The Form and Function of Scallop Mantle Eyes

by

Daniel Isaac Speiser

Department of Biology
Duke University

Date: _____

Approved:

_____
Dr. Sönke Johnsen, Supervisor

_____
Dr. Manuel Leal

_____
Dr. Greg Wray

_____
Dr. Ken Lohmann

Dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy in the
Department of Biology in the
Graduate School of Duke University

2010

ABSTRACT

The Form and Function of Scallop Mantle Eyes

by

Daniel Isaac Speiser

Department of Biology
Duke University

Date: _____

Approved:

_____
Dr. Sönke Johnsen, Supervisor

_____
Dr. Manuel Leal

_____
Dr. Greg Wray

_____
Dr. Ken Lohmann

An abstract of a dissertation submitted in
partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the
Department of Biology in the
Graduate School of Duke University

2010

Abstract

Scallops, a family of swimming bivalve mollusks, have dozens of eyes arrayed along the edges of their valves. Relatively little is known about the form and function of these unusual eyes. To learn more about them, we studied the visually influenced behavior of scallops, as well as the morphology and spectral sensitivity of their eyes. Of particular interest was whether or not the simple neural architecture of these animals constrains the number of visually-influenced behaviors they can perform. We were also interested to learn whether scallop eyes, despite providing relatively poor visual acuity, show optical refinements, such as corrections for spherical and chromatic aberration, that are known from the eyes of animals with better vision. In the following dissertation, Chapter 2 discusses the visually-influenced behaviors of scallops. It has been argued that bivalve mantle eyes only act as predator-detectors, but the behavioral trials described in this chapter suggest that vision may serve additional purposes in scallops. For example, it was found that visual cues relating to flow conditions may influence scallop feeding behavior. Chapter 3 presents a comparative study of scallop eye morphology. Here, it is found that eye morphology varies considerably between scallop species and that highly mobile scallops have better vision than less mobile or immobile species. Evidence is also presented that one of the two scallop retinas may

perform tasks of similar importance to all species, such as predator detection, while the other retina may perform tasks more important to mobile species, such as those associated with the visual detection of preferred habitats. Chapter 4 investigates the spectral sensitivity of the two retinas in the mantle eyes of two scallop species. It is found that there is both inter- and intra-specific variation in scallop spectral sensitivity and that color perception in scallops may be influenced by both environmental light conditions and chromatic aberration caused by their lens. The research in this dissertation provides insight into how vision functions in animals that, like scallops, have a vast number of eyes, but a limited capacity for neural processing. Despite such limitations, it is evident that scallops display a wide range of visual behaviors and have eyes with highly-refined optics.

Dedication

This dissertation is dedicated to my parents, Marc and Mary Speiser.

Contents

List of Tables

List of Figures

Acknowledgements

First of all, I would like to thank my advisor, Sönke Johnsen, for all of his help over the course of my time as a graduate student. His advice regarding both science and life has been invaluable. Thanks as well to Mark Hooper of Smyrna, NC, for teaching me so much of what I know about the distribution and habits of North Carolina's edible sea life. For the work discussed in Chapter Two, I would like to specifically thank Sam Boyarsky, Bill Kirby-Smith, Richard Forward, and Steve Vogel for their help. Chapter Three's completion was aided by Sam Johnson of the Duke Light Microscopy Core Facility, Nina Tang Sherwood, and Eric Warrant. Additional thanks go to Jeanne Serb and Dan Runcie for their help with specimen collection. For the research included in Chapter 4, I would like to thank Ellis Loew for supplying lab space and equipment and helping with the microspectrophotometry (MSP) recordings. Thanks as well to Todd Oakley for his input regarding the proposed experiments described in Chapter 6 of this dissertation. I would like to thank my dissertation committee of Sönke Johnsen, Kathleen Smith, Manuel Leal, Greg Wray, and Ken Lohmann, as well as past and present Johnsen lab members Alison Sweeney, Jamie Baldwin, Cynthia Tedore, Nick Brandley, and Sarah Zylinski. Finally, thanks to my parents, Marc and Mary Speiser, for all of their love, support, and encouragement over the years.

1. Introduction

1.1 Special-purpose and general-purpose eyes

Biologists have long been puzzled by how animals with simple, decentralized nervous systems process sensory input. The amount of information that even a simple eye can gather seems overwhelming for an animal with relatively few neurons. How such animals convert visual stimuli to behavioral responses is also poorly understood. Land and Nilsson (2006) propose that animals with a limited capacity for neural processing have "special-purpose" eyes. As the name suggests, these eyes are thought to gather information relevant to just a single, specific task. Examples of special purpose eyes include those of box jellyfish, which are used for avoiding obstacles (Coates 2003), and those of heteropod mollusks, which are specialized for tracking prey (Land 1982). Other examples include the eyes of tubeworms and bivalves, which are thought to act as simple predator-detectors (Nilsson 1994). In contrast, "general purpose" eyes that are employed for a wide range of complex tasks may be only be found in vertebrates, cephalopods, insects, some crustaceans, and perhaps jumping spiders(Land and Nilsson 2006). If a challenge to this hypothesis is to be found, it will likely be posed by an animal that has an unequivocally simple and decentralized nervous system, yet displays a wide range of visually-influenced behaviors.

1.2 'Matched filters' and decentralized nervous systems

Another way that animals with decentralized nervous systems may deal with sensory input is through a 'matched filters' model. Proposed by Wehner (1987), this model predicts that simple animals will process sensory information on the level of their receptors. For example, some insects navigate by patterns of polarized light in the sky by having photoreceptors arranged to filter out everything but this pattern; these eyes form poor images in a conventional sense, but by filtering sensory input, they let these animals perform a seemingly complex task without much neural investment (Wehner 1987). While many examples of 'matched filters' in animals have been demonstrated, few counter-examples have been proposed. As in the case of 'special-purpose' eyes described above, an animal with a simple nervous system and complex eyes may pose a worthy challenge to an influential theory.

1.3 Introducing the scallop and its unusual eyes

Scallops (Family Pectinidae) are a monophyletic family of filter-feeding bivalve mollusks related to mussels and oysters (Giribet and Wheeler 2002; Puslednik and Serb 2008; Waller 2006). In comparison to other mollusks, scallops, like all bivalves, have a highly atrophied head and cerebral nervous system (Wilkens 2006). Unlike most other

bivalves, however, scallops have a multitude of eyes. These eyes are arrayed along the margins of the valves and run from one end of the dorsal hinge to the other (Fig. 1). By this author's count, different scallops can have mantle eyes that number anywhere from a few dozen to over two hundred. Scallop eyes are lined in back by a concave spherical mirror. At certain angles, light reflected by this mirror may be seen shining through a pupil at the tip of an eyestalk that may be pigmented black, brown, red, or a brilliant blue (Fig. 1) . These lovely eyes have drawn the attention of a long line of invertebrate morphologists (Dakin 1910; Hesse 1900; Poli 1795). It was not until 1965, however, that it was discovered that scallops do not use their lens to form images, as had previously been thought, but instead employ the mirror at the back of their eye (Land 1965). This discovery, aided by behavioral (Buddenbrock and Moller-Racke 1953) and electrophysiological studies (Land 1966; McReynolds and Gorman 1970; Wald and Seldin 1968), made it clear that scallops not only have relatively acute vision for a bivalve, but also have some of the most unusual eyes in the animal world. For example, the scallop eye contains two retinas. Moreover, the distal set of photoreceptors hyperpolarize in response to light and are morphologically similar to those of chordates, whereas the proximal receptors depolarize at the onset of light and resemble those found in most other invertebrate eyes (Barber et al. 1966; Hartline 1938; McReynolds and Gorman 1970).

With their limited capacity for neural processing and their abundant supply of morphologically complex, image-forming eyes, scallops may be an ideal system in which to test the theory that animals with decentralized nervous systems are limited to eyes that can only perform a single function (Land and Nilsson 2006). It is generally believed that scallop eyes evolved for spotting predators not kind enough to cast shadows ahead of themselves (Morton 2000). Indeed, all scallops respond to the presence of predators by closing their valves up tight. There are some indications, however, that scallops use their eyes for more than this one task. For example, scallops are known to extend their tentacles towards novel visual stimuli (Buddenbrock and Moller-Racke 1953; Wilkens 1981; Wilkens 2006). Furthermore, unlike other eyed filter-feeders such as tubeworms and ark clams (Nilsson 1994), scallops are quite mobile. Most species are able to swim by firing jets of water through gaps between their valves (Cheng and DeMont 1996); some species can even reach swimming speeds of up to 100 cm/s (Joll 1989). There is even behavioral evidence that scallops will swim towards preferred habitats, such as grassbeds, using visual cues (Hamilton and Koch 1996). Thus, there is good reason to believe that scallops have a visual repertoire that goes beyond predator-detection.

Furthermore, there is evidence that scallops are able to process visual information: the lateral lobes of their parieto-visceral ganglion (PVG) are activated by

visual input (Spagnolia and Wilkens 1983; Wilkens 2006; Wilkens and Ache 1977). Therefore, one goal of this dissertation project is to explore whether scallops have behaviors, other than predator detection, that are visually-influenced. Many invertebrates with simple nervous systems use visual cues for detecting prey or optimum feeding conditions (Land and Nilsson 2006). By presenting scallops with visual stimuli related to different flow conditions, we tested if these filter-feeders use their eyes in an analogous manner. We also explored whether there is a correlation between a scallop species' swimming ability and its optical resolution. We found a positive correlation between the two, which suggests that vision plays at least some role in habitat selection, as the ability to perform this behavior is dependent on a species' mobility. Finally, we took into account the two separate retinas in the scallop eye. The presence of two different sets of receptors suggests that scallop vision may be sub-funtionalized; *i.e.* the two retinas may be specialized for different sets of tasks (Land 1966; Wilkens 2006).

Scallop eyes may also challenge the 'matched filters' model, which predicts that animals with decentralized or simple nervous systems will tend to filter information at the level of their sensory receptors. For example, there is evidence that scallop eyes correct for various forms of optical aberration. Land (1965) hypothesizes that scallop lenses are unusually shaped because they correct for spherical aberration caused by the

focusing mirror. Scallops' eyes may have thus evolved to produce a sharp image, despite their small size. Such an optical design contrasts with that observed in box jellyfish (*Carybdea*), which have eyes in which an under-focused image appears to form on the retina (Nilsson et al. 2005). Here, we investigated whether scallop eyes, like those of box jellies, filter information by forming sub-optimal images, or whether they are fine-tuned to produce as sharp an image as possible. For example, all biological lenses produce some degree of chromatic aberration (Kroger 2000); it is unlikely that the scallop lens poses any exception. As scallops correct for this form of optical aberration, we may have found strong evidence that scallops are not optically filtering the information that reaches their photoreceptors.

Additional aspects of scallop vision have drawn the attention of biologists in recent years. Scallop visual pigments (Kojima et al. 1997), photoreceptor transduction pathways (Gomez and Nasi 2000) and lens proteins (Carosa et al. 2002) are all recent or on-going topics of research. Our work on scallops thus addresses research questions both long-standing and up to date, as well as scientific interests ranging from morphological specificities to general theories concerning the evolution of animal vision.

2. The behavioral response of scallops to simulated moving particles

2.1 Introduction to scallop behavior

The Bay Scallop *Argopecten irradians* (Lamarck, 1819) has up to one hundred bright blue eyes at the tips of short tentacles on the middle mantle fold (Fig. 1). These eyes line the edges of the right and left valves from the anterior to the posterior ends of the hinge. *A. irradians*' mantle eyes contain a lens, two distinct retinas, and a concave spherical mirror, morphology similar to that of previously described scallop eyes (Barber et al. 1966; Dakin 1910; Morton 2001). Light reflected off the mirror is thought to form a focused image on the distal retina (Land 1965), which appears to be well-suited for the detection of movement (Land 1966).

Because scallops close their valves in the presence of large moving objects, it has been argued that their eyes primarily act as predator detectors (Morton 2000; Nilsson 1994). While predator detection is almost certainly one task of the mantle eyes, evidence suggests that other scallop behaviors are also visually influenced. For example, scallops have been observed extending their tentacles in response to visual stimuli (Buddenbrock and Moller-Racke 1953; Wilkens 2006) and visually navigating toward preferred habitat (Buddenbrock and Moller-Racke 1953; Hamilton and Koch 1996).

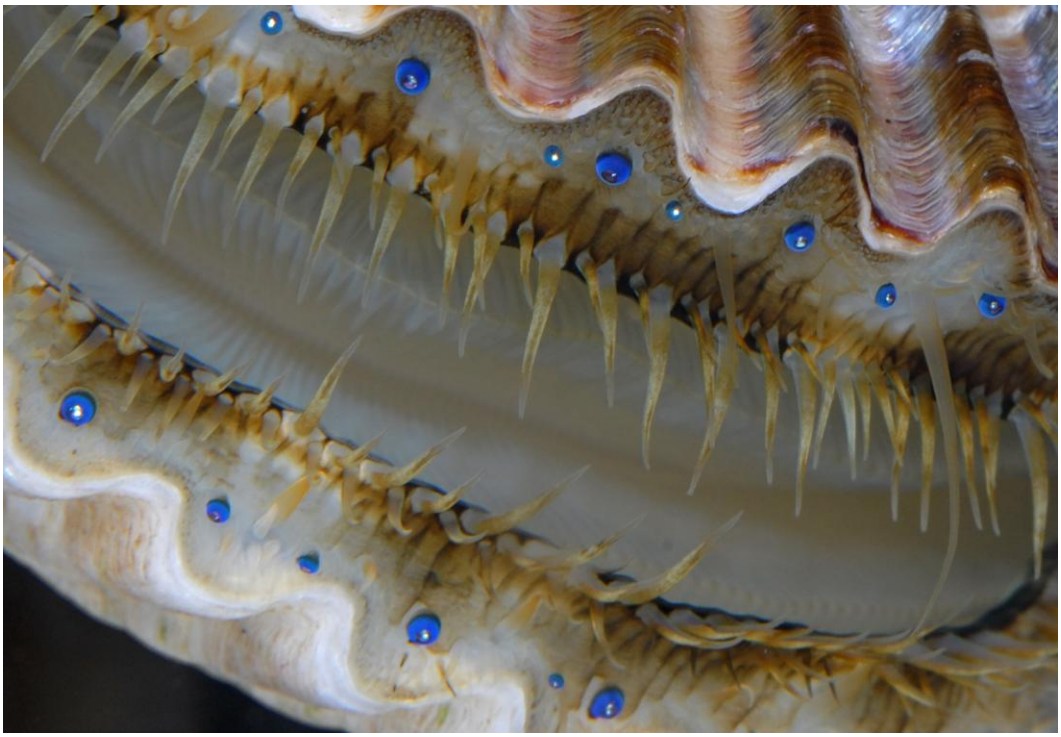

Figure 1: The bay scallop *Argopecten irradians*.

Note the bright blue eyes. Photo credit: Sönke Johnsen.

Measurements of optical resolution also imply that predator detection may be only one of several functions performed by scallop eyes. Behavioral (Buddenbrock and Moller-Racke 1953), morphological (Land 1965), and physiological (Land 1966) studies all conclude that scallop eyes have an angular resolution of around 2º. In comparison, the predator-detecting eyes of other bivalves have angular resolutions (Table 1) ranging from 13º to 40º (Land 2003; Nilsson 1994). While coarse, an angular resolution within this range would still likely allow scallops to spot major predators, such as crabs, gastropods, rays, and starfish (Brand 2006; Myers et al. 2007), at ecologically relevant distances. Therefore, while predator detection may explain some aspects of scallop vision, it does not account for these bivalves' diverse visual behaviors or why they see so relatively well.

In the following study we test the hypothesis that scallops visually detect the presence and speed of moving particles when assessing feeding conditions. Scallops actively feed on suspended organic particles and can open their valves to see without opening their mantle gape. Thus, the visual detection of particle presence and speed may let these animals monitor feeding conditions without exposing the vulnerable structures within their mantle cavity. We placed specimens of *A. irradians* in a flow tank, showed them simulated particles of different sizes moving at a range of different speeds, and recorded and analyzed their responses. We worked with *A. irradians* because it lives

in bright, shallow water and has been used in previous studies of scallop vision (*e.g.* Hamilton and Koch, 1996).

Table 1: The optical resolution of a selection of animal eyes

The optical resolution of a selection of animal eyes

Name	Equivalent inter-receptor angle (degrees)	
Eagle	0.004	
Human	0.007	
Octopus	0.01	Mollusk
Human (legally blind)	0.07	
Rat	0.5	
Honey bee	1.0	
Scallop	1.6	Bivalve mollusk
Wolf spider	1.8	
Sea snail (Heteropod)	4.5	Mollusk
Fruit fly	5	
Nautilus	8	Mollusk
Giant clam	16.5	Bivalve mollusk
Ark shell	20 – 40	Bivalve mollusk

Table adapted from Land and Nilsson (2002).

2.2 Scallop behavior materials and methods

2.2.1 Specimen collection and care

Specimens of *A. irradians* were obtained from Middle Marsh, NC, USA (6 km east of Beaufort, NC, approximately 34.72º N, 76.59º W) on 25 May and 12 July 2006 and on 10, 17, and 31 May 2007. Animals were collected from eelgrass (*Zostera sp.*) beds at low tide (water depth of 30-60 cm). Animals collected in 2006 were immediately transported to Duke University (Durham, NC, USA), where they were kept in 40 l aquaria tanks under natural light with biweekly water changes. Salinity was kept at 28-30 ‰ (Instant Ocean sea salt, Aquarium Systems, Inc, Mentor, OH, USA). Animals collected in 2007 were immediately transported to the Duke University Marine Laboratory (Beaufort, NC, USA), where they were kept in sea tables with continually flowing filtered sea water. At both sites, animals remained in apparent good health for over a month. Experiments were conducted on animals one to three weeks after they were collected.

2.2.2 Experimental apparatus

The experimental set-up included a computer monitor, laptop computer, small flow tank, video camera, and recorder (Fig. 1). The flow-tank was a plexiglass box (64 cm L x 14 cm W x 18 cm H) attached at each end to a curved length of 6 cm diameter

PVC pipe. A 1200 liter-per-hour rated submersible pump (Penguin 1140, Marineland Aquarium Products, Moorpark, CA, USA) and a 13 cm long baffle made of plastic drinking straws created a laminar flow of 5-10 cm/s within the tank, well within the normal range of flow rates encountered by scallops in nature (MacDonald et al. 2006). Flow was used because scallops, in preliminary trials, rarely opened their valves in still water.

The flow-tank was placed in front of a 46 cm monitor attached to a laptop computer that ran the particle simulation program. The behavior of *A. irradians* was recorded with a video camera attached to a time-lapse VHS video recorder. Video camera output was displayed on a second monitor so that proper aperture and focus could be maintained from trial to trial. In both 2006 and 2007, trials were conducted in light-tight rooms. The only illumination in these rooms was provided by the two monitors.

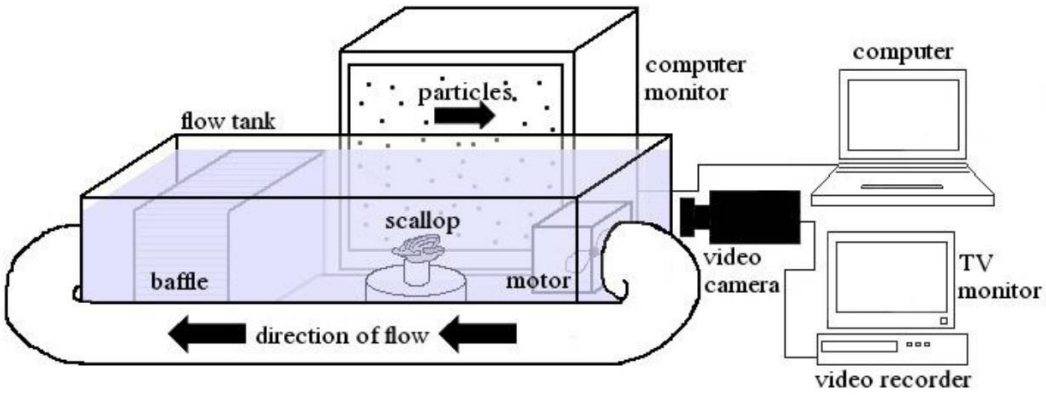

Figure 2: The experimental arena used in behavioral trials

This figure shows a diagram of the flow tank set-up we used to test the behavioral responses of scallops to virtual particles traveling across a computer monitor. Flow direction matched virtual particle direction and scallops were mounted right valve down so that their anterior faced away from oncoming flow.

2.2.3 Experimental procedure

The flow tank was rinsed and filled to a depth of 14 cm with newly-mixed artificial sea water on each day that trials were run. Trials were conducted during daylight hours and with one animal at a time. To prevent scallops from swimming during trials, while allowing a full range of valve motion, specimens of *A. irradians* were glued, right valve down, to a short length of PVC pipe that was then attached to a mount at the bottom of the flow-tank. Specimens were mounted so that the anterior (inhalant) opening faced the video camera and was downstream with regards to flow. The flow-tank was positioned so that the computer monitor was, at most, 2.5 cm from the nearest point on a test animal.

In our experiment, we observed the behavior of scallops shown moving, simulated particles of different sizes and speeds. The particle simulation program was written in JavaScript (Ecma International, Geneva, Switzerland) and run as an HTML file. In our first set of trials, particles in the no particle treatment were grey (grey value = 80 out of 255) and invisible against the grey background (grey value = 80 out of 255) and particles in the 0.6 x 0.6 mm (1.4° angular size) and 1.5 x 1.5 mm (3.4° angular size) particle treatments were black (grey value = 0 out of 255). All particles in our first set of trials moved at 2.5 cm/s. In our second set of trials, black virtual particles were 1.5 x 1.5 mm in size and moved at 2.5, 5, or 10 cm/s against the grey background. In all

treatments, particles appeared at random positions on the left edge of the screen at a rate of 10 per second and moved left to right, the same direction as the flow in the tank. Flow speed was not altered between trials. Monitor refresh rate was 50 Hz and irradiances at the scallop (integrated from 400 to 700 nm) were nearly identical between trials, with readings of 1.20×10^{14} photons/cm^2/s and 1.21×10^{14} photons/cm^2/s for the no particle and particle treatments, respectively. Furthermore, no observed scallop behaviors differed significantly when the monitor background was changed from white (grey value = 255; N = 23) to grey (grey value = 80; N = 23) to black (grey value = 0; N = 24) in an independent set of trials. The irradiance values at the scallop for the white, grey, and black backgrounds were 6.61×10^{14}, 1.20×10^{14}, and 5.11×10^{11} photons/cm^2/s, respectively. No virtual particles were displayed in these treatments. The results from this set of trials suggest that the slight differences in irradiance values between the virtual particle treatments did not influence scallop behavior.

Trials for the no particle and 1.5 x 1.5 mm, 2.5 cm/s virtual particle treatments were conducted in 2006 and trials for the 0.6 x 0.6 mm, 2.5 cm/s and the 1.5 x 1.5 mm, 5 and 10 cm/s treatments were conducted in 2007. Different animals were used for each trial within a given treatment. Because some animals were used in both the 0.6 x 0.6 mm, 2.5 cm/s and the 1.5 x 1.5 mm, 5 cm/s treatments, these two conditions were not compared in our analysis. Trials for the black, grey, and white background conditions

were conducted in 2006 and 2007. Different animals were used for each trial within a treatment and no animals were used in more than one treatment.

2.2.4 Data collection and analysis

The particle simulation program was initiated and behavioral recording began immediately after scallops were placed in the flow tank. All trials lasted 10 minutes, measured from the onset of recording. For our recordings of each trial, scallop mantle gapes were scored as open or closed and tentacles were scored as extended or not extended at 24 second intervals. Mantle gapes were scored as open if there was a gap in the anterior mantle folds and the gills were exposed (Fig. 2A). Mantle gapes were scored as closed if no gap was visible between the anterior mantle folds and the gills were not exposed (Fig. 2B). We also counted the number of times that each scallop clapped its valves during a trial. Scallops can see as long as their valves are open, but it is unlikely that they are able to see when their valves are closed. Therefore, we only analyzed trial data recorded after a scallop first opened its valves. This resulted in a variable number of observations per trial. However, the total number of observations varied little between treatments (Table 1).

