The pupil response of a teleost fish, *Porichthys notatus*: description and comparison to other species

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Abstract

The pupil response of *Porichthys notatus* to different intensities of illumination is described and compared to that of *P. myriaster*, *Cephaloscyllium ventriosum*, and a human. While the fully dark adapted pupil is round, at the highest light intensities it consists of only two small, almost independent, apertures with a total area 4.9% of that observed in the fully dilated animal. The response is at least partially consensual and occurs, albeit at a much reduced rate, in isolated eyes. *P. notatus* also displays retinomotor movements comparable to those seen in most teleosts, suggesting that, contrary to most previous assumptions, pupillary responses and retinomotor migrations are not mutually exclusive. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Pupillary constriction in response to an increase in ambient illumination is widespread among vertebrates, occurring in most mammals and birds [1], as well as in many amphibia [2,3] and reptiles [4–7]. The distribution of pupil responses among fish, however, is less clear. Although iris mobility is well established for elasmobranchs [8–17], its occurrence among bony fish is equivocal. Despite the fact that it has been known for over 150 years that the irises of some teleosts are responsive to light [18], and that teleosts have a variety of muscles within their irises [19], their pupils are still generally regarded as static.

However, some teleosts undoubtedly have responsive pupils. Limited pupillary constriction has been elicited in intact animals and isolated eyes by both electrical stimulation [20–23] and by changes in ambient illumination [24–27]. However, the resulting changes in pupil area have usually been small and their significance for the naturally behaving animal, especially when electrical stimulation has been employed, is uncertain.

Certain groups of bony fish, however, display significant pupil mobility. The eel, for example, was the first teleost shown to have extensive pupil constriction both when intact animals [25,27] and isolated eyes [18,24,26,28–30] were exposed to light. Large-scale pupillary migrations have also been reported in several species of pleuronectiform flatfish and in the bottom dwelling *Uranoscopus scaber* and *Lophius piscatorius* following both electrical stimulation of isolated eyes [20] and exposure of intact animals to light [8,31,32]. Rubin and Nolte [33] found that isolated irises of *Opsanus tau* exhibited an even greater light-induced pupillary constriction than the isolated iris of *Lophius piscatorius*, suggesting that the intact animal probably shows pupillary changes comparable to, or even greater than, those of *Lophius*. Walls [34] also claimed that the commensal pearl-fish, *Encheliophis jordani*, has pupils which can 'close to a mere dot', and suggests that some armoured catfish, such as *Plecostomus*, also show extensive pupillary changes. Despite this relative wealth of data, there have been no detailed quantitative descriptions of pupillary dynamics in response to different intensities of illumination in any intact teleost species.

In this study we investigate the pupil response of the plainfin midshipman, *Porichthys notatus*, which is in many ways a most unusual teleost fish. During the day,

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like most other teleosts with extensive pupillary migrations, it buries itself in the sea floor, rising up only at night to feed on krill, mysids, shrimp and squid [35]. While young individuals live in shallower water, adults are caught at considerable depths (100–400 m) but return to shallow water, even intertidally, in late spring and early summer to spawn. Male *Porichthys* excavate nests under rocks and guard the eggs. The males are also quite vocal, producing loud grunts and hums, especially during the spawning season. Perhaps most surprisingly for a fish that spends much of its time either buried in the substrate or in shallow water, it has an extensive array of ventral photophores typical of those observed in many mesopelagic animals, which it probably uses for counter illumination while feeding in the water column [36].

Here we report the basic dynamics of the *P. notatus* pupil response following stimulation by different intensities of light, and compare it to the pupil movements of three other species; the closely related *Porichthys myriaster*, the swell shark *Cephaloscyllium ventriosum*, and a single human subject. We also show that the *Porichthys* pupil response is at least partially consensual and that it occurs, albeit at a much reduced rate and to a lesser extent, in isolated eyes. This is the first detailed account of pupil movements in a teleost fish that are comparable in extent and speed to those of 'higher' vertebrates.

2. Materials and methods

Two size/age classes of *Porichthys* sp. (Batrachoidiformes: batrachoididae) were used in this study. Smaller *P. notatus* (standard lengths 5.5–8.5 cm) were raised from eggs or recently hatched juveniles contained within nests collected off the Santa Barbara coast using SCUBA and subsequently kept in a laboratory in running sea water on a 12 h light/12 h dark cycle (fluorescent lighting providing 9.84×10^2 $\mu W/cm^2$ at the water surface), for 9–11 months. A single larger *P. notatus* (sl 13.5 cm) and two *P. myriaster* (sls 14.0 and 24.0 cm) were caught locally as adults at 50–75 m using an otter trawl and kept for up to 7 weeks in running sea water aquaria exposed to the natural light/dark cycle. A single swell shark, *Cephaloscyllium ventriosum* (Carcharhiniformes: scyliorhinidae) (total length 25.0 cm) was raised in the laboratory from an egg case and maintained for 9–12 months in the natural light/dark cycle.

All three species proved ideal subjects for the following experiments as they naturally spend a great deal of time motionless, and *Porichthys* sp. will usually bury themselves in the sand with only their eyes protruding. They can thus be virtually immobilised without any form of anaesthesia or paralysis. The smaller *P. notatus*

were placed in a glass tube (diameter 2.5 cm) filled with oxygenated sea water, the bottom of which was covered in sand. Larger individuals of all three species were positioned within a longitudinally semi-sectioned Perspex pipe placed on sand within an aquarium. Under these conditions the animals usually lay on the substrate, moving only rarely during an experiment. Background illumination was provided by an infrared source. Diffuse monochromatic (500 nm) or white light stimuli were delivered to the animal through a fibre optic bundle coupled to an electronic shutter, from either a tungsten light source, in conjunction with a Bausch and Lomb 1350 lines/mm grating monochromator and a neutral density wedge, or a Nikon MkII fibre optic light source whose intensity was controlled by neutral density filters, respectively. One eye of each animal was videotaped with an infrared-sensitive camera placed in a plane parallel to the cornea. Images, with superimposed time signal and voice commentary, were recorded in an adjacent area, separated from the experimental animal by a light-tight curtain. This area also contained the stimulating light and video monitor.

Pupil area was determined from individual video frames captured on an AV-capable Macintosh computer using NIH-image. To allow comparison between animals with different eye sizes, pupillary dimensions were expressed relative to each animal's fully dilated pupil area.

2.1. Dark adaptation

Six small *P. notatus* were removed from their home tank during the light phase of their normal light/dark cycle and their pupil size was monitored throughout 90–120 min of darkness.

2.2. Light adaptation

Four small *P. notatus* were introduced into the experimental set-up at the time the lights would normally have gone off in their home tank and dark adapted in situ for 70–100 min. All experiments were performed during this 'dusk' period to minimise the influence of any endogenous circadian effect on the pupil response. The pupil area at the end of this initial dark adaptation period was taken as the animals dark adapted pupil area to which other areas were compared, even though in some instances, following a series of light/dark exposures, the pupil subsequently dilated even further (see below). Following dark adaptation each animal received a series of 5–10 min 500 nm light exposures. Between each exposure the animal was left in darkness for 5–15 min (the exact time depending on the brightness of the preceding exposure) to allow the pupil to redilate fully. The maximum number of exposures in any series was seven. 500 nm illumination was used as

it closely matches the maximum sensitivity of the scotopic visual pigment extracted from this species (λ_{max} 498 nm) [37].

2.3. Comparative light adaptation

One large *P. notatus*, a single *P. myriaster* and one *C. ventriosum*, were dark adapted for 90–100 min before being subjected to a relatively dim white light stimulus (3.07×10^1 $\mu W/cm^2$ at the cornea) for 10–15 min, followed by 10–25 min in darkness and a second, more intense (6.75×10^4 $\mu W/cm^2$ at the cornea) white light exposure. A single human subject (male, 41 years old) was exposed to the dimmer white light stimulus for 45 s following 40 min dark adaptation.

2.4. Consensual response

Two experiments were performed to ascertain whether stimulation of one eye leads to pupil constriction in the other, 'unstimulated', eye.

