OBSERVATIONS ON GILLS OF PELAGIC AND DEMERSAL JUVENILE *NOTOTHENIA ROSSII*

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ABSTRACT. The gills of pelagic, blue-phase fingerling and demersal, juvenile *Notothenia rossii marmorata*, an Antarctic teleost sampled at the island of South Georgia were compared using light microscopy. The epithelium of the filament in demersal juveniles was thicker and contained more numerous and larger chloride and mucous cells. In juveniles, the lamellar epithelium was at least two cells thick and contained some mucous cells; fingerlings showed only a single layer of epithelial cells in the lamella. However, melanocytes were present under the epithelium of the filament in the fingerlings, but not in the juveniles. Abundant eosinophils and occasional rodlet cells were seen in the filaments of the juveniles only. The gill area/filament and minimal thickness of the water–blood barrier were determined for both groups and compared with the gills of some marine fish reported by others. There were no evident histological or morphometric specializations that could be related to the Antarctic environment.

INTRODUCTION

The basic anatomy of gills is similar among teleost species and is reasonably well understood (Hughes, 1980). Filaments extending from gill arches carry lamellae, which act as the gas-exchange surface in respiration. Furthermore, much ionic and osmotic regulation occurs in the gills. Species differences abound in the shape and number of cellular elements making up the gills.

Antarctic fish have adapted to a highly stenothermal, low-temperature environment. However, the well-oxygenated seas in which they live are quite stable, in that temperatures show relatively little annual variation (DeWitt, 1971).

Notothenia rossii marmorata is endemic to the Scotia arc of the Southern Ocean. Eggs of the species, laid in April–May on the continental shelf, hatch in September and October, with the pelagic larvae and post-larvae swimming offshore in the upper zones of the water column for five to six months. Between January and February the blue phase fingerlings, still pelagic but with visible scales and heavy pigmentation, enter the fjords of islands in the Scotia Arc and rapidly become demersal. By the end of April most of the fingerlings have developed into the brown phase, a demersal stage; the characteristic marbled pattern of the juvenile requires almost 15 months to develop from the larval stage. The animal remains a demersal juvenile for approximately five years before attaining sexual maturity and moving offshore to become a pelagic adult (Burchett, 1983*a*, *b*).

Morphological studies of gills of a few Nototheniiformes have been reported. They include those on the 'white-blooded' *Chaenocephalus aceratus* (Hughes and Byczkowska-Smyk, 1974; Steen and Berg, 1966) and *Champsocephalus esox* (Steen and Berg, 1966), both species lacking circulating erythrocytes and respiratory pigments, and on the 'red-blooded' *Trematomus borchgrevinki* (Boyd and others, 1980) and *Notothenia tessellata* (Steen and Berg, 1966), which possess circulating red blood cells. However, no histological information is available on possible morphological changes between pelagic and demersal phases of the same species.

The following study compares the gills from pelagic fingerling and demersal juvenile *N. rossii*. Some parameters of the gills are measured and the results correlated with activity levels and body weights.

Materials and Methods

Three pelagic blue phase fingerlings (Burchett, 1983*a*) and three demersal juvenile *Notothenia rossii marmorata* were collected from King Edward Cove, South Georgia, in 1978 and 1980. Lengths and weights of the fingerlings were: 3 cm and 0.7 g, 6 cm and 2.5 g, and 6 cm and 1.9 g. Weights of juveniles were 300 g (estimated), 268 g and 280 g (estimated).

Gill arches were excised and fixed in 10% formalin in dilute sea water before being transported to Canada. The gill tissue was dehydrated, embedded in 2-butoxyethanol methacrylate plastic (PolySciences, Inc.), and sectioned with glass knives on a Porter–Blum JB-4 microtome at 2–6 μ. Sections were stained in Harris's haematoxylin and eosin Y-orange G (H&E) for general oversight staining and in the periodic acid–Schiff reaction, counterstained with Harris's haematoxylin (PAS-H) (Humason, 1979) to demonstrate neutral polysaccharides.

Measurements were made under oil immersion of the thickness of the thinnest portion of the water–blood barrier, including epithelium, basement membrane and pillar cell flanges.