We calculated the proportion of observations in each trial in which a scallop's mantle gape was open or its tentacles were extended (Table 1). These proportions were arcsine square-root transformed for analysis and comparisons between treatments were made using one way ANOVAs and Bonferroni pairwise multiple comparison t-tests. The numbers of valve claps per trial were not consistent with a normal distribution, so

Kruskal-Wallis one way ANOVAs on rank order were used to compare valve claps between treatments.

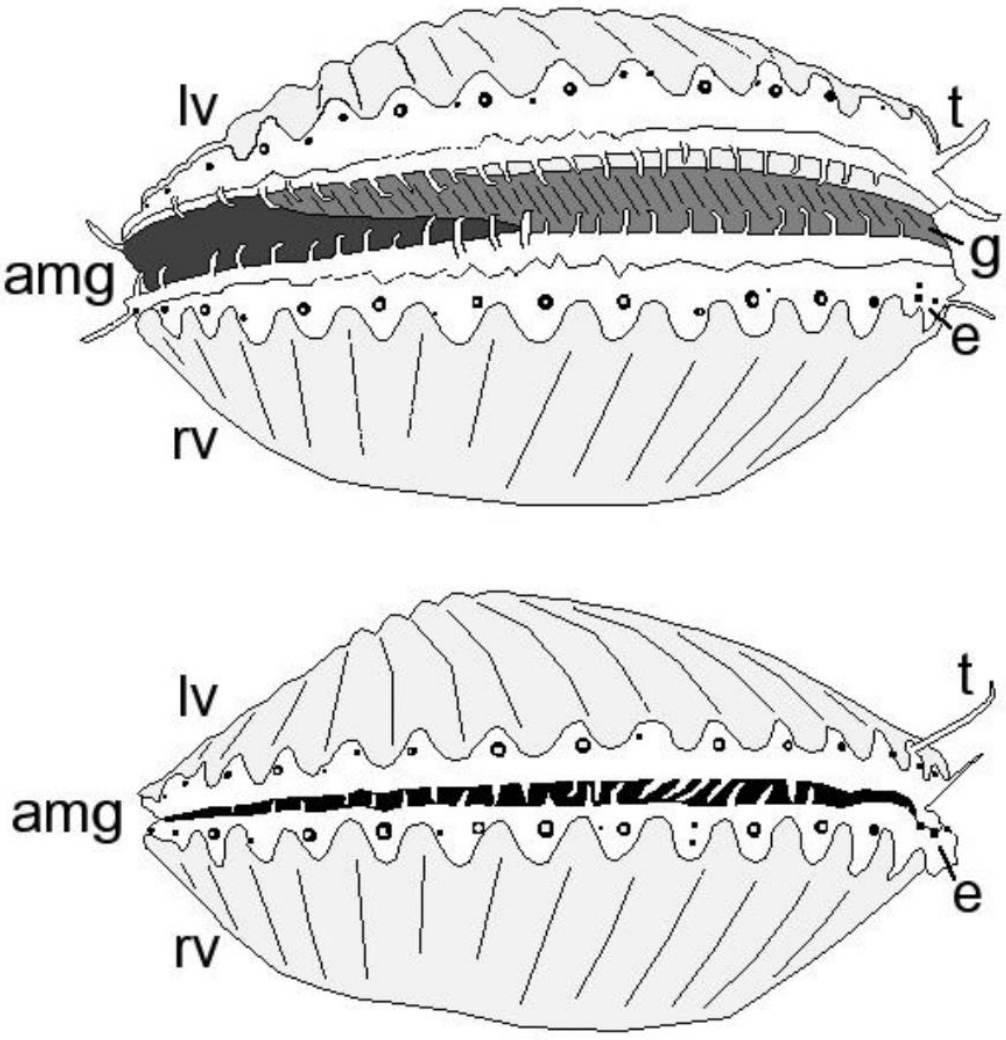

Figure 3: Illustrations of scallop mantle gapes

This figure shows the bay scallop *A. irradians* with (A) open anterior mantle gape and (B) closed anterior mantle gape (amg = anterior mantle gape; e = eye; g = gill; lv = left valve; rv = right valve; t = tentacle).

2.3 Scallop behavior results

2.3.1 Effect of size of virtual particles moving at 2.5 cm/s

Scallop anterior mantle gapes were open in 52 ± 13% (mean ± 2 SE) of the observations per trial for the 1.5 x 1.5 mm particle treatment (N = 25), but in only 23 ± 10% and 29 ± 10% of the observations per trial for the treatments with 0.6 x 0.6 mm particles (N = 24) and no particles (N = 24), respectively (Fig. 3). One way ANOVA revealed that scallop behavior was influenced by virtual particle size ($F_{2, 70} = 7.270$; $P < 0.001$). Bonferroni t-tests indicated that scallop anterior mantle gapes were open significantly more often in the larger particle treatment than in either the smaller particle ($t = 3.660$; $P = 0.001$) or no particle ($t = 2.726$; $P = 0.024$) treatments.

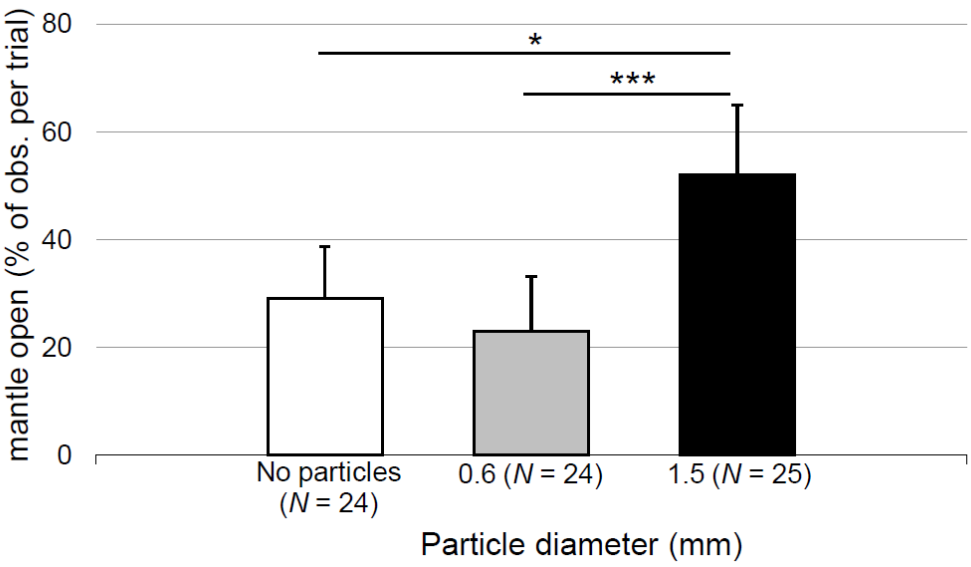

Figure 4: The response of scallops to virtual particles of different size

The above figure shows the percent of observations per trial in which scallop anterior mantle gapes were open. Particle diameter varied between trials, but particle speed was held constant at 2.5 cm/s. Background radiance in each trial was identical. Error bars represent + 2 SE. N = 24, 24, and 25 for the no particle, 0.6 mm particle, and 1.5 mm particle treatments, respectively. *P = 0.05; ***P = 0.001.

2.3.2 Effect of speed of 1.5 x 1.5 mm virtual particles

Scallop anterior mantle gapes were open in 52 ± 13% and 49 ± 11% of the observations per trial for the 2.5 and 5 cm/s particle treatments (N = 25 for both), but in only 26 ± 10% of the observations per trial for the 10 cm/s particle treatment (N = 24; Fig. 4). As previously noted, scallop anterior mantle gapes were open in 29 ± 10% of the observations per trial from the no particle treatment. We found that scallop behavior was influenced by virtual particle speed ($F_{3, 94} = 5.397$; P = 0.002; one way ANOVA) and, by Bonferroni t-test, that scallop anterior mantle gapes were open significantly less often in the 10 cm/s particle treatment than in either the 2.5 (t = 3.305; P = 0.008) or 5 cm/s (t = 2.860; P = 0.031) treatments.

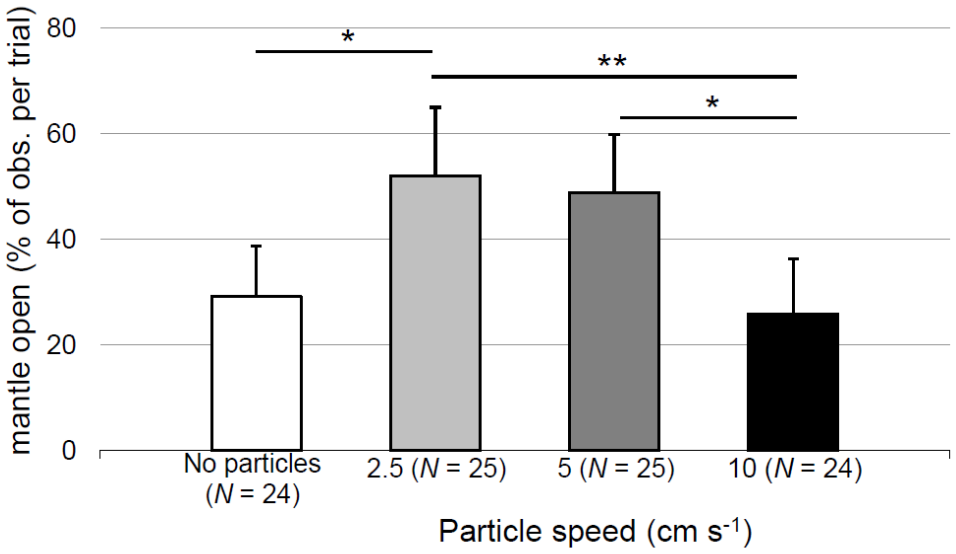

Figure 5: The response of scallops to virtual particles of different speed

The figure above shows the percent of observations per trial in which scallop anterior mantle gapes were open. Particle speed varied between trials, but particle size was held constant at 1.5 x 1.5 mm. Background radiance in each trial was identical. Error bars represent + 2 SE. N = 24, 25, 25, and 24 for the no particle, 2.5 cm/s, 5 cm/s, and 10 cm/s treatments, respectively. *P = 0.05; **P = 0.01.

2.3.3 Tentacle extension and valve claps

Scallops had extended tentacles in 53 ± 14% to 69 ± 12% of the observations per trial for the different particle treatments (Table 1). Particles of different size ($F_{2, 70} = 1.031$; $P = 0.362$; one way ANOVA) or speed ($F_{3, 94} = 1.091$; $P = 0.357$; one way ANOVA) did not have an influence on scallop tentacle extension. Scallops also clapped their valves between 1.4 ± 0.7 and 1.9 ± 0.8 times per trial (Table 1). Kruskal-Wallis ANOVAs revealed that the number of valve claps per trial did not vary significantly between treatments when particle size ($H_2 = 3.330$; $P = 0.189$) or particle speed ($H_3 = 2.032$; $P = 0.566$) varied.

Table 2: Results from scallop behavioral trials.

In our experiment, individual trials yielded a variable number of observations, but treatments had a similar number of total observations. When appropriate, data is given as mean ± 2 SE (to give a 95% confidence interval).

	particle treatments				
	no particles	**0.6 mm, 2.5 cm/s**	**1.5 mm, 2.5 cm/s**	**1.5 mm, 5 cm/s**	**1.5 mm, 10 cm/s**
Number of trials (N)	24	24	25	25	24
Total observations	533	556	555	584	542
Open mantle observations	156	125	287	284	144
% of open mantle observations per trial	29 ± 10	23 ± 10	52 ± 13	49 ± 11	26 ± 10
Extended tentacle observations	342	300	376	365	292
% of extended tentacle observations per trial	63 ± 14	54 ± 14	69 ± 12	64 ± 14	53 ± 14
Valve claps per trial	1.8 ± 1.4	1.4 ± 1.4	1.8 ± 1.2	1.5 ± 1.4	1.9 ± 1.6

2.4 Scallop behavior discussion

Specimens of *A. irradians* opened their anterior mantle gapes significantly more often when they were shown larger, slower virtual particles than when they were shown smaller or faster particles. Scallops open their mantle gape to collect food particles on their gills (MacDonald et al. 2006) and other bivalves, such as mussels, have been found to increase their mantle gape in response to the presence of feeding cues (Riisgard et al. 2003). It is likely, therefore, that the scallops in our study responded to the size and speed of virtual particles with a feeding-related behavior. Scallops must also regularly open their mantle gape to respire (MacDonald et al. 2006), which may account for why open mantle gapes were observed during trials in which no particles, or any other feeding cues, were present. Respiratory conditions were identical between trials, so it is unlikely that respiration accounted for the differences observed between treatments.

Scallop behaviors other than mantle gape position, including tentacle extension and valve clapping, varied little between treatments (Table 1). Scallops extended their tentacles within the first two minutes of most trials, indicating that this behavior may be a general response to new environmental conditions. Valve clapping, on the other hand, did not display a temporal pattern and may have represented behavior related to respiration, swimming attempts, or pseudo-feces expulsion. These findings support our

hypothesis that *A. irradians* responded to different virtual particle sizes and speeds with specific behaviors consistent with increased or decreased levels of feeding activity.

The response of *A. irradians* to virtual particle size was consistent with the estimated 2º inter-receptor angle of its eyes (Speiser and Johnsen 2008). An angular resolution of 2º would likely have let *A. irradians* see the 1.5 x 1.5 mm particles, which had angular sizes of 3.4º. On the other hand, it is less likely that scallops were able to detect the 0.6 x 0.6 mm particles, which had angular sizes of 1.4º. This strongly implies that the differences in scallop behavior we observed between treatments were due to the detection of the virtual particles by visual means.

Scallops may respond to the presence of virtual particles with feeding-related behavior because they visually monitor feeding conditions. Scallops feed on suspended organic particles ranging from 5 – 950 μm in diameter (Mikulich and Tsikhonlukanina 1981; Shumway et al. 1987). Objects in the upper half of this range would be visible to scallops at a distance of a few millimeters, provided that scallop eyes are capable of focusing at such short distances. Alternately, scallops may detect larger, inorganic particles and use this information as a proxy for the presence of smaller, organic particles.

Coastal scallops, such as *A. irradians*, encounter highly variable feeding conditions (Fegley et al. 1992). For example, re-suspensions of bottom sediment by tide

or wind (Grant et al. 1997) and changes in phytoplankton abundance (Frechette and Bourget 1987) may cause food particle concentrations to fluctuate. Previous studies have clearly established that scallops track these fluctuations using tactile and chemosensory cues (MacDonald et al. 2006). However, we hypothesize that the visual detection of suspended particles may be a safe and efficient method for scallops to initially assess new feeding conditions. For example, while scallops may be able to continually test for the presence of food particles by opening their mantle gapes and sampling water with their gills, this action may increase their vulnerability to mantle cavity parasites such as pinnotherid crabs (Krucynzki 1972) and odostomid gastropods (Leibovitz et al. 1984). Because scallops can see even when their mantle gape is closed, visually monitoring for food particles may allow them to avoid these risks. Furthermore, the detection of food particles on the gills may, like feeding in most bivalves (Widdows and Hawkins 1989), incur a metabolic cost in scallops. This cost may be avoided if scallops are able to visually detect food particles.

A. irradians responded to not only differences in particle size, but to differences in particle speed as well. We found that *A. irradians* had open anterior mantle gapes significantly more often when they were shown virtual particles moving at 2.5 or 5 cm/s than when they were shown particles moving at 10 cm/s. Laboratory experiments suggest that scallop feeding may be inhibited by flow rates over 10-15 cm/s (Kirby-Smith

1972; Wildish and Saulnier 1992). Therefore, our findings suggest that *A. irradians* exhibited higher rates of feeding-related behavior when they were shown simulations that indicated favorable feeding conditions. This implies that scallops may visually monitor aspects of their environment related to feeding efficiency, such as particle speed, not just food availability.

Scallops process visual information in the lateral lobes of their visceroparietal ganglion (VPG), an organ that innervates the adductor muscle and likely controls mantle gape position (Wilkens 2006). Processing may be simplified if visual input is filtered at the level of the scallop eye. For example, scallops may optimally respond to a range of environmental conditions if they simply close their mantle gape when they are unable to visually detect suspended particles. Electroretinograms (ERGs) indicate that scallop eyes (*Amusium japonicum*) have an integration time of around 200 ms (Kanmizutaru et al. 2005). Moving at a speed of 10 cm/s and at a distance of 2.5 cm, the 1.5 x 15 mm particles in our study likely traveled the entire distance across *A. irradians* retinas in less than a single visual cycle. It is unlikely, therefore, that the scallops in our study were able to detect the virtual 10 cm/s particles that they were shown. As previously mentioned, flow rates over 10-15 cm/s may inhibit scallop feeding (Wildish and Saulnier 1992), so an inability to distinguish between rapidly moving and non-existent particles may help scallops link a single behavioral output, mantle gape position, to a wide range of visual

conditions. This sort of visual system, which filters information at the level of the eye, may be a common feature in animals that lack the neural complexity to process large amounts of visual input (Nilsson et al. 2005; Wehner 1987).

The position in which scallops were mounted in the flow tank may have influenced our results. Evidence suggests that high flow rates strongly inhibit scallop growth when posterior (exhalant) openings face oncoming flow, as they did in our study, and that juvenile *A. irradians* actively turn their anterior (inhalant) opening to face oncoming flow when flow speeds exceed 9 cm/s (Eckman et al. 1989). This suggests that we may not have observed a decrease in feeding-related behavior at virtual flow speeds of 10 cm/s if *A. irradians* had been positioned in the flow tank in their preferred anterior-to-flow orientation. However, given that scallops are probably unable to detect objects moving faster than 10 cm/s (Kanmizutaru et al. 2005), it is doubtful that the observed visual response of *A. irradians* to virtual particle speed was influenced by flow direction.

2.5 Future directions in scallop visual behavior

Future experiments will explore whether scallops use vision to help assess flow direction. Scallops prefer to filter-feed with their anterior (inhalant) opening turned to face oncoming flow; however, the scallops in our study were fixed in place so that their posterior (exhalant) opening faced oncoming flow, a position associated with lower growth rates (Eckman et al. 1989). It is possible that scallops, placed in a behavioral arena similar to ours but allowed to move, would respond to the direction of virtual particles by changing their position. We predict that, in such a situation, scallops would turn their inhalant opening so that it faced the flow direction of the virtual particles. If this is observed, it would offer strong support for our hypothesis that scallop feeding behavior is influenced by visual cues.

We will also explore how the size of passing objects influences scallop behavior. It is well-known that large passing objects elicit a defensive response from scallops in which they close their valves. As we show here, small passing objects can cause the opposite response; we found that viewing virtual particles led scallops to open their valves. Our goal is to find the dividing line between these two types of response. How scallops determine object size may reveal some details about how they process visual input. We will also explore how other factors relevant to feeding, such as turbidity,

affect scallop behavior and whether the visual responses we observe may continue to be interpreted through a 'matched filters' model of information processing (Wehner 1987).

3. Comparative morphology of the concave mirror eyes of scallops

3.1 Introduction to scallop eye morphology

Scallops have more acute vision than any other bivalve mollusk (Warrant and Nilsson 2006), but it has been argued that their eyes, like those of other bivalves, function merely as "burglar alarms" that trigger valve closure when large passing objects are detected (Nilsson 1994). Scallops are also notable for their ability, in most cases, to swim by a form of jet-propulsion (Cheng and DeMont 1996) and there is some indication that their swimming behavior may be visually influenced. For example, it appears that scallops are able to visually detect and swim towards preferred habitats (Buddenbrock and Moller-Racke 1953; Hamilton and Koch 1996). Arguments have been put forth, however, that scallops are unable to perform visual tasks of such complexity due to the limitations of their decentralized nervous system (Morton 2000). We suspect that these limitations may not be as severe as once thought, given recent findings that other animals with decentralized nervous systems, such as box jellyfish (Coates 2003) and sea urchins (Blevins and Johnsen 2004), use image formation to help guide movement. We therefore believe that the relationship between scallop vision and swimming behavior is one worth continued study.

If scallops are able to visually detect preferred habitats, as we hypothesize, it may be expected that swimming species have more acute vision than non-swimmers. Alternately, if scallops only use their eyes to detect predators, it is likely that little difference exists between the eyes of mobile and immobile species. Little is known about how optical resolution and sensitivity vary among scallop species, but it is thought that eye morphology is largely conserved within Pectinoidea (Dakin 1928a; Morton 2001), a superfamily (Waller 2006) containing both scallops and spondylids (for brevity, we will refer to all members of Pectinoidea as "scallops" in this report). All scallops so far examined have eyes lined with a concave spherical mirror that reflects focused light onto a pair of retinas, as well as a lens that is believed to help correct for spherical aberration caused by the mirror (Land 1965).

To test our hypothesis, we examined eye morphology by immunofluorescent labeling and confocal microscopy in the swimming scallops *Amusium balloti* (Bernardi, 1861; Fig. 1), *Placopecten magellenicus* (Gmelin 1791), *Argopecten irradians* (Lamarck, 1819), *Chlamys hastata* (Sowerby, 1842), and *Chlamys rubida* (Hinds, 1845) and the sessile scallops *Crassadoma gigantea* (Gray, 1825) and *Spondylus americanus* (a spondylid; Hermann, 1781). We calculated inter-receptor angle (a measure of optical resolution) and optical sensitivity for each species and explored the relationships between these

calculations and ecological factors such as a scallop's swimming ability, preferred substrate type, and range of habitat depth.

Figure 6: The left valves of the scallops studied in Chapter 3

Shown above are the left valves of the scallop species examined in this study. Pictured are the swimming scallops Amusium balloti (A), Placopecten magellenicus (B), Argopecten irradians (C), Chlamys hastata (D), and Chlamys rubida (E) and the sessile scallops Crassadoma gigantea (F) and Spondylus americanus (G). The scale bar represents 1 cm.

3.2 Scallop eye morphology materials and methods

3.2.1 Specimen collection and fixation

Four specimens apiece of *Argopecten irradians* and *Placopecten magellenicus* were obtained from Beaufort, NC, USA and Woods Hole, MA, USA, respectively. Three specimens of *Spondylus americanus* were obtained from the Florida Keys (FL, USA), a single specimen of *Amusium balloti* was obtained from Australia's Great Barrier Reef, and single specimens of *Chlamys hastata*, *Chlamys rubida*, and *Crassadoma gigantea* were obtained from Friday Harbor, WA, USA. Animals were anesthetized in a 3% $MgCl_2$ solution prior to dissection. Excised eyes were fixed in buffered 4% formaldehyde for between two and twelve hours and then washed three times in PBTw, a buffer solution containing the mild detergent Tween 20™. Samples were next rinsed three times in 70% ethanol and stored in 70% ethanol, except for *C. hastata*, *C. rubida*, and *C. gigantea* tissue, which was rinsed and stored in 100% methanol. All samples remained in alcohol for less than two months before measurements were taken. Except for *C. gigantea*, in which all eyes were of nearly equal size, all examined species had both large and small mantle eyes. Only large eyes were used for measurements. Eyes from the ventral (middle) section of the left valve mantle margin were used for measurements whenever possible.

3.2.2 Sample preparation and measurements

For sectioning, fixed scallop eyes were cut in half with a scalpel blade. Eyes were only used for measurements if a clean, perpendicular cut was made through the center of the lens. Sectioned eyes were stained with fluorescently-labeled antibodies to alpha-tubulin, a microtubule protein, and Hoescht 33245, a DNA-binding fluorescent dye. Eyes were incubated in the anti-alpha-tubulin primary at 4º C overnight and in an Alexa Flour 488 secondary for 4 hours at room temperature. Both the primary and secondary antibodies were diluted 1:500 in a blocking buffer which contained BSA powder and goat serum diluted in 1 x PBS. After alpha-tubulin staining, 10 mg/mL Hoescht 33245 stock solution, diluted 1:100 in 1x PBS, was used to stain the eyes for five minutes. Stained eye sections were mounted in glycerol on standard microscope slides. Cover-slips were applied with modeling-clay feet so as not to disturb natural eye morphology. Eyes were mounted so that pupils and cover-slips were perpendicular. Images were obtained with the 10 or 20x objective of a Zeiss 510 LSM inverted confocal microscope housed in the Duke University Light Microscopy Core Facility. Illumination was provided by 405, 488, and 561 nm lasers. Images were processed on a Zeiss-built Fujitsu Siemens Intel Xeon CPU using Zeiss LSM 510 version 4.2 software.

