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Quantitative Histological Study of the Optic Nerve in Species of Minnows (Cyprinidae, Teleostei) Inhabiting Clear and Turbid Water

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Abstract
Quantitative analysis of the optic nerve of minnows using light- and electron microscopy demonstrated that anatomical characteristics of the visual system are closely related to habitat turbidity. Species in the genera *Notropis* and *Cyprinella* inhabiting predominantly clear water had larger eyes and almost twice as many optic nerve fibers compared to minnows of turbid habitats. No differences were detected in the thickness of myelination, the axon diameter profile, or the number of optic nerve fibers per retinal area, indicating that the relative number of fibers, as well as their anatomical characteristics, are similar in all species and independent of habitat turbidity. It is therefore hypothesized that quantitative differences in the number of visual elements available for sampling and processing in the retina, optic nerve, and optic tectum are sufficient to account for presumed differences in visual performance.

Introduction

The environment may act upon the brain (and hence behavior) through evolutionary forces as well as epigenetic changes. Therefore, the comparative study of brain structures among ecologically distinct species, even when genetic and environmental influences cannot be isolated, provides a rich source of hypotheses concerning the way the nervous system functions under various environmental conditions and how they have responded to natural selection.

Minnows, especially species in the genera *Notropis* and *Cyprinella*, are useful subjects for studying the relationship between the nervous system and the environment. This family has undergone considerable adaptative radiation, and the ecological diversity represented in the family is reflected in the gross and microscopic structure of a number of brain parts [Kishida, 1979; Kotrschal and Junger, 1988; Northcutt and Wulliman, 1988; Huber and Rylander, 1991, 1992; Kotrschal and Palzenberger, 1992].

The sensory apparatus appears particularly sensitive to environmental variation. Recently we showed that the microscopic structure of the visual part of the optic tectum is better developed in species of minnows that inhabit clear water than in those that live in turbid water [Huber and Rylander, 1991]. However, the optic nerve of minnows has not been studied in relation to ecology; indeed, even descriptive accounts of the ultrastructure of the optic nerve of teleosts in general are surprisingly few [eg. Gaze, 1970; Tapp, 1973, 1974; Anders and Hibbard, 1974; Scholes, 1979; Bunt, 1982; Bunt and Horder, 1983; Easter et al., 1984].

In transverse-section, the optic nerve of most teleosts is a long ribbon-like structure that is folded into a roughly cylindrical shape (fig. 1a). Fibers are mostly myelinated, with estimated numbers ranging from 24,000 in *Ameiurus*

