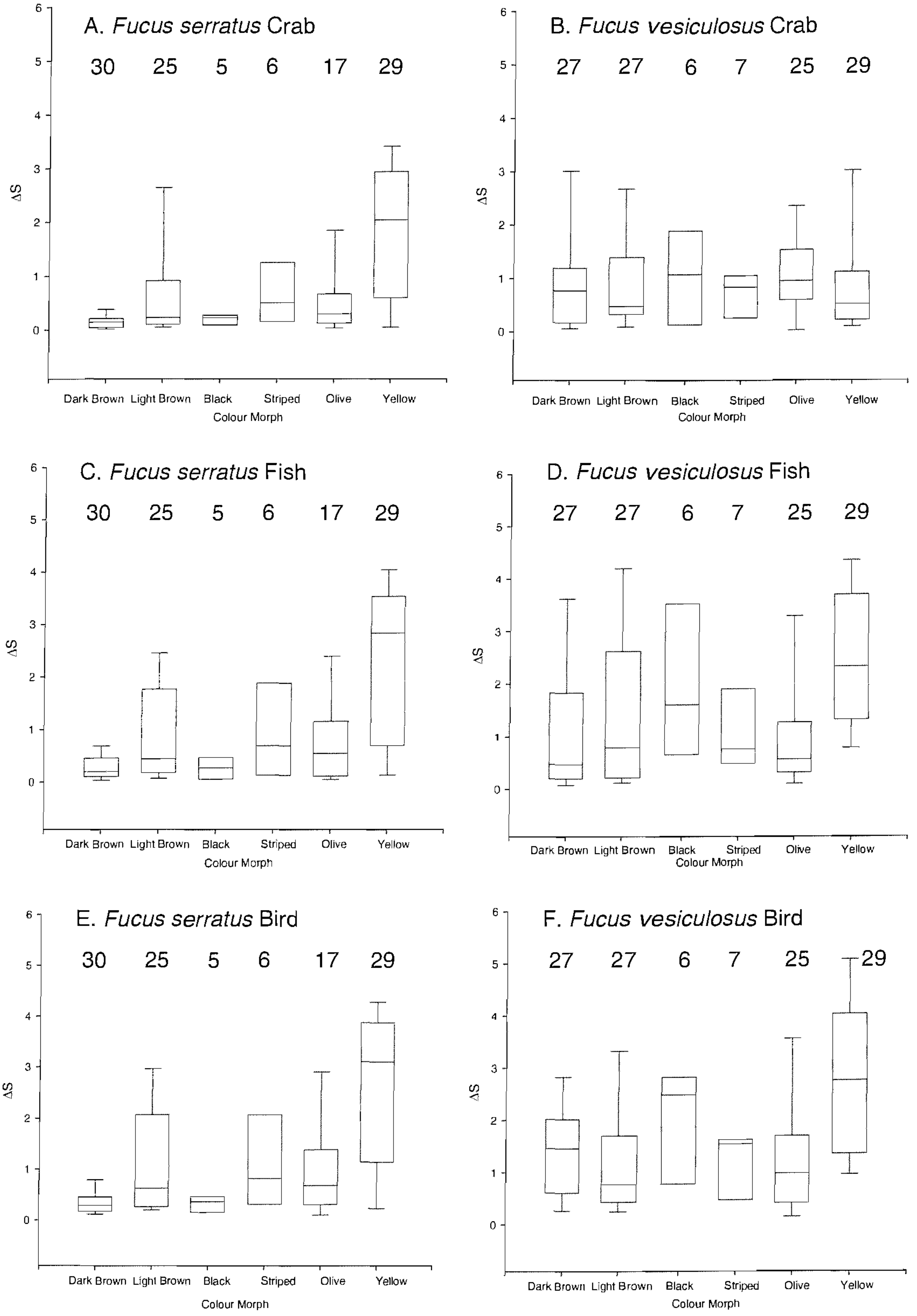


Figure 6.3.6: Median ΔS values and interquartile range for each colour morph. Left, *Fucus serratus*; right, *F. vesiculosus* crab (A, B), fish (C, D), bird (E, F). Number of replicates indicated above each morph, where there were less than 10 individuals error bars are omitted.

Overall these results show that littorinids on *F. vesiculosus* were more conspicuous than on *F. serratus*. Indeed there was a significant interaction between morph and algal species for the crab and the bird. This was probably because dark morphs (dark brown and black morphs) were most conspicuous against *F. vesiculosus* backgrounds and dark morphs were the most common. However, the yellow morph was consistently more conspicuous than other morphs on both algal backgrounds except when viewed by a crab on a *F. vesiculosus* background.

(6.3.5) *Algal Structural Parts*

The extent to which yellow and dark brown littorinids matched different structural components of *F. serratus* and *F. vesiculosus* in the bird visual system is shown in Figure 6.3.7. The most noticeable difference between the two of the most common morphs was that yellow littorinids were more different from all components of both algal species than dark brown littorinids.

