

RESEARCH ARTICLE

A latitudinal cline in the efficacy of endogenous signals: evidence derived from retinal cone contraction in fish

Robert Yammouni¹, Anna Bozzano² and Ron H. Douglas^{1,*}

¹Henry Wellcome Laboratory for Vision Sciences, Department of Optometry and Visual Science, City University London, Northampton Square, London, EC1V 0HB, UK and ²Institut de Ciències del Mar (CSIC), 37-49 Passeig Marítim de la Barceloneta, Barcelona 08003, Spain

*Author for correspondence (r.h.douglas@city.ac.uk)

Accepted 19 October 2010

SUMMARY

Like many physiological systems synchronised to the light:dark cycle, retinomotor movements in 'lower' vertebrates are controlled by both the ambient illumination and input from endogenous circadian oscillators. In the present study, we examine the relative influence of these two signals in various species of teleost fish with different latitudes of origin. We find equatorial species show very strong endogenous control. The cones of the glowlight tetra, for example, continue to go through undiminished cycles of contraction and relaxation that mirror the previous light:dark cycle for at least two weeks in continual darkness. To quantify the relative effectiveness of the ambient light compared with endogenous signals in causing cone contraction, the degree to which seven teleost species responded to light during the dark phase of their light:dark cycle was examined. In this situation the retina receives conflicting instructions; while the light is acting directly to cause light adaptation, any endogenous signal tends to keep the retinal elements dark adapted. The further from the equator a species originated, the more its cones contracted in response to such illumination, suggesting animals from higher latitudes make little use of endogenous oscillators and rely more on ambient illumination to control behaviours. Equatorial species, however, rely on internal pacemakers to a much greater degree and are relatively insensitive to exogenous light signals. Because these data are consistent with published observations in systems as diverse as melatonin synthesis in Arctic reindeer and the behaviour of regional populations of *Drosophila*, latitudinal clines in the efficacy of circadian oscillators may be a common feature among animals.

Key words: retinomotor, endogenous, circadian, cone contraction, latitude, equator, pole.

INTRODUCTION

Many of an animal's physiological systems and behaviours display circadian rhythms synchronised to the light:dark cycle. In order to facilitate preparation for predictable events, such as the breaking of dawn, while still allowing flexible reactions to more irregular, transient, environmental occurrences, such responses are often controlled by both internal circadian oscillators and the ambient illumination (Foster and Kreitzman, 2004).

Most, but by no means all (Shand and Foster, 1999), of the photoreceptors that control an animal's behaviour directly and provide input to its circadian clocks to ensure their synchronicity with the environmental light:dark cycle are located within the retina (Foster and Hankins, 2002). It is therefore perhaps not surprising that the retina displays more circadian rhythms, involving changes in morphology, electrophysiology, biochemistry and gene expression, than most, if not all, other organs in the body (Cahill and Besharse, 1995).

During 24h animals are exposed to a range of light intensities of over 10 log units, which is beyond the dynamic range of any one photoreceptor. Vertebrates retain sensitivity throughout this large range by switching between two differentially sensitive photoreceptors, the rods and cones, active in low and high light levels, respectively. Switching between rod- and cone-based vision is the main reason for the plethora of retinal rhythms.

The sensitivity of rods and cones can be adjusted by a number of biochemical and neurobiological processes (Perlman and Normann, 1998; Lamb and Pugh, 2004), which, in 'lower' vertebrates, are augmented by simple morphological (retinomotor) changes within the outer retina that serve to position the rods and cones optimally for their different functions (for reviews, see Burnside and Nagle, 1983; Wagner et al., 1992; Burnside and Kingsmith, 2010). In the light, cone myoids contract, positioning the cone ellipsoids and outer segments close to the external limiting membrane (ELM), while at the same time the rod myoids elongate, placing the rod ellipsoids and outer segments towards the outer boundary of the retina covered by dispersed melanosomes within the retinal pigment epithelium (RPE). Thus, the cones are the first photoreceptors to absorb incoming illumination in photopic conditions and the sensitive rods are protected from excessive illumination by a blanket of melanosomes (Douglas, 1982a). In darkness these positions are reversed. The cone myoids elongate to position the cone outer segments furthest from the light, the rods are contracted so that their outer segments lie closer to the ELM and can absorb the relatively less intense incoming illumination, while the melanosomes of the RPE are aggregated in a dense band adjacent to the choroid. Such an arrangement makes optimal use of limited retinal space.

Like other physiological systems, retinomotor movements respond both to ambient illumination and are subject to endogenous

control. Because, even in response to very bright light, they take at least 15 min to complete, and significantly longer following dimmer illumination, endogenous signals prepare animals for the forthcoming changes in illumination and prevent them from being visually impaired at dawn and dusk. Consequently, retinomotor movements occur in synchrony with the changes of light level during natural twilight (McFarland et al., 1979; Douglas, 1982a) rather than lagging behind them, as would be the case if they simply reacted to the illumination.

In the laboratory such endogenous control of retinomotor movements is manifest both by them continuing in periods of prolonged darkness and by the anticipation of expected changes in an artificial light:dark cycle with abrupt transitions in illumination. An endogenous component to the control of retinomotor movements has been demonstrated in 14 species of teleost fish (Welsh and Osborn, 1937; Arey and Mundt, 1941; Wigger, 1942; John and Haut, 1964; John et al., 1967; John and Gring, 1968; Olla and Machioni, 1968; John and Kaminester, 1969; Levinson and Burnside, 1981; Douglas, 1982b; Douglas and Wagner, 1982; Lythgoe and Shand, 1983; Burnside and Ackland, 1984; Dearry and Barlow, 1987; McCormack and Burnside, 1991; Douglas et al., 1992; McCormack and Burnside, 1992; McCormack and McDonnell, 1994; Menger et al., 2005). However, among these species the relative degree to which the retinomotor movements are under endogenous control rather than subject to the direct influence of ambient illumination varies widely. In some species, for example, retinomotor movements continue with only slight damping for several days in continual darkness whereas in others the amplitude of movement is severely decreased in such conditions.

