ECOLOGY, OSMOREGULATION AND REPRODUCTIVE BIOLOGY OF THE WHITE STEENBRAS, *LITHOGNATHUS LITHOGNATHUS* (TELEOSTEI: SPARIDAE)*

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ABSTRACT

Over a one-year period 437 steenbras, *Lithognathus lithognathus*, ranging from 8–39 cm fork length were sampled from the Heuningnes River Estuary. The length-weight relationship was linear and there was no fluctuation in the modal size range throughout the year. Steenbras up to the age of six years and over inhabited the estuary, adapting to large salinity fluctuations. Abundance of food items, mainly Crustacea and Annelida, and virtual absence of predators made the estuary an ideal nursery ground. Ectoparasitic infestation by leeches and copepods was moderately intense without causing any apparent deleterious effects.

In a series of experiments designed to study osmoregulation in steenbras, it was found that haematocrits from fish sampled after 48 hours in freshwater were significantly ($P<0,01$) higher than the seawater controls. Two of the five protein fractions, however, as well as total protein, chloride, sodium, potassium and osmolality were all significantly ($P<0,01$) decreased in freshwater. Steenbras were unable to survive more than one week in freshwater. Due to capture diuresis the plasma constituents from a freshly captured sample were all significantly ($P<0,01$) higher when compared to steenbras acclimated for 48 hours in seawater.

Gonads from the entire estuarine sample were all infantile, virtually impossible to sex and showed no macroscopic signs of development. Histology of a representative sample showed them to be all hermaphroditic, with mainly testicular-dominant ovotestes. Maturity indexes assigned on the basis of ovarian and testicular development gave an indication of a cyclic rhythm in activity during prematuration, with a June peak. Marine steenbras ovotestes, on the other hand, were large and could be accurately sexed macroscopically. Histology of 42 gonads again revealed a majority of testicular-dominant hermaphroditic ovotestes. Maturity indexes indicated peak activity in June and a minimum in September, suggesting a spawning period in July and August.

Sex-chromatin (Barr) bodies were detected in liver nuclei of ovarian-dominant ovotestes, but were almost entirely absent from testicular-dominant ovotestes. Prediction of the genetic sex against the actual dominant macroscopic sex, based on the presence or absence of Barr bodies in a sample of steenbras liver sections proved very accurate. This is the first record of sex-chromatin occurring in teleost tissue.

There was no indication of sex-reversal, the steenbras remaining permanently hermaphroditic throughout the study year. It is postulated that the steenbras is a rudimentary hermaphrodite with only one of the two sexes functioning throughout the individual's life span. This is possibly a transitionary stage towards eventual gonochorism.

INTRODUCTION

Although the white steenbras, *Lithognathus lithognathus* Cuvier and Valenciennes 1830, is one of the most widely distributed and popular angling fish in South African waters (Biden 1930), there has been no previous account of its biology apart from Lucks (unpubl.) who made a study of *Lithognathus aureti*, the white steenbras from South West Africa. The taxonomy of the four

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species of *Lithognathus* from the South African coast has been thoroughly described by Smith (1938, 1962) and Penrith and Penrith (1969). The ecology of many South African estuaries has been extensively covered: Day (1951, 1959, 1967); Day, Millard and Harrison (1952); Scott, Harrison and McNae (1952); Day, Millard and Broekhuysen (1954); Millard and Harrison (1954); Millard and Scott (1954), but except for Talbot (1955), only scant detail has been recorded for the teleost fauna of these estuaries. In the present investigation, the ecology of the steenbras in the Heuningnes River Estuary has been studied in an attempt to supplement the information available on estuarine fish life. The steenbras exhibits the characteristics of a typical estuarine fish spending its first years maturing in the estuaries and then migrating seaward to spawn. A special attempt has therefore been made to determine whether the steenbras required protection during any particular stage in its life cycle, to ensure the future abundance of this species.

Atz (1964) reviewed the phenomenon of hermaphroditism found in a few teleost families and concluded that the type of hermaphroditism found in the Sparidae is more complex than in any of the others. Upon first consideration it would appear that the steenbras exhibits several possible types of hermaphroditism. I have studied the histology of estuarine and marine steenbras ovotestes in detail and the major part of the present investigation has been devoted to an attempt to compare these results with the work of earlier investigators and to determine the previously unknown breeding season.

The steenbras is apparently a good osmoregulator in its natural environment, for its presence has been recorded from predominantly freshwater lakes which had become cut off from their estuarine water source for a considerable time. A series of experiments was therefore devised to study the changes occurring in some of the plasma constituents of the steenbras, to determine if this typically marine fish could successfully adapt to an artificial freshwater environment.

Detailed procedures and results are described in the different sections.

HABITAT

This section gives the results of a survey of some of the physical conditions in the Heuningnes River Estuary and a brief outline of the physical conditions of the Strand, False Bay, from where the marine samples were obtained.

PROCEDURE

Monthly surface water samples were collected at stations 1, 2 and 3 (Fig. 1) in the course of the estuary for twelve consecutive months. The water was collected in air-tight plastic bottles, and the chlorinities determined in the laboratory by the silver nitrate titration method. The salinities were then read off from standard tables (Strickland and Parsons 1968). Water osmolalities were determined with a Hi-precision research advanced osmometer, model 67–31 RAS (Advanced Instruments, Inc.). Surface water pH readings were measured at each station, using a Lovibond comparator and B.D.H. indicators, Thymol Blue and Cresol Red. Surface water temperatures were recorded to the nearest 0,5°C at each station, being the average of three readings (morning, noon, and night).

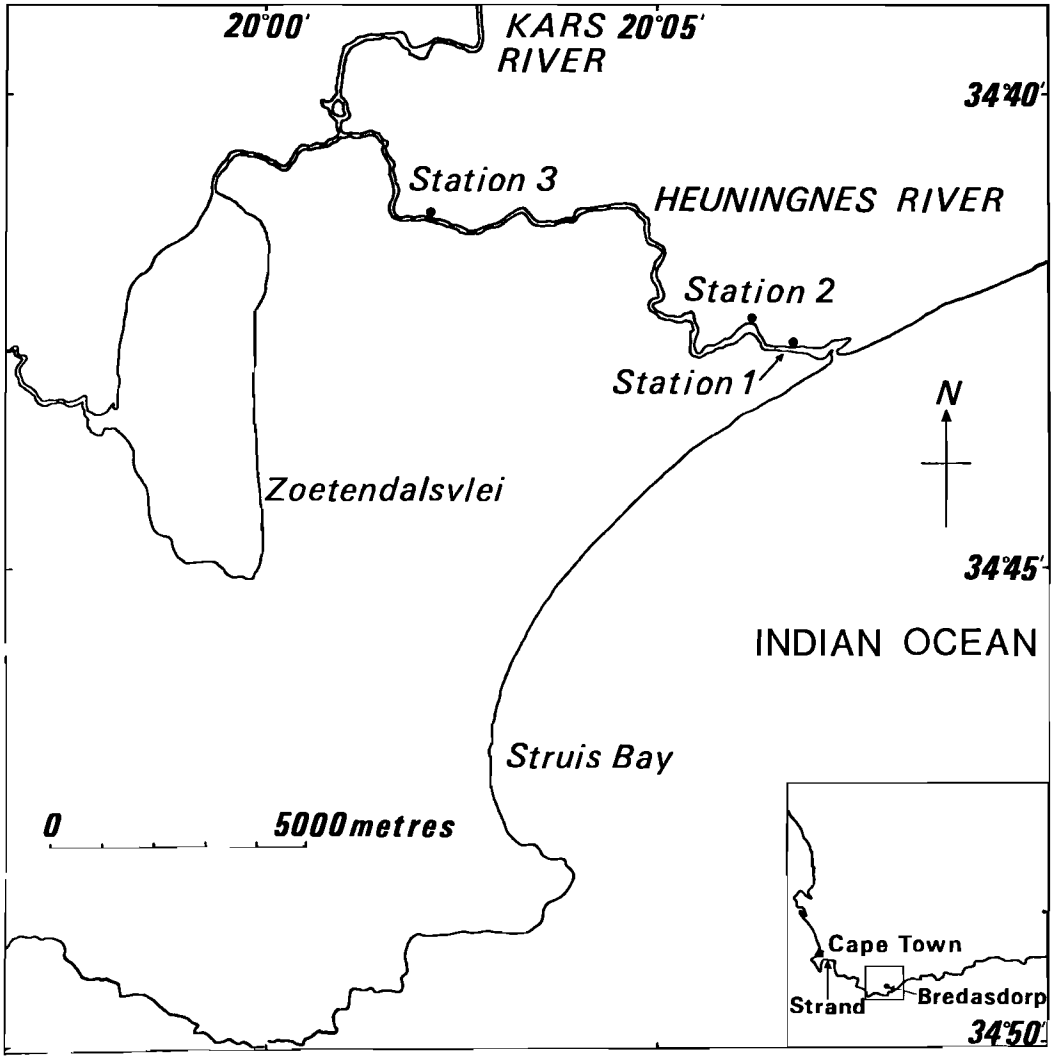

FIGURE 1

The Heuningnes River Estuary, based on sheets 3419 DB and 3420 CA of the 1:50000 topographical series of South Africa.

DESCRIPTION OF THE ESTUARINE SYSTEM

The Heuningnes River Estuary is situated about 24 km from Bredasdorp, southern Cape Province (Fig. 1). The river extends approximately 16 km inland, running from West to East. It drains an area of sandy waste for 2 km from the mouth, to a flat farming district for the rest of its course. It receives a moderate rainfall (Table 1), most of which falls in the winter months from April to August. During different periods the river may be subjected to severe drought, or to winter flood conditions.

TABLE 1

AVERAGE ANNUAL RAINFALL 1931–1960 AND TOTAL RAINFALL DURING THE STUDY YEAR 1971, AT "DIE MOND" (STATION 2), BREDASDORP

Year	*Jan.*	*Feb.*	*Mar.*	*Apr.*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*	*Total (mm)*
1931–60	11,5	24,5	29,2	47,0	70,6	52,5	56,4	63,9	26,4	36,2	19,3	18,4	455,9
1971	0,0	15,0	21,7	57,0	33,5	45,0	73,5	60,5	27,0	22,0	36,7	40,5	432,4

Most of the water in the estuary comes from the sea, the narrow mouth opening permanently into Struis Bay. During normal conditions the estuarine system is very stable and the tidal effects are evident for about half the length of the river. In the upper reaches tidal flow seems to be very limited, and the salinity here is considerably higher than at the mouth, due probably to a small inflow of fresh water. During winter flood periods the river may receive some water from Zoetendalsvlei and a considerable overflow from the Kars River. At these times the estuarine conditions are drastically altered. The tidal inflow from the sea can make no impression at all on the estuary and the fast-flowing muddy water extends right down to the mouth and out to sea for a considerable distance.

Three stations along the estuary and river were chosen as sampling points, their locations are shown in Fig. 1.

Station 1: A point approximately 1,5 km from the mouth, in a broad channel about 10 m in depth at high tide, with a sandy substrate and clear water. Clean sand lines both banks right down to the mouth. There is no vegetation.

Station 2: "Die Mond", a point approximately 3 km from the mouth, in a narrow channel about 10 m deep at high tide, generally with turbid water. Mud banks extend down to the water on both sides and some *Phragmites* sp occurs at the water's edge.

Station 3: Riverside Bridge, approximately 9,5 km from the mouth, in a narrow strip of very muddy water, about 5 m in depth at low tide. Both muddy banks are lined with tall *Phragmites* sp which extends down into the water.

RESULTS AND DISCUSSION

Salinity

The salinity records for 1972 (Fig. 2) showed a considerable variation due to tidal and seasonal changes.

Station 1 showed a stable salinity close to that of normal seawater (35‰) up to July, and station 2 closely followed this pattern. The slightly higher salinities during January to March were probably due to a slightly decreased tidal effect and increased evaporation at the time of sampling. The results suggest a full tidal effect at stations 1 and 2. Station 3 salinities were consistently higher than at the other stations from February to June, which suggests that tidal

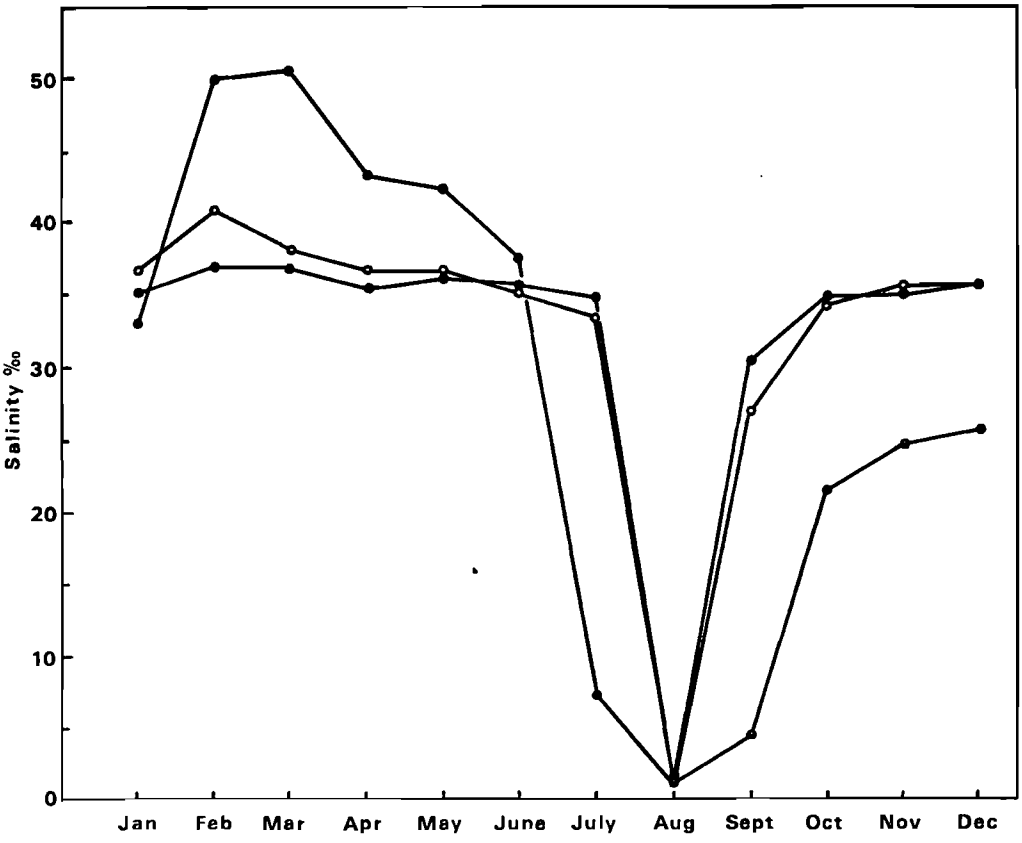

FIGURE 2

Monthly surface water salinities for 1971, from three stations on the Heuningnes River Estuary.
● Station 1
○ Station 2
◒ Station 3

movement had little effect on the upper reaches during this period of drought. The figures indicate a reversed salinity gradient up to July, the salinity increasing from the seaward end to the upper reaches. This was probably the result of evaporation exceeding freshwater inflow, the seawater additions at the mouth keeping salinities down in that area, but having little effect on the upper reaches. Such results are typical of drought conditions and have also been observed in the St. Lucia Estuary (Day 1951; Day, Millard and Broekhuysen 1954).

During July the first effects of an inflow of freshwater from the Kars River into the upper reaches of the Heuningnes River were observed. The salinity at station 3 fell dramatically from 37,13‰ to 7,67‰. In August the full effect of the flood waters sweeping down to the sea was reflected in a drastic drop in salinity at all three stations. At this stage the estuarine water was virtually fresh and the tidal inflow had no effect. From the beginning of September stations 1 and 2 made a quick recovery to normal salinity conditions, and while station 3 followed this trend, it was at a slower rate. This phenomenon has also been observed in the St. Lucia estuary (Day 1951; Day, Millard and Broekhuysen 1954). These results supported the assumption that tidal flow affected the salinities at stations 1 and 2, but had virtually no effect at station 3.

Lucks (unpubl.) recorded a very stable yearly salinity range for *L. aureti*, 34,98–35,43‰ for Sandwich Harbour Lagoon, South West Africa, and a slightly lower but still very stable range, 34,93–35,28‰, from a routine station 16 km west in the open sea. While the latter range compares favourably with that for the Strand (Table 3) the conditions in the lagoon at Sandwich Harbour show none of the fluctuations recorded from the Heuningnes River Estuary.

Osmolality

The osmolalities, a measure of the total dissolved substances in water, showed an identical trend to the salinity readings at all three stations (Fig. 3).

Day (1951) defines an estuary as that part of a river system where there is an appreciable variation of salinity due to the sea, and where the composition of the dissolved salts is essentially similar to that of the sea. The above results comply with his definition.

Hydrogen ion concentration (pH)

The pH readings at all three stations for the year indicated that alkaline conditions existed for the river system. Station 1 recorded 8,0 in August and station 2 recorded 8,6 in December, but these were isolated results. For all the other months the pH readings were remarkably constant at 8,2–8,4, which is close to the normal pH value of seawater 8,1–8,3 (Day 1951).

Day states that an excess of basic radicles, together with some weak acid radicles, gives seawater its buffering characteristics. River waters are seldom buffered so that larger variations will be found at the head of an estuary. My results show that the Heuningnes River is in fact buffered by seawater and the pH range recorded is within the range described for another southern Cape estuary, the Klein River, Hermanus (Scott, Harrison and McNae 1952).

Temperature

Table 2 records the average monthly surface water temperatures for the three stations. The range of 12–24°C is close to that observed for the Klein River Estuary, and is well within that

recorded for bodies of water along the coastal strip of the western Cape Province, 12–28°C (Scott, Harrison and McNae 1952).

Lucks (unpubl.) recorded a yearly temperature range of 12,5–22,9°C from the Sandwich Harbour Lagoon, South West Africa, which is comparable to that recorded for the Heuningnes River Estuary.

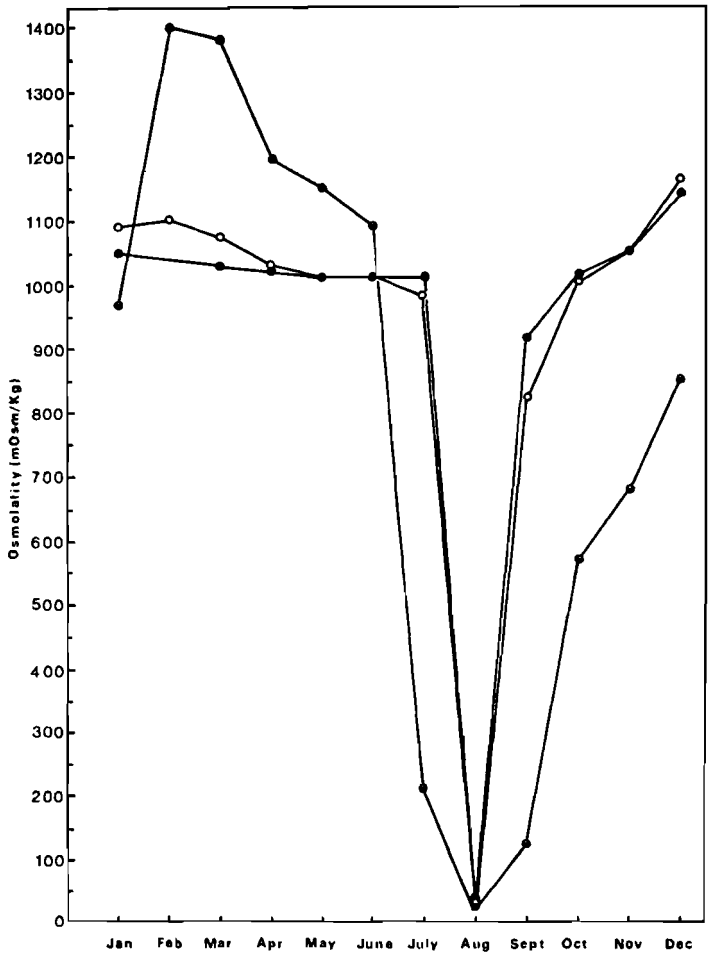

FIGURE 3

Monthly surface water osmolalities for 1971, from three stations on the Heuningnes River Estuary.

● Station 1
○ Station 2
◒ Station 3

TABLE 2

AVERAGE MONTHLY SURFACE WATER TEMPERATURES (°C) FOR 1971, RECORDED FROM THE THREE STATIONS ON THE HEUNINGNES RIVER ESTUARY

Station	*Jan.*	*Feb.*	*Mar.*	*Apr.*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1	24,0	19,0	19,0	18,5	15,0	14,5	13,5	12,0	16,0	16,0	19,5	18,5
2	24,0	20,0	20,0	19,0	14,5	14,0	13,5	13,5	17,5	16,0	23,0	21,5
3	24,0	21,0	21,0	19,0	14,0	14,5	13,5	15,0	16,5	16,0	22,0	19,5

Description of marine environment

Lucks (unpubl.) recorded a yearly temperature range of 12,6–17,1°C from a routine station at sea, off South West Africa, which is a range only slightly lower than that recorded from the Strand coastline in False Bay (Table 3).

TABLE 3

AVERAGE SURFACE WATER TEMPERATURES AND SALINITIES FROM "THE STRAND", 1961–1963. FROM "SARDINOPS" SURVEY, STATION 1144, 34°12'S/18°46'E, (FIGURES FROM RECORDS OF THE DIVISION OF SEA FISHERIES, CAPE TOWN)

	Jan.	*Feb.*	*Mar.*	*Apr.*	*May*	*June*
Temperature (°C)	19,64	16,29	15,52	15,33	14,59	14,51
Salinity (‰)	35,05	34,93	34,92	34,91	34,97	34,91
	July	*Aug.*	*Sept.*	*Oct.*	Nov.	Dec.
Temperature (°C)	14,55	14,56	15,13	15,46	16,79	16,88
Salinity (‰)	35,22	34,92	35,15	35,17	35,27	35,11

NUTRITION

The records of fauna and flora occurring in South African estuaries are remarkably complete, having been extensively studied by those authors mentioned in the Introduction. Numerous factors are responsible for determining the nature of the fauna and flora to be found in any parti-

cular estuary and these have been outlined by Day (1951). It is not the intention of this survey to elaborate on those factors, but rather to outline those food items which constitute the diet of the steenbras in the Heuningnes River. It is hoped the results will provide a guide-line to any future detailed ecological study of the estuary.

