Development of dim-light vision in the nocturnal reef fish family Holocentridae II: Retinal morphology

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Summary statement

Coral reef fishes in the family Holocentridae remodel their retina at the cellular level to adapt to a nocturnal lifestyle during development.

Abstract

Ontogenetic changes in the habitats and lifestyles of animals are often reflected in their visual systems. Coral reef fishes start life in the shallow open ocean but inhabit the reef as juveniles and adults. Alongside this change in habitat, some species also change lifestyles and become nocturnal. However, it is not fully understood how the visual systems of nocturnal reef fishes develop and adapt to these significant ecological shifts over their lives. Therefore, we used a histological approach to examine visual development in the nocturnal coral reef fish family, Holocentridae. We examined seven representative species spanning both subfamilies,
Holocentrinae (squirrelfishes) and Myripristinae (soldierfishes). Pre-settlement larvae showed strong adaptation for photopic vision with high cone densities and had also started to develop a multibank retina (i.e., multiple rod layers), with up to two rod banks present. At reef settlement, holocentrids showed increased investment in their scotopic visual system, with higher rod densities and higher summation of rods onto the ganglion cell layer. By adulthood, they had well-developed scotopic vision with a highly rod-dominated multibank retina comprising 5-17 rod banks and enhanced summation of rods onto the ganglion cell layer. Lastly, the ecological demands of the two subfamilies were similar throughout their lives, yet their visual systems differed after settlement, with Myripristinae showing a more pronounced investment in scotopic vision than Holocentrinae. Thus, it is likely that both ecology and phylogeny contribute to the development of the holocentrid visual system.

**Introduction**

Vision is important to the behaviour and survival of most vertebrates (Cronin et al. 2014). Due to the broad range of habitats and light environments that they experience, marine fishes show great diversity in their visual adaptations. These adaptations are reflected at the cellular level in the structure and organisation of their eye and retina (Walls 1942; de Busserolles et al. 2020; Cortesi et al. 2020). The retina has four key cellular strata (in order of neural processing): the photoreceptor layer (PRL), outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL) (Land and Nilsson 2012). The PRL and ONL house the outer segments (OS) and nuclei of photoreceptors, respectively, of which there are two main types: rods and cones (Lamb 2013). Rods usually mediate scotopic (dim light) vision, while cones mediate photopic (bright light) and colour vision and are divided into single and double cones (i.e., two fused single cones). The synaptic terminals of rods and cones are contained within the outer plexiform layer (OPL) where they communicate with the next cellular layer, the INL.

The INL contains the nuclei of interneurons, such as bipolar, horizontal and amacrine cells, and their synapses are located within the inner plexiform layer (IPL), which represents the primary stage of opponent processing for colour vision (Baden and Osorio 2019). Finally, visual signals are summated in the GCL, which sets the limits of the luminous sensitivity of the eye (i.e., more rods summating onto a single GC increases sensitivity) and spatial resolving power (i.e., acuity) (Warrant 2004). Importantly, the density and distribution of the
different neural cells are usually heterogenous across the retina. Regions of the retina with high densities of a particular cell type, *i.e.*, retinal specialisations, provide higher acuity and/or sensitivity in a specific part of an animal’s visual field (Collin and Pettigrew 1989). Retinal specialisations often facilitate specific behavioural tasks, such as feeding or predator avoidance (Collin and Pettigrew 1988a; Luehrmann *et al.* 2020; de Busserolles *et al.* 2021).

In general, differences in the organisation and densities of the retinal cell types correlate well with ecological demands (Shand 1997; Stieb *et al.* 2016; Luehrmann *et al.* 2020). For instance, fishes that are predominantly active in dim light (*e.g.*, deep-sea habitat or nocturnal lifestyle) have evolved a shared array of cellular adaptations to enhance the sensitivity of their eyes, including high rod densities and low cone densities (Pankhurst 1989; Shand 1994b), high summation of rods onto GC (Shand 1994b; de Busserolles *et al.* 2020; de Busserolles *et al.* 2021), and thick PRL (with longer rods) (Wagner *et al.* 1998). Several species have pushed scotopic adaptations to an extreme level by evolving a pure rod retina (Munk 1966) or a retina with multiple layers of rods (known as a multibank retina) (McFarland 1991; de Busserolles *et al.* 2021), or by combining the characteristics of both rods and cones into a single photoreceptor cell (known as transmutation) (de Busserolles *et al.* 2017). Although some of these adaptations are relatively common, little is known about their development.