It was not possible to determine the total gill area. However, the lamellar area/filament for each fish was estimated using the formula of Hughes (1966), i.e. total area of lamellae/filament $= [2(L/d')]\, bl$, with modifications. The longest filament (L) on the section was measured with an ocular micrometer. At approximately midway along the filament, a region was chosen where the lamellae were longest and of equal length on either side of the filament; the heights of lamellae for each fish were measured using a camera lucida attachment and a Zeiss MOP-3, and averaged to derive b. The number of lamellae/mm on one side were counted to approximate $1/d'$. In the juveniles, the length (l) of the base of an unembedded lamella was measured with an ocular micrometer and found to be 1.07 mm. No unembedded gills of fingerlings were available; assuming that the ratio $1/b$ is constant in the two groups, (l) was estimated to be 0.48 mm for the blue phase fingerlings.

The lamellar area/filament was calculated for some marine fish (active, sluggish and Antarctic fishes) from the total gill areas reported in the literature. The results obtained here for *N. rossii* were compared with other marine species using a log/log plot of gill area/filament versus weight.

Results

Representative filament(s) with lamellae from fingerling and juvenile *N. rossii* are illustrated in Figs. 1 and 2. The epithelium covering the filaments is 3 or 4 cells thick

Fig. 1. Portion of a gill arch (GA) with primary filaments from which extend secondary lamellae. Melanocytes (arrows) are present in the primary filaments; blue phase fingerling. H&E. × 50.

Fig. 2. Primary filaments with secondary lamellae on which mucous cells (arrows) are seen; juvenile. PAS-H. × 50.

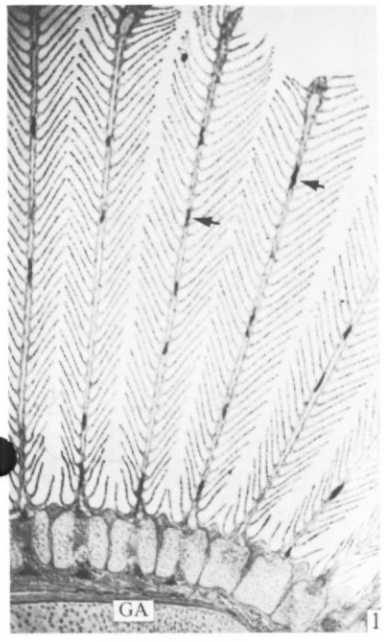
Fig. 3. Melanocyte (arrow) lying beneath epithelium of a primary filament. Chloride cells (c) and an occasional mucous cell (m) are present; blue phase fingerling. H&E. × 525.

Fig. 4. Multilayered epithelium of primary filament with mucous cells (m), chloride cells (c) and numerous eosinophils (e); juvenile. H&E. × 600.

Fig. 5. Secondary lamellae. Note the single layer of epithelial cells whose nuclei (arrows) lie over the pillar cell nuclei (p), a chloride cell (c) and erythrocytes (arrowhead) in the blood channels; blue phase fingerling. H&E. × 1 200.

Fig. 6. Secondary lamellae. Note the bilayered epithelium, whose nuclei may lie adjacent to the blood channel, mucous cells (m), and basal lamina (arrows); juvenile. PAS-H. × 1 200.

Fig. 7. Mucous cells (m) and a rodlet cell (r) in the epithelium of a primary filament; juvenile. PAS-H. × 1 600.



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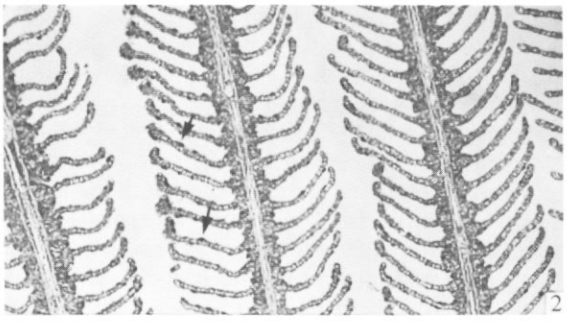
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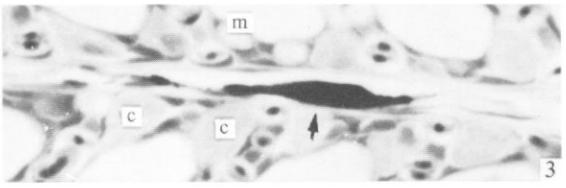

