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# Allometric relations of total volumes of prolactin cells and corticotropic cells to body length in the annual cyprinodont *Cynolebias whitei*: effects of environmental salinity, stress and ageing

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**Summary.** An analysis of the allometric relations of the total volumes occupied by prolactin (PRL) and corticotropic (ACTH) cells (PRL volume and ACTH volume, respectively) to body length and a study of the immunocytochemical staining intensity of PRL and ACTH cells were used to determine the differences in activity of PRL and ACTH cells in freshwater-reared and in saltwater-reared *Cynolebias whitei* during the entire lifespan of this annual cyprinodont fish. An inflection in the allometric relation of PRL volume to body length was observed in fish of one-week old. The relatively large PRL volume in younger fish may be related to PRL cell activity before hatching. No inflections were observed in the allometric relations of PRL volume and ACTH volume to body length at the onset of maturation and the onset of ageing, indicating that the increased pituitary growth in maturing and ageing *C. whitei* is not the result of changes in PRL or ACTH cells. The slope of the allometric relation of PRL volume to body length in freshwater-reared fish was significantly steeper than the slope in saltwater-reared fish. The PRL volume in adult freshwater-reared fish was eight times larger than that in saltwater-reared fish of the same length. The intensity of immunocytochemical staining of saltwater PRL cells was significantly reduced. These volumetric and staining differences correspond to the low functional demand put upon PRL cells in saltwater-adapted fish. In contrast, the slope of the allometric relation of ACTH volume to body length and the intensity of immunocytochemical staining of ACTH cells were similar in freshwater-reared and in saltwater-reared fish. It is concluded that the functional demand put upon ACTH cells is similar in freshwater-reared and saltwater-reared *C. whitei*; the involvement of ACTH cells in the osmoregulation of the fish in both environments is similar.

**Key words:** Prolactin cells – ACTH cells – Allometric growth – Osmoregulation – Stress – *Cynolebias whitei*

Since the observation by Pickford and coworkers (1959, 1965) that a prolactin-like substance from the pituitary gland is indispensable for the survival of teleost fish in freshwater, the importance of fish prolactin (PRL) for osmore-

gulation has been well documented (see Loretz and Bern 1982). Light- and electron-microscopical studies of PRL cells indicate a high activity in freshwater-adapted fish, with considerably lower activities in fish from seawater or diluted seawater (Ball and Ingleton 1973; Holtzman and Schreiber 1975; Olivereau and Olivereau 1983; Wendelaar Bonga et al. 1985).

Compared to saltwater-adapted fish, high PRL levels have been found in the pituitary glands of freshwater-adapted *Poecilia latipinna* (Ball and Ingleton 1973) and *Oreochromis* (formerly *Sarotherodon*) *mossambicus* (Nicoll et al. 1981) and in the blood of freshwater-adapted *O. mossambicus* (Nicoll et al. 1981) and *Salmo gairdneri* (Prunet et al. 1985; Hirano et al. 1985). In *Oncorhynchus keta* plasma PRL levels had significantly increased after transfer from salt water to fresh water (Hirano et al. 1985). It has been suggested that PRL regulates the permeability of the gills to water and ions in freshwater-adapted *O. mossambicus* (Wendelaar Bonga and Van der Meij 1981).

The second hormone produced in the rostral pars distalis of teleosts, ACTH, has also been implicated in the control of osmoregulation. ACTH replacement therapy in hypophysectomized fish has been reported to promote freshwater survival or to restore plasma osmolarity or plasma sodium levels (Maetz et al. 1967; Parwez and Goswami 1985). This role of ACTH is probably mediated mainly by interrenal secretion of cortisol (Singley and Chavin 1975; Fortner and Pickford 1982; Parwez and Goswami 1985). Cortisol has been reported to stimulate differentiation of chloride cells in the gills (Doyle and Epstein 1972) and to increase the Na-K-ATP-ase activity located in these cells (Kamiya 1972; Foskett et al. 1983). Thereby, cortisol promotes salt secretion in seawater-adapted fish and has therefore been considered the dominant hormonal factor in seawater osmoregulation (for review: Dharmamba 1979). However, although in *Oncorhynchus kisutch* and *Carassius auratus* cortisol levels have been reported to be significantly higher in seawater-adapted fish (Redding et al. 1984a, b; Singley and Chavin 1975), in *Cyprinus carpio* and *O. mossambicus* only a transient elevation of cortisol levels has been observed after direct transfer from fresh water to diluted sea water (Assem and Hanke 1981; Abo Hegab and Hanke 1984). From these results, it has been concluded that the main action of cortisol involves the adaptational response to salinity transfer and not the maintenance of the hydromineral balance in salt water.

A similar conclusion has been reached with respect to

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**Table 1.** Age, sex and mean length of the groups of fish used in the study of the development of PRL cells and ACTH cells in freshwater and saltwater-reared *Cynolebias whitei*. The number of fish per group, used to study PRL cells (*no. PRL*) or ACTH cells (*no. ACTH*) are given

Freshwater-reared fish					Saltwater-reared fish				
Age	Sex	Mean length	No. PRL	No. ACTH	Age	Sex	Mean length	No. PRL	No. ACTH
n.h.		3.5	5		n.h.		3.5	5	
1 d		3.9	3	6	1 d		3.9	5	6
2 d		4.1	3	6	1 w		8.3	8	
1 w		6.8	6	4	2 w		13.4	7	4
2 w		10.0	5	5	3 w		9.9		5
3 w		9.4		6	3 w		14.8		5
3 w		11.8		6	3 w		15.6		5
3 w		13.8	5	5	3 w		16.9	8	6
3 w		15.8		5	5 w	♂♀	14.4		6
4 w		18.6	6		5 w	♂♀	24.6	7	3
5 w	♂♀	21.5	6	5	3.5 m	♂♀	47.3	3	3
6 w	♂♀	28.6	3						
2 m	♂♂	32.5	7	5	<i>n.h.</i>	newly hatched			
3 m	♀♀	32.5		4	<i>d</i>	days			
3 m	♂♂	46.1	8	3	<i>w</i>	weeks			
4 m	♂♂	51.0	8	5	<i>m</i>	months			
6 m	♂♂	56.9	7	4					
7 m	♂♂	55.0	3	3					

*C. whitei* matures at 5 weeks of age

pituitary involvement in saltwater adaptation (Dharmamba 1979). In contrast, morphological studies on ACTH cells have indicated that in diluted seawater, ACTH cell activity is higher than in either seawater (Olivereau 1968, as cited by Olivereau and Ball 1970; Cassifour and Chambolle 1976; Malo-Michelle 1979) or fresh water (Mattheij et al. 1971; Leatherland 1972; Goswami et al. 1983).

From the above literature it can be concluded that no consensus has so far been reached concerning the difference in ACTH cell activity in freshwater-adapted and saltwater-adapted fish. To this end, we have used another approach: the analysis of the effects of fresh water and salt water on the development of ACTH cells. A continuous functional demand will lead in most cases to an increase in size of the organ under strain (Goss 1978), and thus one may expect a difference in the volume occupied by ACTH cells when fish are reared under conditions that put different demands on ACTH cells. In a previous paper, we have reported the results of such an approach and demonstrated that PRL cell development is influenced by environmental salinity: after five weeks under iso-osmotic conditions the volume occupied by PRL cells is only 25% of the PRL volume in freshwater-reared fish (Ruijter et al. 1984). The low activity of PRL cells in saltwater-reared fish is also reflected in the low immunocytochemical staining affinity of these cells (Ruijter et al. 1984).