It is a generally accepted fact that those teleost species which spend the early years of their lives in estuaries, do so principally for the variety and abundance of food items present there, as well as for protection from large predators. The stomach contents of steenbras in the Heuningnes River were therefore recorded to analyse the nutritive qualities of the estuary, and to explain the presence of the large numbers of steenbras there.

PROCEDURE

The stomachs and intestines of 437 steenbras sampled over a one-year period from the Heuningnes River Estuary, were dissected out and preserved whole in a 5 per cent formalin solution for laboratory analysis of food items.

RESULTS AND DISCUSSION

Figure 4 shows the relative occurrence of the main food types from all three stations. The data were calculated as the number of fish per monthly sample containing a particular food item and expressed as a percentage. Table 4 shows the classification and relative abundance of all the food items from steenbras stomachs captured at the three stations.

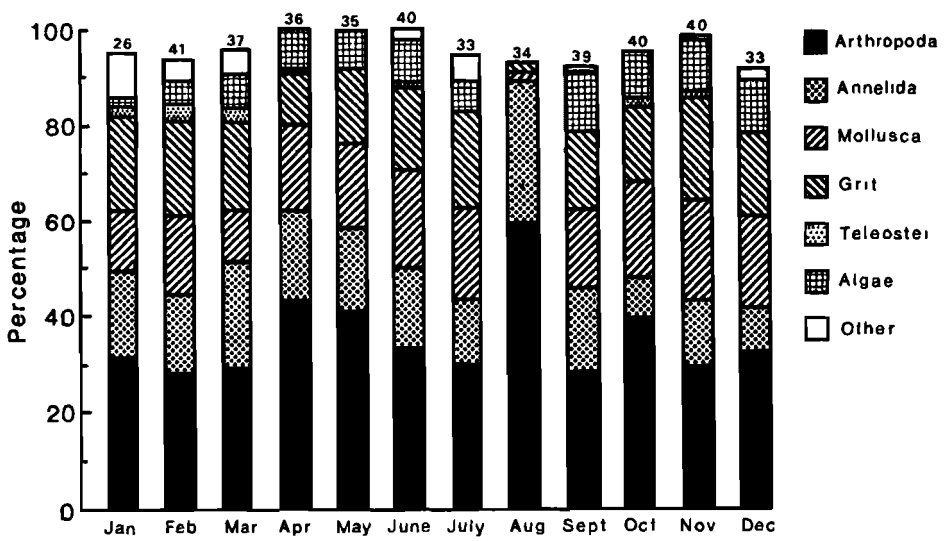

FIGURE 4

Number of estuarine steenbras, expressed as a percentage, containing various food items on a monthly basis. Where percentages do not add up to 100 per cent, the differences represent empty stomachs. Numbers above each column indicate number of fish examined in each month.

TABLE 4

FOOD ITEMS FROM 437 ESTUARINE STEENBRAS STOMACHS. N = NUMBER OF STOMACHS EXAMINED FROM EACH STATION. R = RARE; P = PRESENT; C = COMMON; A = ABUNDANT. (IDENTIFICATION BASED MAINLY ON DAY 1969)

Species	*N = 315 Station 1*	*N = 112 Station 2*	*N = 10 Station 3*
ALGAE:			
Chlorophyceae			
Enteromorpha sp	A	A	P
Ulva sp	A	A	
Rhodophyta			
Arthrocardia sp	R	P	P
PROTOZOA:			
Sarcodina			
Foraminifera sp.	P	R	P
ASCHELMINTHES:			
Nematoda			
Contracaecum sp	P	R	
Acanthocephala			
Rhadinorhynchus sp.	R		
ANNELIDA:			
Polychaeta			
Arenicola loveni	A	P	
Eunice sp	C	R	
Lumbrinereis sp	R		
Nereis sp	C	R	
Pomatoleios kraussii	P	C	
Sthenelais sp	R	R	
Unidentified remains	A	C	P
Echiurida			
Ochaetostoma capense	R		
Sipunculida			
Golfingia capensis	P		
CRUSTACEA:			
Cirripedia			
Chthamalus sp	R		
Amphipoda			
Ampithoe ramondi	A	A	P
Tanaidacea			
Leptochelia sp	R	R	
Macrura			
Betaeus jucundus	R	R	
Palaemon pacificus	R		
Anomura			
Callianassa kraussi	A	C	
Diogenes brevirostris	C	R	
Upogebia africana	C	R	

Brachyura			
Cleistostoma edwardsii		C	
Cyclograpsus punctatus	R		
Hymenosoma capensis	A	C	
Thaumastoplax spiralis	A	A	
INSECTA:			
Unidentified pupae or larvae	A	A	P
MOLLUSCA:			
Amphineura			
Ischnochiton sp	R		
Pelecypoda			
Kellya? sp	C	A	
Psammotellina capensis	C	P	
Tellina sp	P	C	
Gastropoda			
Assiminea globulus	A	A	P
Haminea alfredensis	R	R	
Rissoa capensis		C	
Scala sp	P	C	
Siphonaria capensis	R		
Tricolia capensis	R		
ECHINODERMATA:			
Echinoidea			
Echinocardium cordatum	R		
Parechinus angulosus	C	R	
Holothuroidea			
Epitomapta knysnaënsis	R	R	
UNIDENTIFIED SHELL FRAGMENTS: "Grit"	A	A	P
CHORDATA:			
Pisces			
Psammogobius knysnaënsis	P		
Trulla capensis	R	R	

From Fig. 4 and Table 4 it is evident that the diet of the steenbras is a varied one and that the Heuningnes River provides an abundant choice of food items. The composition of the diet was fairly constant throughout the year, the main food items occurring in approximately equal proportions in each monthly sample. The most common food items throughout the year were the crustaceans *Callianassa kraussi* (sand prawn); *Ampithoe ramondi* (amphipod); *Hymensoma capensis* and *Thaumastoplax spiralis* (brachyuran crabs); many unidentified insect larvae and pupae; the algae *Enteromorpha* sp and *Ulva* sp; the gastropod *Assiminea globulus;* numerous polychaet worms; and a large quantity of unidentifiable shell fragments designated "grit". The latter was probably present due to the feeding habits of the steenbras which include sucking and blowing in the sand with their protrusible lips. Lucks (unpubl.) recorded mussels (*Mytilus* spp), zooplankton (*Ampelisca* spp) and polychaetes as the main food items for *Lithognathus aureti* from South West Africa.

With the advent of flood conditions in August, the only types of food items present were the burrowing sand-prawn *Callianassa kraussi* and a few other crabs, many species of poly-

chaets, including *Arenicola loveni*, and algae. In an estuarine system where the salinity is continually subject to changes, only those animals which have adapted an efficient osmoregulatory mechanism, will be able to withstand drastic changes in salinity (Day 1951). The best examples are the sandprawn *C. kraussi*, which has a known salinity range of 1,25–59,5‰ (Day 1951); other species of crustacea, for example *Cyclograpsus punctatus*, known to tolerate salinity as low as 9,6‰ (Scott, Harrison and McNae 1952); the algae *Ulva* sp and *Enteromorpha* sp (Day 1951); and the polychaetes, for example *Arenicola loveni* (Wells and Ledingham 1940, *in* Day 1951). My results for August confirm the findings of these authors.

It is apparent from the variety of food items that the Heuningnes River Estuary provides an abundant and varied diet for steenbras. Steenbras are present in the river throughout the year in large numbers and it is obvious that this estuary provides an ideal nursery ground for the younger fish.

PARASITISM

There has been no previous record of parasites from the steenbras, *Lithognathus lithognathus*. Lucks (unpubl.) recorded two ectoparasites from *L. aureti* in South West African waters, a monogenic trematode occurring on the fins, and a peracarid crustacean on the gills. Euzet and Oliver (1967) have also recorded monogenic trematodes from the gills of various Sparidae.

PROCEDURE

Ectoparasites were individually counted, and their distribution on individual estuarine steenbras noted. Representative samples were relaxed in pure acetic acid for 30 seconds, washed for several minutes in distilled water, fixed and preserved in 70 per cent alcohol for later identification.

RESULTS AND DISCUSSION

Two ectoparasites were recorded from the steenbras. A copepod (Order Lernaeopodoidea) was found attached mainly to the gill filaments and to a lesser extent on the head and fleshy lips. A single large leech, *Malmiana stellata*, occurred in the buccal cavity of one steenbras, but a smaller leech *Marsupiobdella africana*, occurring in the buccal cavity and over the head of the steenbras, was far more common.

Table 5 shows the monthly percentages of parasitized steenbras. Table 6 shows the average number of both the parasites per fish and the position on the host. Nearly all the steenbras were parasitized by both copepods and leeches. Although the copepod showed a preference for the gills, there was no preference for any of the gill arches on either the left or right sides, the numbers being evenly distributed over all the gill filaments.

In contrast, Tedla and Fernando (1970) found that the copepod, *Ergasilus confusus*, parasitizing the gills of the yellow perch, *Perca flavescens*, occurred in larger numbers on the second and third gill arches than on the first and fourth. Furthermore, both the incidence and intensity of infestation of *E. confusus* were positively correlated with the size of the fish.

Likewise, in the steenbras the leech preferred the buccal cavity, but occasionally occurred in larger numbers on the head of a particular specimen. The expected trend of increased infestation with increasing length was not apparent. Often, the larger fish had very few parasites, while the smaller ones showed a heavy infestation. Lucks (unpubl.) found that only two or three parasites occurred on each, usually mature, specimen of *L. aureti*. He found a 4–7 per cent incidence of parasitism during winter and spring, but none during summer and autumn. Clearly there is no basis for a comparison of the parasites occurring on *L. aureti* and *L. lithognathus*.

The flesh and abdominal cavity of *L. lithognathus* showed no evidence of any endo-parasitic infestation.

The flood conditions in August had drastic results on the copepod infestation, virtually eliminating these parasites during the subsequent months (Table 5). In contrast, the leeches increased substantially during this period. This result would seem to indicate that the copepod

TABLE 5

PERCENTAGE INCIDENCE OF COPEPOD AND LEECH PARASITISM IN ESTUARINE STEENBRAS BY MONTHS, 1971

Month	*Number of fish sampled*	*Total percentage of fish parasitized (Copepod and leech)*	*Percentage parasitized fish with*	
			Copepods	*Leeches*
April	36	53	53	Not recorded
May	35	51	34	34
June	40	67	60	32
July	33	85	76	61
Aug.	34	56	62	21
Sept.	39	79	5	77
Oct.	40	77	2	75
Nov.	40	87	10	85
Dec.	33	79	0	79

was not an efficient osmoregulator and that the great reduction in salinity caused their disappearance. Conversely, the leech, perhaps more dependent on its host, would appear to be a very good osmoregulator and able to withstand fluctuating salinities.

Although *Marsupiobdella africana* is a typical freshwater leech, it is not unusual for it to occur in estuaries. This leech is known to be parasitic on amphibians, although it may use freshwater crabs as transport hosts (*pers. comm.* J. H. Oosthuizen). As several specimens of *M. africana* were observed to have fed on blood, the evidence would seem to indicate that it is parasitic on the steenbras. Due to the large numbers of leeches found, it is unlikely that their presence can be solely attributed to the chance infestation as a result of the fishes' feeding habits.

Mann (1962) records that leeches have an osmotic pressure of body fluids considerably higher than that of the water in which they live, so there must be a constant inward flow of water

TABLE 6

AVERAGE NUMBER OF COPEPODS AND LEECHES PER PARASITIZED FISH, OCCURRING IN DIFFERENT POSITIONS ON ESTUARINE STEENBRAS. N = NUMBER OF PARASITIZED FISH IN EACH PARTICULAR GROUP

Month	*Copepods*				*Leeches*			
	N	*On gills*	*N*	*On head*	*N*	*In buccal cavity*	*N*	*On head*
June	21	4,2	14	2,1	10	1,5	1	1,0
July	17	4,1	15	1,9	14	2,0	13	1,6
Aug.	15	5,1	13	1,5	6	1,0	3	1,3
Sept.	1	1,0	1	1,0	25	2,8	25	2,6
Oct.	0	0	1	1,0	27	3,8	17	2,4
Nov.	4	2,3	3	1,0	28	2,8	26	2,6
Dec.	0	0	0	0	24	4,3	19	4,4

through the integument. He considers it unlikely that the nephridia are completely efficient in removing excess water while retaining as many as possible of the inorganic ions, so that there is a steady drain on the salt content of the coelomic fluid which must be replaced. However, Krogh (1939, *in* Mann 1962) showed that *Haemopis*, a freshwater leech, has an efficient salt uptake mechanism in the epidermis and was able to take up sodium and chloride ions simultaneously. Thus it appears possible that the leeches occurring on steenbras in the Heuningnes River were able to osmoregulate sufficiently well to adapt to the changing salinity conditions.

The infested fish did not appear to be suffering any ill effects from the presence of parasites, irrespective of the degree of infestation.

No records were kept of any parasites occurring on the marine samples of *L. lithognathus*. I have, however, noted the presence of the copepod (Order Lernaeopodoidea) on some marine individuals both on the head and on the gill filaments, usually in small numbers, but leech infestation was entirely absent.

GROWTH

Apart from Talbot (1955), Lucks (unpubl.) and Penrith (1972), no previous record of growth has been made for the Sparidae from South African waters. In the present investigation the length/weight relationship of estuarine *L. lithognathus* was determined and the length and weight frequencies compared against age, the latter determined from scale and otolith readings.

PROCEDURE

A total of 437 steenbras, ranging in size from 8–39 cm fork length, were collected in monthly samples of approximately 35 fish from the three stations on the estuary over a one-year period,

January–December, 1971. Most of the samples were taken by means of a trek-net, while use was also made of angling. Gill-netting provided relatively few fish. A total of 68 marine samples, ranging from 25–83 cm fork length, were caught by rock anglers of the Western Province Angling Clubs along the Strand coastline of False Bay. The availability of these samples fluctuated greatly with weather conditions and season, and some monthly quotas could not be met. Fish were measured to the nearest centimetre fork length and weighed to the nearest gram. Six scales were removed from the left side of each fish from an area just posterior to the pectoral fin and just below the lateral line, washed in water, any foreign matter rubbed off between the thumb and forefinger, and placed in numbered envelopes. In the laboratory, the scales were mounted between two glass slides and the annual rings counted using an enlarging projector. By making a transverse cut anterior to the first vertebra and snapping the head back (after Baird 1970), the two otoliths (sagittae) were removed with forceps, washed, and placed in numbered envelopes. In the laboratory, the untreated otoliths were immersed in a small dish of water and the annual rings counted using a binocular microscope.

RESULTS AND DISCUSSION

Sampling methods

In sampling estuarine fish a 4,5 cm mesh trek-net gave the best results, both for size-range and quantity, and was used throughout the year at station 1. Angling, using the sandprawn *Callianassa kraussi*, and occasionally the bloodworm *Arenicola loveni* as bait, supplemented the catches mainly from station 2. Use of gill nets was discontinued at all stations after a few months, due to the very poor returns per effort required to set and recover the nets.

TABLE 7

TOTAL NUMBER OF STEENBRAS SAMPLED AT THE THREE STATIONS ON THE HEUNINGNES RIVER, 1971, AND THE METHOD OF CAPTURE EMPLOYED

Capture method	*Stations*		
	1	*2*	*3*
Trek net	274	8	—
Angling	29	103	—
Gill nets	12	1	10
Totals	315	112	10

The best conditions for catching marine steenbras appear to be during a south-west wind which has been blowing for a few days preceded by a 3–4 day spell of a south-east wind. This has the effect of producing a strong swell which stirs up the sandy bottom and exposes the favourite food items, namely sandprawn and polychaetes. Anglers by casting their lines, baited with sandprawn (*Callianassa kraussi*) or bloodworm (*Arenicola loveni*), into the clearer patches of water, are usually very successful in these ideal conditions (*pers. comm.* W. S. Morries, False Bay Angling Club).

Effect of season on size distribution

The length frequency of any species is influenced by the fact that many species of fish have a relatively constant and limited spawning season each year, so that the population consists of a series of age groups each with its own size range. When the frequency distribution of length is plotted these age groups are indicated by modes in the distribution. Fig. 5 shows the length frequencies of estuarine steenbras plotted on a monthly basis for one year. Although some modes are apparent there was no significant shift of the modes during any of the months and thus no fluctuation in the size distribution with changing seasons. This is surprising, for one would expect the shift of a predominant single mode in successive months, thus giving an indication of the average size increase of fish. The relatively small monthly sample and the

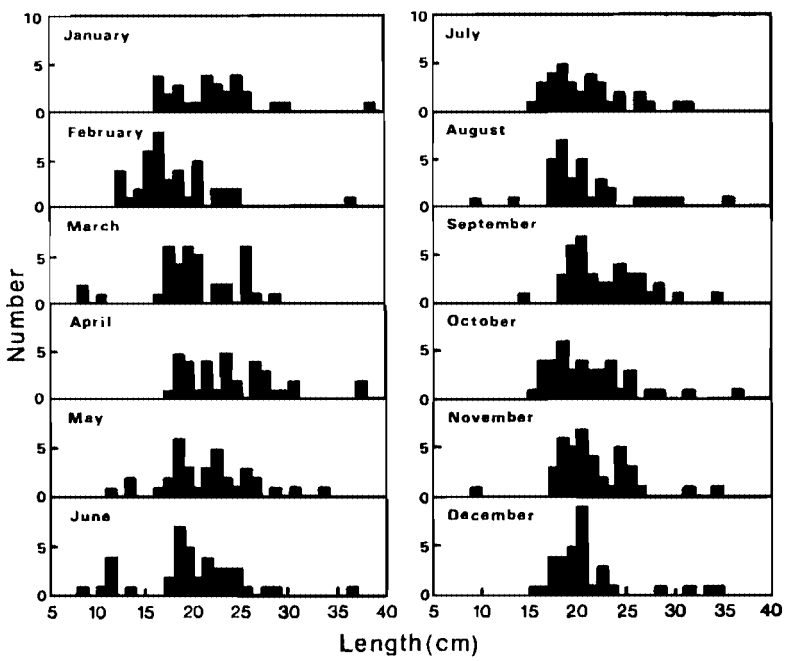

FIGURE 5

Monthly length frequencies of steenbras from the Heuningnes River Estuary, 1971.

possible exchange of young fry between the sea and the estuary could perhaps explain the absence of modal movements.

Fig. 6 shows the length-frequency distribution of 68 marine *L. lithognathus* ranging from 25 to 83 cm fork length, plotted in 5 cm length classes. Lucks (unpubl.) recorded a size range of 28–77 cm for *L. aureti* from Sandwich Harbour, South West Africa, with peaks at 32–36 cm and 58–66 cm. This size range and the second peak for *L. aureti* are comparable to the results obtained for the marine samples of *L. lithognathus*. Lucks also showed that *L. aureti* exhibited a fluctuation in size distribution with changing seasons, but that this was related to the changing sexual ratio. As all the estuarine samples of *L. lithognathus* from the Heuningnes River were found to be hermaphroditic and sexually inactive (see *Reproductive Biology*), the size range of these fish was considerably narrower than that occurring in *L. aureti* at Sandwich Harbour. No valid comparison can therefore be made between the two species.

Length/weight relationship

The lengths of estuarine steenbras in 2 cm classes, plotted against weight, are shown in Fig. 7. As expected, length is directly proportional to weight. The data were further analysed by

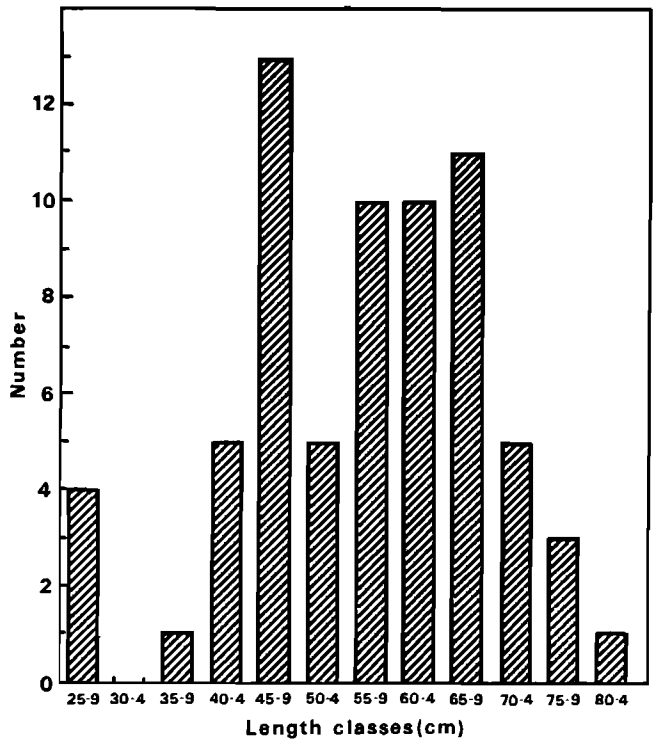

FIGURE 6

Length frequency (in 5 cm classes) of 68 marine steenbras from the Strand coastline, 1971–72.

determining the length/weight relationship in the equation $W=aL^n$, where W=weight, L=length, a=constant, and n an exponent usually lying between 2,5 and 4,0. This relationship has been illustrated by plotting the lengths and weights as a scatter diagram on double logarithmic graph paper (Fig. 8). The regression line, being the logarithmic form of $W=aL^n$, log W=log a $+n$ log L, where n represents the slope of the line and log a its position, was found to be linear. The final calculated regression $W = 0{,}02282\ L^{2{,}8562}$ was within the expected range (Le Cren 1951). As the weights of the marine steenbras sample were not accurately recorded and the sample size was very small, the regression line was not calculated.