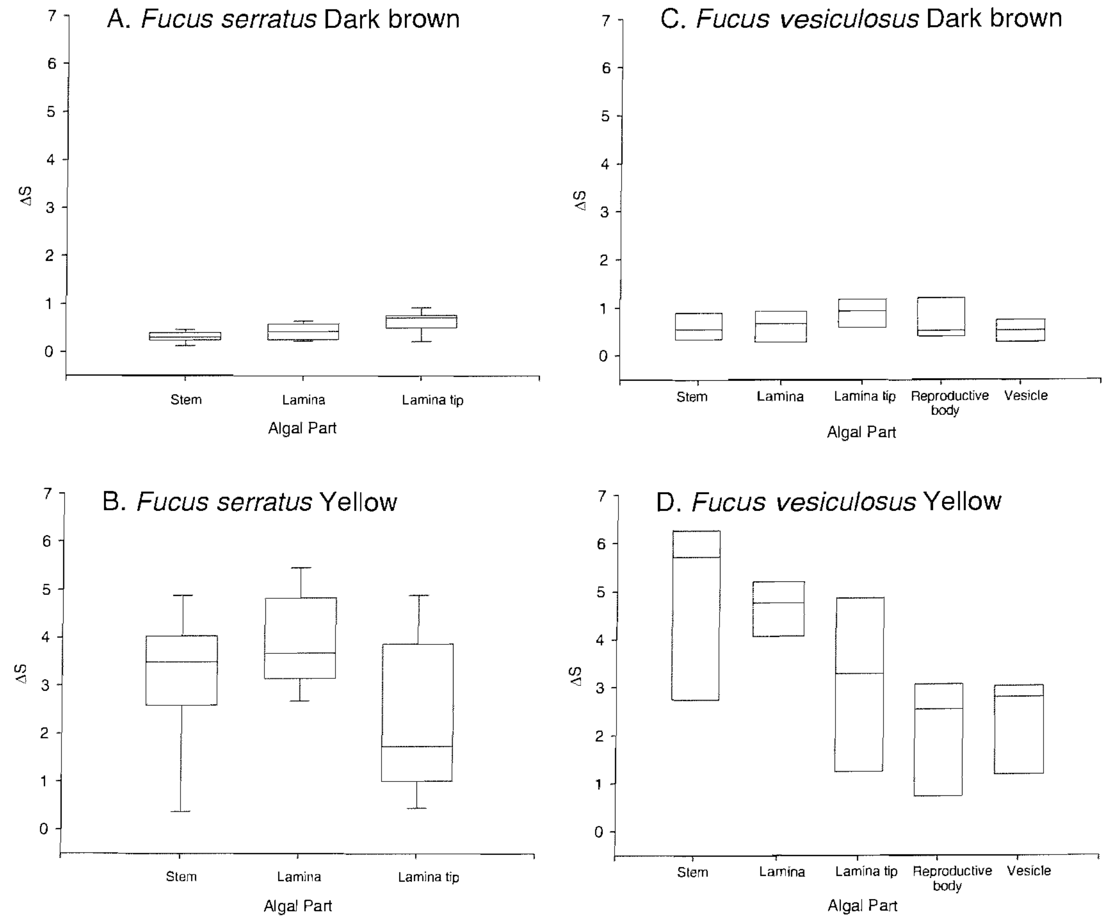


Figure 6.3.7: Median ΔS values and interquartile range for yellow and dark brown colour morphs on the different structural parts of *Fucus serratus* (N=10 paired comparisons per algal part) (A, B) and *F. vesiculosus* (N=6 paired comparisons per algal part) (C, D) in the visual system of a bird.

Using the bird visual model, for yellow and dark brown morphs on *F. serratus* there was a highly significant interaction between the morph and algal part (General Linear Model, Box-Cox transformed data: $F_{(2, 54)}=5.03$, $P=0.01$), a significant effect of colour morph ($F_{(1, 54)}=138.22$, $P<0.001$) and no significant effect of algal part ($F_{(2, 54)}=1.63$, $P=0.205$) (Table 6.3.3). Looking at Figure 6.3.7, the yellow morph was most conspicuous against *F. serratus* laminae whereas the dark brown morph was most cryptic against the stem. On *F. vesiculosus* there was a highly significant difference between the two morphs (General Linear Model, Box-Cox transformed data: $F_{(1, 50)}=71.31$, $P<0.001$), no effect of algal part ($F_{(4, 50)}=1.22$, $P=0.32$) and no interaction between morph and algal part ($F_{(4, 50)}=1.36$, $P=0.261$) (Table 6.3.3). In summary, different structural parts of *F. serratus* provide backgrounds against which yellow and dark brown littorinids are differentially cryptic.

Table 6.3.3: General Linear Model comparing bird visual model colour space distances of yellow and dark brown morphs on different structural parts of *F. serratus* (for both colour morphs N=10 for stem, lamina and lamina tip) and *F. vesiculosus* (for both colour morphs N=6 for stem, lamina, lamina tip, reproductive body and vesicle). Significant results and interactions in *italics*.

Algal species	Source	*d.f.*	Sum of squares	*F*	*P*
Fucus serratus	Morph	1	0.69	138.22	*<0.001*
	Algal part	2	0.02	1.63	0.205
	Morph x algal part	2	0.05	5.03	*0.010*
	Error	54	0.27		
Fucus vesiculosus	Morph	1	0.45	71.31	*<0.001*
	Algal part	4	0.03	1.22	0.316
	Morph x algal part	4	0.03	1.36	0.261
	Error	50	0.84		

(6.3.6) *Effect of turbidity*

There was a significant interaction between turbidity, morph type and algal species on colour space distance in the visual systems of the crab ($F_{(3, 200)}$ =7.12, P<0.001) but not in the fish visual system ($F_{(3, 200)}$ =1.07, P=0.362) or the bird visual system ($F_{(3, 200)}$ =0.7, P=0.552) (Table 6.3.4). Figure 6.3.8 shows the median ΔS values for turbid water conditions and should be compared to appropriate morphs in Figure 6.3.6 (clear water conditions). In clear water conditions the yellow morph was more cryptic on *F. vesiculosus* than in turbid water conditions. Furthermore there was a significant interaction between turbidity and algal species ($F_{(1, 200)}$ =109.23, P<0.001) and also turbidity and morph type ($F_{(3, 200)}$ =5.46, P=0.001) in the bird visual system. The light brown morph when on *F. serratus* in turbid water was more cryptic to a bird visual system than when in clear water.

Table 6.3.4: Repeated measures General Linear Model of Box-Cox transformed ΔS values from different littorinid morphs as they were found in the field on *F. serratus* (dark brown, N=30; light brown, N=25; olive, N=17; yellow, N=29) and *F. vesiculosus* (dark brown, N=39; light brown, N=15; olive, N=26; yellow, N=27) comparing clear and turbid water conditions. Significant results and interactions in *italics*.

Visual Model	Source		*d.f.*	Sum of Squares	*F*	*P*
Crab	Within-subjects	Turbidity	1	0.104	12.87	*<0.001*
		T x Algae	1	0.001	0.18	0.672
		T x Morph	3	0.063	7.86	*<0.001*
		T x M x A	3	0.057	7.12	*<0.001*
		Error	200	0.008		
Fish	Within-subjects	Turbidity	1	0.047	1.83	0.178
		T x Algae	1	0.001	0.006	0.936
		T x Morph	3	0.028	1.075	0.361
		T x M x A	3	0.028	1.072	0.362
		Error	200	0.026		
Bird	Within-subjects	Turbidity	1	0.001	0.43	0.514
		T x Algae	1	0.063	109.23	*<0.001*
		T x Morph	3	0.003	5.46	*0.001*
		T x M x A	3	0.001	0.7	0.552
		Error	200	0.001	5.84	