In this paper, we report on the development of PRL and ACTH cells throughout life in the annual cyprinodont fish *Cynolebias whitei* in fresh water and salt water (260 mOsm/kg) in a combined immunocytochemical and morphometric study. In order to avoid influences of growth differences that result from different rearing conditions, allometric relations of body length and volumes occupied by PRL and ACTH cells are determined. Additional observations concerning the effects of salinity transfer, short-

term handling stress and chronic disturbances during development, on the ACTH cells are reported.

## Materials and methods

### Experimental animals

The annual fish *Cynolebias whitei* (Myers 1942) were reared in our laboratory on a daily schedule of 10 h light – 14 h darkness in 90-liter aquaria. The water temperature (25° C) was close to natural temperature for this species, and the aquaria contained plants to provide sufficient hiding places. Once every two weeks, half of the water was replaced. Young fish were fed freshly-hatched brine shrimp, older fish were fed *Tubifex* and Tetramin tropical fish food. Pre-hatching larvae were dissected from eggs that were stored in moist peat for at least three months (Schoots et al. 1983). Larvae hatched in fresh water and were reared in fresh water (6 mOsm/kg) or iso-osmotic salt water (diluted artificial seawater; 260 mOsm/kg; Ruijter et al. 1984).

The development of PRL cells and ACTH cells was studied in a series of fish from fresh water (from hatching until 7 months old) and salt water (from hatching until 3.5 months old). Age, sex, mean length, and number of the groups of fish are given in Table 1. Fish were killed at different ages by anaesthesia in MS222 (0.3% in tap water) immediately after handnetting from the aquaria.

With respect to the ACTH cells, three additional experiments were carried out. In one experiment, the effect of transfer of the fish from fresh water to salt water, and vice versa, was studied. Fish were killed two days after transfer. In the second experiment the effect of stress (viz. handnetting) on the ACTH cells was studied. Fish from freshwater were netted and killed either immediately, or after 10, 20 or 30 min in another aquarium. In another

experiment the effect of repeated disturbances on the ACTH cells was studied. Fish were reared for three weeks in fresh water and during this period they were transferred at irregular intervals (approximately twice a day) to another tank by handnetting. Siblings reared under normal, undisturbed conditions served as controls. Fish were killed 12 h after the last disturbance.

#### *Histological procedure*

After anaesthesia the standard length of the animals was measured. Small fish were fixed undissected; the mandible was removed from bigger fish and only the head was fixed. When fish were larger than 15 mm, the base of the brain was dissected free by removing the eyes and the base of the skull. All fish were fixed for three days at 4°C in a mixture (1:3) of 25% glutaraldehyde and saturated picric acid (both in aqueous solution) with 1% acetic acid. After being dehydrated and embedded in Paraplast, the tissue was sectioned sagittally at a thickness of 4 µm. The pituitary gland was localized in the Paraplast ribbon by examining every tenth section.

Series of 6–10 sections through the pituitary gland were stained with Cleveland and Wolfe's trichrome stain (C&W) or immunocytochemically with rabbit antiserum raised against ovine PRL (kindly provided by Dr. JAM Mattheij, Agricultural University, Wageningen) or synthetic ACTH (Dr. B Jenks, Catholic University, Nijmegen). Details of immunocytochemical procedures have been described previously (Ruijter et al. 1984).

#### *Densitometry*

Immunocytochemical staining affinity was quantified by densitometry on an automated image-analysis system (IBAS, Department of Image processing and Design, State University Utrecht). Staining intensity was presented as the mean optical density, calculated as the integrated optical density per total area. Thus, mean optical density reflected the average staining intensity. Only sections that had been treated in one staining session were compared, to avoid misinterpretation that might have been caused by differences in staining efficiency. Background-staining intensity of sections from one immunocytochemical staining session deviated  $1.1 \pm 1.0\%$  ( $\bar{x} \pm SD$ ) from the mean background staining level.

#### *Morphometry and statistics*

Volumes occupied by PRL cells and ACTH cells were determined by combined point-counting volumetry and planimetry. Immunocytochemically-stained sections were drawn at a final magnification of  $1334 \times$  for small to  $127 \times$  for large pituitary glands. PRL area in sections of saltwater-adapted fish was drawn from C&W-stained sections.

The percentages of the pituitary volume occupied by PRL or ACTH cells were determined by point-counting volumetry (Weibel 1979). A regular test grid with a point distance of 7 mm was used. Total pituitary volume was computed as the product of the total pituitary area on the drawings (determined by planimetry) and the distance of the sections, divided by the magnification squared. Volumes occupied by PRL and ACTH cells were computed by multiplying pituitary volume and the volume percentages of these cell types.

Allometric relations of volumes to body length were calculated by linear regression (Sokal and Rohlf 1969) on logarithmically transformed volume and body length data (Ricker 1979). Individual volume data were used in this regression. When systematic deviations from the regression line were observed, the fitting of sets of lines was tested. The decrease of the unexplained variance of those sets was used as the measure to decide whether or not a better fit was reached (Sokal and Rohlf 1969). The set of lines with the least unexplained variance and significantly different slopes was used. Points near the intersection of such lines were included in both regressions. The difference in the slopes of the regression lines was tested with an F-test (Snedecor and Cochran 1980). Densitometric data were tested with the two-sample test of Wilcoxon (U-test; Sokal and Rohlf 1969).

