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Cataract occurrence in Micropogonia furnieri (Desmarest, 1822) in the area between Capes Frio and Torres (23° S and 29° S), Brazil : investigation of causes and electrophoretic studies of total proteins of the eye lenses

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CATARACT OCCURRENCE IN *MICROPOGONIAS FURNIERI* (DESMAREST, 1822), IN THE AREA BETWEEN CAPES FRIO AND TORRES (23°S AND 29°S), BRAZIL: INVESTIGATION OF CAUSES AND ELECTROPHORETIC STUDIES OF TOTAL PROTEINS OF EYE LENSES*.

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Synopsis (In English exactly as presented by authors Vazzoler and Phan in original paper).

"The presence of specimens of *Micropogonias furnieri* suffering from cataracts was observed in the area occupied by the population I (Vazzoler, 1971). The frequency and distribution of these specimens were analysed and the electrophoretic patterns of soluble eye-lens proteins were determined. This cataract is not of parasitic origin and was rare in specimens from the coastal area. In the lagunar region it occurs mainly in specimens of the River Baguacu from April to November. Only young fish (200 to 250 mm)

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in immature or in maturation stages were affected. Two main pathways of evolution in the process seems to occur with a "radial" and a "diffuse" type. The analysis of electrophoretic patterns showed 5 steps of evolution with remarkable alterations on the concentration of total soluble proteins as well as on the relative concentration of 8 proteic fractions grouped in 4 aggregates in electropherograms. There is an increase in the relative concentration of the aggregate I (more cathodic) and II, and a decrease of the III and IV (anodic) with the disappearance of the last two fractions of the latter in the final stages of the process. No alterations were determined in the relative concentrations of these aggregates when comparing eye-lens of normal specimens with normal eye-lens of affected fish suggesting that the occurrence of cataract in one eye does not cause any alteration in the normal eye. The results suggest that cataract may be the cause of natural mortality in the area, and its incidence may be due to a physiological or metabolic factor induced by environmental conditions."

Introduction

We have been attempting to establish the degree of reproductive isolation between two contiguous populations of *Micropogonias furnieri* using genetic-biochemical methods. Population I occupies the area (of the Brazilian coast, *nobis*)[1] from Cape Frio to Cape Torres (23°S to $29^{\circ}21'$S) and Population II occupies the area between Cape Torres and Cape Chui ($29^{\circ}21'$S)

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1 Special Notes for the English version: Translation Editor Hargis' notations or insertions are in parentheses and indicated by *nobis*, meaning *by us*. These are intended to clarify the text. We hope that they do.

to $33^{o}S$). Both populations were described by Vazoller in 1971. While carrying out these studies (making the collections for these studies, *nobis*) we observed individuals with various degrees of eye-lens opacity (cataracts).

The specialized literature refers only to the occurrence of cataracts in fresh water fishes. Its incidence is greater in cultivated fish (*i.e.* aquaculture and captive fish) than natural populations. References have been made to traumatic cataract of unknown cause ("grey cataract"), characterized by opacification of the lens. Usually only one eye is involved (Van Duijn, 1956). Another, more common, type is the parasitic or helminthic cataract, "diplostomatosis" or "worm blindness", caused by infestation of the eyeball by endoparasitic digenetic trematode larvae with metagenetic development producing changes in the host (Van Duijn, *op. cit.*; Uspenskaya, 1961; Petrushevski and Shulman, 1961; Reichenbach-Klinke and Elkan, 1965; Amlacher, 1970). This type of cataract...(End text, p. 65: Beginning text, p 66)[2]...causes serious problems, reaching epizootic proportions in some feral populations (Petrushevski and Shulman *op. cit.*). It is easily identified by the presence of small white spots (larvae) against the dark background of the retina: Opacification is not homogeneous.

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2 Parentheses-enclosed notes such as this are intended to allow readers, wishing to do so for purposes of comparison of our translation to the original, to locate appropriate passages in the original text. Bracket-enclosed notes are intended to allow readers to locate tables and figures in the original for the same purposes.

Progressive loss of vision causes a reduction in the ability to locate ("visualize" or see, *nobis*) food, causing a reduction in the nutritional state of infected fish (Uspenskaya, *op.cit.*, Sallman *et al.*, 1966; Sato *et al.*, 1975). Sallman *et al.* (*op. cit.*) conducted studies on cataract induction in *Salmo gairdnieri* fed a prolonged diet of thioacetamide. The most striking feature in the histology of affected lenses was a massive proliferation of the epithelium and its transformation in a "mass" of pleomorphic cells, which sometimes replaced a great portion of the anterior cortex of the lens. Other eye structures did not show abnormalities. Matsusato and Kanazawa (1975) studied the histopathological alterations of the eye of *Oncorhynchus rhodurus f. macrostomus* (Gunter), noting various types of proliferations of the epithelial cells of the lens as well as symptoms (or signs) of edema and liquefaction of the fibrous layers of the lens. In his review of the subject Dukes (1975) refers to cataracts as the second most frequent opthalmological condition described in fishes. However, most reports are incomplete, lacking detail.

Once cataracts were seen in feral populations of... (End text, p. 66)[(Fig. 1 here in original, p. 66)] (Beginning text, p. 67)... *Micropogonias furnieri* we judged it important to determine the distribution of the disease, its incidence and possible causes since we found no references to cataracts in marine fishes. Also, it (cataract disease, *nobis*) may be one factor contributing to an increase in natural mortality. The area in which we observed the greatest incidence of the disease (cataracts) is considered a nursery ground for the species and is located close to the spawning grounds (Vazoller, 1971).

Materials and Methods

Samples were collected at: a) open water region during 4 oceanographic cruises conducted on the R/V "Prof. W. Besnard" in February, May, September and November of 1975, as part of the FAUNEC project (Program, nobis) (Fig. 1); b) the Estuarine-lagoon system of Cananeia (25°01'S) at eight stations and in front of the Island of Bom Abrigo during the period from March 1975 to February 1976 (Fig. 2). Both lenses were taken from each of 744 live individuals with total lengths from 98 to 710 mm. Two-hundred and seventy-five (275) were collected at 36 offshore stations and 469 in the Cananeia - Bom Abrigo region (Tables I and II). To obtain the eye lenses we made a longitudinal incision in the cornea of the living specimen, using forceps to extract them (Smith and Goldstein, 1967; Bon *et al.*, 1968; Eckroat and Wright, 1969). They were then placed on a filter paper and all of the residual (extraneous, nobis) material adhering to their membranes (surfaces, nobis) were removed with forceps. Data taken from each specimen included total length (TL in mm), total weight (TW in g)... (End text, p. 67) [Figure 2 here in the original text, p. 67] (Beginning text, p. 68)...sex, stage of sexual maturity and condition of the left and right left eye lenses. Condition of the lenses was recorded as follows: (N)=Normal, (P)=Partial cataract and (T)=Total cataract. The Normal condition (N) comprised eye lenses which were perfectly transparent[3]; Partial (P) refers

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3 Here we translate the word translucid, employed in the text, not to translucent but to transparent. Translucent implies a certain diffusion or scattering of light rays during their passage through the lens. This is a characteristic of cataractous lenses and not of normal ones, which are transparent, allowing light to pass through without diffusion.

to those with one opaque spot to only one transparent spot; those classified as Total (T) are homogeneously (and totally, nobis) opaque.

Of the 744 individuals examined, 53 had cataracts and from a total of 1488 lenses, 77 were cataractous, Tables I and II. [Tables I and II approximately here, p. 68] Of these cataractous lenses, 13 were examined, dissected or whole, under the Wild M-5 stereomicroscope for presence or absence of parasitic worms. Six were fixed and processed (histological sections) for microscopic examination. (Approximate end text, p. 68: Approximate beginning text, p. 69). With the same objective; 10 cataractous lenses were collected under aseptic conditions and used for bacterial culture in specific (special, nobis) media. The remaining 48 pairs with various degrees of opacity were placed in pro-vials of inert (or neutral, nobis) plastic, separated by a plastic funnel (left before right) and immediately frozen in liquid nitrogen in storage containers. At the laboratory the specimens were transferred to a freezer and held at $-15^{o}C$. They were gradually analyzed over the next 45 days following collection. Electrophoretic analyses for total soluble protein were conducted on the remaining 48 lenses and on 20 normal lenses from affected individuals (i.e. with one lens normal and the other cataractous). Lenses were weighed on a Mettler H-15 analytical balance with 1.0×10^{-4} g (0.000 lg) precision. Extracts were obtained using a solution of 0.9% NaCl at a 4:1 (volume:weight) proportion.

Electrophoretic analyses were conducted in cellulose acetate "Cellogel" in a discontinuous buffering system using barbital at pH 8.6 in the cathode and tris-glycine at pH 9.5 in the anode, with a continuous voltage of 300V for 25 minutes. Total protein concentration in the extracts (g/dl) was determined using an ATAGO refractometer. The patterns thus obtained were stained with Ponceau-S and, after membrane diafanization (after clearing the membrane, nobis), were quantitatively analyzed by ATAGO "Quick" densitometer.