Over ontogeny, many marine fishes experience significant ecological shifts. As larvae, most marine fishes inhabit a bright and broad-spectrum light environment (Boehlert 1996) in the upper epipelagic ocean where they consume (zoo)plankton (Job and Bellwood 2000; Helfman *et al.* 2009). As they grow older and become juveniles and adults, they may switch to a very different habitat (pelagic, estuarine, reef, deep-sea), diet (planktivory, carnivory, herbivory, corallivory), and/or diel activity pattern (diurnal, nocturnal, crepuscular) (King and McFarlane 2003; Helfman *et al.* 2009). These ecological shifts and consequent changes to the light environment are thought to be the main drivers of visual development in marine fishes (Carleton *et al.* 2020; Musilova *et al.* 2021). As such, ontogenetic variation in the organisation and/or structure of their visual systems have previously been correlated with changes in diet [*surgeonfishes* (Tettamanti *et al.* 2019)], diel activity patterns [*several reef fish families* (Shand 1997)], depth [*lanternfishes, hoki, hake, roughy, oreodories* (Pankhurst 1987; Mas-Riera 1991)], habitat [*goatfishes* (Shand 1994a); *mackerel icefish* (Miyazaki *et al.* 2011)], and body morphology [*winter flounder* (Evans and Fernald 1993)].
For species that adopt bright environments, visual development is characterised by typical changes in the cellular architecture of the retina. Specifically, the retina is initially cone-dominated, and the densities of cones, INL cells and GC increase early in development and then decrease slightly (as retinal area expands), while rod densities undergo a minor increase (Fernald 1990; Shand 1997). Contrastingly, in fishes which adopt dim environments, visual development seems to be characterised by a more rapid and extreme version of these changes. For example, some deep-sea fishes seem to possess cones as larvae but progress to having only rods in adulthood (Bozzano et al. 2007; de Busserolles et al. 2014a; Lupše et al. 2021). However, most of the previous studies on visual development in fishes with dim habitats or lifestyles focused on deep-sea fishes. In contrast, how the visual system develops in nocturnal reef fishes is poorly understood [but see (Shand 1997)].

Here, we investigated visual development at the cellular level in the nocturnal reef fish family, Holocentridae. Holocentridae comprises two subfamilies, Holocentrinae (squirrelfishes) and Myripristinae (soldierfishes). As larvae, both subfamilies inhabit the upper pelagic ocean and feed on zooplankton (Tyler et al. 1993; Sampey et al. 2007). During the transition to juvenile life, most holocentrids migrate to a shallow tropical coral reef habitat (Nelson 1994) and adopt a nocturnal lifestyle feeding on benthic crustaceans (Holocentrinae) or zooplankton in the water column (Myripristinae) (Gladfelter and Johnson 1983; Greenfield 2002; Greenfield et al. 2017). Recently, we examined the visual systems of adult holocentrids (de Busserolles et al. 2021). We found that they possess well-developed scotopic vision with a rod-dominated retina arranged into multiple banks. The complexity of their multibank retina resembles that of some deep-sea fishes, with up to 7 and 17 banks in Holocentrinae and Myripristinae, respectively (de Busserolles et al. 2021). Adults also have some level of photopic vision which is more pronounced in Holocentrinae than Myripristinae, with the presence of both single cones and double cones, all well organised into retinal specialisations (de Busserolles et al. 2021).

While the visual systems of adult holocentrids have been described in detail, their development is poorly understood. Hence, we used a histological approach to examine anatomical structure and cell densities in the retina at key ontogenetic stages (pre-settlement larvae, settlement larvae, settled juveniles and adults). We studied shallow reef-dwelling species from three genera (Sargocentron, Neoniphon and Myripristis) covering both subfamilies, as well as an adult for a deeper-dwelling species (Ostichthys sp.). We used this approach to address the following aims: 1) to assess how the holocentrid visual system
changes as they shift from being predominantly active in bright light to dim light, and 2) to study the development of their deep-sea-like multibank retina.