## **Results**

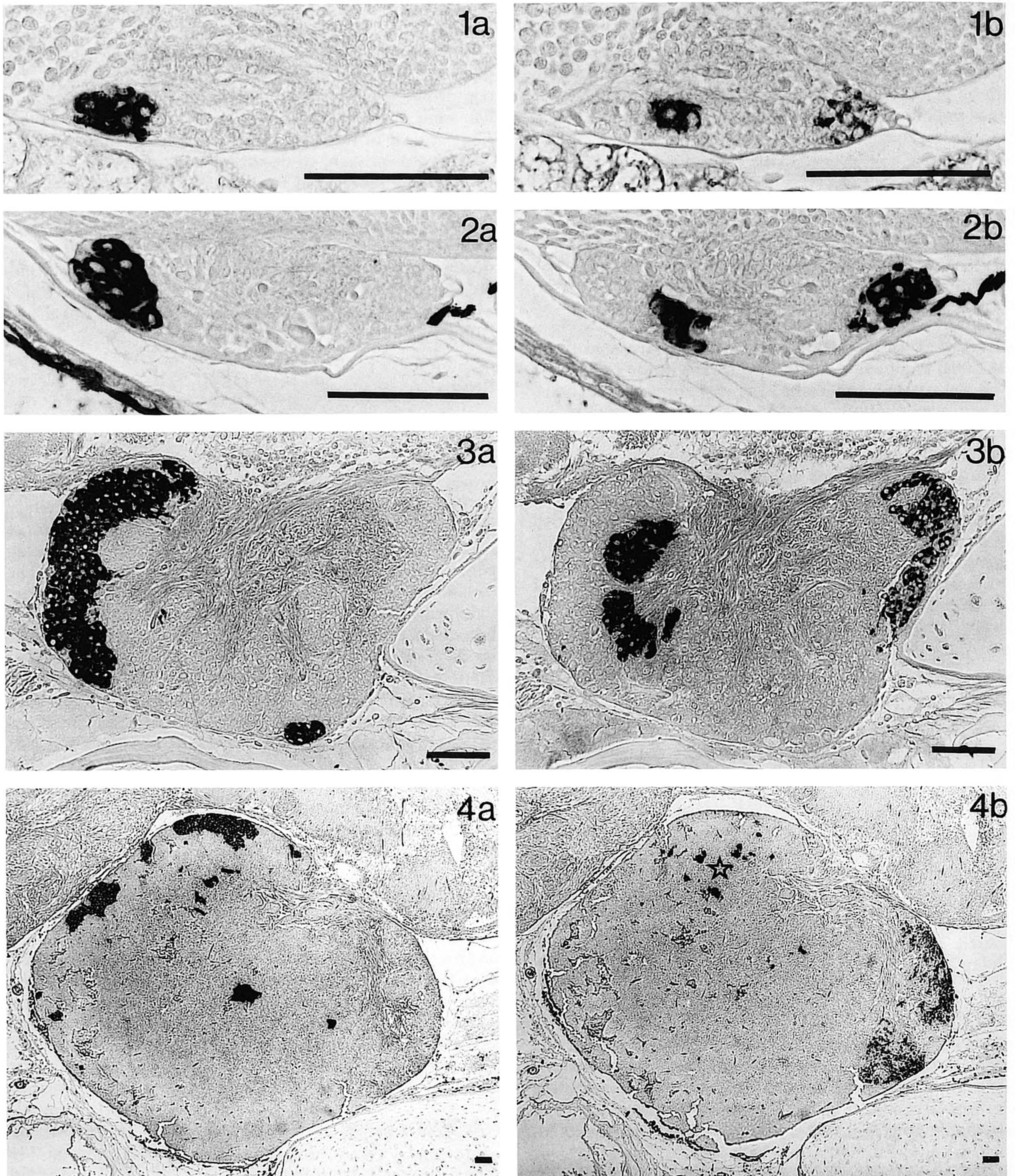
#### *Immunocytochemistry and densitometry*

*Fresh water.* Both PRL immunoreactive and ACTH immunoreactive cells were present in pre-hatching larvae (Fig. 1a, b). PRL cells occupied the most rostral part of the pituitary gland and ACTH cells were located directly caudal to the PRL cells. ACTH cells formed a single cell group, often penetrated by rostral branches of the neurohypophysis. The melanotropic cells in the pars intermedia showed cross reactivity with the ACTH serum at all ages studied (Figs. 1–4b) but could always be distinguished from the ACTH cells by their specific location and less intense staining affinity. The location of PRL cells and ACTH cells remained unchanged until the age of five weeks (Fig. 2a, b). In older fish, small groups of PRL cells were scattered throughout the pituitary gland (Figs. 3a, 4a; Ruijter 1987). ACTH cells also became dispersed, but remained confined to the rostral part of the pituitary gland at all ages (Figs. 3b, 4b). No changes in immunocytochemical staining affinity were observed in either PRL cells or ACTH cells in the course of the lifespan.

*Salt water.* In fish reared from hatching in iso-osmotic saltwater, immunostaining affinity of PRL cells was less than that in freshwater fish (Ruijter et al. 1984). Densitometry of groups of three-week-old fish revealed a significant difference in the mean optical density of the PRL cells between fresh- and saltwater-reared fish (Fig. 5;  $P=0.02$ ). The immunostaining affinity of ACTH cells of fish from fresh- and saltwater, killed immediately after netting, was similar (Fig. 5;  $P>0.2$ ).

#### *Volumetry and allometry*

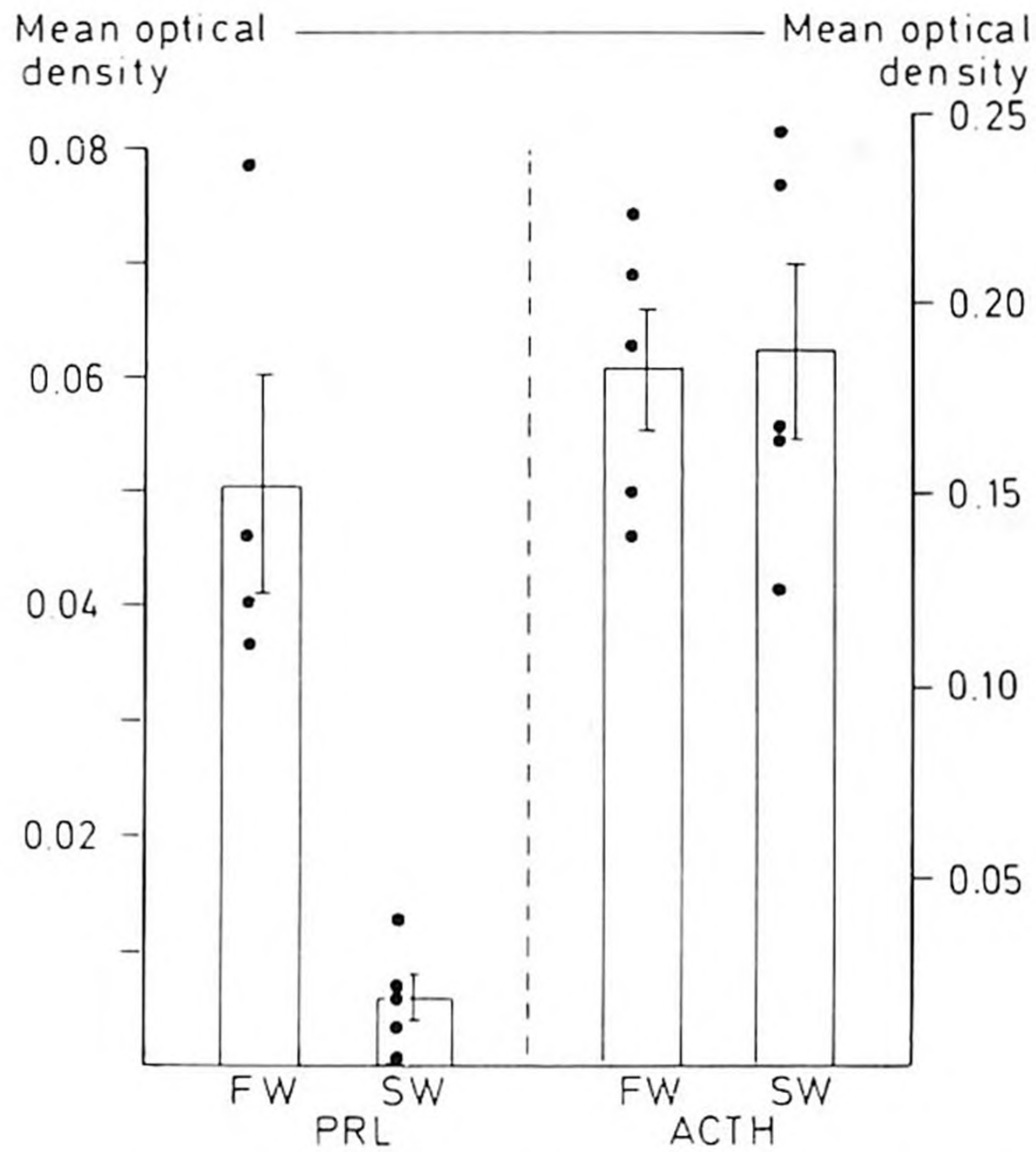
*Fresh water.* The volumes occupied by PRL and ACTH cells were measured in fish reared in fresh water until seven months of age. The results are plotted allometrically in Figs. 6a and 6b. In the slope of the allometric relation of PRL volume to body length, a significant inflection ( $P<0.001$ ; Fig. 6a) occurs in one-week-old fish (7 mm body length). In the youngest fish, the slope of the regression line is 1.446 (correlation coefficient ( $r$ )=0.996;  $n=17$ ). From one week until seven months the slope is 3.092 ( $r=0.998$ ;  $n=64$ ). The slope of the allometric relation of ACTH volume to body length is 2.033 ( $r=0.985$ ;  $n=69$ ; Fig. 6b).