Eye internal diameter, focal length (f), pupil diameter (D), photoreceptor spacing for distal (s_d) and proximal (s_p) retinas, and rhabdom length for the photoreceptors of the distal (l_d) and proximal (l_p) retinas were measured for each eye section. The image in a scallop eye is formed by the reflection of light off a concave spherical mirror (Land 1965), making focal length (f) equal to half the radius of mirror curvature (Halliday and Resnick 1988). We measured focal length by manually fitting circles to the mirror layer at the central section of each eye (the section in which the apparent curvature of the mirror matches its actual curvature), then calculating half the radius of the circle. Pupil diameter (D) was estimated from cornea diameter. Image stacks obtained with the microscope's 20x objective allowed us to study the morphology of individual photoreceptors from each eye's distal and proximal retina. Photoreceptors were distinguished from other cells by their strong staining by alpha-tubulin antibodies. Photoreceptor spacing (s) was calculated as the distance from the center of one photoreceptor's rhabdom to the center of the rhabdom of its nearest neighbor.

3.2.3 Calculations of optical resolution and sensitivity

We calculated inter-receptor angle for the distal ($\Delta\phi_d$) and proximal ($\Delta\phi_p$) retinas of each scallop eye section using the formulas:

$$\Delta\varphi_d = \tan^{-1}\left(\frac{s_d}{f}\right) \cong \frac{s_d}{f}$$

Equation 1: Scallop distal retina inter-receptor angle

and

$$\Delta\varphi_p = \tan^{-1}\left(\frac{s_p}{f}\right) \cong \frac{s_p}{f}$$

Equation 2: Scallop proximal retina inter-receptor angle

In the equations above, s_d and s_p correspond to photoreceptor spacing for the distal and proximal retinas and f is focal length (Land and Nilsson 2002). Rhabdoms were contiguous in the eyes of all species examined, letting $\Delta\phi_d = \Delta\rho_d$ and $\Delta\phi_p = \Delta\rho_p$, where $\Delta\rho_d$ and $\Delta\rho_p$ are the acceptance angles of the photoreceptors of the distal and proximal retina, respectively. The optical sensitivities of distal (S_d) and proximal (S_p) retinas were calculated using the formulas:

$$S_d = (\frac{\pi}{4})^2 D^2 (\Delta\rho_d)^2 P_d (1 - P_d)(1 - P_p)^2$$

Equation 3: Scallop distal retina optical sensitivity

and

$$S_p = (\frac{\pi}{4})^2 D^2 (\Delta\rho_p)^2 P_p (1 - P_d)(1 - P_p)$$

Equation 4: Scallop proximal retina optical sensitivity

In equations 3 and 4, D is pupil diameter and the terms $(1 - P_d)(1\text{-}P_p)^2$ and $(1 - P_d)(1\text{-}P_p)$ account for the light that is absorbed as it passes through both retinas on the way to the mirror and through the proximal retina on the way back to the distal retina. This absorption of unfocused light effectively lowers sensitivity in the scallop eye. P_p and P_d are the fractions of light absorbed by the photoreceptors during one pass through the proximal and distal retinas, respectively. P_p and P_d were calculated using the formula:

$$P_{abs} = \frac{\int_{400}^{700} I(\lambda)(1 - e^{-kA(\lambda)l}) d\lambda}{\int_{400}^{700} I(\lambda) d\lambda}$$

Equation 5: Light absorption by scallop photoreceptors

In equation 5, $I(\lambda)$ is ambient irradiance (Kirschfeld 1974; Land 1981; Warrant and Nilsson 1998), k (=0.0067) is the absorption coefficient of the rhabdom, and l is rhabdom length (measured for distal or proximal photoreceptors where appropriate). For our calculations, we assumed that scallops live in environments dominated by green light, appropriate given estimated habitat depths in coastal waters (Table 1). We also assumed that scallops eyes have peak sensitivity at 480 nm, based on evidence from behavioral trials (Cronly-Dillon 1966).

3.2.4 Statistical analysis

Measurements of eye internal diameter, focal length (f), pupil diameter (D), photoreceptor spacing for the distal (s_d) and proximal (s_p) retinas, rhabdom length for the photoreceptors of the distal (l_d) and proximal (l_p) retinas, inter-receptor angle of the distal ($\Delta\phi_d$) and proximal ($\Delta\phi_p$) retinas, and optical sensitivity of the distal (S_d) and proximal (S_p) retinas were compared between *Placopecten magellenicus*, *Argopecten irradians*, and *Spondylus americanus* using Tukey-Kramer HSD multiple comparison tests (Zar 1999). Comparisons were not made between measurements for other scallop species due to insufficient sample sizes (Table 1).

3.3 Scallop eye morphology results

3.3.1 Comparative scallop eye morphology

Scallop eyes were located on the middle mantle fold at the distal ends of short tentacles. These eye-bearing tentacles lined the edges of the right and left valves from one end of the hinge to the other and were interspersed with longer, extensible sensory tentacles in all species. The eyes were surrounded by a pigmented epithelium, which was brown in *Amusium balloti*, blue in *Argopecten irradians*, and black in *Placopecten magellenicus*, *Chlamys hastata*, *Chlamys rubida*, *Crassadoma gigantea*, and *Spondylus americanus*. The corneas were composed of a monolayer of nucleated cells (Fig. 2). Corneal cells were cuboidal in all species except for *C. gigantea*, in which they were columnar. Lenses were cellular in all species examined. The lenses of *A. balloti* and *P. magellenicus* were the largest observed and had front curvatures that were approximately hyperbolic, causing them to resemble those described (Land 1965) for *Pecten maximus* (Linnaeus, 1758). In contrast, the lenses of the other five species were relatively small and had front curvatures that were relatively spherical (Fig. 2). All scallop eyes contained the distinctive double retina described in detail in a number of past reports (Barber et al. 1966; Dakin 1910). Cells completely negative for alpha-tubulin staining were present in scallop retinas along with the photoreceptor cells. We suspect

that these non-staining cells were glial cells (Barber et al. 1966), which generally serve to support neural cells and are not known to act in signal processing. The backs of all eyes were lined with a concave spherical mirror, again as previously described (Land 1965). Underlying the mirror was a red pigment layer. Contrary to past reports, we found that a cavity was present between the mirror and the retinas in all scallop species examined (Fig. 2). Cavity size varied greatly between species. Relatively small cavities were found in *A. balloti* and *P. magellenicus*, resulting in eyes that were morphologically similar to those of *P. maximus* (Land 1965), while larger cavities were present in the eyes of the other five species. Dissection and whole-mount microscopy revealed that the cavity was filled with a clear fluid.

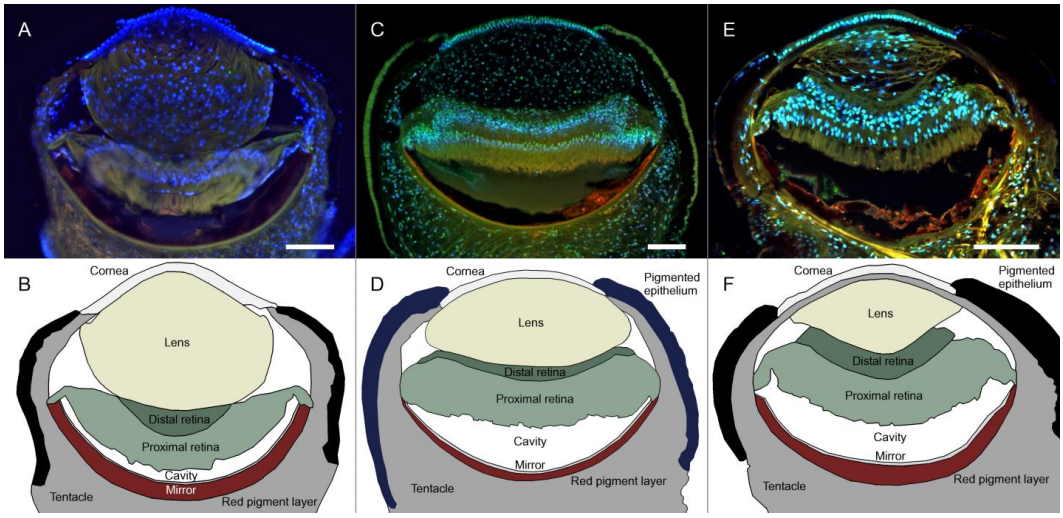

Figure 7: Images and diagrams of sectioned scallop mantle eyes

Shown here are mantle eye sections from the swimming scallops *Placopecten magellenicus* (A) and *Argopecten irradians* (C), imaged under a 10x confocal objective, and the sessile scallop *Spondylus americanus* (E), imaged under a 20x objective. Eyes were stained with Hoescht dye, causing cell nuclei to appear blue, and alpha-tubulin, causing microtubules to appear green. The pigment layer underneath the mirror appears red both in the images and *in vivo*. The diagrams (B and D and F) correspond to the confocal images above and are labeled accordingly. The scale bars represent 100 μm.

3.3.2 Scallop optical resolution and sensitivity

Eye internal diameter, focal length (f), pupil diameter (D), photoreceptor spacing for distal (s_d) and proximal (s_p) retinas, and rhabdom length for the photoreceptors of the distal (l_d) and proximal (l_p) retinas varied between scallop species (Table 1). Swimming species generally had larger eyes, larger pupils, longer focal lengths, and proximal retina photoreceptors that were more closely spaced (Table 1). Rhabdom length and distal retina photoreceptor spacing did not appear to correlate with whether a species could swim or not (Table 1). Our calculations indicated that distal and proximal retina inter-receptor angle and optical sensitivity also differed between scallop species (Table 1). Swimming species tended to have smaller distal and proximal retina inter-receptor angles than sessile species (Table 1). Optical sensitivity did not appear to be related to scallop swimming ability.

Table 3: Scallop eye morphology measurements and calculations

Morphological measurements and calculations of optical sensitivity and inter-receptor angle, a measure of optical resolution, for the eyes of the swimming scallops *Amusium balloti*, *Placopecten magellenicus*, *Argopecten irradians*, *Chlamys hastata*, and *Chlamys rubida* and the sessile scallops *Crassadoma gigantea*, and *Spondylus americanus*. Values represent mean ± 2SE. Measurements and calculations for *P. magellenicus*, *A. irradians*, and *S. americanus* (appearing in bold columns) were compared statistically using Tukey-Kramer HSD multiple comparison tests. Significant differences between one species and the other two are denoted by * (if $\alpha = 0.05$) or ** (if $\alpha = 0.01$). Information regarding shell height, substrate type, habitat depth, and attachment type was adapted from Brand (2006), Lauzier and Bourne (2006), and personal observation (D.I.S.). Shell height refers to the dorsal-ventral length of the valves.

	A. balloti (*n* = 2)	***P. magellenicus* (*n* = 16)**	***A. irradians* (*n* = 16)**	*C. hastata* (*n* = 2)	*C. rubida* (*n* = 2)	*C. gigantea* (*n* = 3)	***S. americanus* (*n* = 16)**
inter-receptor angle, distal retina $\Delta\varphi_d$ (°)	1.7 ± 0.1	**2.5 ± 0.2****	**2.1 ± 0.1****	2.5 ± 0.5	2.8 ± 0.1	3.0 ± 0.1	**3.6 ± 0.2****
inter-receptor angle, proximal retina $\Delta\varphi_p$ (°)	1.0 ± 0.1	**1.3 ± 0.1****	**1.9 ± 0.2****	2.5 ± 0.5	2.7 ± 0.3	3.2 ± 0.2	**4.5 ± 0.3****
optical sensitivity, distal retina S_d (μm² • sr)	4 ± 1	**8 ± 1****	**5 ± 1**	8 ± 5	6 ± 1	2 ± 1	**5 ± 1**
optical sensitivity, proximal retina S_p (μm² • sr)	3 ± 1	**4 ± 1****	**11 ± 3****	21 ± 10	10 ± 6	7 ± 3	**19 ± 4****
eye internal diameter (μm)	570 ± 30	**550 ± 40****	**670 ± 40****	480 ± 20	480 ± 20	450 ± 20	**370 ± 30****
pupil diameter *D* (μm)	390 ± 12	**350 ± 30***	**400 ± 30***	360 ± 40	310 ± 10	170 ± 20	**230 ± 20***
focal length *f* (μm)	170 ± 10	**150 ± 10****	**180 ± 10****	140 ± 20	110 ± 10	110 ± 10	**95 ± 6****
photoreceptor spacing, distal retina s_d (μm)	5	**6.4 ± 0.3**	**6.4 ± 0.2**	6	5.2 ± 0.4	6	**5.9 ± 0.3***
photoreceptor spacing, proximal retina s_p (μm)	3	**3.3 ± 0.2****	**5.8 ± 0.2****	6	5	6.3 ± 0.3	**7.4 ± 0.3****
rhabdom length, distal retina l_d (μm)	15 ± 2	**19 ± 3****	**12 ± 2**	20	12 ± 1	14 ± 4	**13 ± 1**
rhabdom length, proximal retina l_p (μm)	25	**33 ± 6**	**30 ± 10**	45	20 ± 6	40	**25 ± 2**
shell height of specimens examined (cm)	?	**10**	**6**	6	5	10	**9**
preferred substrate	sandy	**sandy**	**sandy**	rocky	rocky	rocky	**rocky**
habitat depth (m)	10-75	**20-110**	**1-12**	2-150	1-200	1-80	**1-150**
attachment type	unattached	**unattached**	**unattached**	byssal	byssal	cemented	**cemented**

3.4 Scallop eye morphology discussion

3.4.1 Scallop eye morphology

Our study revealed several new aspects of scallop eye morphology. First, we found that lens size and shape varied among scallop species (Fig. 2). The lenses of *Amusium balloti* and *Placopecten magellenicus* had shapes similar to those described for *Pecten maximus* (Land 1965). The front of the *P. maximus* lens appears to be curved in such a way as to correct for spherical aberration caused by the reflection of light off the mirror (Land 1965), a function we will also attribute, tentatively, to the lenses of *A. balloti* and *P. magellenicus*. The lenses of the other five species appeared to have front curvatures that were relatively spherical, an indication that they may do little to correct for spherical aberration caused by the mirror. We are currently exploring the functional consequences of these different lens shapes and the phylogenetic distribution of lens types among a wide range of scallop species.

Second, we consistently noted a fluid-filled cavity between the proximal retina and the mirror in the eyes of all seven scallop species examined (Fig. 2). This cavity ranged in size between species. Small cavities were found in the eyes of *Amusium balloti* and *Placopecten magellenicus*, resulting in eyes that closely resembled those of *Pecten maximus* (Land 1965). Conversely, a large cavity was found between the proximal retina

and the mirror in the eyes of the other five scallop species examined. The optics of the scallop eye are greatly influenced by the size of the cavity that exists between the proximal retina and the mirror. Following Land's analysis (1965) of the optics of *Pecten maximus,* which has eyes with a small cavity, it appears that focused light likely falls on the distal retina in the morphologically similar eyes of *Amusium balloti* and *Placopecten magellenicus*. Alternately, due to the presence of a large cavity, it appears likely that focused light falls on the proximal retina in the eyes of the other scallop species we examined. We would be tempted to conclude that focused images simply fall on different retinas in different scallop species, but we have also found that photoreceptor spacing is tighter in the proximal retinas of *A. balloti* and *P. magellenicus* than it is in their distal retinas (Table 1). This is not consistent with a model in which the proximal retinas of *A. balloti* and *P. magellenicus* fail to receive focused light. We also found that *A. balloti* and *P. magellenicus* have the most tightly packed proximal retina photoreceptors of any of the species examined (Table 1), which again suggests that their proximal retinas may be involved in image formation. As an explanation for these inconsistencies, we speculate that scallop eyes are optically dynamic structures that can alternately focus light on to either of the two retinas through slight changes in shape. We are, at this time, exploring possible mechanistic bases for such a process.

3.4.2 Swimming ability and scallop vision

An analysis of scallop visual capabilities provided evidence that swimming scallops have more acute vision than non-swimmers and that the best swimmers have the most acute vision (Fig. 7). Among the scallops included in this study, *Amusium balloti* and *Placopecten magellenicus* were the strongest swimmers, capable of moving at speeds of up to 100 cm/s (Joll 1989) and 67 cm/s (Brand 2006), respectively. These scallops had proximal retina inter-receptor angles of around 1º, the smallest of any we calculated (Table 2). Weaker swimmers like *Argopecten irradians*, able to swim at speeds of 40 cm/s (Brand 2006), had proximal retina inter-receptor angles between 2-3º (Table 1). Our findings in this case concur with past morphological studies that found that *Pecten maximus*, a scallop with swimming abilities comparable to those of *A. irradians* (Brand 2006), had an optical resolution of about 2º. Sessile scallops, which cement to their substrate in a manner similar to oysters (Lauzier and Bourne 2006), had the largest proximal retina inter-receptor angles observed, at around 3-5º (Table 1). Proximal retina inter-receptor angle diversity was a product of differences in both focal length and photoreceptor spacing. For example, tighter photoreceptor packing was largely responsible for *A. balloti* having a smaller proximal retina inter-receptor angle than *A. irradians*, but longer focal length was responsible for *A. irradians* having a smaller proximal retina inter-receptor angle than *Chlamys hastata.* Factors other than swimming

ability may also help explain why some scallop species have better optical resolution than others. For example, scallops from sandy substrates tend to have better vision and be better swimmers than those from rocky habitats (Table 1). Another important caveat is that our methods have led us to estimate the theoretical maximum of visual acuity in each scallop species. Neural processes, like spatial summation, and optical imperfections, such as spherical aberration, may lead to scallops having actual visual acuities that are below these estimates (Land and Nilsson 2002). However, behavioral (Buddenbrock and Moller-Racke 1953) and electrophysiological (Land 1966) studies on *Pecten maximus* provide evidence that actual scallop visual acuity is close to the theoretical maximum derived from focal length and photoreceptor spacing. This suggests that our estimates of inter-receptor angle likely point towards true functional differences between the eyes of mobile and immobile scallop species. Finally, interspecific differences in inter-receptor angle will have little consequence if focused light falls on different retinas in different scallop species, a possibility that we address in detail above.

Distal retina inter-receptor angles, ranging from 1.7 ± 0.1º for *Amusium balloti* to 3.6º for *Spondylus americanus*, only varied two-fold between species, as opposed to the four-fold difference observed between proximal retina inter-receptor angles (Table 1). Distal retina inter-receptor angle also correlated with scallop swimming ability, but not

as strongly as proximal retina inter-receptor angle. For example, proximal retina inter-receptor angle was larger in *P. magellenicus* than it was in *A. irradians*, despite *P. magellenicus* being the stronger swimmer (Brand 2006). Perhaps more tellingly, variation in distal retina inter-receptor angle was largely a product of interspecific differences in focal length, not photoreceptor spacing. Distal retina photoreceptor spacing fell between 5 and 6.5 μm in all species and, unlike proximal retina photoreceptor spacing, a relationship between this measure and a scallop species' swimming ability was not suggested by the data (Table 1).

3.4.3 The subfunctionalization of scallop retinas

It has been suggested that the two scallop retinas perform different visual functions (Land 1966; Wilkens 2006), in part due to evidence that the retinas operate via different opsins and signal-transduction pathways (Kojima et al. 1997) and that the neurons of the distal retina hyperpolarize in response to light, while those of the proximal retina depolarize (Hartline 1938; Land 1966; McReynolds and Gorman 1970). This proposal is supported by our evidence that proximal retina photoreceptor spacing may depend on a scallop species' swimming ability, while distal retina photoreceptor spacing varies little between species (Table 1). This implies that scallop proximal retinas may be involved in visual tasks more important to swimming species, such as those

relating to the detection of preferred habitat, and that the distal retinas are likely involved in tasks of equal importance to both swimming and sessile species, such as predator detection.

Further support for functional differentiation of this sort comes from indications that scallop proximal retinas are better at gathering information about relatively static environmental features (Land 1966), like the eelgrass beds towards which *A. irradians* has been found to swim (Hamilton and Koch 1996), while the distal retinas are better at detecting movement, such as that by potential predators.

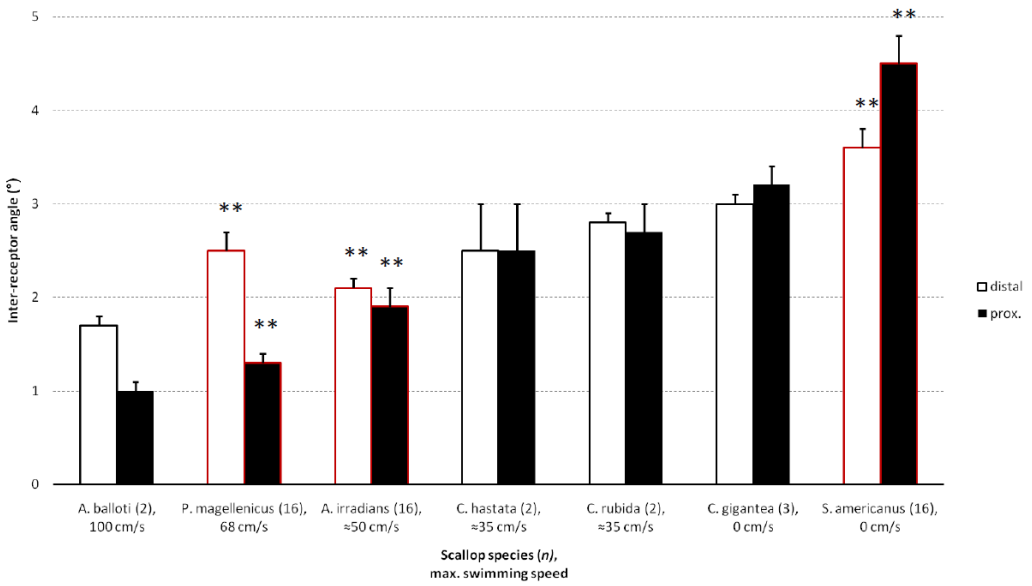

Figure 8: A comparison of scallop swimming speed and optical resolution

Scallop swimming speeds estimated from Brand (2006). ** = significant difference between all species marked, as figured by a multiple-comparison Tukey-Kramer HSD test ($\alpha = 0.01$).

3.4.4 Visual processing in scallops

Unrecognized differences between the eyes of mobile and immobile species have contributed to arguments that swimming scallops do not visually detect preferred habitats, as has the fact that scallops lack a centralized nervous system (Morton 2000). While it is true that scallops do not process much visual information in their brain, their visceroparietal ganglion (VPG) contains optic lobes that likely give these animals the neural capacity to convert a range of visual inputs into behavioral output (Wilkens 2006). It has been noted that information from the proximal retina elicits greater activity in the VPG's optic lobes than information from the distal retina (Wilkens and Ache 1977), a finding seemingly at odds with the claim that focused light only falls on the scallop distal retina (Land 1965). As a potential solution to this problem, we suggest that focused light may fall on the proximal retina in at least some scallop species. This suggests that previously unrecognized interspecific variation may account for inconsistencies between past reports. It also suggests that the scallop optic lobes may, at least in some cases, process visual information from the proximal retina for the sake of complex behavioral tasks like habitat detection.

3.4.5 Scallop optical sensitivity

Scallop optical sensitivity, like optical resolution, differed between retinas and between species (Table 1). However, unlike optical resolution, optical sensitivity did not appear to correlate with swimming ability or, as might be expected, with habitat depth (Table 1). Given that irradiance values in scallop habitats may vary over several orders of magnitude, depending on tide conditions and time of day, the differences we observed between optical sensitivities may have only minor functional consequences for the species examined in this study.

In conclusion, we found that eye morphology varied among scallop species and that swimming scallops tend to have better vision than sessile scallops. This latter discovery is consistent with our hypothesis that mobile scallops may visually detect preferred habitats. We also found evidence that scallop distal and proximal retinas may be functionally differentiated.