2.4.1. Experiment 1
A single *P. myriaster* (sl 24 cm) was dark adapted for 17.5 h, after which, while one eye was kept in darkness and videotaped as before, the other was stimulated with white light from a single optic fibre housed within a light-tight black tube completely covering the eye. This tube appeared to eliminate any scattered light within the aquarium. The only other route for light to reach the 'non-illuminated' eye would have been through the head (intraocular separation ca. 2–2.5 cm). However, we feel this is unlikely, as the light would not only have to pass through the skull and brain, it would also have to traverse the opaque sclera, choroid and retinal pigment epithelium of both eyes.

2.4.2. Experiment 2
Although the above experiment reduces the risk of stray light reaching the 'unstimulated' eye to a minimum, it does not enable one to view both eyes simultaneously. In order to allow a comparison of the responses of the stimulated and 'unstimulated' eyes, both eyes of a smaller *P. notatus* that had been in darkness for 4 h, were videotaped using a mirror placed at an angle above the fish. One eye was then stimulated by low intensity white light through a single optic fibre imaged just below the eye. Although some stray light may have reached the 'unstimulated' eye, it will have been of a very much lower intensity than that reaching the stimulated eye.

2.5. Response of isolated eye

Seven small *P. notatus* were sacrificed 85–160 min into the dark phase of their light/dark cycle by cervical

transection and pithing. Their intact eyes were isolated under dim red illumination and submerged in sea water. Eight eyes were immediately exposed to intense white light (6.12×10^4 $\mu W/cm^2$ at the cornea) for 20 min, followed by 30 min of darkness. A further four eyes were simply maintained in darkness for 50 min.

2.6. Retinal histology

Four of the smaller, laboratory reared, *P. notatus* were used to examine retinal structure. A single animal was killed 30 min before the light would have gone out in its home tank, while another three were killed under dim red illumination 90–140 min into the dark phase of their light/dark cycle. Eyes were immediately removed, hemisected and fixed in 2.5% gluteraldehyde/1% paraformaldehyde in phosphate buffer for at least 24 h, before being dehydrated and embedded in araldite. Sections of 0.5 μm were cut on a Reichert-Jung Ultracut E microtome, stained in toluidine blue and viewed with a light microscope.

3. Results

3.1. Dark adaptation

When *P. notatus* were removed from their home tank during the light phase of their light/dark cycle and placed in darkness, they took around 30 min to reach full pupil dilation (Fig. 1). The size of the pupil at the beginning of the dark adaptation period, however, was only 52% of the dark adapted pupil area, which is far from fully constricted (4.9%, see Section 3.2). This is because of the moderate intensity of the illumination within the home tank. Non-systematic observations suggested that the speed of dark adaptation depended both on the intensity and the duration of the preceding light period; long exposure and brighter lights resulting in slower dark adaptation.

3.2. Light response

On exposure to light the pupil of *P. notatus* rapidly constricted before gradually redilating to a new 'plateau' level (e.g. Fig. 2). Once the lights were extinguished the pupil slowly dilated often slightly 'overshooting' the previous dark adapted pupil area.

The fully dilated pupil was round (Fig. 3(A)). As it constricted, however, the dorsal margin contracted to a greater degree, giving the partially constricted pupil a 'heart-shaped' appearance, until at the highest light intensities the pupil consisted of two small, almost independent, apertures (Fig. 3(B)). The average size of the fully dilated pupil area for the four fish used in this part of the study was 2.09 mm^2. Following stimulation
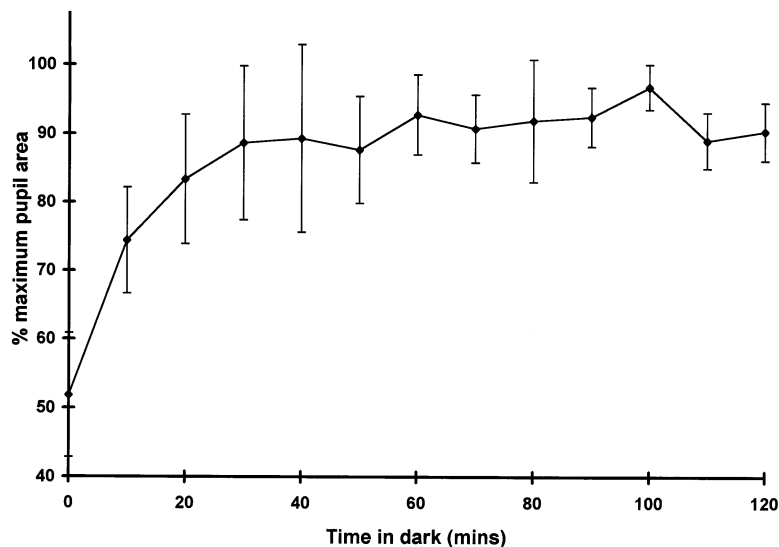
Fig. 1. Average pupil area as a function of time in darkness for six *Porichthys notatus*. The error bars represent 2 S.D.

with intense white light pupils constricted to an average area of 0.10 mm^2, equivalent to 4.9% of the dilated pupil area.

The degree of initial pupil constriction, the level of the eventual stable pupil area, and the time taken to reach this plateau, all depended on the intensity of the stimulating light. Within limits, the brighter the light, the greater the degree of initial pupil constriction (Figs. 4 and 5), and the smaller the eventual stable size of the pupil (Figs. 6 and 7). At the lower light intensities, the pupil fully redilated to dark adapted levels despite the continued presence of light (Figs. 6 and 7).

Since pupil constriction was initially very rapid before approaching maximum constriction more slowly, the exact time that the minimum pupil area was attained was hard to determine accurately. A more precise measure of the speed of constriction is given by the time taken to reach half maximum constriction ($t_{0.5max}$). In all cases, irrespective of light intensity, this was between 0.4 and 0.8 s after light onset.

3.3. Comparative light adaptation

In response to the same, relatively dim, white light stimulus *P. notatus*, *P. myriaster*, and the human subject gave similar initial responses, maximally contracting to 21, 13, and 24% of their dark adapted pupil areas, respectively ($t_{0.5max}$; human, 0.64 s; *P. notatus*, 0.74 s; *P.myriaster*, 0.90 s; Fig. 8(a)). Following maximal constriction the human pupil began to redilate immediately (Fig. 8(a)), while in *P. notatus* and *P. myriaster* this redilation again occurred more slowly (Fig. 8(b)). The human pupil also showed regular oscillations during redilation which none of the fish pupils did. The response of *C. ventriosum* was very much slower than that of the other species, with full constric-

tion (41% of dark adapted pupil area) taking about 14 min ($t_{0.5max}$ 4 min), and with no sign of a redilation during the experiment (Fig. 8(b)).

In response to a more intense white light stimulus, the pupils of both *P. notatus* and *P. myriaster* again behaved similarly, reaching a maximal constriction of 6 and 10% of maximal dilation, respectively ($t_{0.5max}$ 0.76 and 0.86 s; Fig. 8(c)). The swell shark pupil constricted to an even greater degree, reaching 2.1% of its fully dilated area, although this again took significantly longer than in the other fish ($t_{0.5max}$ 21.9 s).

It should be noted that all light levels quoted in this study were measured in the plane of the animal's cornea. Thus, although all species were exposed to the same light levels, the retinal irradiance will have varied since this depends, when viewing an extended source, on both pupil area and focal length which is different in all species. Comparison is further complicated since the spectral sensitivities of the photoreceptors eliciting pupil constriction are likely to be different in all species. However, we calculate that retinal irradiance will be within the same order of magnitude in all species as the larger pupil area of man is compensated for by the shorter focal length of the fish lens.