**Figs. 1–4.** Sagittal sections through the pituitary gland of *Cynolebias whitei* stained immunocytochemically with antiserum to ovine prolactin (Figs. 1a–4a) and ACTH (Figs. 1b–4b). ACTH cells are located immediately caudal to the prolactin cells whereas the melanotropic cells occupy the caudal part of the pituitary gland. Figs. 1a, b: prehatching larvae (640 $\times$ ); Figs. 2a, b: 1 week old (580 $\times$ ); Figs. 3a, b: 3 months old (210 $\times$ ); Figs. 4a, b: 6 months old (*star* indicates ACTH cells; 58 $\times$ ). *Bar* indicates 50  $\mu$ m

Although some groups deviate from the regression line, fitting of a set of lines does not reduce the unexplained variance significantly. No set of regression lines with significantly different slopes can be fitted to these data.

*Salt water.* PRL and ACTH volumes were measured in fish reared in iso-osmotic salt water till 3.5 months of age. The results are plotted in Fig. 7a and b. As in freshwater-reared fish, the slope of the allometric relation of PRL



**Fig. 5.** Mean optical density of immunocytochemically stained PRL and ACTH cells in three-week-old freshwater (FW)- and salt-water (SW)-reared *Cynolebias whitei*. Mean  $\pm$  SEM; points indicate individual fish

volume to body length shows a significant break in one-week-old fish (Fig. 7a;  $P < 0.001$ ). In the youngest fish, the slope is 0.703 ( $r = 0.996$ ;  $n = 16$ ). After the inflection, the slope is increased to 2.396 ( $r = 0.999$ ;  $n = 33$ ). Both slopes are significantly flatter than the slopes in freshwater-reared fish of the same age (Figs. 6a and 7a;  $P < 0.005$ ). In the slope of the allometric relation of ACTH volume to body length in saltwater-reared fish, no significant inflections occur (Fig. 7b). The slope of the regression line is 1.756 ( $r = 0.981$ ;  $n = 42$ ). There is no significant difference in the slopes of the allometric relations of ACTH volume to body length in fish reared in fresh- or saltwater (Figs. 6b and 7b;  $P > 0.1$ ).

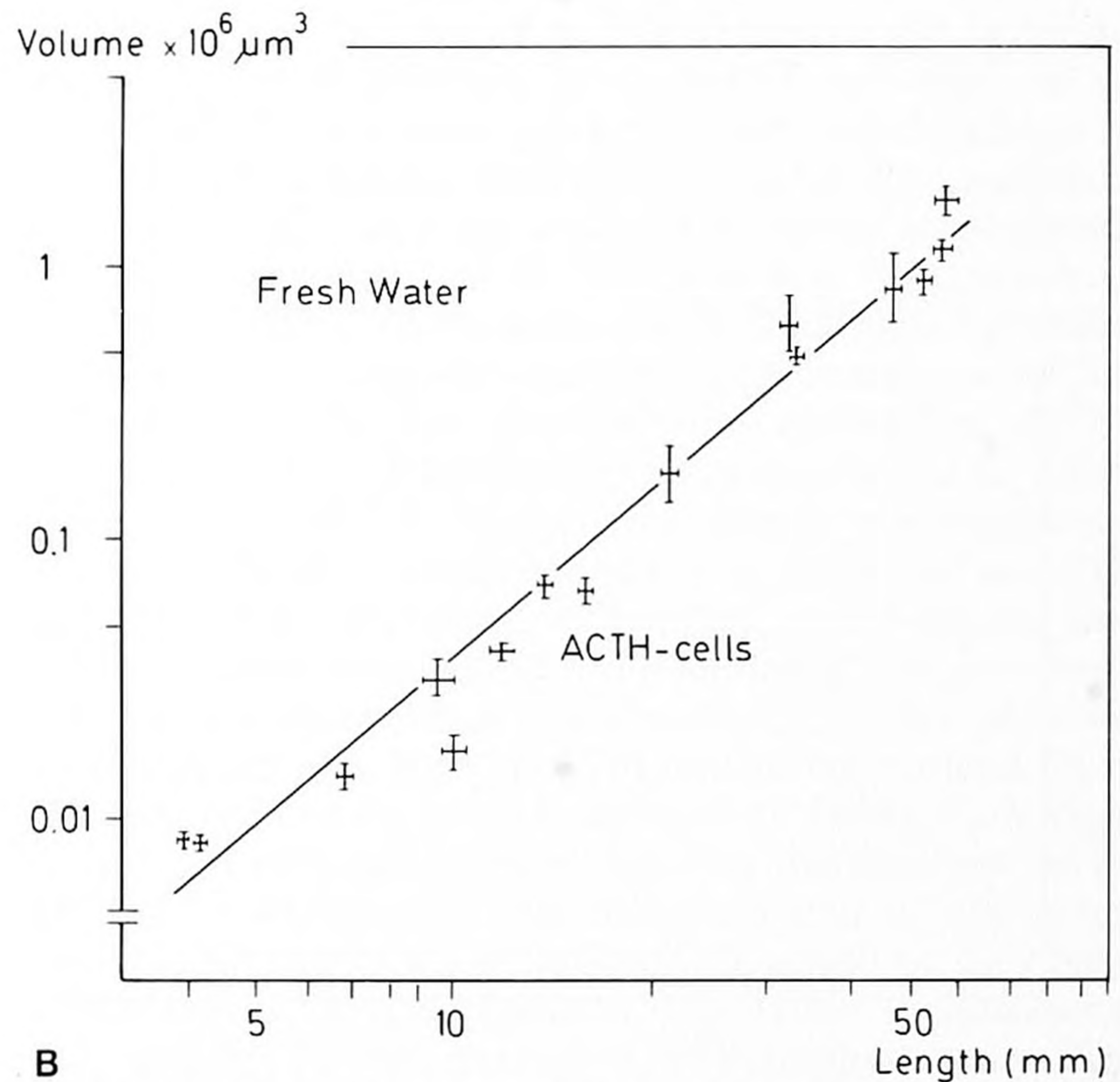
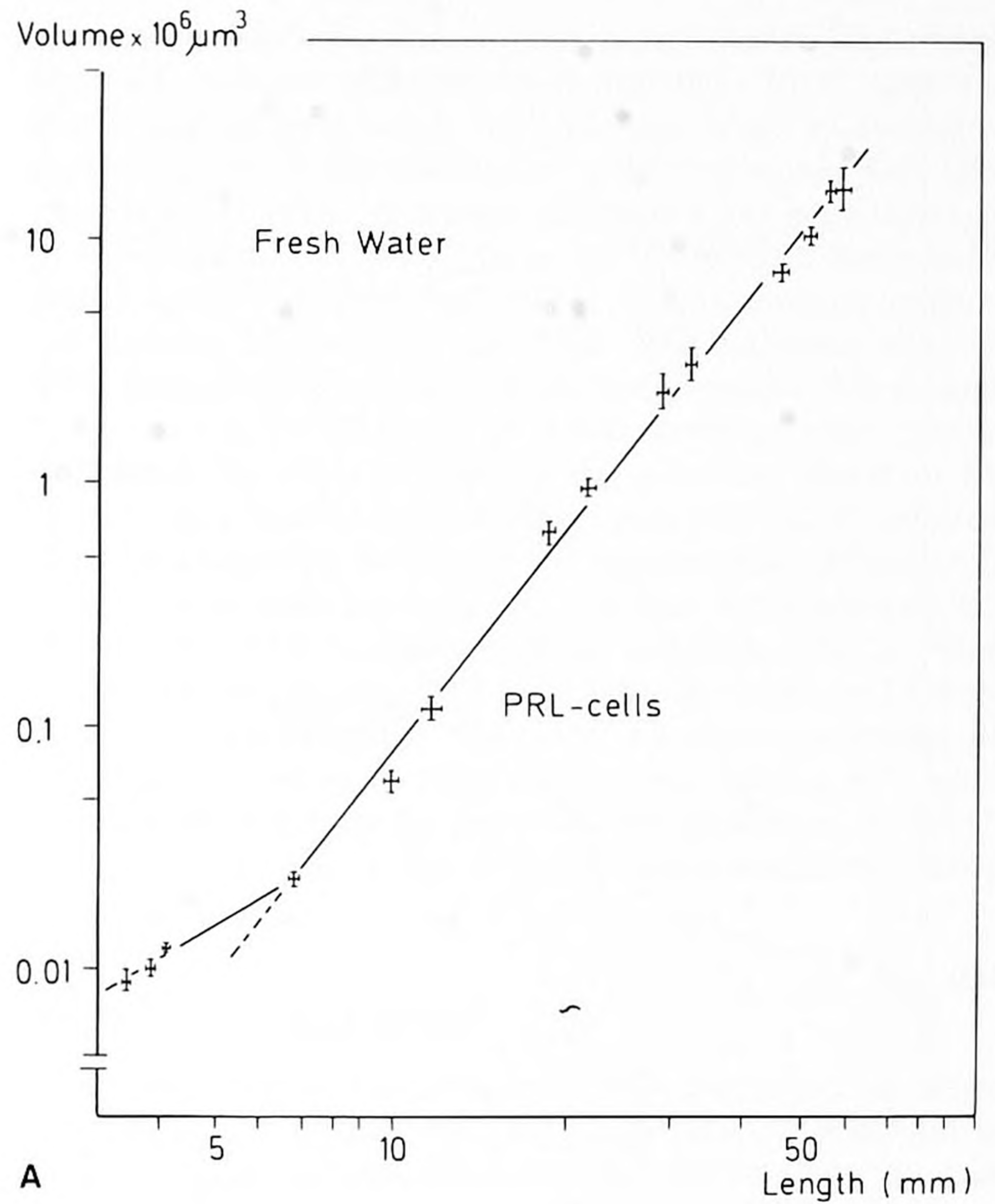
In freshwater-reared fish of three to four months old, the PRL volume is about eight times the PRL volume in saltwater-reared fish of 3.5 months old and similar length (Figs. 6a and 7a). At the same age, ACTH volume is the same in both environments (Figs. 6b and 7b).