This study addresses the question of why different species rely on endogenous signals to differing degrees. We describe the retinomotor movements of the equatorial glowlight tetra

(*Hemigrammus erythrozonus*, Durbin), and find a degree of endogenous control of cone contraction much greater than previously observed in any animal, with cone contraction continuing undamped for at least two weeks in continual darkness. To quantify the relative sensitivity of cone and RPE melanosome migration to ambient illumination as opposed to endogenous control, we exposed seven species of teleost fish belonging to four families and originating from different latitudes to light in the middle of their dark phase. This potentially gives the retinal elements conflicting instructions. While the light is acting directly to cause light adaptation, any endogenous signal will tend to keep the retinal elements dark adapted. The degree to which the retina light adapts in these conditions is therefore an indication of the relative effectiveness of these two signals; the less the retina light adapts, the stronger the endogenous signal. We observe a cline of increased endogenous control in animals originating from lower latitudes. Because these data are consistent with published observations in diverse physiological systems and over a broad range of species, we suggest the correlation between increased latitude and reduced efficacy of circadian oscillators may be a common feature among animals.

MATERIALS AND METHODS

Animals

All fish (Table 1) were obtained from suppliers within London, UK, except for sticklebacks, which were caught in an Essex river, Loughton, UK. The five tropical species were kept at 23°C, while goldfish and sticklebacks were maintained at 19°C, on a 12 h:12 h light:dark cycle (with an abrupt light–dark transition) for at least 30 days prior to experimentation. Light within the tank was provided by a fluorescent tube (Philips, TLD 18W/25D, Eindhoven, The Netherlands) giving an illumination on the water surface of

Table 1. Phylogeny, size and geographical distribution of species

Order	Family	Species	Standard length (cm)	Distribution	Northern/Southern boundary of species distribution	
					Latitude	Annual variation in daylight
Characiformes	Characidae	Glowlight tetra; <i>Hemigrammus erythrozonus</i> (Durbin 1909)	2.0–3.0	Restricted to Essequibo River and its tributaries in Guyana (F. Lima, personal communication)	5°30'N	40 min
		Neon tetra; <i>Paracheirodon innesi</i> (Myers 1936)	2.0–2.4	Upper Amazon basin of Brazil and Peru (Weitzman and Fink, 1983; Kullander, 1986) (F. Lima, personal communication)	6°S	44 min
Perciformes	Cichlidae	Fairy cichlid; <i>Neolamprologus brichardi</i> (Poll 1974)	4.0–5.5	Locally at southern end of Lake Tanganyika (A. Konings, personal communication) (Konings, 1988)	8°48'S	1 h 9 min
Cypriniformes	Cyprinidae	Harlequin rasbora; <i>Trigonostigma heteromorpha</i> (Duncker 1904)	2.0–2.4	Thailand, Malaysia and Indonesia (FishBase)	12°35'N	1 h 19 min
		Zebrafish; <i>Danio rerio</i> (Hamilton 1822)	2.7–3.2	Pakistan, India, Bangladesh, Nepal and Myanmar (FishBase)	33°N	4 h 38 min
		Goldfish; <i>Carassius auratus</i> (Linnaeus 1758)	3.5–4.4	Although now found throughout the world following introduction by humans, originated in China (FishBase)	53°N	8 h 30 min
Gasterosteiformes	Gasterosteidae	Three-spined stickleback; <i>Gasterosteus aculeatus</i> (Linnaeus 1758)	1.2–4.0	Widely distributed in northern hemisphere including areas north of the Arctic Circle (FishBase)	71°N	24 h

0.38–0.47 mW cm⁻². Fish were fed Tetramin flakes (Melle, Germany) five days a week at variable times during the light phase of their light:dark cycle. Care of animals complied with local ethical procedures.

Sampling of fish

Fish were sampled in both light and dark phases of the light:dark cycle. All manipulations in the 'dark' were performed using a dim red torch of an intensity and wavelength that did not induce retinomotor movements. Previous experiments (Kirsch et al., 1989) indicated that if several fish were freely swimming within a tank in darkness and some were removed with a net, this could induce light adaptation in those remaining in the tank. Such 'stress-induced' light adaptation was eliminated here by putting groups of 2–5 fish into individual jars with a mesh lid and replacing them in the home tank in the light phase prior to experimentation. In this way groups of fish could be removed from the tank within a matter of seconds at the appropriate time without significantly affecting them or the other fish in the tank.

Glowlight tetra sampling in a light:dark cycle and in continual darkness

Groups of three glowlight tetra were sampled at various times during a light:dark cycle and subsequently during 40 h of prolonged darkness. This was repeated on a number of occasions and the results were pooled. Sampling was particularly frequent around the times of lights on and off as the positions of the photoreceptors and RPE melanosomes were most variable at these times. Retinae were also sampled less frequently after 7, 14 and 28 days in continuous darkness.

Light exposure in the middle of the dark phase

Groups of three animals of all seven species were sampled in the middle of their light and dark phases to establish baseline positions for fully light- and dark-adapted cones and RPE melanosomes. Subsequently, the lights in the home tanks were turned on in the middle of their dark phase, and groups of three animals were sampled at intervals over the next 100 min of illumination.

Histological procedures

Following removal from the tank fish were immediately killed by cervical transection and pithing. The central cornea was pierced and

either the whole fish (smaller species) or the eyes (larger animals) immersed in a mixture of 1% paraformaldehyde, 2.5% glutaraldehyde in 0.1 mol l⁻¹ phosphate buffer with 3% sucrose. After at least 12 h in fixative, isolated eyes were hemisected and the posterior eyecup was embedded in resin by conventional means. 1 µm radial sections were cut on a Reichert–Jung Ultracut E microtome (Heidelberg, Germany), stained with 1% Toluidine Blue and examined using a light microscope at a magnification of ×1000.

Measurement of cone and RPE melanosome position

Cone and RPE melanosome positions were expressed as indices to compensate for individual differences in retinal thickness and oblique sectioning. Rods were not examined as their positions could not be measured accurately at the light microscopic level. The cone index is the distance from the ELM to the base of the outer segment, divided by the separation between Bruch's membrane and the ELM (Fig. 1). Similarly, the pigment index is represented by the distance between Bruch's membrane and the innermost extension of melanin pigmentation, also expressed relative to the distance from Bruch's membrane to the ELM. The positions of 50 cones and 50 melanosomes were determined from up to four retinal regions within each retina to give mean indices for that retina. As the retinomotor movements are controlled locally within the retina, for example, continuing after the removal of all efferent input (Easter and Macy, 1978; Deary and Barlow, 1987), it is assumed the two eyes of an individual are independent in this regard. The indices for each time point sampled are thus determined from the mean of at least six retinae. To compare the retinomotor movements of different species following illumination in the dark phase (Figs 3–5), the indices for each species were expressed as a percentage of the range between the fully light-adapted index in the middle of the light phase (100% light adaptation) and the index in the middle of the dark phase (0% light adaptation). The glowlight tetra RPE, like that of the neon tetra (Lythgoe and Shand, 1983), contains both rod-shaped melanosomes, which undergo retinomotor movements, and spherical melanosomes, which remain stationary near Bruch's membrane throughout the light:dark cycle. The pigment index of this species therefore only refers to the migratory rod-shaped melanosomes.

