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Circuit-mechanisms for colour vision in zebrafish

Tom Baden

Abstract

The use of spectral information in natural light to inform behaviour is one of the oldest and most fundamental abilities of visual systems. It long-predates animals’ venture onto the land, and even the appearance of image forming eyes. Accordingly, circuits for colour vision evolved under the surface of ancient oceans for 100s of millions of years. These aquatic beginnings fundamentally underpin, and likely constrain, the organisation of modern visual systems. However, in contrast to our detailed circuit level understanding from diverse terrestrial vertebrates, comparatively little is known about their aquatic counterparts. Here, I summarise some of what is known about neural circuits for colour vision in fish, the most species diverse group of vertebrates. With a focus on zebrafish, I will explore how their computational strategies are linked to the statistics of natural light in the underwater world, and how their study might help us understand vision in general including in our own eyes.

Introduction

The perception of “colour” is perhaps one of the most salient aspects of our own sensory experience – despite the fact that a scene can be readily interpreted rather well in its absence, as illustrated by the effectiveness of “black-and-white” photographs. The cognitive importance of colour vision may be illustrated by the frequency with which children ask each other: what is your favourite colour? You rarely hear the question: what is your favourite boundary orientation? And as children grow up their philosophical development can often be seen when they start to ask questions such as: I wonder if your perceptual experience of “red” is the same as mine? So where does our ability to see colour come from?

When one thinks of what is colourful in nature, examples that immediately come to mind are fruits and flowers. These illustrate an important and intuitive function of colour across animals: “meaning”

To a pollinating animal, the colour of a flower is informative about its identity and can lead to attraction or avoidance. However, while flower-colouration co-evolved with colour-discrimination abilities of various visual systems

for example in species of butterflies and birds

in the grand scheme of things flowers and fruits have not been around for all that long

Angiosperms (flowering plants) first appeared around 140 million years ago, during the height of the age of dinosaurs. Before that, the world was mostly covered in algae, mosses and ferns, all of which were presumably shades of green to support photosynthesis, similar to their today’s descendants. In contrast, animal colour vision evolved more than 500 million years ago.

It therefore stands to reason that, hundreds of millions of years later, the dinosaurs would have been able to discriminate the greens of vegetation from the blues of the skies and seas, or the browns of the earth. Indeed, some dinosaurs may have been rather colourful themselves, presumably for the same set of purposes that terrestrial and aquatic animals alike use body colouration today. These include mate attraction, recognition of conspecifics, as a warning signal to fend off potential predators, or for camouflage. However, the use of body coloration to make complex behavioural decisions presupposes a fair degree of
cognitive finesse, and it remains unclear to what extent these might have been met by the budding nervous systems of our non-vertebrate (yet) ancestors when colour vision probably first appeared\textsuperscript{11}.

Perhaps then, how us humans think about colour, and how we use it in our everyday lives, does not necessarily reflect its ancestral purposes. Rather, these are likely linked with more primal gains that can be made\textsuperscript{12}, such as telling water depth, the time of day, or to compensate for the brightness-flicker that is inevitably caused in the shallows by constant movements of the water surface\textsuperscript{13}. Some of these possible primal functions of colour vision are presumably either lost, or at least superseded by other uses in terrestrial animals. However, in the water, which remains home to most vertebrate species till the present day, they are probably still very much alive and kicking. In fact, it is here where primal functions must have first combined, at a circuit level, with more advanced and to us more intuitive “cognitive” uses of colour vision. Perhaps therefore, studying circuits for colour vision in fish can open an important window into the evolutionary past to provide new insights into why our own eyes and brains are organised as they are.

**Cones, spectral opponency, and why zebrafish**

From a sensory-physical perspective, colour vision refers to an animal’s ability to discriminate the wavelength of light from its intensity\textsuperscript{14}. In vertebrates, this usually begins by comparing the signals from at least two spectrally distinct types of photosensitive neurons at the circuit level. For example, the retinas of many mammals comprise two spectrally distinct types of cone-photoreceptors, SWS\textsubscript{1} and LWS, which derive from ancient “UV-” and “red-cones”, respectively (Figure 1A). For colour vision, the signals from these two cones are differentially combined using postsynaptic circuits to yield “colour-opponent” neurons: SWS\textsubscript{1}ON/LWS\textsubscript{OFF} or SWS\textsubscript{1}OFF/LWS\textsubscript{ON}. This general strategy may involve any or all of the retina’s remaining four principal classes of neurons (horizontal, bipolar, amacrine and retinal ganglion cells) as well as diverse brain circuits\textsuperscript{15–17}.

However, many vertebrates including fish feature three or more spectral types of photoreceptors (Figure 1A), and in nearly all these cases our understanding of the circuit mechanisms that serve these animals’ colour vision remains rudimentary at best\textsuperscript{14,18} – except, that is, for a small number of closely related surface-dwelling freshwater bony fish (teleosts) of the carp-family (cyprinids). These include traditional model species such as rainbow trout, carp, goldfish, and – more recently – zebrafish\textsuperscript{19}.

Below I briefly outline the evolutionary origins of the vertebrate retina\textsuperscript{6}, highlighting that the ability to see colour was probably already well established in our earliest jawless ancestors that roamed the Cambrian oceans (Figure 1A). Accordingly, circuits for colour vision evolved first for vision in the water, and I will discuss how this differs from colour vision on land\textsuperscript{10} (Figure 1B-J). I will then summarise our current understanding of the circuits for colour vision in zebrafish. For this, I follow the natural path of the visual signal, from photoreceptors and horizontal cells (Figure 2) via the feature extracting circuits of the inner retina (Figures 3,4) to the brain responses that drive behaviour (Figure 5).
The aquatic origins of vertebrate circuits for colour vision

The fundamental blueprint of the vertebrate retina is as old as the vertebrates themselves\(^6,20,21\), first emerging during the early Cambrian some 540 million years ago (mya). From here, first jawless fish (Agnatha: e.g. lampreys) and later cartilaginous fish (Elasmobranchi: sharks, rays, skates) diverged from the lineage that would eventually give rise to bony fish (Teleostii, ~400 mya), all preceding vertebrates’ emergence onto the land (~370 mya). Accordingly, all terrestrial vertebrate retinas stem from a common motif that had been evolving for probably more than 150 mya in the well-illuminated shallows of the early world’s oceans and rivers\(^20\). This evolutionary history has left its marks on how diverse vertebrates see the world today.

Throughout this early evolution, the basic circuit machinery for colour vision may have already been in place\(^22\). For example, already some species of jawless fish, such as the Australian lamprey *G. australis*, feature multiple spectral cone-like photoreceptor types that underpin vertebrate colour-vision. From here, emerging lineages have repeatedly reduced, sometimes extended, and often spectrally shifted their photoreceptor complement to suit their environments (Figure 1A). For example, while many rays are at least cone-dichromats\(^{23,24}\), sharks are usually cone-monochromats, or even have a rod-exclusive retina\(^24\). Sharks, but not rays, are therefore generally thought to be colour blind. This is reminiscent also of many aquatic mammals, such as whales and dolphins, which only retain at most a single cone-type despite having evolved for more than 300 million years on land\(^25\).