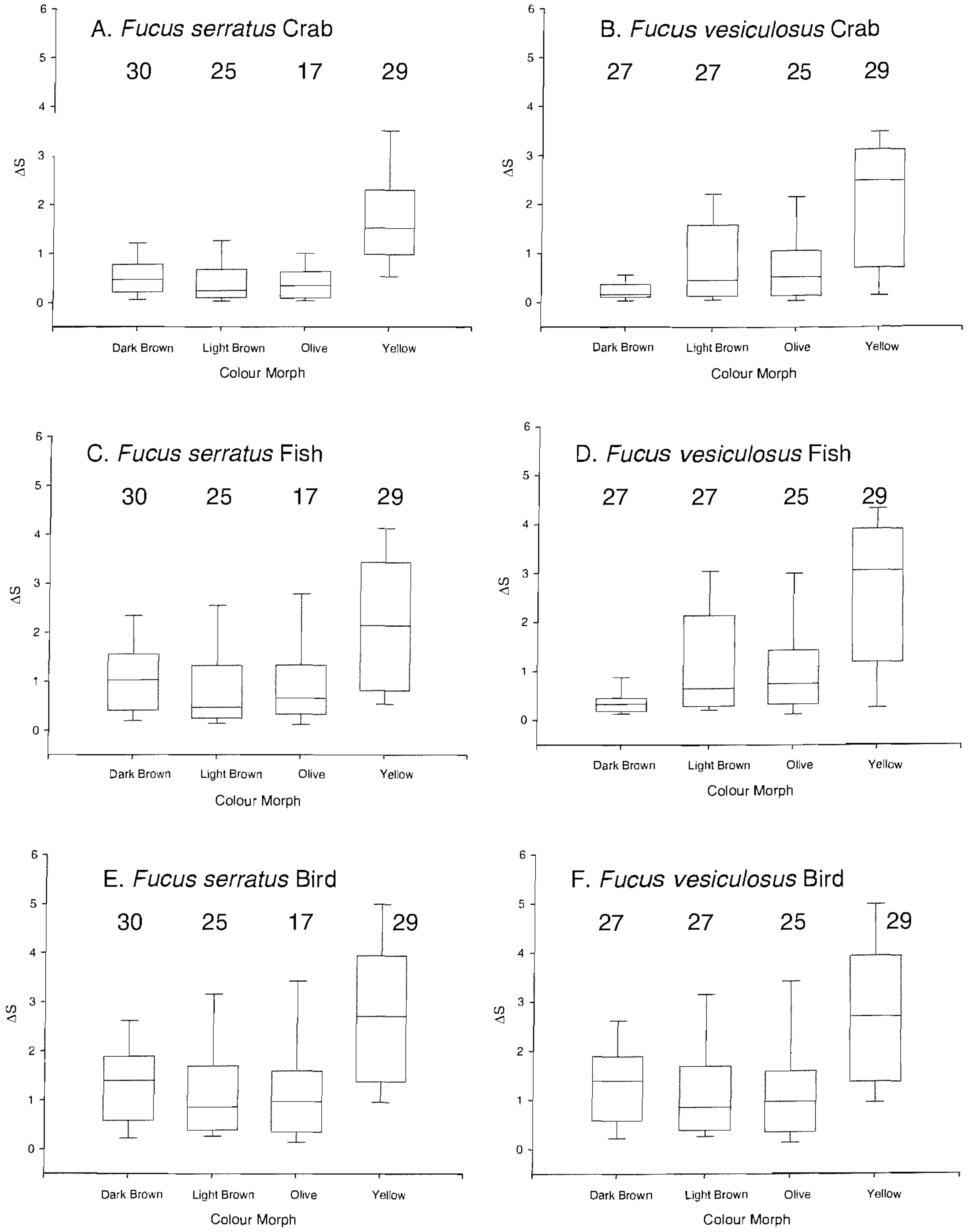


Figure 6.3.8: Median ΔS values and interquartile range for each of the principal colour morphs under turbid water conditions. Left, *Fucus serratus*; right, *F. vesiculosus* crab (A, B), fish (C, D), bird (E, F). Number of replicates indicated above each morph.

(6.3.7) *Differences in Predator Visual Ability*

In terms of differences between the visual models, the crab visual model produced the lowest ΔS values; in other words the lowest discrimination of spectra pairs across all morphs. The bird and the fish visual models produced significantly different ΔS values on *F. serratus* (paired t-test, Box-Cox transformed; $t = 10.59$, $P < 0.001$) however there was a positive correlation between the outputs of the two visual models ($R^2 = 0.986$, Fig.6.3.9A). There were 80.4% of cases where the bird was better than the fish at discriminating the spectra pairs on *F. serratus*. Therefore the bird was best at discriminating littorinids from their backgrounds when viewed against *F. serratus* (Fig. 6.3.9A). There was no relationship between the ability of the fish and bird visual systems in terms of ΔS values of littorinids on *F. vesiculosus* ($R^2 = 0.117$, paired t-test, Box-Cox transformed; $t = 1.71$, $P = 0.09$; Fig. 6.3.9B).

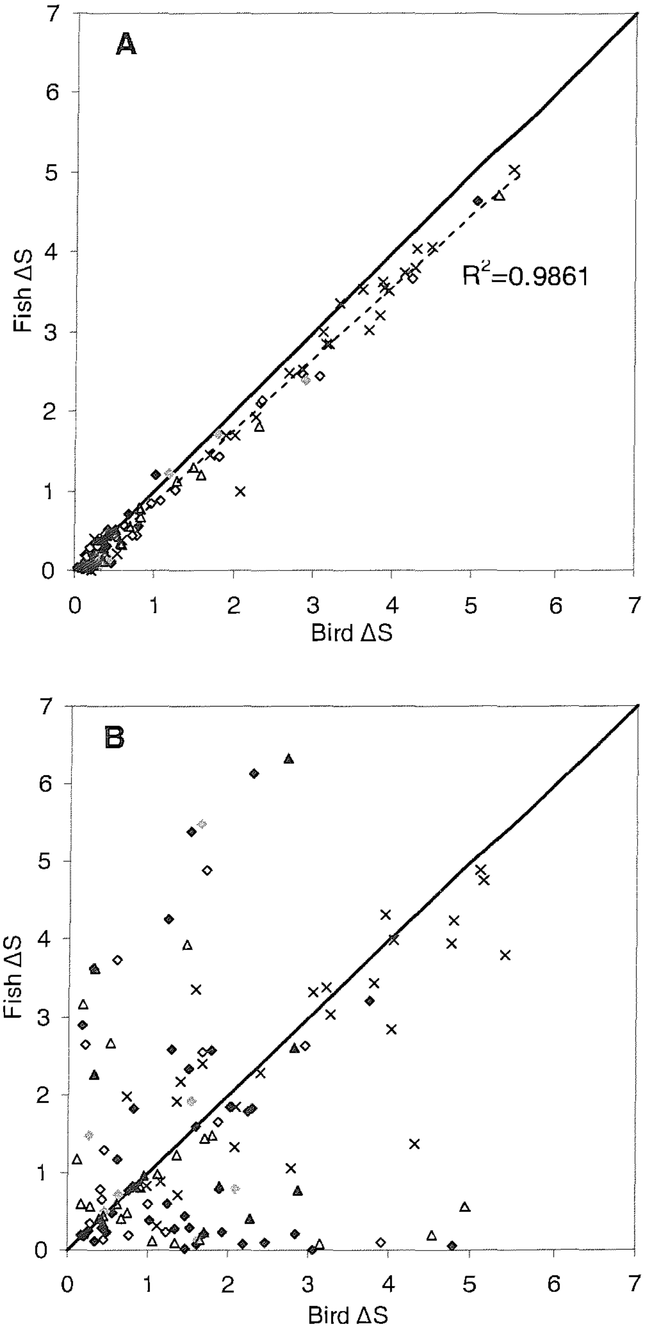


Figure 6.3.9: Differences in the discriminability of littorinid and background spectra pairs when viewed by a bird or a fish on *Fucus serratus* (A) and *Fucus vesiculosus* (B). Solid line is the expectation if no difference between the two systems. Colour morphs: Dark Brown, filled diamonds; Light Brown, empty diamonds; Black, filled triangles; Striped, grey diamonds; Olive, empty triangles; Yellow, crosses. Dashed lines show the regression slopes of the relationships between the signals received by the bird and the fish visual systems when looking at littorinids.

