Chapter 2

Photopic adaptations to a changing environment in two Lake Victoria cichlids

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Abstract

During the past 30 years, Lake Victoria cichlid fishes have encountered severe environmental and ecological changes including an introduced predator and other prey types. Furthermore, increased eutrophication led to reduced water transparency and shifted the spectral composition of underwater light to longer wavelengths. Here, collections of two cichlid species, *Haplochromis pyrrhocephalus* and *Haplochromis tanaos*, from before and after the environmental changes, were compared with respect to their photopic resolution and sensitivity. Eyes of both species were dissected and retinal features were measured from tangential sections. In both species the eyes became smaller, independently of body size. This decrease possibly occurred to make space for other structures in the head that increased in size. In *H. pyrrhocephalus,* a significantly lower resolution was found. However, despite the smaller eyes, the size and thus photon catching ability of the double cones, remained unchanged. In the modern populations of *H. tanaos*, the double cone size increased in relation to eye size, so that the photon catching ability of the smaller modern fishes remained the same. However, no significant decrease in resolution was found. Shortwave sensitivity was found to be lower in both modern populations, because of reduction or complete absence of single cones. Our results imply that these resurgent zooplanktivores are capable of adapting their eye morphology to the changed environmental conditions without losing crucial aspects used for survival and reproduction.

Introduction

Since the 1980s, Lake Victoria's endemic haplochromine cichlids have had to cope with extreme environmental changes in the lake. Nile perch were introduced into the lake and their numbers boomed, resulting in a decline in abundance of the haplochromines (Ogutu-Ohwayo 1990; Witte *et al.* 1992a; Goudswaard *et al.* 2008). At the same time, eutrophication increased, resulting in lower dissolved oxygen (DO) levels and poorer light conditions (Muggide 1993; Hecky *et al.* 1994; Seehausen *et al.* 1997a; Chapter 5 in this thesis). The increased light absorption by dissolved and dispersed organic matter resulted in reduced illumination and less penetration of short-wavelength blue light (Seehausen *et al.* 2008). The multiple stressors mentioned above contributed to the decline of the haplochromines (Kaufman 1992; Witte *et al.* 1992a; Seehausen *et al.* 1997a; Hecky *et al.* 2010). However, after a decline of the Nile perch population in the 1990s, a number of haplochromine species reappeared in the Mwanza Gulf of Lake Victoria. This occurred despite the fact that predation pressure by the remaining Nile perch was still high, DO concentrations were still low, and the light conditions were still poor (Witte *et al.* 2000, 2007). Two of the resurging species were the zooplanktivores *Haplochromis (Yssichromis) pyrrhocephalus* and *Haplochromis tanaos.* In the 1970s, *H. pyrrhocephalus* co-existed with some other zooplanktivores in the open waters of the Mwanza Gulf. It was mainly found near the bottom, at depths of 8-14m during the day, and nearer to the surface at night (Goldschmidt *et al.* 1990). *Haplochromis tanaos* mainly occupied shallow sand bottoms at depths of less than 6m in bays of the Mwanza Gulf (Van Oijen & Witte 1996).

After its resurgence, *H. pyrrhocephalus,* extended its habitat into regions of only 4 m deep (Kishe-Machumu 2012) and became the most common haplochromine cichlid of the Mwanza Gulf (Witte *et al.* 2000). *Haplochromis tanaos* extended its habitat to deeper (13 m) mud bottoms and also became one of the more common species (Van Oijen & Witte 1996; Seehausen *et al.* 1997b; Kishe-Machumu 2012). In addition, both species shifted their diet from zooplankton to insects and other larger and more robust invertebrates (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Chapter 4 in this thesis).

Major morphological changes were observed in the resurgent *H. pyrrhocephalus*. An increase in the surface area of the gills seemed to be an adaptation to the lower DO concentrations (Witte *et al.* 2008). A decrease in head size, which could be an adaptation for escaping Nile perch predation, was also observed (Chapman *et al.* 2008; Witte *et al.* 2008; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). More difficult to explain was a decrease in eye size, despite the decreased light conditions. Witte *et al.* (2008) suggested that the smaller eye size might be due to a trade-off with the increased space needed to accommodate larger gills and a larger buccal cavity depth in a smaller head. They suggested that the structure of the retina should be studied to see whether a decrease in eye size would negatively influence the visual capacities of the fish.

There are many ways to compensate for the above-mentioned decrease in eye size in a turbid environment. Recent studies have shown that opsin gene expression plays a major role in cichlid eye adaptation and speciation in turbid environments (Carleton *et al.* 2005; Seehausen *et al.* 2008; Hofmann *et al.* 2009, 2010; Maan & Seehausen 2010). In addition, behavioural changes in response to turbidity can contribute to the persistence of cichlid species (Gray *et al.* 2011; Gray *et al.* 2012). However, this is beyond the scope of the present study, where we concentrate on morphological changes.

To cope with the environmental changes, the haplochromines, which are visual predators (Fryer & Iles 1972), would require an increased photopic sensitivity. This would mean an enlargement of the cones at the cost of their planimetric and thus angular density (Van der Meer & Anker 1984). Enlargement of the cones is a common feature in growing cichlid eyes (Van der Meer 1993, 1994). So, the reduced eye-size found in the modern population of *H. pyrrhocephalus* was unexpected in relation to the assumed visual demands. Studies of the retina of *H. pyrrhocephalus* from before the environmental changes (old populations) however, revealed exceptionally large long-wavelength-sensitive (LWS) double cones (Van der Meer *et al.* 1995). In addition, Van der Meer & Bowmaker (1995) showed that the spectral sensitivity of this species covered significantly longer wavelengths than in other haplochromines investigated. This suggested a "pre-adaptation" to the new light conditions which may have contributed to the successful recovery of *H. pyrrhocephalus*.

A decrease in eye-size does not necessarily imply a reduction of photopic sensitivity, since the photon catching ability (PhCA) depends on cone-size and not on eye size (Van der Meer & Anker 1984). An increased PhCA (due to increased cone size) compensates for the reduced visibility of nearby objects (predator, prey or congener). The higher the PhCA, the sooner a fish can respond, either by approaching or escaping the object of detection. Following retinal studies on haplochromines by Van der Meer *et al*. (1995), such detection is most profitable in a lateral direction. Therefore, the largest cones were expected to be located in the medial and rostral regions of the retina because the eyes are slightly directed forward. Detail discrimination, e.g. for manipulation of food particles, is determined by resolution, which depends on the angular density of the photopic units (single and double cones, Van der Meer & Anker 1984). Therefore, the highest angular density of LWS double cones (which are red-green sensitive) was expected in the caudal periphery creating a detailed image of objects directly in front of the snout.