Presumably for these large marine vertebrates or their ancestors, a combination of visual environment (e.g. deep water), behavioural patterns (e.g. nocturnal rhythms) and/or the evolution of additional sensory abilities (e.g. electro- and echolocation) have reduced the evolutionary pressure to retain retinal circuits for colour vision. In contrast, many smaller, and often near-surface-dwelling fish are thought to have rich colour vision, based on all or most of the four ancient spectral cone-types. For example, goldfish retain all four, and behavioural work\(^26\) showed that they have tetrachromatic colour vision in bright light (but switch to trichromacy in low light). These same four cone-types, and one rod, are also still present in Australian lungfish\(^27\), which are amongst today’s closest living relatives to the ancestors of all tetrapods (which include all amphibians, reptiles, birds and mammals). However, compared to our detailed understanding of photoreceptor complements and their lineages of probably hundreds of aquatic and semi-aquatic species from all major branches of the vertebrate tree of life (e.g. for fish see Carleton *et al.*\(^28\)), vanishingly little is known about the structure and function of their downstream retinal and central circuits for colour vision\(^14\).

**Seeing colour under water**

The underwater world of natural light presents spectral challenges and opportunities that are only partly overlapping with those from terrestrial visual scenes. Perhaps most obviously, the absolute intensity of light decreases sharply with depth, and its spectral composition becomes increasingly monochromatic. These basic facts of physics have driven a wide range of depth-specific adaptations in the visual systems
of aquatic animals\textsuperscript{28}, and, they can also be exploited. For example, because green light penetrates water more readily than UV-light, their ratio is informative about depth. Exploiting this phenomenon, the eye-less planktonic larvae of the marine annelid worm \textit{Platynereis} adjust their vertical position in the water column based on one of the perhaps simplest spectral-comparison circuits described to date: two photoreceptors, one sensitive to UV-light, and another to green-light, wired up in a hierarchical manner\textsuperscript{29}. Clearly, behaviourally critical spectral discrimination does not categorically require the presence of an eye, as is also evident in pineal circuits that differentially process widefield spectral information for circadian entrainment\textsuperscript{30}.

However, with good eye-optics, the potential uses of spectral information for guiding visual decision making and behaviour become a lot more diverse. For example, the spectral composition of light in underwater scenes is not only informative about depth: the same effect also occurs when looking along the underwater horizon. Objects that provide high spectral complexity cannot be far away, simply as in that case they would appear as monochromatic\textsuperscript{31} (Figure 1B). This means that spectral contrast can be used as a shorthand for physical proximity: If an object is colourful, it must be nearby, and larval zebrafish have the basic processing machinery in place that would at least in theory allow them to make use of this cue\textsuperscript{32}.

Next, the spectral composition of shallow aquatic natural scenes can vary strongly with visual elevation. During the day the ground is well-illuminated by the sun to reveal a colour-rich lower visual field\textsuperscript{33}. In contrast, almost any solid overhead object blocks out the skylight, and thus appears as an approximately achromatic silhouette (Figure 1B). Both this effect, and spectral attenuation with distance mentioned above, do also occur above the water - however in this case they are usually much less obvious because air does not strongly attenuate light with (short) distance: For example, we can usually tell the hue of canopy seen from below, because it is well-illuminated from light reflecting off the ground\textsuperscript{34}. Overall, vertical gradients in spectral variance of natural light have meant that colour-computing circuits are often found in specific eye positions examining specific regions of visual space. In the case of larval zebrafish, this has driven the evolution of profound eye-wide asymmetries in the structure and function of the eye (Figure 1C,D,H-J). For example, their dorsal retinal circuits (looking down) are Off-dominated and perform diverse colour-computations, while their ventral retinal circuits (looking up) are On-dominated and perform mostly achromatic computations\textsuperscript{33,35}. Interestingly, this pattern is approximately inverted in mice\textsuperscript{36-38}.

Finally, UV-light behaves quite differently underwater compared to in air, which brings about additional challenges and opportunities for aquatic vision\textsuperscript{39-41} (Figure 1D-G,J). UV-light is rapidly absorbed and scattered with depth and tends to poorly reflect off the ground, accentuating its vertical brightness gradient. Nevertheless, within the uppermost water column where UV-light does penetrate, it can be a powerful tool to highlight otherwise hidden structures in the scene. UV-scatter near the surface masks distant image structures in a bright haze, thereby highlighting any nearby UV-opaque objects\textsuperscript{39}: In a way, this is “nature’s green-screen” – it simplifies subtraction of potentially complex background structures for spotting object’s silhouettes\textsuperscript{40} (Figure 1E,F). A conceptually related UV-silhouette effect is also
available for terrestrial vision where this background subtraction works well e.g. for removing clouds from the sky, but less obviously so for e.g. delineating an approaching bird against the treetops\textsuperscript{34,42}. In fact, beyond serving colour vision \textit{per se}, differentially combining “visible light” and UV signals at a circuit level (discussed below) could potentially serve to accentuate achromatic contrasts\textsuperscript{34} (Figure 1G). Beyond silhouette detection or potential contrast enhancement, the same UV-scatter can also be exploited at very short distances (millimetres) to highlight some of the particles that scatter UV-light in the first place: many small aquatic animals, including larval zebrafish, feed on approximately transparent microorganisms that however appear as tiny UV-bright objects when illuminated by the sun, ready for the taking\textsuperscript{40,43} (Figure 1D, see also Figure 5J,K).

Taken together, from a visual ecology perspective, animal eyes have much to gain from investing into sophisticated circuits that capitalise on the detection of spectral variance in natural light, rather than simply its intensity. Below I will summarise the current state of the art in our understanding of how this is implemented within the circuits of the zebrafish retina and brain leading to behaviour. I will argue that in zebrafish, large fractions of behaviourally critical visual functions can be rather directly supported by distinct, and perhaps only partially overlapping photoreceptor systems and their downstream circuits (Figure 1H-J).

**Circuits for colour vision**

\textbf{Outer retinal circuits.} In the vertebrate retina, spectral contrasts in incoming light are extracted by the signals from distinct types of photoreceptors. While this can principally occur across any combinations of cone- and rod-types present\textsuperscript{14}, or recruit intrinsically photosensitive non-photoreceptor neurons which are abundant in fish retinas\textsuperscript{44}, I will here focus on the probably dominant role of cone-circuits for supporting zebrafish colour vision.