Results

We found that cataract-bearing individuals (in collections made in ocean waters, nobis) inside of the 100 m isobath in offshore areas between Capes Frio and Torres are rare. Only one diseased specimen (0.36%) occurred (in the ocean, nobis) near Bom Abrigo Island. In contrast, 52 diseased specimens (22.27%), 51 in the Baguacu River and one (1) on the Trincheira River[4] occurred in the estuarine-lagoon region of Cananeia, (Table II; Fig. 2). Considering those with affected lenses, cataract occurrence was almost restricted to the Baguacu River where a frequency of 98.07% was found within the interior region (of the Estuarine-lagoon system of Cananeia, nobis) and 96.23% as compared to the total or entire study area. (Approximate end text, p. 69) [Figure 3 here bottom of p. 69] (Approximate beginning of text p. 70). At the Baguacu River site individuals with cataracts occurred from April to November with a peak in July (47.40%). Considering both eyes (left and right), cataracts at various stages of development occur in all combinations in the individuals within the collection (Tab. II; Fig. 3a).

Frequency of Normal (undiseased or non-cataractous lenses, nobis), Partial cataracts and Total cataracts are shown in Figure 3B. Analysis of

[4] In Fig. 2, this specimen is actually shown as having been captured in the River Tagua which empties into the Bay of Trapande in the upper left-hand corner of the map. The letter at end of the name is slightly obscured in the Figure. We believe it to be an a.

total lengths of the specimens showed that, within the various size classes ranging from 50-100 mm to 700-750 mm, cataracts occur only in individuals up to the 250-300 mm size class, with a predominance in those of the 200-250 mm size class (Fig. 4). [Figure 4, approximately here in text, p. 70]. Detailed analysis of the frequency of cataractous fishes greater than or equal to 200 mm TL shows that the greatest incidence occurs in individuals of the 220-230 mm size class (Fig. 3C). Considering sex and stage of maturity, cataracts occur in both sexes, predominating in females (Fig. 3D) and individuals up to the B stage or maturing gonad stage, with a greater frequency in immature (A) individuals (Fig. 3E). Comparison of condition factor (k) values (Vazzoler and Vazzoler, 1965; Vazzoler and Rossi-Wongtschowski, 1976) did not indicate a loss in weight by diseased individuals; with k = 9.242 for normal individuals and k=9.291 for infected ones.

We observed various stages in the development (evolution) of cataracts which appear to follow two pathways of development. The first (the radial cataract) begins as an opaque nucleus from which bands spread throughout the lens as the cataract develops. In the second (the diffuse cataract) the entire lens becomes slightly opaque in the beginning, with the opacity becoming more severe as the cataract progresses (Fig. 5).

Analysis of dissected, whole cataractous lenses at various stages of development under the stereomicroscope and by histological preparations disclosed no evidence of infection by helminths. (Bacterial culture efforts and examination by stereomicroscopic and histological preparations, nobis) also showed that the cataracts were not caused by bacteria.

Qualitative analysis of the electrophoretic patterns of normal lenses of infected individuals and patterns from lenses in various stages of disease development suggested that alterations occur in the intensity of coloration of the various protein fractions as the cataracts evolve (Fig. 6.) and these reflect variations in the relative concentration of each fraction.

The normal pattern of total soluble proteins from the lenses of M. furnieri, of Population I, shows variation with development and has four groups constituted of eight protein fractions (Phan et al., 1977).

Quantitative data of relative concentration (%) of each fraction obtained from densitometric curves, allowed us to characterize 5 phases of cataract evolution (development, nobis) electrophoretically. These we called the Partials -- (P)1, (P)2, (P)3, (P)4 and Total (T). Calculating the means... (End text, p. 70: Beginning text, p. 71)... of the concentration of total soluble proteins (g/dl) in extracts of normal and infected lenses in each of the five stages of evolution (development) we could observe a real decrease in relative concentration which varied from 6.60 g/dl to 1.15 g/dl (Fig. 7). [Figs. 5, 6 and 7 on this page -- p. 71]

We observed a considerable increase in the relative concentration of Groups I and II, whereas Group III showed a marked decrease in its relative concentration and Group IV disappeared in the final steps of the process (of cataract development, nobis) (Tab. III, Figs. 8 and 9). We verified this by analysing the behavior of each group and their constitutent fractions in parallel fashion during cataract evolution (Table III as follows):

a) Group I, consisting of only one fraction, presented a relative concentration of about 18.0% for normal and P-1 phase lenses, which increased markedly to 44.0% in Phase T (=Total cataract, nobis) with an inflection in the increment rate at Phase P-3 (Fig. 10).

b) For Group II, consisting of Fractions II-1 and II-2, we observed a gradual increase in its concentration from 24.0% to 30.7% up to Phase P-3, when an inflection occurred and it increased to 35.0% in Phases P-4 and T. It was observed that the concentration of Fraction II-2 remained almost stable with a slight decrease from 15.0% to 12.0%, whereas Fraction II-1 showed an increase from 9.0% to 23.5%, being responsible for the pattern of variation, of the groups (Fig. 11);... (End text p. 71: Beginning text p. 72)...

c) Group III, formed by Fractions III-1, III-2 and III-3, showed relative concentrations around 53.0% in normal lenses and in Phase P-1, decreased markedly to 18.0% in Phase T; Fraction III-1 showed slight oscillations in its relative concentration from 4.6% to 5.7%. Variation in Fraction III-2 was not significant up to Phase P-4 (14.8% to 11.0%), but it decreased to 5.5% when it (the cataract stage, nobis) reached Phase T. Fraction III-3 was the one which presented a marked variation, establishing the characteristics of the entire group with a concentration around 34.0% in Normal and P-1 Phase lenses, and 30.8% in Phase P-2, decreasing drastically to 6.7% in Phase T (Fig. 12);... (End text, p. 72). [Table III and Fig. 8 are on p. 72] [Page 73 has no text, consisting entirely of Figs. 9, 10, 11, 12.] (Beginning text, p. 74 (10))...

d) Group IV, consisting of fractions IV-1 and IV-2, showed a marked decrease and was the only group that disappeared completely in the final phases of the process of cataract development. It decreased from 4.2% in normal lenses to 0.36% in Phase P-3 and 0.0% in P-4 and T. Fraction IV-1 showed a slow and even decrease, with a concentration of 1.6% in normal lenses, 1.0% in Phase P-1, 0.4% in P-2 and P-3 and 0% in P-4 and T. Fraction IV-2 was more abrupt, with a relative concentration of 2.6% in normal lenses, dropping to 1.0% by Phase P-1 and 0.0% from P-2 to T (Fig. 13).

Discussion

These results show that, in M. furnieri, the cataracts we have seen are not caused by larval trematode infestation of the ocular globe (eyeball) since they were never observed in any of the preparations. Further, neither the lenses nor the eyeballs presented the characteristics reported for cases of parasitic cataract, i.e. tubular cavities caused by the larvae in the lens (Hughes and Hall, 1928), liquid accumulation causing extrusion of the eyeball (or exopthalmos -- the word in the text was "queratoglobo" -- cheratoglobe , nobis), or corneal rupture and extravasation (Van Duijn Jr., 1956; Amlacher, 1970). We observed that cataracts occur only in juvenile specimens below 275 mm in total length. This "corresponds" to the length at the beginning of the first sexual maturation of individuals of Population I in which the frequency of specimens with maturing gonads (B) is minimal since all individuals of this population, up to 200 mm TL, are immature (Vazzoler, 1971).

Studying the electrophoretic patterns of total proteins in lenses of normal individuals, Phan et al. (1977) observed that the fractions showed marked variability in juveniles, becoming stabilized and remaining so in the adult phases. Comparing the results concerning the relative concentration of the four (protein, nobis) groups for specimens of the 200-250 mm TL size class, considering lenses in individuals bearing both normal and cataractous lenses, we observed that there are no significant differences. The values found were respectively: (Protein, nobis) Group I, 20 and 18; Group II, 23 and 24; Group III, 53 and 53; and Group IV, 4 and 4. In this fashion, occurrence of cataract in one of the eyes does not cause any implications or alteration (of protein groups, nobis) in the other eye.

It is known that cataracts, interfering with vision, cause a decrease of the ability of an individual to locate food causing a reduction in its nutritional state (Uspenskaya, 1961) and its rate of growth in weight (Sallman et al., 1966). This causes significant "great" mortality in fresh water fishes (Petrushevski and Shulman, 1961). Our estimated K values, considering normal and cataractous individuals did not indicate a reduction in the nutritional state of the latter. However, we did not observe any cataractous adults, which suggests that diseased individuals do not reach the mature adult stage. Considering that the percentage occurrence of cataract-bearing individuals at different stages of cataract development, within the combinations shown in Table II, reflects their survival rate we have: 1) for individuals with one normal lens, a rate of 55%; 2) for those with both lenses affected with P-1 a rate of 30%; and, 3) for individuals with both lenses with total (T) cataracts, a rate of 15%. These results suggest that cataracts are a factor of natural mortality of M. furnieri as well. (End of text, p. 74) [Fig. 13 here, p. 74] (Beginning text, p. 75) In this case it seems to us that the development of cataracts may be connected with some physiological or metabolic factor, determined by environmental conditions in the Baguacu River.