(6.4) Discussion

Fucus vesiculosus was the most heterogeneous background in terms of colour. Despite high background heterogeneity, *F. vesiculosus* did not support a more diverse polymorphism than *F. serratus.* Furthermore littorinids matched the *F. serratus* background better than *F. vesiculosus.* Therefore this study does not support the hypothesis that morph diversity is proportional to background pattern complexity in terms of colour (Endler, 1978). However, there was evidence to suggest that in the visual system of a crab, the yellow morph was more cryptic against *F. vesiculosus.* Additionally in all visual systems, the dark brown morph was more conspicuous on *F. vesiculosus* than *F. serratus.* Potentially both the yellow and brown morphs could at certain times gain crypsis from certain background elements. Furthermore the positioning of an individual may be dependent on its requirement to feed (Van Dongen, 1955), potentially giving rise to pleiotropic linkage between habitat requirements and shell colour. However, there is no evidence of differential feeding requirements between morphs of littorinids, although significant differences between morphs in terms of their positioning on algae have been observed (Ekendahl, 1994).

Algal species related differences in background matching

Polymorphism in snails is often associated with background heterogeneity (Endler, 1978; Cook, 1986a). This association is strengthened if frequency-dependent selection is occurring such that the predator removes disproportionately more of the common morph (Hughes and Mather, 1986). Direct, quantifiable relationships between background heterogeneity and polymorphism have seldom been confirmed in natural systems particularly since the background may simply serve to lower the signal:noise ratio and impede detection by a visually hunting predator rather than absolute masquerade of background elements (Cook, 1986a). The yellow morph of *L. obtusata*, however, reaches its highest frequency in habitats dominated by *Ascophyllum nodosum* which has more yellow fronds (Wilbur and Steneck, 1999). It is clear that *F. serratus* and *F. vesiculosus* do indeed differ in background heterogeneity, both structurally and chromatically. However the *L. obtusata* and *L. fabalis* morphs were more cryptic against *F. serratus,* the more uniform background type. *Littorina obtusata* has in fact been found to be most polymorphic on *F. serratus* as opposed to *Ascophyllum nodosum* which is more structurally diverse (Smith, 1976; Wilbur and Steneck, 1999).

In *Cepaea nemoralis* a quantifiable relationship between background elements and morph fitness has not often been identified (*e.g.* Cain and Currey, 1963). Specifically brown, pink and fused banded morphs of *C. nemoralis* have high fitness in woodland habitats, in which case there is no evidence to suggest that any morph should be at an advantage (Clarke, 1960; Jones *et al.*, 1977). Also changes in habitat have resulted in changes in the structure of the polymorphism in *C. nemoralis,* although this correlation was observed at the habitat level rather than by quantification of background coloration (Clarke and Murray, 1962). Yellow morphs of *C. nemoralis* tend to be found in grassland and disturbed areas (Greenwood, 1974). Woodland and grassland habitats differ from each other over large distances (from metres to kilometres) whereas both yellow and brown algal dwelling *L. obtusata* and *L. fabalis* occur together in the same habitat (Smith, 1976). Within the same habitat there is evidence to suggest that yellow morphs are more often found on the surface of the algal canopy (Sacchi, 1974) and also that brown morphs prefer the stem and yellow morphs prefer the lamina of fucoids (Reimchen, 1979; Ekendahl, 1994). However, this habitat selection in littorinids need not have evolved through predation pressure directly. Yellow morphs of the mangrove-dwelling genus *Littoraria* remain cooler in sunlight than brown morphs (Reid, 1987) and therefore habitat selection (and the polymorphism) could have evolved in response to temperature (Cook and Freeman, 1986; Cook and Garbett, 1992; Parsonage and Hughes, 2002).

It is not only the specific morphological structures of the algae that could be important in crypsis for the littorinid morphs but also the general appearance of the algae (Ekendahl, 1994). The general appearance of the algae includes coloration, condition, the thickness of the canopy and its interaction with light (Riemchen, 1979; Cummings and Partridge, 2001). Indeed there is evidence to suggest that algal canopy thickness which is dependent on exposure influences the ratios of morphs of algal dwelling littorinids (Reimchen, 1979). Depending on the thickness of the canopy surrounding the position where a visual signal is being received, the light is altered primarily by transmission through the algae. The method by which background spectra were collected in the field was somewhat artificial in the sense that it could not capture reflectance of backgrounds which are not flat against the substratum and thus it only investigated the 2-dimensional heterogeneity of the algal canopy. It is therefore to be expected that there is a variety of backgrounds which are not accounted for, including the reflectance spectra of algae when light is transmitted through the laminae. A more structurally heterogeneous algal species such as *F. vesiculosus* would have a greater variety of textures and thicknesses which could potentially provide crypsis for the conspicuous yellow morph upon interaction with sunlight.

Despite the differences between the two algal species, the determinant of genetic diversity and polymorphism on a particular shore may in fact be a function of the diversity of algal species which is dependent on exposure and topography of the shore (Smith, 1976). On very sheltered shores (Ballantine grade 6) with *F. serratus, F. spiralis, F. vesiculosus* and *A. nodosum* there should be the highest diversity of littorinid morphs (Sacchi, 1969). Bembridge is a moderately exposed shore, grade 4-5 (Ballantine, 1961) and should therefore support a relatively diverse population of both *L. obtusata* and *L. fabalis.* This however was not the issue under investigation and it was more important to focus on the signals available to predators from the different morphs on the two algal species.

Morph related differences in background matching

Littorina obtusata and *L. fabalis* have long been studied as effectively dimorphic populations given that the brown and yellow morphs are the most common and differ the most in coloration (Reimchen, 1979, 1989; Ekendahl, 1994, 1995). Indeed this study shows that the most striking differences in background matching were between brown and yellow morphs. Overall it did appear that the brown morph was more often cryptic against the algal background irrespective of algal species. The yellow morph should, if it is selected by visually-orientated predators, be at a disadvantage. So why does the yellow morph occur at such high frequencies?

There is no evidence to suggest that yellow morphs are avoided by crab predators from laboratory (Tucker, 1988) and field studies (Reimchen, 1974) or indeed by fish (Reimchen, 1979) or birds (Alerstam *et al.,* 1992; Johannesson and Ekendahl, 2002). In fact yellow is commonly the colour used in selection experiments with artificial pastry baits testing apostatic selection in terrestrial birds (Willis *et al.,* 1980; Greenwood *et al.,* 1981). Birds are very conservative in their visual capabilities (Ödeen and Håstad, 2003) and terrestrial birds, as well as shore birds, are known to forage on rocky shores (Feare, 1967; Pettitt, 1975). Therefore there is no reason to suppose that yellow littorinids should be avoided by bird predators.

Yellow individuals of the *Littorina scabra* group occur at low frequencies but are probably at a selective advantage due to differential selection by predators (Hughes and Mather, 1986). The yellow morph gains this selective advantage in mangrove habitats by appearing cryptic in the foliage whereas the brown morph is restricted to the bark (Reid, 1987; Cook, 1990; Cook and Garbett, 1992). This study has shown a similar pattern in that brown morphs were more cryptic against the stem and the yellow morphs were more conspicuous against the lamina. It was hypothesised in this work that the more heterogeneous *F. vesiculosus* might provide background elements than *F. serratus* against which more morphs are cryptic. Despite the absence of convincing evidence for this relationship it is evident that the brown morph is more conspicuous against *F. vesiculosus*. Thus there is at least evidence to show that at certain times the usually cryptic brown morphs can, like the yellow morph, appear conspicuous. Consequently the visual heterogeneity of the algal canopy could go some way to explaining the existence of quite different morphs of the same species.