Many shallow-water fish, including zebrafish, use up to four spectral types of cone-photoreceptors: LWS, RH2, SWS2 and SWS1\textsuperscript{19} (Figure 2, hereafter referred to as ancestral red-, green- blue- and UV-cones, respectively). In zebrafish larvae, each of these four cone-types forms independent\textsuperscript{45–47} and topographically skewed\textsuperscript{33} mosaics (Figure 2A,B). Red-, green- and blue-cones exhibit increased densities around the visual horizon, with red-cones overall being more numerous than green- or blue-cones, especially for surveying the long-wavelength biased ground\textsuperscript{33}. Vice versa, the yet functionally immature rods\textsuperscript{48} are located at the eyes’ ventral and dorsal poles (Figure 2B). Next, unlike all other photoreceptors, UV-cones exhibit their highest density specifically in a ventro-temporal acute zone\textsuperscript{33,40,49}, which surveys visual space above the frontal horizon (cf. Figure 1J). Beyond this basic numerical anisotropy, UV-cones are also functionally distinct depending on their position in the eye\textsuperscript{40,50}. This includes a long list of both structural and functional aspects, including a ten-fold variation in outer segment size (Figure 2C), molecular tuning of phototransduction biochemistry, diverse differences in the On-Off balance and kinetics of their light responses\textsuperscript{40} and ultrastructural tuning of their synaptic release machinery\textsuperscript{50}. To what extent also the other cones exhibit similarly pronounced regional differences, and if any persist into adulthood, remains to be tested.
In contrast to larvae, photoreceptors form crystalline mosaics in zebrafish adults\textsuperscript{45,51}, as they do in many other species of fish\textsuperscript{52,53} (Figure 2D-F). At this life stage, UV- and blue-cones together form alternating rows, interspersed with “double-cones”, which consist of a pair of red- and green cones in close association (Figure 2D). This yields a fixed stoichiometry of 2:2:1:1, respectively, for red-, green-, blue- and UV-cones in the adult. Beyond cones, the now mature rods arrange in groups of four around each UV-cone\textsuperscript{54}. The spatial arrangement of the photoreceptor-mosaic brings about various consequences for vision, for example in relation to spatio-chromatic aliasing or possible “interval decoding”\textsuperscript{55}, as reviewed elsewhere\textsuperscript{56}. All the while, the comparatively ‘disordered’ patch of larval retina never gets ordered or replaced – instead it simply sits approximately unaltered near the optic disc, as the retina grows at the edges throughout life (Figure 2E,F)\textsuperscript{45,51}. While the adult crystalline cone-mosaic arrangement prevents numerical regional biases in the distribution of cones, adults nonetheless feature a pronounced ventro-temporal acute zone at the level of downstream circuits\textsuperscript{57} which is structurally highly reminiscent of the larval one\textsuperscript{35}. In fact, many (but not all) of the downstream retinal neurons in larvae, such as diverse genetically defined retinal ganglion cell types, have a direct counterpart in adults\textsuperscript{58}. However, to what extent the adult retina exhibits larval-like anisotropies, including in spectral processing, remains largely unknown.

At the level of function, the zebrafish cone-system has been studied extensively in adults, for example by way of electroretinograms\textsuperscript{59–61} (ERGs), microspectrophotometry\textsuperscript{59} (MSP) and single-unit electrophysiological recordings\textsuperscript{62}. These studies have generally converged to confirm that the four genetically defined cone-types give rise to four spectral sensitivity peaks, approximately centred at 360, 415, 480, and 570 nm\textsuperscript{19,63}. Small variation in these estimates between studies are likely part-explained by the differential presence of four RH2-gene variants in green-cones, and two LWS-gene variants in red-cones\textsuperscript{64}, and/or possible chromophore shifts\textsuperscript{65}. By and large, the opsin-derived spectral sensitivity functions of zebrafish cones (Figure 2G) are reasonably matched to capture much of the light for daylight vision in their natural habitat\textsuperscript{33} (Figure 2H). However, understanding how circuit interactions may further shape these spectral sensitivities is perhaps less straightforward. This is because already at their output synapse in the outer retina, cones are interconnected via horizontal cells\textsuperscript{66}, which offer the possibility to invert the sign of one cone’s signal relative to another to set-up a spectrally opponent response\textsuperscript{16}. Accordingly, horizontal cells have long been a key focus for the study of vertebrate colour vision\textsuperscript{14,66,67}.

Like many vertebrates including birds\textsuperscript{68,69}, zebrafish have three types of cone-horizontal cells (H1, H2 and H3) and one rod-horizontal cell (reviewed in Meier et al.\textsuperscript{19}). H1 contacts all four cone types, however with only minor contributions from UV. H2 connects all except red-cones, and finally H3 is largely UV-specific, with minor contributions from blue\textsuperscript{66,70} (see also Figure 3A). Electrophysiological recordings from the horizontal cell somata of zebrafish adults as well as diverse other species of fish revealed the spectral tunings of H1-3 as spectrally mono-bi- and triphasic, respectively\textsuperscript{66,67,71} (for larval zebrafish see Figure 2L), providing key functional evidence of horizontal cells’ involvement in spectral processing. However,
it remained unclear how these horizontal cell tunings were reflected in the cones for transmission to bipolar cells and thus to the rest of the visual system.

This was recently addressed using in-vivo two-photon imaging in larval zebrafish\textsuperscript{70}, which revealed that indeed when stimulated with wide-field flashes of spectrally narrow light, horizontal cells rendered the synaptic output from green- and blue-cones strongly spectrally opponent but left red-cones fully, and UV-cones largely non-opponent (Figure 2I-L). From here, comparison of the cones’ specific tuning functions to the spectral statistics in natural light revealed that red- and green-cones were tuned efficiently to encode achromatic and primary chromatic signals, respectively\textsuperscript{70}. In other words, reading out red-cone signals in isolation should enable downstream circuits to compute based on brightness alone, without being contaminated by “colour”. Vice versa, any green-cone-isolating circuit should be highly informative of spectral contrast but uninformative about brightness. Next, the opponency in blue-cones probably further supports spectral processing of contrasts not already well-captured by green-cones, while UV-cones are then free to serve potentially colour-independent detection of short-wavelength specific signals. The latter is likely further helped by horizontal cell mediated beta-band suppression in red- and green-cones\textsuperscript{70} (Figure 2K,L), which boosts spectral isolation of UV-cone signals. In fact, with H3 UV-cones even have their own nearly-private horizontal cell\textsuperscript{70,72}, which is probably key for their temporal processing\textsuperscript{40}.