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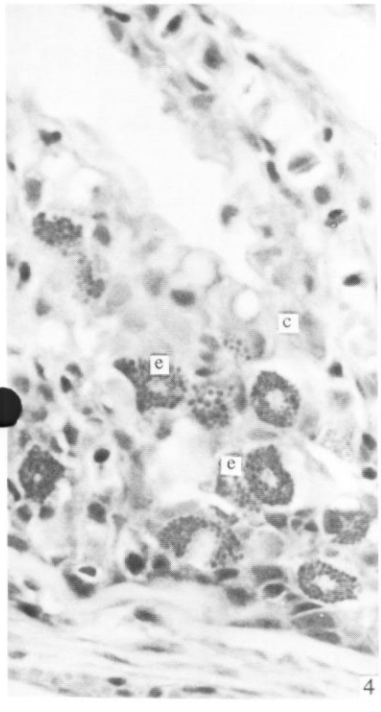

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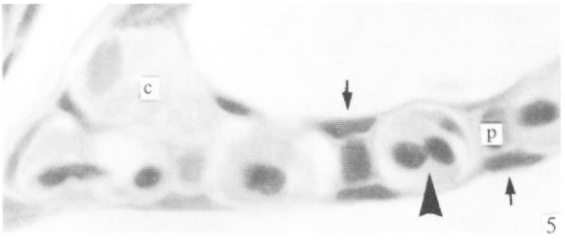

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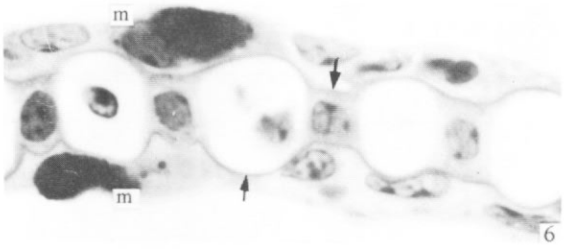

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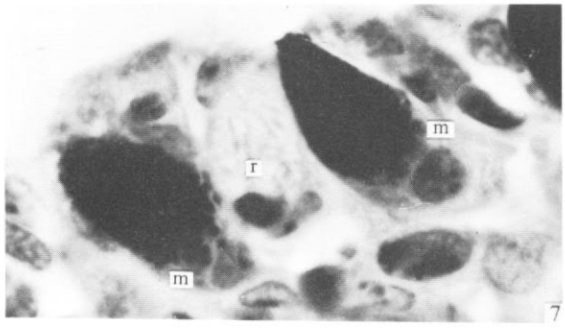

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in fingerlings (Fig. 3) and up to 10 cells thick in the juveniles (Fig. 4). In the lamellae of both groups of fish, a prominent basal lamina separates the epithelium from the blood channels formed by the boundaries of the pillar cell flanges and bodies (Figs. 5 and 6). The epithelium of the lamellae of fingerlings consists of a single layer of flattened cells, whose nuclei, typically, are situated over those of the pillar cells (Fig. 5). In juvenile fish, at least two layers of epithelial cells cover the lamellae; their nuclei, as well as mucous cells, may overlie the blood channels (Fig. 6).

Chloride, mucous, rodlet and granulated cells, as well as the squamous cells, are seen in the epithelium. Chloride cells, recognizable by their finely granular, eosinophilic cytoplasm and basal nuclei, are located on the filaments at the bases of the lamellae (Figs. 3, 4 and 5) in both groups of fish, but are much more numerous in the juveniles. In the juvenile *N. rossii*, the chloride cells are large and columnar and often found with prominent apical pits. In the fingerlings, the shorter and more rounded chloride cells (Figs. 3 and 5) are never seen associated with apical pits.

The mucous cells possess apical globules basophilic after H&E staining and strongly PAS-positive. In the juveniles, the large mucous cells are seen in the epithelium of the primary filaments (Fig. 7) and extend on to the lamellae (Fig. 6). In the fingerlings, the smaller mucous cells are scantily distributed on the filaments, and rarely found on the lamellae.