Summary

Once the occurrence of cataract in M. furnieri was observed, the frequency and distribution of cataract-bearing specimens in the area

occupied by Population I of the species (23°S-29°S) were analyzed. This involved samples from offshore waters inside of the 100 m isobath, where cataracts were rare, as well as from the Estuarine-lagoon region (system) of Cananeia (25°01'S) where they occur at high frequencies in the Baguacu River from April to November, with a peak in July. Cataract disease affected only immature or maturing juveniles with total lengths between 200 and 250 mm, affecting either one or both of the eyes in various possible combinations (of severity, nobis). These cataracts were not of parasitic or bacterial origin: No trematode larvae were found.

Two pathways appear to exist for the process of cataract development, characterized by the "radial" and "diffuse" types.

Analysis of the electrophoretic patterns of total proteins of cataractous lenses allowed us to distinguish five phases of cataract development, from partial (P) in the beginning to total (T). Marked alterations occur during this development, either in the concentration of total soluble proteins in the extracts and in the relative concentration of the eight protein fractions grouped in four groups in normal lens patterns. We confirmed an increase in the relative concentration of Groups I and II (more cathodic) and a decrease in Groups III and IV (more anodic) with the disappearance of the two fractions of the latter groups in the final phases of the process (of evolution of the cataracts, nobis).

When comparing lenses of normal and cataractous individuals, alterations in the relative concentrations of the four Groups were not observed, which means that the occurrence of cataract in one of the eyes does not cause any alteration in the unaffected one. The results (relative to size classes affected or unaffected by cataracts, especially the larger size classes, nobis) suggest that cataract constitutes a cause of natural mortality for the species in the area and that cataract development may be connected to some physiological or metabolic factor induced by environmental conditions. (End text, p. 75)

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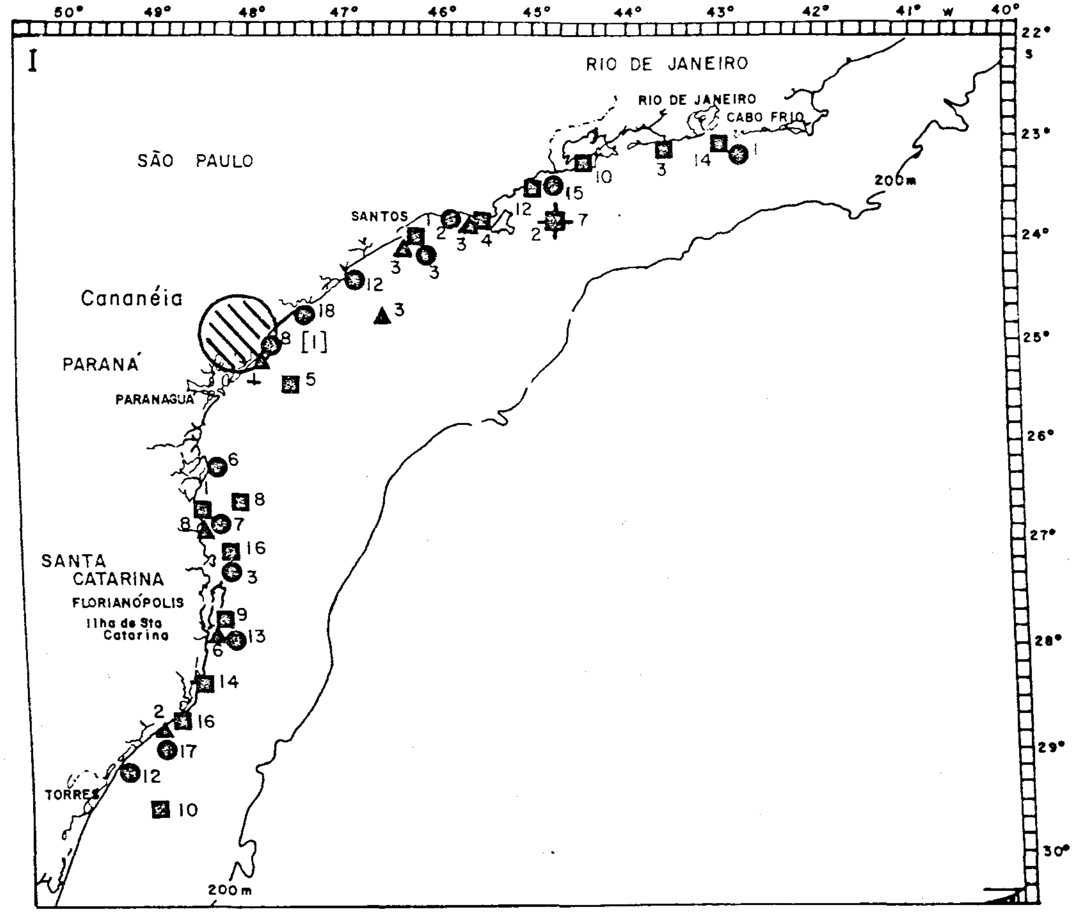
TRANSLATED

TABLES AND FIGURES

To reduce production costs the figures and tables are placed at the end of the translation, where they are presented in the same sequence as in the original text. Their location vis-a-vis the text in the original paper is indicated by appropriately-placed parenthetical notations in our translation.

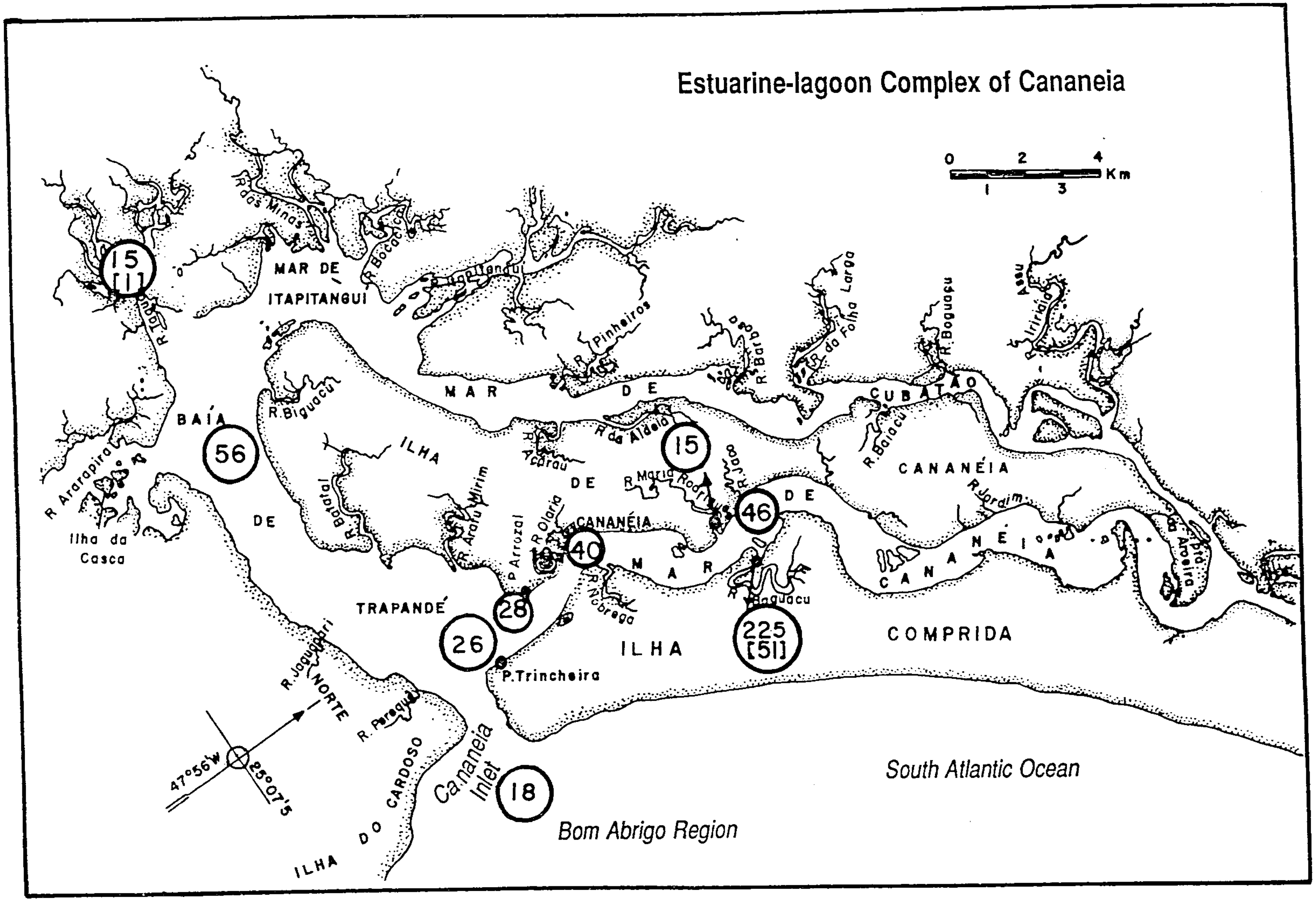


▲ Cruise I (Feb. '75; 7 Stations; 26 Specimens).
✚ Cruise II (May '75; 1 Station; 7 Specimens).
■ Cruise III (Sept. '75; 15 Stations; 125 Specimens).
● Cruise IV (Nov. '75; 13 Stations; 117 Specimens).

(*Numbers beside the station designations show the total number of specimens from which eye-lenses were collected.*)

[ ] Number of individuals (specimens) with cataractous eye-lenses [1].