**Inner retinal circuits.** Beyond the cones, spectral signals are next transmitted to the feature extracting circuits of the inner retina by ~20 types of bipolar cells in case of adult zebrafish\textsuperscript{67,73}. Each morphologically defined bipolar cell makes cone-type specific connections in the outer retina\textsuperscript{73–75} in a manner that appears to conform to a “spectral-block-wiring” principle\textsuperscript{14} (Figure 3A). In short, whenever bipolar cells wire to more than one cone type, they appear to only ever do so for spectrally neighbouring cones (e.g. red+green), rather than “skipping” cones (red+blue, but not green). Moreover, an individual bipolar cell’s dendrites are thought to either conserve or invert the signals from all connected cones, but never a mixture of both\textsuperscript{76}. Accordingly, using dendritic processing alone, bipolar cells are expected to only carry sign-conforming cone-mixtures into the inner retina (Figure 3A,B, Figure 2I), thus dramatically limiting the much larger cone-combinatorial space that might be theoretically possible.

Nevertheless, because already two of the four cone-types are strongly opponent\textsuperscript{70}, some bipolar cell dendrites are expected to inherit opponency in the outer retina. In fact, this chimes well with earlier electroretinogram (ERG) recordings of the adult retina’s bulk spectral sensitivity tuning, which could be reasonably approximated by linear combination, including opposition, of the four opsin templates\textsuperscript{59,60} (Figure 3C,D). This work predicted the dominant spectral opponencies at this stage to occur in the mid- to long-wavelength range, involving the red-, green- and blue-cones, but probably not the UV-cones. Conceptually, this data can now be revisited in the light of the measured spectral sensitivity functions of the cone output from larval zebrafish (Figure 3E, cf. Figure 2K). For example, the longest-wavelength peak of the adult ERG’s b- and d-waves (i.e. On- and Off-components, respectively), is reminiscent of the larval green-cone’s red-opponent fraction ($G_{\text{opp}}$)\textsuperscript{70} (Figure 3F,G). Similarly, the
blue-cone’s long-wavelength opponent response ($B_{\text{opp.}}$) provides a reasonable approximation of the second long-wavelength ERG-peak in the d-wave (Figure 3G), which is absent in the b-wave (Figure 3F). Accordingly, red-cones appear to only be indirectly needed to explain the ERG spectra, by their action on inverting long-wavelength responses of green- and blue-cones. Moving towards the mid-wavelengths, the remaining non-opponent fractions of the green- and blue-cone responses (G’ and B’) provide good matches to the next two ERG-peaks. Interestingly however, the final UV-range peak(s) are poorly matched by the larval UV-cone’s tuning (see also Robinson et al.53). This tentatively hints that UV-cones might spectrally shift and/or sharpen as the animal matures, a possibility that will be interesting to explore in the future.

Looking further downstream, it seems likely that at least some if not all outer retinal opponencies are preserved in the input to the inner retina. For example, zebrafish adults have at least two anatomically defined bipolar cell-types that exclusively wire to green-cones73 (Figure 3A), which therefore presumably inherit their opponent signal. In agreement, early electrophysiological recordings from diverse cyprinid bipolar cells revealed clear spectral opponency at the level of the soma\cite{77,78,79}, including double opponency\cite{80} (Figure 3H). While single-opponency can at least principally be explained by direct inputs from already opponent cones, double opponency might require additional lateral inputs from amacrine cells in the inner plexiform layer. This is because to achieve double opponency requires opposing two opponent signals in a neurons’ centre and in its surround. This could be readily achieved by opposing two ‘intrinsically’ opponent bipolar cells via a sign-inverting amacrine cell. However, the complement of amacrine cells remains poorly understood in zebrafish, and a deep census of amacrine cell spectral processing remains outstanding in any species. Nevertheless, electrophysiological recordings from randomly targeted amacrine cell somata are available in some cyprinids\cite{78}, including larval zebrafish\cite{81}, and these consistently report the presence of diverse forms of spectral biases including opponency. This suggests that amacrine cells add another layer of spectral processing to the already opponent signals in some bipolar cells (Figure 3I-K). Notably, unlike the UV-peak(s) in adult zebrafish ERGs (Figure 3F,G), UV-range responses of larval zebrafish amacrine cells are generally well-approximated by the larval UV-cone (e.g. Figure 3J,K).

Beyond single cell electrophysiology, bipolar cell spectral processing was recently surveyed using 2P imaging in the live larval zebrafish eye\cite{33} (Figure 3L-N). Here, recording light-driven calcium signals from the presynaptic terminals captured the combined result of all previous processing, including cone combinations at bipolar cell dendrites, and lateral inputs from amacrine cells\cite{76}. By and large, this confirmed and extended our previous understanding: About 20% of bipolar cell terminals were strongly colour opponent during wide-field stimulation, carrying mainly relatively simple spectral mixtures (e.g. Figure 3L). Moreover, spectral processing was parsed into neat anatomical layers of the inner plexiform layer, interspaced by non-opponent layers (Figure 3M, left). This strongly suggests that retinal circuits for colour vision in zebrafish involve anatomically, functionally, and therefore probably also genetically well-defined types of bipolar cells and their presynaptic circuits\cite{33,72}. Intriguingly,
some of the functional layers observed in larvae align well with anatomical projection patterns of bipolar cells from adults. For example, the two green-cone-exclusive bipolar cells in adults project to the two inner plexiform layers that in larvae carry most colour opponent responses (Figure 3M) - consistent with a putative BC circuit that simply passes the already opponent green-cone response (Figure 2K) to the inner retina. Similarly, inner plexiform layers 5 and 6 carried the largest fractions of achromatic responses, and this aligns well with the only red-cone exclusive bipolar cell stratifying at this depth, alongside some red-green-mixing bipolar cells (Figure 3M). Beyond setting up this “spectral layering” as a population bipolar cells further build on existing eye-wide cone-asymmetries (e.g. Figure 2B,C) to set-up a profoundly asymmetrical inner retina (Figure 3N, cf. Figure 1H-J). To explore the many possible links between cell types, retinal structure and function, it will be instrumental to further develop our genetic handle on bipolar cell types in zebrafish while in parallel pursuing detailed ultrastructural analysis of their synaptic connections, as is available for mice.