Since blue sensitivity is assumed to have become redundant in the changed spectral environment, we expected a reduction in the size and number of single cones as they are known to contain the SWS photopigments (Van der Meer & Bowmaker 1995). We also expected to find a square mosaic of double and (small) single cones in the old population of *H. tanaos*. This pattern was also found in *H. (Ptyochromis) fischeri* (formerly *H. sauvagei*), that used to coexist with *H. tanaos* in the shallow sand habitat. A reduction in the number and size of single cones may provide more room for double cones in a regular cone mosaic (Van der Meer 1992). Therefore, we expected a modest enlargement of the double cones in the modern population of *H. tanaos* just as in blue-light-deprived specimens of *H. fischeri* (Van der Meer 1993). To investigate if the photopic sensitivity and resolution of the modern populations (collected between 1991 and 2001) of *H. pyrrhocephalus* and *H. tanaos* showed adaptive responses to the new environment, we compared their retinal morphology with those of the old populations (collected between 1977 and 1981).

Materials and methods

Adult specimens of both *H. pyrrhocephalus* and *H. tanaos* were collected with bottom trawls in the northern part of the Mwanza Gulf of Lake Victoria over the period 1977-2001. The fish were initially fixed and preserved in 4% formalin (buffered with borax) and afterwards transferred to 70% ethanol in the Naturalis Biodiversity Center, Leiden, for long-term storage. In total, 22 specimens of *H. pyrrhocephalus* and 12 specimens of *H. tanaos* were selected from the period of 1977-1981 and 22 specimens of *H. pyrrhocephalus* and 13 specimens of *H. tanaos* were selected from the period 1991-2001. As far as the available material permitted, adult fish of equal sizes were selected from the different periods. The standard lengths (SL) of these specimens were measured *sensu* Barel *et al.* (1977). Specimens of *H. pyrrhocephalus* used in the study of Witte *et al.* (2008) were included in this study as well.

The eyes were isolated by dissection and the lens-radius (r) was measured either by using an eye-piece micrometer mounted on a binocular microscope, or from digital photographs (the two techniques gave identical results). Due to damage of the retina, not all eyes were suitable for sectioning. Therefore, we selected 20 specimens of old populations (13 *H. pyrrhocephalus* and 7 *H. tanaos*) and 22 specimens of modern populations (16 *H. pyrrhocephalus* and 6 *H. tanaos*) for further analysis (Appendix Table 2.1, 2.2). Whole eyes were dehydrated and embedded in paraplast® using the position of the falciform process as a means of orientation. Semi-thin (5µm) tangential sections were made on a microtome with a steel knife using a graduated location-device (Van der Meer & Anker, 1986) to determine the original position of the sections. The falciform process, which is always located caudo-ventrally, was used as a reference. Accordingly, data were collected from 12 corresponding retinal areas, *viz.* the medial (4) and peripheral (8) regions of the dorsal, rostral, ventral and caudal areas (Figure 2.1).

All sections were stained with hematoxylin and eosin to obtain sufficient contrast between inter- and intra-cellular spaces. The sections were photographed and stored as TIF (Tagged Image File format) files. With the use of Image Tool 1.28 (*H. tanaos*) and ImageJ 1.44p (*H. pyrrhocephalus*) the mean size of the double cones (S_d; based on five double cones) and their areal density (D_d; number per retinal area in three locations; counting was conducted within a field of 10^3 μm^2) were measured and stored. S_d was actually the area of the cross-section through the semi-combined ellipsoid of a double cone and was considered to be a measure of photopic sensitivity (Van der Meer & Anker 1984). The angular density of the double cones (H_d; number per degree of visual angle), a measure of retinal resolution, was calculated, using:

$$H_d = (2.5 \cdot r \cdot \pi \cdot 360^{-1})^2 \cdot \pi \cdot D_d$$ (Van der Meer *et al.* 1995)

The size and angular density of the double cones in the twelve regions were registered for each fish. Measurements on similar retinal locations allowed us to compare individual specimens by mean values. Also the maximum regional values of size (S_{max}) and angular density (H_{max}) were registered for each specimen, as well as their location.

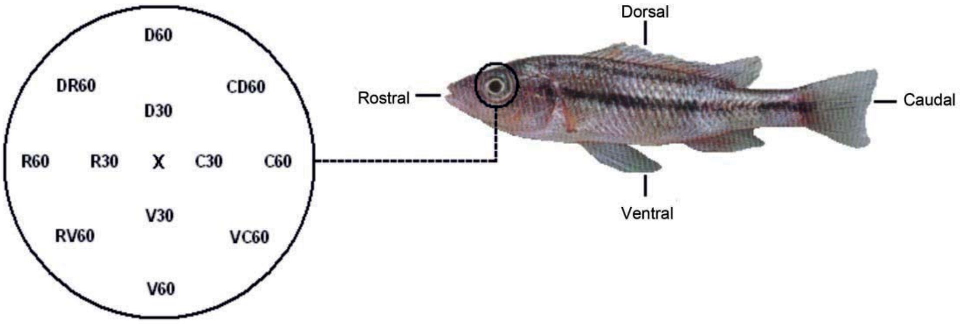

Figure 2.1 The twelve retinal locations sampled in both species (picture of *H. tanaos*). X, centre of retina; C, caudal; D, dorsal; R, rostral; V, ventral; 30, 30°; 60, 60°.

As not all areal densities of each retina could be measured for *H. tanaos*, missing values were interpolated with the use of known mean densities of the double cones of the other individuals of the same group. By calculating mean relative densities (per retinal position) for other individuals and revaluating those according to the other retinal positions, an absolute measure of the unknown areal density was found. Ten missing values for mean density, out of a total of 168 known values, were calculated, assuming an equal retinal distribution amongst individuals.

The size of the inner segments of the single cones S_s was measured in the regions where they were observed. In these regions the relation between the number of single and double cones (s/d) was registered. As in a perfect square mosaic s/d = 0.5, this value was referred to as 100% and the measured single cone occupancy was expressed accordingly. The mean value of S_s in each specimen was derived from the observed single cones, if any, in the entire retina. The mean value of s/d and the percentage of single cone occupancy was derived from the measurements in all twelve regions, including the ones where single cones were absent.