The shape of the *P. myriaster* pupil during constriction was the same as that described above for *P. notatus* (Fig. 3(A, B)). The pupil of *C. ventriosum* also behaved in a similar manner, being almost round when fully dark adapted and taking on the appearance of a narrow slit with two small pinholes at either end when in bright light (Fig. 3(C, D)). However, while the axis of the constricted 'slit' in P*orichthys* sp. was approximately along the fish's body axis, in the shark it was tilted at an angle of about 20° relative to the body axis so that the anterior portion of the pupil lay below the horizontal (Fig. 3(D)).
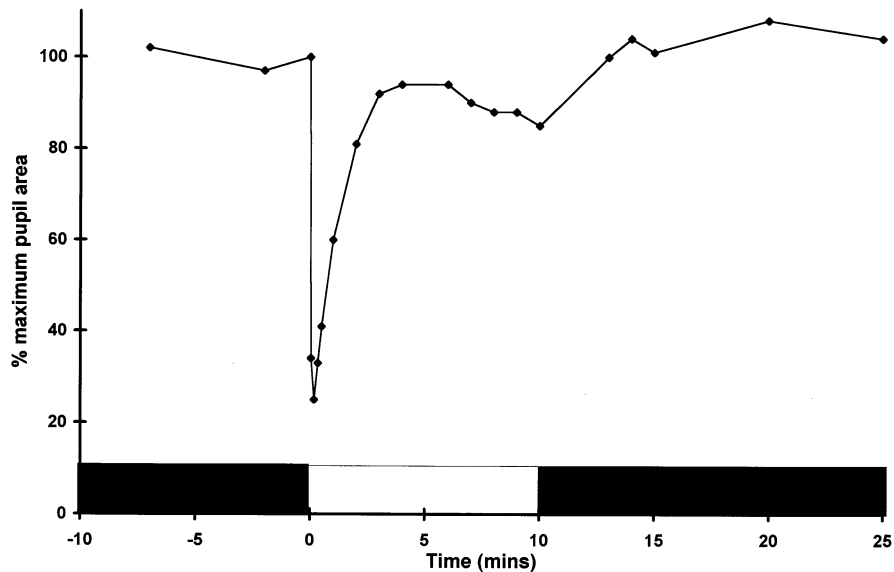
Fig. 2. Typical response of a *Porichthys notatus* pupil to 10 min exposure of 500 nm light (2.71×10^{-1} µW/cm²). Light levels in this and all subsequent figures were measured in the plane of the animal's cornea and therefore do not represent retinal irradiance.

3.4. Consensual response

3.4.1. Experiment 1

When one eye was stimulated by a fibre optic within a light-tight tube, the pupil of other 'unstimulated' eye always showed some constriction (e.g. Fig. 9(a)), thereby indicating that the pupil response of *P. myriaster* is at least partially consensual.

3.4.2. Experiment 2

In this experiment the pupils of both the stimulated and 'unstimulated' eyes constricted, although the response of the 'unstimulated' eye was consistently less than that of the other eye (e.g. Fig. 9(c)). The results of this experiment taken in isolation could be interpreted by assuming the constriction of the 'unstimulated' eye is due to stray light. However, the first experiment indicates that a closely related species of *Porichthys* has a consensual pupil reflex. Assuming that both *P. myriaster* and *P. notatus* have similar light reflexes, which all previous experiments indicate, this second experiment most likely shows that although the pupil response of *Porichthys* sp. is largely consensual, it is not completely so.

3.5. Response of isolated eye

The pupils of isolated eyes constricted when exposed to light (Fig. 10(a)). This response, however, was not due solely to the illumination, as isolated eyes simply placed in darkness also constricted, albeit to a lesser degree (Fig. 10(b)). To reveal that part of the response due only to the illumination, the average response of eyes left continuously in darkness (Fig. 10(b)) was subtracted from the response of each isolated eye ex-

posed to light. The average of the resulting curves (Fig. 10(c)) reveals a response that is significantly slower and less pronounced than that observed in intact animals.

3.6. Retinal histology

The retinae of the young (< 1 year) *P. notatus* examined here are typical of those of a teleost living in relatively low light levels, showing a preponderance of double cones and rods (Fig. 11). Like most other teleosts they also show extensive retinomotor (photomechanical) movements: In light adapted retinae (Fig. 11(a)), cone myoids are contracted, placing the inner segments next to the external limiting membrane (e.l.m.), while the melanin within the retinal pigment epithelium is dispersed thereby covering the rods. After dark adaptation, the epithelial pigment aggregates towards Bruch's membrane and the cone inner and outer segments also migrate towards the back of the eye, leaving the rods exposed to incoming illumination (Fig. 11(b)).

4. Discussion

4.1. Porichthys notatus pupil response in comparison to that of other vertebrates

The pupil response of *P. notatus* is qualitatively similar to that of many other vertebrates, constricting at the onset of illumination before redilating to a steady level in the continued presence of light (Fig. 2). The initial constriction is swift and related in a graded manner to the intensity of the incident illumination, reaching a maximum constriction of 4.9% of the dark
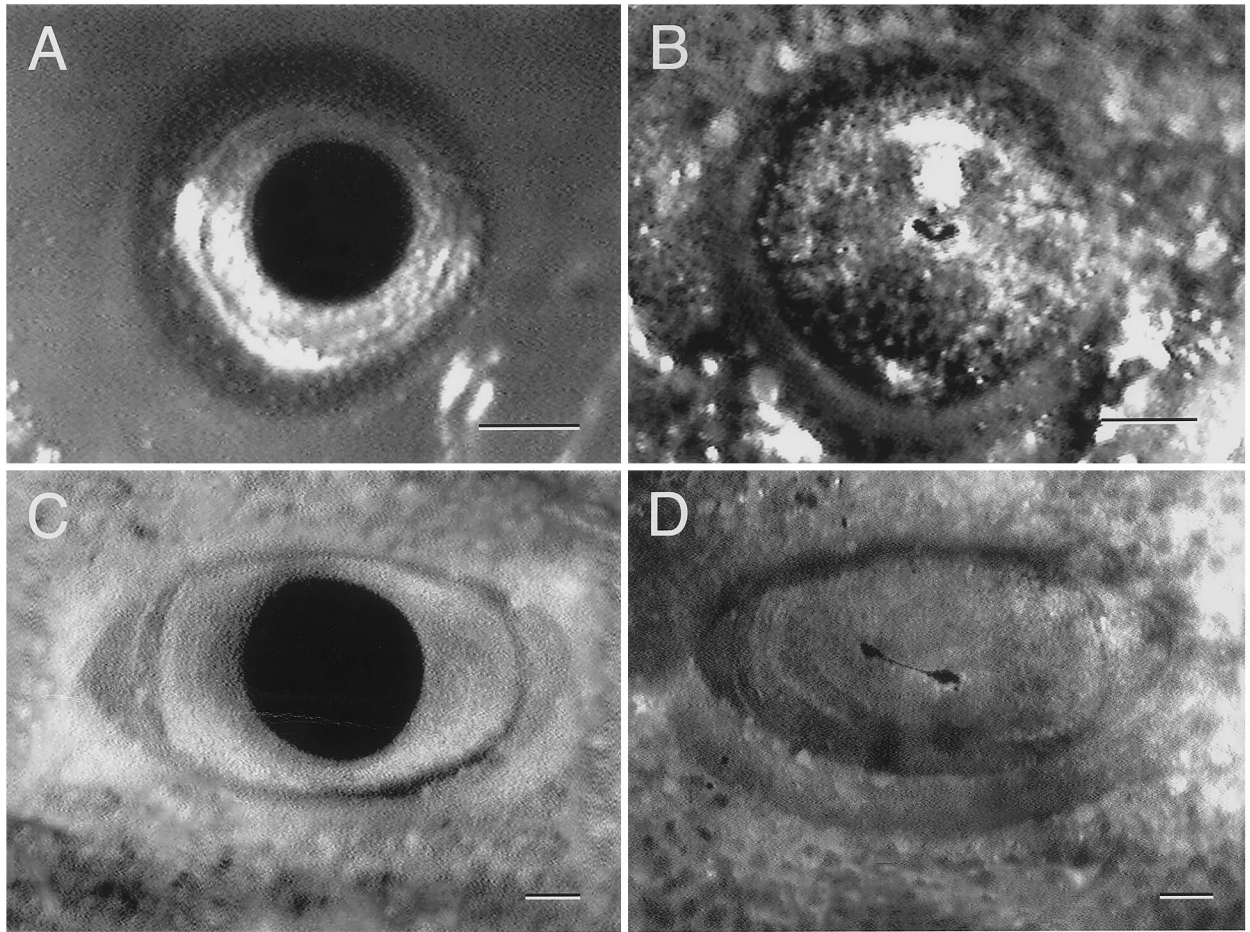
Fig. 3. Fully dilated and constricted pupils of *Porichthys notatus* (A and B) and *Cephaloscyllium ventriosum* (C and D) following stimulation with bright (6.75×10^4 μW/cm^2) white light. The scale bar represents 1 mm. Dorsal is at the top of the figure and rostral on the right.

adapted pupil area in response to the highest light levels. The speed and extent of these initial changes are comparable to those seen in 'higher' vertebrates, such as man (Fig. 8(a)) and are faster and greater than seen in, for instance, amphibia [2,3] and reptiles [7]. Thus, Walls's [34] assertion that in the relatively few teleosts that show pupillary movements, these are always less well developed and occur more slowly than in other vertebrates is clearly not the case for *Porichthys* sp.