Age determination and growth rate

Variations in the pattern of growth during certain periods of the year result in annual rings being formed on some of the permanently hard parts of fishes. Interpretation of the annual layers deposited on the ctenoid scales and otoliths (sagittae) was employed for determining the ages of estuarine steenbras. On all the scales only one type of ring appeared and this was considered to be the true annulus. The rings occurred at regular intervals, suggesting formation by a rhythmical seasonal process. The annual growth rings of the otoliths likewise occurred at regular intervals, appearing as hyaline dark bands in contrast to the white opaque zones. The hyaline zones were counted as the year rings. Baird (1970) and Jessop (1972) also interpreted the hyaline zones as the year rings, whereas Messersmith (1969) and Botha (1971) interpreted the opaque zones as the year rings. Clearly, it is a matter of individual interpretation which zones are accepted. Occasionally large otoliths occurred which were completely opaque lacking the typical zonation. These were discarded. However, in every case where one otolith was abnormal,

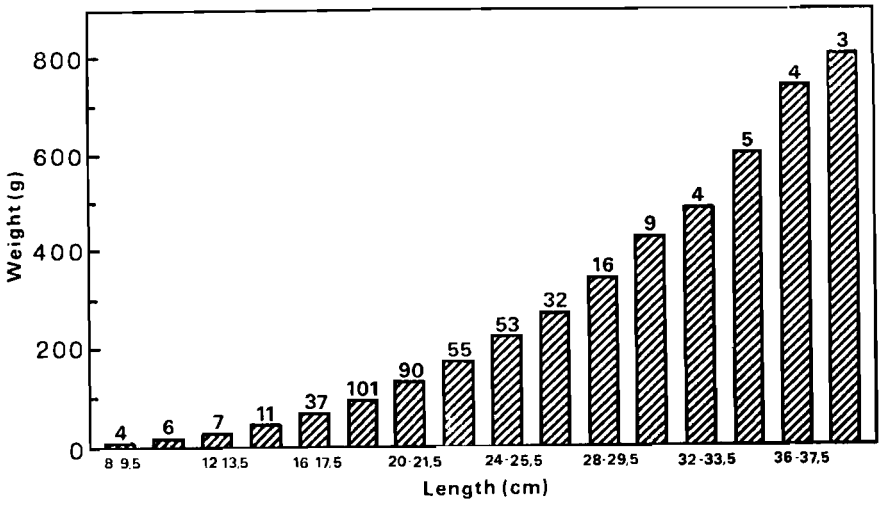

FIGURE 7

Length (in 2 cm classes) plotted against weight, of 437 steenbras from the Heuningnes River Estuary, 1971. The numbers above each column represent the number of fish examined in each length class.

its pair was normal and could be read. Likewise, some scales could not be read, but there always remained at least one scale in a set from which the age could be determined.

Repeated unbiased examination of scales and otoliths from 437 estuarine steenbras showed that both exhibited a pattern of concentric annuli which was consistent, both between different scales and between scales and otoliths. Of the entire sample, 86 per cent were in total agreement and of the remainder only 1% showed discrepancies of more than one year. Most of these differences, which were less than one year (see below), resulted from doubt in interpreting newly formed rings. Clearly, the two methods of ageing estuarine fish are comparable, and

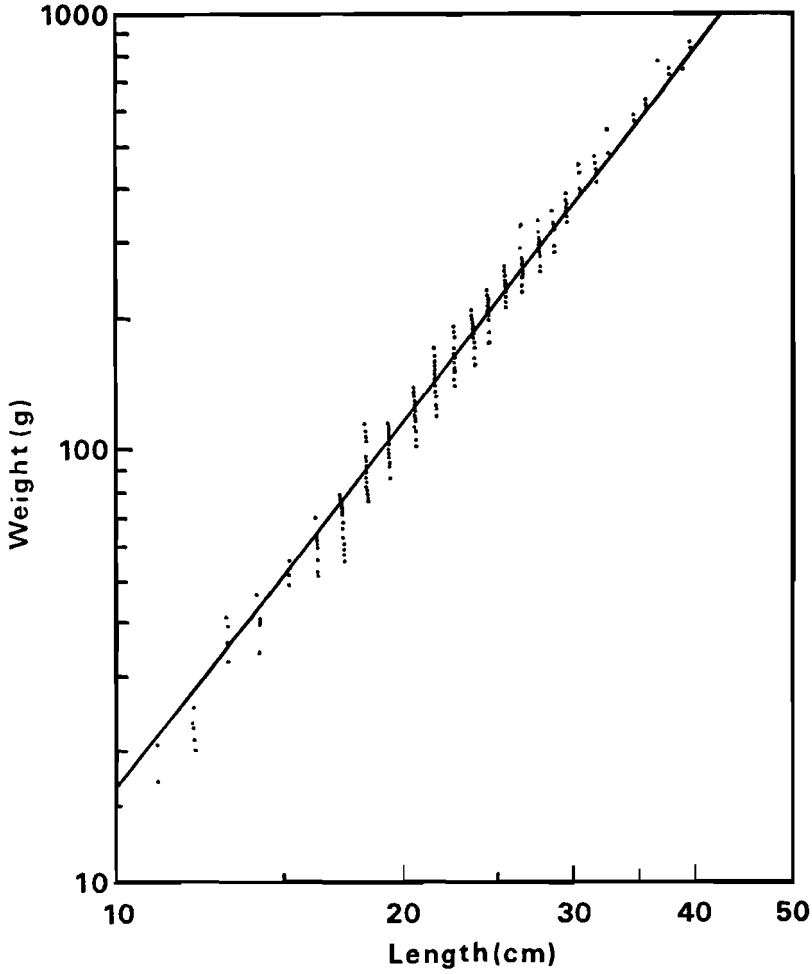

FIGURE 8

Regression of weight plotted against length (logarithmic scales) for 437 estuarine steenbras from the Heuningnes River Estuary, 1971.

both can be used with equal confidence. However, with ages greater than six exact determination from scales, but more especially from otoliths, becomes difficult due to crowding of the annual rings. As it was easier to age the large marine steenbras from scale readings, this method of analysis was employed for both the estuarine and marine samples.

Ages were expressed as 0+, 1+, 2+, etc., with the assumption that the age in years is equal to the number of annuli plus a fraction of a year.

The age of estuarine steenbras plotted against fork length, shows the expected almost linear relationship (Fig. 9). From these data it was found that there was an average annual growth rate of 4,0 cm, after an initial first year growth of 10 cm. Fig. 10 shows that increasing age is also directly proportional to increasing body weight, the increase becoming more marked after 3 years. The ages of the marine steenbras sample in 5 cm length groups (Table 8), showed these fish to have an average annual growth rate of 5,0 cm, which compares closely to the growth rate in the younger estuarine fish.

In *L. aureti*, Lucks (unpubl.) found that scales from females often formed two or three rings per year, described as "breeding" rings, while males formed only one annual ring. These

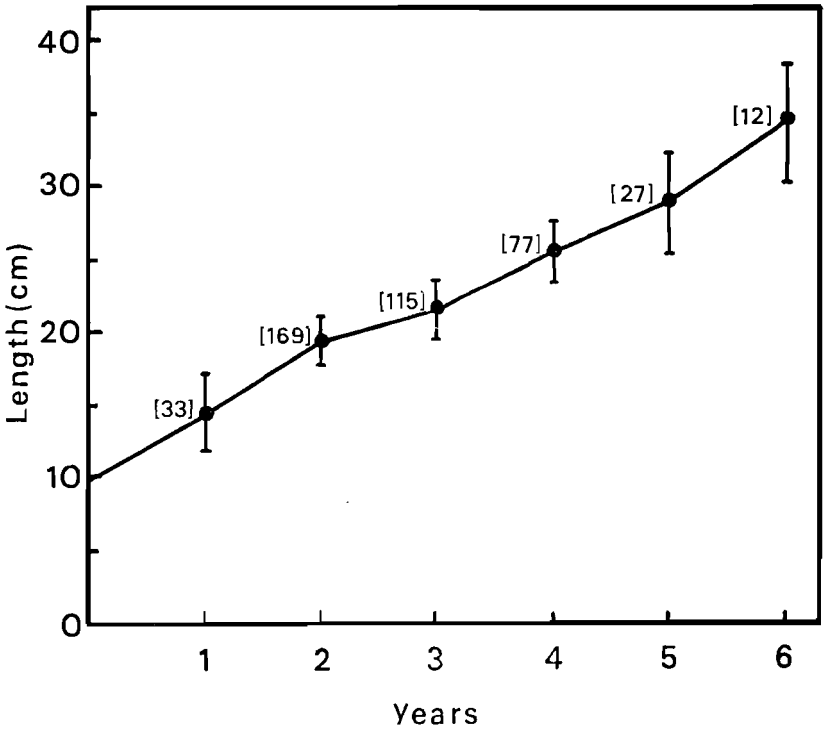

FIGURE 9

Length-age relationship of steenbras from the Heuningnes River Estuary, 1971. Figures in parenthesis indicate number of fish examined in each year class. Standard deviations indicated by T-bars.

fish for the first three years averaged 17 cm growth per annum, but only 7 cm for the next three years. Surprisingly, these results are very different from those obtained for *L. lithognathus*. Lucks aged only 82 fish and did not specify if the ages were determined from scales or otoliths, or both. Moreover, his tabulated results are not conclusive and contain many overlapping readings. Although it is a known fact that fish have a faster growth rate during early life which slows down with increasing age, I find it difficult to accept that *L. aureti* could show such a drastic growth reduction of more than 100 per cent after the first three years of life. Lucks showed samples of *L. aureti* at 19 and 71 cm to be one and seven years old respectively, while I found the corresponding ages for *L. lithognathus* to be 2+ and 13+ respectively. Clearly there is no basis for comparison of the two species and I consider the results obtained by Lucks to be questionable.

Talbot (1955) recorded an annual growth rate of 6 cm in the sparid *Rhabdosargus globiceps*. However, as this fish belongs to a different genus, there is no basis for comparison.

Ageing, whether it be from scales or otoliths, is subject to many errors which may result in biased and therefore false readings. "However, the scale still remains the most trustworthy and dependable means of estimating the age and calculating the growth of fishes. Although scales of different species have peculiarities that can be learned only by observation, there is no substitute

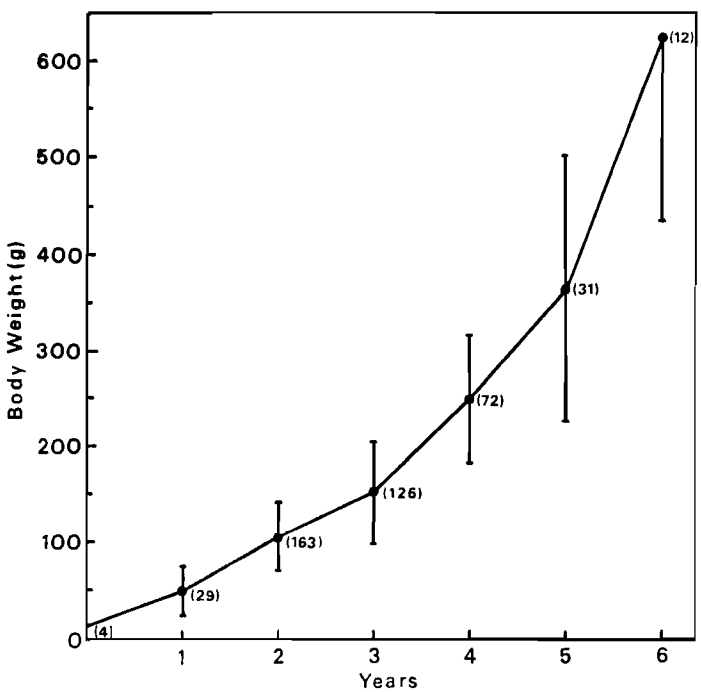

FIGURE 10

Age-weight relationship of steenbras from the Heuningnes River Estuary, 1971. Figures in parenthesis indicate number of fish examined in each year class. Standard deviations indicated by T-bars.

TABLE 8

AGE DETERMINATIONS FROM SCALE READINGS OF 66 MARINE SAMPLES OF *Lithognathus lithognathus* IN 5 CM CLASS INTERVALS. THE NUMBERS REPRESENT INDIVIDUAL FISH AGED IN EACH YEAR GROUP

Length groups (cm)	*Age (years)*												
	3	4	5	6	7	8	9	10	11	12	13	14	15
25–29	2	2											
30–34													
35–39				1									
40–44					5								
45–49				1	1	7	3						
50–54							2	2					
55–59						1	1	7	1				
60–64								2	7	1			
65–69									1	9	1		
70–74										1	4		
75–79												1	2
80–84													1

for experience in actually reading scales" (Rounsefell and Everhart 1953). In the present study, the excellent correlation obtained between the ages from scale and otolith readings, convince me that the data for the estuarine sample in Fig. 9 are reliable. The ages obtained for the marine steenbras (Table 8) do, however, seem high for fishes generally and therefore some doubts must exist as to their validity.

OSMOREGULATION

INTRODUCTION

Like most estuarine fish the steenbras appears to be a fairly good osmoregulator. The fish have been recorded from several land-locked waters, for example Zoetendalsvlei and Verlorenvlei, where the salinity has been $<2‰$; during the whole year from the Milnerton Estuary and Diep River, Cape, in a salinity range of 1,81–57,76‰ (Millard and Scott 1954); and in the present study from the Heuningnes River Estuary, with a salinity range of 1,30–50,59‰.

There have been no previous investigations on the osmoregulation of the steenbras or for that matter of any other member of the Sparidae. However, a considerable literature exists dealing with the levels of common inorganic ions in the blood of many teleost species. For example, Parry (1966) has reviewed the earlier work on the osmotic adaptation of fishes, while Pickford *et al.* (1969) have made a comprehensive study of the serum constituents measured on

parallel groups of the saltwater and freshwater adapted cyprinodont, *Fundulus heteroclitus*, and they have summarized the earlier literature in this field.

A series of experiments was devised to study the effects of gradual transfer of the steenbras from seawater to freshwater on some of the plasma constituents, in an attempt to determine if this fish could survive in an artificial freshwater environment on a long-term basis. The effects of stress conditions on the plasma constituents due to sampling techniques of trek-netting at the estuary have also been analyzed and compared to the existing literature in this field.

PROCEDURE

All experiments were carried out on steenbras captured by trek-net during May and June, 1971, at station 1 on the Heuningnes River. Fish were removed from the net one at a time with the minimum of handling, by pressing only the thumb and forefinger over the opercular flaps, and placed into conical metal containers half-filled with water from the habitat, before being transported back to the Jonkershoek Fisheries Station, Stellenbosch. No artificial air pumps were needed, as the water movement in the containers and the air inflow through the perforated lids was found to provide adequate oxygenation. No more than 10 fish were transported in each container to avoid the effects of overcrowding. This method of transport proved very effective. At Jonkershoek the fish were transferred to concrete tanks, approximately $1{,}5 \times 1 \times 0{,}5$ m in size, half-filled with seawater obtained from the coast along the Strand, for a 48-hour acclimation period. Each tank contained two or three fish of roughly the same size to avoid domination and one or two ordinary aquarium filters which were thoroughly cleaned every day. The steenbras were fed on frozen sandprawn *Callianassa kraussi*, the remains being carefully removed after half an hour. Each tank received normal daily sunlight, the water temperature varying with the air temperature, from 9–14°C.

After 48 hours each tank was reduced to 75% seawater, by adding the appropriate volume of freshwater. This was repeated twice at 24-hour intervals to reduce the water to 25% strength. After a further 24-hour period the tanks were emptied, thoroughly scrubbed, and filled with freshwater. The steenbras were then placed in these tanks using a handnet to avoid any possible damage by handling and a record was kept of their mortality rate until the last of the fish had died. Damaged fish were discarded before transfer to freshwater. The experiment was repeated twice using 20 individuals each time. Blood samples were taken from half this number after 48 hours in 100% seawater and samples were collected from the other half after 48 hours in freshwater. In order to obtain baseline values blood samples were also taken from 20 estuarine fish at the Heuningnes River.

Blood was collected by cardiac puncture using 2 ml heparinized disposable syringes. The point at which the needle was inserted was first carefully washed with distilled water and dried with clean paper tissues. Haematocrits were determined using heparinized micro-haematocrit tubes and spun for 20 minutes in a clinical centrifuge. The plasma was placed in plastic vials, sealed, frozen and stored for later analysis. Plasma proteins and total proteins were determined first, to avoid any possible deleterious effect on the proteins with repeated freezing and thawing.

Total plasma proteins were determined by the Biuret method. Plasma protein patterns were determined with a Microzone Electrophoresis Cell Model R–101 and Densitometer Model

R–110 (Beckman Instruments, Inc.). Plasma chloride was determined using a CMT 10 Chloride Titrator (Radiometer) on 20µl samples of plasma. Plasma sodium and plasma potassium were determined on a 343 flame photometer (Instrumentation Lab., Inc.). Plasma osmolalities were determined with an advanced research osmometer, model 67–31 RAS (Advanced Instruments, Inc.).

For those fish suspected of having fungal attacks, stereomicroscopic and microscopic examinations were made of the affected areas. Isolations were also made from the tank water and from skin scrapings using baiting techniques applying *Drosophila;* sterile tapwater and *Drosophila;* sterile physiological salt solution and *Drosophila;* Labouroud's agar medium and potato dextrose agar medium, to test for fungal growths.

Data are expressed as means $\pm$ standard errors and differences were tested for significance using student's t-test.

RESULTS AND DISCUSSION

Mortality in freshwater

Up until the time of transfer to freshwater, all the fish fed readily on the sandprawn *Callianassa kraussi* and showed no sign of distress in their artificial environment. On being transferred to freshwater the steenbras showed greatly increased activity, swimming around frantically in random directions and often breaking the surface. However, after a two-hour period this hyperactivity ceased and the behaviour returned to normal. None of the fish accepted food once they had been transferred to freshwater. Fig. 11 summarizes the mortality rate in freshwater. The first death occurred after almost three days and thereafter at regular intervals, the last fish surviving for almost one week. With increasing time spent in freshwater the movements of the fish became progressively more sluggish and moribund fish often drifted helplessly upside down. They occasionally swam frantically in unusual attitudes before dying. Conte and Wagner (1965) found similar behavioural stress patterns in juvenile steelhead trout, *Salmo gairdneri*, which were unable to osmoregulate when transferred from fresh- to saltwater. It was also observed that several fish showed subepidermal haemorrhagic blotches especially in the region of the pelvic fins and in the caudal peduncle. The significance of this is discussed in the following sections.

During the course of the experiment scrupulous attention was paid to cleanliness in an effort to lessen infection due to accumulation of metabolic wastes and decaying organic matter which could have promoted fungal development. The filters were regularly cleaned, all food remains were removed after half an hour and the freshwater was changed after three days, when the tanks were thoroughly cleaned. During the experiment most fish developed white spots on the fins and the skin immediately adjacent, and dirty-yellow patches on the skin. These conditions progressively deteriorated, especially during the freshwater phase of the experiment. The last fish to die was covered with yellow patches over its entire body. The fins were also covered with small white spots and growths, and the webbing between the fin rays was torn. It was first assumed that these symptoms were due to fungal growths and that they were responsible to some extent for the inability of the fishes to survive for any length of time in freshwater. Control

fish, maintained in 100% seawater, however, survived indefinitely. The above symptoms did appear after several weeks but far less severely.

The following are the results obtained from a series of tests for fungal infections which were carried out on a single fish which exhibited both of the above symptoms.

1. Stereomicroscopic examination of white spots on skin (x75):
   (*a*) Skin of the fish was damaged and filled with a mucous material which showed up as a white spot.
   (*b*) Yellowish spots occurred on and under the scales of the fish.
2. Microscopic examination:
   (*a*) Scrapings from the skin at white areas:
   These were examined under ordinary light and with phase contrast, both unstained and stained with Ractophenol cotton blue. No recognizable fungal structures were observed. Filaments of fibre-like structures were noticed, which were not typical of fungal hyphae.
   (*b*) Scrapings from the skin at yellow areas:
   These were examined as in 2 (*a*). A yellow substance was noticed in, or on the cells, with filament-like structures not typical of fungal hyphae. Many *Amoeba* spp were present.
   (*c*) Sections from tissues from both areas:
   No fungal structures were observed.

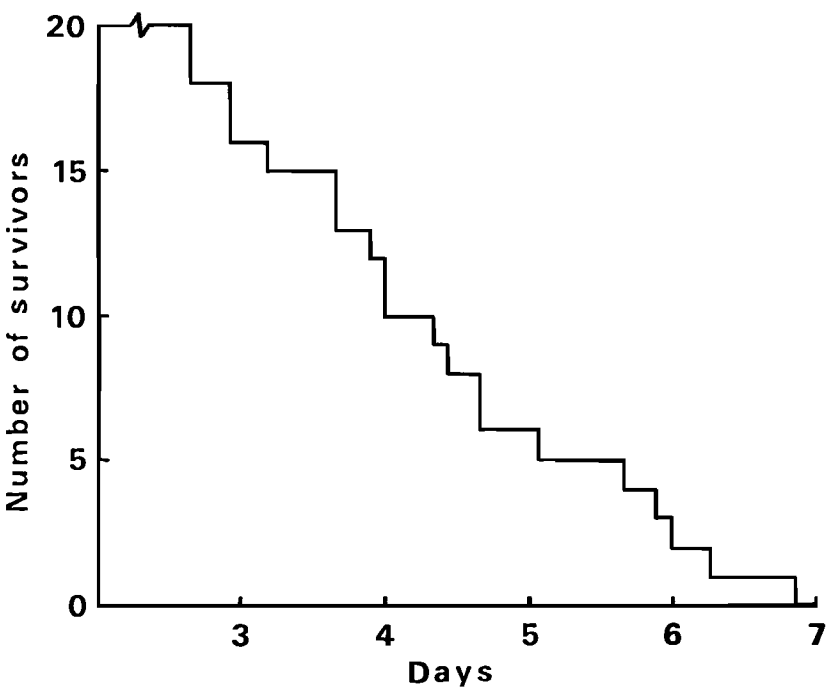

FIGURE 11

Mortality of steenbras in freshwater, after acclimation to decreasing concentrations of seawater.

3. Isolations:
   (*a*) From tankwater:
   Using a baiting technique, applying *Drosophila*. Sexual and asexual structures of *Achyla* spp were observed.
   (*b*) From scrapings of skin of fish:
      (i) A baiting technique using sterile tapwater and *Drosophila* produced no fungal structures.
      (ii) A baiting technique using sterile physiological salt solution and *Drosophila*, showed no fungal structures.
      (iii) Isolation on Labouroud's agar medium resulted in only a few bacterial colonies and sparse colonies of *Cephalosporium* spp and *Penicillium* spp.
      (iv) Isolation on potato dextrose agar medium produced many bacterial colonies, a few yeast colonies and two fungal colonies of *Rhizopus* spp and *Cephalosporium* spp.