Geographical origin of species

The geographical distribution of each species was determined from data on FishBase (<http://www.fishbase.org>), the scientific literature

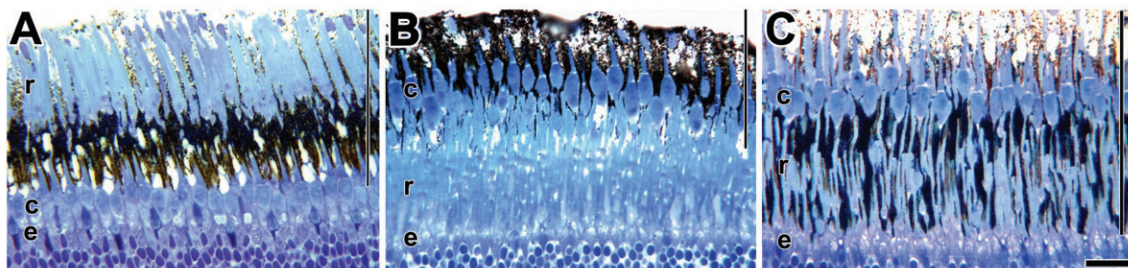


Fig. 1. Transverse retinal sections from glowlight tetra sampled in various light conditions. In all sections the outer boundary of the retina (the position of Bruch's membrane) is at the top of the picture. (A) Retinae of fish sampled in the middle of the light phase have contracted cone myoids, positioning the cone ellipsoids and outer segments (c) close to the external limiting membrane (ELM) (e), while the rod myoids are elongated, placing the rod ellipsoids and outer segments (r) towards the outer boundary of the retina covered by dispersed melanosomes within the retinal pigment epithelium (RPE) (whose extent is indicated by the vertical bar). (B) In retinae sampled in the middle of the dark phase the cone myoids have elongated to position the cone outer segments furthest from the light, the rods are contracted so that their outer segments lie closer to the ELM, while the RPE melanosomes are aggregated adjacent to Bruch's membrane. (C) A retina sampled 100 min after bright light exposure in the middle of the dark phase. The RPE melanosomes have migrated to light-adapted positions near the ELM, but unusually the cones and rods remain in their dark-adapted positions. The horizontal thick dark bar=20 µm.

and by consultation with species experts. Although the general ranges of each species are well known, the precise most northerly/southerly boundary is often harder to determine. Where more detailed information was not available we have used the latitude furthest from the equator listed in the species occurrence data on FishBase to indicate the extremes of an animal's distribution (Table 1).

RESULTS

Retinomotor movements in the light:dark cycle

When sampled in the middle of their light and dark phases all seven species studied here showed cone, rod and RPE melanosome positions qualitatively similar to those of most light- and dark-adapted teleost retinae (Fig. 1A,B).

When the positions of the retinal elements of glowlight tetra were quantified more frequently during a laboratory light:dark cycle, both cones and RPE melanosomes commenced their light-adaptive migrations 2.25 h before the lights came on, reaching almost full light adaptation by the time of lights on (Fig. 2A). They remained in light-adapted positions until 0.5 h after the end of the light phase of the cycle and did not reach full dark adaptation until 2.0–2.5 h after the onset of darkness (Fig. 2A).

Glowlight tetra retinomotor movements in prolonged darkness

The commencement of glowlight tetra cone contraction and RPE melanosome dispersal before the onset of illumination (Fig. 2A) shows that these events are triggered by endogenous oscillators. This is further indicated by the continuation of such migrations during 40 h of darkness that were no different to those observed during a light:dark cycle (Fig. 2A). In fact, endogenous movements for cones in this species continued in a virtually undiminished manner for at least 14 days in continual darkness (Fig. 2B). Melanosomes within the RPE, however, ceased normal movements sooner; the melanosomes failing to become fully dark adapted at 'night' on both the seventh (data not shown) and the 14th day in darkness (Fig. 2B). By the 28th day of darkness all cone and RPE melanosome movements had ceased and they remained fixed in their light-adapted positions (data not shown).

Light adaptation in the middle of the dark phase

In response to illumination in the dark phase of the light:dark cycle, the melanosomes within the RPE of all species light adapted fully (Fig. 3A). The response of cones to such illumination, however, was very species dependent (Fig. 3B).

The effectiveness of light in the middle of the dark phase in causing cone contraction is reflected by both the degree of light adaptation after 100 min of light and the speed of contraction. The areas under the curves in Fig. 3B combine both of these measures and therefore quantify the extent to which the cones of each species are influenced by such 'inappropriate' illumination in the dark phase (Fig. 4).

Species variation in the strength of endogenous signals for cone contraction and latitude of origin

The greater the degree of light adaptation following illumination in the dark phase of the light:dark cycle, the greater the sensitivity of the cones to ambient illumination and the less effective the endogenous signals are.

The degree to which the cones of a species are influenced by endogenous signals correlates well with its geographical origin. The further a species most northerly/southerly distribution boundary is from the equator, the greater the amount of cone contraction in response to illumination in the middle of the dark phase of their light:dark cycle (Fig. 4). Thus, the cones of the stickleback, whose range extends north to within the Arctic Circle (Table 1), light adapt fully within 15 min (Fig. 3B), representing the most rapid retinomotor movements ever recorded, and indicating relatively little influence of an endogenous oscillator on cone position. The cones of the glowlight tetra, however, which occurs over a geographically very restricted region close to the equator (Table 1), seem to be entirely controlled by an internal clock, as they are completely unresponsive to such illumination and remained fully elongated in their dark-adapted position even after 100 min illumination (Fig. 1C, Fig. 3B).