Rodlet cells, characterized by their basal nuclei, thick cell border and cytoplasmic rodlike structures, are occasionally observed in the epithelium of the filaments in juvenile fish (Fig. 7) but never in blue phase fingerlings.

Large cells with eccentric nuclei and cytoplasm filled with large round eosinophilic and PAS-negative granules are present in the epithelium of the filaments (Fig. 4), and occasionally in the lamellae of the juvenile only.

In each fingerling, flattened, elongate cells containing black pigment are observed in the connective tissue of the gill arch and extending along the filament just beneath the epithelium (Figs. 1 and 3); similar pigmented cells are not seen in the filaments of juvenile fish.

Table I. Gill areas (and dimensions measured) and minimal thickness of the water–blood barrier in two groups of *N. rossii*

	Fingerling			*Juvenile*		
	1	2	3	1	2	3
Weight (g)	0.7	2.5	1.9	300	268	280
Filament length, L (mm)	2.5	2.3	2.5	9.15	9.5	8.68
Lamellae/mm ($N = 10$)						
$1/d'$	23	24	24	15	15	14
Range	22–25	22–27	22–26	14–16	14–16	12–16
Height of lamellae, b (mm)	0.20	0.23	0.19	0.46	0.50	0.40
s.e.m.	±0.003	±0.004	±0.002	±0.01	±0.005	±0.008
N	49	47	50	50	50	23
Length of lamellar base, l (mm)	0.48*	0.48*	0.48*	1.07*	1.07	10.7*
Gill area/filament (mm²)	11.04	12.19	10.76	134.21	142.94	103.92
Minimal thickness of water–blood barrier ($N = 10$) (μm)	0.43	0.46	0.39	1.64	1.66	1.63
s.e.m.	±0.02	±0.02	±0.02	±0.12	±0.08	±0.08
Range	0.33–0.53	0.37–0.67	0.33–0.53	1.33–2.47	1.27–2.10	1.23–1.93

* Estimated.

Table II. Gill areas/filament of some marine active, sluggish and Antarctic fishes.

Species	*Body weight (g)*	*Area/filament (mm^2)*	*Ref.*
ACTIVE FISH			
Trachurus trachurus	12	5.42	1
	26	12.23	2
	40	19.57	1
	125	70.21	1
	135	80.91	1
Clupea harengus	11	5.27	4
	85	58.30	1
Gadus virens	1200	297.06	4
Gadus merlangus	51	20.04	1
Katsuwonus pelamis	3258	725.07	2
Thunnus thynnus	26600	3632.87	2
Scomber scombrus	800	174.29	4
	226	83.53	1
[illegible]mo trutta	705	66.67	1
Mugil labrosus	250	115.30	1
Brevoortia tyrannus	525	283.52	1
SLUGGISH FISH			
Acanthocottus scorpius	60	12.86	4
Taurulus [Cottus] bubalis	40	30.20	1
	52	21.24	1
Trigla gurnardus	17.8	3.24	1
Zeus faber	300	37.29	1
Lophius piscatorius	1550	143.93	1
Pleuronectes platessa	86	42.70	1
Callionymus lyra	24	8.40	1
	39	16.81	2
	46	19.97	1
	64	37.68	1
Opsanus tau	251	73.02	2
	305	70.20	1
	326	85.58	1
Platichthys flesus	370	147.00	4
Paralichthys dentatus	404	58.49	1
ANTARCTIC FISH			
Chaenocephalus aceratus	815	97.46	3
	860	54.13	2
	1040	213.17	4
	1227	121.79	3
	1820	165.77	3
Chaenichthys rugosus	450	39.79	3
Champsocephalus esox	66	43.05	4
Notothenia tessellata	50	27.55	4
Notothenia rossii	0.7	11.04	
	2.5	12.19	
	1.9	10.76	
	300	134.21	
	268	142.94	
	278	103.92	

(1, Hughes, 1966; 2, Hughes and Morgan, 1973; 3, Jakubowski, 1982; 4, Steen and Berg, 1966.)