Although the initial iris constriction in response to light is similar in *Porichthys* sp. and man, the redilation in continued illumination occurs more rapidly in humans (Fig. 8(a)). This might be explained by the fact that while man has both iris sphincter and dilator muscles, initial histological observations suggest that *P. notatus*, like many lower vertebrates, appears only to posses a sphincter muscle. Pupil dilation thus probably results from relatively slow passive processes such as elastic recoil.

The pupillary responses of both species of *Porichthys* and man noted here are very much faster than those of the swell shark, *Cephaloscyllium ventriosum* (Fig. 8), which is a solitary animal that usually lives in shallow

water, but has been caught down to 500 m [35], and spends most of the daylight hours immobile in caves and crevices, feeding mainly at night. This agrees with previous observations on elasmobranchs, where pupil movements of nocturnal species are considerably slower than those of diurnal animals, which appear almost as rapid as those noted here for *Porichthys* sp. [14–17].

4.2. Distribution of extensive pupillary responses among fish

With two exceptions, all teleost fish that have been shown to have extensive pupillary movements (several pleuronectiform flatfish [8], *Uranosopus scaber* [20,31,32], *Lophius piscatorius* [20,32], *Opsanus tau* [33], *Plecostomus* sp. [34], *Porichthys* sp. present study) have a dorso-ventrally flattened body, and spend much of their time on, or buried in, the substrate with only their eyes, which are positioned near the top of the head, protruding. Phylogenetically, however, these species are quite distinct, residing in four different orders. Only *Opsanus* and *Porichthys* are closely related, sharing the same family (Batrachoididae). The only non-bottom
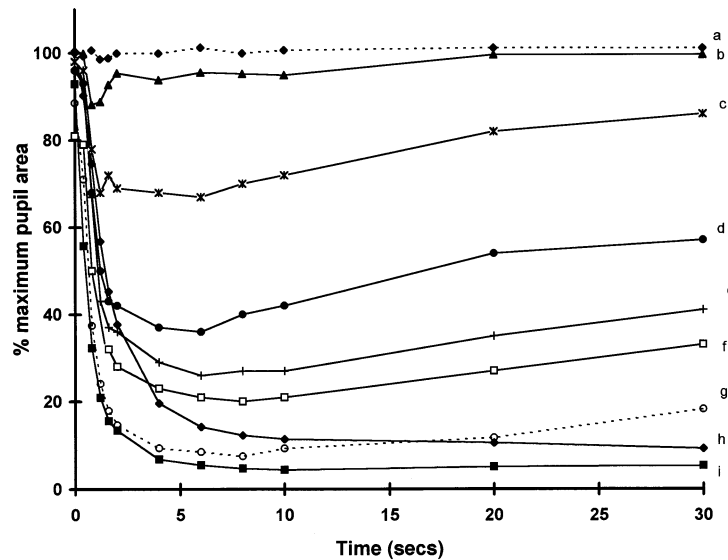
Fig. 4. Pupil responses of *Porichthys notatus* during 30 s exposure to different intensities of 500 nm light: (a) complete darkness; (b) 2.72×10^{-3} $\mu W/cm^2$; (c) 1.44×10^{-2} $\mu W/cm^2$; (d) 7.07×10^{-2} $\mu W/cm^2$; (e) 2.14×10^{-1} $\mu W/cm^2$; (f) 2.71×10^{-1} $\mu W/cm^2$; (g) 2.85 $\mu W/cm^2$; (h) 3.27×10^2 $\mu W/cm^2$. Also shown is the response to maximum intensity (6.75×10^4 $\mu W/cm^2$) white light exposure (i). All curves are individual responses, except for (b) and (d), which are the average responses of two and three fish, respectively.

dwelling teleosts with extensive pupil responses, the eel [27] and *Encheliophis jordani* [34], are grouped into a further two orders. The eel, like *Porichthys* sp., spends a proportion of its life in deep water, while frequently returning to shallower depths. *Encheliophis jordani*, on the other hand, is an eel-like commensal fish that lives inside the body cavity of invertebrates.

Given their lifestyle, it is not surprising to find that fish with extensive pupillary movements also have retinae adapted to optimising sensitivity. Anguilliform eels, for instance, have heavily rod dominated retinae [38]. Similarly, the retinae of elasmobranchs, most of whom show large pupillary responses, although they do contain a small proportion of cones, consist primarily of rods [39]. Although the retinae of *Uranoscopus*, *Lophius* and most pleuronectiform flatfishes appear to contain relatively more cones than eels and elasmobranchs, these are generally large and most form double cones [38]. Similarly, *P. notatus*, like its close relative *P. porosissimus* [38], has a retina consisting primarily of rods and large double cones [37] (Fig. 11). Double cones, although photopic receptors, probably code luminosity rather than wavelength information [40] and are often characteristic of animals living in deeper water [41].

4.3. Function of Porichthys pupil movements

The primary purpose of pupillary movements in most vertebrates is to regulate the light flux incident on the retina, thereby optimising visual resolution in all ambient lighting conditions. Pupillary constriction in response to light may also, among other things, enhance

acuity by decreasing the effect of optical aberrations and increase the depth of field when viewing close objects [42]. It has also been suggested that in the gecko the pupil may be a means of intraspecific signalling [43] and the human pupil is also known to reflect emotions to some extent [42].

Beyond these more or less well documented functions, pupillary constriction may convey an additional advantage to teleosts with highly contractile pupils, most of which, like *Porichthys* sp., try to conceal themselves during daylight hours by blending into the substrate. The most difficult part of an animal to conceal is usually its eye and in particular its large dark circular pupil [44]. Many animals, most notably several species of fish, have therefore evolved various mechanisms, such as pigment stripes running through the eye, for masking the pupil [34,44]. Pupillary constriction in substrate resident animals might be another such mechanism, as on constriction the dark pupil is replaced by a reflective iris that blends well with the body of the animal and the substrate.

Such a concealment function for pupillary closure will only be applicable to younger *Porichthys* sp., which live near the shore, and to adults returning to shallower water to spawn. Mensinger [36] has proposed an alternative function for the pupillary constriction of adult *P. notatus*, which have a mesopelagic lifestyle living at depths of up to 400 m. At these depths the bioluminescence produced both by *Porichthys* and most other mesopelagic animals, rather than down-welling sunlight, may be the primary visual stimulus. Pupil constriction in response to a bioluminescent flash could help preserve the sensitivity of the retina to subsequent
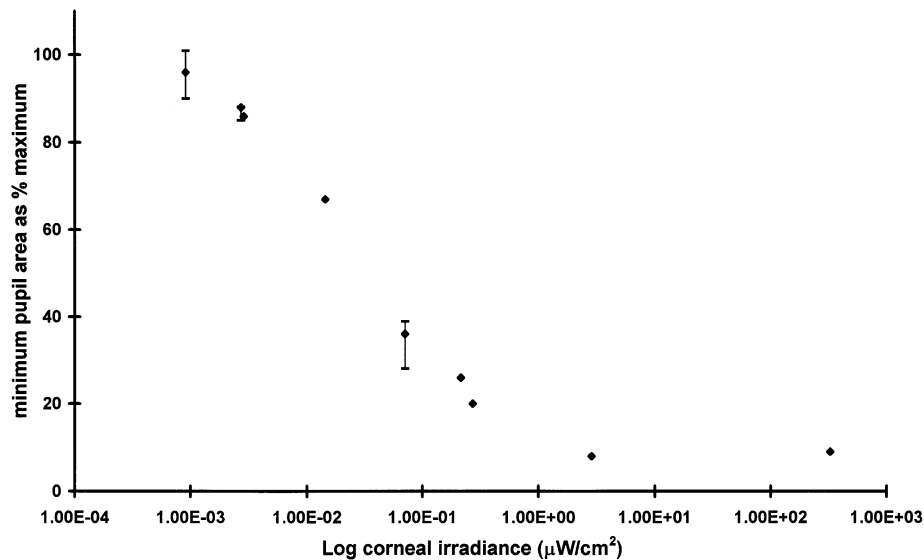
Fig. 5. Maximum pupil constriction of *Porichthys notatus* in response to different intensities of 500 nm light. All points represent data from individual fish except those indicated by the error bars. The error bars indicate the total range of values. For the two lowest intensities with error bars two fish were used, while three were used for the remaining point.

flashes. If this were the case one might expect mobile pupils to be widespread among both bioluminescent and non-bioluminescent mid-water fish. Preliminary observations on several mesopelagic species suggests this is not so (R.H. Douglas, personal observation).