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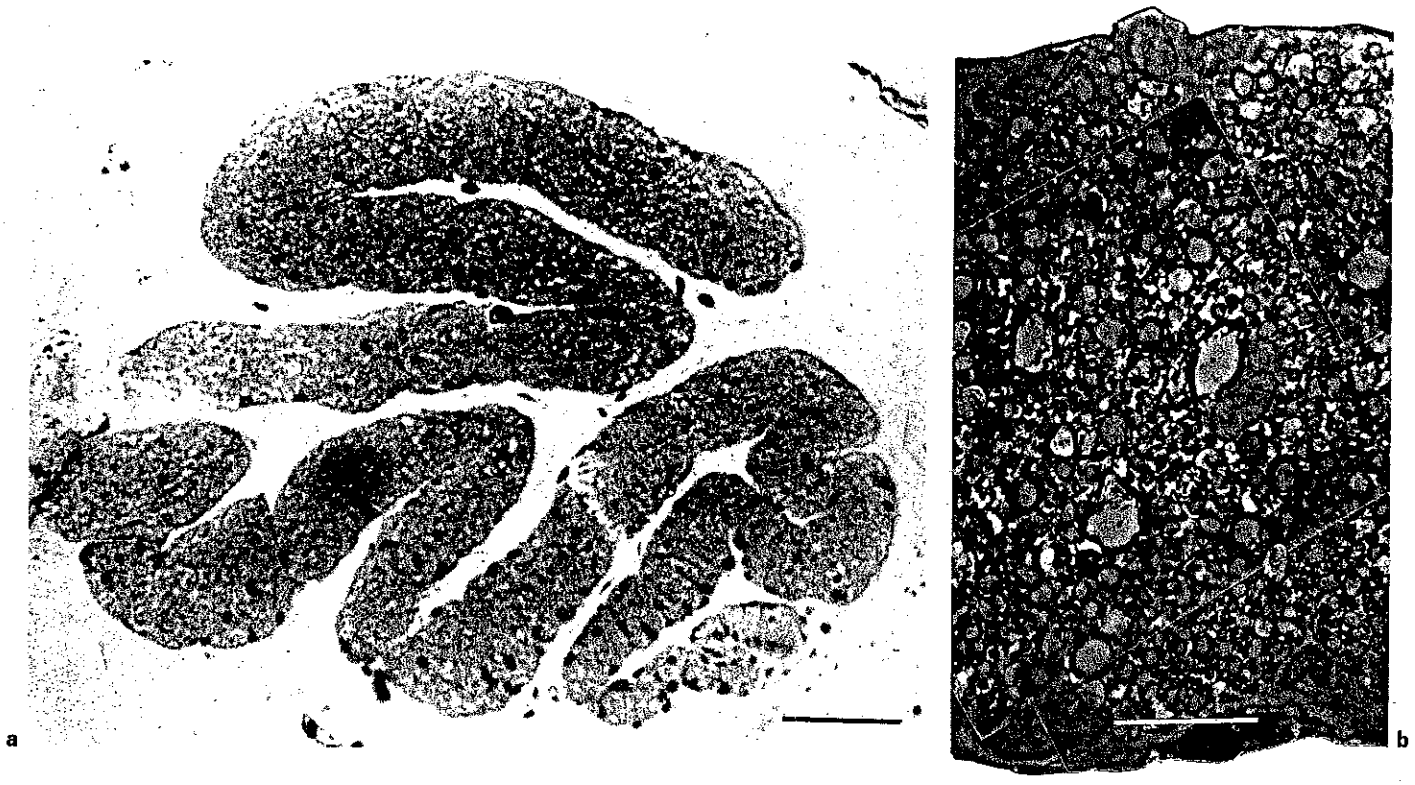


Fig. 1. *Notropis atherinoides,* optic nerve cross section. **a** Entire cross section. Scale bar represents 50 μm. **b** Mosaic of electron micrographs showing a short segment of the optic nerve. Scale bar represents 5 μm.

melas [Bruesch and Arey, 1942] to 200,000 in *Eugerres plumieri* [Tapp, 1974]. Fibers within the optic nerve are arranged in a chronotopic and retinotopic fashion; fibers of similar age and retinal origin travel alongside each other in the optic nerve and terminate in adjacent areas of the optic tectum [Dawnay, 1979a, b; Bunt, 1982; Easter et al., 1981, 1984; Rusoff, 1984; Bernhardt and Easter, 1985, 1986; Easter, 1985; Springer and Mednick, 1986]. Optic nerve fibers vary widely in internal diameter and thickness of the myelin sheath (fig. 1b), which, as in other anamniotic vertebrates, may correspond closely to functional differences [Maturana et al., 1960; Chung et al., 1974; Grüsser and Grüsser-Cornehls, 1976]. In some teleosts, optic nerve fibers of different diameters project to different targets within the central nervous system [Ito et al., 1984; Northcutt and Wullimann, 1988].

The goal of the present study was to determine if the structure of the optic nerve is related to habitat turbidity, as was shown to be the case for overall brain morphology [Huber and Rylander, 1992] and the histology of the optic tectum [Huber and Rylander, 1991]. Thus we employed light microscopy and transmission electron microscopy to analyze three parameters in six species of *Notropis* and *Cyprinella* that live in either clear or turbid water: (1) the size and myelination of optic nerve fibers; (2) the overall numbers of fibers; and (3) the relationship between retinal surface area and number of optic nerve fibers.