### Stress

**Transfer experiments.** No differences were observed in the immunocytochemical staining affinity of ACTH cells between control fish and fish killed two days after transfer by handnetting from fresh water to salt water or vice versa.

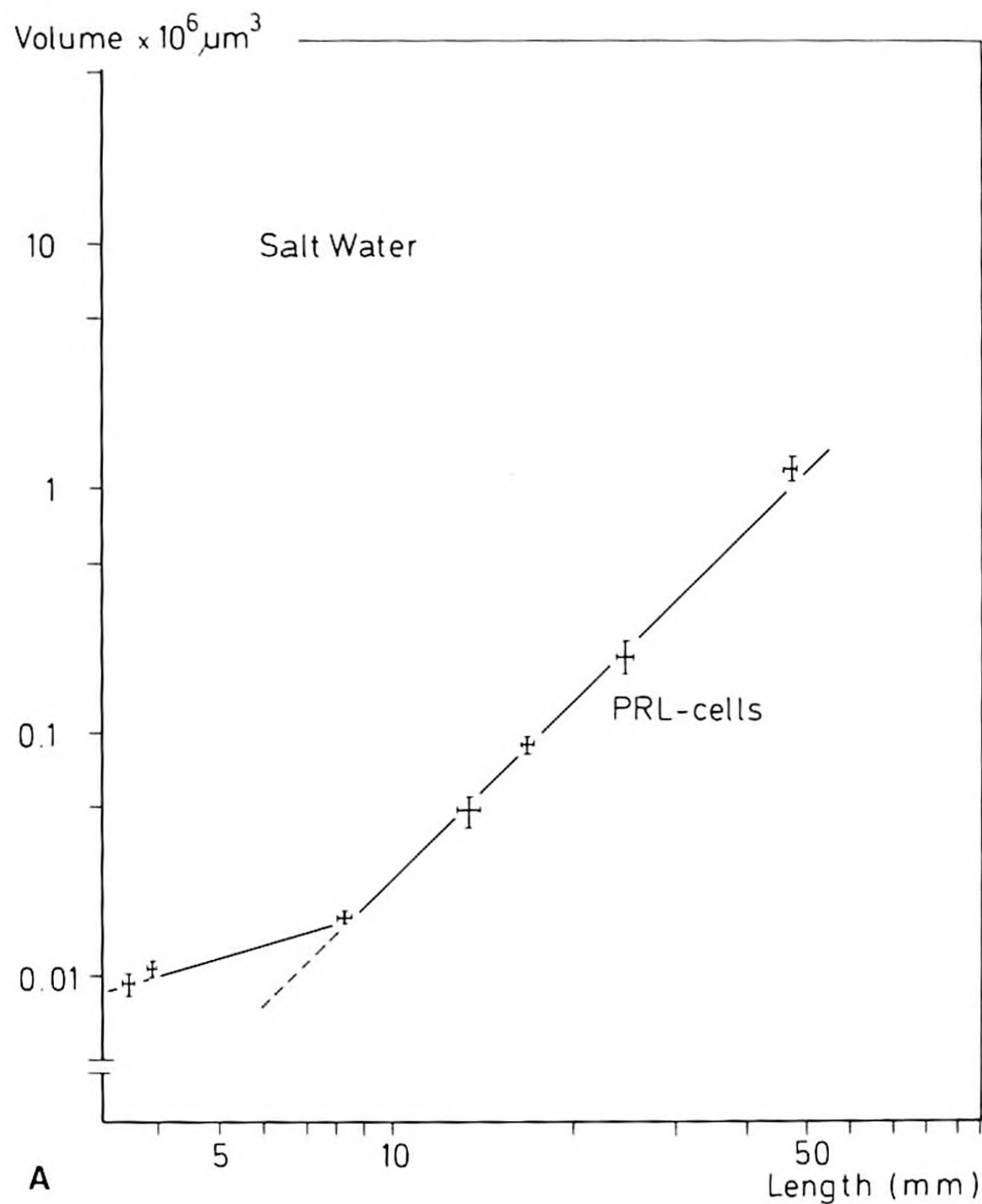
**Handling stress.** The immunostaining affinity (mean optical density) of ACTH cells was significantly reduced in fish killed 10 min after netting (Fig. 8;  $P = 0.05$ ). In the following 20 min the mean staining affinity of the group remained at a similar level (Fig. 8). The variability among the fish, however, was increased and the difference from the unstressed controls was no longer significant.