Fig. 1. Map of the region occupied by Population I of *M. furnieri* indicating collection stations occupied during the four (4) oceanographic cruises of the "FAUNEC" program, conducted in 1975.



◯ Number of specimens (individuals) from which eye lenses were collected (469)

◯ [ ] Number of specimens with cataractous lenses [52].

Fig. 2. Map of the estuarine-lagoon of Cananeia, showing the locations of collections of *M. furnieri* during the period from March, 1975 to February, 1976.

Table 1. Summary of the relative occurrence of *M. furnieri* individuals with lens cataract from Population I during the period February 1975 to February 1976.

Locality Area Population I	No. of specimens collected	No. with lens cataracts (%)	Frequency of occurrence of lens cataracts	Stages of development of cataracts (left lens - right lens)
Cape Frio to Cape Torres 36 stations in the open sea (i.e. coastal waters)	275	1 (1.9)	0.36%	PP
Region of the estuarine lagoon of Cananeia (8 Locations)	244	1 (1.9)	0.41%	NP
Baguacu River (region of Cananeia)	225	51 (96.2)	22.67%	NP-NT-PP-TT-PN-TN PT-TP
Total Study Area (23°S - 29°S)	744	53 (100.0)	7.12%	All Combinations

Stages: N = normal; P = partial; T = total

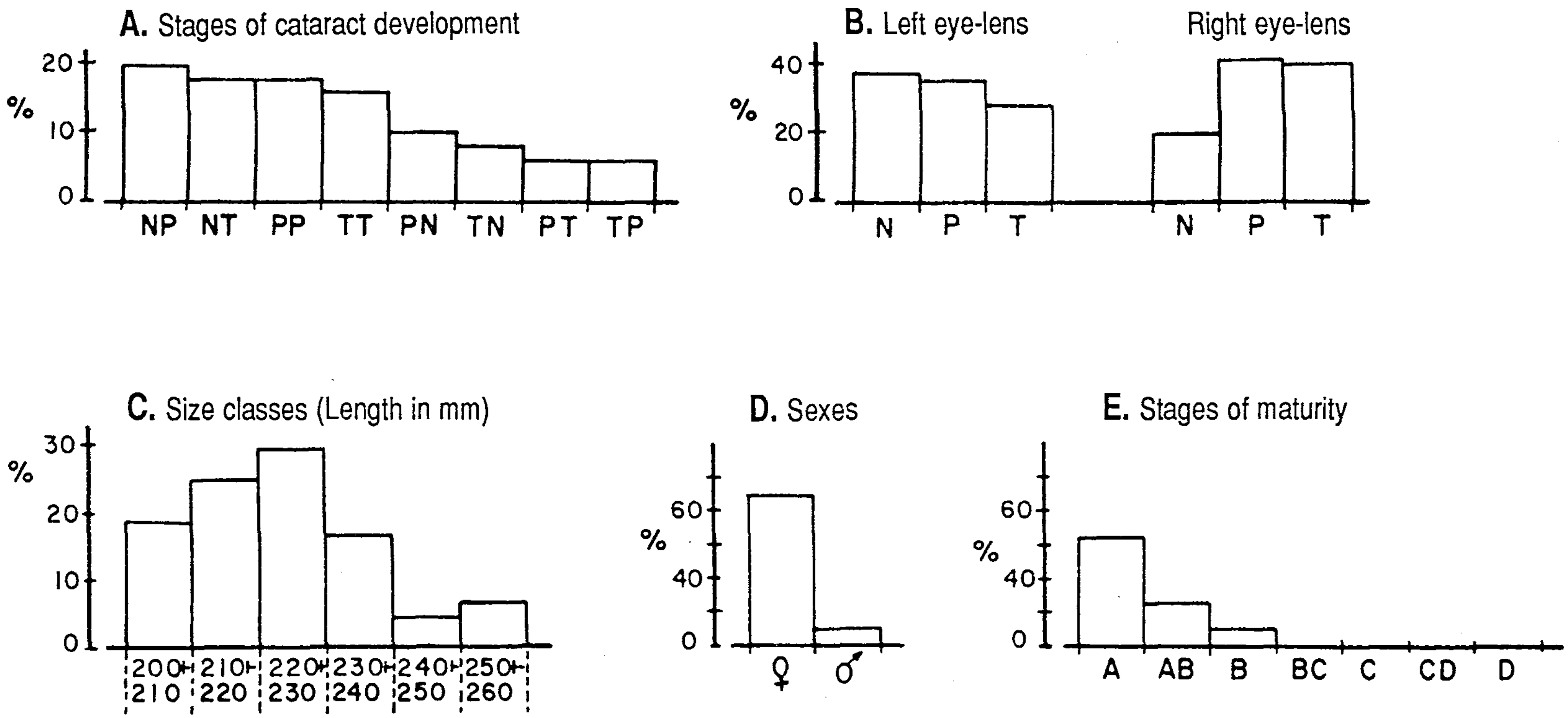


Fig. 3. Graphic representation of the results obtained from 51 specimens of *M. furnieri* with cataracts collected from the Baguacu River: (A)Frequency of left and right eye-lenses with different stages of cataract development (N=normal; P=partial; T=Total); (B)Frequency of normal eye-lens versus those bearing partial or total cataracts (by left and right eye); (C)Length-frequency distribution of disease-bearing individuals; (D)Frequency of females and males with cataract; (E)Frequency of cataractous individuals by stage of maturity (A=virgin or immature; B=maturing; C=mature or ripe; D=spent or spawned).

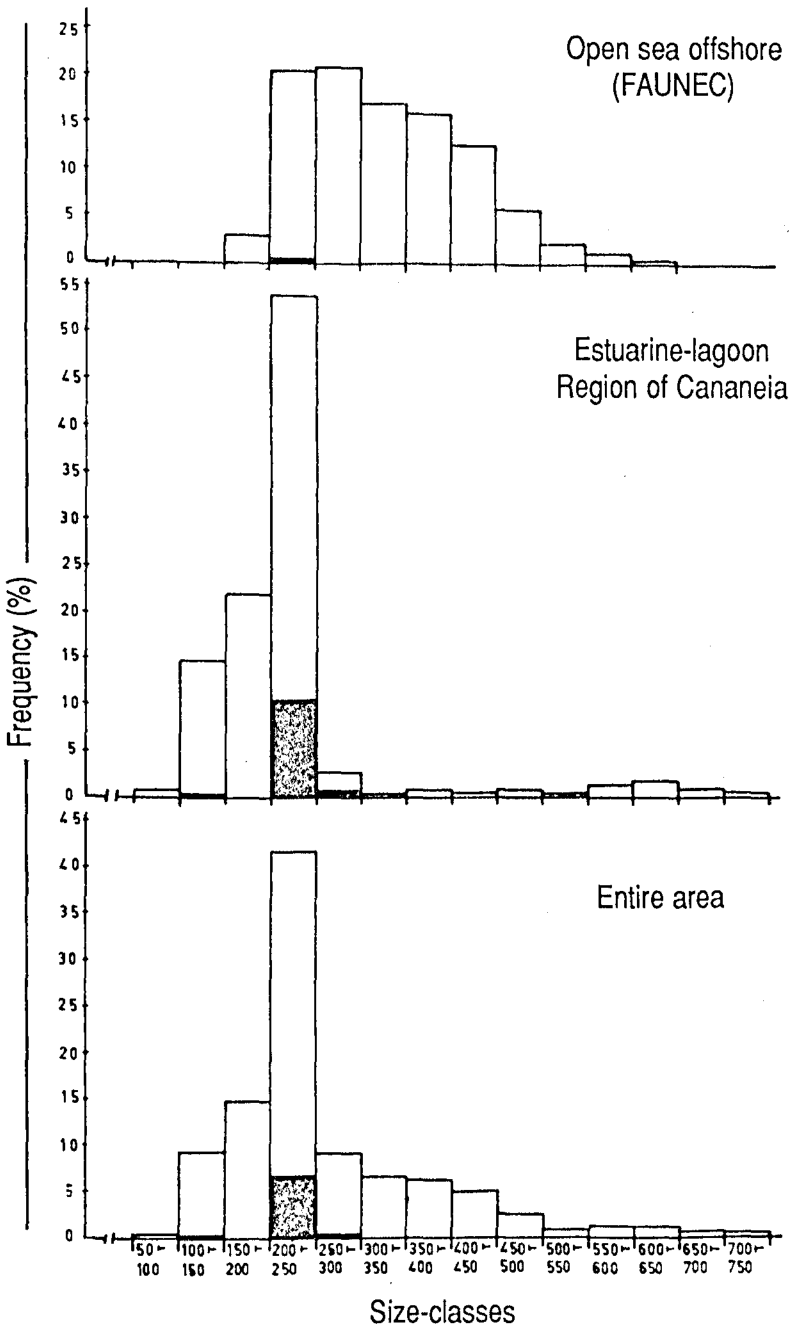


Fig. 4. Length-frequency distribution (TL in mm) of individual M. furnieri from which eye-lenses were collected (entire column or bar) and those bearing cataractous eye-lenses (black portion of bar) for the offshore region, estuarine-lagoon region and the entire area or region.