**Retinal ganglion cells.** Next, bipolar cells and amacrine cells together drive retinal ganglion cells which form the optic nerve. Already early electrophysiological work on goldfish showed clear evidence of diverse forms of spectral opponency at this level, including double opponency (Figure 4A,B). In agreement, two recent two-photon population imaging studies report a rich complement of spectral response profiles amongst larval zebrafish ganglion cells, both at the level of the retina (Figure 4C-F) and at their axon terminals in the brain. Importantly, ganglion cell opponencies were already clearly evident at the level of their dendritic calcium signals (Figure 3D, bottom). While principally these dendritic signals might reflect backpropagation of spikes from the axon hillock, they are also consistent with the possibility that much of ganglion cell spectral processing is simply inherited from upstream. In support, compared to bipolar cells, retinal ganglion cells do not generally seem to add much further spectral complexity in the sense that both populations of neurons encode a variety of similar short-versus-long wavelength opponencies with a single zero crossing (Figure 4C,D). That is, with one important difference amongst “blue”-responses. While amongst bipolar cells, blueON and blueOFF responses occur in approximately equal measure (Figure 3N), amongst ganglion cells most blueON responses are lost (Figure 4E). Presumably related, only ganglion cells, but not bipolar cells, exhibit a substantial fraction of opponent responses with two zero crossings of the form UVON, blueOFF, redON (Figure 4D). In fact, broadly-tuned On-ganglion cells, which would require the presence of blueON responses, are rare. Instead, most broadly tuned ganglion cells are Off-type. Beyond biasing the polarity of spectral responses, which may be linked to their strong rectification at the level of spike trains exiting the eye, retinal ganglion cells also add substantial temporal and spatial complexity to the inherited signal from bipolar cells. For example, UV-responses in ganglion cells are generally sped-up, while blue-responses are noticeably slowed, (Figure 3E). One possible explanation for the consequent “slow blueOFF” effect could be that it constitutes a form of background subtraction against which faster UV- and/or green/red circuits can compute. In the future it will be intriguing to experimentally address this possibility,
also in view of testing for any systematic links between spectral and spatial processing.

**Spectral processing in the brain.** Ultimately, retinal ganglion cells project to the brain to innervate the pretectum and tectum. In zebrafish, these retinorecipient areas are divided into ten arborisation fields (AFs). Of these, AF1-8 are small, and mostly served by ganglion cell axon collaterals that travel on to also innervate AF9 and/or the tectum itself (AF10). In view of its large size, experimental accessibility, and its homology to the superior colliculus in ‘higher’ vertebrates, the tectum has received most attention to date. For example, field potential measurements from this site in adult zebrafish (Figure 5A), like ERGs (Figure 3C,D), suggested the presence of non-opponent and opponent cone-contributions from UV- and blue-/green-/red-cones, respectively. Moreover, tectal responses were generally weighted towards shorter wavelengths, reiterating the dominant role of UV-cone inputs across both the eye and brain. Nevertheless, mid-/long-wavelength peaks did remain prominent and included a notable relative enhancement of the longest-wavelength peaks in On-responses compared to Off (Figure 5A). Similarly, 'voluntary' behavioural sensitivity, determined by means of an appetitive conditioning paradigm, was dominated by a mid-wavelength peak (Figure 5B). Spectral responses in the early visual brain were also probed in other cyprinids. For example, single unit recordings from the torus semicircularis (Figure 5C) and tectum (Figure 5D) of rainbow trout displayed clear spectral opponency that was most frequently of the form: UV\textit{ON}, blue/green\textit{OFF}, red\textit{ON-OFF}. This specific spectral arrangement is remarkably reminiscent of larval zebrafish retinal ganglion cells and, as discussed below, also of the larval zebrafish brain.

Until very recently, spectral processing in the larval zebrafish brain had received little attention – that is, until 2020, which saw three independent imaging studies appear quasi-simultaneously, all asking the same key question: how do neurons in the tectum, and the rest of the brain, respond to spectrally distinct stimuli? The results from these studies, which are largely in agreement with each other, confirmed and extended our previous understanding from diverse species of adult cyprinids: Sensitivity is far from uniform over the fish-visible spectrum, instead showing a clear dominance of UV- and long-wavelength responses over mid-wavelengths (Figure 5E,F). UV-responses had the highest gain overall, were distributed most widely over the brain including in the spinal cord and were heavily skewed to encoding On-rather than Off-transitions. In contrast, blue-/green- responses near exclusively encoded Off-transitions, while red-responses encoded both On- and Off transitions in approximately equal measure (Figure 5F).

Accordingly, like in rainbow trouts, the dominant brain filter of larval zebrafish brain is of the form: UV\textit{ON}, blue/green\textit{OFF}, red\textit{ON-OFF}. From here, two major questions emerge: One, how is this “brain filter” linked to retinal processing starting with the cones, and two, how does it link with behaviour?

**From cones to behaviour**

To begin addressing these questions, superposition of full larval cone-spectral tunings functions (Figure 2K), or of their monophasic fractions (Figure 3E), with
those of the brain proves to be highly instructive. For example, the short-wavelength peak of the brain’s On-response is essentially a perfect copy of the UV-cone response (Figure 5G, left), strongly suggesting that in this case, the UV-cone simply filters through all the way to the brain in an approximately unperturbed manner. Similarly, the long-wavelength peak of the brain’s On-filter is well approximated by the opponent fraction of green-cones ($G_{\text{opp}}$) - rather than being directly explained by red-cones (Figure 5G, left). Notably, this also aligns well with the pervasive presence of a $G_{\text{opp}}$-like signal in adults (e.g. Figure 5A,B). Principally, this On-peak could be explained by a hypothetical green-cone-like retinal circuit that is “clipped” along the way, for example by rectification in retinal ganglion cells. Next, unlike On-, the brain’s Off-filter is a monotonic function that is highly reminiscent of the red-cone tuning, and also of the mean spectrum of light in the zebrafish natural habitat\textsuperscript{32,35,70} (Figure 5G, middle). This suggests larval zebrafish primarily rely on Off-responses to encode truly achromatic contrasts, rather than leveraging both On- and Off-channels – incidentally a clear textbook violation of how we traditionally think about pathway splitting into On- and Off-component in a more general sense\textsuperscript{93}. Finally, putting the larval zebrafish brain’s On- and Off-responses together yields an approximately triphasic filter which can be principally approximated by the opposition of the “complete” green- and blue-cone tunings (Figure 5G, right). Alternatively, however, it could also be approximated by opposing the aforementioned UV-cone and $G_{\text{opp}}$ signals against red-cones. This latter possibility would present an interesting conundrum: what then is the purpose of the blue-cones? Here, one answer may potentially come from adults, where the largest peak of the behavioural spectral sensitivity is well-approximated by the opponent fraction of the blue-cone ($B_{\text{opp}}$). In fact, spectral sensitivity peaks that closely match both the opponent and non-opponent fractions of larval blue-cone responses prominently feature across adult the brain (Figure 5A, B). Could it therefore be that in zebrafish, blue-cone processing becomes increasingly important with age, perhaps alongside a possible spectral sharpening of the UV-system as noted above? It will be fascinating to directly address this possibility experimentally in the future. Importantly, unlike in larvae, any such experiments will in addition need to consider possible contributions from rods, which peak in the same “low-500 nm” wavelength range covered by the $B_{\text{opp}}$ component, although they are much broader\textsuperscript{94}.