3.5 Future directions in scallop eye morphology

We are currently working to clarify the relationship between vision and swimming ability in scallops. To do so, we will survey the eyes of a wider range of scallop species. This will expand our phylogenetic coverage and improve the statistical support for our hypothesis that highly mobile scallops have better vision than less mobile or sessile species. We will also compare the eyes of scallops to those of related bivalves, such as limids (*e.g. Lima scabra*). This may tell us more about how the unique eyes of scallops have evolved; limid eyes have a double-retina similar to the one in scallop eyes (Mpitsos 1973), but some limid eyes lack a lens and none are known to use a focusing mirror like a scallop's for image-formation.

The first priority of our future work on scallop eye morphology will be to gather live images of the inside of scallop eyes. This will tell us whether a cavity actually exists between the mirror and proximal retina of some scallop species, or whether this cavity is an artifact caused by fixation and sectioning. Live imaging may also give us information about scallop lens shape, which will let us perform more detailed analyses on the ways that different scallop species may or may not correct for the spherical aberration caused by their focusing mirror. Live imaging will also help us generate new hypotheses regarding how scallops may be able to form focused images on both of their retinas.

4. Spectral sensitivity of scallop eyes

4.1 Introduction to scallop optics

Scallop eyes are unlike any other in the animal world (Fig. 1A). They are single-chambered and contain a large lens, but they do not function like the camera eyes that they superficially resemble. Instead of using a lens to form images, the scallop eye uses a concave spherical mirror (Land 1965). The main purpose of the scallop lens is to correct for the spherical aberration that this mirror produces (Land 1965). Aside from the deep-sea fish *Dolichopteryx longipes* (Wagner et al. 2009), scallops are the only animals known to use mirrors for image formation. Scallops also have an unusually arranged pair of retinas (Fig. 1B). Other animals with multiple retinas, including the alciopid worms *Vanadis* and *Torrea* (Wald and Rayport 1977), the deep-sea squid *Bathyteuthis* (Chun 1903), and several species of mesopelagic fish (Collin et al. 1997; Warrant and Locket 2004), have laterally arranged retinas that gather information from different visual fields. Scallops, in contrast, have a distal and proximal retina arranged as a stack (Fig. 1B). In this way, scallop eyes resemble the multibank retina eyes of jumping spiders such as *Phidippus* (Land 1969), the firefly squid *Watensia* (Michinomae et al. 1994), and some deep sea teleosts (Denton and Locket 1989). Multibank retinas offer several potential advantages over single-stack retinas: they improve rates of photon

capture within eyes by increasing optical path length (Warrant and Locket 2004); they can provide color vision, even in the absence of multiple visual pigments, by using distal photoreceptors as spectral filters for more proximal receptors (Warrant and Locket 2004); and, finally, they may help compensate for longitudinal chromatic aberration (LCA) produced by a lens (Blest et al. 1981; Kroger and Gislen 2004).

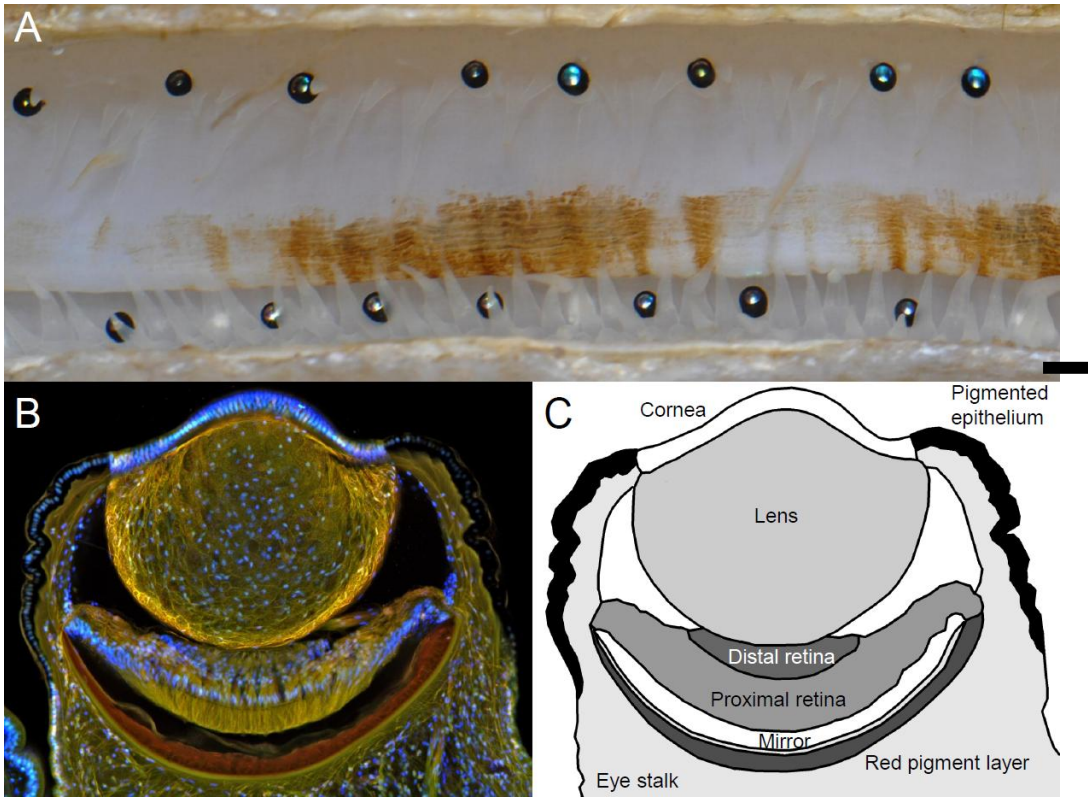

Figure 9: The eyes of the sea scallop *Placopecten magellenicus*

Shown here are the eyes of the sea scallop *Placopecten magellenicus*. (A) Eyes arrayed along the valve mantle margins of a live *P. magellenicus*. The scale bar represents 1 mm. (B) Cross-section of an eye from *P. magellenicus* under a 10x confocal objective. The sample was stained with Hoescht dye, causing cell nuclei to appear blue, and alpha-tubulin, causing microtubules to appear green. The pigment layer underneath the mirror appears red in the image and *in vivo*. The scale bar represents 100 μm. (C) A labeled diagram corresponding to (B).

4.2 Spectral sensitivity and scallop optics

Molecular evidence suggests that the two sets of photoreceptors in the scallop eye express different visual pigments (Kojima et al. 1997). If these visual pigments have different wavelengths of peak maximum absorbance (λ_{max}), scallop vision may be enhanced in one of several (not necessarily exclusive) ways. If we consider scallop eyes as multi-retina eyes, having proximal and distal photoreceptors that differ in λ_{max} may make the two scallop retinas better suited for specific tasks. Many aquatic animals have visual pigments with a λ_{max} that closely matches the dominant wavelength of down-welling light in their environment (Clarke 1936; Munz 1958). This is ideal for a number of tasks, but, under some conditions, a visual pigment with a λ_{max} offset from this peak may be more useful for detecting reflective objects (Lythgoe 1968).

Alternately, if we consider scallop eyes as multibank retina eyes, visual pigments that differ in λ_{max} may provide a different set of potential advantages. First, two different visual pigments may grant scallops dichromatic vision. It is unlikely that they could ever do so, however. There is no evidence that information received separately by the two scallop retinas is integrated within the scallop eye or optic lobes, a necessary event if these animals are to possess color vision in any conventional sense (Spagnolia and Wilkens 1983; Wilkens and Ache 1977).

Second, differences in the λ_{max} of scallop visual pigments may limit the amount of self-screening that occurs within the scallop eye. Self-screening occurs in scallop eyes because the focused light that reaches each retina is modified by having passed, unfocused, through both sets of photoreceptors on the way to the mirror and then back through the proximal receptors on the way to the distal receptors. If scallop visual pigments differ in λ_{max}, the amount that one retina screens the other will be decreased, thereby increasing the total amount of focused light available for absorption by each set of photoreceptors.

Third, differences in the spectral sensitivities of the two scallop retinas may compensate for longitudinal chromatic aberration (LCA) caused by the scallop lens. All known biological lenses have higher refractive indices at shorter wavelengths of light than at longer wavelengths, a property that causes them to bend short wavelengths more sharply than long wavelengths (Kroger 2000). In camera eyes, the outcome of LCA is that shorter and longer wavelengths have focal planes that are relatively closer and further from the lens, respectively (Fig 2A). The mirror in the scallop eye does not produce chromatic aberration, but it reverses the pattern described above by folding light paths within the scallop eye. Because of the mirror, short wavelengths are focused further from the scallop lens (and closer to the mirror) than longer wavelengths (Fig. 2B). We hypothesize that scallops may limit the effects of chromatic aberration by having a

visual pigment in their distal retina that has a longer λ_{max} than the pigment in their proximal retina. In this scenario, both scallop retinas are relatively sensitive to the focused wavelengths of light that they receive from the mirror, and relatively insensitive to unfocused light.

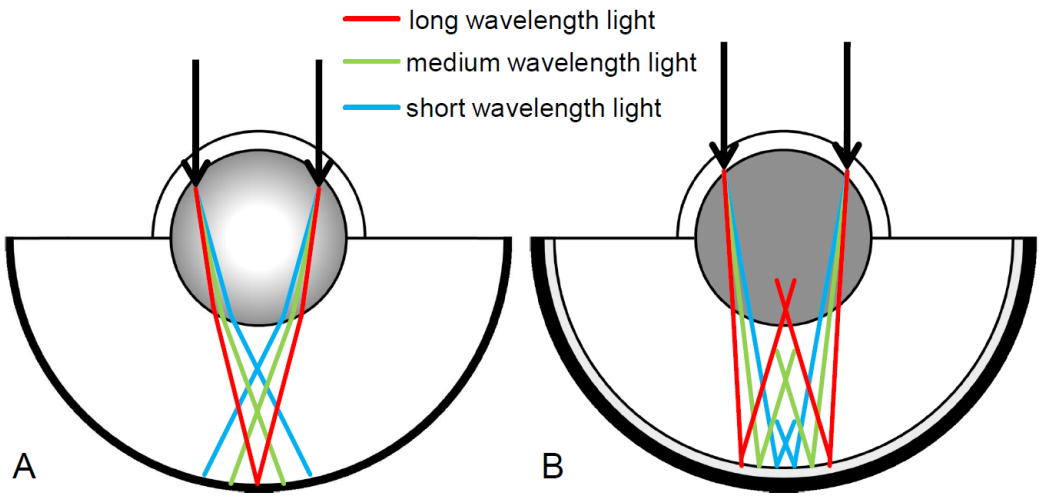

Figure 10: Chromatic aberration in different types of eye

Examples of longitudinal chromatic aberration in A) a camera eye, like those of fish or cephalopods, in which there is a lens with a high refractive index, a retina at the back of the eye, and no image-forming mirror and B) a scallop eye in which there is a lens with a low refractive index and an image-forming, concave spherical mirror overlying a pigment layer at the back of the eye. In a camera eye, longitudinal chromatic aberration (LCA) causes shorter (bluer) wavelengths to be focused closer to the lens than longer (redder) wavelengths. Assuming that the scallop lens, like all biological lenses, produces LCA, shorter wavelengths come into focus further away from the lens than longer wavelengths.

4.3 Spectral sensitivity and scallop environments

In the following report, we use microspectrophotometry (MSP) to measure the absorbance spectra of photoreceptors from the distal and proximal retinas of the deep-dwelling (20 - 110 m) sea scallop *Placopecten magellenicus* and the shallow-dwelling (1-12 m) bay scallop *Argopecten irradians* (Brand 2006). We chose these species so that we could explore the relationship between a scallop's light environment and its spectral sensitivity. Due to higher concentrations of phytoplankton, inshore water tends to be greener than offshore water (Jerlov 1976; Loew and McFarland 1990). Water also acts as a spectral filter that absorbs long wavelengths, so water generally gets bluer with depth as well (Jerlov 1976; Tyler and Smith 1970). The visual pigments of marine animals tend to reflect these environmental differences; λ_{max} is often shifted towards longer (greener) wavelengths in species from shallow coastal water and towards shorter (bluer) wavelengths in species from deeper offshore water (Denton and Warren 1957; Lythgoe 1972; Munz 1958; Partridge 1990). We therefore expect that scallops from inshore and offshore environments will have visual pigments with λ_{max} values that are consistent with this well-established pattern, despite the optical complications posed by their unique, double-retina, concave mirror eyes.

4.2 Spectral sensitivity materials and methods

4.2.1 Specimen collection and care

Adult scallops of the species *Placopecten magellenicus* and *Argopecten irradians* were obtained from Woods Hole, MA, USA (41.53°N, 70.66°W) and Smyrna, NC, USA (34.76°N, 76.53°W), respectively. Specimens of *P. magellenicus* were delivered to Duke University on 12 January 2009 and were kept in a 200 liter aquarium, which was initially set at 10º C and was brought up to 20° C over a two-week period. Specimens of *A. irradians* were transported to Duke University (Durham, NC, USA) on 6 February 2009 and kept at 20° C in a 950 liter flow-through seawater system. Both aquaria were kept under natural light and salinity was maintained at 32 ‰ (Instant Ocean sea salt, Aquarium Systems Inc., Mentor, OH, USA). Three adults of each species were transported by car to Ellis Loew's lab at Cornell University (Ithaca, NY, USA) on 24 February 2009. There, the animals were split between two 40 liter aquaria and were again kept at 20° C and a salinity of 32 ‰.

4.2.2 Microspectrophotometry

Prior to experimentation, scallops were dark-adapted for 10-12 hours overnight and then dissected under dim red light. The largest eyes from the ventral portion of the

left valve mantle margin were identified, excised with surgical scissors, and placed in small dishes of seawater. Retinas were isolated from the eyes using forceps via the following procedure: Starting at the pupil, a small tear was made down the side of the eye and gentle pressure, exerted from below, was used to expel the lens, followed by the retinas, through the tear. Isolated, intact retinas appeared as a shallow bowl, with the distal receptors forming the concave inner surface and the proximal receptors constituting the convex outer surface. The distal and proximal retinas were loosely attached and were separated from one another in some cases. In other cases, retinas were cut into pieces with a scalpel so that proximal and distal photoreceptors could be observed together more easily. Excised retinas were washed with several changes of seawater to remove any loose pieces of tissue that had attached to them. They were then placed between two cover-slips edged with silicone grease.

Microspectrophotometry was performed using the single-beam, computer-controlled microspectrophotometer (MSP) described in McFarland and Loew (1994). This MSP used a Leitz (Oberkochen, Germany) 180X quartz mirror objective and a Zeiss 100X Ultrafluar (0.85 NA) objective, which was used to focus light for collection by the photomultiplier. A 2 μm X 3 μm beam was used for measurements. Spectra for baseline and sample recordings were taken from 750 to 350 nm and back again at a rate of 100 nm s^{-1} and a wavelength accuracy of 1 nm (McFarland and Loew 1994). To position the

MSP's beam, samples were viewed using infrared illumination and an image converter. Photoreceptors were easily identified within retinas due to their morphology, described for *A. irradians* and *P. magellenicus* by Speiser and Johnsen (2008), as well as their palisade-like arrangement. Proximal and distal receptors were distinguished from one another based on their relative position within the retina (as described above) and their length and width. Proximal receptors, in both species, are longer and more tightly packed than receptors from the distal retina (Speiser and Johnsen 2008) and have ends that are more sharply tapered (personal observation).

Recordings from *A. irradians'* retinas were taken on both 27 February 2009 and 1 March 2009. Retinas from *P. magellenicus* were studied on 28 February 2009 and 2 March 2009. Eyes from freshly dissected animals were used on each day. Recordings were always taken from at least three eyes from the same individual, and 100 – 200 different retinal cells were tested on each of the four days. We found no evidence that visual pigments differed among retinas of the same type from scallops of the same species. Similarly, no differences were found among retinas of the same type taken from different eyes found on the same individual scallop. Also, photo-bleaching, lasting from 30 to 300 s, did not appear to have an effect on the absorbance spectra of any of the visual pigments we examined in this study. We have, therefore, not included difference spectra in our results.

Absorbance spectra for individual photoreceptors were included in calculations of λ_{max} (the wavelength at which the maximum absorbance of a template-derived photopigment best matches the experimental data) if they displayed a single clear peak between 400 and 700 nm. The long wavelength limbs (470-700 nm) of the spectra were fit to an A1 rhodopsin template (Stavenga et al. 1993) via a least squared algorithm implemented using Solver (Excel 2003, Microsoft Inc. Redmond, WA, USA) that varied λ_{max}, peak height, baseline level, and optical path length.

4.2.3 Absorption and self-screening in the scallop eye

The optical configuration of the scallop eye, which involves a tiered double-retina and a mirror, is such that the focused light that reaches each retina is modified by self-screening. This occurs because unfocused light must pass through both sets of receptors before reaching the mirror and back through the proximal receptors on the way to the distal receptors. The fraction of incident light (at one wavelength) that arrives in focus at the proximal retina, modified by the absorption of unfocused, incoming light by the proximal and distal receptors, can be estimated by:

$$V_{prox} = e^{-A_{dist} cl_{dist}} e^{-A_{prox} cl_{prox}} \left(1 - e^{-A_{prox} cl_{prox}}\right)$$

Equation 6: The absorption of light by scallop proximal photoreceptors

In Equation 6, A and l are the normalized absorbance spectrum and the length of the rhabdoms of each set of receptors, respectively, and c is the absorption coefficient of the rhabdoms. In the case of the distal retina, focused light is modified by both the absorption of unfocused incoming light by the proximal and distal receptors and the absorption of unfocused, mirror-reflected light by the proximal receptors:

$$V_{dist} = e^{-A_{dist}cl_{dist}} e^{-A_{prox}cl_{prox}} e^{-A_{prox}cl_{prox}} \quad 1 - e^{-A_{dist}cl_{dist}}$$

Equation 7: The absorption of light by scallop distal photoreceptors

From Speiser and Johnsen (2008), the lengths of proximal and distal rhabdoms in *Placopecten magellenicus* were 33 and 19 μm long, respectively; in *Argopecten irradians*, the corresponding rhabdoms were 30 and 12 μm. The absorption coefficient c was taken to be 0.0067 μm^{-1} (Warrant and Nilsson 1998).

4.2.4 Modeling scallop light environments

Horizontal radiance (*i.e.* the background light in a scallop's field of view) was modeled using measured inherent optical properties and a sophisticated radiative transfer software package (Hydrolight 5.0, Sequoia Scientific). Given the depth profiles

of the absorption coefficient, beam attenuation coefficient, and chlorophyll concentration, the software calculates the underwater radiance distribution as a function of depth and wavelength. The software also takes into account solar elevation and azimuth, atmospheric parameters, bottom reflectance, sea surface conditions, chlorophyll fluorescence, and Raman scattering by the water. The ability of the software to accurately model radiance distributions has been validated by *in situ* measurements of selected radiances and irradiances in numerous studies (Maffione et al. 1998; Mobley et al. 1993; Stramska et al. 2000).

Depth profiles of inherent optical properties for oceanic water (approximately Jerlov oceanic type I) were obtained from Drs. Andrew Barnard, Scott Pegau and Ronald Zaneveld (College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA), who collected them using a dual path, multiband absorption/attenuation meter (ac-9, Wetlabs Inc.) and fluorometer in the Equatorial Pacific (10:05 AM local time, 30 April, 1996; 0°0'N 177°21'W). Absorption and beam attenuation coefficients (at 412, 440, 488, 510, 532, 555, 650, and 676 nm) and chlorophyll concentration were measured at 1 m intervals to a depth of 138 m. Depth profiles of inherent optical properties for coastal water were obtained using an ac-9 deployed at a site 80 km from the coast of Portsmouth, New Hampshire (42°47'N 70°05'W, 11:06 local time, 30 June, 2000). Absorption and beam attenuation coefficients (at 440, 488, 510, 532,

555, and 650 nm) were averaged over 1 m intervals to a depth of 92 m. All data were collected on upcasts to limit artifacts due to bubbles, etc. In addition, discrete samples were collected from 3 depths (1, 20, 40 m), filtered onto Whatman GF/F filters, and extracted overnight in cold 90% acetone for standard fluorometric determination of chlorophyll concentration. Both sets of measurements were corrected for temperature and salinity, and absorption measurements were corrected for scattering errors (Pegau et al. 1997; Pegau and Zaneveld 1994)

These two profiles were input into Hydrolight. In both cases, solar elevation was set at 70°, and the sky was considered to be clear. The sky irradiance was calculated using the Radtran model (Gregg and Carder 1990), and the sky radiance distribution was calculated using the model given in Harrison and Coombes (1988). Both sky models account for atmospheric effects, such as the reddening of the sun as it approaches the horizon, and are well established. Pure water absorption was taken from Pope and Fry (1997), and the scattering phase function was Petzold's average particle (Petzold 1977). Chlorophyll fluorescence was calculated from chlorophyll absorption taken from Prieur and Sathyendranath (1981) and a fluorescence efficiency of 0.02.

For the oceanic environment, we assumed that water was 100 m deep with a dark sediment bottom. This environment was meant to match the continental slope off of St. George's Bank, MA, USA, a site known for its abundant sea scallop populations

(Brand 2006). For the coastal environment, we assumed that the water was 1 meter deep and had a sea grass bottom. This hypothetical environment closely matched the shallow eelgrass (*Zostera*) beds from which we collected specimens of *A. irradians* for this study. In both cases, radiance was calculated from 400-700 nm at 10 nm intervals with an angular resolution of 15° (azimuth) by 10° (elevation).

4.2.5 Estimating scallop quantum catch

We calculated N_o, the number of photons absorbed by a single photoreceptor within its integration time Δt (in sec) when a scallop eye experiences an illumination spectrum of quantal intensity $L_h(\lambda)$ in units of photons sec^{-1} nm^{-1} m^{-2} $steradian^{-1}$. We modeled quantum catch for proximal and distal receptors from the eyes of *Placopecten magellenicus* and *Argopecten irradians* using a formula adapted from Warrant & Nilsson (1998), Warrant (Warrant 1999) and Kelber et al. (2003):

$$N_o = 1.13(\frac{\pi}{4})(\frac{R\pi}{180})^2 D^2 \Delta t \int_{400}^{700} \kappa \tau L_h(\lambda) V(\lambda) d\lambda$$

Equation 8: Quantum catch by scallop photoreceptors

The terms before the integral determine the number of photons that pass through the optics of the scallop eye and reach individual photoreceptors. These parameters

include: the acceptance angle R of scallop photoreceptors, which equals 1.3 and 2.5° for the proximal and distal receptors of *P. magellenicus*, respectively, and 1.9 and 2.0° for the corresponding receptors in *A. irradians* (Speiser and Johnsen 2008); pupil diameter D, which respectively equals 0.035 and 0.04 cm in *P. magellenicus* and *A. irradians* (Speiser and Johnsen 2008); and integration time Δt, which we set at 0.2 seconds for both sets of photoreceptors in both species based on recordings from the scallop *Amusium japonicum* (Kanmizutaru et al. 2005).

The integral term describes the number of photons that will be absorbed in a photoreceptor of spectral sensitivity $V(\lambda)$ viewing an illumination spectrum $L_h(\lambda)$, where λ is wavelength. Spectral sensitivities for scallop proximal and distal receptors were calculated in equations 1.1 and 1.2 above, giving $V_{prox}(\lambda)$ and $V_{dist}(\lambda)$, and the illumination spectra were those previously calculated for the respective oceanic and coastal habitats of *P. magellenicus* and *A. irradians*. The integral is calculated between two wavelength limits: λ_1 and λ_2 (Warrant and Nilsson 1998), which we set at 400 and 700 nm, respectively. The final parameters included are the quantum efficiency of photoreceptor transduction κ and the transmission of scallop optics τ. These values were set at 0.5 and 0.8, respectively, for all scallop photoreceptors modeled. Radiance, for all calculations, was pooled over 10 nm intervals, giving $d\lambda$, the final term in our equation.