Materials and methods

Animal collection and retinal tissue preservation

Details of all animals used in this study are given in Table S1. Animal collection and developmental staging followed methods outlined in greater detail in Fogg et al. (2022). Briefly, pre-settlement larvae were collected using light traps and settlement larvae were collected using a crest net (Lecchini et al. 2004; Besson et al. 2017). Settled juveniles were larvae caught in light traps which were allowed to metamorphose and further develop for two weeks in outdoor aquaria exposed to natural light. Adults were collected with either spearguns, pole and lines or clove oil and hand nets, or were sourced from a supplier, Cairns Marine (Cairns Marine Pty Ltd, Cairns, Australia; https://www.cairnsmarine.com/).

Fish collection and euthanasia followed procedures approved by the University of Queensland Animal Ethics Committee (QBI 304/16). Briefly, fish were first anesthetised by immersion in a solution of 0.2 mL clove oil per litre of seawater until respiration and all response to light and touch had ceased and were then euthanised by swift decapitation. All collections within Australia were conducted under a Great Barrier Reef Marine Park Permit (G17/38160.1) and Queensland General Fisheries Permit (180731) and all collections in French Polynesia were conducted in accordance with French regulations. Following euthanasia, all individuals were photographed adjacent to a ruler and their body size [total length (TL) and standard length (SL)] and eye diameter were subsequently measured from photographs using Fiji v1.53c [National Institutes of Health, USA; (Schindelin et al. 2012)]. Eyes were immediately enucleated, the cornea and lens removed, and the eye cup preserved in 4% paraformaldehyde [PFA; 4% (w/v) PFA in 0.01M phosphate-buffered saline (PBS), pH 7.4] depending on the analysis (see below for details).

Histology

Retinal histology was conducted on PFA-fixed eyes from the following individuals: three pre-settlement larvae (Sargocentron rubrum, n=3), five settlement larvae (S. microstoma,
n=1; Myripristis berndti, n=1; M. kuntee, n=3), two settled juveniles (S. rubrum, n=2) and ten adults (S. rubrum, n=3; S. microstoma, n=1; S. diadema, n=1; M. berndti, n=2; M. kuntee, n=1; M. violacea, n=1; Ostichthys sp., n=1). All animals were sampled in the light-adapted state except for the pre-settlement larvae and the adult Ostichthys specimen, which were dark-adapted. To account for intraretinal variability (de Busserolles et al. 2021), two (dorsal and ventral) or five (dorsal, ventral, central, nasal and temporal) retinal regions were sampled for pre-settlement larvae and later stages, respectively (Fig. 1). Notably, for the Ostichthys sp., tissue quality was only sufficient to examine one region (ventral). Briefly, a small square of retina was dissected from each region, post-fixed in 2.5% glutaraldehyde and 2% osmium tetroxide, dehydrated in ethanol and/or acetone, and embedded in EPON resin (ProSciTech). All tissue processing was done in a BioWave Pro tissue processor (PELCO).

Radial 1 μm-thick retinal sections were cut with a glass knife on a Leica ultramicrotome (Ultracut UC6) and stained with a solution of 0.5% toluidine blue and 0.5% borax. Retinal sections were viewed with a 63X objective (oil, 1.4 numerical aperture, 0.19 mm working distance, 0.102 μm/pixel) on a Zeiss Axio upright microscope (Imager Z1) and brightfield images acquired with Zeiss Axiocam 506 mono and 512 colour microscope cameras. Rod outer segment (ROS) length, PRL thickness, and whole retinal thickness were then measured from micrographs using Fiji. A body size range at which the full complement of banks (i.e., the maximum number of rod banks detected across all individuals/stages for each species) was reached was determined by comparing TL in individuals with the full complement to the maximal TL published on FishBase (https://www.fishbase.se) (Froese and Pauly 2019).