**Chronic stress.** ACTH volumes were measured in fish reared for three weeks under stressful conditions. Compared to fish reared under normal conditions, a small, non-significant, increase of ACTH volume was observed (Fig. 9;  $P >$

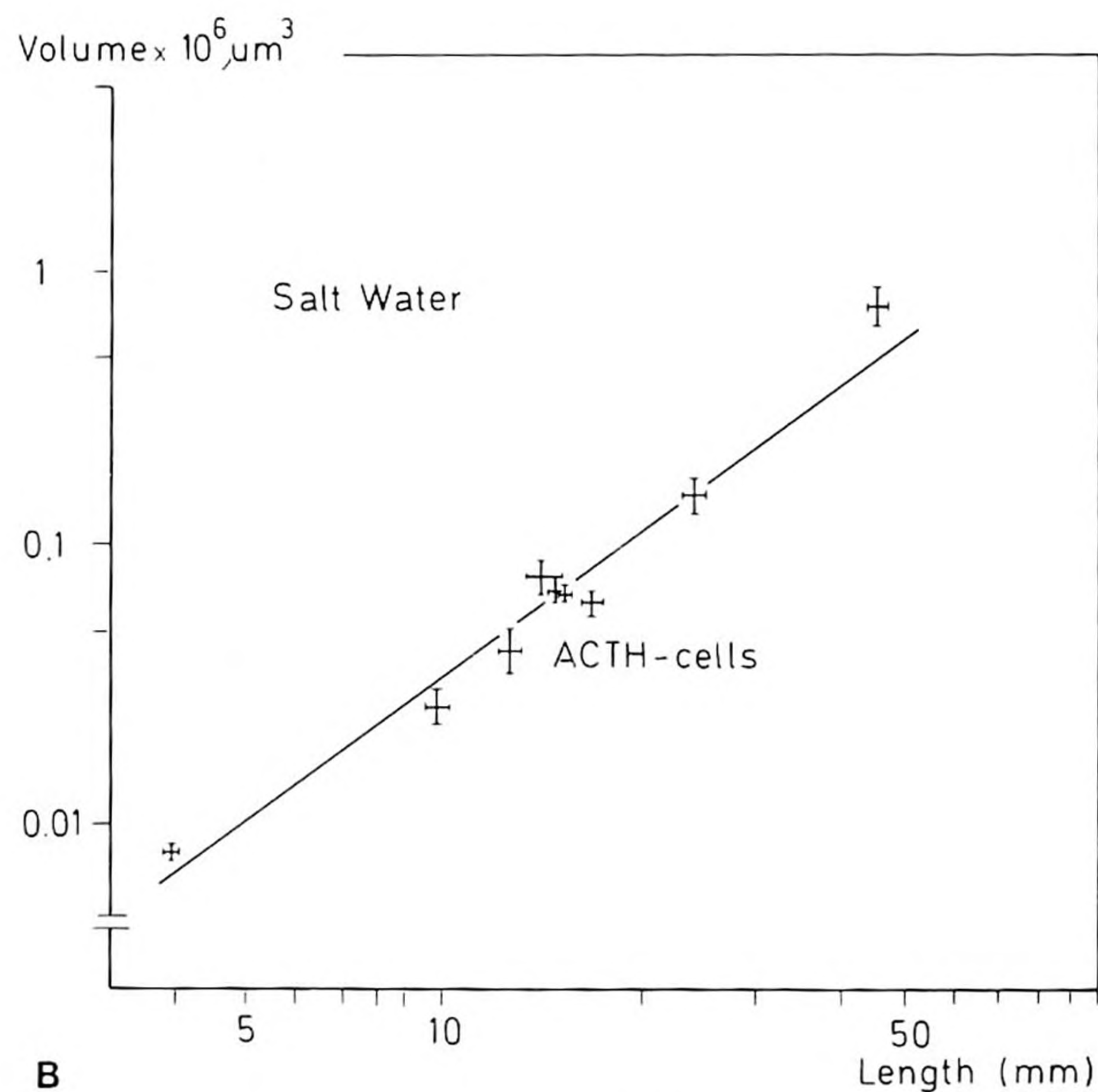


**Fig. 6a, b.** Allometric relations of PRL volume (Fig. 6a) and ACTH volume (Fig. 6b) to body length in freshwater-reared *Cynolebias whitei*, ranging in age from 1 day to 7 months

0.4). Mean fish length was  $13.9 \pm 0.8$  mm in normal ( $n = 6$ ) and  $15.0 \pm 0.8$  mm in stressed fish ( $n = 7$ ;  $P > 0.4$ ). Fish were killed immediately after netting, 12 h after the last disturbance to prevent short-term handling-stress effects. No differences in the immunostaining affinity of the ACTH cells were observed.