DISCUSSION

Retinomotor movements are influenced both directly by the ambient illumination and by signals from endogenous oscillators. The degree

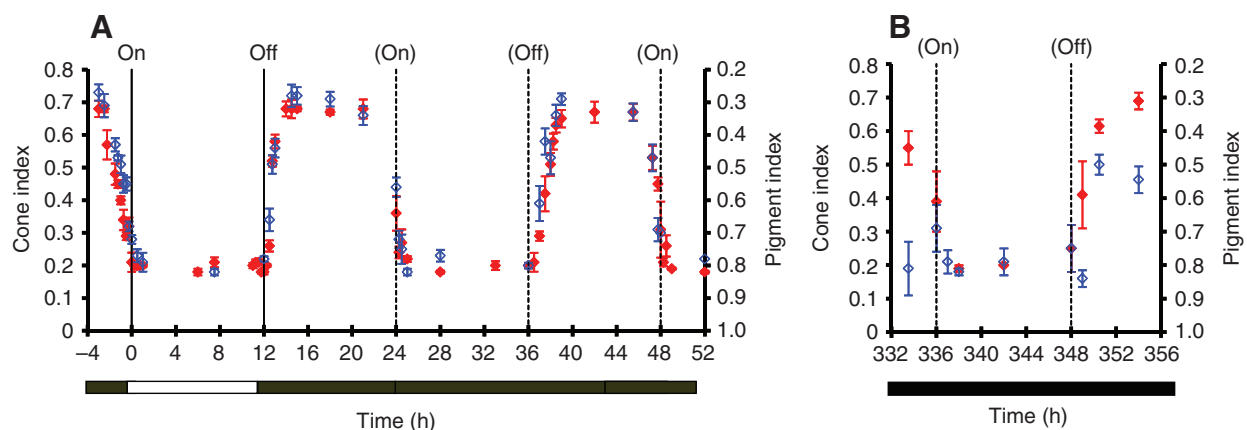


Fig. 2. Indices showing the retinomotor positions of retinal cones (solid red symbols) and retinal pigment epithelium (RPE) melanosomes (hollow blue symbols) of the glowlight tetra during a 12:12 h light:dark cycle and in prolonged darkness. Data obtained during (A) a light:dark cycle and the immediate subsequent period of continuous darkness, and (B) after 14 days of continuous darkness. The vertical solid lines show the times the lights went on and off and the dotted lines show the times they would have done so had the light:dark cycle been maintained. The light and dark bars under the graphs represent the ambient lighting conditions. Each point represents the mean \pm 1 s.e.m. of at least six retinae.

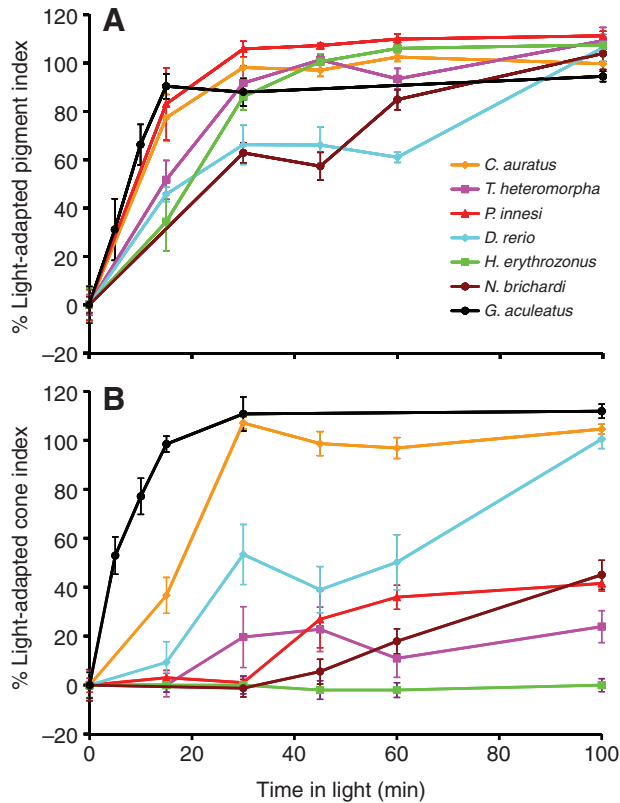


Fig. 3. Relative positions of the (A) retinal pigment epithelial melanosomes and (B) cones at various times after light exposure in the middle of the dark phase of the light:dark cycle in seven species of fish. Cone and pigment positions were initially quantified using indices and have been expressed here relative to maximal light- and dark-adapted values. The error bars represent ± 1 s.e.m. from at least six retinæ. Degrees of light adaptation below 0% and above 100% represent slight differences to control values that were not significant.

to which the cones are subject to endogenous control varies between species. This study shows that the closer a species originates to the equator the more strongly it appears to rely on endogenous signals to determine cone position.

Although most animals possess a 'master clock', such as the suprachiasmatic nucleus, that is responsible for generating many of the body's rhythms, it is clear that many peripheral tissues have their own self-sustaining 'peripheral' pacemakers capable of controlling rhythms within that organ (Whitmore et al., 1998) (for a review, see Hastings et al., 2003). The first description of a retinal pacemaker was in the photoreceptors of *Xenopus* (Besharse and Iuvone, 1983; Cahill and Besharse, 1993). Although retinal clocks have now been demonstrated in all classes of vertebrate (Tosini and Fukuhara, 2002; Green and Besharse, 2004; Iuvone et al., 2005; Tosini et al., 2008), including fish (Whitmore et al., 1998; Ribelayga et al., 2003; Iigo et al., 2006), the number of oscillators and the identity of the cells involved in different animals is not always clear (Tosini and Fukuhara, 2002). It is likely, however, that the retina contains multiple clocks, some of which are located in the photoreceptors (Green and Besharse, 2004). Since both light-driven (Easter and Macy, 1978; Deary and Barlow, 1987) and endogenous (McCormack and Burnside, 1992; McCormack and McDonnell, 1994) retinomotor movements continue when the retina is isolated from the rest of the central nervous system, it appears likely that

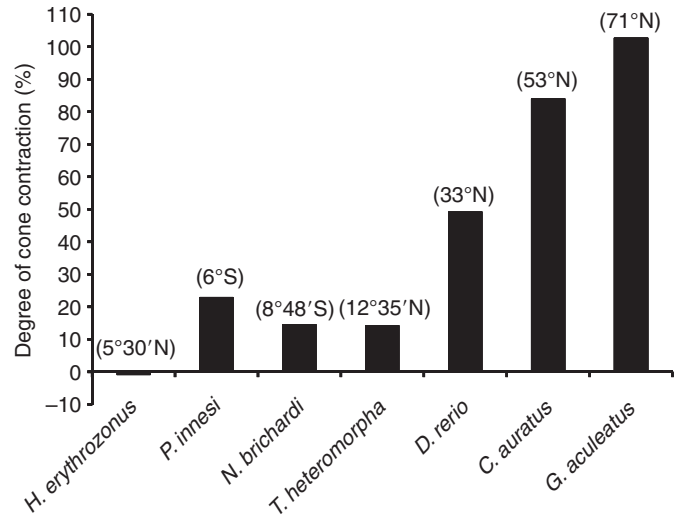


Fig. 4. The degree to which cones contract following illumination in the dark phase of their light:dark cycle expressed by the area under the curves in Fig. 3B. A small area indicates strong endogenous control. The numbers above the bars indicate the boundary of the species' distribution range furthest from the equator.

retinomotor movements are influenced by such intra-retinal oscillators.