The above results, which showed an absence of pathogenic fungal growths, suggested that these secondary infections resulted from mechanical damage and bruising caused either during transport from the estuary, or while in the experimental tanks. Van Duijn (1967) records that dermatomycosis is a common disease attacking only those fishes that have been wounded, or whose resistance has been weakened. Of such fungi, *Achyla* spp is one of the most common affecting fishes. Fungus is not an obligatory parasite and can live equally well without fishes, provided there is sufficient organic material present. In the experimental tanks there was a minimum of such organic material, and any fish that had been damaged, provided an ideal host. Van Duijn lists the probable causes for fungal growths as lack of cleanliness of ponds and very alkaline waters, which tend to have a deleterious effect on the protecting mucous coat of the fishes. These conditions are frequently found where the concrete of the tanks has not properly matured. Van Duijn cites the ideal pH range as 6,8–7,2 for freshwater and 7,8–8,0 for saltwater. Although intensive care was taken to ensure cleanliness of the tanks, it is possible that the precautions taken were not fully effective. Moreover, the pH values in both the freshwater and seawater tanks were very alkaline, higher than the range cited by Van Duijn, and it is very likely that this factor was the main cause of infection and its acceleration with time.

It is concluded that these infections were caused by mechanical damage, but that they were not responsible as such for the deaths of the steenbras. Undoubtedly, however, they would have accelerated the deterioration of the fishes, making them more susceptible to an earlier death. More probably, the failure of the fish to adjust to the osmotic changes is responsible for their inability to survive for any length of time in freshwater. This factor is discussed in the following sections.

Plasma analysis

The literature on osmoregulation in fish is vast. Parry (1966), however, has written a thorough review of the subject and no purpose will be served in reviewing it here.

Although the steenbras is able to withstand a wide salinity range in nature and is thus to some extent euryhaline, it is unable to survive in freshwater. A study of some of the main plasma constituents was therefore undertaken, in an attempt to explain why this breakdown in osmoregulatory ability occurred.

Haematocrit

Table 9 shows the haematocrit percentages obtained from steenbras maintained in 100% seawater for 48 hours and in freshwater for a similar period.

TABLE 9

THE EFFECT OF EXPOSURE TO FRESHWATER ON HAEMATOCRIT VALUES IN STEENBRAS

Treatment	*n*	*Mean (percentage)*	*Standard deviation*
Seawater	21	23,7	3,67
Freshwater	19	34,9	4,80

The significantly ($P < 0,01$) higher haematocrit obtained in the freshwater sample was totally unexpected. Marine teleosts transferred to freshwater will tend, by osmosis, to suffer from excessive hydration. This should have the effect of diluting the body fluids and the plasma resulting in a lower haematocrit reading. However, it was noticed that blood sampled from steenbras in freshwater was in fact much more viscous and difficult to collect than from the fish in seawater. The reason for this is not clear and the increased haematocrit values must at this stage be attributed to either stress or other unknown causes. In any event, it is apparent from the haematocrit values that the steenbras were not able to adjust physiologically to the change from seawater to freshwater in an artificial environment.

Pickford *et al.* (1969) found no difference in the haematocrit values of *Fundulus heteroclitus* between freshwater and saltwater groups, except for one group of "freshwater trained" fish whose haematocrit (54%) was significantly higher than its saltwater counterpart (45%). This was attributed to the higher gonosomatic index of the sexually mature males, a factor which was not applicable in this study.

Plasma proteins

The electrophoretic pattern of steenbras plasma on cellulose acetate showed five distinct fractions, which have been arbitrarily labelled $\alpha 1$, $\alpha 2$, $\alpha 3$, $\beta 1$ and $\beta 2$ (Fig. 12). Although human serum also displays five fractions, these are not comparable to steenbras plasma as there is no trace of the characteristic albumin fraction of mammals. Pickford *et al.* (1969) report that in some species of marine teleosts there occasionally exists a fraction with a low solubility comparable to that of human serum albumin, but usually it is entirely lacking. The total absence of serum albumin in *F. heteroclitus* has been recorded by Umminger (1970b). The steenbras conforms to this pattern.

Although two of the protein fractions changed significantly in steenbras maintained in freshwater, namely the $\alpha 3$ fraction ($P < 0,10$) and the $\beta 2$ fraction ($P < 0,01$), no special significance can be attached to these changes. It is, however, to be expected that when normal osmoregulation is disrupted, some change in plasma protein patterns will occur.

TABLE 10

THE EFFECT OF EXPOSURE TO FRESHWATER ON PLASMA PROTEIN FRACTIONS IN STEENBRAS

Treatment	*Plasma protein fractions (percentage)*									
	α1		*α2*		*α3*		*β1*		*β2*	
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*
Seawater	6,50	3,69	9,43	4,45	25,57	5,82	41,52	4,33	16,95	2,99
Freshwater	7,29	4,31	8,34	5,28	29,95	8,52	40,68	4,41	14,32	2,05

TABLE 11

THE EFFECT OF EXPOSURE TO FRESHWATER ON TOTAL PLASMA PROTEIN VALUES IN STEENBRAS

Treatment	*n*	*Total protein (g/100 ml)*	
		Mean	*Standard deviation*
Seawater	22	1,93	0,26
Freshwater	19	3,05	0,45

Most of the literature records little difference between the total serum proteins in marine and freshwater teleosts, with the exception of the Anguillidae. Pickford *et al.* (1969) found that the total serum proteins of *F. heteroclitus* showed a trend towards higher levels in freshwater, being 7% greater than that of the saltwater controls.

Table 11 shows that the total protein in the freshwater steenbras was 58% greater ($P < 0,01$) than that of the seawater fish. This drastic increase, the highest recorded for such an experiment, is further indication of the severe disturbance of osmoregulation in freshwater steenbras. Due to the greater viscosity of the blood, it would be expected that there would be increased protein per unit volume of plasma, and a general increase of non-excretable substances (such as protein) per unit volume of plasma. This would result in a retarded vascular flow, especially through the capillaries. The gills would then receive less blood per unit time with a consequent overall decrease of oxygen uptake and ultimate death by asphyxiation.

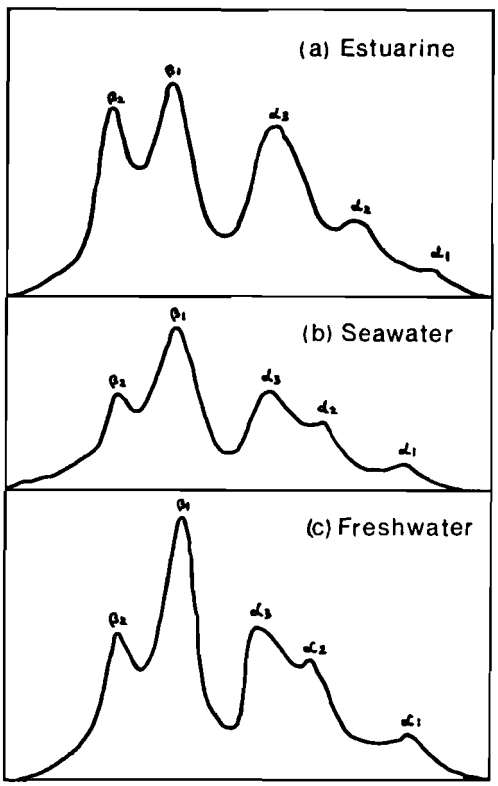

FIGURE 12

Electrophoretograms of plasma protein fractions: (a) from estuarine sample of steenbras; (b) from steenbras maintained for 48 hours in 100% seawater; (c) from steenbras maintained for 48 hours in freshwater, after a gradual acclimation period through decreasing salinities.

There was good evidence available to support such an assumption. During the experiment on mortality in freshwater, the steenbras were observed to first become very sluggish, with a rapid increase in the respiratory movements of the operculi. The frantic swimming just before death was apparently a reflection of an acute stress condition caused by respiratory failure, due to the lack of sufficient oxygen.

Plasma chloride

It has been established (Pickford *et al.* 1969) that serum chlorides are lower in freshwater than in seawater teleosts and subsequent studies of adaptation to seawater or freshwater in euryhaline species has confirmed this difference. Pickford *et al.* recorded a 13,31% decrease of serum chloride in freshwater-maintained *F. heteroclitus*, while Lange and Fugelli (1965) recorded

a 31,5% decrease in plasma chloride in freshwater for *Pleuronectes flesus*. Other authors have recorded decreases in serum chloride from various species as high as 36,7% (in Pickford *et al.* 1969).

However, the euryhaline teleost *F. heteroclitus* is able to osmoregulate in very dilute media, regulating like a typical freshwater teleost: "drinking" is negligible; copious very dilute urine is produced; and branchial absorption of salt occurs (Bergeron 1956). In contrast, Black (1951) found that chum salmon fry, *Oncorhynchus keta*, were able to successfully regulate their body chloride concentrations when transferred from seawater to freshwater, and vice versa. However, salmon are physiologically adapted to successfully osmoregulate in changing water due to the peculiar secretory activity of the chloride-secreting cells of the gills. Moreover, Baggerman (1960) found that the ability of Pacific salmon, *Oncorhynchus* spp, to tolerate the osmotic changes resulting from migrations to and from freshwater was associated with changes in endocrine activity, especially the pituitary-thyroid system. This has been confirmed by Burden (1956), who found that serum chloride in hypophysectomized *F. heteroclitus* was significantly lower in freshwater than in seawater samples. Replacement therapy using *Fundulus* pituitary brei, enabled the hypophysectomized fish to survive in freshwater, and this suggested that the pituitary of *F. heteroclitus* secreted an unknown factor(s) which regulated salt balance of that fish in freshwater. Burden also recorded that some investigators found increased thyroid activity in the euryhaline *F. heteroclitus* in hypotonic media, while survival of marine teleosts in freshwater may be prolonged by the administration of thyroxin or pituitary growth hormone before transfer.

The freshwater steenbras confirmed the findings of Pickford *et al.* (1969), showing a significant ($P < 0,01$) decrease of plasma chloride of 39% when compared to the seawater fish (Table 12).

TABLE 12

THE EFFECT OF EXPOSURE TO FRESHWATER IN PLASMA CHLORIDE CONCENTRATIONS IN STEENBRAS

Treatment	*n*	*Chloride (mEq/litre)*	
		Mean	*Standard deviation*
Seawater	22	153	9,58
Freshwater	19	94	10,39

Nevertheless, in spite of a reduction in plasma chloride similar to that exhibited by euryhaline fish, as shown by Pickford *et al.* (1969), the steenbras was not able to survive in freshwater. This was probably due to insufficient salt absorption by the chloride cells of the gills.

Plasma sodium

Pickford *et al.* (1969) have reviewed the results of various authors showing a decrease in serum sodium in freshwater-adapted fishes. The freshwater-maintained steenbras conformed to these findings. There was a 26% decrease in plasma sodium, significantly different ($P < 0,01$) from the related seawater fishes (Table 13).

TABLE 13

THE EFFECT OF EXPOSURE TO FRESHWATER IN PLASMA SODIUM CONCENTRATIONS IN STEENBRAS

Treatment	*n*	*Sodium (mEq/litre)*	
		Mean	*Standard deviation*
Seawater	22	168	8,07
Freshwater	19	124	9,39

Water excretion by the kidneys is a function of sodium excretion (Langley 1966). Therefore, as the plasma sodium concentration was significantly lower in freshwater steenbras, this suggests that overcompensation by the kidneys was occurring to excrete excess water. However, the failure of the secretory cells of the gills to successfully reverse their function to adapt to the changing osmotic concentrations would appear to be the main reason for the significant decrease in plasma sodium, as was shown for plasma chloride. This has been confirmed by Burden (1956) who recorded that Grafflin (1937) concluded that the failure of freshwater teleosts to adapt to seawater or *vice versa* must be attributed to extrarenal factors.

Plasma potassium

TABLE 14

THE EFFECT OF EXPOSURE TO FRESHWATER IN PLASMA POTASSIUM CONCENTRATIONS IN STEENBRAS

Treatment	*n*	*Potassium (mEq/litre)*	
		Mean	*Standard deviation*
Seawater	22	3,93	0,71
Freshwater	9	3,54	0,93

Pickford *et al.* (1969) record from various investigators of euryhaline teleosts, a significant lowering of serum potassium in freshwater-adapted fish. However, Parry (1961) has noted a 20% increase in plasma potassium concentration in the adult salmon *Salmo salar*. Pickford *et al.* found that freshwater-adapted *F. heteroclitus* showed a mean lowering of serum potassium by 36%. The freshwater-maintained steenbras followed this trend, showing a significant ($P < 0,01$) decrease of plasma potassium of 10% when compared to the seawater fish (Table 14).

These results confirm the conclusions made for the previously discussed cations.

Plasma osmolality

It has been established that serum osmolality is lower in freshwater-adapted than in seawater-adapted euryhaline fish (Pickford *et al.* 1969). Steenbras from freshwater likewise showed a significant ($P < 0,01$) decrease in plasma osmolality of 15% when compared with the seawater fish (Table 15).

TABLE 15

THE EFFECT OF EXPOSURE TO FRESHWATER IN PLASMA OSMOLALITY IN STEENBRAS

Treatment	*n*	*Osmolality (mOsm/kg)*	
		Mean	*Standard deviation*
Seawater	10	312	32
Freshwater	10	265	17

Pickford *et al.* (1969) found that the euryhaline cyprinodont *F. heteroclitus* showed an average drop of 8% in serum osmolality when adapted to freshwater, but the absolute values varied from one experiment to another. Pickford *et al.*, and other authors, have also reported a decrease in serum osmolality from ripe or spawning teleosts. The decrease in plasma osmolality in the freshwater steenbras is not related to the sexual cycle, but must be attributed to the osmotic changes resulting from the transfer from seawater to freshwater.

The steenbras is capable of adapting to wide salinity fluctuations. Reports of this fish being found high up river systems or living successfully in landlocked waters is proof indeed of its adaptability. However, these waters were probably not entirely fresh, and this adaptability to freshwater is probably due to the more gradual acclimation period to changing salinities occurring under natural conditions.

The significant lowering of the plasma osmolality concentrations of the experimental freshwater steenbras may be related to the haemolysis of some of these blood samples. With the transfer of this typically marine fish to freshwater excess hydration will occur. The red blood cells, however, also have differentially permeable membranes and this could result in their dilation and ultimately in their rupture (Schmidt-Nielsen 1970).

Andre *et al.* (1972) recorded acute haemorrhagic septicaemia in the captive European eel, *Anguilla vulgaris*, and found that this was simply the result of bad husbandry. The condition was caused by a bacterial septicaemia, resulting in generalized erythma of the fins and ventral surface of the body. Haemorrhagic ulcers developed usually on the head and around the anus and an oedomatous swelling behind the gills, causing the fish to become extremely sluggish and depressed. Recovery was achieved simply by improving the environmental conditions, thus suppressing the bacteria which had merely acted as opportunistic pathogens. The stress effects resulting presumably from bad husbandry (see Van Duijn 1967) during the course of the present investigation, fully support the findings of Andre *et al.*

In addition, the plasma samples from the freshwater steenbras and to a far lesser extent from a few of the seawater fish, had a light green colouring which was assumed to be the bile pigment biliverdin. Bile pigments result mainly from the breakdown of the haemoglobin of erythrocytes and wherever extravasation of the blood occurs, a conversion of blood haemoglobin to bile pigment slowly takes place (Hawk *et al.* 1954). Thus, the fact that bile pigments occurred in those steenbras which showed the effects of subepidermal haemorrhages probably due to mechanical damage, confirms the findings of Hawk *et al.* (1954).

The increase in plasma haematocrit of the freshwater steenbras indicates haemoconcentration brought about by stress conditions, possibly as a result of renal malfunction. However, the reduction in plasma osmolality and cation concentrations suggests that the gill secretory cell function was not reversed, and the failure of the latter mechanism is probably the main cause of the fish's inability to osmoregulate in freshwater. Black (1957) records that some authors defined laboratory diuresis in marine teleosts transferred to aquaria, as an abnormally high loss of salts and water resulting in ultimate death. Although the cause of this was unknown, injury and shock in handling was suggested. However, other authors recorded laboratory diuresis causing an elevation in plasma cation and anion concentrations. The effects of laboratory diuresis could not be determined in the present investigation because the freshwater steenbras had 48 hours before sampling, in which time cation concentrations would have had time to level off. The increased pH of the water in the experimental tanks may also have contributed somewhat to the impaired osmoregulation in the fishes. Nevertheless, the effects of laboratory diuresis, capture stress, and increased pH of the tank water are not considered as important as osmoregulatory failure because in the previous pilot experiment steenbras, which had been captured and transported in an identical manner, survived indefinitely in 100% seawater in the same tanks.

STRESS

INTRODUCTION

The effects of stress due to factors other than water and salt imbalance, have been extensively studied in teleost fishes. The effect of severe muscular activity and lactic acid accumulation in the blood has been thoroughly examined (Black 1955, 1957 a, b, c; Black *et al.* 1959, 1962, 1966; Stevens and Black 1966; Gronlund *et al.* 1968). Haematological responses to thermal shock have been examined by Heinicke and Houston (1965); and Umminger (1969, 1970 a, b). Kamra (1966)

has investigated the starvation effect on blood constituents, while Bouck and Ball (1966) and Umminger (1970 a) have examined the effects of capture methods on some serum constituents.

The present investigation records the effect of prolonged stress on some plasma constituents in trek-netted steenbras. The results are compared to the seawater-maintained fish studied in the previous section.

PROCEDURE

Nineteen steenbras were captured in a single operation by trek-net at station 1 on the Heuningnes River. The net was drawn up so that only the "cod-end" containing the fish was submerged in a few centimetres of water. The fish were removed from the net at approximately ten-minute intervals. The blood sampling method and analyses were conducted as described previously.

RESULTS AND DISCUSSION

Table 16 compares the analyses of some of the plasma constituents of the estuarine sample with those obtained for the steenbras maintained in 100% seawater tanks for an acclimation period of 48 hours. All the results from the estuarine sample were significantly higher ($P < 0,01$) than those of the seawater-acclimated sample.

TABLE 16

THE EFFECT OF CAPTURE STRESS ON VARIOUS PLASMA CONSTITUENTS IN STEENBRAS

Medium	*Haemato-crit (percentage)*		*Total Protein (g/100ml)*		*Sodium (mEq/litre)*		*Potassium (mEq/litre)*		*Chloride (mEq/litre)*		*Osmo-lality (mOsm/kg)*	
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*
Freshly captured estuarine	33,3	8,66	2,88	0,47	188	9,39	6,27	0,97	167	10,27	413	25
Acclimated in seawater for 48 hrs. after capture	23,7	3,67	1,93	0,26	168	8,07	3,93	0,71	153	9,58	312	32

Black (1957 a, b, c), Black *et al.* (1959, 1962, 1966), Parker and Black (1959), Parker *et al.* (1959), Stevens and Black (1966) and Gronlund *et al.* (1968) all report significant increases in blood lactate levels following severe muscular exercise. The levels were especially high in the first two hours following exercise and then gradually returned to the initial level. This increase of lactic acid in blood following severe muscular activity was reported as likely to lead to death

(Black 1957 a, c; Parker and Black 1959; Parker *et al.* 1959). The prolonged stress, caused by severe muscular activity in attempting to escape from the net would presumably also have resulted in increased blood lactate levels in the steenbras.

The energy required for excess muscular activity comes from the anaerobic metabolism of carbohydrates, forming lactic acid (Black 1955). There is thus an increase in the number of osmotically active particles so that water diffuses into the muscle cells from the extracellular fluid, including the blood, until osmotic equilibrium is re-established. The blood volume is reduced with an increase in blood haemoglobin per unit volume (Black *et al.* 1959). In this investigation the haematocrit reading was significantly higher in the estuarine sample and this supports the findings of Black *et al.* (1959). However, whereas Black (1955) and Black *et al.* (1966) also record higher haemoglobin levels as the result of severe muscular activity, Black (1957 b) showed that there was no significant change in the haemoglobin level, and Black (1958) recorded a reduced volume of erythrocytes. Hochachka (1963) found that fish were not capable of buffering the large amount of lactic acid produced in the blood. He found five factors necessary for a given determination to have any particular relevance, namely: age, diet, physical training, exercise and oxygen tension, and concluded that a haemoglobin index of buffering capacity was impractical for most purposes. Possibly, the level of blood haemoglobin is closely related to the degree of muscular hyperactivity. Certainly, it must have been intense in the present investigation.

The literature records an increased osmotic concentration of the blood in fish sampled immediately after capture. My results support these findings. Forster and Berglund (1956) showed an increasing plasma osmolality for *Lophius americanus* with increasing time, after otter trawl net capture. Umminger (1970a) showed that capture of the flounder *Pseudopleuronectes americanus* by trawl elevated the serum osmolality. Bouck and Ball (1966) showed that the blood osmolality was significantly higher in seined rainbow trout, *Salmo gairdneri*, than in non-seined fish. Umminger (1970a) also noted that the serum osmolality was elevated in fish unaccustomed to handling when compared with fish "trained" to be familiar with the handling procedures. Pickford *et al.* (1969) also found that serum osmolalities were higher in "untrained" than in "trained" fish. Umminger (1970a) showed that the serum osmolality increased from 412 mOsm/l in fish killed 1–2 hours after capture to 461 mOsm/l in fish killed 5–6 hours after capture. In the present investigation a similar result was obtained. The plasma osmolality of steenbras sampled one hour after capture, 460 mOsm/Kg, was significantly higher than for those sampled immediately from the net, 368 mOsm/Kg. Furthermore, Umminger found that the serum osmolality of the freshly-caught fish (437 mOsm/l) was significantly higher than that of fish acclimated to the laboratory (389 mOsm/l). I found exactly the same result for steenbras, the values being 368 and 312 mOsm/Kg, respectively.

It was previously postulated that a possible cause of death in freshwater was asphyxiation, due to the decreased flow of the blood resulting from its increased viscosity. The present situation is comparable and it is conceivable that some of the fish in the net would have died from the delayed effects of hyperactivity, were they to have been transferred to holding tanks. Death due to the delayed effects of hyperactivity has been recorded by Black (1957a, c), Parker and Black (1959), Parker *et al.* (1959) and Bouck and Ball (1966). There is also support for the effects of a reduced oxygen supply, as a result of stress, in Black (1955, 1958), Black *et al.* (1962) and Stevens

and Black (1966). In addition, Black (1955) stated that the increase in the blood level of lactic acid could be due to the effects of excess carbon dioxide on oxygen utilization—the Bohr effect.