Next, the sharp spectral tuning of long-wavelength On-responses is intriguing (Figure 5G, left). It suggests that “true” On-Off processing, often considered a fundamental pillar of all image-forming vision\textsuperscript{93,95}, in zebrafish is implemented only very sparingly (i.e. at and above ~580 nm, Figure 5F). Notably, this happens to be a wavelength range where shallow-underwater natural light is particularly abundant\textsuperscript{33}, and which moreover reflects rather well off the only “reliable” image-structure in the zebrafish natural habitat: the typically sandy riverbed (e.g. Figure 1C). Coincidentally, and perhaps fortuitiously, it also happens to be the wavelength range that is most frequently used for visual stimulation in optical imaging experiments on larval zebrafish, to avoid spectral crosstalk with the mid-wavelength excitation and emission spectra of many fluorescent biosensors\textsuperscript{96,97}. Technical considerations aside, putting the above observations together suggests that computationally demanding visual tasks that benefit from the combination of On- and Off-signals,
such as motion vision, should specifically draw on circuits at this wavelength range. In striking agreement, the spectral sensitivity function of adult zebrafish optokinetic behaviour is essentially an in-between of the larva’s broad red-cone tuning and the more narrow red-opponent fraction of the green-cones (Figure 5H, see also Deveau et al.99). In fact, such a sharper-than-opsin long-wavelength tuning of optokinetic behaviour occurs in diverse vertebrates, including in goldfish but also in frogs and turtles, suggesting that this is a widespread organisational principle. In further agreement, also the larval zebrafish optomotor reflex draws primarily on long-wavelength circuits (Figure 5I), however in this case the detailed spectral tuning of this behaviour remains to be established.

Remarkably, what this also implies is that zebrafish might have co-opted the red-opponent fraction of green-cones to effectively establish their “red-On” response prior to the dendrites of the bipolar cells! Potentially, this is “doubly efficient” – on the one hand, the full green-cone response supports “primary colour” computations, but when the non-opponent green-fraction of this response is clipped in downstream circuits, it could double up as the counterpart to red cones for extracting On-Off contrasts at long wavelengths. This notion will be exciting to test in the future, for example by physiological and behavioural measurements in animals with individual or sets of cone-types genetically ablated – an experimental possibility that is now well within reach.

Moving on from long-wavelength vision, of all photoreceptor signals emerging in the outer retina, those of the UV-cones are perhaps most readily linked with behaviour. Specifically, in larvae they serve visual prey-capture of brighter-than-background prey, such as paramecia when illuminated by the sun. This link was already suggested based on the dominant presence of UV-On circuits in the retina’s acute zone and the demonstration that the corresponding upper-frontal part of visual space is critical for behavioural performance. In fact, this acute-zone UV-dominance begins as early as the cones, and is one of the most pervasive features of zebrafish spectral processing throughout the entire visual system including the spinal cord. The critical role of UV-cones in larval zebrafish prey capture was recently directly confirmed by combining behaviour, spectrally restricted illumination, and genetic cone-ablation (Figure 5J). Specifically, head-mounted zebrafish larvae in the presence of free-swimming paramecia exhibited prey-capture behaviour much more readily when the experimental chamber was illuminated with UV-light, compared to power-matched yellow light, and this difference was abolished following genetic ablation of UV-cones. Importantly, this also demonstrates that it is not UV-light per se (which also activates blue-cones), but in fact the UV-cones that underpin this behaviour.

Interestingly, prey-capture performance in adult zebrafish, which are not known to routinely feed on paramecia, is also markedly affected by UV-cone ablation. This suggests that UV-vision for prey-capture is useful also for larger prey that is suited for adults, which includes diverse types of organic matter such as smaller fish and invertebrates, including their own larvae and eggs. In fact, also larvae are expected to have a much richer diet in nature than “just” paramecia – anecdotally, like adults, they will eat essentially anything organic of the right size. However, what
exactly all these diverse pieces of organic matter look like from the fish’s point of view remains poorly understood. Conceivably, some of these food-items will be darker than the background, which would then perhaps explain why larval zebrafish readily exhibit prey-capture behaviour in response to darker-than-background stimuli\textsuperscript{104}. However, in the case of Off-circuit based prey capture, the spectral and cone-dependence remains to be established. It can however be speculated already now that spectral tuning will be much broader than for On-events, and at least in part require the presence of red-cones. This is because unlike On-ganglion cells, the only Off-ganglion cells present in the acute zone (used for prey capture) are broadly tuned\textsuperscript{35}, and moreover darker-than-background prey detection works well in the absence of UV-light\textsuperscript{110}. Notably, this also implies that visual circuits for detecting bright- and dark-prey may be distinct from each other, at least up to and including the ganglion cells. If and how these putative circuits are combined at the level of the brain, presumably involving the “prey-capture” centre AF7\textsuperscript{106}, will be important to address in the future. This should be readily enabled by the recent availability of a transcriptomic atlas for zebrafish retinal ganglion cells\textsuperscript{58} which already identified one marker that specifically targets an acute-zone biased and diffusely On-stratifying population of retinal ganglion cells. In hand, probing the spectral tuning of the “AF7-adjacent KalTA4u508” neurons\textsuperscript{111}, whose activity suffices to trigger prey-capture-like behaviour, would provide another important puzzle piece. However, unlike for the tectum and AF9, the spectral tunings of deeper pretectal arborisation fields AF1-8 and their immediate downstream circuits, which are thought to critically underpin diverse but specific behavioural functions\textsuperscript{112}, remain largely unknown. Of these, data is currently only available for AF4, AF5 and AF8, all of which carry a perhaps surprisingly diverse complement of spectral responses\textsuperscript{88}. Nevertheless, the general preponderance mostly mid/long-wavelength responses in AF5\textsuperscript{88} is consistent with its role in the processing of optic flow\textsuperscript{113}.

**Putting it all together**

Taking a step back and looking at the overall organisational logic of circuits for colour vision in zebrafish, it seems that much spectral processing in this animal is already established in the outer retina. At this step, interactions between the four cone-types with three horizontal cells (Figure 2G-L) effectively set up a total of six mono-phasic tuning functions: UV- and red-cones, being largely non-opponent, each carry a single Off-type signal that is spectrally broad, and largely explained by their respective opsin. In contrast, the strong opponency of blue- and green-cones means that both these cones effectively carry two potentially useful signals each, one Off and one On, which are also narrower than the underlying opsins (Figure 3E). Together, and with notable exceptions discussed in the preceding sections, these six tuning functions at least qualitatively match all physiological and behavioural spectral peaks in both larval and adult zebrafish (Figures 3C-G, 5A,B,G-H). Based on these considerations, a possible and non-exhaustive functional wiring logic is suggested (Figure 5K). In this view, it might be specifically the role of the outer retina to set-up the primary spectral channels of the visual system, perhaps thereby enabling the majority of their downstream circuits to focus on non-spectral aspects of vision, such as spatiotemporal processing.
Tri- or tetrachromacy?