An Analysis of Variance (ANOVA) was used to test if there was a difference in SL among the selected samples. Since both cone-size and angular density increase during growth, SL and r were chosen as covariates for the General Linear Model (GLM), with the population period (old or modern) as independent factor, to test for the effect of period on the morphological characters. Dependent variables, independent factors and the interactions between them were inserted in this selective model. With the use of Multivariate Analyses of Covariance (MANCOVA), non-significant interactions were removed stepwise from each model and estimated marginal means (EMMs, the means of the morphological characters corrected for the used covariate) were calculated. Significant interactions between SL and population period were plotted to determine the effect of each factor. The unstandardized residuals of each GLM were used to test for normality with the Shapiro-Wilk test. *P*-values of the GLM were corrected with a sequential Bonferroni test. All statistical tests on the morphological characters were performed with SPSS version 16.

Results

H. pyrrhocephalus

The mean SL of the modern *H. pyrrhocephalus* studied (61.4 mm) was slightly, but significantly smaller (4.4 %) than that of the specimens of the old population (64.2 mm; $P = 0.025$). The lens-radius of the modern population ($r = 1.21$ mm) was also significantly smaller compared to that of the old population ($r = 1.46$ mm; $P < 0.001$; Table 2.1). Due to a constant ratio between lens diameter and eye diameter (Matthiessen's ratio; Otten, 1981) the lens radius serves as a measure for eye size. The relation between r and SL (Figure 2.2; Table 2.1) showed consistently smaller lenses (17.1 %) in similar sized specimens of the modern population, indicating a smaller eye-size compared to the old population.

In seven out of 13 specimens (54%) of the old population, small single cones were sporadically observed and were randomly allocated within the retinal regions (Figure 2.3A). In the modern population, except for a few single cones in one specimen, the inner segments or ellipsoids of single cones were never observed (Figure 2.3B). In eight out of 16 specimens (50%) of the modern population, relics of single cones (possibly the remains of their nuclei), were sporadically observed in several regions (Appendix Table 2.1). In the retinas of both the old and modern populations, the double cones were more or less irregularly arranged in rows (Figures 2.3A, B). In both populations, the highest densities of double cones were predominantly found in the caudal periphery, whereas the largest cones were mostly measured in the rostral part of the retina (Appendix Table 2.1).

The H_d and H_{max} (the latter in the caudal periphery) have significantly decreased in the modern population (by 28% and 33%, respectively, $P < 0.001$, Figure 2.2; Table 2.1). Both the S_d and S_{max} of modern specimens with a small standard length ($SL \leq 60$ mm) tend to be larger compared to those of the old population (by 15% and 17% respectively). This occurred while S_d and S_{max} of larger specimens ($SL > 60$ mm) had decreased in the modern population (by 13% and 19%, respectively), which altogether results in a significant interaction (Figure 2.4). Estimated marginal means (EMMs) of S_d and S_{max} differed only slightly between the old and modern populations (Table 2.1; Figure 2.4). Concerning H_d and H_{max} in relation to r, there was no significant effect of period, but there was a significant effect of r as covariate (Table 2.1). Both S_d and S_{max} showed a significant interaction, their EMMs differing only slightly (Table 2.1, Figure 2.5).

H. tanaos

The mean SL of the modern *H. tanaos* (64.7 mm) studied did not differ significantly from that of the specimens of the old population (66.3 mm; $P = 0.289$). The r of the modern population of *H. tanaos* was 1.10 mm. This value is 9.0% smaller ($P < 0.001$) than that of the old population (1.21 mm; Figure 2.2; Table 2.2). The single cone size (S_s) of the modern population was significantly smaller than in the old population ($P < 0.001$, with SL and r as covariate; Figure 2.4, 2.5). The mean single cone occupancy decreased from 88% to 56% ($P < 0.001$, with SL as covariate) which affected the ratio between single cones and double cones (s/d). There was a significant decrease of s/d from 0.44 in the old population to 0.29 in the modern population ($P < 0.001$, Table 2.2).

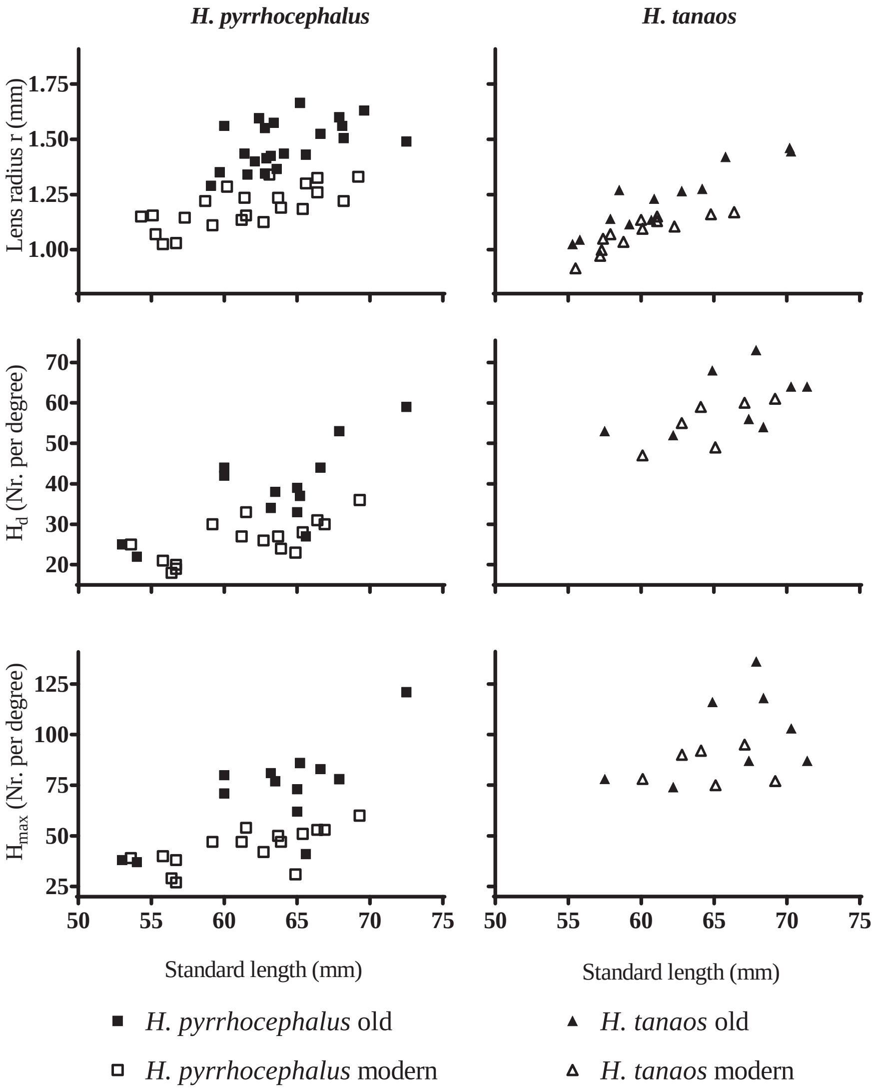

Figure 2.2 Plots of r, H_d, H_{max}, as a function of SL.