RPE melanosome migration exhibits little endogenous control in all species

Following illumination in the dark phase of the light:dark cycle, while the melanosomes within the RPE of all species light adapted fully (Fig. 3A), the cones of most species did not. This indicates a relatively minor influence of endogenous signals on the RPE in comparison with the cones. This is in line with the observation that although cones continue their rhythmic migrations to some degree in continual darkness in most species, RPE melanosome movements are comparatively reduced or absent in such conditions (Fig. 2B) (Welsh and Osborn, 1937; Arey and Mundt, 1941; Olla and Marchinoi, 1968; Burnside and Ackland, 1984). Similarly, while cones usually begin to contract towards their light-adapted positions towards the end of their dark phase before the lights come on, RPE melanosomes often do not predict dawn (Lythgoe and Shand, 1983; Burnside and Ackland, 1984; Douglas et al., 1992). This implies either that endogenous RPE melanosome dispersion and cone contraction are driven by separate endogenous oscillators with differing effectiveness or that a single oscillator affects both processes but has less influence on the RPE, such as might be the case if the single oscillator was located in the photoreceptors.

Species variation in the strength of endogenous control of cone contraction

A degree of endogenous control of cone contraction has prior to this study been demonstrated in 14 teleost species. However, in most species the amplitude of migration in continuous darkness is diminished to varying degrees within the first 24 h of darkness compared to a normal light:dark cycle. In comparison with other species, therefore, the glowlight tetra described here has a comparatively strong endogenous influence on cones as they continue to migrate in a manner similar to that seen in the normal light-driven rhythm during 40 h of continuous darkness. In fact,

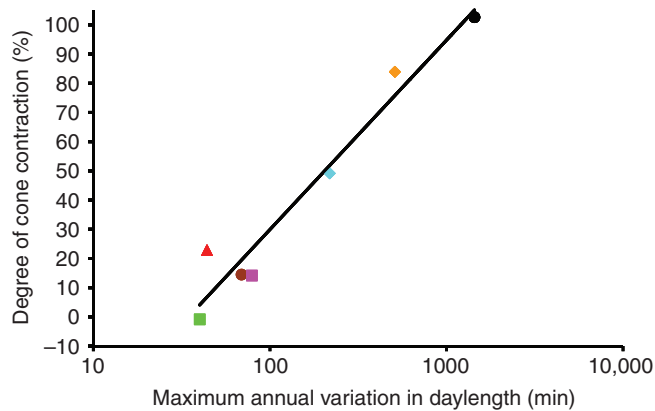


Fig. 5. Relationship between the maximum annual variation in day length that could be experienced by a species and the degree of cone contraction following illumination in the dark phase of the light:dark cycle. A small degree of contraction is indicative of strong endogenous control. The colours of the individual points represent the species as shown in Fig. 3A. The data are well described by the relationship $y=28.167\ln(x)-99.802$ ($R^2=0.9491$).

undamped endogenous movements for cones continue for at least 14 days, representing the most robust endogenous retinomotor rhythm in any species described to date.

This species variation in the degree of endogenous control of cone position is clearly demonstrated by the differential sensitivity of cones to ambient illumination in the middle of the dark phase (Fig. 3B). The observed hierarchy of the reliance of cone contraction on endogenous signals (Fig. 4) is supported by the behaviour of the cones of these species in continuous darkness. While the retinomotor movements of the glowlight tetra (*H. erythrozonus*) cones continue in an undiminished fashion for many days, previous work has shown neon tetra (*Paracheirodon innesi*) (Lythgoe and Shand, 1983) and zebrafish (*Danio rerio*) (Meneger et al., 2005) cones to display a somewhat reduced amplitude of contraction in continual darkness whereas the rhythm of goldfish (*Carassius auratus*) cones is much reduced in such conditions (Wigger, 1941; John et al., 1967). Interestingly, the only other species that has a similarly strong endogenous rhythm of cone contraction in continual darkness to the glowlight tetra, the goldeneye cichlid (*Nannacara anomala*), whose cones continue to contract in an undamped manner for at least three days (Douglas and Wagner, 1982), comes from a similar geographical location to the glowlight tetra (the Aruka River in Guyana, latitude 8.2°; FishBase). The fact that the goldeneye and the glowlight tetra belong to different families suggests their strong endogenous rhythm may be related to their distribution rather than phylogeny.

Relationship between reliance on endogenous signals and latitude

There is a clear correlation between the degree to which the cones of different species are influenced by ambient illumination and their geographical origin (Fig. 4) (Douglas and Wagner, 1982). The closer a species originates to the equator the more strongly it appears to rely on its endogenous signal to determine cone position.

An obvious variable associated with latitude is the annual variation in day length. Equatorial species will experience a virtually constant 12 h:12 h light:dark cycle throughout the year, while further from the equator the day length becomes more variable, with the polar animals experiencing prolonged periods of continual light or

dark. Thus, the less yearly variation in day length an animal is exposed to the more strongly it appears to rely on its endogenous signal to determine cone position (Fig. 5).