A



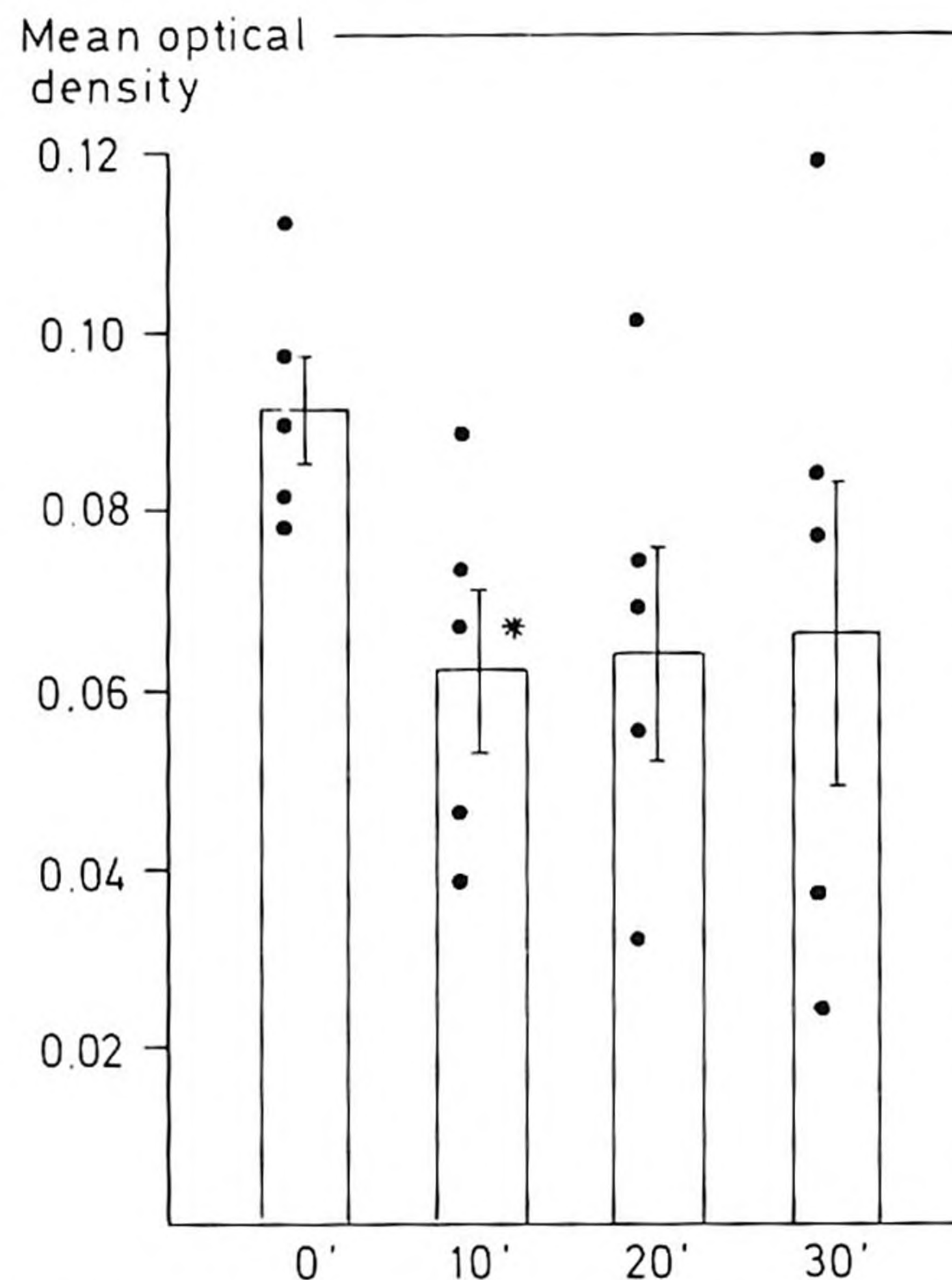
B

**Fig. 7a, b.** Allometric relations of PRL volume (**Fig. 7a**) and ACTH volume (**Fig. 7b**) to body length in saltwater-reared *Cynolebias whitei*, ranging in age from 1 day to 3.5 months

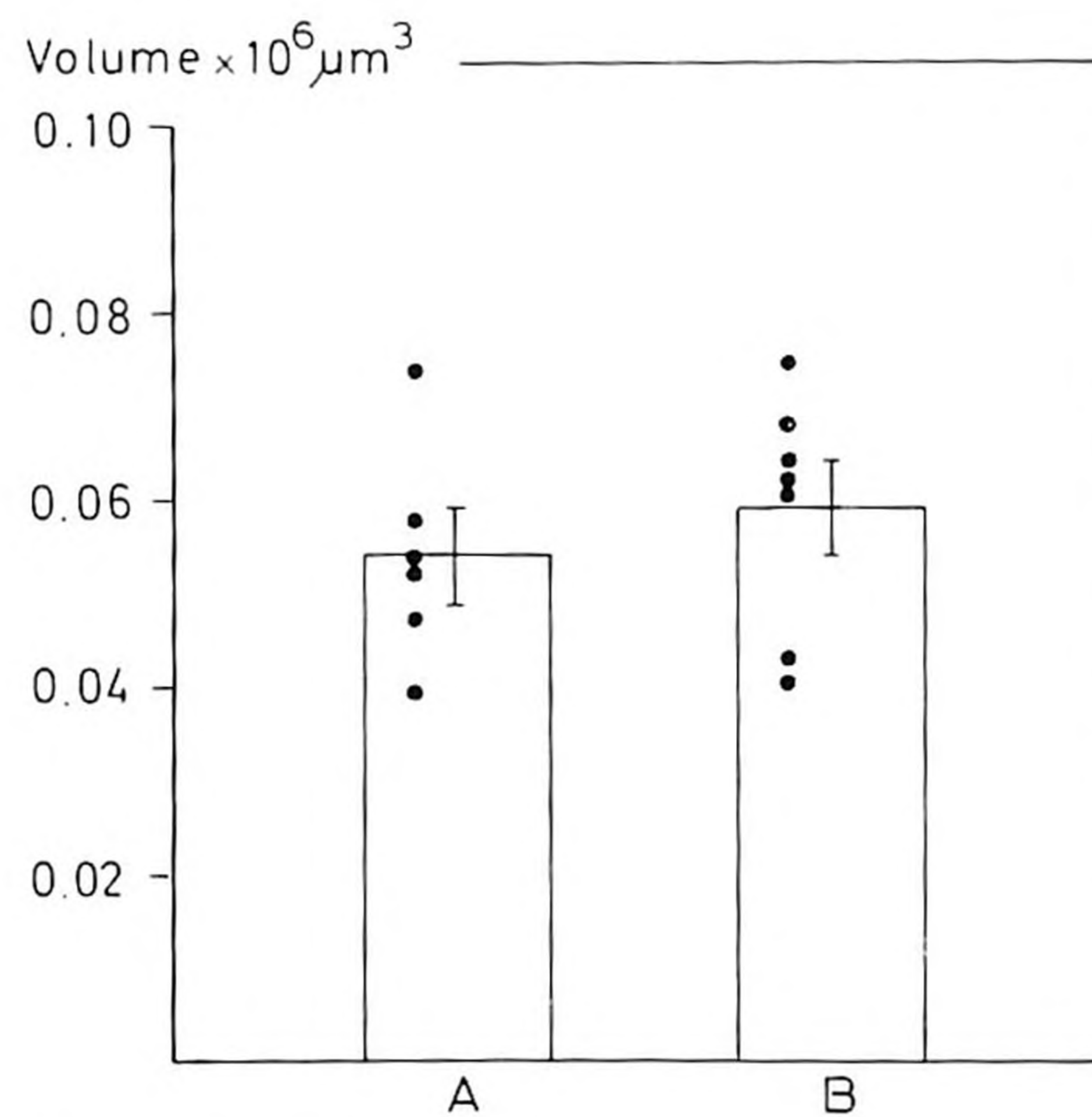
## Discussion

### Identification of PRL and ACTH cells

The immunocytochemical localization of PRL cells in *C. whitei* from prehatching larvae up to fish of five weeks of age has been discussed previously (Ruijter et al. 1984). Up



**Fig. 8.** Mean optical density of immunocytochemically stained ACTH cells in three-week-old *Cynolebias whitei*, immediately before and 10, 20 and 30 min after handling stress. Mean  $\pm$  SEM; points indicate individual fish; asterisk: U-test;  $P=0.05$



**Fig. 9.** ACTH volume in control (A) and chronically disturbed (B) three-week-old *Cynolebias whitei*. Mean  $\pm$  SEM; points indicate individual fish

to that age, PRL cells are located in the rostral pars distalis with an occasional group of cells outside this area. The present data show a further dispersion of the PRL cells with age. It should be noted that with the light-microscopical method employed, the non-granulated cells between the PRL cells could not be distinguished. However, the contribution of these cells to the measured PRL volume is very small (unpublished electron-microscopical observations).