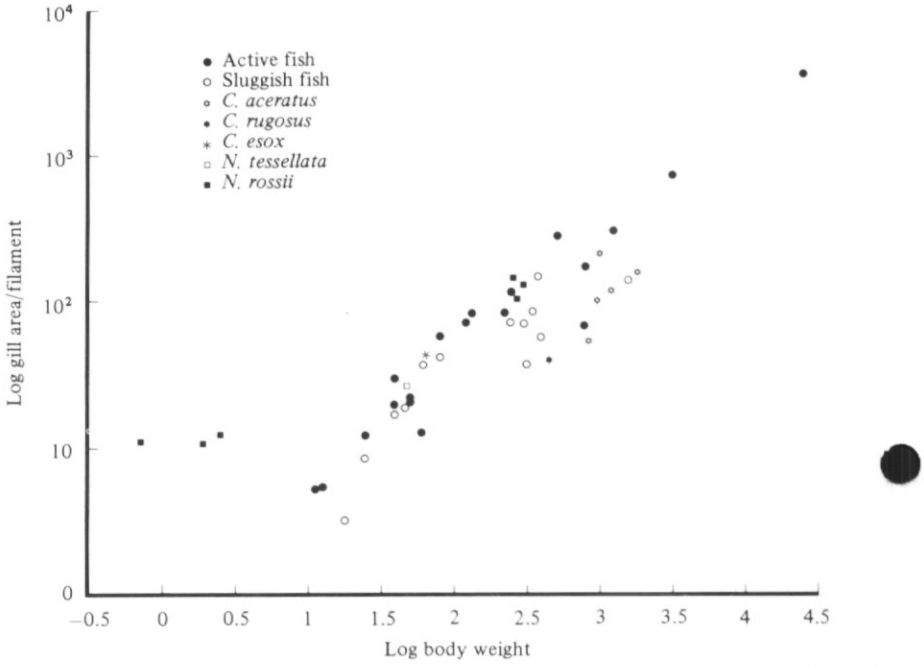

Fig. 8. Log/log plot to show the relationship of lamellar areas and body weights in some marine fish (active, sluggish, Antarctic) and *N. rossii*.

Gill lamellae are regularly arranged on the filaments in both fingerlings and juveniles. In fingerlings, the number of lamellae/mm filament (Table I) shows a mean of 24 as compared with 15 in the juveniles. The minimal thickness of the water–blood barrier (Table I) shows a mean of 0.43 μm in the fingerlings and 1.64 μm in the juveniles.

Tables I and II record the lamellar areas/filament for *N. rossii* and some other marine fish; the log/log plot of the areas/filament versus body weight gives a straight-line plot (Fig. 8). The value for juvenile *N. rossii* lie along the same general axis and in the range of the more active fish. The values for fingerling fish lie well above the axis.

Discussion

Gills function in respiration and as extrarenal osmoregulatory organs. Hughes and co-workers, in particular, have made extensive measurements of morphological parameters of gills from a variety of fish (e.g. Hughes, 1966, 1972, 1980; Hughes and Morgan, 1973). In general, more active fish possess larger gill areas/unit body weight, more and longer filaments with closer spacing of the smaller individual lamellae, and thinner water–blood barriers than more sluggish fish.

The tissue available in this laboratory precluded estimations of total gill areas and very reliable sampling methods for lamellar areas. On the assumption that lamellae measured were cut at right angles to the filament, measurements for the lamellae are minimal areas. Fig. 8 shows that the $\log_{10}$ lamellar area/filament versus body weight gives a straight-line plot similar to that of total gill area versus body weight reported by Hughes (1980) in teleosts. This implies that in the absence of more complete

morphometric information for the gills, measurements of filaments may provide some useful information.

The values for *N. rossii* juveniles lie in the same general area as those of previously measured species, suggesting that the Antarctic environment has not affected their ratios of gill area/body weight. The values for juveniles appear close to those of the more active marine fishes; values for fingerlings lie well above what might be expected. However, this may be related to size (age), since Hughes and Morgan (1973) pointed out the disproportionately larger gill areas/g in fish weighing less than 50 g.