**Cell density estimations**

Retinal cell densities were estimated from transverse retinal sections, a method widely employed for marine teleosts for over 50 years (Munk 1965; Locket 1980; Shand 1997; Taylor et al. 2015). Cell densities were compared at different stages in the same species for Holocentrinae (S. rubrum), and in two species in the same genus for Myripristinae due to a limitation in the number of specimens at specific stages (settlement: M. kuntee, adult: M. berndti). However, to make sure that the data were comparable between the different species from Myripristinae, the densities in settlement M. kuntee were also compared to one settlement M. berndti and the densities in adult M. berndti were compared to one adult M.
Briefly, using Fiji, images were cropped to obtain retinal strips of 250 μm (horizontal length) for lower-density cell types (i.e., cones and GCL cells) in adults, 100 μm for lower-density cell types in larvae, and 40 μm for higher-density cell types (i.e., ONL and INL cells) for all life stages. These counting frames were optimised by conducting trials with several frame sizes and taking the minimum frame size that produced counts ≥95% similar to those attained with the largest frame (assumed to be the most accurate). The number of cone OS, ONL nuclei, INL nuclei and GCL nuclei were counted for three sections per sample using the cell counter plugin in Fiji. Subsequently, counts were corrected for section thickness using Abercrombie’s correction (Abercrombie 1946) and the density of each retinal cell type per 0.01 mm$^2$ of retina was calculated. Rod densities were calculated as the difference between ONL nuclei and cone OS densities, while rod:GCL summation was calculated by dividing the densities of rods by the densities of cells in the GCL (Shand 1994a). Graphs throughout the manuscript were generated using GraphPad Prism software v8.3.1 (www.graphpad.com).

Results

Multibank retina structure

Retinal sections were taken at different life stages from species in each subfamily in Holocentridae to assess the structure of their multibank retina. In all species and stages, rods were arranged in banks in at least part of the retina. Moreover, rod banking increased with size/age. Pre-settlement larvae of *S. rubrum* (the only species obtained at this stage) had two rod banks in the dorsal retina but only one bank in the ventral retina (Fig. 2). In settled juveniles from Holocentrinae, this increased to 3 or 4 rod banks depending on the region, and in adults, increased to five banks in the dorsal, nasal and ventral regions and 7 banks in the temporal and central retina (Fig. 2). Settlement larvae of *M. kuntee* and *M. berndti* had 3-4 rod banks in all regions, while adults possessed 12-13 banks in all regions except the ventral retina, which had 17 banks (Fig. 2). Finally, the adult specimen of the deeper dwelling soldierfish, *Ostichthys* sp., had approximately 10 rod banks in the ventral retina (Fig. S2). Across the family, the full complement of banks was attained by the time fish reached 40-60% of maximal size.
In species from both subfamilies (Holocentrinae: *S. microstoma*; Myripristinae: *M. berndti*), the addition of rod banks between settlement larvae/settled juveniles and adults resulted in an increase in the PRL thickness (Table 1). The regions with the greatest increase in rod banks over ontogeny showed the greatest increase in PRL thickness and maximal PRL thickness matched maximal rod banking in adults. However, the ontogenetic increase in rod banking did not result in a linear increase in PRL thickness due to concurrent shortening of the ROS with age (Table 1).

**Retinal cell densities**

The densities of different retinal cell types (rods, cones, INL cells and GCL cells) were estimated in different regions of the retina for species in Holocentrinae (*S. rubrum*) and Myripristinae (*M. kuntee, M. berndti*) (Fig. 3). In *S. rubrum*, between pre-settlement larval and settled juvenile stages, mean cone, INL and GCL densities decreased across the retina, by 65-75%, 19-42% and 31-39%, respectively (% range for the different regions) (Fig. 3, Table 3). Concurrently, rod densities and rod:GCL summation increased in all regions, by 34-63% and 120-136%, respectively. Between settled juvenile and adult stages, cone, INL and GCL cell densities continued to decrease across the retina, by 81-92%, 77-90% and 83-95%, respectively. Concurrently, rod densities and rod:GCL summation further increased by 10-44% and 663-2,073%, respectively.