With regards to the two species, *Littorina obtusata* and *L. fabalis*, juvenile *L. obtusata* are much more susceptible to predation by *C. maenas* than adult *L. obtusata* or adult *L. fabalis* (Reimchen, 1982). The distribution patterns of the two species on well zoned shores reflect the differential predation pressures such that *L. fabalis* persists lower on the shore and *L. obtusata* is found mainly higher on the shore (Williams, 1992). This pattern is also a reflection of the feeding preferences of the two species: *L. obtusata* prefers to feed on *Ascophyllum nodosum* where available, whereas *L. fabalis* preferentially feeds on *F. serratus* (Van Dongen, 1955; Watson and Norton, 1987; Williams, 1990). Additionally the two species exhibit different levels of desiccation tolerance, *L. obtusata* tolerating more extreme conditions (Williams, 1990). Therefore *L. obtusata* is more commonly dormant when the algae is not covered by the tide whereas *L. fabalis* tends to crawl about on the surface. Although the two sibling species are known to be quite different in certain aspects of their ecology, a shore such as Bembridge where there is a lack of discrete zonation would provide less opportunities to observe behavioural traits of each species in their strictest sense and there would be a significant overlap in their distributions (Watson and Norton, 1987; Little and Williams, 1989). On moderately exposed shores there is convergence in the shell form, behaviour and colour morphs of *L. obtusata* and *L. fabalis*. However in the terrestrial snails in the genus *Cepaea* there is evidence to suggest that mixed colonies of the sibling species *C. nemoralis* and *C. hortensis* diverge more from each other as compared to separate colonies (Clarke, 1962b). This may be because visual predators form search images for the common morph of one species and selected apostatically which tends to have a different effect on the two species (Clarke, 1962b). It is known that the thrush (*Turdus philomelos*), the main visual predator of *Cepaea nemoralis* and *C. hortensis*, does not discriminate between the two sibling species (Bantock and Bayley, 1973). Therefore it is likely that avian predators would not be able to discriminate between *L. fabalis* and *L. obtusata* by sight, assuming that their shell morphology is appropriate for consumption

Predator related differences in background matching

Overall the crab was poor at discriminating littorinid and background spectra pairs. The fish was better but the bird was the most able since it possesses tetrachromatic vision. There were, however, departures form this expectation which are now discussed.

Interestingly the fish and the bird were very similar in their ability to discriminate littorinids from their backgrounds on *F. vesiculosus*. Yet on *F. serratus* the bird was better. Therefore littorinids on *F. vesiculosus* might be as conspicuous to fish as they are to birds. This statement is accompanied by the caveat that fish would be preying on littorinids at when the algal canopy is submerged. When the tide is in the algal canopy is no longer effectively a 2-dimensional habitat and the background would be far more heterogeneous as light is transmitted through the algae and also reflected from the various surfaces of the algae. Furthermore weather conditions and the state of the sea would alter the turbidity of the water and giving rise to temporal and geographical variations in turbidity dependent the substrate type and topography of the shore (Hovárth and Varjú, 1995).

This work demonstrated that the crab has the ability to discriminate littorinids of all morphs despite the relatively low chromatic distances found for all comparisons (<2ΔS). The green shore crab consistently removes the greatest number of littorinids from the rocky shore compared to birds which tend to resort to feeding on littorinids only when their preferred food source is depleted (Pettitt, 1975). Whether or not crabs forage in a selective manner based on colour is questionable (Elner and Hughes, 1978; Tucker, 1988; Ekendahl, 1998) despite evidence for colour vision in this species (Martin and Mote, 1982).

Turbidity was found to have no effect on the ability of fish to discriminate littorinids of any morph. The fish visual model used was that of the blenny, *Lipophrys pholis* (Loew and Lythgoe, 1978; Partridge, 1986). *Salaria pavo,* a blenny which lives in predominately coastal habitats, benefits from short-wavelength cut off property of the lens (White *et al.,* 2004). This lens filtering effect was incorporated into the fish visual model. Short-wavelength (UV) filters are common in shallow water fish (Siebeck and Marshall, 2000; Cummings and Partridge, 2001). These filters improve vision by cutting out veiling short-wavelength light from small organic and inorganic particles (Lythgoe, 1988; Partridge, 1990). In turbid conditions the UV filter may allow the fish to maintain its ability to detect objects.

If an organism is subjected to visual selection by a variety of visual systems it is unlikely that it will be maximally cryptic or conspicuous at any one time unless the contrast between it and its background is zero under all lighting conditions. This situation would not occur in natural systems because multiple visual systems, even if monochromatic, constitute a form of colour vision (Johnsen, 2002). Additionally, polymorphic coloration is not only prominent in littorinids but also in other intertidal organisms (for example, juvenile crabs *Cancer irroratus* Palma and Steneck, 2001; bivalves, *Donacilla cornea,* Whiteley *et al.,* 1997; isopods, *Idotea baltica,* Merilaita, 1998; chitons, *Ischnochiton striolatus*, Rodrigues Gonçalves and Silva Absalão, 2005). Hence in a habitat where multiple marine and terrestrial predators forage it is beneficial to exploit the diversity of backgrounds and incident light conditions to gain crypticity in some places, some of the time. Therefore it is likely that the system controlling the polymorphism of the snails is dynamic both spatially and temporally. This was also the conclusion of a model simulating the effects of multiple predators (Endler and Mappes, 2004).

Importantly, the data presented here only represent chromatic differences between littorinids and their 2-dimensional backgrounds and do not take into account the behaviour of light through the algal canopy or the shape or pattern of the objects in question. Furthermore this work has been concerned with morphs present in two sibling species and cannot explain the evolution and maintenance of the polymorphism in these two species. Despite this, interpretation of visual signals within the visual systems for which they were designed is an important contribution to understanding the polymorphism in *Littorina* species.

Overall yellow is the most conspicuous morph against the algal background but dark morphs can, despite being generally cryptic, sometimes appear conspicuous. Although it is clear from observation in the field that yellow littorinids are the most conspicuous, quantification by visual modelling is a useful exercise to explore the visual environment through the eyes of non-human observers particularly since these littorinids are at risk from predation by species with visual abilities which differ from that of a human.

Chapter 7

General Discussion

(7.1) Introduction

The preceding chapters of this thesis provide a detailed and comprehensive discussion of the findings in relation to other studies. Therefore this chapter serves to bring together these findings with respect to the patterns observed and their ecological importance, the problems with technique used and future directions. This thesis has for the first time examined the visual properties of littorinid polymorphisms in two intertidal habitats by the quantitative, objective measurement of colour and quantification of the signals available to predators.