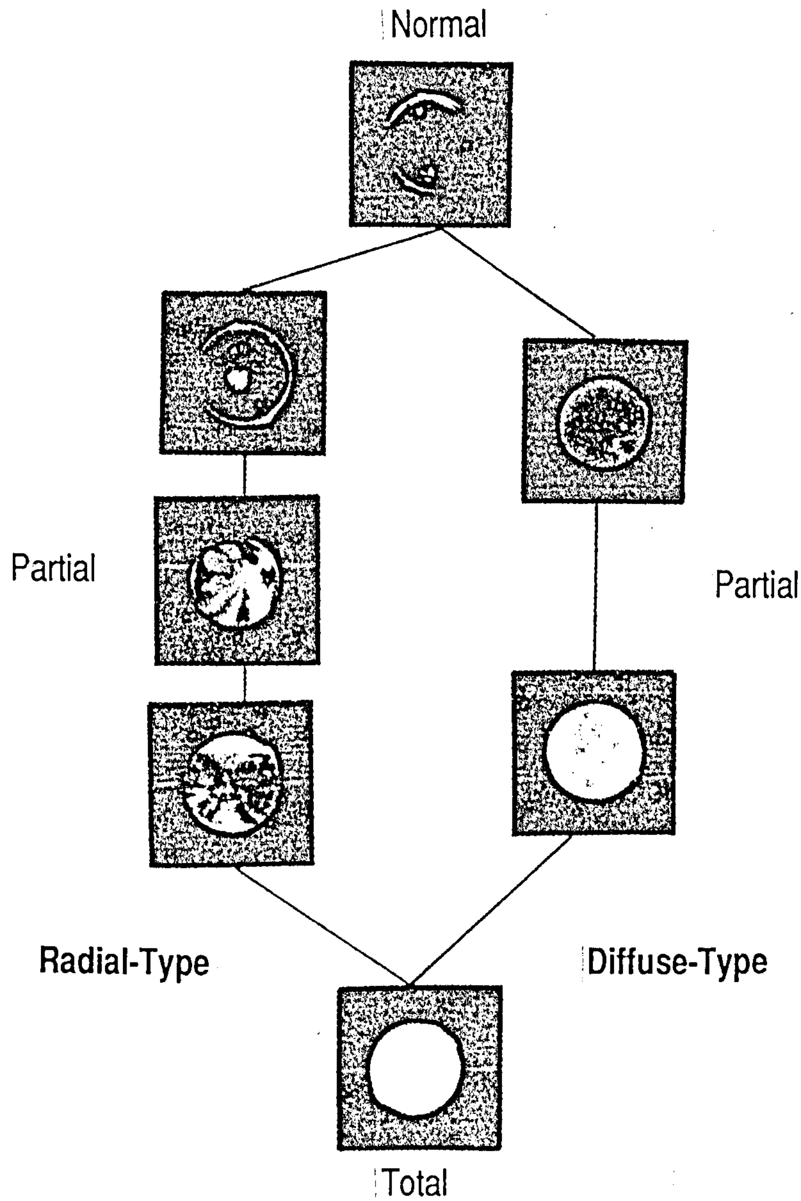


Fig. 5. Two possible pathways of cataract development or evolution in the eye-lenses of *M. furnieri*.

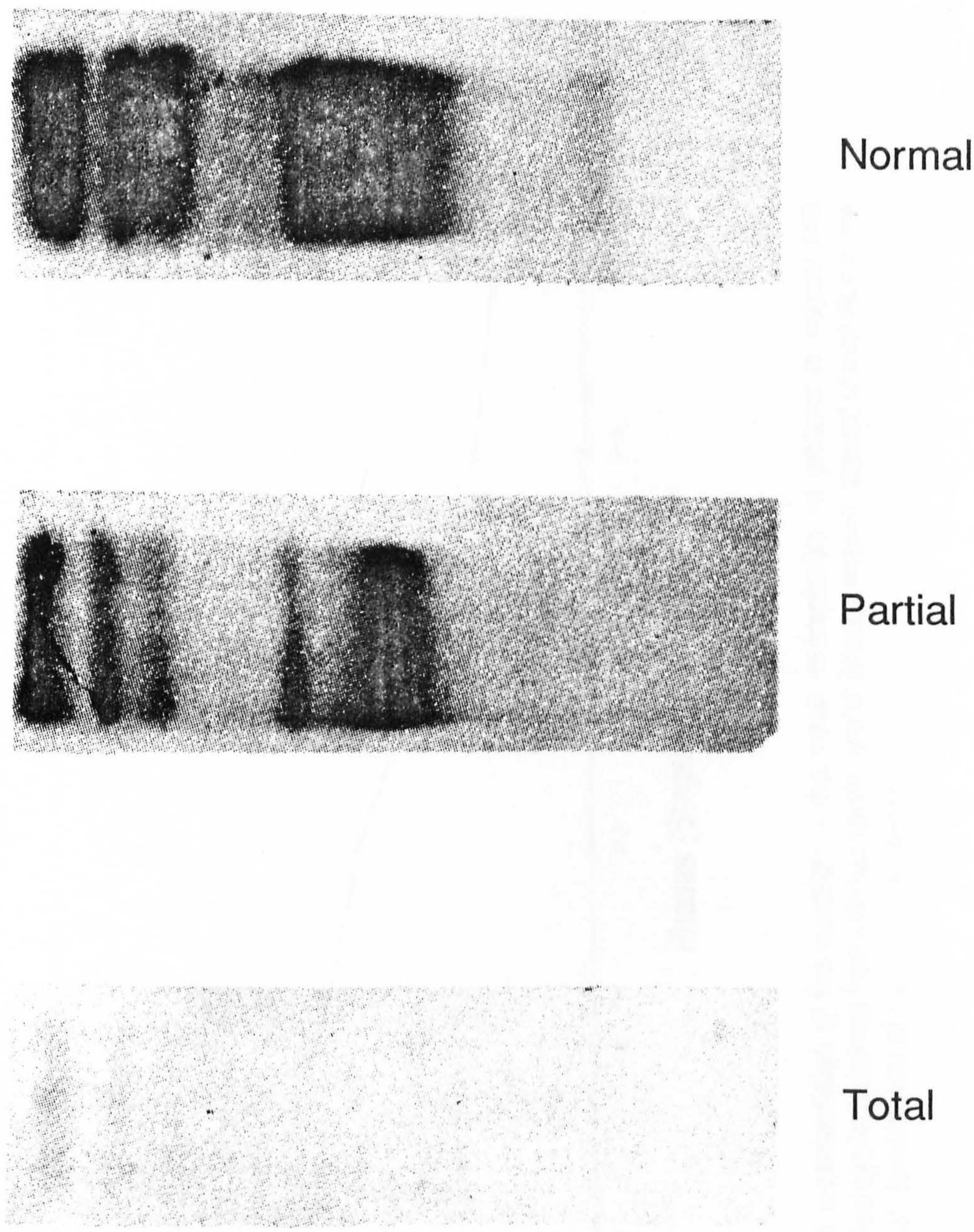


Fig. 6. Electrophoretic patterns of total proteins from normal and cataractous (partial and total) eye-lenses of *M. furnieri*.

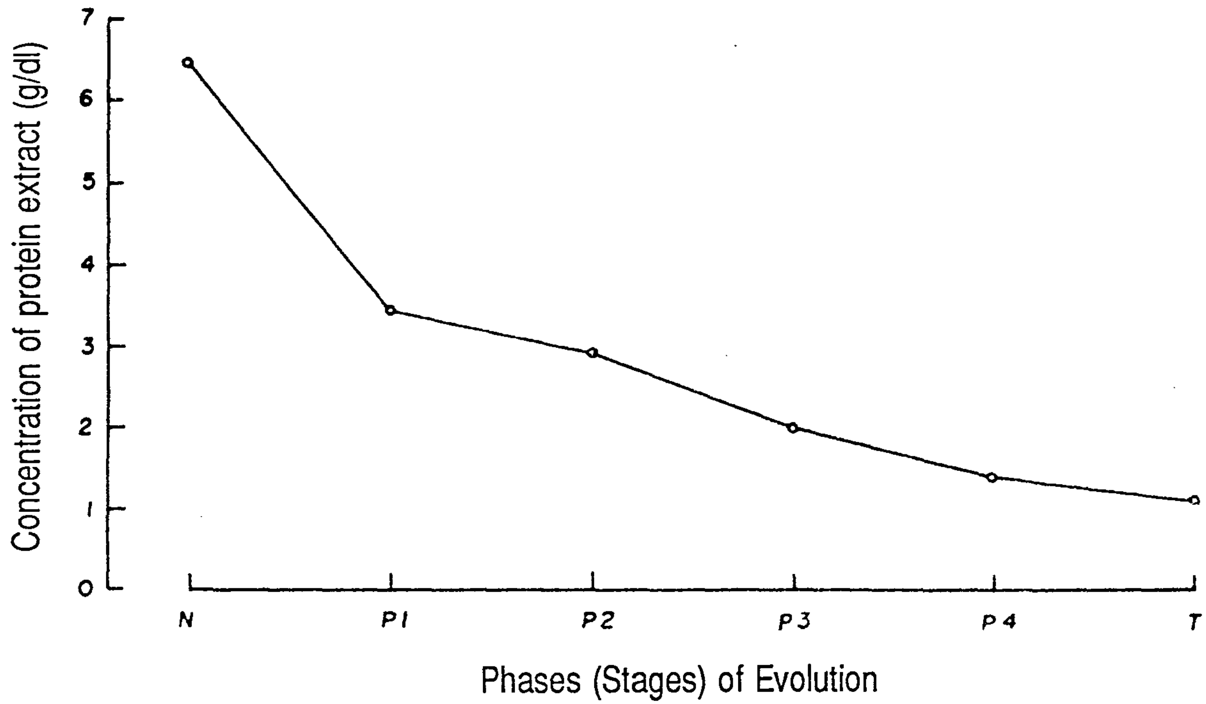


Fig. 7. Graphical representation of the variation in the mean concentration of proteins in normal and cataractous eye-lens extracts from infected specimens at different phases (stages) of evolution in the process of development of cataract in *M.furnieri.*