The loss of single cones did not coincide with a changed configuration from a square pattern into a row pattern (Figure 2.3C, D). Square patterns with small or absent single cones were usually transformed into diamond patterns (intermediate between square and row patterns, Van der Meer 1992). A clear row pattern was found only on a few occasions in the modern population and only in rostral areas. In both populations, the highest densities of double cones were found predominantly in the dorsal periphery. In the old population, the largest cones were usually observed in the rostral regions of the retina. In the modern population, the largest cones were not strictly confined to a specific region although they were mainly observed in the medial parts (Appendix Table 2.2).

Table 2.1 Results of the GLM analyses on the morphological characters of *H. pyrrhocephalus* with SL and lens radius (r) as covariates. Significant interactions are shown in italics, significant values after sequential Bonferroni corrections are shown in bold.

H. pyrrhocephalus			Cov SL					Cov r				
Character	Pop	N	Mean	Difference (%)	*P* Population	*P* SL	*P* Pop * SL	Mean	Difference (%)	*P* Population	*P* r	*P* Pop * SL
Lens-radius	old	22	1.46									
(r, mm)	modern	22	1.21	**-17.1**	**0.000**	**0.000**	NS	X	X	X	X	X
Angular density	old	13	33.6					31.3				
(H_d, Nr. per degree)	modern	16	24.1	**-28.3**	**0.000**	**0.000**	NS	31.8	1.6	NS	**0.000**	NS
Max. angular density	old	13	68.8					56.3				
(H_{max}, Nr. per degree)	modern	16	46.4	**-32.6**	**0.000**	**0.000**	NS	56.5	0.4	NS	**0.000**	NS
Double cone size	old	13	*44.3*					*41*				
(S_d, μm^2)	modern	16	*42.4*	*-4.3*	*0.028*	*NS*	*0.024*	*39.4*	*-3.9*	*0.029*	*NS*	*0.035*
Max. double cone size	old	13	*70.1*					*63.8*				
(S_{max}, μm^2)	modern	16	*64.7*	*-7.7*	*0.001*	*NS*	*0.001*	*59*	*-7.5*	*0.009*	*NS*	*0.01*
Single cone size	old	7	12.2									
(S_s, μm^2)	modern	1	8									
Ret. Occ.	old	13	7									
(%)	modern	16	4									
Ratio single cones -	old	13	0.04									
double cones (s/d)	modern	16	0									

Means are estimated marginal means derived from the GLM. Pop, population.

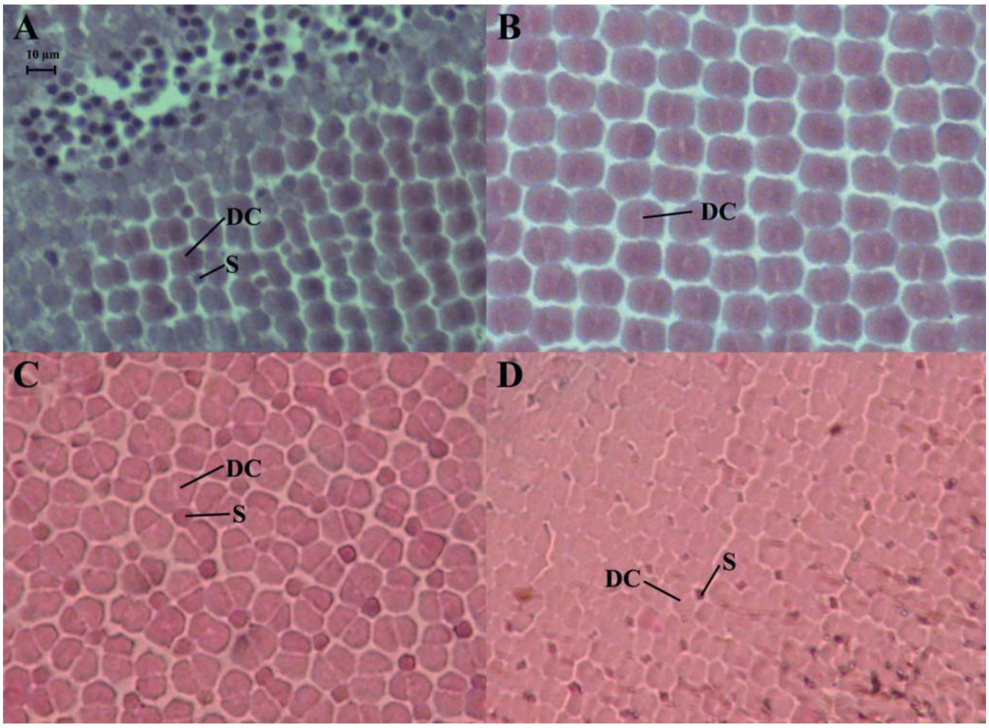

Figure 2.3 Double cone patterns; DC, double cone; S, single cone. A: Row pattern of double cones with presence of some single cones in caudal region of *H. pyrrhocephalus* from 1978 (spec. nr 316-131). B: Row pattern of double cones with no single cones present in the rostral-ventral region in *H. pyrrhocephalus* from 2001 (316-205). C: Square pattern of double cones around single cones in the rostral region in *H. tanaos* from 1978 (320-09). D: Diamond pattern of double cones around tiny single cones in the rostral-ventral region in *H. tanaos* from 2001 (320-15).