Next, we measured how the λ_{max} of scallop visual pigments affected the quantum catch of proximal and distal photoreceptors. To do so, we varied the λ_{max} values used to calculate the normalized absorbance spectra (A) employed in our estimates of V_{prox} and V_{dist} above (see equations 1.1 and 1.2). We then compared the number of photons absorbed by scallop proximal and distal photoreceptors (N_{prox} and N_{dist}) in the context of different pairs of visual pigments. For example, by holding the λ_{max} of a proximal retina constant, we were able to calculate how changes to distal retina λ_{max} affected the quantum catch of both retinas. These steps were repeated for λ_{max} values ranging from 460 to 600 nm, at 5 nm intervals, for proximal and distal photoreceptors from *P. magellenicus* and *A. irradians*. This range of λ_{max} values was chosen because it includes the radiance peaks of the coastal and oceanic environments we modeled. This range of values also covers the λ_{max} of most known visual pigments from marine invertebrate, following a review by Cronin (2006). When calculating quantum catch, we assumed that *P. magellenicus* lives in the oceanic environment we modeled and that *A. irradians* lives in a coastal habitat.

4.3 Spectral sensitivity results

4.3.1 Microspectrophotometry

We found that the wavelengths of peak absorbance (λ_{max}) of scallop visual pigments depended on both the species and the receptor (proximal or distal) that was examined. Visual pigments from the proximal and distal retinas of the sea scallop *Placopecten magellenicus* maximally absorbed shorter (bluer) wavelengths than the pigments from the corresponding retinas in *Argopecten irradians*. We also found that, in both species, receptors of the proximal retina maximally absorbed shorter wavelengths than those of the distal retina (Fig. 3).

Receptors from the proximal retina of *P. magellenicus* contained a visual pigment with an average λ_{max} of 488 ± 1 nm (mean ± S.E; N = 20; Table 1). A representative absorbance spectrum for a photoreceptor of this type may be seen in Fig. 4A, along with a rhodopsin template fit to the right-hand (long wavelength) portion of the curve. For *P. magellenicus* distal receptors, the average λ_{max} was 513 ± 3 nm (N = 26). It therefore appears that these receptors maximally absorb greener light than those of the proximal retina (Fig. 4B).

The proximal receptors of *A. irradians* had an average λ_{max} of 506 ± 1 (N = 21), slightly higher than the λ_{max} recorded for the proximal receptors of *P. magellenicus* (Fig

4C). Receptors from the *A. irradians* distal retina had an average λ_{max} of 535 ± 3 (N = 14). This set of receptors provided absorbance spectra that were the least clean of those observed (Fig. 4D). We suspect that the extra peaks that we observed were caused by photo-stable pigments, as they were far too narrow to have been caused by opsin-based visual pigments.

Table 4: MSP results for *Placopecten magellenicus* and *Argopecten irradians*

Results from the microspectrophotometric (MSP) analysis of proximal and distal retina photoreceptors from the mantle eyes of the scallops *Placopecten magellenicus* and *Argopecten irradians*. Each value seen above refers to λ_{max}, the wavelength of peak absorbance for each photo-pigment studied, and is presented as mean ± SE. Statistical comparisons were made using a Student's t-test (two-tailed). Significant differences (α = 0.01) were found across both rows, which compare homologous retinas between species, and down both columns, which compare different retinas from the same species.

	Placopecten magellenicus	*Argopecten irradians*
Proximal retina λ_{max} (mean ± SE)	488 ± 1 (N = 20)	506 ± 1 (N = 21)
Distal retina λ_{max}, (mean ± 2 SE)	513 ± 3 (N = 26)	535 ± 3 (N = 14)

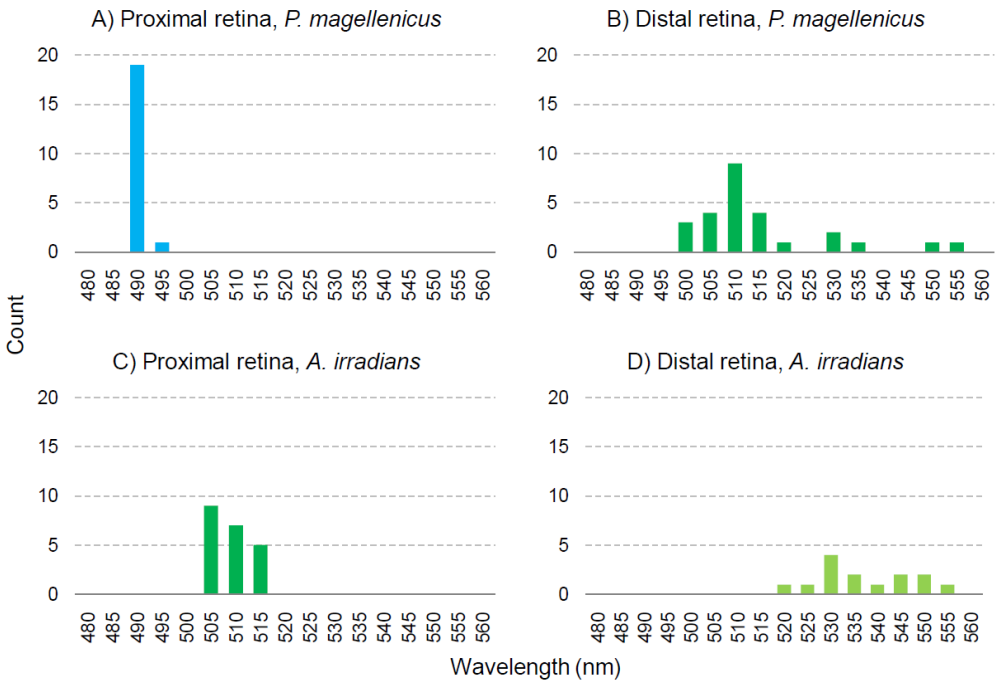

Figure 11: Histograms of MSP recordings

Histograms of the results from the microspectrophotometric (MSP) analysis of individual photoreceptors from the distal and proximal retinas of the sea scallop *Placopecten magellenicus* and the bay scallop *Argopecten irradians*.

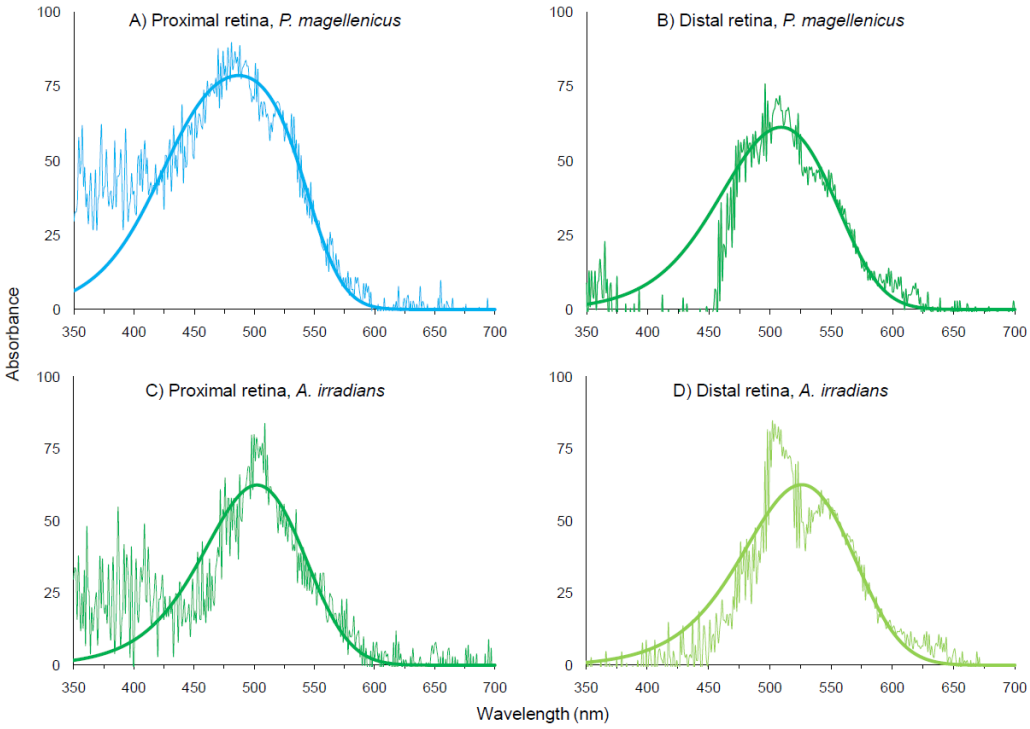

Figure 12: Examples of MSP recordings

Results from the microspectrophotometric (MSP) analysis of individual photoreceptors from the distal and proximal retinas of scallop eyes. One recording is displayed for each of the two retinas from the scallops *Argopecten irradians* and *Placopecten magellenicus*. The graphs present raw MSP data (baseline stripped) for a single representative photoreceptor from the retina and species indicated. The raw data is overlain with a smooth, best-fit curve derived from an A1 rhodopsin template. (A) Proximal retina photoreceptor from *P. magellenicus* (λ_{max} = 487 nm). (B) Distal retina photoreceptor from *P. magellenicus* (λ_{max} = 509 nm). (C) Proximal retina photoreceptor from *A. irradians* (λ_{max} = 502 nm). (D) Distal retina photoreceptor from *A. irradians* (λ_{max} = 526 nm). The values presented here do not necessarily match those seen in Table 1, which presents the mean λ_{max} for each set of photoreceptors.

4.3.2 Absorption spectra of scallop retinas after accounting for self-screening

The absorption spectra of proximal and distal photoreceptors were influenced by self-screening within the scallop eye. Once we accounted for self-screening, the peak sensitivities of scallop proximal receptors shifted slightly towards shorter wavelengths, while the λ_{max} values of the distal receptors showed a more dramatic shift towards longer wavelengths. As a result, self-screening increased the differences in spectral sensitivity between scallop proximal and distal receptors. For example, proximal receptor λ_{max} shifts from approximately 488 to 485 nm in *P. magellenicus* and from 506 to 504 nm in *A. irradians* (Fig. 5). Larger shifts are seen in the distal receptors, with λ_{max} moving from 513 to 528 nm in *P. magellenicus* and from 535 to 549 in *A. irradians* (Fig. 5). Our results also suggest that self-screening changes the shape of the absorption curve for the distal receptors so that there is a relatively long tail on the short wavelength side and a short tail on the long wavelength side (Fig 5).

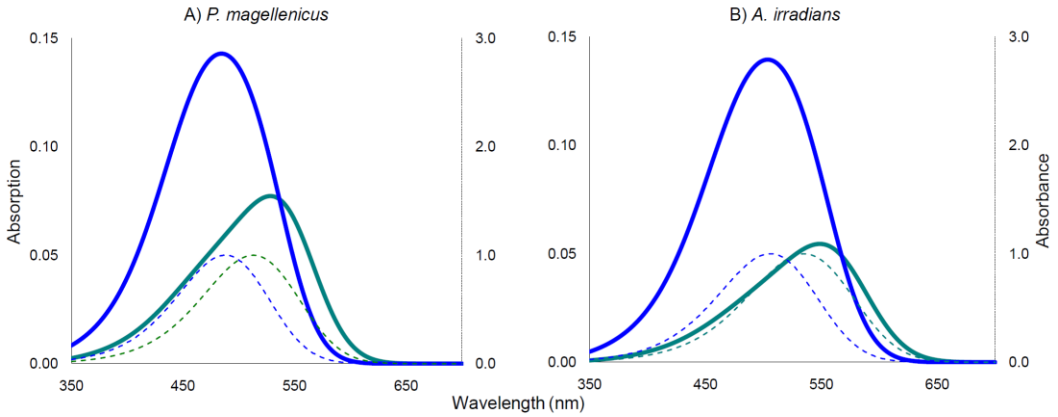

Figure 13: Absorption spectra of scallop photoreceptors after self-screening

The absorption of light by photoreceptors from scallop proximal (solid blue line) and distal (solid green line) retinas after we accounted for self-screening. Both graphs also show the proximal (dashed blue line) and distal (dashed green line) visual pigment absorbance spectra that were used to calculate absorption by the photoreceptors. In both cases, the absorbance curves have been normalized to 1. As can be seen in both A) *Placopecten magellenicus* and (B) *Argopecten irradians*, the absorption of unfocused light by the retinas, prior to their absorption of focused light, causes a shift in their peak sensitivities. The proximal and distal retina in *P. magellenicus* have peak sensitivities of 485 and 528 nm, respectively, while these retinas have peak sensitivities of 504 and 549 nm, respectively, in *A. irradians*. Self-screening is also seen to cause a much greater effect on the absorption spectrum of the distal retina than on that of the proximal retina.

4.3.3 Scallop light environments

Our models revealed that *Placopecten magellenicus* (Fig. 14) and *Argopecten irradians* (Fig. 15) live in light environments that are considerably different. The sea scallop *P. magellenicus* lives in an environment that, during the day, is around 100 times dimmer than *A. irradians*' habitat. Total horizontal radiance for *P. magellenicus'* offshore environment, modeled at 90 m and integrated from 400 to 700 nm, came to 9.6×10^{12} photons cm^{-2} s^{-1} nm^{-1} sr^{-1}, while total horizontal radiance for *A. irradians*' shallow, inshore habitat, modeled at 0.8 m, was estimated at 1.0×10^{15} photons cm^{-2} s^{-1} nm^{-1} sr^{-1}. Due to the selective absorption of long-wavelength light by water, *P. magellenicus* lives in an environment that is not only much dimmer than *A. irradians'*, but much bluer as well. We found that radiance in *P. magellenicus*' habitat reached a maximum at wavelengths between 475 and 485 nm and rapidly declined after 500 nm (Fig. 6). In comparison, radiance in *A. irradians*' environment did not peak until 555 to 565 nm and declined relatively slowly thereafter (Fig. 7). The inshore environment of *A. irradians* had peak radiance in the green part of the visual spectrum due to the presence of phytoplankton, which absorbs both long and short wavelength light and tends to occur at higher concentrations in coastal water.

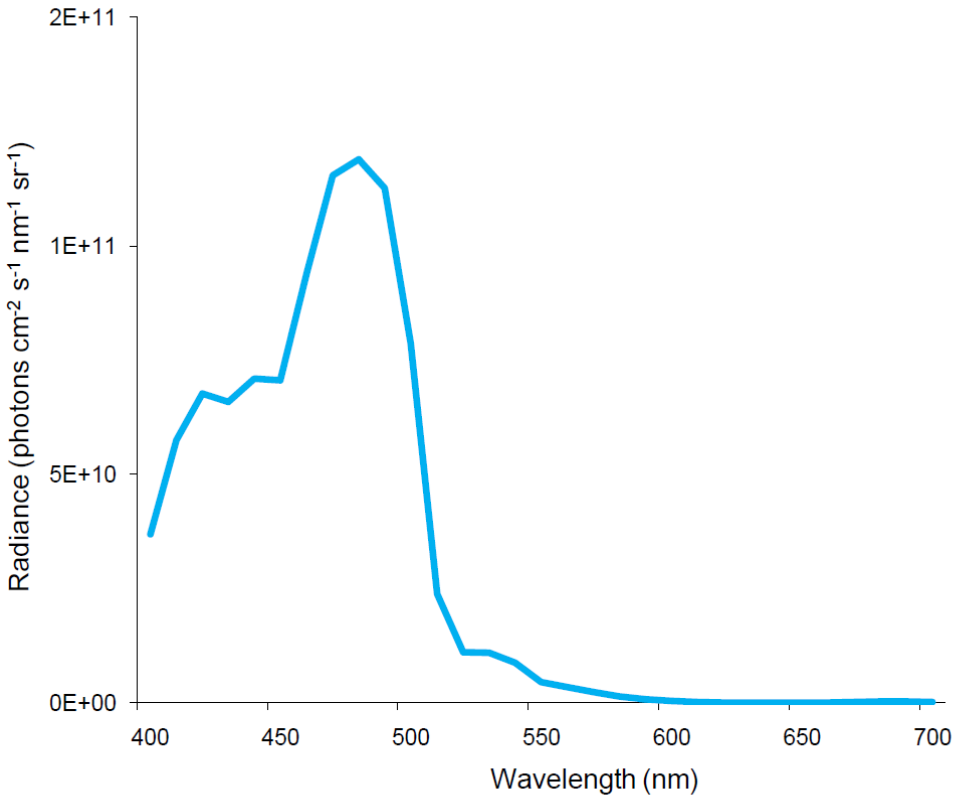

Figure 14: The light environment of the sea scallop *Placopecten magellenicus*

Radiance spectrum for the offshore habitat of the sea scallop *Placopecten magellenicus*. As illustrated here, this scallop tends to live in deep, dim oceanic water that has a narrow radiance peak in the blue part of the visual spectrum.

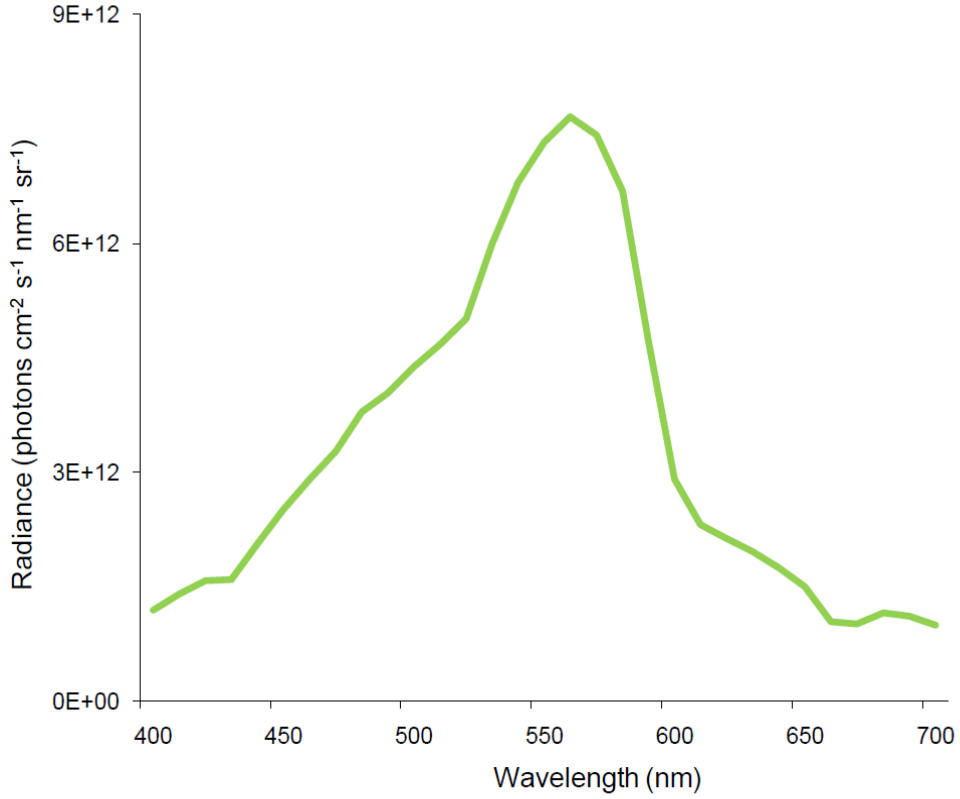

Figure 15: The light environment of the bay scallop *Argopecten irradians*

Radiance spectrum for the inshore habitat of the bay scallop *Argopecten irradians.* Note that this shallow water light environment is much brighter and greener than the deep water environment of the sea scallop *Placopecten magellenicus* (Fig. 12).

4.3.4 Scallop quantum catch

We found that proximal and distal photoreceptors in the sea scallop *P. magellenicus* collected 4.8×10^4 and 7.0×10^4 photons per integration time, respectively, when vision in this species is modeled at 90 m deep in an oceanic environment. Quantum catches for the proximal and distal photoreceptors of the bay scallop *A. irradians* were 9.2×10^6 and 4.8×10^6 photons per integration time, respectively, when this animal is at a depth of 0.8 m in its native coastal habitat (Table 2). Differences in quantum catch between the two scallop species were caused, for the most part, by differences in environment; quantum catch was lower in *P. magellenicus* because this species lives in deeper, dimmer water than the shallow-dwelling *A. irradians*.

The λ_{max} of visual pigments in *P. magellenicus* and *A. irradians* are consistent with these species' respective environments. The sea scallop *P. magellenicus* lives in relatively blue water (Fig. 6) and its proximal (Fig. 8) and distal (Fig. 9) photoreceptors would gather fewer photons if they had visual pigments that were maximally sensitive to longer wavelength light, like those of *A. irradians*. Likewise, the bay scallop *A. irradians* lives in relatively green water (Fig. 7) and its proximal (Fig. 10) and distal (Fig. 11) photoreceptors would be less efficient at photon-gathering if they had shorter λ_{max} values, like those of the sea scallop *P. magellenicus*.

Table 5: Estimates of scallop quantum catch

Quantum catch estimates for the deep-dwelling sea scallop *Placopecten magellenicus* and the shallow-dwelling bay scallop *Argopecten irradians*. Estimates of quantum catch for *P. magellenicus* were made using radiance values modeled for an oceanic habitat at 90 m; estimates for *A. irradians* were made using an inshore habitat 0.8 m deep. The λ_{max} values presented here were obtained from proximal and distal photoreceptors from both species using MSP. All estimates also account for the self-screening that occurs within the scallop eye.

	Proximal retina λ_{max} (nm)	Distal retina λ_{max} (nm)	Proximal retina quantum catch	Distal retina quantum catch;
Placopecten magellenicus	488	513	4.8×10^4	7.0×10^6
Argopecten irradians	506	535	9.2×10^4	4.8×10^6

4.3.5 Optimizing scallop quantum catch

Although the λ_{max} values of photoreceptors in *P. magellenicus* and *A. irradians* are relatively well-suited for oceanic and coastal environments, respectively, these values are not optimized, with regards to quantum catch, for the specific light conditions we modeled. For example, we found that both sets of photoreceptors in the sea scallop *P. magellenicus* would gather more photons if they had peak sensitivities at shorter wavelengths. Such a shift would give *P. magellenicus* photoreceptors with absorbance spectra better matched to the available light in an oceanic environment (Fig 6). For example, proximal photoreceptor quantum catch in *P. magellenicus* would increase to 5.1 x 10^4 photons per integration time, an increase of about 6%, if the λ_{max} of the visual pigment in this retina shortened from 488 to 470 nm (Fig. 8). A λ_{max} of 470 nm for *P. magellenicus* proximal photoreceptors remained optimum over distal photoreceptor λ_{max} values ranging from 503 – 523 nm. Furthermore, maximizing proximal photoreceptor quantum catch in *P. magellenicus* had a negligible effect on distal retina quantum catch (Fig. 8). Similarly, shifting the λ_{max} of *P. magellenicus* distal photoreceptors from their observed peak at 513 nm to a hypothetical peak at 465 nm would increase the quantum catch of these photoreceptors by 34%, while only decreasing proximal photoreceptor quantum catch by about 2% (Fig. 9). As in the preceding example, changing the λ_{max} of

proximal photoreceptors did little to influence our results, despite the self-screening that occurs in scallop eyes.

The bay scallop *A. irradians* would also gather more photons if the peak sensitivities of its visual pigments were different than those we measured. In this species, however, it was shifts in λ_{max} towards longer wavelengths that increased quantum catch. Such shifts in λ_{max} would give *A. irradians* visual pigments better matched to the radiance spectrum we modeled for this species' shallow, coastal habitat (Fig. 7). We found that proximal photoreceptor quantum catch in *A. irradians* would increase to 1.2×10^7 photons per integration time, an increase of about 26%, if the λ_{max} of the proximal retina visual pigment increased from 506 nm to an optimum at 555 nm (Fig. 10). Our findings here were consistent for distal retina λ_{max} values between 525 and 545 nm. It also appears that an increase in proximal retina λ_{max} in *A. irradians* would have relatively little effect on the quantum catch of this species' distal photoreceptors. Despite self-screening, a proximal photoreceptor λ_{max} of 555 nm would only decrease distal photoreceptor quantum catch by about 7% (Fig. 10). Likewise, distal photoreceptor quantum catch in this species would increase by 7%, jumping to 5.2×10^6 photons per integration time, if the λ_{max} of the distal retina visual pigment increased from 535 nm, as measured by MSP, to 565 nm (Fig. 11). Distal photoreceptors in *A. irradians* had an optimal λ_{max} at 565 nm when the proximal photoreceptors had a λ_{max}

between 496 to 516 nm and a shift in distal photoreceptor λ_{max} in this species caused a negligible (less than 1%) change in proximal photoreceptor quantum catch (Fig. 11). Optimizing λ_{max} for distal photoreceptors in *A. irradians* had less of an effect on quantum catch than the same procedure performed on proximal photoreceptors. This was largely due to the λ_{max} of the distal visual pigment already being closer to the peak radiance we modeled for this species' environment.