Materials and Methods

Five adult female specimens each of three turbid water species *(Cyprinella lutrensis, Notropis bairdi, N. atherinoides)* and three clear water species *(C. venusta, N. amabilis, N. boops)* were collected from streams in Texas and Oklahoma [cf. Huber and Rylander, 1992] according to guidelines established by the NIH. Nomenclature follows the recent revision of related genera by Mayden [1989]. Primary fixation was in 10% phosphate-buffered formalin.

The brain, eyes, and optic nerves were removed from the skull, and a 0.5 mm section of the optic nerve, halfway between the eye and the optic chiasm, was postfixed in 0.1% OsO_4 in 0.1 *M* phosphate buffer. This section was later dehydrated in a graded series of ethanols, embedded in EmBed 812, and sectioned using glass knives.

Semithin sections (0.1 μm) were mounted on glass microscope slides, stained with toluidine/methylene blue, photographed using a compound microscope, and printed at a final magnification of 182 × (fig. 1a). The outline of the transverse-section was entered into an Apple Macintosh with a digitizing pad, and the area of the ribbon-shaped cross-section [Scholes, 1979] was calculated using morphometric software [R. Huber, unpubl. obser.].

Ultrathin sections (90–130 nm) were collected on Formvar-coated, mesh-100 grids and stained with 2% aqueous uranyl acetate and lead citrate [Reynolds, 1963]. One transverse-section from each individual was examined by transmission electron microscopy (Hitachi HS-8 at 50 kV and Hitachi HU-11E at 75 kV), and nine sample areas were cho-

Table 1. Mean lengths, weights, and estimated optic nerve fiber number in six species of minnows (mean ± 1 standard deviation)

Species	N	Length (mm)	Weight (g)	Fiber number
Turbid water species:				
C. lutrensis	5	69.60 ± 4.72	3.90 ± 1.15	60,800 ± 8,115
N. bairdi	5	52.00 ± 2.65	1.50 ± 0.27	45,000 ± 3,907
N. atherinoides	5	56.80 ± 7.98	1.39 ± 0.75	53,500 ± 14,989
Clear water species:				
C. venusta	5	70.20 ± 5.89	3.07 ± 0.98	106,600 ± 17,689
N. amabilis	5	55.60 ± 2.97	1.53 ± 0.15	87,000 ± 4,947
N. boops	5	60.40 ± 4.72	1.70 ± 0.21	90,200 ± 16,419

sen that contained fibers originating in central, intermediate and peripheral areas of the ventral, lateral and dorsal retinal sector.

Electron micrographs of these nine sample areas in each of five individuals of the six species studied (a total of 270 micrographs) were taken at magnifications of 4,000 and 4.500 × and printed at a final magnification of 11,200 ×. All subsequent measurements were performed without knowledge of the respective species or retinal area. A coordinate grid was randomly placed onto these micrographs and, in the 20–100 fibers closest to the origin of this grid, the maximum internal diameter (MD), the internal diameter at a right angle to MD (RD), and the thickness of the myelin sheath (MY), were measured to the nearest 0.01 μm (total N of 10,900 fibers). Mean fiber diameter was calculated as the average of MD and RD. The fiber density in each micrograph was quantified by counting the number of axons within 100 μm^2.

Nested multivariate analysis of variance (MANOVA) with MD, RD, and MY as dependent variables was used to evaluate differences in anatomical fiber characteristics between individuals, species and turbidity groups. All variables were sine-transformed to achieve homogeneity of variances.

The number of fibers per μm^2 in each individual was averaged across the nine sample areas. The total number of fibers for each individual was estimated as the product of the average number of fibers per μm^2 and the area of the cross-section. Analysis of variance (ANOVA) was performed to test for differences in the number of optic nerve fibers. For this analysis, species were nested within turbidity groups.

To test for a possible relationship between optic nerve fiber number and retinal area (RA), the latter was calculated from the measured eye diameter (d) using the formula $RA = 2(d/2)^2\pi$. To evaluate if species and turbidity groups differ in the number of optic nerve fibers when retinal area is taken into account, an ANOVA was performed on the residuals of this regression analysis.