Table III. Results of the statistical analysis performed dealing with the relative concentration of each group and (versus) the protein fraction at different phases (stages) of cataract evolution in *M. furnieri.*

Phase	CPSE (g/dl)	Value	Group I	Group II	II-1	II-2	Group III	III-1	III-2	III-3	Group IV	IV- 1	IV-2
Normal (N = 20)	6,50	$\bar{x}$	18,27	23,98	9,16	14,82	53.50	4,62	14,86	34,02	4,22	1,64	2,55
		$s_{\bar{x}}$	0,2526	0,3520	0,3667	0,3787	0,4677	0,2829	0,7559	0,7139	0,1419	0,0858	0,1674
		$I_{\bar{x}}$	17,74-18,80	23,25-24,71	8,40-9,92	14,03-15,61	52,52-54,48	4,03-5,21	13,28-16,44	32,53-35,51	3,92-4,52	1,46-1,81	2,20-2,90
Intermediates: P - 1 (N = 17)	3,48	$\bar{x}$	18,82	25,44	11,44	14,00	51,90	5,87	14,35	33,45	2,04	1,06	0,98
		$s_{\bar{x}}$	0,4364	0,9489	0,5684	0,9476	1,6759	0,4428	1,0662	1,9068	0,5475	0,2822	0,3568
		$I_{\bar{x}}$	17,90-19,74	23,44-27,44	10,24-12,64	12,00-16,00	48,37-55,43	4,94-6,80	12,10-16,59	29,42-37,47	0,89-3,19	0,47-1,65	0,23-1,73
Intermediates: P - 2 (N = 15)	2,96	$\bar{x}$	26,50	28,18	15,11	13,07	44,78	4,42	9,75	30,86	0,38	0,38	0,00
		$s_{\bar{x}}$	0,3866	0,8115	0,4809	0,4770	0,9565	0,2575	0,6205	1,6446	0,1717	0,1717	-
		$I_{\bar{x}}$	25,68-27,32	26,46-29,90	14,09-16,13	12,06-14,08	42,75-46,81	3,88-4,96	8,43-11,07	27,36-34,36	0,02-0,74	0,02-0,74	-
Intermediates: P - 3 (N = 8)	2,02	$\bar{x}$	35,13	30,68	17,56	13,18	34,52	3,93	8,56	22,04	0,36	0,36	0,00
		$s_{\bar{x}}$	1,1256	1,4603	0,8990	0,7421	1,5771	0,3484	0,8715	2,0974	0,3625	0,3625	-
		$I_{\bar{x}}$	32,54-37,72	27,32-34,04	15,49-19,63	11,47-14,89	30,89-38,15	3,13-4,73	6,56-10,56	17,21-26,87	-0,47-1,19	-0,48-1,20	-
Intermediates: P - 4 (N = 4)	1,42	$\bar{x}$	38,49	35,27	20,19	15,08	26,21	2,63	11,11	12,46	0,00	0,00	0,00
		$s_{\bar{x}}$	1,4444	2,1440	0,5430	1,6694	0,9897	1,0168	0,3923	0,6336	-	-	-
		$I_{\bar{x}}$	34,48-42,50	29,32-41,22	18,68-21,70	10,45-19,71	23,46-28,96	-0,19-5,45	10,02-12,20	10,70-14,22	-	-	-
Total (N = 4)	1,15	$\bar{x}$	44,10	35,34	23,54	11,80	18,03	5,74	5,54	6,74	0,00	0,00	0,00
		$s_{\bar{x}}$	2,5005	1,6127	1,5061	2,0298	1,5904	1,3382	1,0391	1,5437	-	-	-
		$I_{\bar{x}}$	41,60-46,60	33,73-36,95	19,36-27,72	6,17-17,43	13.62-22,44	2,02-9,45	2.66-8,42	2,45-11,02	-	-	-

CPSE = Mean concentration of soluble proteins in extracts.
N = Number of eye-lenses analyzed.
x = Mean relative concentration of fraction or group.
s_x = Standard error
I_x = Confidence interval

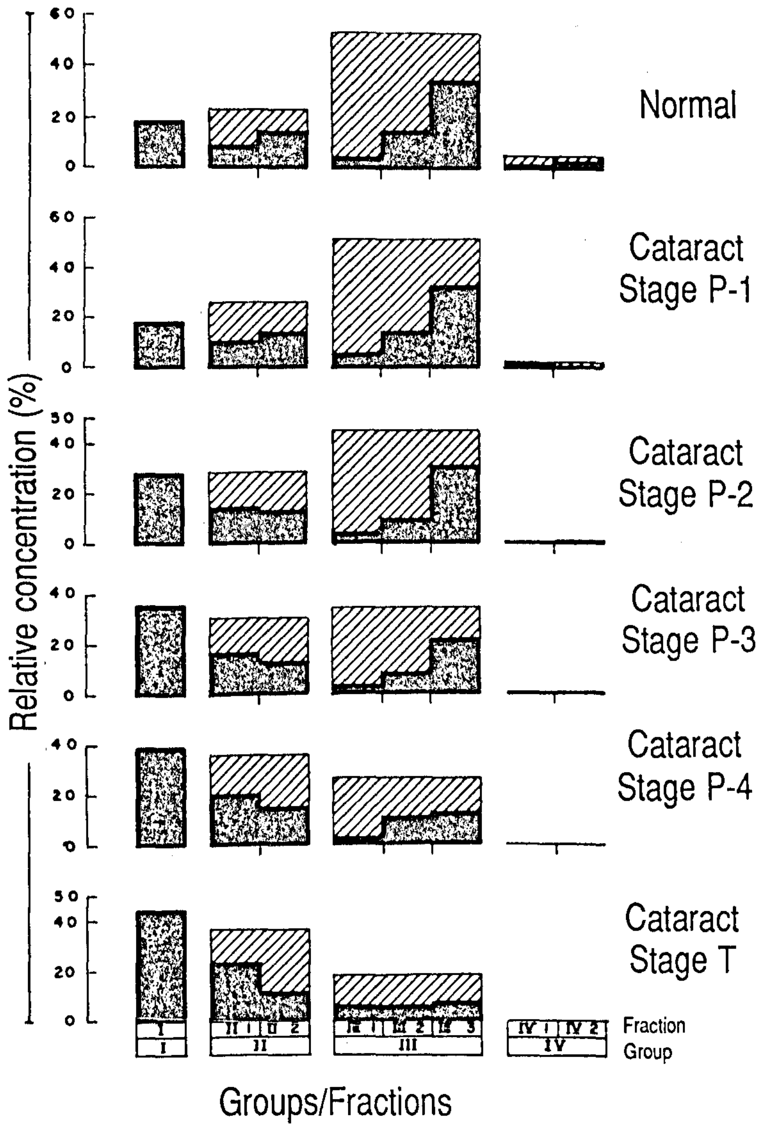


Fig. 8. Graphical representation of the variations in the relative concentrations of the separate (different) groups of protein fractions and for each isolated fraction, from the Normal (N) stage (phase) to the Total cataract stage (T).