As in many teleosts, the ACTH cells of *C. whitei* were localized immunocytochemically immediately caudal to the PRL cells (Doerr-Schott 1976; Batten 1986). The slight affinity of the melanotropic cells in the pars intermedia to the anti-ACTH serum is a result of small amounts of ACTH that are produced in melanotropic cells (Van Eijs and Van den Oetelaar 1981). This staining can be eliminated by fur-

ther diluting the antiserum (Margolis-Kazan and Schreiber 1981).

### Allometry

Allometric relations can be used to determine stanzas (stages or periods) in the life of a fish (Ruijter 1987). Within each stanza, the growth rate of the "organ" studied is constant in relation to the growth rate of the whole fish. At the transition from one stanza to the next, the ratio of these growth rates may change. This is reflected in a break in the slope of the regression line in the allometric plot.

In the allometric relation of pituitary volume to body length in *C. whitei*, inflections have been found at the onset of maturation and at the onset of ageing, at 12 mm and at 50 mm, respectively (Ruijter 1987). The increased pituitary growth in both maturing and ageing *C. whitei* cannot be attributed to changes in PRL or ACTH cells because the allometric relations of PRL volume and ACTH volume to body length do not show inflections at 12 or 50 mm body length. These observations support our conclusion that the increased pituitary growth in these stanzas is caused by the proliferation of the cells of the proximal pars distalis, in particular gonadotropic and thyrotropic cells (Ruijter et al. 1987).

When the allometric relations of PRL volume to body length in fish older than one week are extrapolated to younger, i.e. smaller, fish, the allometric plots (Figs. 6a and 7a) indicate that the pituitary glands of the youngest fish contain relatively more PRL cells than those of fish older than one week. Although the data indicate that the same may be true for ACTH cells (Fig. 6b) this could not be demonstrated by regression statistics. The surplus of PRL volume may be related to an osmoregulatory function of PRL in prehatching larvae.

### PRL and osmoregulation

In freshwater-reared *C. whitei* older than one week, the relation of PRL volume to body length is represented by an allometric equation with a single slope constant. This indicates that the ratio between the specific growth rates of PRL volume and body length is constant and independent of fish size or age.

The osmoregulatory activities of PRL in freshwater-adapted fish probably involve regulation of the permeability of body and gill surface (Wendelaar Bonga et al. 1983, 1985). Because body and gill surface are allometrically related to body size (Hughes 1972; Oikawa and Itazawa 1985), the functional demand for PRL is expected to be related to body size. Such a direct relationship is clearly indicated by our data. We conclude that the allometric relation of PRL volume to body length in *C. whitei* older than one week reflects the need for the production of an adequate amount of PRL at all body sizes in order to maintain osmotic homeostasis.

In saltwater-reared fish of 1 week to 3.5 months old, the slope of the allometric relation of PRL volume to body length is significantly less than in fish from fresh water. Since the allometric exponent represents the ratio of the specific growth rates of PRL volume and body length (Ricker 1979), it can be calculated that the growth rate of PRL volume in saltwater fish is 20% lower than that in freshwater fish. As a result of this difference the PRL

volume in adult *C. whitei* from freshwater is eight times the PRL volume of saltwater-reared fish. In *P. latipinna*, fish reared in fresh water until the age of six months have only twice the PRL volume of saltwater-reared fish (Ball and Ingleton 1973). A similar difference has been observed in three species of *Mugil* from sea water and fresh water (Blanc-Livni and Abraham 1970). The immunocytochemical staining intensity of the PRL cells indicates that the PRL content of these cells in saltwater-reared fish is much lower than in the PRL cells of fish from fresh water. Similar differences in PRL content of the pituitary gland in fish from different salinities have been reported for *P. latipinna* (Ball and Ingleton 1973) and *O. mossambicus* (Nicoll et al. 1981). These differences in PRL content correlate well with the activity of PRL cells in different salinities (see e.g., Wendelaar Bonga and van der Mey 1981; Olivereau and Olivereau 1983). Apparently, the different osmoregulatory demand of freshwater or saltwater environments on *C. whitei* is reflected not only in the different growth rates of the PRL cells, but also in the different immunostaining intensity of these cells.

### ACTH and osmoregulation

The relation of ACTH volume to body length in freshwater-reared fish is best described by one allometric equation including all age groups from one day up to seven months. In contrast to the allometric relation of PRL volume to body length, the allometric relations of ACTH volume to body length of fish reared in fresh water or salt water are not significantly different. When fish of the same size are compared, both groups have the same total volume of ACTH cells. A similar conclusion holds for the densitometric results: when fish are killed immediately after netting, the hormone content of the ACTH cells in fish from fresh water or salt water is the same. From this absence of differences in ACTH volume and hormone content of ACTH cells, we conclude that the functional demand on ACTH cells is similar in freshwater- or saltwater-adapted *C. whitei*. Consequently, ACTH cells are involved in osmoregulation of the fish in these environments to a similar extent. This contrasts with data from the literature, which indicate that the ACTH-interrenal axis is more important for osmoregulation in salt water than it is in fresh water. Interrenal activity (Benjamin and Ireland 1974) and blood cortisol levels (Singley and Chavin 1975; Redding et al. 1984a, b) in freshwater- and saltwater-adapted fish may therefore not be regulated by ACTH cells. An indication that ACTH is not essential for seawater adaptation is suggested by the observation that in *Anguilla japonica*, adaptation to seawater is only delayed but not prevented by hypophysectomy (Kamiya 1972). Apparently, the activation of the interrenals in eels (induced by seawater adaptation) is not mediated by ACTH. The stimulation of cortisol secretion by arginine-vasotocine and isotocine in dexamethasone-blocked *C. auratus* (Fryer and Leung 1982) may indicate a direct action of these neurohypophyseal peptides on the interrenal. Delayed seawater adaptation of hypophysectomized fish may therefore be related to the well-known regeneration of the neurohypophysis after hypophysectomy (Sathyanesan and Gorbman 1965).

However, as mentioned in the introductory paragraph, differences in ACTH cell activity have been described in fish from different salinities. On the other hand, Mattheij



and coworkers (1971) conclude that the moderate stimulation of ACTH cells in *Cichlasoma biocellatum* in salt-water is caused by non-specific stress. In other reports ACTH cell morphology has been studied after prolonged handling before fixation (Leatherland 1972; Goswami et al. 1983). Handnetting of *C. whitei* is followed by an instantaneous decrease of immunostaining intensity of the ACTH cells, indicating a release of ACTH. A stress-induced increase of ACTH in the blood has been found in *S. gairdneri* (Gilham and Baker 1985) and *O. kisutch* (Sumpter and Donaldson 1986). A rapid increase of serum ACTH has also been reported in *C. auratus* when salt is added to the aquarium with freshwater-adapted fish (Singley and Chavin 1975). Thus, the activation of ACTH cells in response to acute stress, including salinity changes, is well established. However, when *C. whitei* is handnetted twice a day for three weeks, the total volume occupied by ACTH cells does not increase significantly. The short-term responses of ACTH cells to handnetting are apparently insufficient to induce an increase in volume and number of these cells.

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