In Part I, Chapter 1 highlighted the lack of objective classification of animal coloration and hence Chapter 2 provided a comprehensive review of the techniques available, both historically and currently, to measure colour using spectroradiometry and to take account of the visual systems of predators. In short, in light of the current rapid developments in colour measurement and knowledge about visual systems, this study aimed to:

1. Ascertain whether spectroradiometry was a suitable technique for studying the adaptive significance of polymorphism in intertidal littorinids.
2. Examine the extent of background matching in polymorphic species of littorinids at different scales of background heterogeneity.

To accomplish these aims, in Part II littorinid polymorphism was investigated at different scales of background heterogeneity in different predator visual systems in terms of the geology of the shore (Chapter 3), the topography and exposure of the shore (Chapter 4), heterogeneity caused by lichen growth (Chapter 5), and in Part III heterogeneity of different algal species (Chapter 6). A summary of the results and the wider ecological significance of these investigations will be discussed in the following sections.

(7.2) Levels of Heterogeneity and Predator Types

Correlations between background elements and morphs are fundamental to understanding the adaptive significance of polymorphisms. Background elements in a habitat are often formed because of physical and biological conditions which can act at the macro- and microhabitat levels making backgrounds, against which a prey item would be observed, more or less heterogeneous dependent on the range of the prey item.

Large scale habitat differences were tested in Chapter 3 by examining the effect of geology on the polymorphism in *L. saxatilis*. The littorinids which were recorded at the two shores of similar geology with low background heterogeneity were more similar to each other in terms of background matching and also matched their backgrounds to a lesser extent than the polymorphism at Kimmeridge which was the more visually heterogeneous shore. Given that the two geologically similar shores were both exposed boulder shores, whereas Kimmeridge is a moderately sheltered rocky ledge, it is perhaps unsurprising that the levels of background matching are in accordance with the physical factors affecting their ecology. Between shore differences and the importance of predators in a well studied system in Sweden was the focus of Chapter 4 where exposure dictated by geology and topography was the factor under investigation. Unlike Chapter 3, the Swedish ecotypes are heavily affected by selection for morphological characteristics over relatively short distances to the extent that morphological intermediates are present in hybrid zones at interfaces of the two exposure types (Janson, 1982; Janson and Sundberg, 1983). Chapter 3 sought to find spectral differences between populations dependent on geology reinforced by exposure, whereas Chapter 4 focused more on the effect of certain predators known to be foraging at the two exposure types. Overall the exposed shore ecotype was more cryptic and indeed the supposedly conspicuous exposed morphs (red, white and banded) were more cryptic against the exposed shore backgrounds. The fact that visually-orientated predators are more important on exposed shores could explain this difference since no quantitative evidence of differences in background heterogeneity was found.

Chapter 5 addressed background heterogeneity caused by lichen growth. Background heterogeneity within each habitat patch (bare and lichen) was easily quantifiable but showed that the uniform lichen background was better for littorinids to appear cryptic against in the crab visual system. Although I treated these two background types separately, within shore heterogeneity may be more important in maintaining the polymorphism. The experiment could therefore have been improved by studying red sandstone shores with and without *Verrucaria maura* present. Indeed, *V. maura* is common on exposed shores in Sweden and forms a discrete band (Johannesson, 1989), yet on sheltered shores it is less common and patchily distributed. Clearly a suitable way to test whether *V. maura* presents a background against which littorinids are more detectable, and whether conspicuousness is dependent on morph, would be to conduct field based selection experiments. These could include tethering live littorinids or sticking empty shells to rocks or cage experiments to exclude predators (Ekendahl, 1998; Ekendahl and Johannesson, 2002). To judge whether a particular morph has been preyed upon due to its conspicuousness, spectroradiometry and visual modelling could be used to quantify discriminability. Additionally these techniques could be applied to geological transition areas such as the problems tackled by Heller (1975), Raffaelli (1979) and Byers (1990) rather than to shores with uniform geology (Chapter 3) or heterogeneity within shores caused by lichen encrustations (Chapter 5). It is clear that there is great potential for further application of the techniques described here, however it is important that background heterogeneity caused by, geology, exposure and patchy lichen growth have been investigated and quantified with consideration to predator vision.

Realistically birds may be the only predator that could possibly exert visual selection on littorinids given that crab colour space distances were generally low for all *L. saxatilis* morphs. A consistent mode of potential visual selection by birds was not observed across the three habitats in which *L. saxatilis* occurred. Specifically a correlation between the common morph and crypsis was not seen, therefore selection against conspicuousness may not be important. However there was evidence in Chapter 3 to suggest that more heterogeneous backgrounds allowed better background matching which is in agreement with Endler's work on background matching theory (Endler, 1978). It may be the case, however, that the morph classifications used in this thesis (Pettitt, 1973a) are not relevant to visually-orientated predators given that in all visual models there was the opportunity for all morphs to be indistinguishable from the background. Indeed it is possible that in littorinids absolute background matching (a scenario close to masquerade) would not be beneficial on the rocky shore. Although littorinids travel small distances whilst foraging, their mobility means that they may be seen against a variety of backgrounds especially since rocky shores are variable both temporally and spatially. Therefore the morphs may be a 'compromised' coloration whereby no particular morph is a perfect colour match to any particular background colour element. This theory has been shown using great tits (*Parus major*) and three types of artificial prey on two backgrounds (Merilaita *et al.*, 2001). Specifically, the prey item that was an intermediate between the two background types was fittest overall (Merilaita *et al.*, 2001). In which case, trying to quantify coloration and hence background matching may be futile.

As an ecological system, the high shore splash zone proved to be a suitable system for the use of spectroradiometric equipment. However, the results shown in this thesis do not provide a clear explanation for the polymorphism in *Littorina saxatilis.* This in part due to the fact that there is high variability within each system which may mask the fine scale background matching patterns predicted. The aim of this thesis was, however, not to determine a universal model for polymorphism in littorinids. Indeed in accordance with other polymorphic molluscs, Jones *et al.,* (1977) published an important review paper entitled '*Cepaea*: A problem with too many solutions' to indicate the complexity of factors involved.

The intertidal splash zone is markedly less structurally complex than the habitats where *Cepaea* species are found. Therefore the differences in visual ecology may be more subtle on the rocky shore. Furthermore other studies relating mollusc polymorphism to background elements have dealt with heterogeneity of vegetation (Heller, 1981; Cowie, 1990; Chang and Emlen, 1993). Indeed examples exist outside of molluscs where vegetation has influenced the morphs found and this is due to the structural environment created. For example, morphs of the stick insect, *Timema cristinae* closely resemble the colour of their food plants and in field selection experiments morph fitness was highly correlated with positioning within the habitat (Sandoval, 1994). Additionally grasshoppers are known to find escape from predation by positioning themselves against certain background elements such as leaves, rocks or stones (Eterovick *et al.,* 1997). Therefore the algal habitat considered in Part III was a more challenging background to study but was similar to the focus of many other investigations of animal coloration.