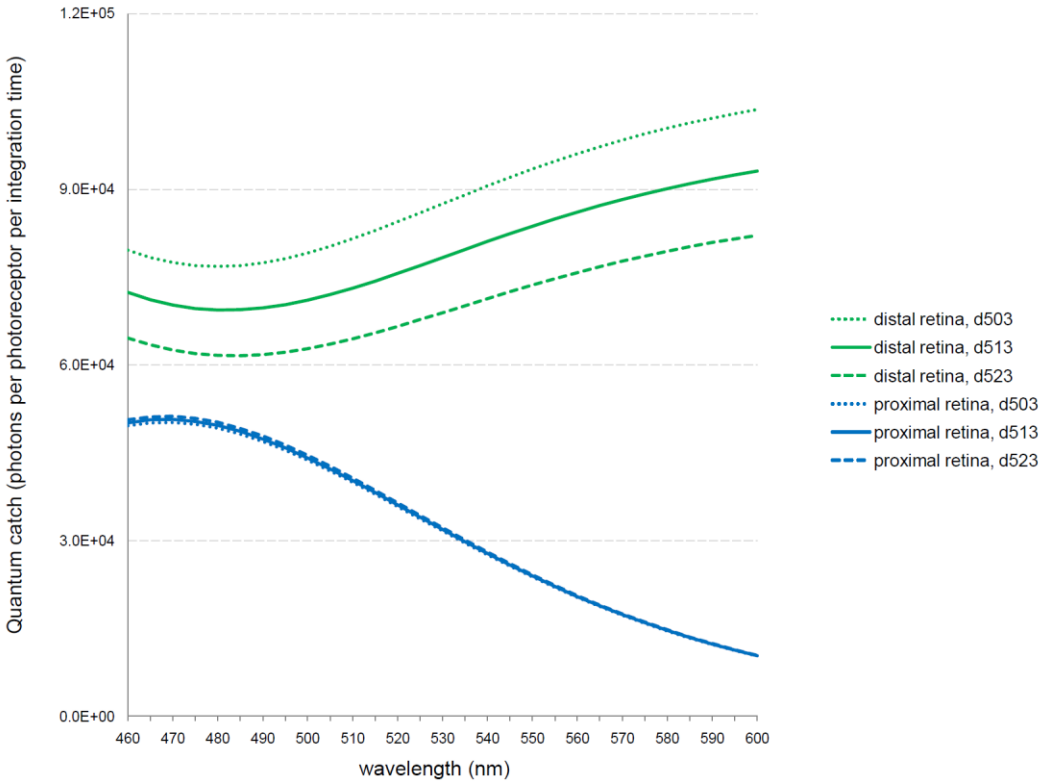

Figure 16: Quantum catch for the proximal retina of *Placopecten magellenicus*

Photoreceptor quantum catch, per integration time, in the eye of the sea scallop Placopecten magellenicus, when proximal receptor λmax varies at 5 nm intervals between 460 and 600 nm. The blue lines represent quantum catch, in photons, for proximal receptors; the green lines show this value for distal receptors. The continuous line represents quantum catch values when the distal receptors have the empirically determined λmax of 513 nm. The dotted and dashed lines represent quantum catch when the distal retina has hypothetical λmax values of 503 and 523 nm, respectively. Quantum catch was estimated using a radiance spectrum modeled for P. magellenicus' oceanic habitat.

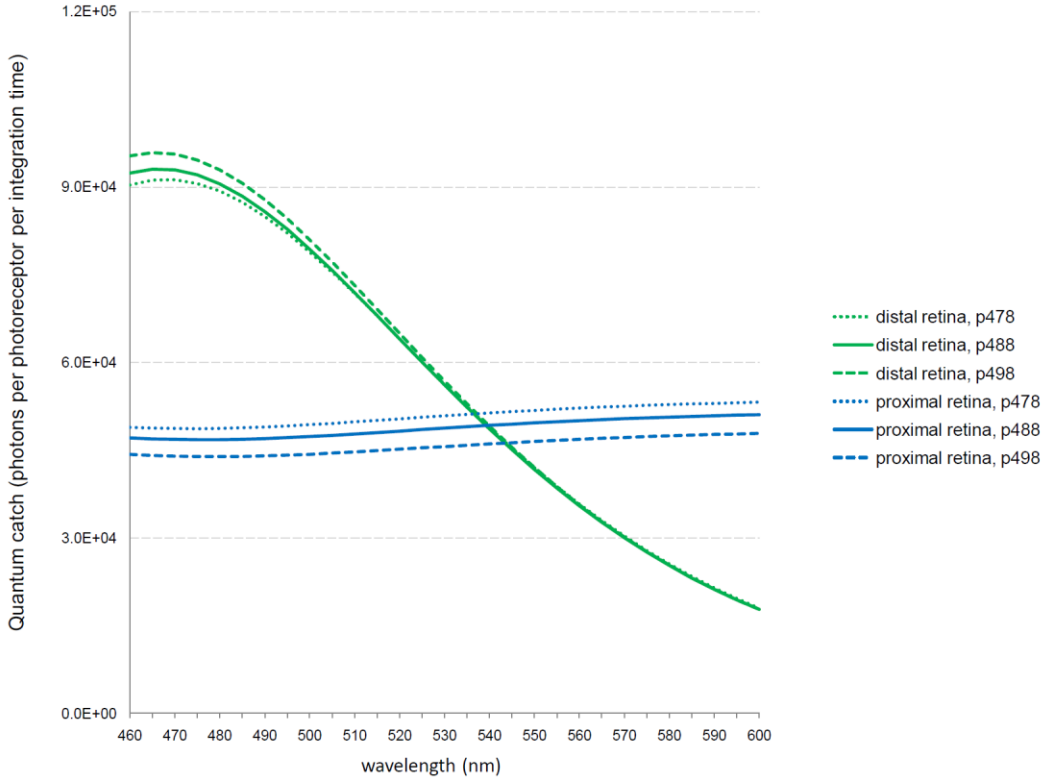

Figure 17: Quantum catch for the distal retina of *Placopecten magellenicus*

Photoreceptor quantum catch, per integration time, in the eye of the sea scallop Placopecten magellenicus, when distal receptor λ_{max} varies at 5 nm intervals between 460 and 600 nm. The blue lines represent quantum catch, in photons, for proximal receptors; the green lines show this value for distal receptors. The continuous line represents quantum catch values when the proximal receptors have the empirically determined λ_{max} of 488 nm. The dotted and dashed lines represent quantum catch when the distal retina has hypothetical λ_{max} values of 478 and 498 nm, respectively. Quantum catch was estimated using a radiance spectrum modeled for P. magellenicus' oceanic habitat.

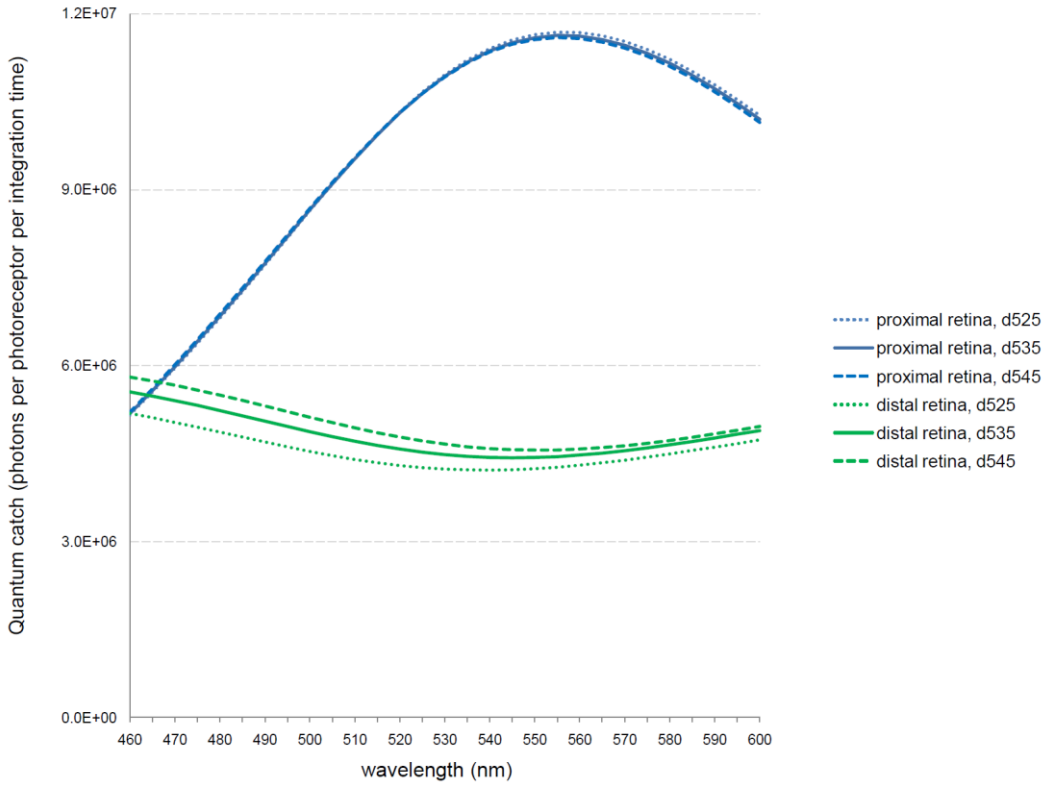

Figure 18: Quantum catch for the proximal retina of *Argopecten irradians*

Photoreceptor quantum catch, per integration time, in the eye of the bay scallop *Argopecten irradians*, when proximal receptor λ_{max} varies at 5 nm intervals between 460 and 600 nm. The blue lines represent quantum catch, in photons, for proximal receptors; the green lines show this value for distal receptors. The continuous line represents quantum catch values when the distal receptors have the empirically determined λ_{max} of 535 nm. The dotted and dashed lines represent quantum catch when the distal retina has hypothetical λ_{max} values of 525 and 545 nm, respectively. Quantum catch was estimated using a radiance spectrum modeled for *A. irradians*' coastal habitat.

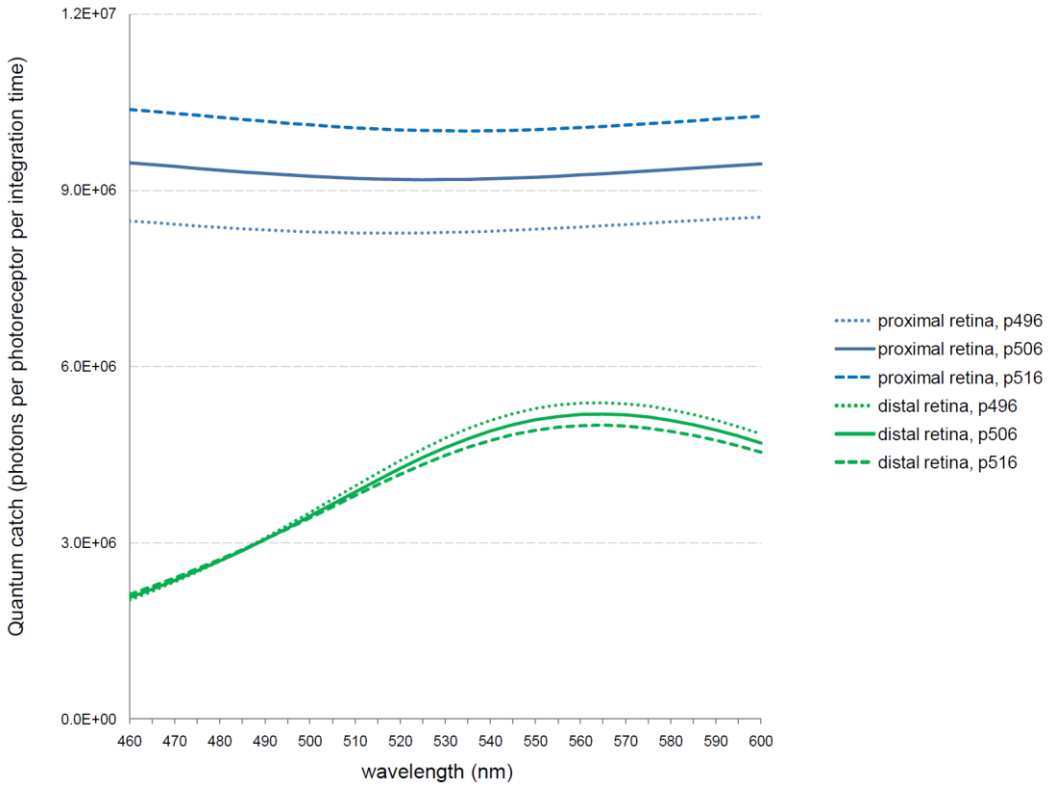

Figure 19: Quantum catch for the proximal retina of *Argopecten irradians*

Photoreceptor quantum catch, per integration time, in the eye of the bay scallop *Argopecten irradians*, when distal receptor λ_{max} varies at 5 nm intervals between 460 and 600 nm. The blue lines represent quantum catch, in photons, for proximal receptors; the green lines show this value for distal receptors. The continuous line represents quantum catch values when the proximal receptors have the empirically determined λ_{max} of 506 nm. The dotted and dashed lines represent quantum catch when the distal retina has hypothetical λ_{max} values of 496 and 516 nm, respectively. Quantum catch was estimated using a radiance spectrum modeled for *A. irradians*' coastal habitat.

4.4 Spectral sensitivity discussion

4.4.1 Estimates of scallop spectral sensitivity

We found that spectral sensitivity differs between scallop species and between the two retinas found within the same scallop eye. Our results are consistent with past behavioral experiments that show that the scallop *Pecten maximus* has spectral sensitivity peaks at 480 nm and 540 nm (Cronly-Dillon 1966). This species lives at depths similar to those of the sea scallop *Placopecten magellenicus* (investigated here) and the spectral sensitivities of the two species are similar, at least once self-screening in the *P. magellenicus* eye is considered (Fig. 5). A caveat here is that the earlier behavioral study did not account for extra-ocular photoreception in *P. maximus*. Many eyeless bivalves, such as mussels, oysters, and clams, have a dermal light sense based in their mantle tissue (Morton 2001), so it is quite possible that photoreceptors outside of *P. maximus*' eyes produced one of the two spectral sensitivity peaks we observed. This interpretation may be supported by results from electroretinography (ERG) which indicate that both the proximal and distal photoreceptors in the scallop species *P. maximus* (Wald and Seldin 1968) and *P. magellenicus* (McReynolds and Gorman 1970) return a maximum electrical response at 500 nm. Inconsistencies between past ERG studies and the current MSP recordings may be explained by the ERG studies examining

scallop spectral sensitivity at wavelength intervals of 10 to 25 nm (McReynolds and Gorman 1970). Our investigation by MSP recorded spectral sensitivity at 1 nm intervals. Thus, our finer-scale examination may have revealed differences in scallop proximal and distal photoreceptor sensitivity that were too small to be captured by prior, coarser-grain methods. Although we found that scallop visual pigments were offset, they were only offset, prior to self-screening, by about 20 nm. These differences could easily have been missed by the earlier ERG experiments.

4.4.2 Scallop visual pigment λ_{max} and habitat depth

Differences in spectral sensitivity between *Placopecten magellenicus* and *Argopecten irradians* suggest that environment influences the λ_{max} of scallop visual pigments. Like most animals outside of the deep ocean, *P. magellenicus* and *A. irradians* experience light conditions that are highly variable. However, the depth ranges of these species are quite different (Brand 2006), so it may be confidently predicted that the deeper-dwelling *P. magellenicus* lives in bluer water, on average, than the shallow-dwelling bay *A. irradians*. The radiance spectra we modeled for representative *P. magellenicus* and *A. irradians* environments support this prediction (Fig. 6). Using the spectra we generated, we estimated that *P. magellenicus* does better in its deep water habitat with its blue-shifted visual pigments than it would with the green-shifted pigments of *A. irradians* (Figs 8 and

9). Similarly, *A. irradians* gathers more photons in its home environment using its own visual pigments than it would if it had visual pigments with longer λ_{max} values, like those of *P. magellenicus* (Figs. 10 and 11). Our results suggest that scallop visual pigments, like those of many other marine animals, have λ_{max} values that are influenced by the pressure to maximize photon-gathering in particular habitats (Denton and Warren 1957; Lythgoe 1972; Munz 1958; Partridge 1990).

The observed inter-specific differences in visual pigment λ_{max} imply, further, that the optical sensitivities of the distal *and* proximal retina are important to scallop vision. While it is unclear whether the proximal retina receives a focused image (Land 1965; Speiser and Johnsen 2008), our observation that environment influences the λ_{max} of both scallop visual pigments suggests that both the distal and proximal retina gather information important enough for relatively small gains in sensitivity to be of adaptive consequence. Given our sample size of just two species, spectral sensitivity data must be gathered from additional scallops, preferably representing a diversity of habitats, if more is to be made of this observation.

4.4.3 Offset visual pigments and the functions of scallop photoreceptors

If scallop eyes are considered multi-retina eyes, differences in λ_{max} between proximal and distal photoreceptors may help the two scallop retinas perform different

tasks. In the sea scallop *P. magellenicus*, the proximal retina has a λ_{max} of 488 nm (or 485 nm once we account for self-screening) that closely matches the dominant wavelengths of horizontal light at 100 m in oceanic water (Fig. 6). The photoreceptors in the *P. magellenicus* distal retina, in comparison, have a λ_{max} that is shifted away from the dominant wavelengths in this light field by at least 30 nm. Photoreceptors with this kind of non-matching λ_{max} are thought to benefit aquatic animals by increasing the visual contrast of reflective objects (Lythgoe 1968). Provided these objects reflect down-welling light, which is bright and spectrally broad, they will stand out against the background light, which is dimmer and has a spectrum narrowed by scattering (Cronin 2006). Thus, *P. magellenicus*' distal photoreceptors are probably better than its proximal receptors at detecting reflective objects, a category which includes an array of other animals. This is consistent with the hypothesis that scallop distal retinas are specialized for predator detection (Land 1966; Speiser and Johnsen 2008). The scallop proximal retina may also be specialized for particular tasks. One hypothesis is that the proximal retina is specialized for habitat selection (Speiser and Johnsen 2008; Wilkens 2006). In scallops, this behavior involves the detection of non-reflective objects such as eelgrass beds (*Argopecten irradians*; Hamilton and Koch 1996) and dark crevices (*Pecten varius*; Buddenbrock and Moller-Racke 1953). These features are best detected by receptors with a λ_{max} that closely matches the peak radiance of the horizontal light field in an

environment (Lythgoe 1968). Photoreceptors of this sort are found in *P. magellenicus*' proximal retina. The spectral sensitivities of *P. magellenicus'* photoreceptors may, therefore, offer further evidence that scallop distal and proximal receptors are specialized for predator detection and habitat selection, respectively.

The spectral sensitivities of *A. irradians*' photoreceptors offer little evidence that the two scallop retinas are specialized for different tasks, however. Neither retina in *A. irradians* has a λ_{max} that closely matches the most abundant wavelengths of down-welling light in this species' inshore environment. From a functional standpoint, this may be due to how much brighter this shallow habitat is than *P. magellenicus's* deeper home; it is possible that shallow water is bright enough for there to be relatively little pressure on an animal's visual pigments to match the most abundant wavelengths available or, alternately, provide enhanced visual contrast by being shifted away from this peak. Light conditions in *A. irradians*' shallow, inshore habitat may also vary enough over short time periods, due to factors like waves, cloud cover, and the sun's position in the sky, for there to be no radiance peak stable enough for the λ_{max} of this species' photoreceptors to track.

We find the second explanation to be more convincing than the first, as maximizing photon absorption helps animals deal with the problem of image contrast (Cronin 2006). Contrast may be a particular issue for *A. irradians*, which lives in coastal

waters often made turbid by re-suspended bottom sediment (Grant et al. 1997) and phytoplankton blooms (Frechette and Bourget 1987). Turbidity can greatly decrease image contrast, making it harder for prey to spot predators (Abrahams and Kattenfeld 1997). Enhancing the optical sensitivity of an eye increases image contrast, so it may be predicted that *A. irradians*, like the deeper-dwelling *P. magellenicus*, would gain an advantage from visual pigments that gather the most possible photons. A final possibility is that the λ_{max} values of *A. irradians'* visual pigments are influenced by the evolutionary history of this species. The fossil record suggests that *A. irradians* evolved from ancestors morphologically similar to *Argopecten gibbus*, an extant species restricted to open, oceanic habitats (Waller 1969). A similar evolutionary history for *A. irradians* is supported by recent molecular phylogenies (Puslednik and Serb 2008) and, perhaps, by our discovery that this species' visual pigments are better suited for slightly deeper environments than the ones it normally inhabits.

4.4.4 Offset visual pigments and self-screening

When we think of scallop eyes as multibank retina eyes, offset visual pigments may provide several additional advantages. Color vision is probably the most obvious benefit an animal can gain from visual pigments with different λ_{max} values, but dichromacy is probably not an option for scallops. Unless multiple pigments are

expressed in the same scallop retina, which appears unlikely given the results of opsin expression studies (*Patinopecten yessoensis*; Kojima et al 1997), a lack of neural integration between scallop retinas likely prevents these animals from combining information from their two separate sets of photoreceptors into a single, dichromatic reconstruction of their visual environment.

A more likely possibility is that offset visual pigments help counter the effects of self-screening in the scallop eye. We found, however, that distal and proximal visual pigments with offset λ_{max} values do little to improve estimates of quantum catch for scallop photoreceptors. In other words, scallop retinas are not more sensitive when they have visual pigments with different λ_{max} values. In terms of quantum catch, it is more important for the λ_{max} of scallop visual pigments to match the wavelengths of peak radiance in natural environments than it is for them to counter the sensitivity costs associated with self-screening. For example, in *P. magellenicus*, two visual pigments with the same λ_{max} may be better than two that are offset (Table 3). Due to the rapid drop-off of long wavelength light in *P. magellenicus'* offshore habitat (Fig. 6), a visual pigment in this animal with a λ_{max} longer than 500 nm will fare poorly in terms of photon capture (Figs. 8 and 9). Given the context of our radiance model, proximal and distal photoreceptors in *P. magellenicus* would both gather the most photons if they had a λ_{max} at about 470 nm (Figs. 9 and 10). If this λ_{max} is used to calculate quantum catch for both

sets of photoreceptors in *P. magellenicus*, we find that the proximal receptors absorb 4.9 x 10^4 photons per integration time, while the distal receptors absorb 9.1 x 10^4. These values represent improvements of 3 and 30% for proximal and distal receptor quantum catch, respectively. Offset visual pigments may, therefore, be associated with decreased quantum catch in *P. magellenicus*, a curious finding given the importance of optical sensitivity to deep sea animals.

We found that offset visual pigments also fail to enhance quantum catch in *A. irradians*. In this species, proximal and distal photoreceptors are optimized for quantum catch when they have a λ_{max} at around 560 nm, at least when the other retina has a λ_{max} close to the one measured empirically. If we set λ_{max} for both sets of photoreceptors in the *A. irradians* eye at 560 nm, we find that the proximal and distal receptors respectively capture 1.2 x 10^7 and 4.6 x 10^6 photons per integration time. In comparison to quantum catch estimates that we calculated using empirically determined values for λ_{max}, these matched visual pigments provide a relative gain in photon absorption of 26% for proximal receptors and a relative decrease of 4% for distal receptors. This suggests that offset visual pigments in *A. irradians*, as in *P. magellenicus*, pose some cost, though this time it is only observed for the quantum catch of proximal photoreceptors. Our estimates of quantum catch are only accurate for the specific light environments we modeled, of course, and they may not represent biologically meaningful improvements

to optical sensitivity. It is clear from our results, however, that visual pigments with an offset λ_{max} do not improve scallop quantum catch. In fact, offset visual pigments may actually hinder photon capture in the scallop eye. When their λ_{max} values closely track the dominant wavelengths of light available in a particular environment, matched visual pigments appear to optimize scallop quantum catch.