Zebrafish clearly display a rich complement of colour opponent circuits across both the retina and brain which fundamentally underpin their behaviour. However, the vast majority of observed spectral computations are of a relatively simple nature: most have either a single zero crossing in wavelength (e.g. red vs. green), or at most two (red/green vs. blue vs. UV). In contrast, triple zero crossings (i.e. red vs. green vs. blue vs. UV) are vanishingly rare. The number of zero crossings is important in relation to information theoretical considerations of efficient spectral encoding. In this view, visual systems benefit from being organised into orthogonal spectral axes that inevitably have increasing numbers of zero crossings (otherwise they would not be orthogonal). These, in order of relative importance, yield one achromatic channel (no zero crossings) followed by increasingly complex opponent channels (one, two, etc. zero crossings). A system with maximally two zero crossings is then trichromatic, while tetrachromacy presupposes a third crossing. Combining this idea of orthogonal components with currently available physiological and behavioural data therefore suggests that the zebrafish colour vision system as a whole is “functionally trichromatic”, rather than truly tetrachromatic. This is reminiscent of Neumeyer’s 1989 behavioural work which highlighted that while cone-tetrachromat goldfish can make truly tetrachromatic colour choices in bright light, this ability breaks down at lower illumination levels, effectively rendering goldfish tri- rather than tetrachromats in all but the brightest environments.

Notably, much of the above discussion rests on results obtained from wide-field stimulation, which is expected to differ in important ways from responses to spatially more structured stimulation. Similarly, also temporal stimulus aspects are likely to play into colour-circuit functions, as for example observed at the level of bipolar and retinal ganglion cell time-colour kernels. Accordingly, in the future it will be critical to assess how responses of visual neurons and behavioural outcomes hinge on the combination of spatial, temporal, and spectral aspects in stimuli.

From fish to humans

The finding that in zebrafish already the cones represent many of the key spectral features that dominate the brain suggests that fish use a fundamentally different circuit strategy for colour vision compared to mammals including humans. This is likely part-related to the fact that mammals lost the ancient green- and blue-cones (RH2 and SWS2, respectively: Figure 1A), which in zebrafish carry much of the spectral information (Figure 2). Instead, they were left with the ancient and possibly mostly non-opponent red- (LWS) and UV-cones (SWS1 - but see Packer et al.). From here, it is tempting to speculate how early dichromatic mammals might have computed spectral contrasts based on circuits inherited from their presumably cone-tetrachromatic ancestors. For example, in view of “spectral block wiring” (Figure 3A), to compute spectral contrast ancient mammals might have co-opted existing circuits that originally connected all four cone types. In many mammals, the dominant retinal type of spectral opponency is achieved by combining two mostly non-opponent bipolar cells at the level of a bistratified retinal ganglion cell. Such a circuit may principally be explained by an ancestral retinal ganglion cell circuit that
effectively compared SWS1(+RH2+SWS2)\textsubscript{ON} versus LWS(+RH2+SWS2)\textsubscript{OFF} signals, as without RH2 and SWS2 this would simply become the familiar SWS1\textsubscript{ON}/LWS\textsubscript{OFF}. Understanding if and how such circuits exist in fish, and how they relate to the well-known mammalian ones will be important to pursue in the future. Interestingly, like in zebrafish, SWS1-Off responses are generally sparse in mammals compared to SWS1-On\textsuperscript{117–119}.

Finally, in our own lineage, existing cone-dichromatic circuits were supplemented with a \textit{de-novo} duplication of LWS cones to yield primate trichromacy. However in this case, circuits contrasting the two LWS-cone variants remain far from understood, in part because there is no known way by which outer retinal dendrites might distinguish between them\textsuperscript{14}. These two LWS cones are often referred to as “green” and “red”, which is not to be confused with ancestral green- (RH2) and red-cones (LWS) (Figure 1A). Notably, a possibly similar thing happened more than once in vertebrate evolution, for example in elephant sharks, who duplicated LWS to regain cone-trichromacy after initially losing both SWS1 and SWS2 in early evolution\textsuperscript{24} (Figure 1A). In view of the obvious parallels with human trichromacy, it would be fascinating to understand if and how these chimeras can differentially read out their “old” and “new” LWS signals.

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Figure 1 – The aquatic origins of vertebrate colour vision.

(A) Approximate phylogeny of vertebrates (based on a schematic by David Lin, creative commons) and photoreceptor lineage (based on papers\textsuperscript{14,20,22,24}, with approximate key photoreceptor gain- and loss- events highlighted. For example, mammals lost the ancestral green- and blue-cones, but later old-world monkeys (here: apes) regained cone-trichromacy by duplicating the remaining LWS cone. mya: million years ago. (B) Example photograph from a coral reef (creative commons), highlighting how under water different parts of the visual field tend to highlight different spectral features. (C) Example photograph from zebrafish natural habitat in northern India (modified from papers\textsuperscript{18,33}, highlighting further systematic variation of visual features with elevation more specific to this shallow freshwater habitat. (D) Comparison of a shallow-water (<10 cm) visual scene photographed with a yellow (left) and UV-filter (right) (modified from Yoshimatsu et al.\textsuperscript{40}). Note the Paramecia in the upper part of the UV image (light “dots”, which are particularly visible in video, available in the original publication\textsuperscript{40}). (E-G) Example photographs of the same reef-scene (from Cronin and Bok\textsuperscript{39}) with “visible light” (E) and UV (F) filters in close succession, and semi-transparent superposition of both images (G) with the UV (top left) or the red (bottom right) image inverted (G) to illustrate possible contrast
gains that could be made by opposing these two signals. (H-J) Schematic summary of how different types of visual functions dominate different parts of the larval zebrafish eye, and which cone systems are likely of particular importance in each case (modified from Zimmermann et al.33). Larval zebrafish schematic here, and in subsequent figures, by Lizzy Griffith.

Figure 2 – Spectral processing in the zebrafish outer retina.

(A) Schematic representation of the typical cone-distribution in larval zebrafish, with cone types indicated, based on Allison et al.45. (B,C) Distribution of photoreceptor-type densities across the larval zebrafish eye, from Zimmermann et al.33 (B) and mean length of UV-cone outer segments with example images from Yoshimatsu et al.40 (C), plotted on a circular x-axis by corresponding direction of view. (D) Schematic of typical photoreceptor-mosaic in adult zebrafish, based on Salbreux et al.51. (E) Crop from retinal whole-mount-view of an adult zebrafish with UV-cones labelled (purple) and "age-ring"-manipulations as indicated (E, from Allison et al.45) to illustrate gradual transition from 'disordered' larval patch to crystalline adult mosaic. dpf: days post fertilisation. (F) Zoomed in version of (E) from a different animal, with UV- and blue-cones labelled to illustrate how the mosaic gets gradually ordered (from Salbreux et al.51). (G,H) Zebrafish log-opsin templates (G) and mean±SD daylight spectrum of light measured in zebrafish natural habitat (from Yoshimatsu et al.70). (I) Schematic representation of the larval zebrafish retina, indicating the five principal neuron classes (modified from Baden et al.18). (H) Averaged calcium-responses of larval zebrafish cone-pediciles in vivo to spectrally narrow widefield flashes of light from dark at different wavelengths (based on Yoshimatsu et al.70). From top: red-, green- blue-, UV-cones. (K,L) Mean in-vivo spectral tuning of cones (K) and horizontal cells (L), modified from Yoshimatsu et al.70. Note that the order of spectra between (H) and (K,L) is inverted, because in the experiment shown in (H) the stimulus sequence was red to UV rather than UV to red, as is traditionally plotted. For simplicity, in (H) one “low-power control” UV-stimulus was graphically removed compared to the original publication.