It thus seems most likely that pupil movements of *Porichthys* sp. serve to regulate the light flux incident on their sensitive retinae when they migrate into shallower water and may additionally aid in camouflaging their eyes when buried in the substrate.

4.4. Pupil shape

The fully dilated *Porichthys sp*. pupil, like that of most vertebrates, is round. However, when constricted it forms two almost independent, horizontally elongated, apertures (Fig. 3(B)). The fully constricted pupil of the swell shark, *Cephaloscyllium ventriosum*, also consists of little more than a slit with a small pin hole aperture at either end (Fig. 3(D)). Such slit pupils are common among nocturnal animals and Walls [34] suggests that they may serve to protect their highly sensitive retinae which are nevertheless sometimes exposed to brighter light, since they are able to close down to a greater degree than a more conventional circular pupil. This explanation could also account for the presence of an horizontally elliptical pupil in the species examined here, both of which have retinae adapted for optimising sensitivity (see Section 4.2) although at times they are exposed to relatively high levels of illumination.

However, the fully constricted pupils of both *Porichthys* sp. and the swell shark are not merely simple ellipses as they consist largely of two small apertures (Fig. 3). Multiple pupillary apertures are not uncom-

mon in vertebrates, occurring, for example, in elasmobranch fish [10–12,15,16] (Fig. 3(D)) and many other vertebrates with sensitive retinae inhabiting relatively low light level environments [34,45]. Such pupils may benefit the animal in a number of ways, for example decreasing the degree of spherical aberration [34]. A more intriguing function for such pupils has, however, been proposed [4,45,46]. A single small aperture results in an eye with a large depth of field. This is of little consequence to animals such as primates, which primarily use binocular cues, such as retinal disparity, to judge distance [47]. However, many animals, including amphibia [48] and some fish [49], use monocular cues, such as monitoring their accommodative state, to measure distance. These animals will be severely disadvantaged by an eye with a large depth of field, as it will not have to accommodate to focus objects at quite different distances. This problem might be alleviated by a pupil that on constriction results in two or more small apertures, which would result in a greatly reduced depth of field. While this explanation is unlikely to apply to feeding behaviour of the two species of *Porichthys* and the shark studied here, as they feed almost exclusively at night, when their pupils are dilated, it is conceivable they may still want to judge the distance of other objects, such as potential predators, when immobile or nest-guarding in shallow water during daylight with constricted pupils.

The shape of the partially constricted pupil of *Porichthys sp*. with the intrusion of a dorsal 'flap', is similar to the iris 'operculum' described in several species of ray [10,11,20,50] and some bottom dwelling teleosts with upward pointing eyes [8,20,34,51]. Underwater, the distribution of illumination is very uneven,
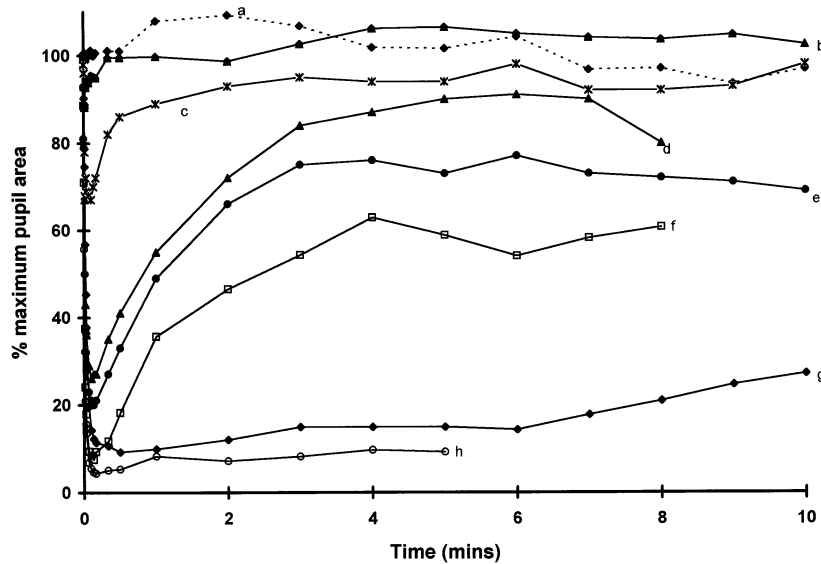
Fig. 6. Pupil responses of *Porichthys notatus* following up to 10 min exposure to different intensities of 500 nm light: (a) complete darkness; (b) 2.72×10^{-3} μW/cm²; (c) 1.44×10^{-2} μW/cm²; (d) 2.14×10^{-1} μW/cm²; (e) 2.71×10^{-1} μW/cm²; (f) 2.85 μW/cm²; (g) 3.27×10^2 μW/cm². Also shown is the response to maximum intensity (6.75×10^4 μW/cm²) white light exposure (h). All curves shown are individual responses, except for (b) which is the average response of two fish.
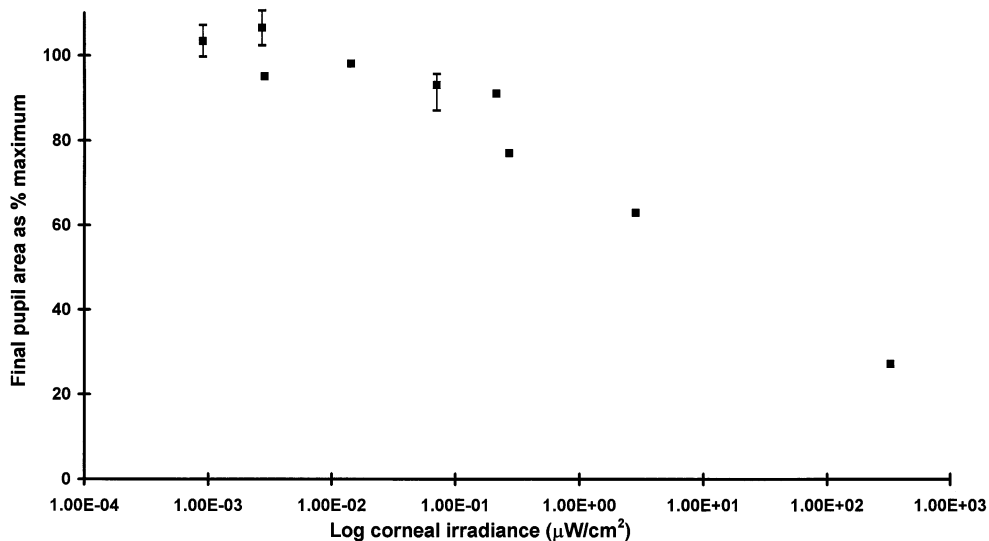

Fig. 7. Maximum re-dilation of *Porichthys notatus* pupils in the presence of different intensities of 500 nm light. All points represent data from individual fish except those indicated by error bars. The error bars indicate the total range of values. For the two lowest intensities with error bars two fish were used, while three were used for the remaining point.

light coming from above the animal being relatively more intense. A dorsal intrusion into the cornea will shade the sensitive *Porichthys* retina from this bright down-welling light, although a number of other functions for similar 'crescent-shaped' pupils have been proposed [45]. Whether the irregular pupil shape of *Porichthys sp.* relates to any regional variation in retinal structure is not known.