TABLE 17

THE EFFECT OF CAPTURE STRESS UPON PLASMA PROTEIN FRACTIONS IN STEENBRAS

Medium	*Plasma protein fractions (percent)*									
	a1		*a2*		*a3*		*β1*		*β2*	
	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*	$\bar{x}$	*S.D.*
Freshly captured estuarine	4,22	2,40	10,06	4,49	31,83	5,69	31,80	2,35	21,86	3,08
Acclimated in seawater for 48 hrs. after capture ..	6,50	3,69	9,43	4,45	25,57	5,82	41,52	4,33	16,95	2,99

From Table 17, four of the plasma protein fractions, namely *a*3, *β*1, and *β*2 ($P < 0,01$) and *a*1 ($P < 0,05$) were significantly different when compared to the "controls" (Fig. 12). There was also a highly significant increase ($P < 0,01$) in the total plasma protein in the estuarine sample. There is every reason to believe that these significant changes are also related to the general stress syndrome. The literature supports this assumption. Bouck and Ball (1965) noted that significant differences in blood plasma profiles resulted from conditions of low oxygen. They found that the method of capture of the rainbow trout, *Salmo gairdneri*, accounted for the high degree of variability in physiological parameters and that certain changes in the composition of plasma proteins was indicative of stressful conditions. Bouck and Ball (1966) found that protein concentrations were higher in seined fish (2,47 g/100 ml) than in non-seined fish (1,97 g/100 ml), in the latter case—from hooked or electroshocked fish. The comparable results obtained from the present study, namely 2,88 and 1,93 g/100 ml respectively, fully support the findings observed by Bouck and Ball. Thurston (1967) noted variations within ten blood serum proteins in the rainbow trout subjected to the different capture methods of hook and line, gill netting, electroshocking and dip netting. Thurston stated that an increased endocrine flow into the blood stream, tissue destruction releasing cellular proteins into the blood stream, and the effects of haemolysis could all have had some bearing on the changes in serum protein patterns. Bouck and Ball (1967) found that any of several factors such as stress, different temperature regimes, and different methods of capture, could change the amount of low-mobility protein. However, they concluded that the amount of low-mobility protein change was primarily controlled by genetic factors.

Apart from Forster and Berglund (1956), no other references exist on the effects of capture-method on plasma chloride, sodium or potassium. In the present investigation all three of these ions were present in significantly ($P < 0,01$) higher concentrations in the trek-netted estuarine sample, than in the 48 hour-acclimated seawater "controls". The possible reason for this could

well be that "capture diuresis" conditions caused increased secretions of the adrenal catecholamines, resulting in the haemoconcentration of the steenbras plasma. Increased haematocrit and osmolality would naturally follow, with an elevation of total plasma proteins and plasma electrolytes.

Forster and Berglund (1956) found an increased amount of chloride in the urine of the aglomerular teleost, *Lophius americanus*, which they directly attributed to the amount of rough handling received by the fish in the trawl net and the length of time spent in the net before capture. These authors also found significant increases of plasma chloride, sodium, and potassium with increased time spent in the net during capture. The results obtained from the present investigation support the findings of Forster and Berglund.

I therefore feel that a recovery acclimation period, to offset the effects of capture diuresis, is of the utmost importance for any haematological study of teleost osmoregulation under controlled experimental conditions.

REPRODUCTIVE BIOLOGY

INTRODUCTION

The Sparidae is a numerically large family of the Teleostei. Smith (1965) listed 34 species and 19 genera in South African waters alone. This did not include the new species *Lithognathus aureti* (Smith 1962), and more recently Penrith and Penrith (1969) described another new species, *L. olivieri*. There are four species of *Lithognathus* in South Africa, *L. lithognathus*, *L. mormyrus*, *L. aureti* and *L. olivieri*. There has been no detailed account of the reproductive biology of *L. lithognathus*, only brief notes occur in Scott *et al.* (1952), Millard and Scott (1954), Talbot (1955) and Day (1967). The only accounts of any biological detail (including reproduction) of sparid fishes from South Africa are by Talbot (1955) on the white stumpnose, *Rhabdosargus globiceps*, Lucks (unpubl.) on the steenbras, *L. aureti*, from South West Africa, and Penrith (1972) on the red roman, *Chrysoblephus laticeps*.

Hermaphroditism among the Sparidae is a well-known phenomenon and during the course of the present investigation it has been found that the steenbras is also hermaphroditic. Reproduction in the Sparidae has been studied by many authors, for example Van Oordt (1929), Kinoshita (1936), D'Ancona (1949 a, b), Reinboth (1962) and Lissia-Frau (1968, 1970) to mention only a few. Atz (1964), although himself not undertaking research on the intersexuality of fishes, has written an excellent and critical review of this subject and summarized the valuable contributions of D'Ancona—who first established the peculiar nature of sparid sexuality. The only previous accounts of hermaphroditism in the genus *Lithognathus* are by D'Ancona (1949 a, b), Suau (1955), Reinboth (1962) and Lissia-Frau and Casu (1968)—all for *L. mormyrus* (=*Pagellus mormyrus*); and Lucks (unpubl.)—for *L. aureti*. Lissia-Frau and Casu stated that *L. mormyrus* was a protandrous hermaphrodite. However, while establishing the undoubted hermaphroditism of *L. mormyrus*, both Suau and Reinboth (1962, 1970) considered its exact nature to be an open question. In the present investigation I examined four specimens of *L. mormyrus*, which were all gonochoristic. It would appear that this South African species is, therefore, not hermaphroditic. Lucks (unpubl.) gave an account of the hermaphroditism of *L.*

aureti from South West Africa, which was unfortunately based only on macroscopic examination of the gonads. Penrith (1972) has, however, described sex-reversal in another genus of sparid fish from South African waters, *Chrysoblephus laticeps*. Penrith also records that eight species of Sparidae show normal heterosexual development. However, two of the above species, *Polysteganus undulosus* and *Petrus rupestris*, which are listed as Sparidae, are classified by Smith (1965) as belonging to the Denticidae—a closely related family.

In the present study, the histology of the ovotestes from both estuarine and marine steenbras has been studied in detail in an attempt to determine the exact type of hermaphroditism exhibited. A new and successful technique, based on the research of Lang and Hansel (1959), was used to show that sex chromatin bodies (Barr bodies) are present in liver sections of the steenbras and can be used to determine its genetic sex. This is the first record of the presence of Barr bodies in teleosts. Comparisons have been made with earlier work on sparid hermaphroditism and a review of the possible origin and significance of teleost hermaphroditism is given. Evidence based on gonad histology is presented in an attempt to establish the breeding season.

PROCEDURE

The gonads from all the steenbras which were collected, both from estuarine and from marine samples, were dissected out, fixed in a 5% solution of formol saline for 48 hours and preserved in 70% alcohol. The intestinal fat index for each fish was visually determined and recorded (after Nikolsky 1963). In the laboratory the gonads were thoroughly blotted, the superficial fat and connective tissue trimmed off, and the weights recorded to the nearest 0,005 g. The heaviest estuarine gonads for each year class in each month, and the five heaviest marine gonads in each month (where available) were examined histologically. A small central piece of each gonad was sectioned at 7–10μ, and stained with azocarmine and azan. Maturity indexes for both sexes of the estuarine and marine samples were determined microscopically (after Braekevelt and McMillan 1967, Hyder 1969, Cala 1970, and Ruby and McMillan 1970), and representative samples of each stage were photographed.

RESULTS AND DISCUSSION

Morphology of the gonads

The gonads of all the estuarine samples were extremely small, thread-like, and virtually impossible to sex macroscopically. Not a single gonad showed any degree of development when examined macroscopically. Histological examination did, however, reveal the hermaphroditic nature.

The ovotestes of the typical marine steenbras are generally compact, rarely elongated, organs, situated in the posterior-dorsal portion of the body cavity. An ovotestis showing about equal male and female development consists of an outer testicular region which is white, solid and uniform in consistency and an inner ovarian part. The latter is reddish, partly lobulated, always extends farther anteriorally than the testicular portion and is well supplied with blood vessels from the gonadial artery and vein. The ovarian portion is usually surrounded in part by

the testicular portion and lies in a "groove" within it (Figs. 17, 20). In the more male-dominant ovotestes, the testicular sections envelope the ovarian sections to a considerable extent, the latter often being reduced to a thin central strip (Figs 18, 21). The rare female-dominant ovotestes are orange in colour, the testicular portion being reduced to very thin, white strips along the lateral margins of the ovary (Figs. 19, 22). Subsequent histological analyses showed that the above results support the findings of Atz (1964).

The testicular and the ovarian portions of the ovotestes open separately via sperm ducts and oviducts into the cloaca. Histological examination confirmed that the two portions of each ovotestis are separated by connective tissue from each other (Figs. 20–22) which is in agreement with the observations of Van Oordt (1929), Kinoshita (1936), Hoar (1957a) and Atz (1964). In total, there are therefore four openings into the cloaca. Forbes (1961) records that D'Ancona also noted this arrangement in the sparid, *Sparus longispinus*.

Hermaphroditism

Palaeontology provides no information on sexual status of fossil fishes. In the most primitive living vertebrates (the Cyclostomata) Walvig (1963) records that *Myxine* is a rudimentary hermaphrodite, indicating an apparent transitional stage from an originally effective hermaphroditism to dioecism. However, due to the lack of ducts for eggs and spermatozoa and the complete lack of connection between the genital organ and the nephric system, Walvig recorded that Schreiner (1955) considered these to be secondary phenomena and that the Cyclostomata, therefore, are not primitive but secondarily modified. If this is the case (which does seem probable), then hermaphroditism in the cyclostomes would appear to be a more recent evolutionary phenomenon. Atz (1964) records Stephan as stating that regardless of whether the ancestors were hermaphroditic or not, present-day normal hermaphroditic fishes are in the minority with a larger number of gonochoristic species, and it would appear that hermaphroditism must represent an entirely new evolutionary development. This latter view is also accepted by Harrington (1971). It is now generally accepted by most authors that the original vertebrates were gonochoristic (Atz 1964), and for the purpose of the following section I have accepted this hypothesis.

There has, likewise, been speculation as to whether fishes first evolved in freshwater or in a marine environment. Robertson (1957) is strongly in favour of a marine origin. He bases his hypothesis on the fact that the earliest vertebrate remains found in the sedimentary rocks of the Ordovician and Silurian periods were mostly associated with marine invertebrate fossil deposits, and stated that as the present-day lower chordates are marine, so probably were the first vertebrates. Conversely, Romer (1966) maintains that the fossil records of the late Silurian and early Devonian vertebrates suggest that the oldest fishes lived and evolved in fresh, inland waters. Robertson agrees that some of the early ostracoderms did inhabit freshwater but that they had secondarily invaded the rivers from the marine environment in which they had evolved. Romer, however, appears to attach little significance to the very strong palaeontological evidence presented by Robertson which supports a marine vertebrate origin. Romer bases his theory of freshwater origin on the glomerular kidney of fishes, which strongly favours osmotic regulation in a freshwater medium.

Black (1957) supports Romer's theory. Robertson argues that the glomerular kidney may

already have existed in marine protovertebrates and similarity between the salt concentration of present-day myxinoid plasma and seawater might be a primitive characteristic derived from marine chordate ancestors. Robertson's first argument is problematical and cannot be substantiated, while the second assumption does not take into account that the cyclostomes are probably secondarily modified and not at all primitive in relation to their ostracoderm ancestors. I find it difficult to accept his theory on the glomerular kidney, in view of the importance in osmoregulation of the specialized secretory cells of the gills. I therefore support the current line of belief (Black 1957) that the first fishes probably evolved in freshwater.

Ghiselin (1969) proposed that hermaphroditism evolved under the following conditions: (*a*) where it was hard to find a mate; (*b*) where one sex benefited from being larger or smaller than the other; (*c*) where there were small, genetically isolated populations. Assuming that the teleost ancestors were gonochoristic and evolved in freshwater, it is not difficult to incorporate all three of Ghiselin's conditions in the development of hermaphroditism. With the invasion of the sea, the teleost ancestors would have been faced with a vastly increased diversity of niches in their new environment, such that speciation and adaptive radiation, resulting from prolonged geographical isolation, would have given rise to genetically isolated populations. With speciation and the resulting barriers which were created, mutations, appearing in one race of the population, would almost certainly not be introduced into others so that the gene pool of each of them would acquire ever-increasing differences in the course of time. Therefore, those fish which were hermaphroditic would be favoured by natural selection (Kosswig 1963).

In the eventuality of any one of Ghiselin's situations occurring, the advantages of hermaphroditism over gonochorism are manifold and only a few examples are given here. The different forms of hermaphroditism, other than synchronous, would compensate for and decrease the chances of inbreeding by self-fertilization and the possible crosses and lowering of the effective population size, caused by the numerical excess of one sex over the other (Ghiselin 1969). Obviously, self-fertilization would be vital for survival in a fish which is alone in an isolated body of water. However, only one known fish, the cyprinodont *Rivulus marmoratus*, exhibits the ultimate mode of hermaphroditism, namely synchronous hermaphroditism with self-fertilization (Harrington 1971).

Penrith (1972) presents a very interesting theory for the presence of hermaphroditism, in this case protogynous, in the sparid teleost *Chrysoblephus laticeps*. This species is characterized by the fact that the larger the individual grows, the less efficient it becomes at swimming. Large specimens are invariably found in caves or rock shelters on reefs. Penrith believes it is doubtful whether the large specimens, lacking a shelter, would have the speed and manoeuvrability to avoid large predators, such as sharks and seals. Sex-reversal results in the larger fish becoming functional males. Consequently, a large male could be expected to have the potential to fertilize several females, the latter being small and better able to avoid predation. Gonad evolution has therefore proceeded to the point where the still mobile size range is composed of functional females and the hydrodynamically less efficient larger size range of functional males. In this way survival of the species has been favoured. Not only are all three of Ghiselin's (1963) postulates, especially the second, incorporated in this theory—but I believe that one need look no further than this impressive argument for a possible reason for the evolution of hermaphroditism in fishes. Certainly, it is the best argument yet presented in the literature in this vast field.

Atz (1964) has defined the different types of hermaphroditism in fishes and although the terminology is sometimes confusing and has often been the subject of new, inexact definitions (Harrington 1971), Atz's definitions have been used in the present investigation.

Of all the vertebrates, teleosts show the most divergent expressions of sex, and hermaphroditism in the Sparidae is the most complex of all teleost families (Atz 1964). Atz states emphatically that the hermaphroditic Sparidae evolved from gonochoristic forms, but believes that the hermaphroditism of the closely related Sparidae and Maenidae is an example of parallelism, involving a common, but not ancient ancestor that was already hermaphroditic or had hermaphroditic tendencies.

According to Atz D'Ancona refers to the Sparidae as intersexes, rather than hermaphrodites. Although by definition all hermaphrodites are intersexes but not all intersexes are hermaphrodites (Atz 1964), D'Ancona distinguishes between the two. He states that instead of mutual tolerance existing between the two sexual tendencies as in hermaphroditism, intersexuality is the result of interference with the two opposing sexual tendencies, neither of which can therefore exert itself decisively. However, D'Ancona added that in the intersexes the testicular and ovarian elements are thoroughly mixed together in the gonad, although probably not entirely at random. In the steenbras, the testicular and ovarian elements are always clearly separated from each other by connective tissue and there can be no doubt at all that the steenbras at least, is one sparid fish that is hermaphroditic and at no stage an intersex.

Oöcytes or oöcyte-like cells have occasionally been found in the testes of normally gonochoristic teleosts and have frequently been observed in the gonads of the hermaphroditic Serranidae and Sparidae (for summary, see Atz 1964). Among these occasional hermaphrodites occurring in a normally gonochoristic species, the same phenomenon has been observed by James (1946) in the large mouth bass, *Huro salmoides*, and by Takahashi (1970) in the gold fish, *Carassius auratus*. Among the Sparidae, in addition to the authors listed by Atz, it has also been recorded by Lissia-Frau (1968) in the protogynous hermaphrodite, *Boops boops*, and Kinoshita (1936) in the protandric hermaphrodite, *Sparus longispinus*.

In the steenbras, this phenomenon was observed in several individuals, the usually primitive Stage I oöcytes lying in well-developed testicular fields (Fig. 23). That this phenomenon could occur in species in which the sexual products are intermingled in the ovotestes as in some serranids (Atz 1964, Smith and Young 1966), or in transformed males of a protogynous species (Smith and Young 1966), is understandable. However, in the steenbras the testicular and ovarian fields are always clearly separated by connective tissue, which makes the presence of oöcytes in the testis difficult to explain.

There are various theories on the significance of this phenomenon (Atz 1964) none of which, however, has been sufficiently substantiated. Takahashi (1970) believes that the answer lies in the mechanism of gonadial sex differentiation during the larval stages, when the germ cells of the sexually indifferent gonad are bipotential. According to Atz, Kolmer and Scheminsky (1922), Kinoshita (1933) and Singe and Sathyanesan (1961) believe that this occurrence is *bona fide* evidence of hermaphroditism. I am in agreement with this hypothesis and also with Takahashi's theory.

The embryological development of the gonads in bony fishes is distinctly different from that of other vertebrates. Instead of the foetal gonads arising from the embryological cortex and

medulla, there is only a single primordium equivalent to the cortex (D'Ancona quoted by Atz 1964). D'Ancona suggested that this lack of separation of the male and female elements during development could be an important factor in the development of hermaphroditism among teleosts. Many prominent authors agree that this is indeed a distinct possibility, including Gorbman and Bern (1962) and Van Tienhoven (1968).

There is little knowledge on the basic type of hermaphroditism in the Sparidae. According to Atz (1964) both Kosswig and D'Ancona reason that it should be rudimentary, i.e. the individual functions only as a male or only as a female. Kosswig stated that this occurred only in species with a genotypically determined sex as evidenced cytologically by the presence of sex chromosomes or genetically by sex linkage, while D'Ancona based his reasoning on the structure and development of the gonads. In a subsequent section I will show that the steenbras probably possesses a genotypically determined sex, and so Kosswig's reasoning finds some support from the present work. Nevertheless, the fact is that the Sparidae exhibit every possible form of hermaphroditism. The possible types are outlined and discussed in a later section, but as Atz (1964) has stated: "the Sparidae seemingly provide a step-wise progression from species in which all the individuals are protandrous or protogynous hermaphrodites, through rudimentary hermaphrodites of different forms and frequencies, to species that are structural as well as functional gonochorists". The results from this study lend support to Atz's observations and present no evidence to the contrary.

That the development of hermaphroditism in fishes must be influenced endocrinologically, is almost certain. However, marine fish have proved poor experimental subjects because of the difficulty of prolonged maintenance in aquaria, so it is not surprising that there is virtually no literature pertaining to endocrine studies on hermaphroditic teleosts, and practically nothing is known about the hormones of the protogynous, protandrous or synchronous hermaphroditic fishes (Atz 1964).

Some evidence is available to show that teleost testes and ovaries secrete androgens and oestrogens respectively, and that manipulation of these hormones can affect the secondary sex characters in some cases (Reinboth 1972a). It has also been established that hormonal action is probably under the control of secretions from the anterior pituitary (Forbes 1961, Gorbman and Bern 1962, Atz 1964, Rai 1966a, b, Van Tienhoven 1968). However, although many typically mammalian sex steroids, especially testosterone and oestradiol-17β, have now been isolated from different fish species (Atz 1964, Reinboth 1972b), treatment of fishes with synthetic sex steroids often produces results which are not expected on the basis of mammalian physiology and it is often impractical to use them (Hoar 1957b). Although Forbes (1961) stated that many structures resembling corpora lutea occur in teleost ovaries, he doubted whether these could be compared to mammalian corpora lutea, as no progesterone had been isolated from them. However, Van Tienhoven (1968) presents evidence that progesterone has been found in the blood, ovaries, and ovotestes of some species, but it is nevertheless thought that the acquisition of an endocrine function by the corpora lutea is an evolutionary recent event (Forbes 1961).

According to Atz (1964), D'Ancona postulated the presence of two embryonic hormones, "androgenine" and "gynogenine", produced by the gonadial medulla and cortex, one supposedly influencing the sex elements of its own primordium and having an inhibitory influence on the opposite one. D'Ancona believed that the Sparidae offered the best example of how these two

hormones might work. Since the male and female portions are clearly demarcated, he suggested it was possible for gynogenine to first exceed some threshold in protogynous hermaphroditic sparids, with androgenine subsequently taking its place, and that only one of the two hormones would ever reach the threshold level. Harrington (1971) records that Reinboth has refuted the above theory, as androgenine and gynogenine were considered to operate merely as embryonic sex differentiators, and not in adults. The postulation that the non-steroid sex differentiators, androgenine and gynogenine, rather than the steroids are the active agents in sex succession has become increasingly doubtful.

Atz (1964) records that Reinboth (1962) injected the serranid, *Serranus cabrilla*, intramuscularly with testosterone. This resulted in the ordinarily flattened epithelial lining of the ovotestis cavity becoming columnar with young oöcytes appearing within the epithelium even where it covered the testicular tissue. Ovarian degeneration was rare while spermatogenesis was only slightly enhanced. Injections of oestradiol distended the gonads and ducts and considerable degeneration of the ovarian tissue occurred. The larger oöcytes were destroyed but young ones formed, while spermatogenesis was stimulated. In summary, the oestradiol appeared to have had a negative effect on the ovarian part but a positive effect on the testicular part, the reverse results expected from mammalian physiology (*cf.* Hoar 1957b). If, as Atz believed, there was an apparent lack of specificity by the androgens and oestrogens, then the suggestion that this was indicative of a synchronous hermaphrodite has some validity.

Reinboth treated the sexually undifferentiated protandrous hermaphroditic sparid, *Sparus auratus*, with oestrogen and found that both male and female development was suppressed. Androgen treatment of the protogynous hermaphrodite *Maena maena* resulted in degeneration of the ovarian part of the ovotestis, enlargement of the testicular parts and ducts, and a commencement of active spermatogenesis. Both of these experiments confirmed the fact that the effects of oestrogens on hermaphroditic teleosts seem to be much less specific than that of androgens, while the administration of androgen can cause precocious sex-inversion in protogynous species (Reinboth 1970).

The remaining endocrine research performed on sex-reversible and hermaphroditic teleost species is worthy of brief summary. According to Forbes (1961), Berkowitz (1938, 1941) and Querner (1956) administered female sex hormones to male *Lebistes*, which transformed the testes to ovotestes and feminized the secondary sex characters of immature, but not of mature males, Oestrogen had no effect on the females. However, Forbes (1961) records that Okada (1943) and Egami (1955) found that various oestrogens administered to *Oryzias latipes* caused the appearance of "testis-ova". Forbes (1961) records that Vallowe (1957) found that similar treatment apparently had the same result in immature, but not in mature male *Xiphophorus*, while Padoa (1939) found that treatment of the sparid *Serranus hepatus* with oestrogen stimulated the growth of the hermaphrodite gonad.