Latitudinal variations in circadian physiology and behaviour both within a species and between species are not uncommon. For example, aspects of leaf movement in various plants vary systematically with latitude (Mayer, 1966; Michael et al., 2003), as do emergence rhythms in midges (Pflüger and Neumann, 1971). Furthermore, the structure of the genes driving the circadian oscillator varies within different populations of the same species depending on their latitude, in species as diverse as fruit flies (*Drosophila melanogaster*) (Costa et al., 1992; Sawyer et al., 1997; Weeks et al., 2006; Kyriacou et al., 2007) and blue tits (*Cyanistes caeruleus*) (Johnsen et al., 2007). Most recently, in Arctic reindeer, unlike in more temperate species, melatonin synthesis was found to be driven exclusively by ambient illumination, which, coupled to the absence of circadian rhythmicity of two clock genes, suggests the circadian clock is weak or absent in Arctic animals (Lu et al., 2010). However, probably the best described latitudinal clines in various fundamental properties of the circadian pacemaker controlling rhythmicity concern aspects of behaviour in *Drosophila* [eclosion (Lankinen, 1986; Lankinen, 1993; Pittendirgh and Takamura, 1989; Pittendirgh et al., 1991; Joshi and Gore, 1999); oviposition (Allemand and David, 1976; Allemand and David, 1984; Satralkar et al., 2007a; Satralkar et al., 2007b; Keny et al., 2008); and locomotion (Joshi, 1999; Simunovic and Jaenike, 2006)]. Most of the effects of latitude on *Drosophila* behaviour can be interpreted as an increased strength of the circadian pacemaker in equatorial populations.

Thus, in systems as diverse as retinal cone contraction in teleosts, *Drosophila* behaviour and reindeer melatonin synthesis, animals originating from near the equator rely on endogenous pacemakers to a greater extent and have less flexible rhythms than animals originating from higher latitudes, whose pacemakers are more malleable and respond to environmental illumination to a greater degree. As it occurs in such diverse systems, the correlation between increased latitude and reduced efficacy of circadian oscillators may be a common, or even universal, feature among animals.

Such a relationship is perhaps not unexpected. At the equator the light:dark cycle is almost constant, and pacemakers need little adjustment and can be relatively inflexible and insensitive to ambient illumination. Animals from greater latitudes, however, have to continually reset their circadian clock to synchronise it to the ever changing light:dark cycle and will need to be more sensitive to exogenous light. Equatorial species will thus be able to take advantage of the predictive abilities afforded by an endogenous clock, ensuring, for example, that cone contraction always occurs in synchrony with dawn. This is especially significant in equatorial regions as dawn and dusk are faster here compared with higher latitudes. Furthermore, an endogenous clock in a stable photoperiodic environment will buffer the visual system from transient changes in illumination.

Relationship to infradian rhythms?

Although many of an organism's rhythms are related to daily changes in light levels, some, such as annual migration, molting and reproduction, are aligned to longer term seasonal changes in the environment (Goldman et al., 2004; Foster and Kreitzman, 2009). Just as daily cycles can be triggered by both endogenous circadian clocks and exogenous stimuli, yearly events can be a response to both circannual oscillators or a specific photoperiod. In both invertebrates and vertebrates, including fish (Yeates-Burghart et al.,

2009), the direct influence of day length is more pronounced in animals from higher latitudes. For example, although some tropical birds are able to make use of the relatively modest annual changes in photoperiod to initiate reproduction (Hau et al., 1998), the relative paucity of photoperiodic cues results in many tropical species making more use of endogenous cues to ensure migration and reproductive events occur at an appropriate time than those resident in temperate clines, which are more influenced by photoperiodic conditions (Lofts, 1962; Gwinner, 1986; Goldman et al., 2004).

Thus, a latitudinal cline in efficacy of endogenous oscillators may be a general feature for both circadian and infradian rhythms; the physiology and behaviour of equatorial species are subject to a high level of endogenous control whereas species from more temperate clines are more influenced by the direct effects of light.

ACKNOWLEDGEMENTS

We are indebted to Professors Chris Hull and David Crabb for statistical advice, and Professors Daniel Osorio, Jochen Wagner, Russell Foster, Rob Lucas and Karl-Arne Stokkan for useful scientific discussion. Professor Michaela Hau supplied helpful information concerning circannual rhythms in birds, and Professor Dilip Joshi provided an explanation of latitudinal clines in *Drosophila* behaviour. Drs Flávio Lima and Ad Konings provided valuable information about the distribution of South American and African species, respectively. Thanks also to Konstantin Chittka for the supply of sticklebacks. A.B. was funded by a postdoctoral fellowship from the Spanish Ministry of Science.