4.5. Consensual response

Our experiments suggest that the pupil response of *Porichthys sp.* is consensual, although not completely so. This is in agreement with observations on the eel [24], but at odds with findings on other teleosts [21,25,31], none of which observed true consensual responses. The situation is similarly diverse among elasmobranchs where consensual responses were not observed in sharks [10,12,16], while they were seen among rays [8,10,12]. Similarly, in certain amphibia the pupils appear to be independent [3], while in others they are not [24,52]. In reptiles, such as the gecko, the pupils are independent [4,43] as they appear to be in birds [1], while many mammals display a consensual response [42].
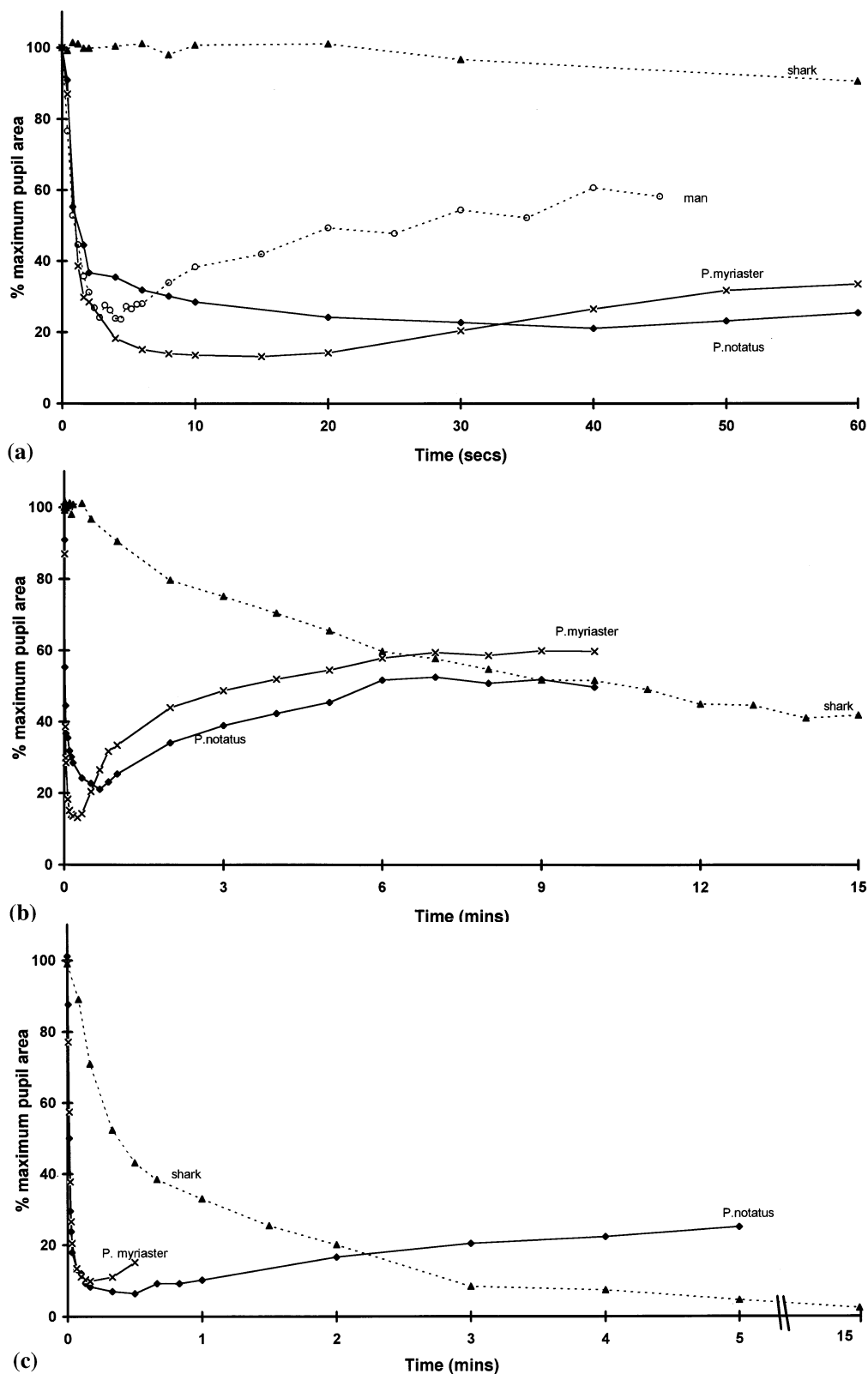
Fig. 8. (a, b) Pupillary response to a relatively dim white light stimulus (3.07×10^1 μW/cm^2) of *Porichthys notatus*, *Porichthys myriaster*, the swell shark *Cephaloscyllium ventriosum* and a human subject. (c) Pupillary responses of the 3 fish species to a brighter (6.75×10^4 μW/cm^2) white light stimulus. The fully dilated pupil areas of all four species were; *P. notatus*, 4.73 mm^2; *P. myriaster*, 5.11 mm^2; *C. ventriosum*, 7.78 mm^2; and human, 31.68 mm^2.
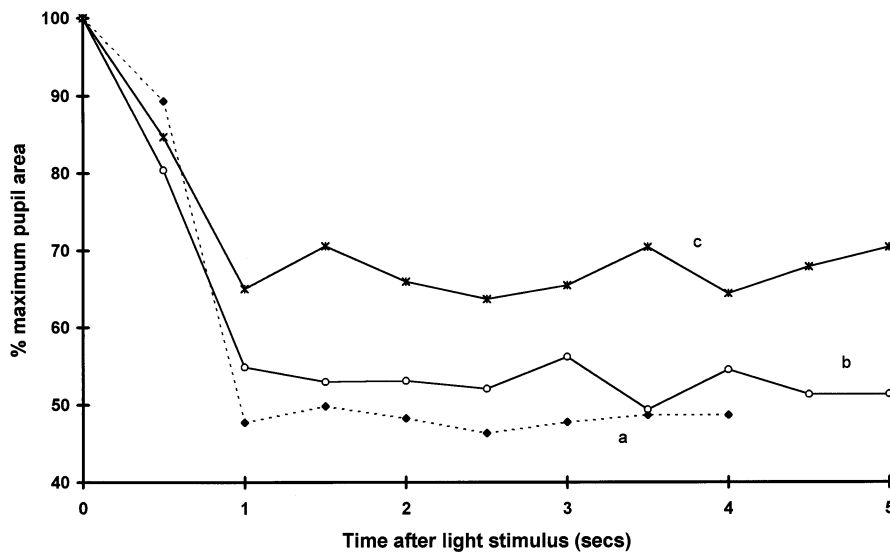
Fig. 9. Consensual responses of *Porichthys* sp. pupils. (a) Pupil response of the unstimulated eye of *Porichthys myriaster* (experiment 1). (b, c) Pupil response of the stimulated and unstimulated eye of *Porichthys notatus*, respectively (experiment 2).
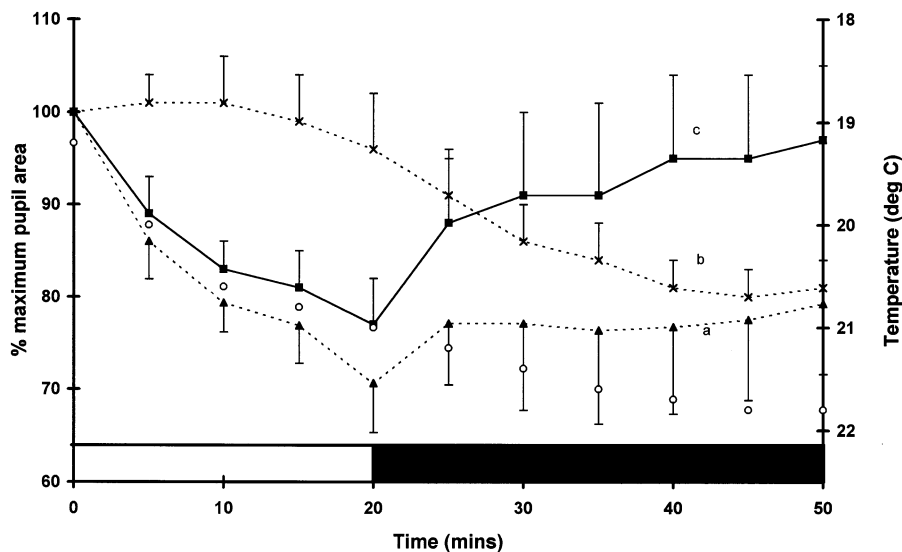

Fig. 10. (a) Average pupil area of eight isolated eyes subjected to 20 min illumination (6.12×10^4 µW/cm^2) followed by 30 min darkness. (b) Average pupil area of four isolated eyes maintained in darkness for 50 min. (c) Average response of isolated eyes to 20 min light followed by 30 min darkness accounting for the constriction observed in control animals kept in darkness. The error bars represent one standard deviation. The open circles show the temperature of seawater in a Petri dish exposed to the same lighting conditions described for (a).