In the following section I will show that the dominant sex of the steenbras, whether it be male or female, is genetically determined. It follows that if the testicular part of the ovotestis is maintained genetically and there is little or no endogenous testosterone as yet in the immature fish, then the oestrogens could have a direct effect on the secondary sex characteristics as well as the gonads – transforming them to ovotestes. Furthermore, the effects of the oestrogens would presumably not be transhypothalamic because the pituitary hormones released, *e.g.* LH and

FSH, would cause secretion of androgens and spermatogenesis respectively, or in unison – and would not produce a hermaphroditic gonad. Oestrogens could, however, block the secretion of the hypothalamic-releasing factors. This hypothesis, is, however, unsubstantiated by any experimental evidence and must at this stage remain pure conjecture.

According to Takahashi (1970), Miyamori (1961, 1964) studied the effects of oestrogens and androgens on the behaviour of testicular cells in *Lebistes reticulatus*, which aggregate at the gonad hilum at an initial stage of gonadogenesis and proliferate to surround the cluster of gonia during testicular differentiation. He found that their development was entirely suppressed by treatment with oestrogen, but was promoted by androgens. Takahashi suggested that an androgenic-inductor-system for gonadial sex differentiation, possibly comprised of somatic cells of different origin in the teleost fishes, may have been maintained as a vestige in the developing ovary, to exert an incomplete androgenic influence upon the ovarian primordial germ cells which are still bipotential in sex differentiation.

Clearly, further research is required along the lines of the above experiment to elucidate the endocrine mechanisms involved in teleost hermaphroditism.

SEX CHROMATIN

Introduction

The presence of intranuclear chromatin bodies (the sex-chromatin bodies) in the female nerve cells of the cat, but only rarely in the male, was first established by Barr and Bertram (1949). Since then research has shown that this condition of sexual dimorphism at the cellular level exists in man and many other mammals (Lang and Hansel 1959, Gardner 1972); in birds (Arora and Dharamarajan 1970); and in two reptilian species (Cock 1964). Cock also reports that Smith (1945) found the same phenomenon in the Lepidoptera. This difference has provided a decisive criterion for the determination of genetic sex (Gorbman and Bern 1962). As far as I could ascertain there is no previous record of sex chromatin being present in teleosts.

Procedure

Small pieces of liver were dissected from 10 estuarine and 10 marine steenbras, fixed in Davidson's fixative for 24 hours and then preserved in 70% alcohol. After dehydration and paraffin-wax embedding, sections were cut at 7–10μ and stained for sex-chromatin with thionin (after the method of Klinger and Ludwig 1957; *In* Humanson 1966). Five areas on each section of each slide were randomly selected and examined under oil-immersion giving a magnification of 1000. Resolution at this magnification was very good. The percentage of nuclei containing the sex-chromatin bodies was recorded from each grid area.

Results and discussion

Barr and Bertram (1949) established a sex difference in cat neural cells based on the presence of an intranuclear chromatin body in the female cell. This body was located adjacent to the nucleolus and was termed the "nucleolar satellite". Barr and Bertram suggested that the somatic cells of various tissues, characterized by large nucleoli, would display distinctive nuclear differences according to sex. This has been substantiated by all the investigators of sex-chromatin,

who have recorded this phenomenon from a variety of somatic tissues such as liver, pancreas, adrenal, duodenum and skin. However, this chromatin body was found rather to be apposed to, and more or less flattened against, the inner surface of the nuclear membrane, and not associated with the nucleolus. Accordingly, the term "nucleolar satellite" has been replaced by the term Barr body (Ham 1965). In some liver cell nuclei of the steenbras Barr bodies were detected, attached to the inner surface of the nuclear membranes. These are shown in Figs. 24 and 25.

Table 18 records the prediction of the genetic sex from the liver tissues of estuarine and marine steenbras. The male tissue had from 0–2,5% of the nuclei showing the sex-chromatin bodies, a range significantly lower $P < 0{,}01$) than that exhibited for the female tissue, 11–41%. It was decided to take an arbitrary figure of 10% and classify all those samples showing a greater percentage of sex-chromatin bodies as female, and those showing less than 10% as males. In all cases the postulated sex agreed with the actual, dominant sex in the marine samples. The latter diagnosis was based on the macroscopic and microscopic examination of the dominant gonadial area of each ovotestes. In the macroscopic determination of the dominant sex of the estuarine steenbras ovotestes, six individuals in the sample of ten were in agreement with the postulated genetic sex. However, as was previously pointed out the macroscopic determination of the actual, dominant sex of estuarine steenbras gonads was virtually impossible, due to their infantile state. Therefore, some disparity between the two sets of predictions was to be expected. The percentage incidence of sex-chromatin in female and male steenbras liver tissue was appreciably lower than that recorded by Lang and Hansel (1959) for cattle, and Arora and Dharamarajan (1970) for birds. However, the amount of sex-chromatin present would seem to be clearly species-specific, and no special significance can be attributed to these apparent differences. Nevertheless, the difference between the two sexes in steenbras was still so significant, that I consider the results obtained to be a clear case for genetic sex determination.

The discovery that the genetic sex is apparently predetermined before development is strong evidence in favour of "rudimentary hermaphroditism" namely, where the individuals function only as a male or female (Atz 1964). Atz recorded that Kosswig (1932) applied this type of hermaphroditism only to species with a genotypically determined sex, a procedure supported by the present results. Presumably, if the steenbras were protandrous or protogynous hermaphrodites and displayed sex-reversal then the sex-chromatin bodies would either not be present, or if so, would occur randomly with no relation to the actual dominant sex. Many of the investigators of sparid hermaphroditism, as summarized by Atz, postulate rudimentary hermaphroditism in many of the species studied.

Although the information available on the genetic mechanisms for sex-determination in laboratory and natural populations of fishes is based almost entirely on the presence or absence of sex-linked colour patterns and other secondary sex-characters (Atz 1964), this information is far more extensive and critical than in any other vertebrate group (Gordon 1957). Unfortunately, however, there is virtually no conclusive evidence as to the genetics of sex determination in hermaphroditic teleosts. The major complication seems to be that the chromosomes of fishes are small, not well differentiated and too numerous to be suitable for cytogenetic studies (Atz 1964). All research in this field remains purely speculative. However, I maintain that a teleost species exhibiting any of the many forms of hermaphroditism, must have the genetic make-up and potential for a particular form of hermaphroditism to develop. In addition, en-

TABLE 18

PREDICTION OF GENETIC SEX IN STEENBRAS AS DIAGNOSED BY PRESENCE OF SEX CHROMATIN

Environment of Samples	*Percentage nuclei including sex chromatin*	*Genetic sex Postulated*	*Actual, dominant macroscopic Sex*
Marine	35	F	F
	23	F	F
	18	F	F
	14	F	F
	11	F	F
	2,50	M	M
	0,60	M	M
	0,35	M	M
	0	M	M
	0	M	M
Estuarine	41	F	F ?
	31	F	M ?
	27	F	M ?
	25	F	M ?
	22	F	F ?
	21	F	M ?
	15	F	F ?
	2,50	M	M ?
	1,00	M	M ?
	0	M	M ?

vironmental stimuli must play a vital role and Gorbman and Bern (1962), Atz (1964) and Montalenti (1956) express similar views. Unfortunately, how this is accomplished is largely unknown, although Gorbman and Bern believe the most critical environmental factor regulating reproductive activity and development is photoperiod, and Harrington (1971) has experimentally substantiated this.

In an interesting study of protogynous sex-reversal in the anthiid teleost, *Anthias squamipinnis*, Fishelson (1970) found that the regulation of sex-reversal was apparently activated by a visual stimulus. He found that when a group of males and females were kept together no changes occurred in the morphology or behaviour of the females to indicate a sex change, although histology of the ovaries showed advanced follicle degeneration. When all the males were removed, one of the females underwent a sex-reversal to become a male and by removing each new male as it developed, Fishelson induced the whole female population to undergo sex-reversal, one by one. Naturally, this development has adaptative implications for survival, as the males are only produced when needed, while the majority of the population consists of reproducing females. However, that the sole environmental stimulus that induced sex-reversal was a visual one is unlikely. Presumably, some genetic influence was involved.

Barr and Bertram (1949) stated that in the neural cells of the cat the nucleolar chromosomes are frequently the sex-chromosomes. They postulated that the sex-chromatin spots were possibly derived from the heterochromatin of the sex-chromosomes. Furthermore, they stated that the cells of the female cat, because of the duplicated X-chromosomes, could have been endowed with a greater quantity of nucleolar associated heterochromatin than were the cells of the male cat. Barr and Bertram concluded that whether or not sex differences in nuclear structure would be found in a given species would probably be determined, in part, by the relationship of the nucleolus to the sex-chromosomes and by the disparity in size and composition between the X- and Y-chromosomes. Lang and Hansel (1959) predicted that the X-chromosomal heterochromatin should be discernible in male cattle cells. However, they found that the large number of autosomal chromocentres of similar size precluded the identification of the X-chromosome, and suggested that the weakly chromotropic mass in the periphery of the male cattle cells could be the heterochromatic remains of the X-chromosomes.

However, it is now known that the sex-chromatin is derived from only one of the 2X-chromosomes, the other X-chromosome behaving as an autosome (Ham 1965, Swanson *et al.* 1967, De Robertis *et al.* 1970). One of the 2X-chromosomes of females is heterochromatic during interphase and is a dense enough body when stained to constitute a visible Barr body (Ham 1965). The remaining X-chromosome is euchromatic and is not visible at interphase. As male nuclei contain only one X-chromosome, which is euchromatic, male cells do not display Barr bodies. Occasionally, bits of chromatin close to the nuclear membrane in male cells are interpreted as Barr bodies. However, these are probably due to chromatin particles not belonging to sex-chromosomes, being accidently shifted to a position close to the nuclear membrane (Ham 1965).

De Robertis *et al.* (1970) stated that the number of sex-chromatin bodies visible at interphase is one less than the number of X-chromosomes. In the steenbras, never more than one sex-chromatin body was detected in the liver nuclei. Therefore, it would seem that the genetic female steenbras is XX, while the genetic male could be either XY or XO.

Gorbman and Bern (1962) stated that the genetic basis of sex could be related to a single pair of sex-chromosomes, the embryological process apparently being one of selective development of one of only two alternative sets of tissue in the gonad. How this genetic information, transmitted by the embryonic nuclei, is translated into sex differentiation is unknown, but Gorbman and Bern suggested that a hormone-like factor produced in one gonad would influence or reverse the sex differentiation in a slightly younger gonad. I concur with these statements.

Clearly, the solution to the whole nature of sex differentiation and development in hermaphroditic species lies in the endocrine and genetic approach.

Effect of season on sexual activity in estuarine steenbras

The abundance of steenbras in the Heuningnes River Estuary system did not fluctuate over the one-year study period. There was always a plentiful supply available. Evidence that steenbras, especially the juveniles, are plentiful in many of the estuarine systems of the Cape Province is provided by Biden (1930), Scott *et al.* (1952), Millard and Scott (1954), Talbot (1955), Day (1967) and J. J. R. Louw (*pers. comm.*). Macroscopic examination of the gonads from 420

estuarine steenbras showed that at no stage of the year was there any indication of maturation, the gonads remaining permanently thread-like and extremely light in weight. Fig. 13 shows that there was an increase in gonad weight with age, but the overall gonad weights were still extremely low for all the age groups when compared to most teleost species.

The five heaviest, and presumably the best-developed gonads, from each age group (when available) of each month were histologically examined. All the gonads were found to be hermaphroditic and on the basis of their development were assigned a maturity index based on either four ovarian classes or four testicular classes. The four ovarian classes (after Braekevelt and McMillan 1967, Cala 1970) and the four testicular classes (after Hyder 1969, Ruby and McMillan 1970) are described below and illustrated in Figs. 26–32.

Ovarian Classes (Figs. 26–28):

1. Only undifferentiated oögonia present, roughly spherical, and with a prominent nucleus.
2. Ovary forming germinal epithelium along ovarian cavity. Most of gonad containing undifferentiated oögonia, but a few primitive oöcytes present in the germinal epithelium. The latter, irregular in shape but with a large, undifferentiated nucleus.
3. Increase in size and extent of germinal epithelium, showing typical ovarian lamellae and increased differentiation of the oögonia (Fig. 26). Stage I oöcytes are present in the epithelium, these being larger than the primitive oöcytes, with the nucleus containing a single, prominent nucleolus (Fig. 27).
4. As for Class 3, but many more Stage I and II oöcytes in the germinal epithelium, the latter being distinguishable from the Stage I oöcytes by the presence of many small nucleoli in the nucleus (Fig. 28).

Testicular Classes (Figs. 29–32):

1. Only undifferentiated spermatogonia present, irregularly shaped and with a well-defined nucleus. No seminiferous tubules apparent.
2. Small seminiferous tubules, if present, irregular in shape and empty (Fig. 29). A few small clumps of early spermatocytes present in the slightly more differentiated spermatogonial field. These spermatocytes are more prominent than the spermatogonia, due to a deeply-stained nucleus occupying almost the entire cell. Many spermatogonia show mitotic stages of division in the nuclei, the black chromatin threads being clearly visible.
3. Seminiferous tubules more differentiated, either round or elongated, containing a few primitive spermatids, these being small and round, with minute deeply stained nuclei (Fig. 30). Many spermatogonial cells still show mitotic activity.
4. As for Class 3, but with seminiferous tubules well differentiated, containing many spermatids (Fig. 31), some exhibiting traces of tails (Fig. 32). Many clumps of well stained spermatocytes present.

Fig. 14 shows the average maturity indexes, based on both the ovarian and the testicular classes, per year groups. There is evidence of the expected trend in increasing maturity with increasing age, this being more pronounced from the testicular area of the ovotestes. However, the extremely light weights of the gonads even in those fish showing the highest maturity index, and the fact that the intestinal fat index (after Nikolsky 1963), always a good indicator of gonad

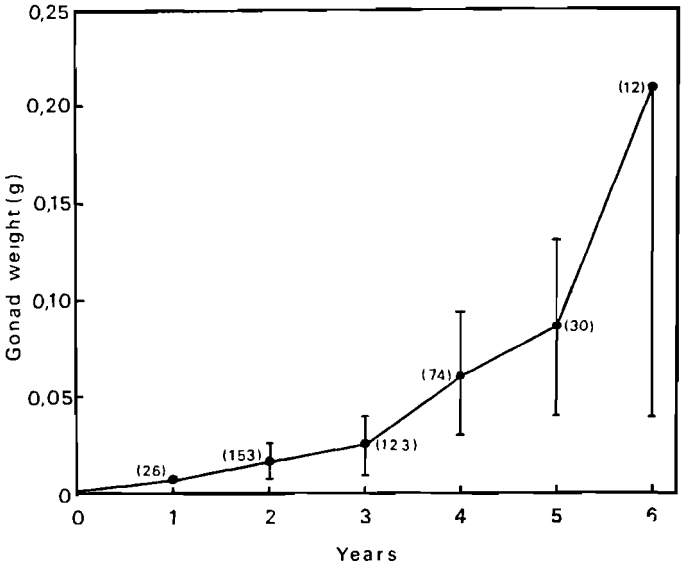

FIGURE 13
Mean gonad weight plotted against age for 420 estuarine steenbras. Figures in parenthesis indicate number of fish examined in each year class. Standard deviations indicated by T-bars.

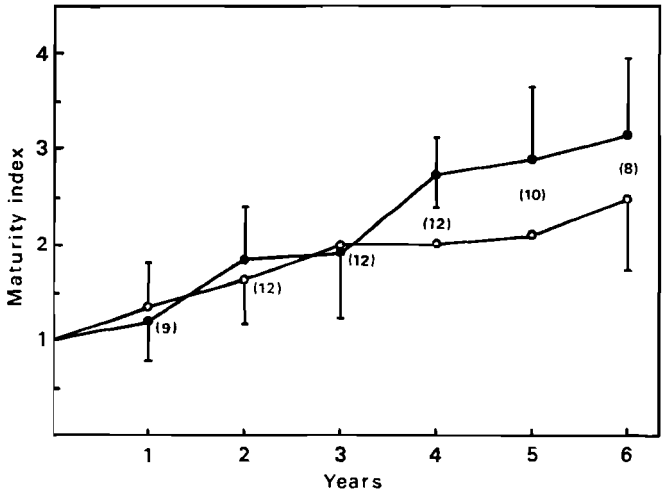

FIGURE 14

Average maturity indexes of 68 hermaphroditic gonads from estuarine steenbras per year class. Figures in parenthesis indicate number of fish examined in each year class. Standard deviations indicated by T-bars.
● Testicular portion of ovotestes
○ Ovarian portion of ovotestes

maturation, was rarely above one unit, indicates that the estuarine steenbras did not exhibit true sexual activity. This is remarkable in view of the relatively advanced age of some individuals (six years). It is possible, however, that the small measure of sexual development which does occur may act as a stimulus for migration to the ocean. As will be discussed later, the estuarine fish, although never showing complete activity, do show maximum sexual development at the same time as the marine fish, which would substantiate the above presumption. Scott *et al.* (1952), Millard and Scott (1954) and Day (1967), all record that the gonads of steenbras are undeveloped in estuarine samples. The statement by Day (1967) that the roes never ripen in the estuary and that the steenbras migrate to the sea to spawn, is fully substantiated from the evidence of this investigation.

Of interest, is the fact that Biden (1930) recorded large white steenbras netted in various Eastern Province rivers in winter, when the roes were found to be fully mature. Smith (1965) records that the range of the white steenbras does extend to the Eastern Cape and also that in 1944 large numbers of steenbras, averaging 25 kg were stranded in a Transkei river mouth. These fish would almost certainly have had well-developed gonads. These cases, however, probably represent unusual situations.

Fig. 15 shows the maturity indexes of both sexes of the ovotestes, for the year classes 4–6 inclusive. The fluctuation of the testicular indexes in the 4 and 6 year age groups follows the same trend, namely, a general rise during the first six months of the year, and then an abrupt regression from July to September. The 5 year age group also exhibits this trend, but at an earlier period of the year. The ovarian indexes are stable throughout the year in the 4 and 5 year classes, but in the 6 year old fish the fluctuation follows that of the testicular indexes. The data from Fig. 15 suggest a possible cyclic rhythm even in these immature fish, especially on the evidence from the testicular part of the ovotestes. Atz (1964) records that Reinboth (1962) found similar situations in some protandrous forms, the oöcytes present in the ovotestes during the male phase of the fish's ontogeny showing annual cyclic changes even though they never passed beyond an early stage of growth. The data from Fig. 15 are in agreement with a possible winter spawning in July and August for the larger marine fish. The latter aspect is fully discussed in the next section.

TABLE 19

DOMINANT SEX IN ESTUARINE STEENBRAS OVOTESTES AS DIAGNOSED BY HISTOLOGICAL EXAMINATION

	Ovotestes		
Number of fish	*Male-dominant*	*Female-dominant*	*Equal development*
65	68%	31%	1%

The histological evidence summarized in Table 19 is indicative of a protandrous hermaphroditism in the steenbras. By definition, a protandrous hermaphrodite is an individual which functions first as a male and later in life as a female (Atz, 1964). Since nearly 70% of the gonads, which were histologically examined, showed testicular-dominant ovotestes, it was at first assumed that the steenbras would undergo a sex-reversal later in life and then function as a female. The significance of this postulation is fully discussed in the following section.

That the young steenbras should seek out an estuary to live the first years of their lives is not surprising. The variety and abundance of food items as well as the unlikely presence of large predators in the Heuningnes estuary, makes this a logical occurrence. The Heuningnes River is permanently open to the sea and it was noticed that the steenbras moved up and down stream with the tidal fluctuations. It is a well-known fact that most estuarine fish leave their habitat to spawn at sea and it seems as if steenbras can be included in this category. At some stage in the

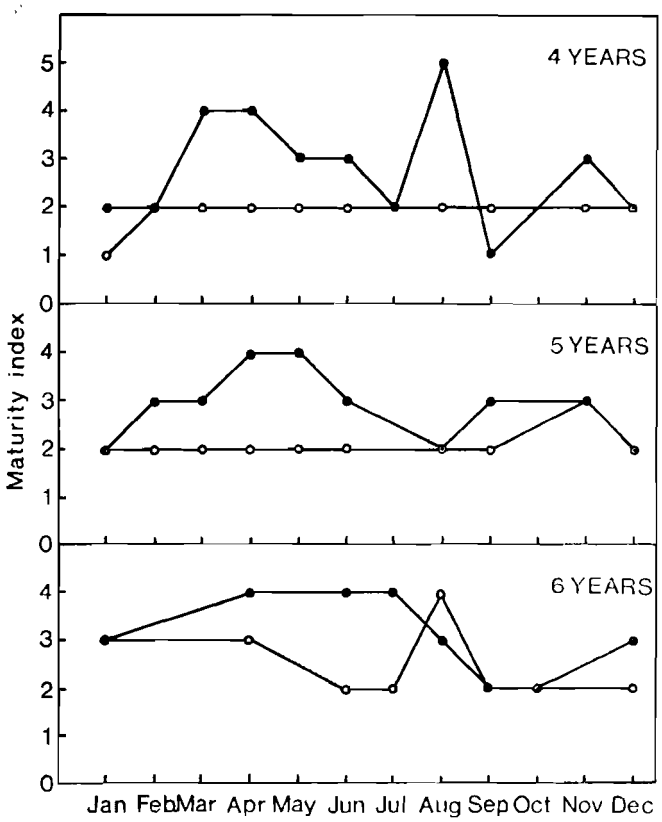

FIGURE 15

Monthly average maturity indexes of ovotestes of estuarine steenbras at 4,5 and 6 years.
● Testicular portion of ovotestes
o Ovarian portion of ovotestes

life-cycle, a "trigger-mechanism" must be activated, which causes the fish to migrate to the sea where they mature and eventually breed, probably never to return to the estuary, at least not in significant numbers. What this "trigger-mechanism" is and how it operates, is unknown. Logically, some environmental influence such as photoperiod, salinity fluctuation, nutrition, or water temperature change should be involved, but the most likely cause would probably be an endocrine one. From the histological examination, no evidence was found of any interstitial cells or their piscine equivalent – seminiferous tubule boundary cells (Gorbman and Bern 1962). However, some pituitary hormone activity must be involved, but it is apparently below the threshold value required for the steenbras to reach full maturity in the estuary, but perhaps sufficient to trigger the pre-maturation and migration to the sea. Chan and Phillips (1967) have recorded the extensive development of interstitial (Leydig) cells among the interstitial fibroblasts in the gonadial lamellae of the protogynous synbranchiform, *Monopterus albus*, concomitant with the rapid proliferation of the male germ cells. This was not observed in the steenbras.