Results

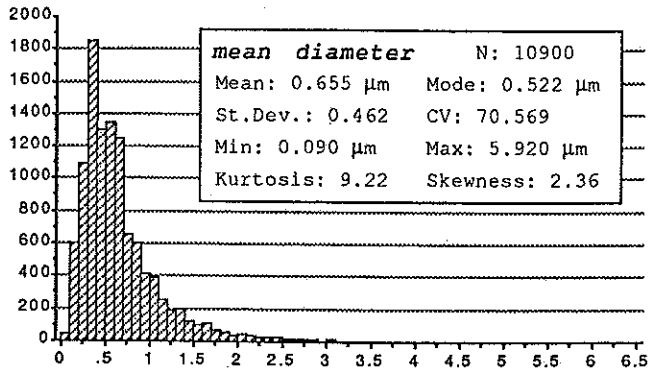
Weights and standard lengths of the specimens studied are listed in table 1. In *Notropis* and *Cyprinella,* mean internal fiber diameter ranged from 0.09 to 5.92 μm, and myelin thickness ranged from 0.01 to 0.80 μm. Histograms and descriptive statistics for the distribution of fibers in each species and all species combined are included in figure 2. Fiber counts for the six species are listed in table 1.

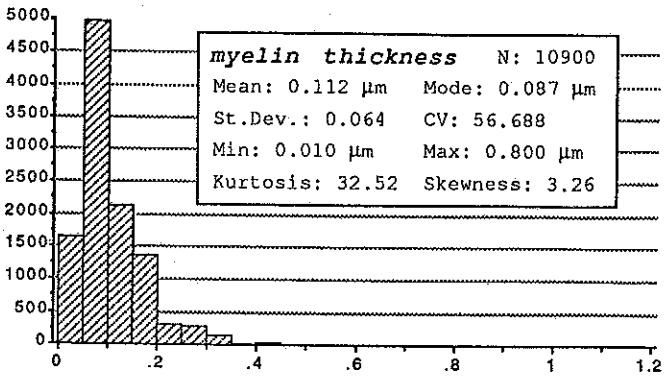
No significant differences in fiber diameter or myelin thickness were detected within or among turbidity groups, but significant differences were found among individuals within species ($p<0.001$). All applied tests, ie. a univariate test for each variable, as well as Pillais', Hotellings', and Wilks' criteria of the multivariate analysis, yielded similar F-values and identical levels of significance (table 2).

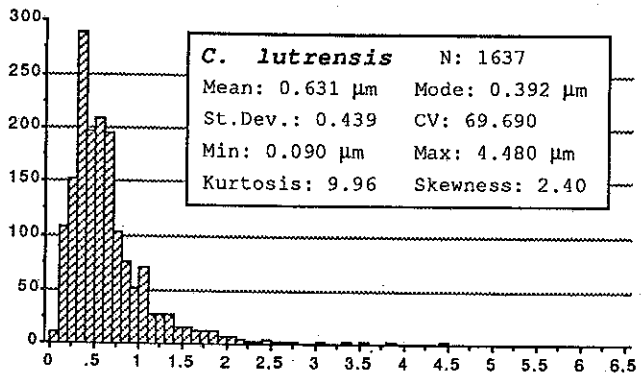
The optic nerve of clear water species contained significantly more fibers than that of turbid water species ($F=29.78$; $DF=1,24$; $0.01>p>0.001$). On the average, clear water species were characterized by almost twice as many optic nerve fibers (mean 94,583 fibers) as were turbid water species (mean 53,134 fibers) (fig. 3). However, neither within the clear not the turbid water group did the three species differ significantly from each other in the number of optic nerve fibers ($F=0.57$; $DF=4,24$; $p>0.05$).