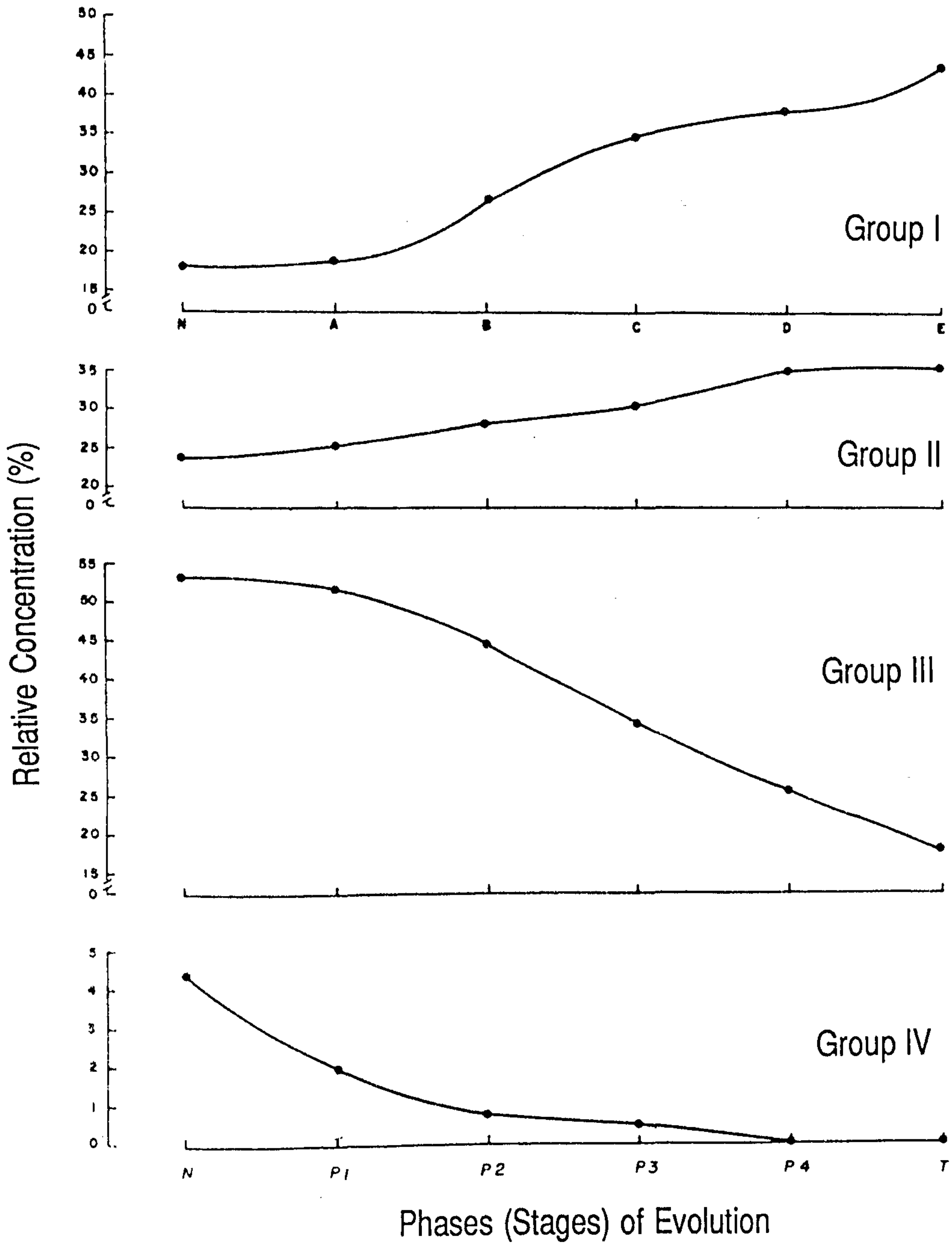


Fig. 9. Graphical representation comparing the variation in the mean concentration of the four groups of protein fractions during evolution of cataracts in *M. furnieri*.

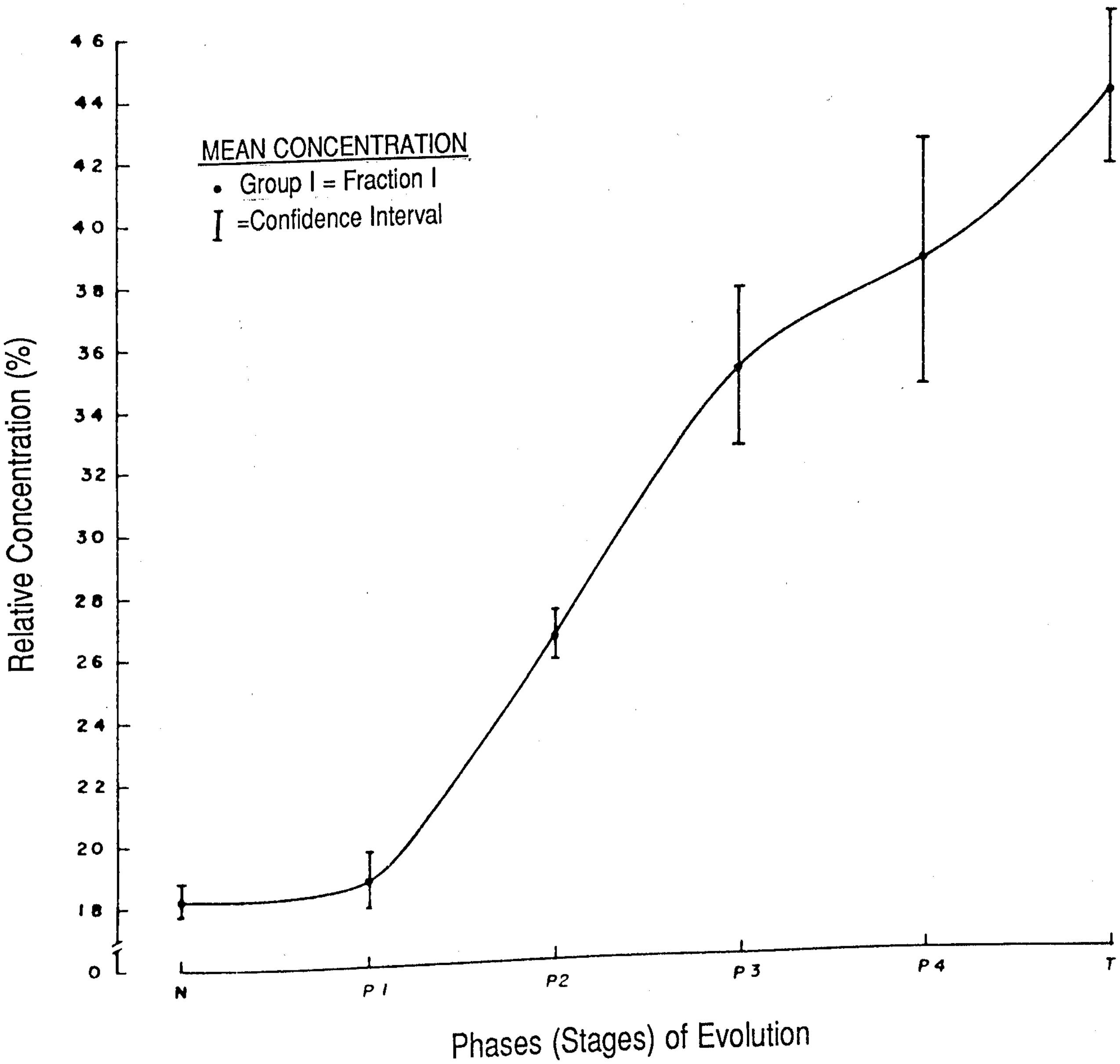


Fig. 10. Graphic representation of the variation in the relative mean concentration of the single protein fraction from Group I during evolution of cataracts in *M. furnieri.*

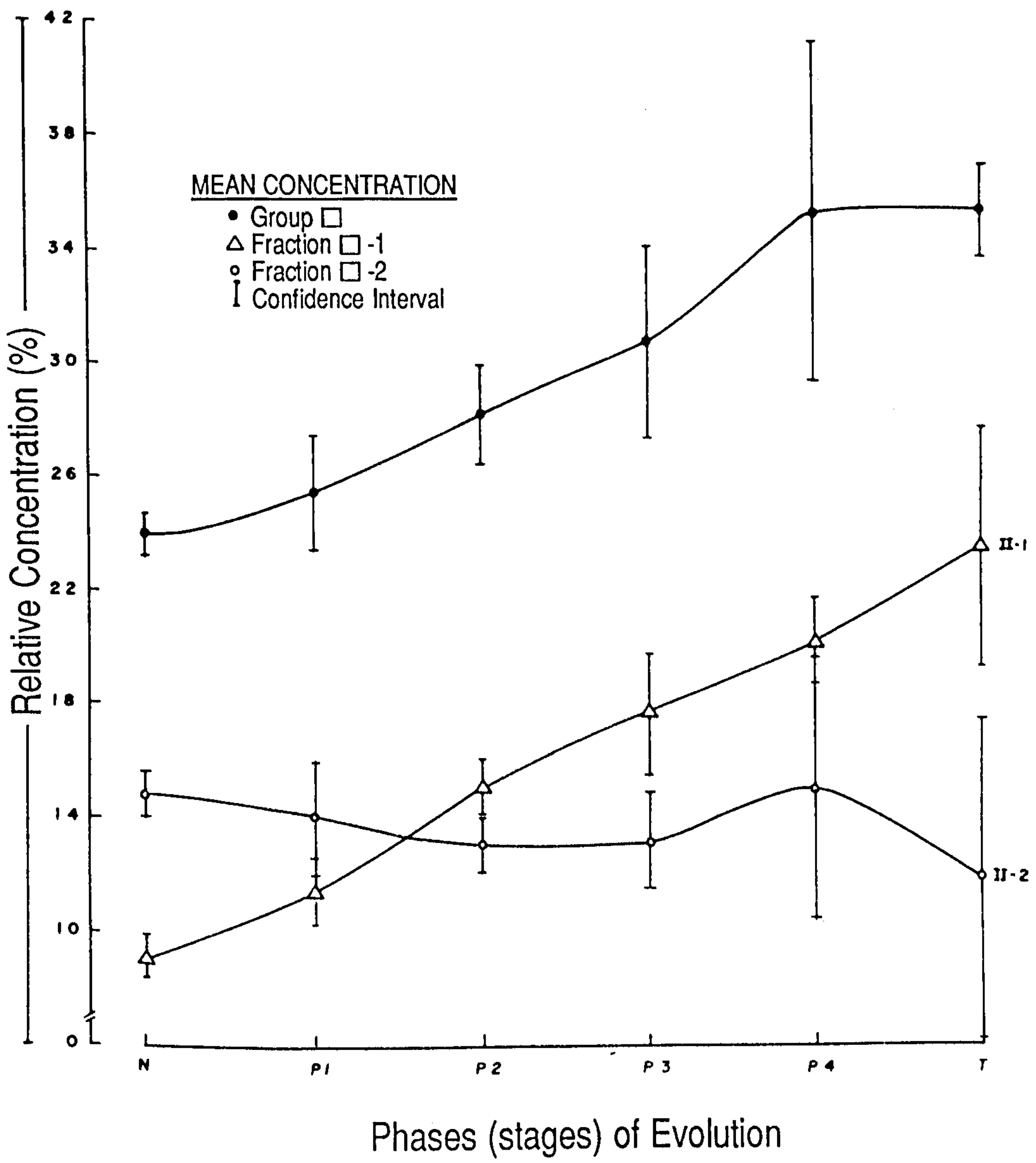


Fig. 11. Graphic representation of the variation in the relative mean concentration of fractions II-1 and II-2 and Group II during evolution of cataracts in *M. furnieri*.