It is concluded that because of the abundance of food available and the absence of many natural enemies, the estuarine stage in the life-cycle of the young steenbras is a vital one. Every effort should therefore be made to protect the estuaries of the Cape Province to ensure the future stocks of marine steenbras, which is such an important angling species.

Effect of season on sexual activity in marine steenbras

Although the steenbras is a common angling species along the southern Cape coastline, its capture is subject to considerable fluctuation depending on weather conditions. Despite every effort to sample as many as possible only 68 specimens of 25–83 cm fork length and 0,3–8,0 kg weight were captured during the study year.

Macroscopic examination of the gonads from the marine steenbras showed them all to be hermaphroditic. Forty-two were examined histologically and placed in the ovarian and testicular classes based on Braekevelt and McMillan (1967) and Hyder (1969), respectively. These are described below and illustrated in Figs. 33–40.

Ovarian Classes (Figs. 33–37):

5. Germinal epithelium extensively folded into ovarian lamellae and containing many Stage I, II and III oöcytes. The Stage III oöcytes could be easily distinguished by the fact that the nucleus occupies most of the cell and the numerous nucleoli were pressed up against the nuclear membrane. In the cytoplasm small yolk globules are visible (Fig. 33).
6. A breakdown of the ovarian lamellae, with the oöcytes lying free in the ovarian cavity. Many Stage II, III and IV oöcytes present, the last characterized by the increased size and number of the yolk globules which had migrated to the periphery of the cell and the beginning of disintegration of the nucleus (Fig. 34).
7. Appearance of Stage V oöcytes in the ovarian cavity, along with Stage II, III and IV oöcytes. The Stage V oöcytes are characterized by the presence of a distinct vitelline membrane at the periphery of the cell. The nucleus and nucleoli have coalesced into a single prominent spherical ball (Fig. 35).
8. Early stage of "atresia" of oöcyte, the roughly spherical ball consisting of atretic cells. The vitelline membrane is still discernible (Fig. 36). Most of the ovary consists of Stage I and a few Stage II oöcytes.

9. Presence of a few collapsed follicles in the ovarian cavity following "atresia". The follicle is an irregularly shaped conglomeration of follicular cells. This stage was only rarely observed (Fig. 37). Most of ovary consists of Stage I oöcytes.

Testicular Classes (Figs. 38–40):

5. Seminiferous tubules now extensive and branching, packed with masses of spermatids and spermatozoa. It was difficult to distinguish between the spermatids and spermatozoa but under high magnification spermatozoa, especially at the periphery of the mass, displayed prominent, deeply stained tails (Fig. 38). Pockets of spermatocytes are still present in the tissue between the tubules, some having burst open into the seminiferous tubules (Fig. 39).
6. General appearance of "spent" testis. Seminiferous tubules empty, or containing only traces of spermatogenic debris (Fig. 40). Only spermatogonial cells and a few pockets of primitive spermatocytes still present in the intertubular tissue.

Fig. 16 shows the average maturity indexes, based on the ovarian and testicular classes, on a monthly basis. The data from Fig. 16 confirm the trend shown in Fig. 15, with an assumed

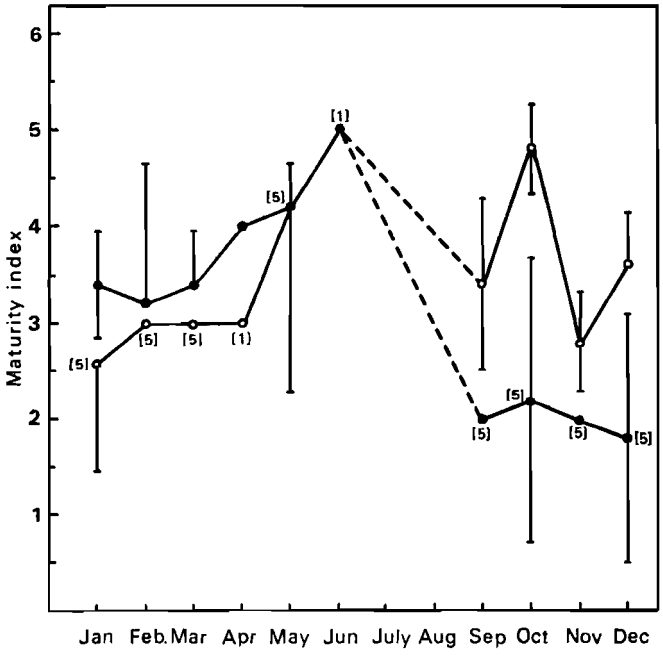

FIGURE 16

Monthly average maturity indexes of 42 ovotestes of marine steenbras. Numbers in parenthesis indicate number of fish examined in each month. (No sample July and August). Standard deviations indicated by T-bars.
● Testicular portion of ovotestes
○ Ovarian portion of ovotestes

spawning period in the winter months of July and August. The fluctuations of the testicular maturity indexes strongly support this assumption as there is a steady increase in maturity up to June and a definite decrease from September onwards. The ovarian maturity indexes follow this pattern to some extent, but remain at a higher level from September.

It is a well-known fact that many fish cease to feed before spawning and utilize energy from the body fat depots for the purpose of gonad maturation. This was also apparent in the steenbras. The intestinal fat indexes remained consistently high throughout the year and although isolated individuals had an unexpectedly high fat index, the fluctuations of the monthly averages also indicated a winter spawning. The peak fat indexes occurred in May and June, being 3,4 and 3,5 units respectively. The October sample averaged 2,7 units, which then began to rise again in the following months. Unfortunately, during July and August no marine samples were obtained in spite of every effort to capture fish by angling and alerting the Western Province Underwater Club. Requests were also made to several commercial fishing companies to report and preserve steenbras. This was also unproductive and supported the generally held belief among fishermen that steenbras are extremely rare during these months and are seldom, if ever, caught (*pers. comm.* W. S. Morries).

It is possible that the fish breed in their normal habitat close inshore amongst the rocks and merely "go off the bite", but more likely they migrate to deeper water to spawn. Biden (1930) stated that the general spawning season was unknown, but that steenbras caught in various Eastern Province rivers, especially during winter, had fully mature roes, which points to winter spawning in this locality. Biden suggested that the spawning places may be wherever the females happened to be when the eggs were ready for shedding, but there is no proof of this. Day (1967) postulated that steenbras spawn at sea and then return to the estuarine waters in summer to feed. This would also imply a winter spawning, but there was certainly no evidence during the present investigation that "spent" fish return to the Heuningnes estuary in summer.

Lucks (unpubl.) found that the main breeding period for *Lithognathus aureti* in South West Africa was from October to January, but he also found ripe or almost ripe fish throughout most of the year. Lucks stated that steenbras spawned in the Sandwich Harbour lagoon and apparently leave the lagoon after spawning.

TABLE 20

DOMINANT SEX IN MARINE STEENBRAS OVOTESTES AS DIAGNOSED BY HISTOLOGICAL EXAMINATION

	Ovotestes			
Number of fish	*Male-dominant*	*Female-dominant*	*Equal development*	*Uncertain*
42	75%	19%	4,5%	1,5%

The histological data summarized in Table 20 are similar to those obtained for the estuarine steenbras, with an even stronger inclination towards male-dominant ovotestes (*cf.* Table 19). This fact immediately precludes any assumption that the steenbras is a protandrous hermaphrodite. For this to be the case, most of the individuals would have to be either true females, or to possess strongly ovarian-dominant ovotestes. Also, there would have to be definite histological evidence of sex-reversal during a certain intermediate length range and this was certainly not the case. The other possibility, namely that of protogynous hermaphroditism, also has to be excluded because of the absence of histological indications of sex-reversal and the fact that some estuarine ovotestes were ovarian-dominant. Furthermore, by definition (Atz 1964) a protandrous or protogynous individual must first function either as a male or as a female. From the histological examination, there was absolutely no indication that steenbras up to six years of age had functioned as either males or females. In fact, none of the individuals had even reached sexual maturity.

Many of the Sparidae previously studied (Atz 1964) show protandry or protogyny and in such cases there is usually clear-cut evidence to support the fact. Depending on whether the species is protogynous or protandrous, the young fish will clearly be one sex and the older fish the other. At some intermediate length there should be evidence of an indifferent state of the gonad, such that the population will exhibit a 1:1:1 male : bisexual : female relationship. Further growth then results again in a sex ratio inbalance. Sexual dimorphism is also often apparent in size, such that males of protogynous species are larger than the females (Smith and Young 1966, Penrith 1972). Kinoshita (1936) found these characteristics in the protandrous sparid, *Sparus longispinus;* as did Zei (1950) in three species of protogynous Perciformes; Liem (1963) and Chan and Phillips (1967) in the protogynous synbranchiform, *Monopterus albus;* Lissia-Frau (1968, 1970) in the protogynous sparid, *Boops boops* and the protandrous sparid *Boops salpa* respectively; and Penrith in the protogynous sparid *Chrysoblephus laticeps*. The steenbras exhibited none of the characters outlined by Smith and Young or Penrith.

From the histological analysis of the ovotestes of the marine-sampled steenbras it was established that this fish permanently remains in a morphologically hermaphroditic condition. Although a few individuals had ovotestes which, except for a minute testicular attachment, were almost exclusively ovarian, by far the greatest number of individuals had ovotestes with well developed ovarian and testicular parts. One of the sexes was, however, usually dominant. The histological analysis also showed that variations in the type of hermaphroditism were possible. For example, the ovotestes often showed: (i) both testicular and ovarian sections ripening simultaneously; (ii) one sex ripening at a faster rate than the other; and (iii) that either the male or the female, or both sexes were "spent". It must be stressed that not one individual in the sample was captured in a "ripe-running" condition, but three specimens did show evidence of atretic follicles in the ovary in the months immediately following the postulated spawning period. This is illustrated in Fig. 37.

Surprisingly, not one ripe ovum was found in any of the ovarian sections and this is somewhat puzzling. However, this may be the result of low sample numbers or because the functional females mature extremely rapidly during July and August and shed their eggs exclusively during these months. In the three samples with atretic follicles, the remainder of the ovary consisted of only early Stage I and II oöcytes, which is indicative of a "spent" condition. The atretic follicles

were probably the result of degeneration of a small number of ova which had not been shed at spawning. Atz (1964) records that D'Ancona described atretic follicles and corpora lutea in four species of Sparidae, which often seemed to be associated with the regression of the female portion of the ovotestis, at which time the degeneration and destruction of unovulated eggs might well have been expected. My results give some support to D'Ancona's findings, although no corpora lutea were observed in the steenbras. However, Atz recorded that Pickford and Atz (1957) pointed out there is no good evidence that any of the so-called corpora lutea of bony fishes are anything more than corpora atretica, and this view is also substantiated by the present investigation.

The testicular cycle was far more predictable than the ovarian cycle. Maturation occurred steadily to reach a peak in June, when the seminiferous tubules were packed with spermatids and spermatozoa, while from September on, many testes had the characteristic "spent" appearance and the seminiferous tubules were empty.

Gonad weights proved unreliable indicators of spawning and in only two specimens taken in September were the typically flaccid, empty gonads of a spent fish, found. The ovarian-dominant ovotestes invariably were the heavier, the largest found in the present investigation being 33,75 g. However, many ovotestes which weighed only 2 or 3 g also had well developed sexual products, and fluctuations in gonad weight as a criterion for determining the spawning season are clearly not tenable for the steenbras.

Lucks (unpubl.) studied the reproductive biology of *Lithognathus aureti* from South West Africa and found this species also to be hermaphroditic. According to Lucks, it starts life as male. It either remains permanently male, or passes through a hermaphroditic state to become female, or changes permanently to hermaphroditic. This theory was apparently based entirely on macroscopic examination of the gonads and must, therefore, be questioned.

Also of interest, was the histological appearance of an ovotestis from a 10-year-old steenbras (62 cm fork length, sampled in July), which had been trapped in Verlorenvlei for approximately four years (*pers. comm.* J. J. R. Louw). The ovotestes were well developed, showing equal male and female portions, and weighed 7,34 g. Hislotogical examination showed that the testicular section was in an advanced stage of maturation, again indicative of a possible spawning during July or August. The testicular configuration was normal in appearance, conforming with testes of the marine samples, but the ovarian section showed definite signs of degeneration. The ovarian lamellae had largely disappeared and their products filled most of the ovarian cavity. Most of the oöcytes were tightly clumped together and many showed distinct evidence of degeneration of the nuclei (Fig. 41). Although it is possible that this was only a functional male, it is most likely that without the stimulation of other fish present to elicit spawning, the testicular products would have also degenerated in time. The possibility that this individual, probably isolated from a potential mate, could have functioned synchronously, I consider unlikely. Despite the fact that possible self-fertilization would be better than not spawning at all, such an event would result in inbreeding – a genetically undesirable occurrence from an evolutionary point of view (Ghiselin 1969). The above evidence also lends some support to the hypothesis that steenbras must leave the estuaries and river systems to breed at sea.

Despite the fact that the histology of the ovotestes indicates that a few steenbras could be synchronous hermaphrodites, *i.e.* individuals capable of functioning both as male and female

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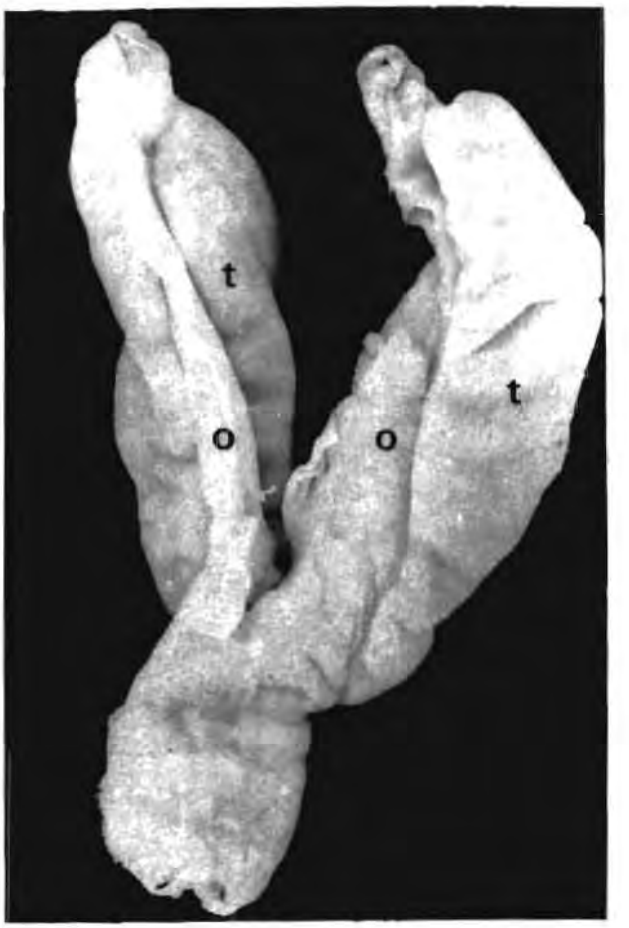

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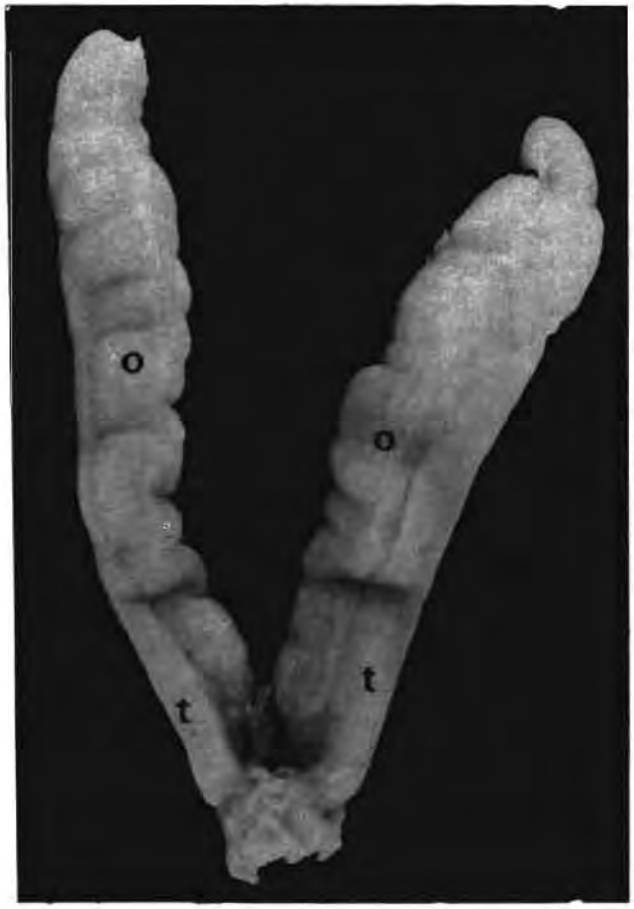
FIGURES 17–19

Ovotestes with equally well-developed ovarian and testicular portions; with testicular portion dominant; with ovarian portion dominant. (Actual size).
t = testis, o = ovary.



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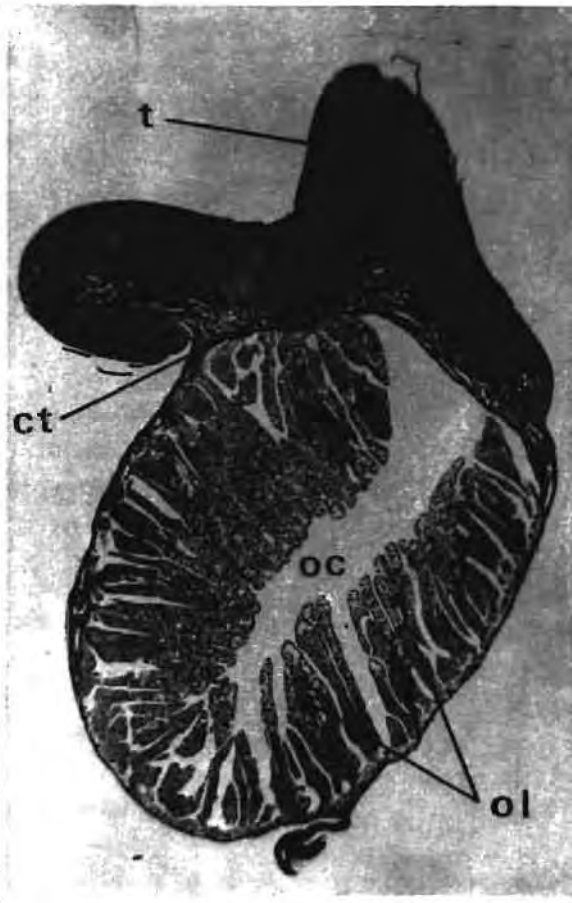

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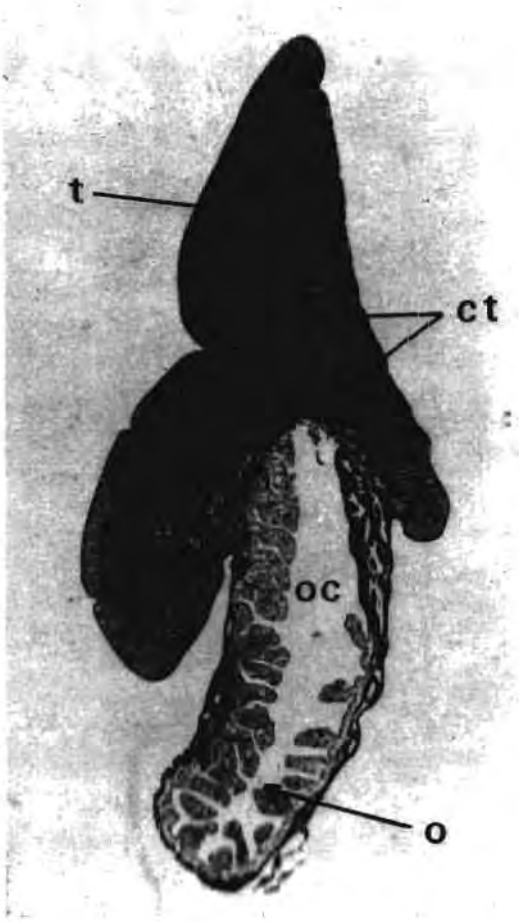
FIGURES 20-22

Cross sections through ovotestes with equally well-developed ovarian and testicular portions (Figure 20, ×6); with testicular portion dominant (Figure 21, × 12); with ovarian portion dominant (Figure 22, × 12).

t = testis, o = ovary
ct = connective tissue
ol = ovarian lamellae
oc = ovarian cavity



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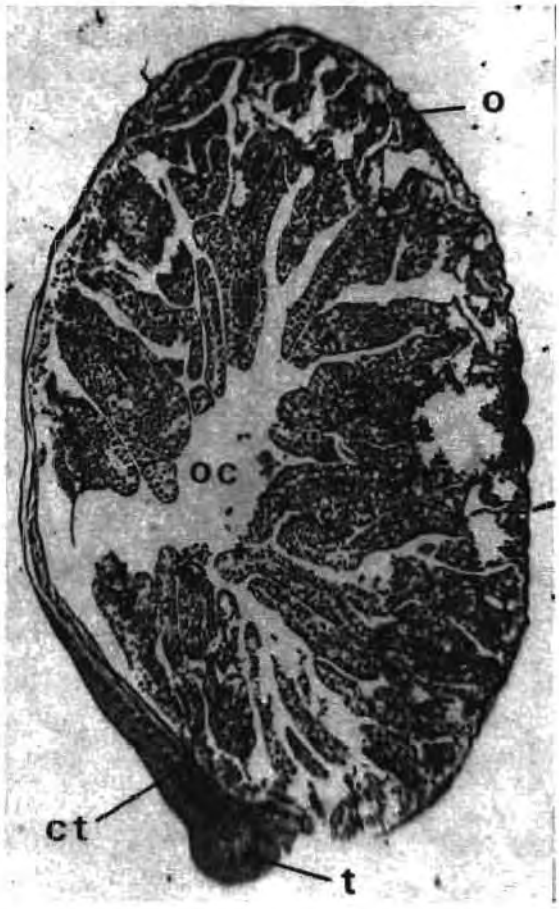

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FIGURE 23
Primitive oöcytes in early class V testicular field. (× 1200). Note seminiferous tubules containing spermatids and a few spermatozoa.