Background heterogeneity differences between algal species were the focus of Chapter 6. This work also investigated the colour matching between different structural parts of two algal species and the common morphs found at the shore. Furthermore the effect of turbidity on the discriminability of littorinids was investigated. Although the two sibling species were considered together, this work focused on the morphs and the extent to which they matched the various algal backgrounds. Conclusions based on selection and evolution in either of the species could not be made but patterns between the two algal backgrounds could be observed. Interestingly, the results of Chapter 6 were in accordance with those in Chapter 5, whereby the more uniform background correlated with a higher degree of crypsis in the population. It is of course possible that the dominant darker morphs in both systems made the overall matching greater in a particular habitat merely because that particular morph was sampled more frequently. Therefore the shore related background matching results throughout this thesis could indeed be influenced by the most common morph. Perhaps morph frequency weighting could have been applied to limit the effects of unbalanced numbers of each morph. In all chapters investigating patterns of matching in the field, consideration was given to specific morphs and significant differences were observed between them.

It is important to note that the significant differences between the human vision classified morphs determined by conventional statistics may not be biologically important to a predator making a decision (Bennett *et al.,* 1994). In this thesis low chromatic distances were representative of low discriminability yet psychophysical testing of visual ability is required to determine whether or not an organism can discriminate an object from its background in 'just noticeable differences' (jnds). Unfortunately jnds could not be used because of problems in parameterising the visual model used (J. Hadfield, *pers. comm.*). Therefore I have attempted to discuss discriminability in a relative sense using colour space distances (*i.e.* one spectra pair is more conspicuous than another).

Other studies which have looked at the fucoid canopy include investigations into isopod coloration. Indeed to the human observer 'plain' variants of *Idotea baltica* appear cryptic on plain *Fucus vesiculosus* and patterned variants appear cryptic on normal *F. vesiculosus* through disruptive coloration (Jormalainen *et al.,* 1995; Merilaita, 1998). Although these observations may be true, considering a background to be plain does not provide any quantifiable relationship between background elements and colour morphs or variants. Perhaps then, the fucoid canopy is too complex to accurately quantify the diversity of colours present given that the canopy changes with tides, cloud cover, sea state and turbidity.

Overall the general theme in littorinid polymorphism research is that correlations between morphs and backgrounds occur in natural populations (Pettitt, 1973b; Reimchen, 1979; Ekendahl, 1995; Cook, 1986a; Hughes and Mather, 1986; Cook, 1990a; Cook and Garbett, 1992). Conversely littorinid polymorphisms have also been explained by non-selective agents. Raffaelli (1979) concluded that the maintenance of shell colour polymorphism in *L. rudis* is most likely mediated by selection on pleiotropic characters rather than shell colour alone. Furthermore Byers (1990) could not confirm that predation was the sole agent in determining shell coloration but rather that consistent matching of littorinid and background colour is also a product of the species' biology. In my opinion these examples of human-based morph classifications have been sufficient to describe polymorphisms where bird vision is concerned. If, however, reflectance spectra had shown significant ultraviolet reflectance, interpretation of visual signals available to birds when making a foraging decision would not be adequate.

Regarding the question of how the polymorphism evolved, it could of course have been the product of some other selective agent which does not act any more. However, the population would have potentially reached equilibrium by now. Additionally, the rocky shore has remained relatively unchanged for thousands of years as human impact has been relatively limited due to a lack of resources and unfavourable conditions for development. There is however evidence to suggest that human intervention by introduction of littorinid species and novel predators is of considerable importance in marine intertidal communities (Vermeij, 1982; Johannesson, 2003; Knight *et al.*, 1987).

(7.3) Spectroradiometry and Visual Modelling: Suitable Techniques?

Birds are renowned for their acute visual capabilities and in fact have one of the most complex retinas of any vertebrate (Hart, 2001b). It is more likely that bird, rather than crab, predation may have a selective effect on shell colour in *L. saxatilis*. This is because birds possess four classes of receptor and are consistently higher discriminators of different hues (Hart, 2001a). Crabs, however, are only maximally sensitive to two areas of the visible spectrum (Martin and Mote, 1982). Furthermore crabs may be more reliant on chemosensory and tactile cues for foraging (Crothers, 1968; Elner and Hughes, 1978), although the use of vision, and indeed colour vision, cannot be refuted as it has been proven to exist (Martin and Mote, 1982). I believe that when considering intertidal fish as predators of littorinids (Chapter 6), the human visual system is adequate. This is not to say that other more complex visual interactions may have occurred, given that I could not account for the entire visual scene underwater.

The surfaces measured in this work do not reflect ultraviolet radiation and therefore the human visual system is adequate for estimating the conspicuousness of littorinids to birds.

Since the spectral sensitivities of human receptor cells enable trichromatic colour vision (Schnapf *et al.,* 1987), the judgement of colour on behalf of organisms with lower discrimination of hues is potentially misleading. I believe that although crabs may not often use vision for detecting littorinid prey, the quantification of the signals has been useful particularly as crabs are such important predators of littorinids world wide (Boulding, 1990). It seems that although fine scale differences between shore and snail coloration have been detected, the human visual sense is adequate for the most important visual predators even if their predation events occur seldomly. However in some cases in nature the human visual system is clearly not sufficient for interpreting ecologically important signals. In birds particularly, interpreting signals in intraspecific communication and foraging has yielded important information particularly since birds use ultraviolet signalling (Burkhardt, 1989; Burkhardt and Finger, 1991; Derim-Oglu and Maximov, 1994; Maier, 1993, 1994; Church *et al.,* 1998 a, b; Smith *et al.,* 2002; Hausmann *et al.,* 2003). Spectral reflectance analysed using segment classification method for colour (Endler, 1990) led to the validation of a sub-species in the Kerguelen Tern, *Sterna virgata* (Gomez and Voisin, 2002). UV sensitivity is also known to be important to some fish species (Cummings *et al.,* 2003; Browman *et al.,* 1994; Losey *et al.,* 1999). Human vision has been known to fail to distinguish sexual dichromatism in birds (Eaton, 2005). The rocky shore system provides no 'hidden' channels of communication between predators and their prey and therefore I believe that the human judgement of colour morphs is sufficient as long as quantitative consideration is given to how much less or more other organisms such as birds and crabs may be able to detect. In an experiment investigating conspicuousness of prey, painted bird mounts were presented to Sparrowhawks (*Accipiter nisus*), UV reflectance was not detected using spectroradiometry therefore the human visual sense was accepted as sufficient (Götmark, 1996). However a wealth of experiments exist where UV vision has not been considered where objects may well reflect UV light (Dittrich *et al.,* 1993; Götmark and Olsson, 1997; see Bennett *et al.,* 1994 for a review).

Although spectroradiometry and visual modelling enables quantification of discriminability, it is quite a lengthy and expensive technique to implement initially. Furthermore it does not take account of the shape of shells and how this affects their contrast against the background (Johnsen, 2002). In my opinion spectroradiometry limits the information detectable in a visual scene, particularly if objects are patterned. In terms of the background, spectral measurements are a small spatial snapshot and given the spatial and temporal complexity of both the rock and algae it would be impossible to capture all positional variability. Recent advances in the use of digital photography to produce pattern frequency indices are a positive step in the direction of creating a measure of colour pattern in the visual system of a potential predator (Stevens *et al.*, in prep.).