The H_d and H_{max} (in the dorsal periphery) of the double cones did not show a significant decrease with SL and r as covariate (Table 2.2; Figures 2.2, 2.5). With SL as covariate, S_d showed a significant interaction, with EMMs of the modern population being slightly larger. No significant difference was found for the S_{max} (Figure 2.4; Table 2.2). The S_d and S_{max} were significantly larger in the modern population with r as covariate. However, after Bonferroni correction, the difference for S_{max} was no longer significant (Table 2.2).

Discussion

In the resurging populations of *H. pyrrhocephalus* and *H. tanaos*, lens size, and thus eye size, decreased, possibly to permit changes in head morphology for other functions than vision (e.g. Witte *et al.* 2008). Both species showed a lower resolution and a decreased blue SWS light sensitivity. In addition, despite the smaller eyes, *H. tanaos* showed an increase of their photopic sensitivity. These changes are in accordance with the increased turbidity, the larger prey types included in the diet of both modern species and the shift to greater depths in *H. tanaos*.

Cone size comparison

There is some discrepancy regarding the double cone size of the old *H. pyrrhocephalus* population from this study, and that of the sample used by Van der Meer *et al*. (1995). The average double cone size in the old population of *H. pyrrhocephalus* from the latter study was larger (52 μm^2) than that of the old population used in the present study (45.5 μm^2, both not corrected for SL). This difference may have several explanations. Retinal rods and cones exchange places under the influence of the environmental light by contraction or elongation of their myoids (light- and dark-adaptation; Ali 1975). The specimens used in the earlier publication were super-exposed to light when caught. This exposure makes sure they were completely light-adapted, *i.e.* the cone myoids were maximally contracted which compressed the ellipsoids towards the outer limiting membrane. This may have been less intense in the light-adapted specimens used in the present study. Moreover, the number of samples from one retina in Van der Meer *et al*. (1995) was much larger (over 30 regions) than in the present study. This affects the balance between the periphery and the centre of the retina for the benefit of the latter, where also the larger cones are located. Furthermore, the fish specimens (and their eyes) used in the study by Van der Meer *et al*. (1995) were stored in 10% formalin (buffered with borax), and were only exposed to alcohol during a relatively short dehydration step. By contrast, the specimens used in the present study were stored in alcohol for many years, and this may have caused more shrinkage of retinal tissue. However, it should be stressed that in the present study, the techniques used for both old and modern fish were identical, thus making the samples comparable.

Eye size

The observed reduction in eye-size in the modern population of *H. pyrrhocephalus* (Witte *et al.* 2008) was confirmed by the present data on lens size in *H. pyrrhocephalus* and *H. tanaos*. The reduction of eye size can be explained by the changed environment. Environmental conditions, especially low dissolved oxygen levels, have a major influence on body shape and cause an increase in gill surface area (Chapman *et al.* 2000; Rutjes *et al.* 2009; Crispo & Chapman 2010a). The first two studies found, under lab conditions, larger gills in a *larger* head. By contrast, Witte *et al*. (2008) found larger gills in a *smaller* head in wild modern *H. pyrrhocephalus*. The smaller head may have been caused by the increased predation pressure of Nile perch (Chapman *et al.* 2008; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]). Moreover, Witte *et al.* (2008) suggested that the observed larger buccal cavity depth (cheek depth) could be relevant for eating larger prey items. Consequently, it is likely that the smaller eyes are caused by predation pressure, diet change and hypoxia. Thus, the smaller eyes may act as a trade-off for larger gills and a larger buccal cavity in smaller heads (Witte *et al.* 2008). However, Gray *et al*. (2011, 2012) found that behavioural changes are also important in the survival of cichlids in a turbid environment. Furthermore, female sticklebacks rely more on olfactory than on visual cues in turbid waters when choosing a mate (Heuschele *et al.* 2009), which might be the case for cichlids too. These studies suggest that there may be multiple strategies to cope with a turbid environment.

Angular density

As visual resolution primarily depends on the number of receptors per visual angle, the lower angular density in the modern populations indicates a lower resolution in the modern populations, compared to the old population. Since cichlids are known to be visual feeders (Fryer & Iles 1972), the lower visual resolution is in agreement with the diet shift of *H. pyrrhocephalus* and *H. tanaos* from zooplankton to larger prey types (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu 2012; Chapter 4 in this thesis). Differences between old and modern populations in angular density in relation to eye size of both species were caused by the smaller retina.

SWS single cones

The reduction of the SWS single cones in both species agrees with the stronger absorbance of shortwave light due to eutrophication of the environment (Seehausen *et al.* 2003) and also with the shift of *H. tanaos* to deeper water over mud bottoms. Apparently, there was no "need" anymore for single cones because the short wavelengths are absorbed by the turbid water. The absence of functional elements of single cones in the studied sections of *H. pyrrhocephalus* does not imply their complete disappearance, as indicated by the occasional nuclei observed in the sections.

A recent study on cone opsin expression in Lake Malawi cichlids revealed that phyto/zooplanktivores had higher SWS opsin gene expression than species feeding on fish or benthic invertebrates (Hofmann *et al.* 2009). However, SWS opsin gene sequence of some Lake Victoria cichlid species show hardly any variability between species, in contrast with LWS opsin gene sequence (Carleton *et al.* 2005). These LWS opsin genes have shown to have a high differentiation rate between two sympatric *Pundamilia* phenotypes in association with water clarity (Seehausen *et al.* 2008). This difference resulted in longer LWS pigments for the deep water red species compared to the shallow water blue species. Multiple studies suggest that the variation in the expression of opsin genes might be adaptive and driven by variation in ambient light (Carleton *et al.* 2005; Seehausen *et al.* 2008; Hofmann *et al.* 2009; Maan & Seehausen 2010). In addition, Hofmann *et al.* (2010) found evidence suggesting sensory plasticity played a role in cichlid diversifications in Lake Malawi. Selection on and/or plasticity of opsin genes might also have resulted in the reduction or absence of single cones found in the present study. Unfortunately, our sample did not allow us to study opsin gene expression as fish were preserved in formalin.

Furthermore, it cannot be ruled out that in the modern populations, the reduction of single cones was the result of elongation of their myoids (as in dark-adaptation, Ali 1975) In this scenario, myoid elongation screens the single cones from incoming light and reduces their function. Such a reversible phenotypic phenomenon, however, is not supported by earlier studies of the retina in *H. pyrrhocephalus* raised in brightly illuminated tanks for several generations (Van der Meer & Bowmaker 1995).