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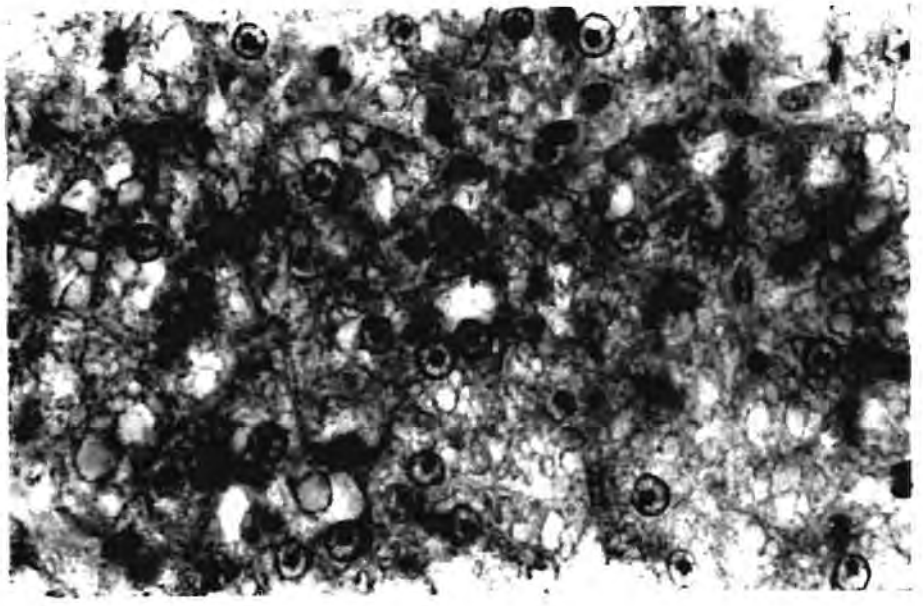
FIGURES 24–25
Typical sex-chromatin bodies (arrowed) in nuclei of female liver tissue of steenbras (Figure 24), absent from male nuclear tissue (Figure 25). (× 3500).



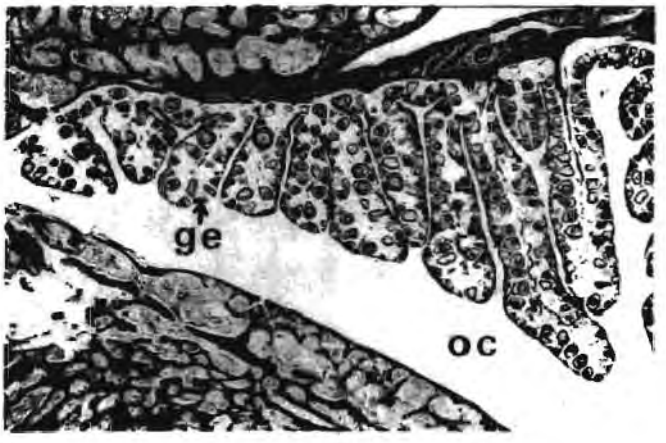


FIGURE 26

Well-developed germinal epithelium (ge) containing Stage I oöcytes. Note ovarian lamellae surrounded by the testicular field. (× 80). oc = ovarian cavity.

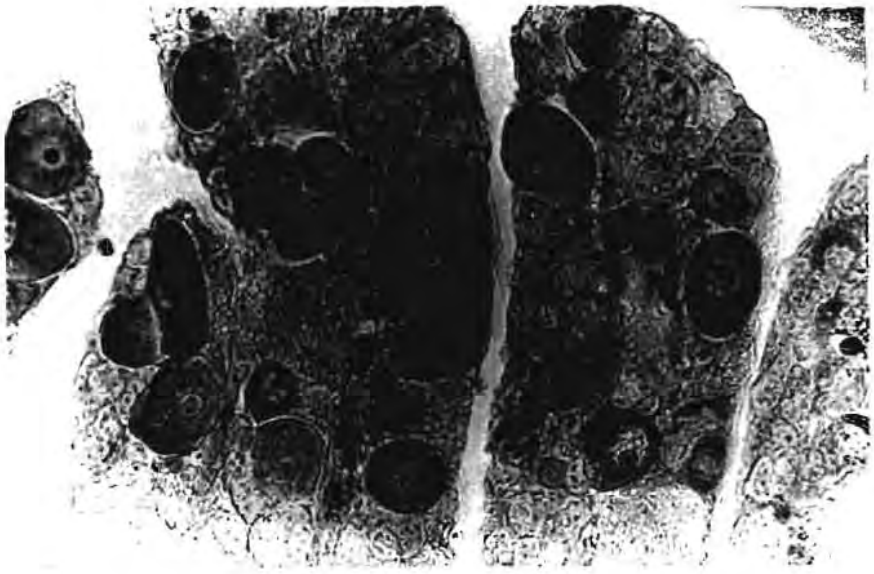


FIGURE 27

Stage I oöcytes in ovarian lamellae. (× 1200).

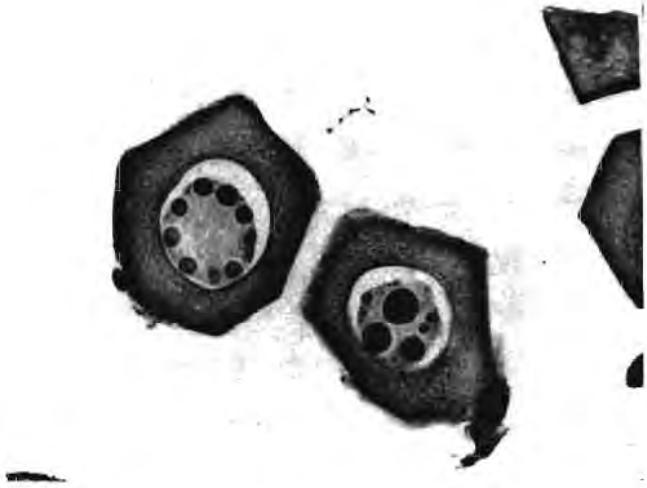
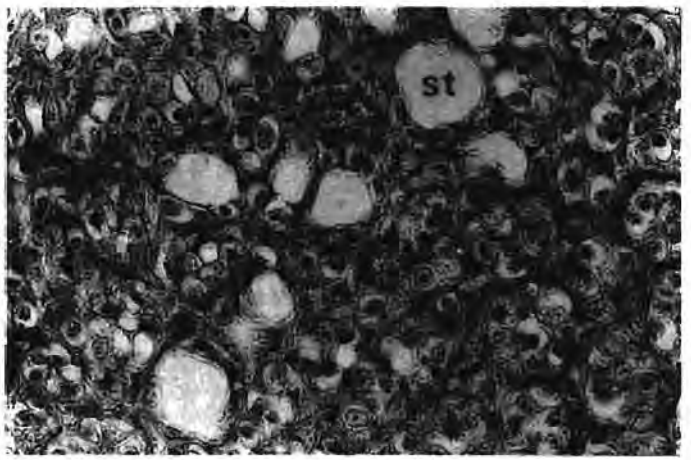


FIGURE 28

Typical Stage II oöcytes. Note the numerous nucleoli in the nucleus. (× 800).



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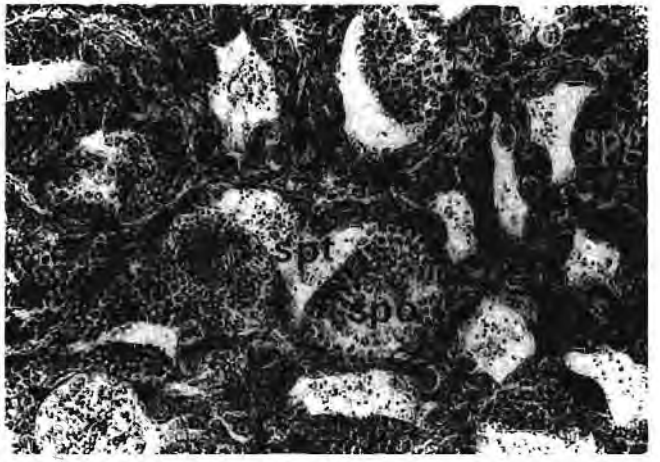

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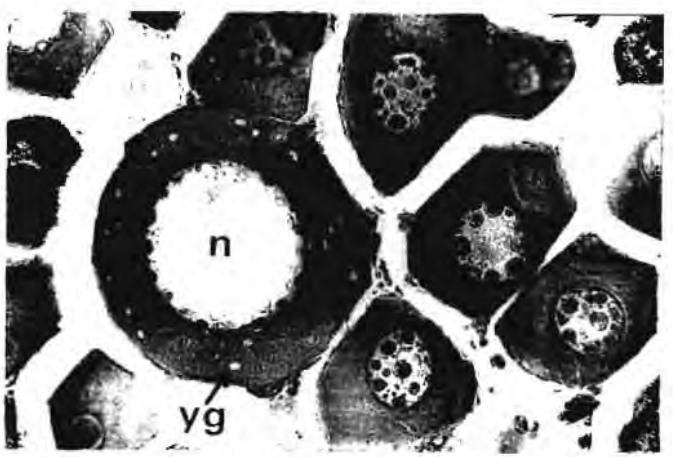
FIGURE 29 Class II testis. Note small, empty seminiferous tubules (st). (× 300).
FIGURE 30 Class III testis, showing spermatogonia (spg), pockets of spermatocytes (spc), and seminiferous tubules containing a few spermatids (spt). (× 800).
FIGURE 31 Class IV testis, showing spermatogonia, pockets of spermatocytes, and seminiferous tubules packed with spermatids. (× 500).
FIGURE 32 As for Figure 31. Note some spermatids showing tails. (× 2000).



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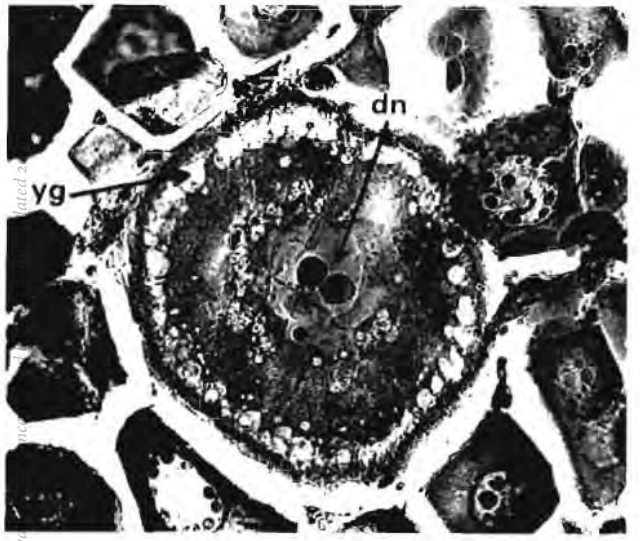

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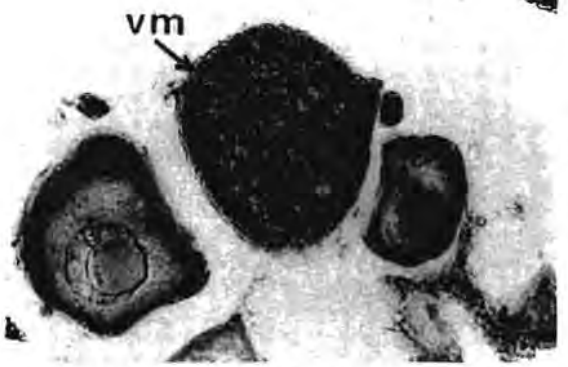

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FIGURE 33 Stage III oöcyte, showing a few yolk globules (yg) in cytoplasm. Note nucleoli in periphery of nucleus (n). (× 800).
FIGURE 34 Stage IV oöcyte showing accumulation of yolk granules in periphery of cytoplasm. Degeneration of nucleus (dn) has begun. (× 750).
FIGURE 35 Stage V oöcyte showing presence of vitelline membrane (vm). (× 1200).
FIGURE 36 Early stage of atresia of oöcyte, the sphere contains atretic cells. Vitelline membrane still present, although reduced. (× 1200).

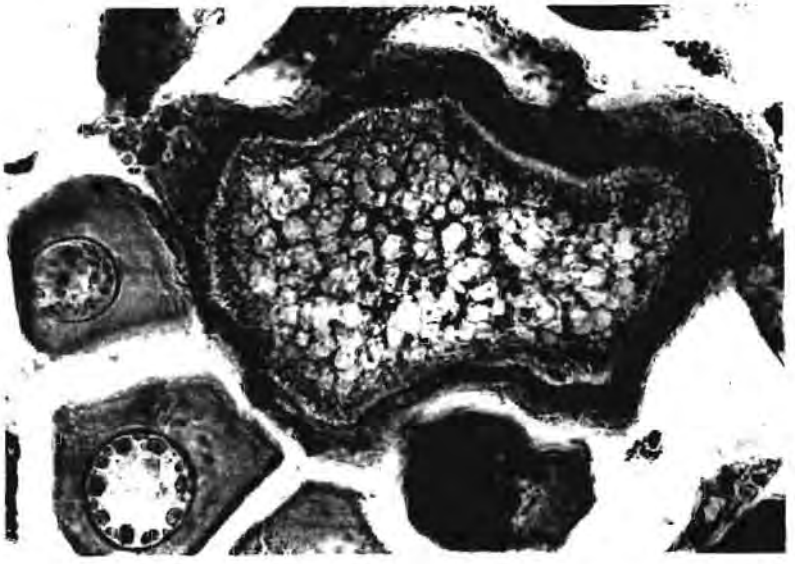

FIGURE 37

Collapsed "follicle" containing atretic follicular cells. (× 1200).

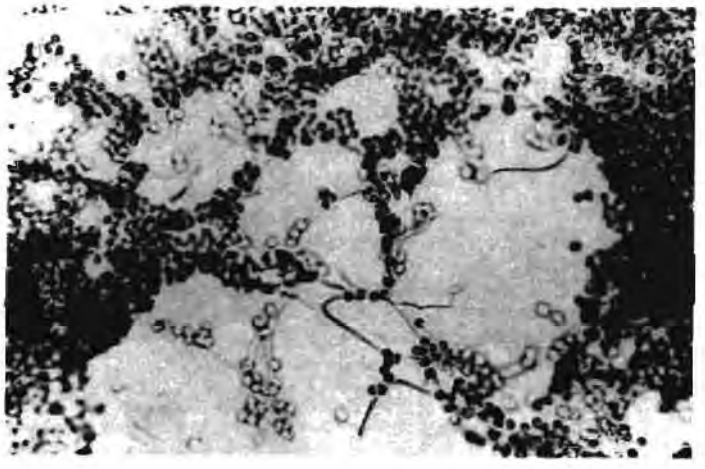

FIGURE 38

Mass of spermatids and spermatozoa in seminiferous tubules. Note tailed spermatozoa lying free from the clumps. (× 2000).

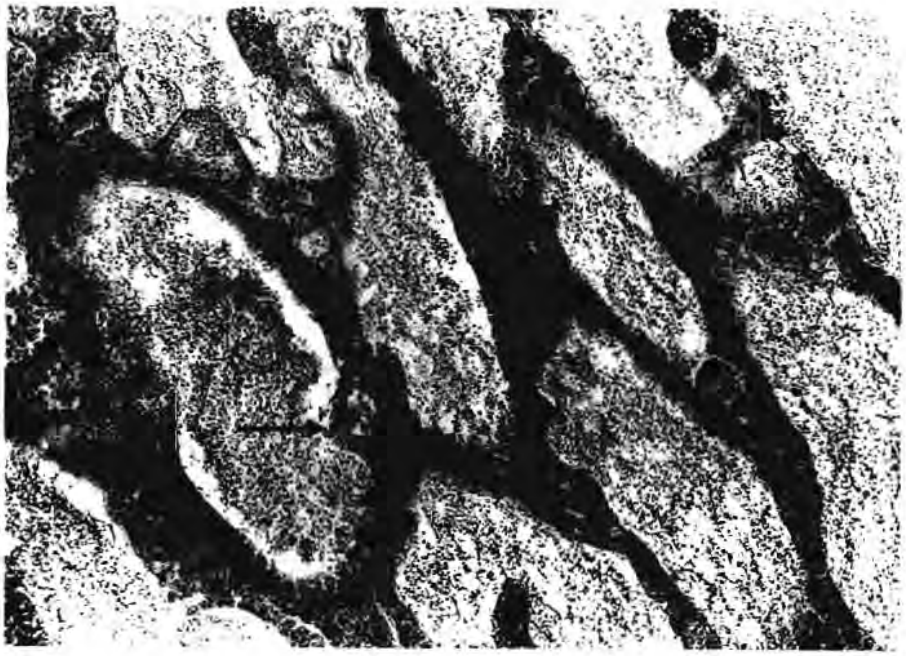

FIGURE 39

Class V testis, showing seminiferous tubules packed with clusters of spermatids and spermatozoa. Note small pockets of spermatocytes in intertubular tissue, one of which (arrowed) has burst open into tubule. (× 750).

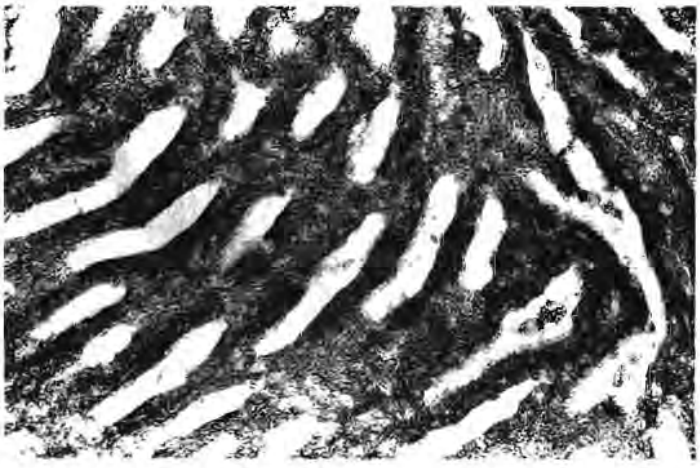

FIGURE 40

Class VI testis, typical of "spent" condition. Note the empty seminiferous tubules. (× 500).

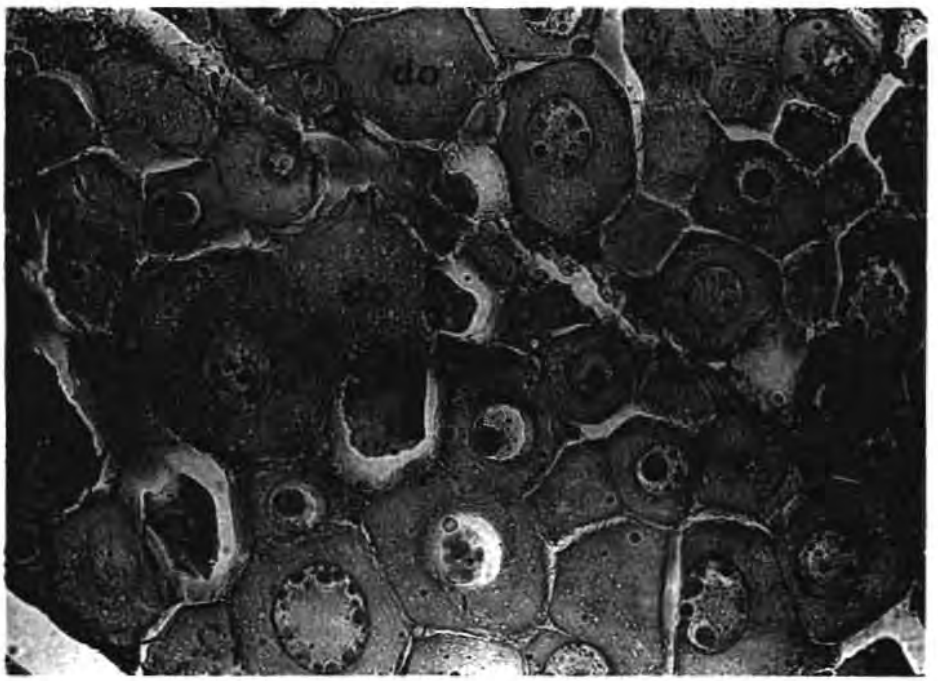

FIGURE 41

Cross section of ovary from ovotestis of a 10 year old steenbras trapped in a landlocked lake for four years. Note poorly developed oöcytes, with many degenerating oocytes (do). (× 300).

simultaneously, I believe that these specimens make up a very small percentage of the population. The conclusions reached from the detection of sex-chromatin (Barr) bodies, which showed that the steenbras is genetically male or female, must favour a rudimentary hermaphroditism. According to Atz (1964), Kosswig and D'Ancona both reasoned that, basically, rudimentary hermaphroditism should be the rule for Sparidae. Regarding the evidence from the steenbras, I agree with this statement.

There is some confusion as to the precise application of the term "rudimentary hermaphroditism". Liem (1963) states that the rudimentary part of a bisexual gonad does not remain rudimentary and latent during the entire life cycle, and applies the term to protogynous hermaphrodites in which the entire gonad changes from female to male by sex-reversal. However, this was certainly not the case in the steenbras, and the simpler definition of Atz (1964) has been adhered to in this investigation.

I therefore conclude that the steenbras is a rudimentary hermaphrodite, the "non-functional" sex of each individual remaining latent and possibly the relic of some earlier type of hermaphroditism in the phylogeny of the steenbras. Furthermore, from an evolutionary point of view, the steenbras would seem to be in a transitionary state of rudimentary hermaphroditism tending towards eventual gonochorism. The evidence for this belief, however, is very slender at this stage.

Atz (1964) states "that the gonads of individual sparidae often change fundamentally during their ontogeny and because different individuals of the same species sometimes exhibit quite different life-histories – any definitive study of sparid sexuality must include good sized samples of fish of all ages. Prodigious labour is necessary to study them all histologically but there is no substitute for histological analysis". I fully agree with this statement. Undoubtedly, the histological analysis of a larger marine sample than that examined in this investigation will ultimately provide the answers to most of the unresolved histological anomalies.

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