A similar developmental pattern was observed in *Myripristis* spp. (Fig. 3, Table 3). Since cell densities were found to be similar between *M. kuntee* and *M. berndti* (Fig. S1), a comparison between stages was done using the two species to increase sample size. Between settlement and adulthood, cone, INL cell and GCL cell densities decreased across the retina by 92-96%, 77-90% and 90-95%, respectively. Concurrently, rod densities and rod:GCL summation increased by 104-294% and 2,123-6,592%, respectively.

Intraretinal shifts in peak cell densities were also found in all holocentrids examined (Fig. 3, Table 3). Around settlement, all species had higher cone, INL cell and GCL cell densities in the temporal retina. At adulthood, *S. rubrum* retained these peak densities in the temporal retina, while *Myripristis* spp. shifted its peak cone and GCL cell densities centrally, and its peak INL cell densities ventrally. Conversely, rod densities did not peak in the same regions for *S. rubrum* and *Myripristis* spp. at either stage (Fig. 3). Lastly, the highest densities for each cell type were similar around settlement, irrespective of subfamily, but by adulthood,
S. rubrum had much lower peak rod densities and higher peak cone and GCL cell densities than Myripristis spp.

Discussion

Development of the multibank retina

The multibank retina is one of the most common visual specialisations in deep-sea fishes, found in at least 38 families from across the teleost phylogeny (de Busserolles et al. 2020; Awaiwanont et al. 2001). Based on the few studies on multibank retina development, it appears that rod banks are added as fish grow (Locket 1980; Pankhurst 1987; Frohlich and Wagner 1998; Wagner et al. 1998; Omura et al. 2003; Taylor et al. 2011, 2015), either continually (for mesopelagic fishes and one catadromous elopomorph species, Anguilla japonica), or until 20 – 47% of the maximal size is reached (for bathypelagic fishes) (Locket 1980; Pankhurst 1987; Frohlich and Wagner 1998; Omura et al. 2003). Similar to bathypelagic fishes, the present study showed that in holocentrids, banks were added as the fish grew (Fig. 2, Table S1), up until they reached 40-60% of their maximal size. However, this may be found to be even earlier if more intermediate sizes were examined. Moreover, most banks were added after holocentrids settled on the reef and transitioned to a dimmer environment. Whether the addition of rod banks was driven by the exposure to dim light is still unknown. However, light environment has consistently been shown to be the dominant driver of visual adaptations in marine fishes (Shand et al. 2008; Cortesi et al. 2016; Luehrmann et al. 2018; Schweikert et al. 2018) and thus, represents a convincing possibility.

Further evidence for light environment as a driver of multibank retina development comes from an examination of intraretinal and interspecific variability in bank numbers. This variability has been reported in some adult deep-sea fishes (Locket 1985; Denton and Locket 1989) as well as some adult holocentrids (de Busserolles et al. 2021). Similarly, this study showed that the number of banks in adult holocentrids varied with both retinal region and species (Fig. 2). However, at earlier stages, rod banking did not vary greatly with either factor. Thus, the holocentrid multibank retina only became specialised later in life once they had adopted a nocturnal lifestyle. This implies that the multibank adaptation does not become fully active until maturity and/or under dim conditions.
Despite the prevalence of multibank retinas, their function remains a mystery. Two main non-mutually exclusive hypotheses have been proposed: 1) multibank retinas enhance luminous sensitivity (Frohlich and Wagner 1998) and/or, 2) they allow colour vision in dim light (Denton and Locket 1989). Results from this study seem to support both ideas. Support for the sensitivity hypothesis comes from the co-localisation of peak rod:GCL convergence and peak rod banking in Myripristinae (Fig. 2, Fig. 3), suggesting that summation of visual signals is prioritised in their multibank retina. Conversely, support for the colour vision hypothesis comes from the co-localisation of peak INL cell densities with peak cone densities at settlement but peak rod densities in adulthood (Fig. 3). Given that the INL contains the nuclei of cells involved in the primary stages of opponent processing (Baden and Osorio 2019), this potentially suggests a developmental switch in opponent processing of cone- to rod-derived signals. Although these are intriguing insights, future electrophysiological and behavioural studies are required to confirm the function of the multibank retina throughout ontogeny.