Juvenile *N. rossii* possess 15 lamellae/filament and a minimal water–blood barrier of 1.64 μm; these figures are comparable to 17.5 lamellae/mm filament and a barrier of 2 μm reported by Steen and Berg (1966) for a 50 g *N. tessellata*, a known pelagic species (Moreno and Jara, 1984). Other Southern Ocean species for which measurements have been made include the sub-Antarctic *Champsocephalus esox* (66 g) and the Antarctic *Chaenocephalus aceratus* (1040 g), with a water–blood barrier of 1 μm and 6 μm and 18 and 8 lamellae/mm respectively (Steen and Berg, 1966). The differences between the figures for *C. aceratus* and the other Antarctic fish may reflect the sedentary habit of *C. aceratus* compared to the more active habits of *N. rossii*, *N. tessellata* and *Champsocephalus esox* (Moreno and Jara, 1984). Comparison of *N. rossii* with other teleosts, whose gill parameters are known (Hughes, 1972), indicates that *N. rossii* is more like active pelagic temperate zone species such as *Katsuwonus pelamis* and *Trachurus trachurus* in the thickness of the water–blood barrier, although the number of lamellae/mm is smaller.

There is no previously published information on water–blood barriers in larval or fingerling nototheniids. Their very thin water–blood barrier, as compared with that of the juveniles, may reflect their higher activity level during this pelagic phase, which could protect them from predators and enable them to compete effectively for food, or it may simply be related to their size (age), such a relationship having been shown by Hughes (1972).

The differences in thickness of the water–blood barriers of the two groups of *N. rossii* are reflected in the histology of the gills. In both fingerlings and juveniles the water–blood barrier consists of epithelium, a basal lamina and pillar cell flanges. In the fingerling the epithelium is one cell thick, and the epithelial nuclei are located over the pillar cell nuclei; thus, the epithelial component of the water–blood barrier consists of a thin layer of cytoplasm. In the juveniles the epithelium is at least two cells thick; their nuclei may lie adjacent to the blood channels and mucous cells are also present.

Chloride cells are recognized as being the site of NaCl secretion in the gills (Maetz, 1971). In both fingerling and juvenile *N. rossii* they are abundant. Boyd and others (1980) found that chloride cells were absent in lamellae but present on gill filaments in interlamellar pits in *T. borchgrevinki*.

Several functions have been ascribed to mucous cells, including facilitating gaseous exchanges across the water–blood barrier (Hughes, 1980) and protection against physical abrasion and bacterial action (Hughes and Wright, 1970). The more numerous mucous cells seen in the juvenile may relate to the thickened water–blood barrier; however, their presence on the secondary lamellae reduces the area available for gaseous exchange and contributes to the increased thickness of the barrier. Boyd and others (1980) reported that mucous cells are abundant in *T. borchegrevinki* and other Antarctic fishes.

The difference in size of both chloride and mucous cells of blue phase fingerling and juvenile fishes may simply reflect the ability of the thicker epithelium of the juvenile to accommodate larger cells.

Rodlet cells, whose function(s) and origin are unknown (Barber and others 1979;

Barber and Westermann, 1983) are found in a wide variety of freshwater and marine teleosts. Although Hughes and Byczkowska-Smyk (1974, plate IIIb) figured rodlet cells in the gills of *C. aceratus*, they interpreted them as a second type of mucous cell. The presence of rodlet cells in several ecologically isolated species of Antarctic fish is further evidence of their ubiquity, whatever their identity.

The cells with large granules seen in the filament epithelium of juveniles are similar in morphology and staining properties to eosinophilic granulocytes observed in haemopoietic tissues of the spleen and kidney (Westermann and Barber, unpublished observations). However, the epithelial granulocytes are larger and possess larger granules than blood eosinophils. Their origins and functions are unknown.

No histochemical tests were done to determine the identity of the pigments in the cells seen in the filaments of the fingerlings; we strongly suspect that these cells are melanocytes on the basis of their morphology but cannot suggest any function of them that would account for their presence in the fingerlings and their absence in the juveniles.

Comparisons of the various measurements made, as well as the histological structure, of the gills of *N. rossii* with some other fishes do not show recognizable specializations that can be directly attributed to the Antarctic environment.

Note: *Chaenichthys rugosus* Regan 1913, as used by Jacubowski (1982) has been subsumed under the name *Chaenichthys rhinoceratus* Richardson 1844 (Hureau, 1964).

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