Scallops could potentially increase the optical sensitivities of their two retinas by having offset visual pigments, but this could only happen if two requirements were met: First, the λ_{max} values of these pigments would have to be far apart, due to the broad absorbance spectra of opsin-based visual pigments; second, scallops would have to live under bright white light, an unlikely scenario for a benthic marine invertebrate. As is, the distances that visual pigment λ_{max} values may be offset are constrained by natural radiance spectra which, in marine environments, peak in the blue or green. These radiance peaks are generally broad, except in very deep water, but they are not broad enough. Our radiance models and calculations of quantum catch indicate that scallop visual pigments cannot be offset far enough to solve the problem of self-screening without falling outside the range of λ_{max} values useful for collecting photons in real-life coastal and oceanic environments.

4.4.5 Offset visual pigments and longitudinal chromatic aberration

So why do scallops have offset visual pigments? We hypothesize that the visual pigments of scallops are tuned to correct for the longitudinal chromatic aberration (LCA) produced by their lens. Chromatic aberration is a particular problem in eyes that have low f-numbers, like those of scallops. The f-number of an eye is the ratio of its focal length to the diameter of its aperture; in both *P. magellenicus* and *A. irradians* it is about 0.5 (Speiser and Johnsen 2008). Eyes with low f-numbers have shallow depths of field, which means that small differences in the focal lengths of different wavelengths of light produce non-overlapping images (Kroger 2000). In comparison to scallops, humans have an f-number that ranges from about 4, when the pupil is contracted, to 2.5 when the pupil is completely dilated. Given that an f-number of 2.5 is low enough to cause problems associated with LCA, one can easily see why scallops, with f-numbers around 0.5, may need to compensate for this optical defect.

While we did not measure the LCA produced by scallop lenses, it is possible to estimate, roughly, the amount of aberration that these lenses produce. The focal length of scallop lenses has been measured at between 1200 and 1800 microns for *Pecten maximus* (Land 1965), a species with an eye similar in size to those of the species studied here. If scallop lenses produce the same amount of chromatic aberration as fish lenses, they produce LCA on the order of 2 - 4% of their focal length over a spectral range of

486-656 nm (Kroger and Campbell 1996). This suggests that the focal planes for blue and red light fall somewhere between 24 – 72 microns apart in scallop eyes that are similar in size to those of *P. maximus* (as are the ones studied here). Given that the rhabdoms of the photoreceptors in scallop distal and proximal retinas are separated by as little as 50 microns (see Fig. 1B), LCA may be a major enough issue for scallops that it requires some form of optical correction. Our results from MSP suggest that scallops may perform this correction by having offset visual pigments that match the direction of chromatic aberration in their eye. This form of correction would enhance the sensitivity of scallop distal and proximal photoreceptors to the wavelengths of light that are focused upon them. Due to unfocused light passing through both retinas on the way to the mirror and back through the proximal retina on the way to the distal retina, image contrast is also a major problem for scallops. Offset visual pigments may help scallops improve image contrast by decreasing the sensitivity of their photoreceptors to unfocused light.

4.5 Spectral sensitivity conclusions

In conclusion, it appears that the λ_{max} of scallop photoreceptors is influenced by environmental light conditions. As predicted, we found that the deeper-dwelling scallop *Placopecten magellenicus* sees bluer light than the related coastal species *Argopecten irradians*. This difference in spectral sensitivity is likely due to the evolutionary pressure on these species to maximize photon capture in their respective environments. Differences in λ_{max} between the proximal and distal photoreceptors within the same scallop eye indicate that these photoreceptors may be specialized for different tasks. Alternately, offset visual pigments may help scallops correct for LCA produced by their lens. Obtaining visual pigment λ_{max} values for a broad range of scallop species and an estimate of the LCA caused by scallop lenses will be necessary if the relative merits of these two hypotheses are to be weighed.

We are also intrigued by the possibility that offset visual pigments decrease scallop optical sensitivity by shifting at least one visual pigment away from the λ_{max} that is optimal for photon capture. The steps that scallops have taken to limit LCA may, therefore, come with some costs. As this LCA is caused by a lens that probably evolved to correct for spherical aberration produced by the scallop's focusing mirror (Land 1965), the scallop eye provides an excellent example of how functional trade-offs can influence eye design. Just as there are inevitable trade-offs between resolution and sensitivity in

any eye of a given size (Land and Nilsson 2002; Warrant and Locket 2004), various forms of aberration within an eye cannot be corrected without some costs being incurred. The evolution of eyes and vision should not be considered an on-going journey towards optimum results; instead, it should be seen as a constant weighing of the relative costs and benefits of particular optical refinements in the context of specific eye designs in specific light environments. Eyes, therefore, provide one more example that, for all living things, no trait is without trade-offs and all adaptations are, inevitably, context-dependent.

4.6 Spectral sensitivity future directions

As noted earlier, it has been hypothesized that scallop retinas perform specific tasks; the distal retina is thought to detect predators, while the proximal retina may be used for habitat selection. In the case of the distal retina, this hypothesis is supported by evidence that a focused image falls in this region of the eye (Land 1965). Although electrophysiological recordings suggest that the distal receptors only provide information about object position and movement (Land 1966), information about light intensity may be unnecessary for predator detection. Scallops use chemosensory and tactile cues to distinguish predators from other animals (Wilkens 1981) and may simply use visual cues to know if a potential threat is approaching.

The proximal retina's role in scallop vision is less clear. It has been argued that the proximal retina simply does not receive focused light (Land 1965), but we suspect that there is more to this story. The photoreceptors of the scallop proximal retina are narrower, more tightly packed, and more numerous than those of the distal retina (Speiser and Johnsen 2008), which makes it appear, at least morphologically, that it is the proximal retina, not the distal retina, that is specialized for the reception of focused images. Furthermore, it is clear that scallops use visual cues for habitat selection (Hamilton and Koch 1996), which implies that these animals are somehow gathering information about static features in their environment. Scallops cannot do this with

their distal receptors, as explained earlier, and it is unlikely that scallops do this using unfocused light that falls on their proximal retinas. Eyes with low f-numbers, like those of scallops, have a shallow depth of focus. In such an eye, objects cast focused light on a very narrow image plane. Thus, unfocused light is of very little use to scallops. Objects either appear as a focused image in their eye or they are received as light that is entirely out-of-focus. Information about light intensity, which is potentially useful for habitat selection, is gathered by scallop proximal receptors, but recordings from proximal receptor optic nerves suggest that these receptors are not involved in image formation (Land 1966). However, it is input from the proximal receptors, not the distal receptors, that stimulates activity in the optic lobes of the parieto-visceral ganglion (PVG), a nerve center located on the scallop adductor muscle (Dakin 1928a; Spagnolia and Wilkens 1983; Wilkens and Ache 1977). This suggests that recordings from scallop optic nerves may be misleading when it comes to whether or not the proximal retina receives a focused image. Information from the proximal retina is clearly processed in the scallop central nervous system, which makes sense functionally: the distal receptors are involved in a predator response that would be aided by a simple, rapid neural circuit; the proximal receptors, used for habitat selection, could operate effectively under a slower, more selective neural regime. If information from the proximal receptors is processed in the scallop optic lobes, it is not surprising that a tonic response was all that

was recorded from the proximal receptor optic nerve. This tonic response, as measured by ERG, may contain a great deal of information. Each proximal receptor axon carries light intensity information for its respective field of view. Thus, evidence from behavioral and neural studies is equivocal with regard to whether scallop proximal receptors do or do not receive focused light. What remains to be discovered is a plausible mechanism by which scallops can somehow cause focused images to simultaneously fall on both of their tiered retinas.

We propose that the two scallop retinas may separately receive focused light from objects that are different distances away. This possibility arises as an inevitable consequence of the scallop eye's optical design. It is based on the relationship between the distance of an object from a scallop and the location within the scallop eye where this object forms a focused image. The relationship between object distance and image distance for a concave spherical mirror, such as the one used by the scallop eye to focus light, is explained by the Gaussian mirror equation (Halliday and Resnick 1988):

$$\frac{1}{f} = \frac{1}{d_o} + \frac{1}{d_i}$$

Equation 9: The Gaussian mirror equation

In equation 9, d_o is object distance, d_i is image distance and f is the focal of length of the mirror. As a consequence of this equation, objects that are closer to the scallop eye produce images further from the mirror than objects that are far away. This would provide an elegant solution to how scallops make the most out of their two retinas; the distal retina would only detect predators that are very close (and hence, more of a threat) and the proximal retina would receive focused light from more distant objects. Unfortunately, mirror-to-retina distances estimated from sectioned scallop eyes are rather unreliable. In some cases, as in *Argopecten* (Speiser and Johnsen 2008), both the proximal and distal retina appear to be far enough from the focusing mirror to receive focused light from an object at some real distance; *i.e.*, d_o is positive when an estimated value of f for an eye is used and d_i is set equal to the distance between the focusing mirror and the rhabdoms of proximal or distal photoreceptors. Our case here is bolstered by the scallop eye's shallow depth of focus, a consequence of its low f-number. In this case, a shallow depth of focus implies that objects at slightly different distances away from a scallop will produce non-overlapping focused images in the scallop eye.

The problem with this hypothesis is that, in the scallops *Patinopecten* (Speiser and Johnsen 2008) and *Pecten* (Land 1965), the proximal retina may be too close to the mirror to receive focused light from an object at any real distance; *i.e.*, d_o is negative when d_i is set as the distance between the focusing mirror and the rhabdoms of the proximal

receptors. Until mirror-to-retina distance can be estimated from live scallop eyes, it is difficult to say which, if any, of the samples mentioned above were marred by artifacts caused by fixing and sectioning (or if scallop eyes are simply morphologically diverse). Therefore, the live imaging of scallop eyes is the top priority in our on-going investigation of scallop optics.

The chromatic aberration of the scallop lens further confounds the model proposed above. Objects at two different distances will always form focused images on two different image planes in the scallop eye, but LCA, which is almost certainly produced by the scallop lens, will cause the different wavelengths of light associated with a single object to appear on multiple image planes within the eye. Calculating the LCA of the scallop lens will thus be another goal of our future work, as will understanding how interactions between the Gaussian mirror equation and chromatic aberration influence image formation within the scallop eye.

5. Conclusions

5.1 Eye morphology varies between scallop species

We found that eye morphology varies between scallop species (see Chapter 3). Prior to the work reported here, it was thought that all scallops had morphologically similar eyes (Dakin 1928b; Morton 2000). Indeed, the scallop eyes we surveyed all had a lens, a double-retina, and a concave mirror at the back. However, we also found that scallops that were better swimmers had larger eyes with longer focal lengths and wider pupils than scallops that were poor swimmers or non-swimmers. Our calculations suggest that these longer focal lengths and wider pupils imply greater optical resolution and sensitivity, respectively. Differences in eye morphology between scallop species may thus equate to functional differences in vision. Our interpretation of these results is that habitat selection in scallops is visually-influenced, which could explain why better swimmers tend to have better vision. Scallops that were strong swimmers also had proximal photoreceptors that were narrow and tightly packed; in comparison, scallops that were poor swimmers or non-swimmers had eyes with proximal receptors that were broader and more widely spaced. We did not see any differences between the distal retinas of different species. This pattern suggests that the morphology of scallop proximal retinas correlates with swimming ability, while that of the distal retinas does

not. Based on this, we hypothesize that the scallop proximal retina is used for tasks related to swimming and that the distal retina is used for tasks of equal importance to all species, such as predator detection.

Lens shape also varied between scallop species. Some scallops had lenses that were shaped in such a way as to correct for the spherical aberration produced by their focusing mirror. Other scallops had lenses that did not appear to be shaped in such a way. Correlations between scallop lens shape and other optical characteristics may offer additional evidence that scallops are using their lenses to correct for spherical aberration, as has been hypothesized previously (Land 1965). Our findings also suggest that there is a cavity between the mirror and the proximal retina in the eyes of at least some scallop species, although further work will be necessary to verify its presence or absence. If this cavity is real, focused images may fall on different retinas in different scallop species. Alternately, if this cavity is a fixation artifact, it suggests that different scallop eyes respond much differently to fixation. Either way, morphological differences between scallop eyes may reveal as-yet-undiscovered aspects of vision in these animals. Our research in this area indicates that comparative morphology is a valuable tool for learning about the optics and function of poorly-characterized visual systems, such as the unique concave mirror eyes of scallops.

5.2 Scallop eyes are multi-functional

Scallops use spatial vision for multiple tasks. It has long been known that scallops close their valves in response to large passing objects. This behavior has been interpreted as a defensive response to approaching predators. It has been hypothesized that this is the sole function of scallop eyes (Morton 2001; Nilsson 1994), despite past reports that scallops extend their tentacles towards novel objects they visually detect (Buddenbrock and Moller-Racke 1953; Wilkens 1981) and will swim towards objects representing potential shelter, such as eelgrass beds (Hamilton and Koch 1996). To these reports we add the observation that scallops open their valves in response to small passing objects (see Chapter 2).

We found that scallops only opened their valves when the passing objects were large and slow enough for their low-resolution, slowly-integrating eyes to detect. Our interpretation of this finding is that scallops visually detect suspended particles and use this information to decide whether or not to filter-feed. While our hypothesis may not be correct, our work clearly demonstrates that spatial information prompts scallops to open their valves, a heretofore unreported behavior in these animals. The small moving objects we displayed to scallops did not change the radiance in our behavioral arena; *i.e.* scallops had to use spatial vision to detect them. Provided that scallops use spatial

vision, not just changes in overall illumination, to detect predators and/or preferred habitats, it may be concluded that these animals have eyes that are multi-functional.

Furthermore, it cannot be argued that the two scallop retinas each have a single function. While we do find evidence that the two scallop retinas are subfunctionalized (see Chapters 3 and 4), our argument here is that the different retinas perform different sets of tasks. The distal retina is specialized for detecting moving objects; the proximal retina does not collect information about object movement (Land 1966). Thus, scallop distal photoreceptors must be collecting the information that causes scallops to both open their valves in response to small passing objects and close their valves in response to larger passing objects.

Thus, at the level of the eye or photoreceptor, we conclude that scallop vision is multifunctional. A relative lack of neural integration does not mean that scallops are only able to use vision for a single, specialized task, as has been previously argued, both for scallops in particular (Morton 2000) and about animals with decentralized nervous systems in general (Land and Nilsson 2006).

5.3 Scallop eyes and 'matched filters'

The 'matched filters' model predicts that animals with simple nervous systems may filter sensory information at the level of their receptors (Wehner 1987). We found

that scallop eyes, at least optically, do not fit the predictions of this model, despite scallops having a relatively simple, decentralized nervous system (Wilkens 2006). Past (Land 1965) and present (see Chapter 3) work suggests that scallop lenses correct for the spherical aberration produced by their focusing mirror. Furthermore, differences in spectral sensitivity between scallop distal and proximal receptors may correct for the chromatic aberration that is produced by the scallop lens (see Chapter 4). Thus, the evolution of scallop eyes appears to have involved a number of steps associated with producing a sharp image.

Our findings here are contrary to those of past studies. For instance, it was found that the eye of the box jellyfish *Carybdea* appears to be under-focused; *i.e.* the optics of the box jellyfish eye do not produce an image sharp enough for this animal's tightly-packed photoreceptors to return the full optical resolution they could potentially provide (Nilsson et al. 2005). In contrast, we find that scallops may have taken rather elaborate steps to take full advantage of the optical resolution potentially provided by the spacing of their photoreceptors.

While we suspect that the 'matched filters' model does not apply to scallop optics, it may apply to processes that occur on the level of individual photoreceptors, such as the speed of sensory transduction. For example, we found that scallops responded to small passing objects traveling at speeds below 10 cm/s, but did not

respond to similarly sized objects that were traveling more quickly (see Chapter 2). We hypothesize that scallops responded to the presence of these particles with feeding-related behavior. Scallop growth rates are inhibited at flow rates above 10 cm/s (Kirby-Smith 1972), so by being unable to detect quickly-moving particles, scallops may be making an appropriate feeding response based on an inability to detect a stimulus; such information-filtering at the level of a photoreceptor fits the predictions of the 'matched filters' model (Wehner 1987). Further work will be necessary to determine whether some aspects of vision, such as transduction speed, are more amenable to information-filtering than others, such as the optical production of a focused image.

6. Future directions in the study of bivalve eyes

6.1 Bivalves as a model system for the study of evolutionary innovation

Evolutionary innovations are driven by an organism's ecology and constrained by the underlying architecture of its genome. To understand why and how an innovation evolved, we must simultaneously study its phenotypic and genotypic history in an ecologically-informed, phylogenetic context. Eyes are evolutionary innovations that may be studied in such a rigorous manner. Eyes are easy to identify and categorize, the genes that mediate visual function are well-characterized and conserved across phyla, and the field of visual ecology allows us to generate specific hypotheses regarding the relationship between eyes and the environment.

The eyes of pteriomorph bivalves, a monophyletic lineage that includes mussels, ark clams, oysters, and scallops (Giribet and Wheeler 2002), are a particularly promising target for the study of evolutionary innovation. These eyes are found at a range of anatomical locations (Fig. 1A) and they vary widely in phylogenetic distribution (Morton 2001). For example, most pteriomorphs have simple pigment-cup eyes on their head ("cephalic eyes"), while only specific lineages have eyes positioned along the mantle margins of their shells ("mantle eyes"). These mantle eyes are morphologically

diverse and include the concave mirror eyes of scallops (Land 1965) and the compound eyes of ark clams (Nilsson 1994). Bivalve mantle eyes also vary in their molecular construction and physiological response to light. They may contain receptors that depolarize to light via a rhabdomeric-type transduction pathway ("r-receptors") or receptors that hyperpolarize to light through a ciliary-type pathway ("c-receptors"). Furthermore, even eyeless bivalves, such as mussels and oysters, respond to passing shadows through a dermal light sense based in their mantle (Morton 2001).

I hypothesize that cephalic eyes and a dermal light sense are ancestral features of pteriomorph bivalves, but that mantle eyes are not. Instead, I predict that mantle eyes have evolved multiple times in lineages that dwell in clear, well-lit water. I also hypothesize that bivalve mantle eyes have evolved from the diffuse receptor cells that confer the dermal light sense in eyeless taxa.

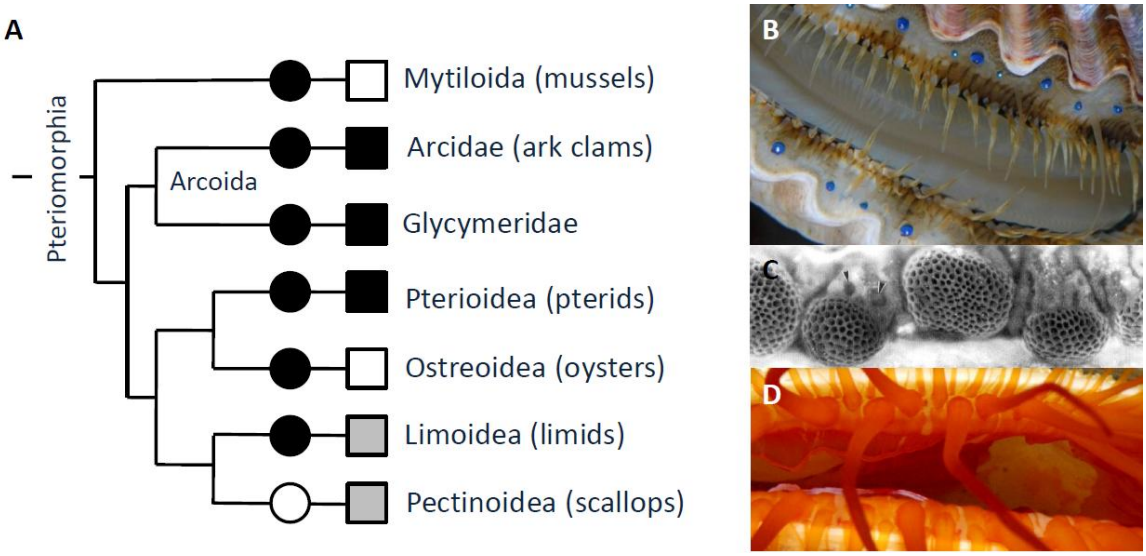

Figure 20: Eyes across the pteriomorph bivalves

The above phylogeny shows the distribution of eyes among pteriomorph bivalves. A). Phylogeny from Giribet and Wheeler (2002); eye data from Morton (2001). Black circles = cephalic eyes present; white circle = cephalic eyes absent; Black squares = mantle eyes on the outer mantle-fold; Grey squares = mantle eyes on the middle mantle fold; White squares = mantle eyes absent. B). Mantle eyes of the scallop Argopecten irradians (photo: S. Johnsen). C). Compound eyes of the ark clam Arca zebra (photo: D-E. Nilsson). D. Eyes of the limid Lima scabra, which appear as small, red spots between the tentacles (photo: Alison Sweeney).

6.2 Research Objectives

6.2.1 A new bivalve phylogeny

Objective 1: I will integrate all available molecular, morphological, and genomic information on bivalves into a concatenated phylogenetic data set that will be used to investigate specific questions regarding the ancestry and homology of different bivalve eyes. Hypothesis: Cephalic eyes and a mantle-based dermal light sense were present in the ancestral pteriomorph bivalve, but mantle eyes were not.

I will integrate all available molecular, morphological, and genomic information on pteriomorph bivalves into a concatenated phylogenetic data set. Mollusc phylogenetics is an active area of research for many labs, but the study of bivalve eye evolution requires a particular melding of new genomic resources and older morphological data sets. As both these sources of information are limited with regards to species sampling, I will produce a robust phylogeny suited to the specific needs of this study. I hypothesize that, by including all relevant data on pteriomorph bivalves, I will be able to build a phylogeny with higher nodal confidence than those produced from previous, smaller data sets (Rokas et al. 2003). I will use this data set and phylogeny to address specific questions regarding the ancestry and homology of different eye types within the pteriomorphs. For example, I will work to resolve several

key phylogenetic relationships within this group that influence our understanding of bivalve eye evolution. For example, it is unclear whether mussels, which lack mantle eyes, or ark clams, which possess mantle eyes (Fig. 1A), are the earliest branching pteriomorph family (Giribet and Wheeler 2002). The relationship between scallops and limids may also be influential (Fig. 1A). If these groups are sister taxa (Giribet and Wheeler 2002), it is likely that their unusual, double-retina eyes are homologous. If they are not, these eyes may represent a fascinating case of convergent evolution.

6.2.2 Bivalve eyes and environment

Objective 2: I will use the phylogeny constructed in Objective 1 to test for correlations between mantle eyes in pteriomorph bivalves and different environmental conditions. Hypothesis: Bivalves with mantle eyes tend to dwell in shallow, well-lit water with low turbidity, while bivalves without mantle eyes tend to live in darker or more turbid environments.

Mantle eyes allow bivalves to visually detect predators that fail to cast direct shadows (Nilsson 1994). The functional advantage provided by this ability prompts the question why many pteriomorph bivalves, such as oysters and mussels, only possess a dermal light sense. I hypothesize that environmental factors, such as water depth and turbidity, influence the phylogenetic distribution of mantle eyes in pteriomorph

bivalves. Specifically, I predict that mantle eyes are found in pteriomorph taxa that live in shallow, well-lit environments with low levels of turbidity. In dim environments, the small eyes of bivalves likely lack the optical sensitivity necessary for object detection (Land and Nilsson 2002). In turbid water, image contrast is decreased and prey species have a harder time spotting predators (Abrahams and Kattenfeld 1997). This suggests that, under certain environmental conditions, mantle eyes may not offer bivalves any functional advantages and are therefore less likely to be observed.

6.2.3 Bivalve eye evolution

Objective 3: I will evaluate whether bivalve mantle eyes have evolved from diffuse photoreceptors that confer the dermal light response in eyeless species.

Hypothesis: I predict that I will find homologous genes that are specialized for visual function in both bivalve mantle eyes and the mantle tissue of eyeless bivalves.