Retinal cell densities over development

Most teleosts commence life with a pure cone retina, with rods added later (Evans and Fernald 1990; Raymond et al. 1995). While most diurnal shallow-water fishes follow this developmental trajectory (Blaxter and Staines 1970), it may be adjusted when faced with different ecological demands. For example, deep-sea or nocturnal fishes show more rapid and pronounced increases in rod densities and decreases in cone densities over development (Shand 1997; Locket 1980; Pankhurst 1987; Bozzano et al. 2007). In line with their ecology, holocentrids followed a nocturnal pattern, rapidly decreasing cone densities and increasing rod densities (Fig. 3, Table 3). These retinal changes were particularly pronounced post-settlement correlating with the timing at which holocentrids are thought to become nocturnal (Shand 1994b). Moreover, the extent of developmental changes differed between the two subfamilies. At settlement, both subfamilies had similar visual systems. However, in adults, higher rod densities and lower cone densities were found in Myripristinae compared to Holocentrinae, similar to findings from retinal wholemounts (de Busserolles et al. 2021). Thus, Holocentrinae retained more of their photopic visual system, the reason for which requires further studies on their day-time activities. In summary, the holocentrid visual
system is remodelled at the cellular level over development to suit their nocturnal lifestyle, while still maintaining some adaptation for daytime activity.

Shallow-water holocentrids are thought to have emerged from a deep-water existence and some of their extant relatives are still found at greater depths, down to 640 metres (Yokoyama et al. 2008; Greenfield et al. 2017). Given this phylogenetic connection to the deep-sea, it is not surprising that some aspects of their visual development were comparable to deep-sea fishes while others more closely resembled shallow-water fishes. In terms of the cones, a steep decline in densities was evident during development (Fig. 3, Table 3) and the adult population was mainly composed of double cones, similar to what is found in some deep-sea fishes (Boehlert 1979; Munk 1990; de Busserolles et al. 2021). However, holocentrids retained cones in all retinal regions throughout life while these are often lost at early developmental stages (Bozzano et al. 2007) or become restricted to certain retinal regions (Munk 1990) in some deep-sea fishes. With respect to rods, adult holocentrids (particularly in Myripristinae) possessed peak densities that rival those of some deep-sea fishes (e.g., *Myripristis berndti*: $\approx$2.6 million rods mm$^{-2}$ vs. *Myctophum brachygnathum*: $\approx$2 million rods mm$^{-2}$, and *Hoplostethus atlanticus*: $\approx$1.7 million rods mm$^{-2}$) (Pankhurst 1987; de Busserolles 2013) and their maximal rod:GCL summation even exceeds that of many deep-sea species (e.g., *Myripristis berndti*: 3800:1 vs. *Lampanyctodes* spp.: 2000:1, and *Chauliodus sloani*: 200:1) (Locket 1980; Pankhurst 1987). Finally, the developmental decrease in GCL cell densities in holocentrids is intermediate compared to the very steep decrease observed in deep-sea fishes (Locket 1980; Pankhurst 1987) and the more subtle change found in diurnal shallow-water species (Johns and Easter 1975; Shand et al. 2000).