Table 2.2 Results of the GLM analyses on the morphological characters of *H. tanaos* with SL and lens radius (r) as covariates. Significant interactions are shown in italics, significant values after sequential Bonferroni corrections are shown in bold.

H. tanaos			Cov SL					Cov r				
Character	Pop	N	Mean	Difference (%)	*P* Population	*P* SL	*P* Pop * SL	Mean	Difference (%)	*P* Population	*P* r	*P* Pop * SL
Lens-radius	old	12	1.21									
(r, mm)	modern	13	1.10	**-9.1**	**0.000**	**0.000**	**NS**	X	X	X	X	X
Angular density	old	8	59.9					57.6				
(H_d, Nr. per degree)	modern	6	56	-6.5	NS	0.045	NS	59.1	2.6	NS	**0.01**	NS
Max. angular density	old	8	98.9					94.4				
(H_{max}, Nr. per degree)	modern	6	85.8	-13.2	NS	NS	NS	91.8	-2.8	NS	0.072	NS
Double cone size	old	8	*27.4*					26.2				
(S_d, µm^2)	modern	6	*29.5*	*7.7*	*0.029*	*0.034*	*0.035*	31.7	**21.0**	**0.001**	**0.000**	NS
Max. double cone size	old	8	39.3					37.8				
(S_{max}, µm^2)	modern	6	43.3	10.2	NS	NS	NS	45.3	19.8	0.019	**0.018**	NS
Single cone size	old	8	7					6.6				
(S_s, µm^2)	modern	6	4.2	**-40.0**	**0.000**	0.014	NS	4.7	**-28.8**	**0.001**	**0.001**	NS
Ret. Occ.	old	7	88.5					*87.9*				
(%)	modern	6	55.8	**-36.9**	**0.000**	NS	NS	*40.8*	*-53.6*	*NS*	*0.050*	*0.038*
Ratio single cones -	old	7	0.44					*0.44*				
double cones (s/d)	modern	6	0.29	**-34.1**	**0.000**	NS	NS	*0.22*	*-50.0*	*0.047*	*0.028*	*0.021*

Means are estimated marginal means derived from the GLM. Pop, population.

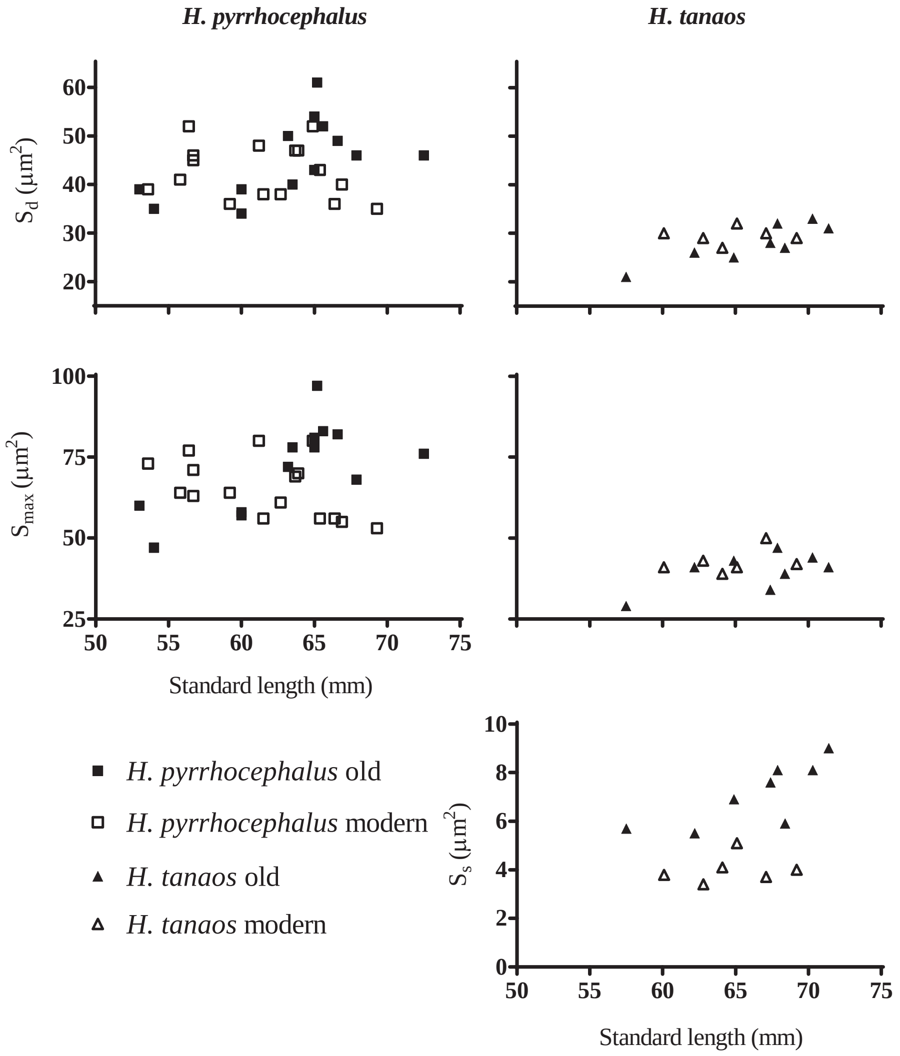

Figure 2.4 Plots of S_d, S_{max} and S_s as a function of SL.

LWS double cones

Even though the eyes became smaller in the modern populations of both species, the double cones remained of a similar size in the adult fish, or even increased for modern *H. tanaos* in relation to the smaller lens-radius. Consequently, the presumed photopic sensitivity did not decrease. The increase in double cone size in *H. tanaos* is likely facilitated by the reduction in size, and decrease in number, of single cones relative to eye-size.

The larger size of double cones in the smaller eyes of the modern population of *H. tanaos* suggests a shift in retinal growth from addition of cone cells to stretching of cone cells. *Haplochromis pyrrhocephalus* shows no increase of photon catching ability (by enlargement of its double cones) as an adaptive adjustment to the decreased light conditions. We can confirm that the already large cones and LWS photopigments of this species (also observed by Van der Meer & Bowmaker 1995) might be a "pre-adaptation" to the turbid environment.