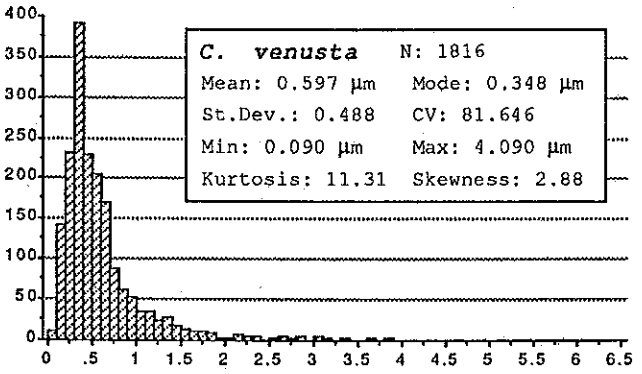
The number of optic nerve fibers was closely related to retinal area, as shown by regressing fiber number onto retinal surface ($F=25.001$; $DF=1,28$; $p<0.001$; cf. fig. 4). To evaluate whether species or turbidity groups displayed differences in the number of optic nerve fibers that remained once retinal area was taken into account, an ANOVA was performed on the residuals from this regression analysis. Differences were not detected between turbidity groups ($F=0.77$; $DF=1,24$; $p>0.05$), nor among the three clear or turbid water species ($F=1.80$; $DF=4,24$; $p>0.05$). This indicates that the relative number of optic nerve fibers per retinal area is similar in all species and independent of habitat turbidity.

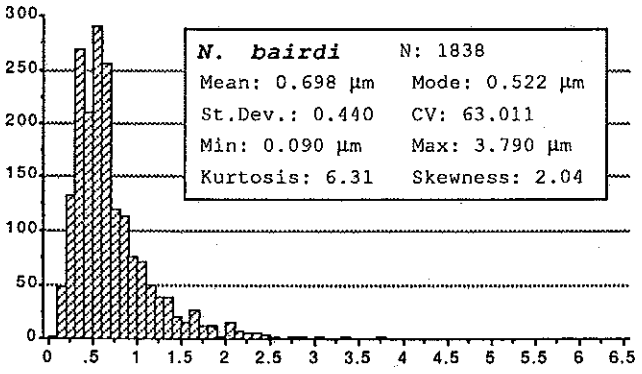
Fig. 2. Frequency distributions of mean internal diameter and thickness of the myelin sheath of optic nerve fibers for all species combined, and of mean internal diameter for three turbid water species *(C. lutrensis, N. bairdi, N. atherinoides)* and three clear water species *(C. venusta, N. amabilis, N. boops).*

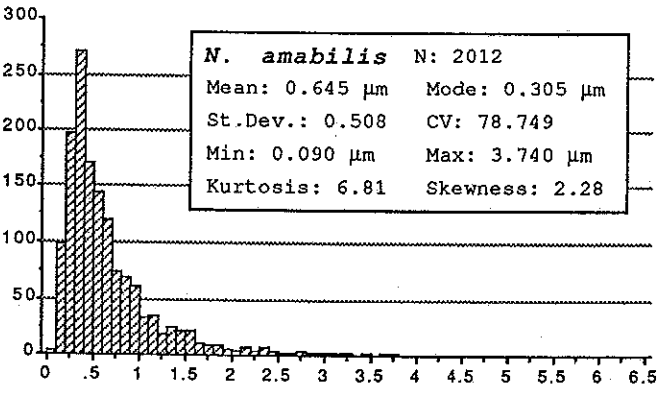

mean diameter N: 10900
Mean: 0.655 µm Mode: 0.522 µm
St.Dev.: 0.462 CV: 70.569
Min: 0.090 µm Max: 5.920 µm
Kurtosis: 9.22 Skewness: 2.36

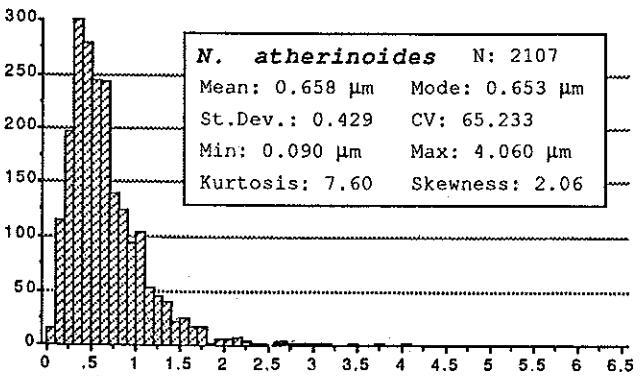

myelin thickness N: 10900
Mean: 0.112 µm Mode: 0.087 µm
St.Dev.: 0.064 CV: 56.688
Min: 0.010 µm Max: 0.800 µm
Kurtosis: 32.52 Skewness: 3.26