**Ontogenetic shifts in retinal specialisations**

Retinal specialisations in teleosts usually reflect ecological demands (Collin and Pettigrew 1988a, 1988b; Luehrmann et al. 2020; de Busserolles et al. 2014b; Collin 2008) and, accordingly, have been shown to shift during ontogeny (Shand et al. 2000; Tettamanti et al. 2019). This is also the case in the holocentrids. At settlement, all species had similar retinal specialisations (Fig. 3). The region with greater acuity (i.e., highest GCL cell densities) and better adaptation for bright light vision (i.e., highest cone densities) was found in the temporal retina. This area surveys the visual field immediately in front of the fish, which may help the larvae to see their small zooplankton prey in the brightly lit surface layers of the
After holocentrids have settled on the reef and adopted their nocturnal lifestyle, their retinal specialisations shift accordingly (Fig. 3). In adults, the regions with the highest acuity (i.e., highest GCL cell densities) were located temporally in Holocentrinae (i.e., looking forward) and ventro-temporally in Myripristinae (i.e., looking forward and upwards). These specialisations are likely linked to their nocturnal feeding ecologies. As benthic feeders, prey would be viewed in front of Holocentrinae when the mouth is angled towards the seafloor, while Myripristinae feed in the water column where food items usually occur in front of and above fishes [also see: de Busserolles et al. (2021)]. The regions which are best adapted for sensitivity (i.e., highest rod densities) overlapped with the regions of higher acuity in both subfamilies (Holocentrinae: central and temporal; Myripristinae: ventral) and so may also facilitate nocturnal feeding. Finally, the regions with the greatest adaptation for bright light vision (i.e., highest cone densities) were located temporally in Holocentrinae and centrally in Myripristinae, surveying the area in front of or lateral to the fish, respectively. Little is known about the day-time activities of holocentrids; however, these areas may be linked to social interactions and identification of safe havens for refuge during the day (Winn et al. 1964; Carlson and Bass 2000).

Conclusion
The holocentrid visual system adapted to life in dim light over ontogeny. At the morphological level, they increased rod banks from 1-2 to 5-17, adopted a rod-dominated retina and increased visual summation. Despite the early emergence of the multibank retina, substantial topographic specialisations in bank number were only evident after the transition to a dimmer environment. Together, this suggests that ecology drives visual development in Holocentridae. However, subfamily-specific differences in the degree of scotopic specialisation emerged over development (i.e., more rod banks, higher rod densities and greater summation in Myripristinae) and these were correlated with phylogenetic relatedness to deep-water representatives rather than ecology. This suggests that the development of the holocentrid retina may also be somewhat driven by phylogeny. Future studies on visual
development in other nocturnal reef fishes as well as other marine fish families with both shallow- and deep-water forms, such as Anomalopidae (flashlight fishes) and Engraulidae (anchovies), may provide further insights into the ecological and phylogenetic drivers of the development of dim-light vision.

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Competing interests

No competing interests declared.

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Data availability

All data are provided in the main text and/or supplementary information.

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Fig. 1. **Histological sampling of the retina.** (A) Schematic illustrating the locations of the dorsal (D), ventral (V), central (C), nasal (N) and temporal (T) regions of the retina in an intact fish. (B-C) Representative radial sections of the entire retina from different life stages in Holocentrinae (B) and Myripristinae (C) illustrating the different retinal layers for which cell densities were estimated. A representative rod and cone photoreceptor is indicated in each section by an arrow and an asterisk, respectively. PRL, photoreceptor layer; INL, inner nuclear layer; ONL, outer nuclear layer; GCL, ganglion cell layer; SLa, settlement larva; SJ, settled juvenile. Scale bars: 30 µm.
Fig. 2. Development of the multibank retina in different retinal regions in Holocentrinae and Myripristinae. Representative radial sections of the retina in key retinal regions showing photoreceptor layer with multiple banks of rods at different ontogenetic stages in Holocentrinae (pre-settlement *Sargocentron rubrum*, settled juvenile *S. rubrum* and adult *S. diadema*) and Myripristinae (settlement larval *Myripristis kuntee* and adult *M. berndti*). Rod banks are numbered as $B_{1-n}$. Scale bars in central row of images are accurate for all images. PL, pre-settlement larva; SJ, settled juvenile; SLa, settlement larva. Scale bars: 50 µm.
Holocentridae

- Rods (cells/0.01 mm²)
- Cones (cells/0.01 mm²)
- INL cells (cells/0.01 mm²)
- GCL cells (cells/0.01 mm²)
- Rod/GCL