The observed maximum double cone size in the medial and rostral regions of the retinas of both species, and the maximum angular density in the caudal periphery of *H. pyrrhocephalus*, are both in accordance with our expectations (see the introduction of this chapter). They seem to be characteristic for pelagic zooplanktivorous fish (Browman *et al.* 1990). The high dorsal resolution in *H. tanaos* (dorsal location of the maximum angular density in both the old and modern populations), suggests a detailed scanning of the bottom. This would categorise this species as a bottom-dweller as was also suggested by Van Oijen & Witte (1996) because *H. tanaos* was never caught in surface trawls. The observed retinal findings agree with the relation between retinal cell topography and feeding behaviour in other fishes (Shand *et al.* 2000).

Mechanisms behind retinal changes

The retinal changes in the modern populations of both species may have been the result of phenotypic plasticity as observed in shortwave light-deprived specimens of *H. fischeri* in laboratory experiments (Van der Meer 1993). But there are several other possibilities.

Based on mitochondrial DNA, Mzighani *et al.* (2010) suggested that in the relatively murky Mwanza Gulf, modern *H. pyrrhocephalus* hybridises with *H. laparogramma*, in contrast to three other locations with clearer water. If the modern specimens of *H. pyrrhocephalus* from the Mwanza Gulf were hybrids, this may have influenced the size of their double cones. As larger double cones were expected in the modern population of *H. pyrrhocephalus* and the double cones of *H. laparogramma* with the same SL were smaller (before the environmental changes, adult *H. laparogramma* were larger than adult *H. pyrrhocephalus*; Van der Meer *et al*. 1995), hybridization might have resulted in relatively smaller double cone sizes for *H. pyrrhocephalus*.

In addition to phenotypic plasticity and hybridization, natural selection might have played a role in the observed retinal changes as was suggested for several opsin genes (Carleton *et al.* 2005; Seehausen *et al.* 2008; Hofmann *et al.* 2009; Maan & Seehausen 2010).

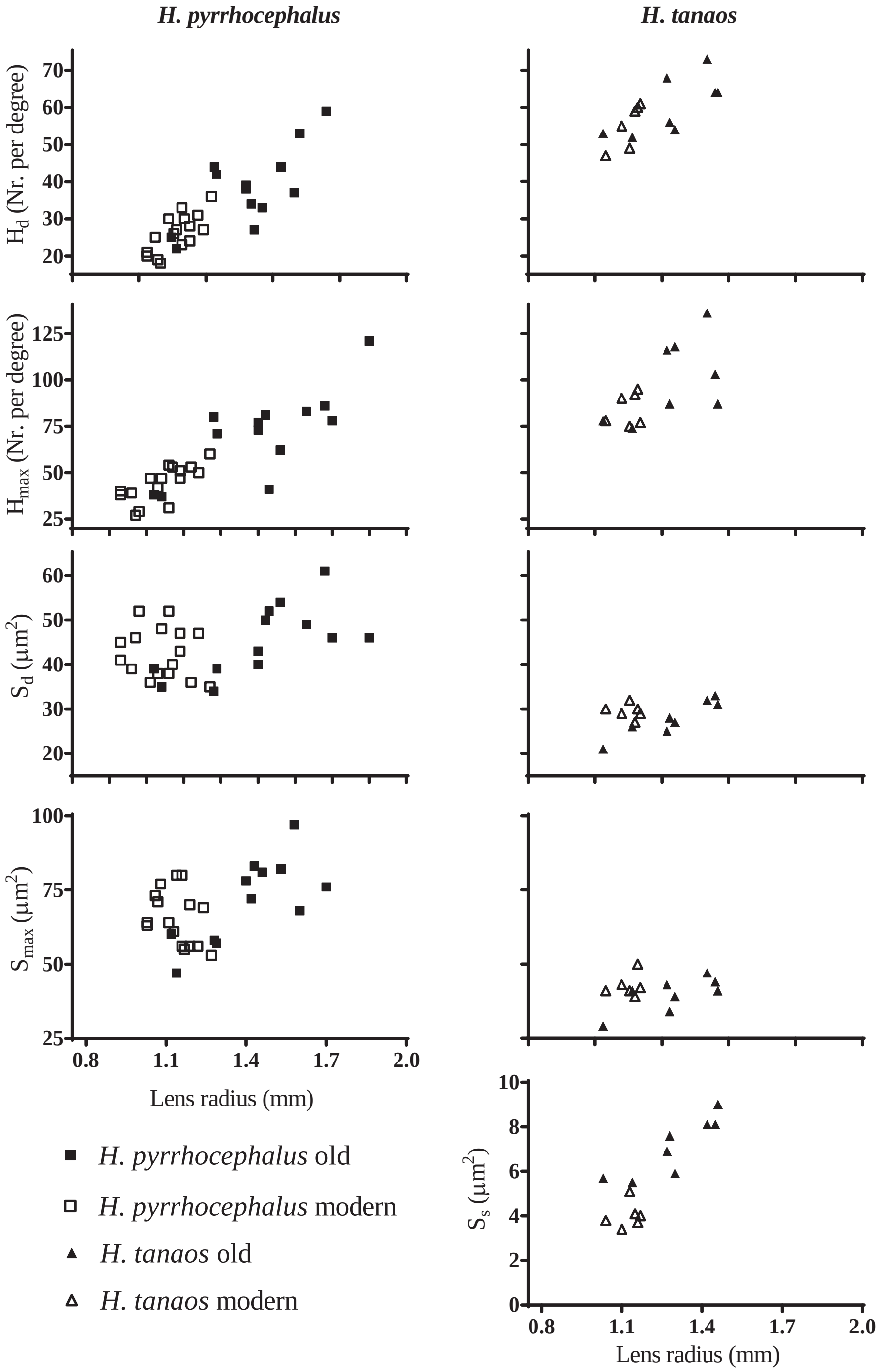

Figure 2.5 Plots of H_d, H_{max}, S_d, S_{max} and S_s as a function of r.

Morphological and ecological convergence

In addition to the reduction of single cones, both species show other changes in the same direction (morphological convergence). For example, the mean difference in lens size (and thus eye size) between the old populations of both species was almost twice as large (17.1%) as that between the modern populations (9.1%). There was also some convergence for cone size between both species as the difference became slightly smaller for the modern populations (30.1%) compared to the difference between the old populations (38.1%). The morphological convergences agree with the overlap in diet and habitat found for the resurgent populations of both species. Moreover, body shape convergence was found between *H. pyrrhocephalus* and *H. laparogramma* (J. C. van Rijssel & F. Witte unpublished data); the latter species shifted its habitat and diet towards that of *H. pyrrhocephalus* (Witte *et al.* 1995; Chapter 4 in this thesis).