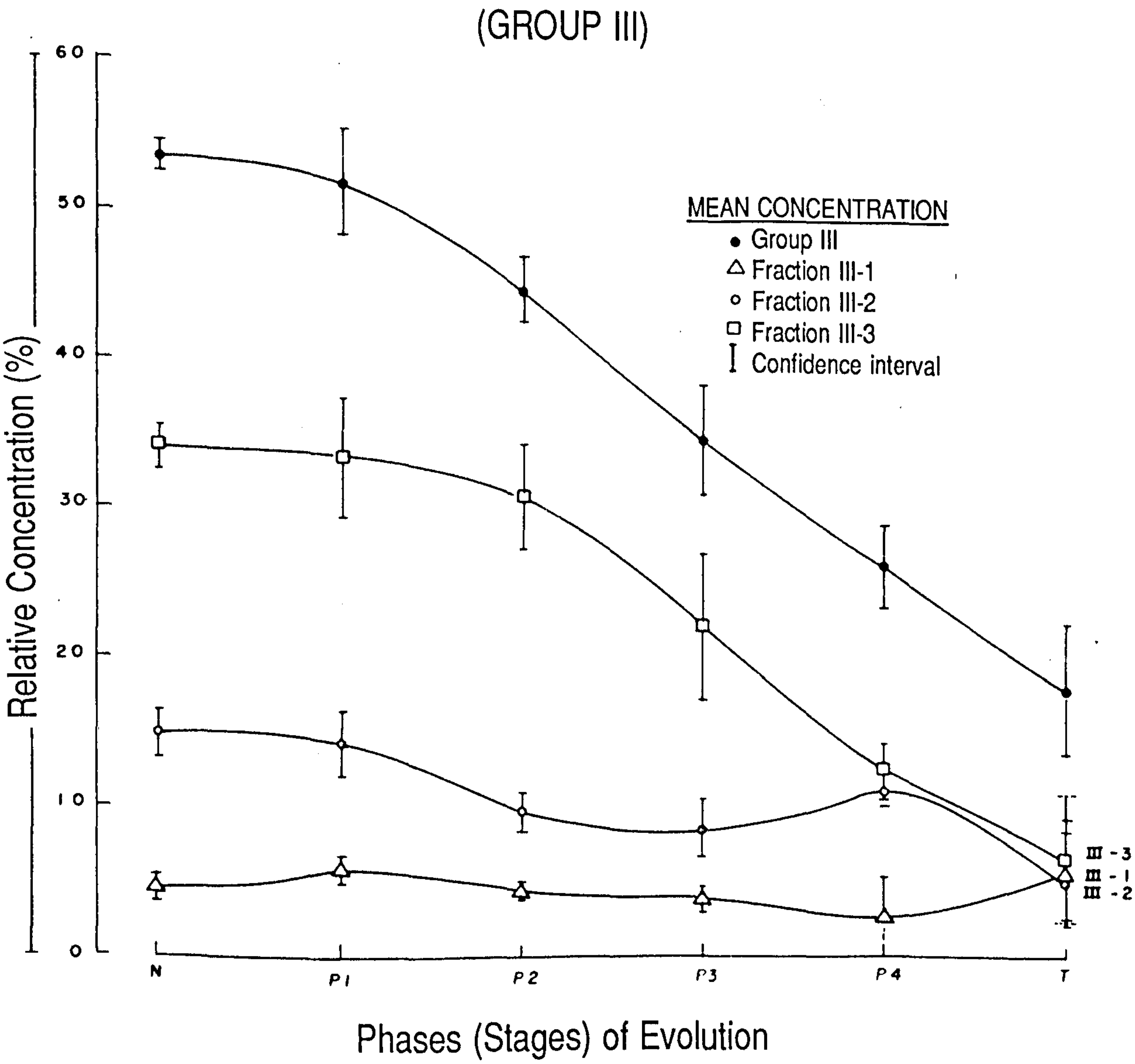


Fig. 12. Graphical representation of the variation in the relative mean concentration of fractions III-1, III-2 and III-3, and of Group III during evolution of cataracts in *M. furnieri*.

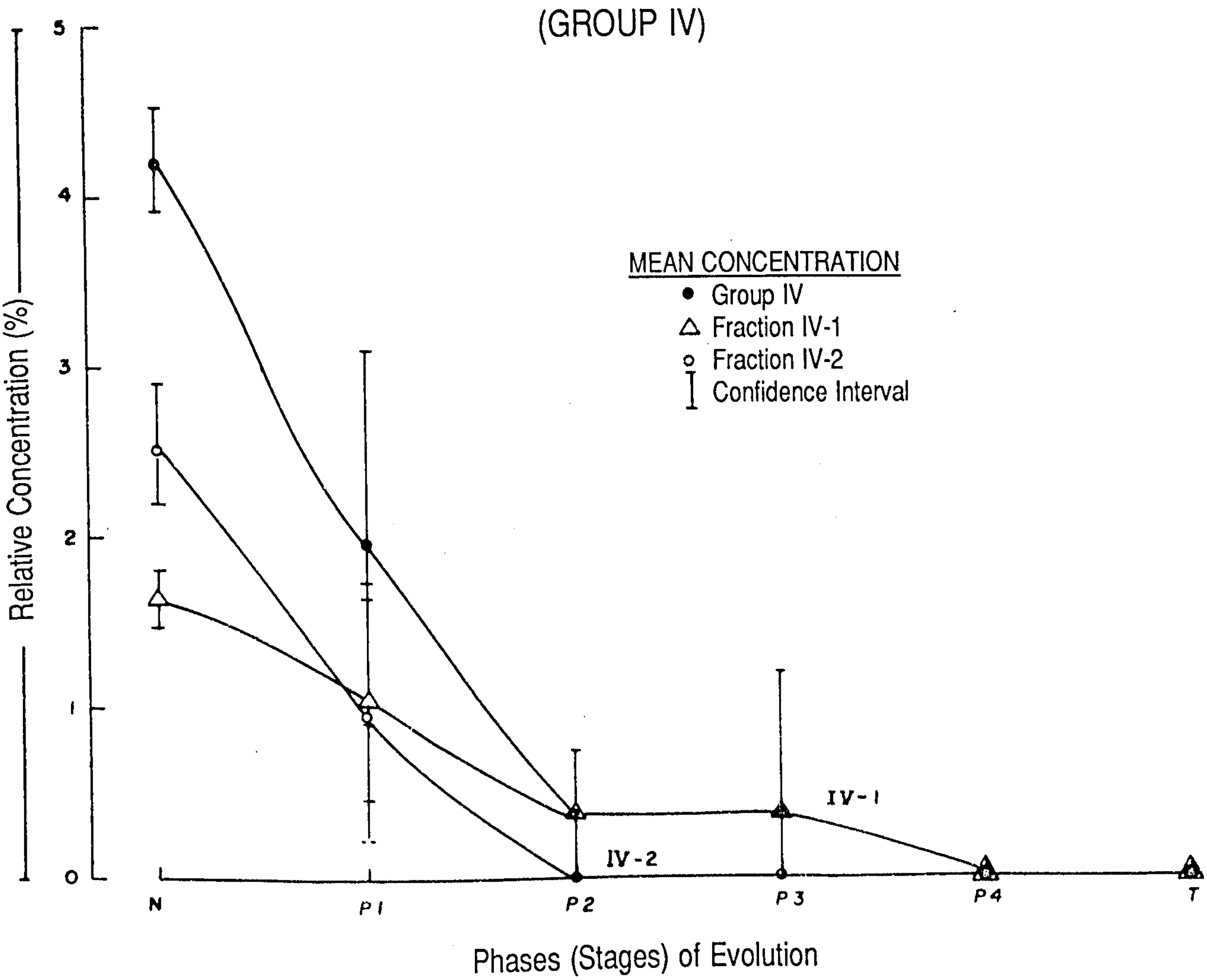


Fig. 13. Graphic representation of the variation of the mean relative concentration of fractions IV-1 and IV-2 and Group IV during cataract evolution in *M. furnieri*.