Myripristinae

- SLa
- Adult

Retinal region
Fig. 3. Retinal cell densities in holocentrids over ontogeny. Abercrombie-corrected densities of rods, cones, inner nuclear layer (INL) cells and ganglion cell layer (GCL) cells, and rod:GCL summation in the dorsal (D), ventral (V), central (C), nasal (N) and temporal (T) retina in Holocentrinae [Sargocentron rubrum pre-settlement larvae (n=3), settled juveniles (n=2) and adults (n=3)] and Myripristinae [Myripristis kuntee settlement larvae (n=3) and M. berndti adults (n=2)]. Cell densities are cells per 0.01 mm$^2$ of retina given as mean ± s.e.m. Green, pre-settlement larvae (PL); orange, settlement larvae (SLa; Myripristinae) or settled juveniles (SJ; Holocentrinae); purple, adults. Cell measurements used for Abercrombie’s correction are given in Table 2.
Table 1. Retinal measurements from species in Holocentridae over development.

Average thickness of the photoreceptor layer (PRL) and retina (in µm), % of retinal thickness occupied by the PRL, and number of rod banks is given for different stages of *Sargocentron* (Holocentrinae) spp and *Myripristis* (Myripristinae) spp (*n*=1 for all). ROS length (in µm) was also measured in one settlement larva and one adult for one species from each subfamily. Individuals for which particular measurements were not taken are denoted by n.a. Note that not all sampled individuals were used for retinal measurements and that only the highest quality sections were used.

<table>
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<tr>
<th>Species</th>
<th>Ontogenetic stage</th>
<th>Retinal region</th>
<th>PRL thickness</th>
<th>Retinal thickness</th>
<th>% PRL (of retina)</th>
<th>ROS length</th>
<th>Rod banks</th>
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<td>505</td>
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Table 2. Retinal cell measurements used for Abercrombie’s correction. Correction factors are mean (in µm) of six measurements per cell type and individual for the dorsal, ventral, central, nasal and temporal retina in Holocentrinae and Myripristinae (see legend of Fig. 3 for number of individuals). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segment.

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<th>Myripristis spp.</th>
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<td>Cone OS</td>
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<td>GCL nuclei</td>
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Table 3. Retinal cell densities in holocentrids over ontogeny. Abercrombie-corrected retinal cell densities in different life stages/species in Holocentrinae and Myripristinae (see legend of Fig. 3 for number of individuals). Values are given mean ± s.e.m. in µm for each retinal region (i.e., dorsal, ventral, central, nasal or temporal). Dorsal and ventral regions were sampled for pre-settlement larvae (due to eye size) and other regions are marked as n.a. Abbreviations: INL, inner nuclear layer; GCL, ganglion cell layer.

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<th>Settled juveniles – S. rubrum</th>
<th>Adults – S. rubrum</th>
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<tr>
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<td>Ventral 56.8 2.9  3803.4 0.0</td>
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| Region | Mean | s.e.m. | |
|--------|------|-------| |
| Central | 51.4 | 9.3 | 1141.8 | 75.7 |
| Nasal | 58.6 | 8.5 | 2253.0 | 408.2 |
| Temporal | 30.6 | 8.7 | 1700.1 | 620.4 |
Fig. S1. Comparison of retinal cell densities in two species in Myripristinae. Graphs showing that Abercrombie-corrected cell densities are similar between *Myripristis kuntee* and *M. berndti* for each stage and retinal region. INL, inner nuclear layer; GC, ganglion cell.
Fig. S2. The multibank retina in Ostichthys sp. Representative transverse section of the ventral retina showing photoreceptor layer with multiple banks of rods (up to approximately 10) in an adult deep-dwelling soldierfish (Ostichthys sp). Banks are numbered B_{1-n}. Scale bar: 30 µm.
Table S1. Details of animals used in study. This study used a total of 20 individuals from the family Holocentridae, 18 of which were collected in the current study and 2 of which were collected in de Busserolles et al. (2021). Locations: LI, Lizard Island; MI, Moorea Island; CM, Cairns Marine. Analyses: Cell densities, cell densities in each retinal region quantified; Histology, rod banking examined in histological sections. Standard length was not recorded for one individual and therefore, is marked as n.a.

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<th>Species</th>
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<th>SL (cm)</th>
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<th>Eye used</th>
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