4.6. Response of isolated eyes

Numerous studies using, for instance, eyes removed from the head, isolated irises, intact eyes within the body following optic nerve section or small spots of light directed at the irises of intact animals, have shown that the irises of amphibia [3,52–56], some teleosts [18,21,24,26–30,33,57,58] and elasmobranchs [9–12,16] and even some mammals [59–61], are directly sensitive to light. However, even among the limited number of teleosts studied in this respect, iris photosensitivity is not universal [31–33].

Our observation of pupillary movement in the isolated eyes of *P. notatus* is most probably also due to a photosensitive iris, although we cannot exclude the possibility that it is mediated via a local reflex involving the retina. The pupil response of the isolated *P. notatus* eye, which takes at least 20 min for constriction (Fig. 10), is very much slower than any of the directly photosensitive responses in other species, all of which occurred within seconds. Furthermore, several authors [12,26,27,57] have found the pupil responses of isolated irises and intact animals to be indistinguishable, which is clearly not the case for *P. notatus*. This discrepancy
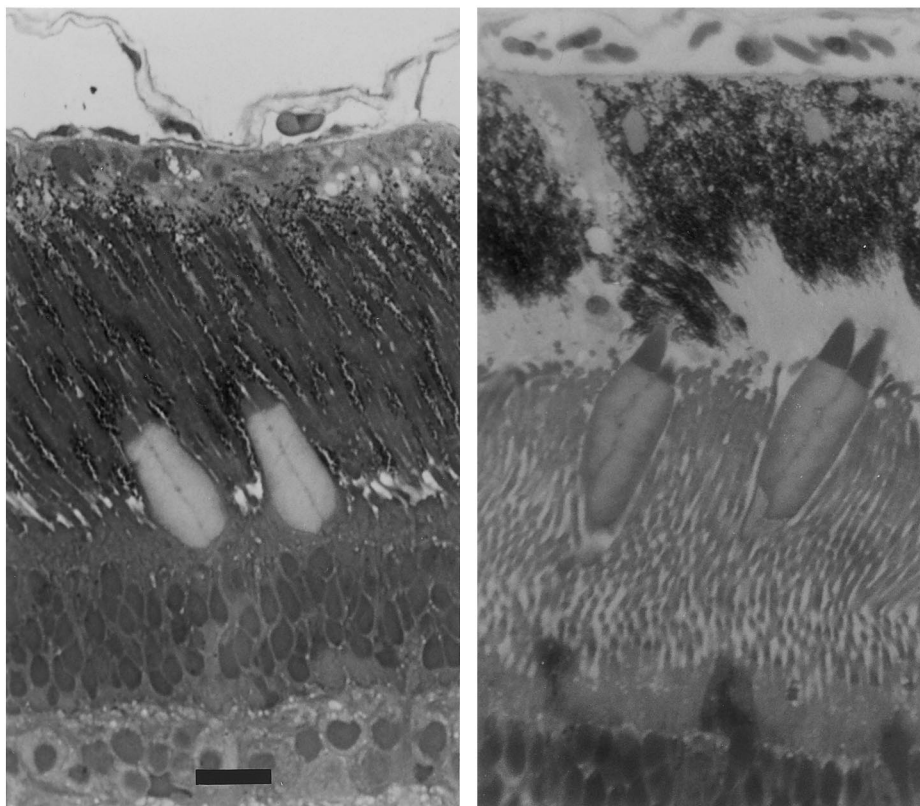
Fig. 11. Transverse section of a (a) light and (b) dark adapted *Porichthys notatus* retina displaying typical retinomotor movements of the cones and RPE. In the dark adapted retina the RPE has become slightly separated from the neural retina. The scale bar represents 10 μm.

between our results and those of others might point to a real difference in the pathways controlling the CNS independent pupillary responses, although possible experimental artefacts need to be considered.

The most likely light-independent trigger for pupil constriction is temperature, which has been shown to influence the response of isolated irises [18,24,54,55]. The temperature of the sea water bathing the isolated eyes in our experiments does in fact rise by 1.8°C during the 20 min of illumination in which the pupil constricts (Fig. 10). However, while pupillary constriction ceases once the lights are extinguished, the temperature continues to rise as the sea water is still below room temperature. The relatively small temperature change observed and the divergence of the temperature and pupillary response patterns after the stimulating light is extinguished (Fig. 10) make it unlikely that the temperature of the bathing solution is affecting our results.

Although the pupil of *P. notatus* does seem to be able to contract independently of the central nervous system in response to light, the slow time course of the response compared to that of the intact animal makes it likely that the pupil response of this species is primarily controlled by the autonomic nervous system [21,31–33].

4.7. Pupillary movements and retinomotor movements

Retinomotor movements of the rods, cones and retinal pigment epithelium (RPE), which serve to adapt the retina to photopic and scotopic conditions and to protect the sensitive rods in high levels of illumination [62], are most pronounced in fish and amphibia, occur to a lesser extent in birds and reptiles and are virtually absent in mammals. Pupillary movements, on the other hand, are most well developed in 'higher vertebrates', and less pronounced in the other vertebrate classes. Retinomotor responses and pupillary movements are therefore usually seen as functionally mutually exclusive, with pupillary mechanisms representing the more 'advanced' form of adaptation [34,63]. However, both forms of sensitivity control have for some time been known to occur to varying degrees in a variety of amphibia and birds. Von Studnitz [27] also noted extensive pupil responses, as well as light mediated rod elongation and RPE dispersal, but not cone contraction, in the eel. The presumed incompatibility of retinomotor and pupillary movements is made even less tenable by our observation that *P. notatus* displays both retinomotor migrations of its cones and RPE comparable to those seen in most teleost fish (Fig. 11)

and pupillary responses as well developed as those of higher vertebrates. In fact the possession of both forms of adaptation would seem desirable, as pupillary mechanisms are effective in a matter of seconds, while retinomotor light adaptation takes a minimum of around 15 min. Thus, at least in *P. notatus*, retinal sensitivity will be influenced by both retinomotor changes and pupillary movements, as well as by other biochemical, structural and physiological events within the retina [64,65]. The differential phylogenetic distribution of retinomotor responses and pupil mobility among vertebrates can therefore not be explained by the functional mutual exclusivity of these two processes.

Acknowledgements

References

[1] Schaeffel F, Wagner H. Barn Owls have symmetrical accommodation in both eyes, but independent pupillary responses to light. Vis Res 1992;32(6):1149–55.

[2] Cornell EA, Hailman JP. Pupillary responses of two *Rana pipiens*-complex anuran species. Herpetologica 1984;40(4):356–66.

[3] Henning J, Henning PA, Himstedt W. Peripheral and central contribution to the pupillary reflex control in amphibians: pupillographic and theoretical considerations. Biol Cybernet 1991;64:511–8.

[4] Denton EJ. The response of the pupil of *Gekko gecko* to external light stimulus. J Gen Physiol 1956;40:201–16.

[5] Werner YL. Extreme adaptability to light in the round pupil of the snake *Spalerosophis*. Vis Res 1970;10:1159–64.

[6] Frankenberg E. Pupillary response to light in Gekkonid lizards having various times of daily activity. Vis Res 1979;19:235–45.

[7] Granda AM, Dearworth JR, Kittila CA, Boyd WD. The pupillary response to light in the turtle. Vis Neurosci 1995;12:1127–33.

[8] Bateson W. Contractility of the iris in fishes and cephalopods. J Mar Biol Assoc UK 1890;1:215–6.

[9] Franz V. Beobachtungen am lebenden Selachierauge. Jenaische Z Naturwiss 1906;41:429–71.

[10] Franz V. Die Akkommodation des Selachierauges und seine Abblendungsapparate, nebst Befunden an der Retina. Zool Jahrb Abt Allg Zool Physiol Tiere 1931;49:323–462.

[11] Young JZ. Comparative studies of the physiology of the iris. I. Selachians. Proc R Soc London, Ser B 1933;112:228–41.

[12] von Studnitz G. Studien zur vergleichenden Physiologie der Iris III. Selachier. Z Vgl Physiol 1933;19:619–31.

[13] Gruber SH. A behavioural measurement of dark adaptation in the Lemon shark, *Negaprion brevirostris*. In: Rall DP, Gilbert PW, Mathewson RF, editors. Sharks, Skates and Rays. Baltimore: John Hopkins Press, 1967:479–90.