REFERENCES

- Allemand, R. and David, J. R. (1976). The circadian rhythm of oviposition in *Drosophila melanogaster*: a genetic latitudinal cline in wild populations. *Experientia* **32**, 1403-1405.
- Allemand, R. and David, J. R. (1984). Genetic analysis of the circadian oviposition rhythm in *Drosophila melanogaster*: effects of drift in laboratory strains. *Behav. Genet.* **14**, 31-43.
- Arey, L. B. and Mundt, G. H. (1941). A persistent diurnal rhythm in visual cones. *Anat. Rec. (suppl)* **79**, abstract 41.
- Besharse, J. C. and Iuvone, P. M. (1983). Circadian clock in *Xenopus* eye controlling retinal serotonin N-acetyltransferase. *Nature* **305**, 133-135.
- Burnside, B. and Ackland, N. (1984). Effects of circadian rhythm and cAMP on retinomotor movements in the Green Sunfish, *Lepomis cyanellus*. *Invest. Ophthalmol. Vis. Sci.* **25**, 539-545.
- Burnside, B. and Kingsmith, C. (2010). Fish retinomotor movements. In *Encyclopedia of the Eye* (eds D. A. Dart, J. Besharse and R. Danz), pp. 142-150. Oxford: Academic Press.
- Burnside, B. and Nagle, B. (1983). Retinomotor movements of photoreceptors and retinal pigment epithelium: mechanisms and regulation. *Prog. Ret. Eye Res.* **2**, 67-109.
- Cahill, G. M. and Besharse, J. C. (1993). Circadian clock functions localized in *Xenopus* retinal photoreceptors. *Neuron* **10**, 573-577.
- Cahill, G. M. and Besharse, J. C. (1995). Circadian rhythmicity in vertebrate retinas: regulation by a photoreceptor oscillator. *Prog. Ret. Eye Res.* **14**, 267-291.
- Costa, R., Peixoto, A. A., Barbujani, G. and Kyriacou, C. P. (1992). A latitudinal cline in a *Drosophila* clock gene. *Proc. R. Soc. Lond. B Bio.* **250**, 43-49.
- Dearry, A. and Barlow, R. B. (1987). Circadian rhythms in the Green Sunfish retina. *J. Gen. Physiol.* **89**, 745-770.
- Douglas, R. H. (1982a). The function of photomechanical movements in the retina of the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **96**, 389-403.
- Douglas, R. H. (1982b). An endogenous crepuscular rhythm of rainbow trout (*Salmo gairdneri*) photomechanical movements. *J. Exp. Biol.* **96**, 377-388.
- Douglas, R. H. and Wagner, H. J. (1982). Endogenous patterns of photomechanical movements in teleosts and their relation to activity rhythms. *Cell Tissue Res.* **226**, 133-144.
- Douglas, R. H., Wagner, H. J., Zaunreiter, M., Behrens, U. D. and Djamgoz, M. B. A. (1992). The effect of dopamine depletion on the light-evoked and circadian retinomotor movements of the teleost retina. *Visual Neurosci.* **9**, 335-343.
- Easter, S. S. and Macy, A. (1978). Local control of retinomotor activity in the fish retina. *Vision Res.* **18**, 937-942.
- Foster, R. G. and Hankins, M. W. (2002). Non-rod, non-cone photoreception in vertebrates. *Prog. Retin. Eye Res.* **21**, 507-527.
- Foster, R. G. and Kreitzman, L. (2004). *Rhythms of Life*. London: Profile Books.
- Foster, R. G. and Kreitzman, L. (2009). *Seasons of Life*. London: Profile Books.
- Goldman, B., Gwinner, E., Karsch, F. J., Saunders, D., Zucker, I. and Ball, G. F. (2004). Circannual rhythms and photoperiodism. In *Chronobiology; Biological Timekeeping* (eds J. C. Dunlap, J. J. Loros and P. J. DeCoursey), pp. 107-142. Sunderland: Sinauer Associates.
- Green, C. B. and Besharse, J. C. (2004). Retinal circadian clocks and control of retinal physiology. *J. Biol. Rhythms* **19**, 91-102.
- Gwinner, E. (1986). *Circannual Rhythms. Zoophysiology* **18**. Heidelberg: Springer-Verlag.
- Hastings, M. H., Reddy, A. B. and Maywood, E. S. (2003). A clockwork web: circadian timing in brain and periphery, in health and disease. *Nature Rev. Neurosci.* **4**, 649-661.
- Hau, M., Wikelski, M. and Wingfield, J. C. (1998). A neotropical forest bird can measure the slight changes in tropical photoperiod. *Proc. R. Soc. Lond. B. Biol. Sci.* **265**, 89-95.
- Iigo, M. I., Ikeda, E., Sato, M., Kawasaki, S., Noguchi, F. and Nishi, G. (2006). Circadian rhythms of ocular melatonin in the wrasse *Halichoeres tenuispinnis*, a labrid teleost. *Gen. Comp. Endocrinol.* **145**, 32-38.
- Iuvone, P. M., Tosini, G., Pozdeyeva, N., Haquea, R., Kleind, D. C. and Chaurasiaa, S. S. (2005). Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. *Prog. Retin. Eye Res.* **24**, 433-456.
- John, K. R. and Gring, D. M. (1968). Retinomotor rhythms in the bluegill *Lepomis macrochirus*. *J. Fish. Res. Board Can.* **25**, 373-381.
- John, K. R. and Haut, M. (1964). Retinomotor cycles and correlated behaviour in the teleost *Astyranax mexicanus* (Phillipi). *J. Fish. Res. Board Can.* **21**, 591-595.
- John, K. R. and Kaminester, L. H. (1969). Further studies on retinomotor rhythms in the teleost *Astyranax mexicanus*. *Physiol. Zool.* **42**, 60-70.
- John, K. R., Segall, M. and Zawatzky, L. (1967). Retinomotor rhythms in the goldfish, *Carassius auratus*. *Biol. Bull.* **132**, 200-210.
- Johnsen, A., Fidler, A. E., Kuhn, S., Carter, K. L., Hoffmann, A., Barr, I. R., Biard, C., Charmanier, A., Eens, M., Korsten, P. et al. (2007). Avian Clock gene polymorphism: evidence for a latitudinal cline in allele frequencies. *Mol. Ecol.* **16**, 4867-4880.
- Joshi, D. S. (1999). Latitudinal variation in locomotor activity rhythm in adult *Drosophila ananassae*. *Can. J. Zool.* **77**, 865-870.
- Joshi, D. S. and Gore, A. P. (1999). Latitudinal variation in eclosion rhythm among strains of *Drosophila ananassae*. *Indian J. Exp. Biol.* **37**, 718-724.
- Keny, V. L., Vanlalngaha, C., Hakim, S. S., Khare, P. V., Barnabas, R. J. and Joshi, D. S. (2008). Latitude dependent arrhythmicity in the circadian oviposition rhythm of *Drosophila ananassae*. *Biol. Rhythm Res.* **39**, 143-150.
- Kirsch, M., Wagner, H. J. and Douglas, R. H. (1989). Rods trigger light adaptive retinomotor movements in all spectral cone types in a teleost fish. *Vision Res.* **29**, 389-396.
- Konings, A. (1988). *Tanganyika Cichlids*. Verduijn cichlids: Zevenhuizen.
- Kullander, S. O. (1986). *Cichlid Fishes of the Amazon River Drainage of Peru*. Stockholm: Swedish Museum of Natural History.
- Kyriacou, C. P., Peixoto, A. A., Sandrelli, F., Costa, R. and Tauber, E. (2007). Clines in clock genes: fine-tuning circadian rhythms to the environment. *Trends Genet.* **24**, 124-132.
- Lamb, T. D. and Pugh, E. N. (2004). Dark adaptation and the retinoid cycle of vision. *Prog. Ret. Eye Res.* **23**, 307-380.
- Lankinen, P. (1986). Geographical variation in circadian eclosion rhythm and photoperiodic adult diapause in *Drosophila littoralis*. *J. Comp. Physiol. A* **159**, 123-142.
- Lankinen, P. (1993). North-south differences in circadian eclosion rhythm in European populations of *Drosophila subobscura*. *Heredity* **71**, 210-218.
- Levinson, G. and Burnside, B. (1981). Circadian rhythms in teleost retinomotor movements; a comparison of the effects of circadian rhythm and light condition on cone length. *Invest. Ophth. Vis. Sci.* **20**, 294-303.
- Lofts, B. (1962). Photoperiod and the refractory period of reproduction in an equatorial bird, *Quelea quelea*. *IBIS* **104**, 407-414.
- Lu, W., Meng, Q. J., Tyler, N. J. C., Stokkan, K. A. and Loudon, A. S. I. (2010). A circadian clock is not required in an arctic mammal. *Curr. Biol.* **20**, 1-5.
- Lythgoe, J. N. and Shand, J. (1983). Endogenous circadian retinomotor movements in the neon tetra (*Paracheirodon innesi*). *Invest. Ophth. Vis. Sci.* **2**, 1203-1210.
- Mayer, W. (1966). Besonderheiten der circadianen Rhythmik bei Pflanzen verschiedener geographischer Breiten. *Planta* **70**, 237-256.
- McCormack, C. A. and Burnside, B. (1991). Effects of circadian phase on cone retinomotor movements in the Midas cichlid. *Exp. Eye Res.* **52**, 431-438.
- McCormack, C. A. and Burnside, B. (1992). A role for endogenous dopamine in circadian regulation of retinal cone movement. *Exp. Eye Res.* **55**, 511-520.
- McCormack, C. A. and McDonnell, M. T. (1994). Circadian regulation of teleost retinal cone movements in vitro. *J. Gen. Physiol.* **103**, 487-499.
- McFarland, W. N., Ogden, J. C. and Lythgoe, J. N. (1979). The influence of light on the twilight migrations of grunts. *Environ. Biol. Fishes* **4**, 9-22.
- Menger, G. J., Koke, J. R. and Cahill, G. M. (2005). Diurnal and circadian retinomotor movements in zebrafish. *Vis. Neurosci.* **22**, 203-209.
- Michael, T. P., Salomé, P. A., Yu, H. J., Spencer, T. R., Sharp, E. L., McPeck, M. A., Alonso, J. M., Ecker, J. R. and McClung, C. R. (2003). Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* **302**, 1049-1053.
- Olla, B. L. and Marchioni, W. N. (1968). Rhythmic movements of cones in the retina of Bluefish, *Pomatomus saltatrix*, held in constant darkness. *Biol. Bull.* **135**, 530-536.
- Perlman, I. and Normann, A. (1998). Light adaptation and sensitivity controlling mechanisms in vertebrate photoreceptors. *Prog. Retin. Eye Res.* **17**, 523-563.
- Pflüger, W. and Neumann, D. (1971). Die Steuerung einer gezeitenparallelen Schlüpfrythmik nach dem Sanduhr-Prinzip. *Oecologia* **7**, 262-266.
- Pittendrigh, C. S. and Takamura, T. (1989). Latitudinal clines in the properties of a circadian pacemaker. *J. Biol. Rhythms* **4**, 217-235.
- Pittendrigh, C. S., Kyner, W. T. and Takamura, T. (1991). The amplitude of circadian oscillations: temperature dependence, latitudinal clines, and the photoperiodic time measurement. *J. Biol. Rhythms* **6**, 299-313.
- Ribelayga, C., Wang, Y. and Mangel, S. C. (2003). A circadian clock in the fish retina regulates dopamine release via activation of melatonin receptors. *J. Physiol. (Lond.)* **554**, 467-482.
- Satralkar, M. K., Keny, V. L., Khare, P. V., Vanlalngaha, C., Kasture, M. S., Shivagaje, A. J., Barnabas, R. J., Iyyer, S. B. and Joshi, D. S. (2007a). Latitudinal variation in oviposition rhythm of *Drosophila ananassae* strains originating from the equator to subtropics. *Biol. Rhythm Res.* **38**, 391-398.
- Satralkar, M. K., Keny, V. L., Khare, P. V., Vanlalngaha, C., Kasture, M. S., Shivagaje, A. J., Barnabas, R. J., Iyyer, S. B. and Joshi, D. S. (2007b). Latitude