Although digital photography analysis of visual scenes would provide more information about the background heterogeneity it would still only provide a 'snap-shot' of the situation. Indeed spectroradiometry in this thesis has provided a series of snap-shots of littorinid positions. It is likely that the situation would vary dependent on climatic variables such as cloud cover, precipitation, solar intensity (Little, 1989; Lee and Williams, 2002).

None of these techniques are, however, dealing with perception (Chiao and Cronin, 2002). Visual modelling goes as far as quantifying the signals available to the brain of the observer. We have no way of detecting the capacity of the nervous system to determine the quality of the signal (Goldsmith, 1990). Variation between individuals of the same species may also be an important factor (Lythgoe and Partridge, 1989). Indeed microspectrophotometry (MSP) by its nature is an invasive technique and requires a dead organism. Therefore characterisation of visual systems is often carried out using a small number of individuals. For example the spectral sensitivity and ocular filter data used in this thesis from the work of White (2003) on the Peacock blenny, *Salaria pavo* used the eyes of only 12 individuals. In addition, it is quite possible that the MSP procedure itself could be subject to variability. Despite this, it can be accepted that the magnitude of differences between two separate spectra in terms of their coloration should be perceived to be different to the same magnitude (Stevens *et al.*, in prep.). Combining visual modelling with behavioural experiments has long been undertaken to test the assumptions of visual modelling (Vorobyev *et al.*, 2001). For example spectral reflectance and visual modelling combined with feeding trials in birds showed the importance of UV signals in detecting artificial, UV reflecting prey (Church *et al.*, 1998b; Smith *et al.*, 2002). In this thesis selection experiments were conducted in conjunction with spectroradiometry and visual modelling and they confirmed the findings of previous authors (Ekendahl, 1995; Reimchen, 1979). Specifically, the visual differences between morphs on different backgrounds were of the same magnitude as their fitness differences when presented to a predatory blenny. Therefore visual modelling provides a relative measure of the realistic discriminability of two spectra, however, whether that object will actually be chosen is dependent on a) the presence of a predator, b) the predator's motivation, c) the incident light conditions, d) viewing distance, e) viewing angle and f) interference of the medium through which the predator is viewing the prey (*i.e.* turbid water, mist or fog).

(7.4) Future Directions

In my opinion it is fundamental to continue considering the visual systems of predators when studying the adaptive significance of coloration in molluscs. There are, however, more appropriate techniques being developed such as digital photograph analysis. Although it is not present in this thesis I have undertaken work using digital photography to calculate the colour pattern frequency of images of littorinids in their natural habitats. This technique allows a more flexible and precise method to obtain data from a visual scene including colour patch size and distribution measures, brightness and colour metrics and broadband reflection values (such as in the long-, medium- , and short-human visible wavebands) (Stevens *et al.*, in prep.). The result is an index of pattern difference between two photos, one with the object of interest present and one with the object absent. This index represents the sum of differences at each spatial frequency. The spatial frequencies measured are determined by the colour space used and the spectral sensitivities of the observer. This allows calculation of the visual signals available to a predator in an entire visual scene, rather than measuring pin point colour measurements.

Changes in morph frequencies in mangrove-dwelling littorinids have been observed over the seasons (Hughes and Mather, 1986; Reid, 1987; Cook 1986b). Indeed littorinid behaviour is affected by weather conditions (Little, 1989; Lee and Williams, 2002). Therefore it would be interesting to investigate the affect of weather on background matching in littorinid and how these weather conditions may coincide with times that predation might occur. Furthermore, two behavioural morphs of *Littorina brevicula* have been shown to differentially exploit niches for breeding dependent on the seasons (Takada, 2003). Therefore I believe that the temporal differences between morphs would be a valid investigation in which to apply spectral techniques. Indeed there are marked changes in the algal canopy throughout the year (Williams, 1996). Throughout my PhD I collected algal spectral data from a local shore but found that spectral techniques were unable to detect changes in algae given their natural heterogeneity. Perhaps digital image analysis would be more suitable to capture and quantify seasonal changes in background coloration.

Colour pattern is known to have many functions in nature including warning and disruptive coloration (Endler, 1978, 1986, 1988; Merilaita, 1998; Chiao and Hanlon, 2001a, b; Cuthill *et al.,* 2005). Some of the littorinids studied in this thesis were patterned (tessellations and banding) but spectroradiometry is unable to quantify pattern. Again digital image analysis of these patterned shells against a variety of backgrounds would be useful to understand the evolutionary forces that created and maintains them.

I believe there is much scope for many more experimental tests of selection rather than for assuming information on associations between morphs and backgrounds (see Cook and Garbett, 1992 for a review). For example transplant experiments combined with spectral analysis would yield useful information about changes in coloration of littorinids throughout their life stages and the susceptibility to predation or indeed how an individual snail of a particular morph changes its position over the course of a day. I also believe that there is a level of background heterogeneity, particularly in algal habitats, which may not be quantifiable by any technique. However it would be very interesting to accurately measure within-canopy reflectance and irradiance when the tide is in to gain more information about the visual environment of crabs and fish. Zeil and Zanker (1997) recorded scenes in a crab colony to enable the reconstruction of another animal's visual world. Although this was a highly technical experiment it gave the researchers specific information on detection and the importance of different types of visual information termed 'local' and 'global' in intraspecific communication of the Fiddler crab (genus *Uca*) (Zeil and Zanker, 1997).

Regarding the sampling technique used throughout this thesis, with hindsight it may have been more useful to randomly select an equal number of each colour morph present on each shore/habitat type/algal species. This would allow improved statistical analysis with balanced factors and would correct for the occasions when only one individual of a morph was present. Although this method of spectral data collection would enable balanced statistical analyses, data collection would be subject to bias. The method used was entirely random and therefore all littorinids in a given area were sampled therefore I was unable to be biased towards those littorinids that either conformed to my hypothesis or alternatively were easier to sample. Furthermore, laboratory-collected shell reflectance spectra for each shore were available but its seemed more appropriate to use shore collected shell spectra with shore collected background spectra to minimise the difference in the quality of the spectra, as laboratory spectra were often less prone to noise. In other words, using the littorinid and background spectra both collected in the field meant that both types of spectra were susceptible to the same level of experimental error. The chromatic contrasts would be more representative of field conditions. However, experiments using small samples of background spectra have been successfully conducted (Norris and Lowe, 1964; Stuart-Fox, 2004; Siddiqi *et al.,* 2004) and perhaps this technique should be considered in the future. Additionally it would be interesting to account for different viewing angles adopted by predators given that crabs, birds and fish vary greatly in their visual field.

(7.5) Concluding Remarks

Although this thesis demonstrates the same techniques of spectral data modeling and analysis throughout, all shores and systems were treated in a consistent manner. I have also presented data to enable judgement of the usefulness of spectroradiometry as a technique in this particular system for the first time. In general, previous studies have focused on more flamboyant spectra, often involving ultraviolet communication channels, in systems where both predation and intraspecific communication are important. This work has, for the first time, quantified visual signals in a system comprising of only one visually-orientated party, the predator and quite ‘ordinary’ spectra. Therein this work contributes to understanding the interactions of predators and their prey on the rocky shore.

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