Eyeless bivalves, such as mussels and oysters, have a dermal light sense based in their mantle tissue (Morton 2001). In Objective 1 of this study, I expect to find that the dermal light sense is an ancestral trait in pteriomorph bivalves and that mantle eyes are a derived trait. I hypothesize that bivalve mantle eyes have evolved from the diffuse photoreceptors that confer the dermal light sense. Here, I will test this hypothesis by using 454 pyrosequencing to compare gene expression between various bivalve mantle

eyes and mantle tissue from eyeless species (Fig. 1A). If my hypothesis is correct, homologous genes specialized for visual function will be expressed in both bivalve mantle eyes and the mantle tissue of eyeless species. Aspects of visual function, such as the transduction cascade of different photoreceptor types, are well-characterized (Bao and Friedrich 2009; Larhammar et al. 2009), conserved across phyla (Ogura et al. 2004), and have been used to trace the ancestry of eyes that are unusual in both their morphology and phylogenetic distribution (Kozmik et al. 2008). It is also known that genes familiar for their role in visual function, such as opsins and G-proteins, are expressed in the mantle eyes of scallops (Kojima et al. 1997). Therefore, I believe that we will be able to distinguish genes involved with visual function from the widely-expressed housekeeping genes that will likely be expressed in both bivalve mantle eyes and mantle tissue. It is also possible that I will not find genes associated with visual function in the mantle tissue of eyeless bivalves, or that the vision-specific genes expressed in bivalve mantle eyes and mantle tissue are not homologous. If this is the case, it will suggest that bivalve mantle eyes have not evolved from the ancestral dermal light sense and that they may have, alternately, evolved from the cephalic eyes through a process of duplication and divergence.

6.3 Significance and Justification

This project uses bivalve eyes as a target for the study of evolutionary innovation. No other animal lineage includes a greater diversity of eye types and in no other group is it more likely that eyes have evolved multiple times. Here, we will learn about the ecological conditions that influenced the origin of bivalve mantle eyes and the molecular mechanisms that have shaped their evolution. We will learn how bivalve mantle eyes have evolved through the co-option of existing molecular components and, thus, how other such novel, complex traits may have originated. As part of this project, I will design new methods for super-matrix analysis that will address issues specific to concatenated datasets. These will include tools for generating better branch length estimates that will help researchers reconstruct ancestral states and perform correlation tests. I will also leverage full genome sequences and bioinformatic techniques for a comparative study of gene expression in bivalve eyes and mantle tissue and I will work on improved ways to use 454 pyrosequencing to generate EST data. For example, I will develop new methods to build phylogenies using full gene trees that include all paralogous loci, not partial trees that only include orthologues, *e.g*. Dunne et al. (2008). Such methodological improvements will help biologists use EST data to test, identify, and utilize new loci for phylogenetic analysis.

References

Abrahams, M., and M. Kattenfeld. 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. Behavioral Ecology And Sociobiology 40:169-174.

Bao, R. Y., and M. Friedrich. 2009. Molecular Evolution of the Drosophila Retinome: Exceptional Gene Gain in the Higher Diptera. Molecular Biology And Evolution 26:1273-1287.

Barber, V. C., E. M. Evans, and M. F. Land. 1966. The fine structure of the eye of the mollusc *Pecten maximus*. Cell Tissue Res. 76:295-312.

Blest, A. D., R. C. Hardie, P. McIntyre, and D. S. Williams. 1981. The Spectral Sensitivities Of Identified Receptors And The Function Of Retinal Tiering In The Principal Eyes Of A Jumping Spider. Journal Of Comparative Physiology 145:227-239.

Blevins, E., and S. Johnsen. 2004. Spatial vision in the echinoid genus Echinometra. Journal Of Experimental Biology 207:4249-4253.

Brand, A. R. 2006. Scallop ecology: distributions and behaviour. Pp. 651-744 *in* S. E. Shumway and G. J. Parsons, eds. Scallops: Biology, Ecology, and Aquaculture. Elsevier, New York.

Buddenbrock, W. v., and I. Moller-Racke. 1953. Uber den lichtsinn von Pecten. Pubbl. Staz. zool. Napoli 24:217-245.

Carosa, E., Z. Kozmik, J. E. Rall, and J. Piatigorsky. 2002. Structure and expression of the scallop Omega-crystallin gene - Evidence for convergent evolution of promoter sequences. Journal Of Biological Chemistry 277:656-664.

Cheng, J. Y., and M. E. DeMont. 1996. Jet-propelled swimming in scallops: Swimming mechanics and ontogenic scaling. Canadian Journal Of Zoology-Revue Canadienne De Zoologie 74:1734-1748.

Chun, C. 1903. Verh. Dtsch. Zool. Ges. 13:67-.

Clarke, G. L. 1936. On the depth at which fish can see. Ecology 17:452-456.

Coates, M. M. 2003. Visual ecology and functional morphology of Cubozoa (Cnidaria). Integrative And Comparative Biology 43:542-548.

Collin, S. P., R. V. Hoskins, and J. C. Partridge. 1997. Tubular eyes of deep-sea fishes: A comparative study of retinal topography. Brain Behavior And Evolution 50:335-357.

Cronin, T. W. 2006. Invertebrate vision in water. Pp. 211-249 *in* E. J. Warrant and D.-E. Nilsson, eds. Invertebrate vision. Cambridge University Press, New York, New York.

Cronly-Dillon, J. R. 1966. Spectral Sensitivity of the Scallop Pecten maximus. Science 151:345-346.

Dakin, W. J. 1910. The eye of *Pecten*. Quarterly Journal of Microscopical Science 55:49-112.

Dakin, W. J. 1928a. The Anatomy and Phylogeny of Spondylus, with a Particular Reference to the Lamellibranch Nervous System. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character 103:337-354.

Dakin, W. J. 1928b. The Eyes of Pecten, Spondylus, Amussium and Allied Lamellibranchs, with a Short Discussion on their Evolution. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character 103:355-365.

Denton, E. J., and N. A. Locket. 1989. Possible Wavelength Discrimination By Multibank Retinae In Deep-Sea Fishes. Journal Of The Marine Biological Association Of The United Kingdom 69:409-435.

Denton, E. J., and F. J. Warren. 1957. The Photosensitive Pigments In The Retinae Of Deep-Sea Fish. Journal Of The Marine Biological Association Of The United Kingdom 36:651-662.

Dunn, C. W., A. Hejnol, D. Q. Matus, K. Pang, W. E. Browne, S. A. Smith, E. Seaver, G. W. Rouse, M. Obst, G. D. Edgecombe, M. V. Sorensen, S. H. D. Haddock, A. Schmidt-Rhaesa, A. Okusu, R. M. Kristensen, W. C. Wheeler, M. Q. Martindale, and G. Giribet. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452:745-U5.

Eckman, J. E., C. H. Peterson, and J. A. Cahalan. 1989. Effects Of Flow Speed, Turbulence, And Orientation On Growth Of Juvenile Bay Scallops Argopecten-Irradians-Concentricus (Say). Journal Of Experimental Marine Biology And Ecology 132:123-140.

Fegley, S. R., B. A. Macdonald, and T. R. Jacobsen. 1992. Short-Term Variation In The Quantity And Quality Of Seston Available To Benthic Suspension Feeders. Estuarine Coastal And Shelf Science 34:393-412.

Frechette, M., and E. Bourget. 1987. Significance Of Small-Scale Spatiotemporal Heterogeneity In Phytoplankton Abundance For Energy-Flow In Mytilus-Edulis. Marine Biology 94:231-240.

Giribet, G., and W. Wheeler. 2002. On bivalve phylogeny: a high-level analysis of the Bivalvia (Mollusca) based on combined morphology and DNA sequence data. Invertebrate Biology 121:271-324.

Gomez, M. D., and E. Nasi. 2000. Light transduction in invertebrate hyperpolarizing photoreceptors: Possible involvement of a G(o)-regulated guanylate cyclase. Journal Of Neuroscience 20:5254-5263.

Grant, J., P. Cranford, and C. Emerson. 1997. Sediment resuspension rates, organic matter quality and food utilization by sea scallops (Placopecten magellanicus) on Georges Bank. Journal Of Marine Research 55:965-994.

Gregg, W. W., and K. L. Carder. 1990. A Simple Spectral Solar Irradiance Model For Cloudless Maritime Atmospheres. Limnology And Oceanography 35:1657-1675.

Halliday, D., and R. Resnick. 1988. Fundamentals of Physics. John Wiley and Sons Inc., New York.

Hamilton, P. V., and K. M. Koch. 1996. Orientation toward natural and artificial grassbeds by swimming bay scallops, Argopecten irradians (Lamarck, 1819). Journal Of Experimental Marine Biology And Ecology 199:79-88.

Harrison, A. W., and C. A. Coombes. 1988. Angular-Distribution Of Clear Sky Short Wavelength Radiance. Solar Energy 40:57-63.

Hartline, H. K. 1938. The discharge of impulses in the optic nerve of *Pecten* in response to illumination of the eye. Journal of Cellular Comparative Physiology 11:465-478.

Hesse, R. 1900. Die Augen einiger Mollusken. Zeit. f. wiss. Zool. lxviii

Jerlov, N. G. 1976. Marine Optics. Elsevier, Amsterdam.

Joll, L. M. 1989. Swimming Behavior Of The Saucer Scallop *Amusium balloti* (Mollusca, Pectinidae). Marine Biology 102:299-305.

Kanmizutaru, T., K. Anraku, and S. Toyoda. 2005. Light perception capability of pallial eyes in Japanese moon scallop Amusium japonicum as determined by electroretinogram. Nippon Suisan Gakkaishi 71:928-934.

Kelber, A., A. Balkenius, and E. J. Warrant. 2003. Colour vision in diurnal and nocturnal hawkmoths. Integrative And Comparative Biology 43:571-579.

Kirby-Smith, W. W. 1972. Growth of the bay scallop: the influence of experimental water currents. J. Exp. Mar. Biol. Ecol. 8:7-18.

Kirschfeld, K. 1974. Absolute Sensitivity Of Lens And Compound Eyes. Zeitschrift Fur Naturforschung C-A Journal Of Biosciences C 29:592-596.

Kojima, D., A. Terakita, T. Ishikawa, Y. Tsukahara, A. Maeda, and Y. Shichida. 1997. Novel G(o)-mediated phototransduction cascade in scallop visual cells. Journal Of Biological Chemistry 272:22979-22982.

Kozmik, Z., J. Ruzickova, K. Jonasova, Y. Matsumoto, P. Vopalensky, I. Kozmikova, H. Strnad, S. Kawamura, J. Piatigorsky, V. Paces, and C. Vlcek. 2008. Assembly of the cnidarian camera-type eye from vertebrate-like components. Proceedings Of The National Academy Of Sciences Of The United States Of America 105:8989-8993.

Kroger, R. H. H. 2000. Optical and developmental constraints on colour vision with lens eyes. Journal Of Optics A-Pure And Applied Optics 2:R39-R43.

Kroger, R. H. H., and M. C. W. Campbell. 1996. Dispersion and longitudinal chromatic aberration of the crystalline lens of the African cichlid fish Haplochromis burtoni. Journal Of The Optical Society Of America A-Optics Image Science And Vision 13:2341-2347.

Kroger, R. H. H., and A. Gislen. 2004. Compensation for longitudinal chromatic aberration in the eye of the firefly squid, Watasenia scintillans. Vision Research 44:2129-2134.

Krucynzki, W. L. 1972. The effect of the pea crab, *Pinnotheres maculates* Say, on the growth of the bay scallop, *Argopecten irradians concentricus* Say. Chesapeake Sci 13:218-220.

Land, M. F. 1965. Image formation by a concave reflector in the eye of the scallop, Pecten maximus. J Physiol 179:138-53.

Land, M. F. 1966. Activity In Optic Nerve Of Pecten Maximus In Response To Changes In Light Intensity And To Pattern And Movement In Optical Environment. Journal Of Experimental Biology 45:83-&.

Land, M. F. 1969. Structure Of Retinae Of Principal Eyes Of Jumping Spiders (Salticidae - Dendryphantinae) In Relation To Visual Optics. Journal Of Experimental Biology 51:443-&.

Land, M. F. 1981. Optics Of The Eyes Of Phronima And Other Deep-Sea Amphipods. Journal Of Comparative Physiology 145:209-226.

Land, M. F. 1982. Scanning Eye-Movements In A Heteropod Mollusk. Journal Of Experimental Biology 96:427-&.

Land, M. F. 2003. The spatial resolution of the pinhole eyes of giant clams (Tridacna maxima). Proceedings Of The Royal Society Of London Series B-Biological Sciences 270:185-188.

Land, M. F., and D.-E. Nilsson. 2002. Animal eyes. Oxford University Press, New York .

Land, M. F., and D.-E. Nilsson. 2006. General-purpose and special-purpose visual systems. Pp. 167-209 *in* E. J. Warrant and D.-E. Nilsson, eds. Invertebrate vision. Cambridge University Press, New York, New York.

Larhammar, D., K. Nordstrom, and T. A. Larsson. 2009. Evolution of vertebrate rod and cone phototransduction genes. Philosophical Transactions Of The Royal Society B-Biological Sciences 364:2867-2880.

Lauzier, R. B., and N. F. Bourne. 2006. Scallops of the west coast of North America. Pp. 965-989 *in* S. E. Shumway and G. J. Parsons, eds. Scallops: Biology, Ecology, and Aquaculture, New York, New York.

Leibovitz, L., E. F. Schott, and R. C. Karney. 1984. Diseases of wild, captive, and cultured scallops. J. World Maricul. Soc 15:269-283.

Loew, E. R., and W. N. McFarland. 1990. The underwater visual environment. Pp. 1-43 *in* R. H. Douglas and M. B. A. Djamgoz, eds. The Visual System of Fish. Chapman and Hall, London.

Lythgoe, J. N. 1968. Visual Pigments And Visual Range Underwater. Vision Research 8:997-&.

Lythgoe, J. N. 1972. The adaptation of visual pigments to their photic environment. Pp. 566-603 *in* H. J. A. Dartnall, ed. Handbook of Sensory Physiology, Vol. VII/I: The Photochemistry of Vision. Springer-Verlag, Berlin.

MacDonald, B. A., V. M. Bricelj, and S. E. Shumway. 2006. Physiology: energy acquisition and utilization. Pp. 417-492 *in* S. E. Shumway and G. J. Parsons, eds. Scallops: Biology, Ecology, and Aquaculture. Elsevier, New York.

Maffione, R. A., J. M. Voss, and C. D. Mobley. 1998. Theory and measurements of the complete beam spread function of sea ice. Limnology And Oceanography 43:34-43.

McFarland, W. N., and E. R. Loew. 1994. Ultraviolet Visual Pigments In Marine Fishes Of The Family Pomacentridae. Vision Research 34:1393-1396.

McReynolds, J. S., and A. L. F. Gorman. 1970. Membrane conductances and spectral sensitivities of *Pecten* photoreceptors. The Journal of General Physiology 56:392-406.

Michinomae, M., H. Masuda, M. Seidou, and Y. Kito. 1994. Structural Basis For Wavelength Discrimination In The Banked Retina Of The Firefly Squid Watasenia Scintillans. Journal Of Experimental Biology 193:1-12.

Mikulich, L. V., and E. A. Tsikhonlukanina. 1981. Food Composition Of The Sea Scallop. Okeanologiya 21:894-897.

Mobley, C. D., B. Gentili, H. R. Gordon, Z. H. Jin, G. W. Kattawar, A. Morel, P. Reinersman, K. Stamnes, and R. H. Stavn. 1993. Comparison Of Numerical-Models For Computing Underwater Light Fields. Applied Optics 32:7484-7504.

Morton, B. 2000. The function of pallial eyes within the Pectinidae, with a description of those present in *Patinopecten yessoensis*. Pp. 247-255 *in* E. M. Harper, J. D. Taylor and J. A. Crane, eds. Evolutionary biology of the Bivalvia. The Geological Society, London.

Morton, B. 2001. The evolution of eyes in the bivalvia. Pp. 165-205. Oceanography And Marine Biology, Vol 39.

Mpitsos, G. J. 1973. Physiology Of Vision In Mollusk Lima-Scabra. Journal Of Neurophysiology 36:371-383.

Munz, F. W. 1958. The Photosensitive Retinal Pigments Of Fishes From Relatively Turbid Coastal Waters. Journal Of General Physiology 42:445-459.

Myers, R. A., J. K. Baum, T. D. Shepherd, S. P. Powers, and C. H. Peterson. 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315:1846-1850.

Nilsson, D. E. 1994. Eyes As Optical Alarm Systems In Fan Worms And Ark Clams. Philosophical Transactions Of The Royal Society Of London Series B-Biological Sciences 346:195-212.

Nilsson, D. E., L. Gislen, M. M. Coates, C. Skogh, and A. Garm. 2005. Advanced optics in a jellyfish eye. Nature 435:201-205.

Ogura, A., K. Ikeo, and T. Gojobori. 2004. Comparative analysis of gene expression for convergent evolution of camera eye between octopus and human. Genome Research 14:1555-1561.

Partridge, J. C. 1990. The colour sensitivity and vision of fishes. Pp. 167-184 *in* P. J. Herring, A. K. Campbell, M. Whitfield and L. Maddock, eds. Light and Life in the Sea. Cambridge University Press, Cambridge.

Pegau, W. S., D. Gray, and J. R. V. Zaneveld. 1997. Absorption and attenuation of visible and near-infrared light in water: dependence on temperature and salinity. Applied Optics 36:6035-6046.

Pegau, W. S., and J. R. V. Zaneveld. 1994. Temperature Dependence Of The Absorption Coefficient Of Pure Water In The Visible Portion Of The Spectrum. Pp. 597-604. Ocean Optics Xii.

Petzold, T. J. 1977. Volume scattering functions for selected ocean waters. Pp. 150–174 *in* J. E. Tyler, ed. Light in the Sea. Dowden, Hutchinson, and Ross, Stroudsberg, PA.

Poli, J. 1795. Testacea utrinsque Siciliae eorumque historia et anatome aeneis illustrata, Parmae.

Pope, R. M., and E. S. Fry. 1997. Absorption spectrum (380-700 nm) of pure water.2. Integrating cavity measurements. Applied Optics 36:8710-8723.

Prieur, L., and S. Sathyendranath. 1981. An Optical Classification Of Coastal And Oceanic Waters Based On The Specific Spectral Absorption Curves Of Phytoplankton Pigments, Dissolved Organic-Matter, And Other Particulate Materials. Limnology And Oceanography 26:671-689.

Puslednik, L., and J. M. Serb. 2008. Molecular phylogenetics of the Pectinidae (Mollusca: Bivalvia) and effect of increased taxon sampling and outgroup selection on tree topology. Molecular Phylogenetics And Evolution 48:1178-1188.

Riisgard, H. U., C. Kittner, and D. F. Seerup. 2003. Regulation of opening state and filtration rate in filter-feeding bivalves (Cardium edule, Mytilus edulis, Mya arenaria) in response to low algal concentration. Journal Of Experimental Marine Biology And Ecology 284:105-127.

Rokas, A., B. L. Williams, N. King, and S. B. Carroll. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425:798-804.

Shumway, S. E., R. Selvin, and D. F. Shick. 1987. Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): a quantitative study. J. Shellfish Res. 6:89-95.

Spagnolia, T., and L. A. Wilkens. 1983. Neurobiology Of The Scallop.2. Structure Of The Parietovisceral Ganglion Lateral Lobes In Relation To Afferent-Projections From The Mantle Eyes. Marine Behaviour And Physiology 10:23-55.

Speiser, D. I., and S. Johnsen. 2008. Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). American Malacological Bulletin 26:27-33.

Stavenga, D. G., R. P. Smits, and B. J. Hoenders. 1993. Simple Exponential Functions Describing The Absorbency Bands Of Visual Pigment Spectra. Vision Research 33:1011-1017.

Stramska, M., D. Stramski, B. G. Mitchell, and C. D. Mobley. 2000. Estimation of the absorption and backscattering coefficients from in-water radiometric measurements. Limnology And Oceanography 45:628-641.

Tyler, J. E., and R. C. Smith. 1970. Measurements of Spectral Irradiance Under Water. Ocean series 1. Gordon and Breach, New York.

Wagner, H. J., R. H. Douglas, T. M. Frank, N. W. Roberts, and J. C. Partridge. 2009. A Novel Vertebrate Eye Using Both Refractive and Reflective Optics. Current Biology 19:108-114.

Wald, G., and S. Rayport. 1977. Vision In Annelid Worms. Science 196:1434-1439.

Wald, G., and E. B. Seldin. 1968. Electroretinogram And Spectral Sensitivity Of Common Scallop. Biological Bulletin 135:441-&.

Waller, T. R. 1969. The Evolution of the Argopecten gibbus Stock (Mollusca: Bivalvia), with Emphasis on the Tertiary and Quaternary Species of Eastern North America. Memoir (The Paleontological Society) 3:i.

Waller, T. R. 2006. New phylogenies of the Pectinidae (Mollusca: Bivalvia): Reconciling morphological and molecular approaches. Pp. 1-44 *in* S. E. Shumway and G. J. Parsons, eds. Scallops: Biology, Ecology, and Aquaculture. Elsevier, New York.

Warrant, E. J. 1999. Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. Vision Research 39:1611-1630.

Warrant, E. J., and N. A. Locket. 2004. Vision in the deep sea. Biological Reviews 79:671-712.

Warrant, E. J., and D.-E. Nilsson. 2006. Invertebrate vision. Cambridge University Press, New York.

Warrant, E. J., and D. E. Nilsson. 1998. Absorption of white light in photoreceptors. Vision Research 38:195-207.

Wehner, R. 1987. Matched-Filters - Neural Models Of The External World. Journal Of Comparative Physiology A-Sensory Neural And Behavioral Physiology 161:511-531.

Widdows, J., and A. J. S. Hawkins. 1989. Partitioning Of Rate Of Heat Dissipation By Mytilus-Edulis Into Maintenance, Feeding, And Growth Components. Physiological Zoology 62:764-784.

Wildish, D. J., and A. M. Saulnier. 1992. The Effect Of Velocity And Flow Direction On The Growth Of Juvenile And Adult Giant Scallops. Journal Of Experimental Marine Biology And Ecology 155:133-143.

Wilkens, L. A. 1981. Neurobiology of the Scallop. I. Starfish-Mediated Escape Behaviours. Proceedings of the Royal Society of London. Series B, Biological Sciences 211:341-372.

Wilkens, L. A. 2006. Neurobiology and behaviour of the scallop. Pp. 317-356 *in* S. E. Shumway and G. J. Parsons, eds. Scallops: Biology, Ecology, and Aquaculture. Elsevier, New York.

Wilkens, L. A., and B. W. Ache. 1977. Visual responses in the central nervous system of the scallop *Pecten ziczac*. Experientia 33:1338-1339.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey.

Biography

Daniel Isaac Speiser was born in Riverton, New Jersey on March 5th, 1981. Dan later attended public school in East Lansing, MI and graduated from East Lansing High School in 1999. The following fall, Dan began his undergraduate education at Carleton College in Northfield, MN. There, he studied biology and literature and was a member of the varsity swim team. In the spring of 2003, Dan graduated *magna cum laude* from Carleton with a Bachelor's of Arts in Biology. He was also inducted into Phi Beta Kappa at this time. In the fall of 2004, Dan started in the Ph.D. program in Biology at Duke University as a member of Sönke Johnsen's lab. While a graduate student, Dan studied the form, function, and evolution of the concave mirror eyes of scallops. He also studied the evolution of opsins and the origins of the metazoan eye development network, as well as the function and morphology of the eyes of polyplacophoran mollusks (or "chitons"). As a graduate student, Dan published "Comparative morphology of the mirror-based eyes of scallops (Pectinoidea)" in the *American Malacological Bulletin* (2008) and "Scallops visually respond to the presence and speed of virtual particles" in the *Journal of Experimental Biology* (2008). Dan was awarded a James B. Duke Fellowship by Duke University in the fall of 2004 and a National Science Foundation Pre-Doctoral

Fellowship in the spring of 2006. He also became a student member of Sigma Xi in 2007. After his graduation from Duke, Dan will continue teaching and studying biology.