- dependent lability of phase response curve for oviposition rhythm of *Drosophila ananassae*. *Biol. Rhythm Res.* **38**, 421-426.
- Sawyer, L. A., Hennessy, J. M., Peixoto, A. A., Rosato, E., Parkinson, H., Costa, R. and Kyriacou, C. P.** (1997). Natural variation in a *Drosophila* clock gene and temperature compensation. *Science* **278**, 2117-2120.
- Shand, J. and Foster, R. G.** (1999). The extraretinal photoreceptors of non-mammalian vertebrates. In *Adaptive Mechanisms in the Ecology of Vision* (eds S. N. Archer, M. B. A. Djamgoz, E. R. Loew, J. C. Partridge and S. Vallergera), pp. 197-222. Dordrecht: Kluwer Academic Publishers.
- Simunovic, A. and Jaenike, J.** (2006). Adaptive variation among *Drosophila* species in their circadian rhythms. *Evol. Ecol. Res.* **8**, 803-811.
- Tosini, G. and Fukuhara, C.** (2002). The mammalian retina as a clock. *Cell Tissue Res.* **309**, 119-126.
- Tosini, G., Pozdeyev, N., Sakamoto, K. and Iuvone, P. M.** (2008). The circadian clock system in the mammalian retina. *BioEssays* **30**, 624-633.
- Wagner, H. J., Kirsch, M. and Douglas, R. H.** (1992). Light dependent and endogenous circadian control of adaptation in teleost retinae. In *Rhythms in Fishes* (ed. M. A. Ali), pp. 255-291. New York: Plenum Press.
- Weeks, A. R., McKechnie, S. W. and Hoffmann, A. A.** (2006). In search of clinal variation in the *period* and *clock* timing genes in Australian *Drosophila melanogaster* populations. *J. Evol. Biol.* **19**, 551-557.
- Weitzman, S. H. and Fink, W. L.** (1983). Relationships of the neon tetras, a group of South American fishes (Teleostei, Characidae), with comments on the phylogeny of New World characiforms. *Bull. Mus. Comp. Zool.* **150**, 339-395.
- Welsh, J. H. and Osborn, C. M.** (1937). Diurnal changes in the retina of the catfish, *Ameiurus nebulosus*. *J. Comp. Neurol.* **66**, 349-359.
- Whitmore, D., Foulkes, N. S., Strahle, U. and Sassone-Corsi, P.** (1998). Zebrafish clock rhythmic expression reveals independent peripheral circadian oscillators. *Nat. Neurosci.* **8**, 701-707.
- Wigger, H.** (1941). Diskontinuität und Tagesrhythmik in der Dunkelwanderung retinaler Elemente. *Z. Vgl. Physiol.* **28**, 421-427.
- Yeates-Burghart, Q. S., O'Brien, C., Cresko, W. A., Holzapfel, C. M. and Bradshaw, W. E.** (2009). Latitudinal variation in photoperiodic response of the three-spined stickleback *Gasterosteus aculeatus* in western North America. *J. Fish Biol.* **75**, 2075-2081.