[14] Kuchnow KP, Gilbert PW. Preliminary in vivo studies on pupillary and tapetal pigment responses in the Lemon shark, *Negaprion brevirostris*. In: Rall DP, Gilbert PW, Mathewson RF, editors. Sharks, Skates and Rays. Baltimore: John Hopkins Press, 1967:465–77.

[15] Kuchnow KP. Threshold and action spectrum of the elasmobranch pupillary response. Vis Res 1970;10:955–64.

[16] Kuchnow KP. The elasmobranch pupillary response. Vis Res 1971;11:1395–406.

[17] Gilbert PW, Sivak JG, Pelham RE. Rapid pupil change in selachians. Can J Zool 1981;59(3):560–4.

[18] Brown-Séquard E. Recherches expérimentales sur l'action de la lumière et sur celle d'un changement de temperature sur l'iris, dans les cinq classes d'animaux vertébrés. C R Acad Sci Paris 1847;25:482–3.

[19] Grynfeltt E. Les muscles de l'iris chez les teleostiens. Bibl Anat 1909;20:265–332.

[20] Beer T. Die Akkommodation des Fischauges. Pflüg Arch Gesammt Physiol 1894;58:523–650.

[21] Nilsson S. Sympathetic nervous control of the iris sphincter of the Atlantic cod, *Gadus morhua*. J Comp Physiol A 1980;138:149–55.

[22] Somiya H. Dynamic mechanism of visual accommodation in teleosts: structure of the lens muscle and its nerve control. Proc R Soc London, Ser B 1987;230:77–91.

[23] Fujimoto M, Kunioka H, Katayama H. Pharmacology of the retractor lentis muscle of the bluegill (*Lepomis macrochirus*). J Comp Physiol A 1995;176:673–7.

[24] Brown-Séquard E. Recherches experimentales sur l'enfluence excitaire de la lumiere, du froid et de la chaleur sur l'iris, dans les cinq classes d'animaux vertebres. J Physiol Path Gen 1859;2:281–94.

[25] Steinach E. Untersuchungen zur vergleichenden Physiologie der Iris. Pflügers Arch Gessamten Physiologie 1890;47:289–340.

[26] Steinach E. Untersuchungen zur vergleichenden Physiologie der Iris. Pflügers Arch Gessamten Physiologie 1892;52:495–525.

[27] von Studnitz G. Beträge zur Adaptation der Teleosteer. Studien zur vergleichenden Physiologie der Iris II. Z Vg Physiol 1932;18:307–38.

[28] Brown-Séquard E. Note complimentare d'un memoire sur l'action de la lumiere et d'un changement de temperature sur l'iris. C R Acad Sci Paris 1847;25:508.

[29] Magnus R. Beiträge zur Pupillarreaction des Aal- und Froschauges. Z Biol 1899;38:567–606.

[30] Guth E. Untersuchungen über die directe motorische Wirkung des Lichtes auf den Sphincter pupillae des Aal- und Froschauges. Pflüg Arch Gessamt Physiol 1901;85:119–42.

[31] Young JZ. The pupillary mechanism of the teleostean fish, *Uranoscopus scaber*. Proc R Soc London, Ser B 1931;107:464–85.

[32] Young JZ. Comparative studies of the physiology of the iris.-II. *Uranoscopus* and *Lophius*. Proc R Soc London, Ser B 1933;112:242–9.

[33] Rubin L, Nolte J. Autonomic innervation and photosensitivity of the sphincter pupillae muscle of two teleosts: *Lophius piscatorius* and *Opsanus tau*. Curr Eye Res 1982;1(9):543–51.

[34] Walls GL. The Vertebrate Eye and its Adaptive Radiation. New York: Hafner, 1963.

[35] Love M. Probably More Than You Wanted to Know About the Fishes of the Pacific Coast, 2nd edition. Santa Barbara: Really Big Press, 1996.

[36] Mensinger AF. Ecomorphological adaptations to bioluminescence in *Porichthys notatus*. Environ Biol Fishes 1995;44:133–42.

[37] Fernandez HR, Tsuji FI. Photopigment and spectral sensitivity in the bioluminescent fish, *Porichthys notatus*. Mar Biol 1976;34:101–7.

[38] Ali MA, Anctil M. Retinas of Fishes: An Atlas. Berlin: Springer Verlag, 1976.

[39] Cohen JL. Vision in elasmobranchs. In: Douglas RH, Djamgoz MBA, editors. The Visual System of Fish. London: Chapman and Hall, 1990:465–90.

[40] Maier EJ, Bowmaker JK. Colour vision in the passeriform bird, *Leiothrix lutea*: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. J Comp Physiol A 1993;172:295–301.

[41] Boehlert GW. Intraspecific evidence for the function of single and double cones in the teleost retina. Science 1978;202:309–11.

[42] Loewenfeld IE. The Pupil: Anatomy, Physiology and Clinical Applications. Detroit: Wayne State University Press, 1993.

[43] Werner D. Beobachtungen an *Ptyodactylus hasselquistii guttatus* (Geckonidae). Verh Naturforsch Ges Basel 1972;82:54–87.

[44] Cott HB. Adaptive Coloration in Animals. London: Methuen, 1966.

[45] Murphy CJ, Howland HC. The functional significance of crescent-shaped pupils and multiple pupillary apertures. J Exp Zool Suppl 1991;5:22–8.

[46] Murphy CJ, Howland HC. On the Gekko pupil and Scheiner's disc. Vis Res 1986;26(5):815–7.

[47] Collett TS, Harkness L. Distance vision in animals. In: Ingle DJ, Goodale M, Mansfield JW, editors. Advances in the Analysis of Visual Behaviour. Cambridge: MIT Press, 1982:111–76.

[48] Douglas RH, Collett TS, Wagner HJ. Accommodation in anuran amphibia and its role in depth perception. J Comp Physiol A 1986;158:133–43.

[49] Douglas RH, Eva J, Guttridge N. Size constancy in Goldfish (*Carassius auratus*). Behav Brain Res 1988;30:37–42.

[50] Franz V. Zur Anatomie, Histologie und functionellen Gestaltung des Selachierauges. Jenaische Z Naturwiss 1905;40:697–840.

[51] Nicol JAC. The Eyes of Fishes. Oxford: Oxford University Press, 1989.

[52] von Campenhausen C. Quantitative Beziehungen zwischen Lichtreiz und Kontraktion des Musculus sphincter pupillae vom Scheibenzüngler (*Discoglossus pictus*). Kybernetik 1963;1(6):249–67.

[53] Barr L, Alpern M. Photosensitivity of the frog iris. J Gen Physiol 1963;46:1249–65.

[54] Glaus-Most L. Zur Lichtreaktion der isolierten Froschiris. Rev Suisse Zool 1969;76(43):799–848.

[55] Armstrong PB, Bell AL. Pupillary responses in the toad as related to innervation of the iris. Am J Physiol 1968;214(3):566–73.

[56] Morris JL. Motor innervation of the toad iris (*Bufo marinus*). Am J Physiol 1976;231:1272–8.

[57] Seliger HH. Direct action of light in naturally pigmented muscle fibres I. Action spectrum for contraction in eel iris sphincter. J Gen Physiol 1962;46:333–42.

[58] Bell AL. The fine structure of the iris of the eel. J Cell Biol 1965;27:9A–10A.

[59] Bito LZ, Turansky DG. Photoactivation of pupillary constriction in the isolated in vitro iris of a mammal (*Mesocricetus auratus*). Comp Biochem Physiol 1975;50A:407–13.

[60] Suzuki R, Yoshino H, Kurimoto S. Effects of ultraviolet radiation and visible light on hamster pupil. Doc Ophthalmol 1991;77:81–7.

[61] Lau KC, So KF, Campbell G, Lieberman AR. Pupillary constriction in response to light in rodents, which does not depend on central nervous pathways. J Neurol Sci 1992;113:70–9.

[62] Douglas RH. The function of the photomechanical movements in the retina of the rainbow trout (*Salmo gairdneri*). J Exp Biol 1982;96:389–403.

[63] Ali MA. Les résponses rétinometrices: caractères et mécanismes. Vis Res 1971;11:1225–88.

[64] Wagner HJ, Djamgoz MBA. Spinules: a case for retinal synaptic plasticity. Trends Neurosci 1993;16(6):201–6.

[65] Dowling JE. The Retina: An Approachable Part of the Brain. Cambridge: Belknap Press, 1987.