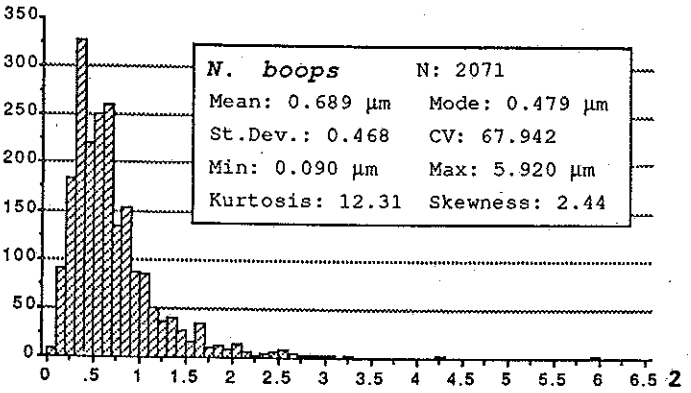

C. lutrensis N: 1637
Mean: 0.631 µm Mode: 0.392 µm
St.Dev.: 0.439 CV: 69.690
Min: 0.090 µm Max: 4.480 µm
Kurtosis: 9.96 Skewness: 2.40


C. venusta N: 1816
Mean: 0.597 µm Mode: 0.348 µm
St.Dev.: 0.488 CV: 81.646
Min: 0.090 µm Max: 4.090 µm
Kurtosis: 11.31 Skewness: 2.88


N. bairdi N: 1838
Mean: 0.698 µm Mode: 0.522 µm
St.Dev.: 0.440 CV: 63.011
Min: 0.090 µm Max: 3.790 µm
Kurtosis: 6.31 Skewness: 2.04


N. amabilis N: 2012
Mean: 0.645 µm Mode: 0.305 µm
St.Dev.: 0.508 CV: 78.749
Min: 0.090 µm Max: 3.740 µm
Kurtosis: 6.81 Skewness: 2.28


N. atherinoides N: 2107
Mean: 0.658 µm Mode: 0.653 µm
St.Dev.: 0.429 CV: 65.233
Min: 0.090 µm Max: 4.060 µm
Kurtosis: 7.60 Skewness: 2.06


N. boops N: 2071
Mean: 0.689 µm Mode: 0.479 µm
St.Dev.: 0.468 CV: 67.942
Min: 0.090 µm Max: 5.920 µm
Kurtosis: 12.31 Skewness: 2.44

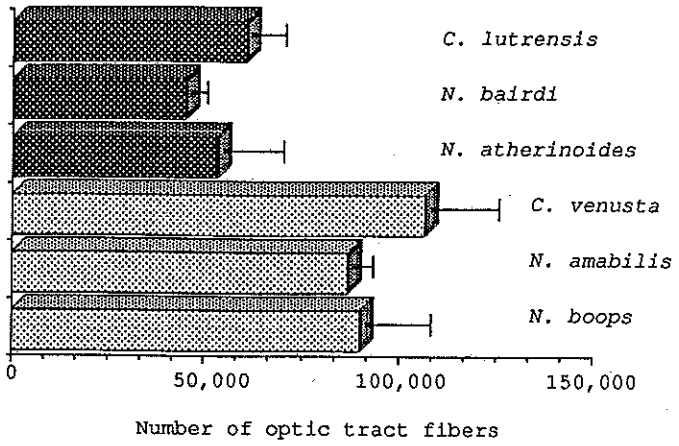


Fig. 3. Bar charts (mean ± 1 standard error) illustrating the number of optic nerve fibers in six species of minnows in *Notropis* and *Cyprinella*. Dark bars identify turbid-water species, whereas light bars indentify clear-water species.