Figure 3 – Spectral processing in the inner retina.

(A) Summary of adult photoreceptor-wiring to horizontal cells (left, based on Klaassen et al.66) and bipolar cells (right, based on Li et al.73). Each vertical stack of boxes denotes one anatomical HC/BC type. Shadings indicate approximate anatomical connection weights. (B) Schematic of adult zebrafish retina (modified from Baden et al.18). OS, outer segment; ONL/OPL/INL/IPL, outer/inner nuclear/plexiform layer; GCL, ganglion cell layer. Note that unlike in larvae, rods are functional in adults. (C,D) Mean spectral sensitivity functions of adult zebrafish electroretinogram (ERG) b- and d-waves (grey dots, based on McDowell et al.89 but here replotted on linear y-axis) and free-hand fit to illustrate the peaks (dashed line). Originally proposed underlying cone-contributions as indicated. (E) Measured cone-sensitivity functions (from Figure 2K) from larval zebrafish, but clipped at zero
(indicated as e.g. G', rather than G). For green- and blue-cones, the sub-zero opponent fractions are included as two additional above-zero peaks (G_{opp}, B_{opp}) to set-up a total of six monophasic basis functions, hereafter referred to a “cone-fractions”. (F,G) superposition of adult ERG-tunings (from C,D) and larval cone-fractions (E), scaled to approximate the ERG-peaks. (H) Example responses of a carp bipolar cell to small spots (top) and annuli (bottom) of different wavelengths (modified from Kaneko and Tachibana). (I,J) example single-unit recording from an amacrine cell in larval zebrafish in response to flashes of light as indicated, and summary of its spectral tuning (J) with possible corresponding “cone-fractions” (from E) scaled and superimposed. (K) A second example amacrine cell; (L-K) modified from Torvund et al. (L) Example bipolar cell terminal cluster means of linear filters estimated from white noise analysis at four wavelengths as indicated (shown as max. normalised per wavelength, modified from Zimmermann et al.). (M) Comparison of example zebrafish adult bipolar cell axonal stratifications (black middle, based on Li et al.) with functional layers determined from in vivo two-photon imaging of bipolar cell terminals in larval zebrafish (left/right, modified from Zimmermann et al., showing dorsal retina). The anatomical morphotype based on cone-connections is indicated above each cell (coloured boxes, cf. A). For example, the two green-cone exclusive BCs stratify in layers 1 and 3 of the IPL, which is also where most colour opponent responses were found (orange histogram), tentatively suggesting that these bipolar cells might inherit the already opponent response from the green-cones (cf. Figure 2K). (N) Relative abundance of responsive bipolar cell terminals to white noise stimulation at four wavelengths (cf. L) plotted by wavelength (four sub-panels), kernel polarity (two distributions per sub-panel), and corresponding direction of view (polar axis). Modified from Zimmermann et al.

Figure 4 – Spectral processing in retinal ganglion cells.

(A,B) Single-unit electrophysiological demonstrations of double opponency in goldfish retinal ganglion cell (A modified from Daw, B modified from Mackintosh et al.). (C,D) as (Figure 3L), but here for larval zebrafish retinal ganglion cell dendrites (C) and summary of typical types of spectral opponency observed in dendrites (grey) and somata (white). Scale bar in % of all regions of interest in the dataset, including non-opponent responses which are not shown here. (E) As (Figure 3N), but here for ganglion cell dendrites. (F) Summary ganglion cell temporal properties, by way of their spectral centroid following Fourier transformation of linear kernels at the four tested wavelengths (as e.g. in C). On- and Off-kernels plotted separately, as indicated. (C-F) modified from Zhou et al.

Figure 5 – Brain and behaviour.

(A,B) Spectral tuning of adult zebrafish tectum measured based on field potentials (A, modified from McDowell et al.) and of behavioural sensitivity (B, based on Risner et al., but replotted on a linear y-axis and with suggested larval cone fractions from Figure 3E scaled and superimposed). (C,D) Typical example spectral
tuning functions of single neurons in torus semicircularis (C) and tectum (D) of rainbow trout (modified from Coughlin and Hawryshyn\textsuperscript{91}). (E,F) Summary of responsive neurons in the larval zebrafish brain to upper-frontal (approximately aligned with acute zone) wide-field stimulation at different wavelengths as indicated. Note the different duration flashes used in (E, modified from Fornetto \textit{et al.}\textsuperscript{92}) and (F, modified from Bartel \textit{et al.}\textsuperscript{32}) which meant that On- and Off components could not be discerned in the former, and which likely masked some slower responses at mid-wavelengths. (G) From left, summary of spectral tuning of the larval brain’s bulk On-, Off- and On-Off responses (grey lines, based on F), with cones (from Figure 2K) and/or cone-fractions (from Figure 3E) superimposed. Note that the green-cone tuning is y-inverted in the third panel to illustrate how their hypothetical opposition to blue-cones could capture the brain’s dominant On-Off response. (H) Sensitivity of optokinetic reflex (OKR) as a function of wavelength (thick line, modified from Krauss and Neumeyer\textsuperscript{98}, here replotted on a linear y-axis) and suggested cone-fractions superimposed. I, Optomotor behaviour in larval zebrafish switches from blue- to red-driven at very low red-contrast (modified from Orger and Baier\textsuperscript{103}). (D) Increased prey-capture performance of live paramecia by larval zebrafish in the presence of UV-light compared to yellow light (indicated in shadings) with UV-cones intact (left) and after UV-cone ablation (right). Modified from Yoshimatsu \textit{et al.}\textsuperscript{40}. (K) Possible non-exhaustive and approximate functional circuit organisation of zebrafish visual system underpinning colour vision and spectral behaviours.

**In Brief.** Baden reviews how zebrafish visual circuits extract and use spectral information from their natural surroundings to guide behaviour. He also puts these findings from fish in an evolutionary context, linking functional circuit motifs across the vertebrate tree of life up to and including humans.