Conclusions

Haplochromis pyrrhocephalus and *H. tanaos* have shown apparently adaptive ecological responses including a probable reallocation of internal head structures (Witte *et al.* 2008). In addition, retinal features have changed in such a way that only the resolution, and not the sensitivity for the ambient wavelengths in the modern environment, was negatively affected. Whether the morphological and retinal adjustments found in this study are the result of response to selection, phenotypic plasticity or hybridization remains unclear. Nonetheless, these presumably adaptive responses might have contributed to the recovery of these two zooplanktivores while other species went extinct.

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Appendix Table 2.1 General data of *H. pyrrhocephalus* specimens used in this study.

H. pyrrhocephalus

Period	Number	Catch	SL (mm)	r (mm)	H_d (Nr. per degree)	H_{max} (Nr. per degree)	S_d (µm^2)	S_{max} (µm^2)	S_s (µm^2)	Ret. Occ. (%)	s/d	Loc. H_{max}	Loc. S_{max}
Old	316-121	1977	72.5	1.7	59	121	46	76		0	0	C60/CV60	R60
Old	316-100	1981	65	1.46	33	62	54	81	9.1	18	0.9	C60	R30
Old	316-131	1978	66.6	1.53	44	83	49	82	3.1	4	0.02	CV60	R60
Old	316-126	1978	65.2	1.58	37	86	61	97		0	0	C60	R30
Old	316-105	1981	65	1.4	39	73	43	78	5.3	28	0.14	CV60	D30
Old	316-?	1980	53	1.12	25	38	39	60	19.6	4	0.02	C60	R30
Old	316-?	1980	54	1.14	22	37	35	47		0	0	CV60	R30
Old	316-96	1981	60	1.29	42	71	39	57	20.4	2	0.01	C60	RV60
Old	316-98	1981	63.5	1.4	38	77	40	78	6.6	34	0.17	CV60	R30
Old	316-127	1978	65.6	1.43	27	41	52	83		0	0	C60	R30
Old	316-130	1977	67.9	1.6	53	78	46	68		0	0	C60	R30
Old	316-?	1980	60	1.28	44	80	34	58	21.2	1	0.005	C60	R30
Old	316-128	1978	63.2	1.42	34	81	50	72		0	0	C60	R30
Modern	316-132	1993	56.4	1.08	18	29	52	77		0	0	C60	R30
Modern	316-133	1993	56.7	1.07	19	27	46	71	<1	2	0.01	C60	RD60
Modern	316-134	1993	55.8	1.03	21	40	41	64		0	0	V60	D30
Modern	316-135	1993	53.6	1.06	25	39	39	73		0	0	RV60	R30
Modern	316-136	1993	56.7	1.03	20	38	45	63		0	0	C60	RD60
Modern	316-144	1991	64.9	1.16	23	31	52	80		0	0	C60	R60
Modern	316-147	1999	66.9	1.17	30	53	40	55		0	0	CD60	R30
Modern	316-149	1999	66.4	1.22	31	53	36	56	8	6	0.03	C30	V60
Modern	316-150	1999	69.3	1.27	36	60	35	53	<1	2	0.01	D30	RV60
Modern	316-205	2001	61.2	1.14	27	47	48	80	<1	1	0.005	CV60	V30
Modern	316-196	2001	59.2	1.11	30	47	36	64	<1	12	0.06	V60	R30
Modern	316-192	2001	63.7	1.24	27	50	47	69	<1	28	0.14	CD60	RV60
Modern	316-189	2001	63.9	1.19	24	47	47	70	<1	9	0.04	CD60	R60
Modern	316-154	1999	62.7	1.13	26	42	38	61		0	0	CD/CV60	RD60
Modern	316-152	1999	65.4	1.19	28	51	43	56	<1	9	0.04	C60	D60
Modern	316-151	1999	61.5	1.16	33	54	38	56		0	0	C60	R60

Ret. Occ. = Retinal occupation of single cones. Loc. H_{max} = Location of the H_{max}, Loc. S_{max} = Location of the S_{max}, s/d = ratio single cones / double cones. The <1 in single cone size stands for the absence of functional components, in spite of observed nuclei.

Appendix Table 2.2 General data of *H. tanaos* specimens used in this study.

H. tanaos

Period	Number	Catch	SL (mm)	r (mm)	H_d (Nr. per degree)	H_{max} (Nr. per degree)	S_d (μm^2)	S_{max} (μm^2)	S_s (μm^2)	Ret. Occ. (%)	s/d	Loc. H_{max}	Loc. S_{max}
Old	320-5	1978	67.4	1.28	56	87	28	34	7.6	90	0.45	D60	C30
Old	320-6	1978	70.3	1.45	64	103	33	44	8.1	86	0.43	D30	RD60
Old	320-7	1978	71.4	1.46	64	87	31	41	9	92	0.46	CD60	R60
Old	320-8	1978	62.2	1.14	52	74	26	41	5.5	88	0.44	D60	R30
Old	320-9	1978	67.9	1.42	73	136	32	47	8.1	88	0.44	D60	R60
Old	320-10	1978	64.9	1.27	68	116	25	43	6.9	88	0.44	D30	R30
Old	320-11	1978	57.5	1.03	53	78	21	29	5.7	86	0.43	CD60	R60
Old	320-12	1978	68.4	1.3	54	118	27	39	5.9			CD60	R60
Modern	320-13	2001	62.8	1.1	55	90	29	43	3.4	54	0.27	D60	R60
Modern	320-14	2001	65.1	1.13	49	75	32	41	5.1	46	0.23	D60	V30
Modern	320-15	2001	69.2	1.17	61	77	29	42	4	48	0.24	D60	C30
Modern	320-16	2001	67.1	1.16	60	95	30	50	3.7	64	0.32	D60	C30
Modern	320-17	2001	64.1	1.15	59	92	27	39	4.1	48	0.29	C60	C30
Modern	320-20	2001	60.1	1.04	47	78	30	41	3.8	76	0.38	CD60	V30

Ret. Occ. = Retinal occupation of single cones. Loc. H_{max} = Location of the H_{max}, Loc. S_{max} = Location of the S_{max}, s/d = ratio single cones / double cones. The retina section of specimen W320-012 was not suitable for determination of the retinal occupation and the s/d ratio.


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