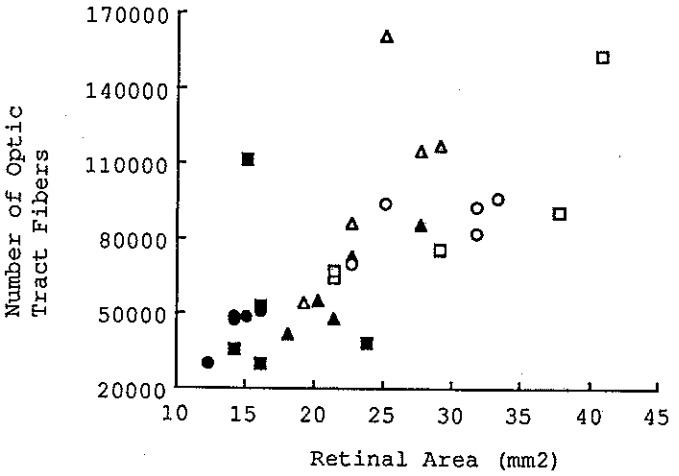


Fig. 4. Relationship between retinal area (in mm^2) and number of optic nerve fibers (N = 30) in six species of minnows in *Notropis* and *Cyprinella*. A regression line was fitted to the data ($y = 2.434 + 31.209x$; $R^2 = 0.472$). ▲, *C. lutrensis*; ●, *N. bairdi*; ■, *N. atherinoides*; △, *C. venusta*; ○, *N. amabilis*; □, *N. boops*.

Table 2. Nested MANOVA and ANOVA results for a comparison of the maximal internal diameter (MD), the internal diameter at right angles to this measurement (RD), and the thickness of the myelin sheath (MY) between turbidity groups, among three species within each turbidity group, and among five individuals in each species of *Notropis* and *Cyprinella*. In each individual, 300 to 450 fibers were measured (Total N = 10,900).

Multivariate test:

Source	df	F	P
Turbidity	3	6.833	ns
Species within turbidity	12	0.435	ns
Individuals within species	72	3.359	***

Univariate tests:

Source	df	F	P
MD			
Turbidity	1	0.034	ns
Species within turbidity	4	0.531	ns
Individuals within species	24	1.992	***
RD			
Turbidity	1	1.197	ns
Species within turbidity	4	0.659	ns
Individuals within species	24	2.752	***
MY			
Turbidity	1	1.778	ns
Species within turbidity	4	0.426	ns
Individuals within species	24	5.277	***

ns = Non-significant; *** = $p < 0.001$.

Discussion

The minnows that inhabited predominantly clear-water had larger eyes and almost twice as many optic nerve fibers compared to those of turbid habitats. Evidence was not found for species-specific differences in fiber characteristics of the optic nerve, nor for differences in the number of axons per retinal area. It is therefore hypothesized that differences in visual performance of minnows are exclusively a result of quantitative differences in the number of visual elements available for sampling and processing in the retina, the optic nerve, and the optic tectum. Predictions concerning absolute visual threshold and resolving power can be formulated according to this hypothesis and may be tested in future psychophysical experiments. More specifically, absolute visual threshold, the lowest stimulus intensity that can be detected, is a function of the number of rods per retinal area and should be independent of eye size. Moreover, resolving power depends on the number of retinal receptors per visual angle and thus should be a function of eye size.

Estimates of fiber number ranging from 45,000 in *N. bairdi* to 106,600 in *C. venusta* are not unusual in teleosts [Bruesch and Arey, 1942; Gaze, 1970; Tapp, 1974; Scholes, 1979]. However, internal diameters of optic nerve fibers in *Notropis* and *Cyprinella* are generally smaller than those reported for other species [Tapp, 1974; Witkovsky, 1971].

Although species did not differ in their anatomical fiber characteristics, significant differences among individuals within a species were detected. It is unlikely that this result is an artifact of sampling from a non-random distribution of fiber diameters, because a large number of fibers were quantified per individual (between 300–700 fibers). The distribution of fiber diameters of the optic nerve of cyprinids changes throughout life [Easter et al., 1981; K. Kotrschal, pers. commun.] and, although only adult specimens were used, the detected morphological differences could correspond to age differences.

The present study does not allow us to evaluate whether the detected differences are the result of functional adaptations to the respective habitats through evolution or of environmental plasticity during development. This question is the focus of a future publication.

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