Seasonal histological and macroscopic changes in the gonads of *Cheimerius nufar* (Ehrenberg, 1820) (Sparidae: Pisces)

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Seasonal histological and macroscopic changes in the gonads of the hermaphroditic fish, *Cheimerius nufar* are described. The ovo-testis of the immature fish differentiates into either a functional ovary or a functional testis, with rudimentary tissue of the atrophied latent sex possibly present. Functional males and females reach maturity at *ca.* 340 mm total length and have a sex ratio of 1:2. The peak spawning period, early December to late January, correlates well with maximum sea temperatures and daylength. A revised classification system for the description of oocyte development, based on oogenetic and vitellogenetic changes, is also presented.

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Seisoenale histologiese en makroskopiese veranderinge in die gonades van die hermafroditiese vis, *Cheimerius nufar* word beskryf. Die ovo-testis van ongeslagsrype vis differensieer in óf 'n funksionele ovarium óf 'n funksionele testis. Rudimentêre weefsel van die geatrofeerde latente geslag kan moontlik aanwesig wees. Funksionele mannetjies en wyfies bereik geslagsrypheid by *ca.* 340 mm totale lengte en het 'n geslagsverhouding van 1:2. Die piek kuitskietperiode, vroeë Desember tot laat Januarie, korreleer goed met die maksimum seetemperature en dagliglengtes. 'n Hersiene klassifikasiesisteem vir die beskrywing van oösiet-ontwikkeling, gebaseer op oögenetiese en vitellogenetiese veranderinge, word ook voorgestel.

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Hermaphroditism in animals is well documented (Ghiselin 1969) and of all vertebrates, the fishes exhibit the most divergent display of sex (Atz 1964). Various types of sexuality, from synchronous hermaphroditism to gonochorism, have been reported for teleosts in general by several authors, *inter alia* Van Oordt (1929), Kinoshita (1939), Bullough (1940), D'Ancona (1948, 1949, 1956), Zei (1950), Bacci & Razzauti (1958), Clark (1959), Harrington (1961), Yamamoto (1969) and Jellyman (1976). A synoptic review of hermaphroditism and other forms of intersexuality is given by Atz (1964) and Reinboth (1962, 1970). More recently, literature on hermaphroditism in South African sparids has become available, *Chrysoblephus laticeps* (Penrith 1972), *Lithognathus lithognathus* (Mehl 1973), *Chrysoblephus laticeps* (Robinson 1976) and *Pterogymnus laniarius* (Hecht & Baird 1977).

The present study investigates seasonal histological and macroscopic changes in the gonads of *Cheimerius nufar*. In addition, construction of the breeding cycle is presented, based on the above results.

Materials and Methods

Material was obtained from anglers' catches off St Croix Island during the period December 1975 to February 1978 (Coetzee & Baird 1981a). St Croix Island (33°48′S/25°46′E) is the largest of three offshore islands in Algoa Bay, on the south-east coast of South Africa (Figure 1).

A total of 323 fish were examined to establish the breeding

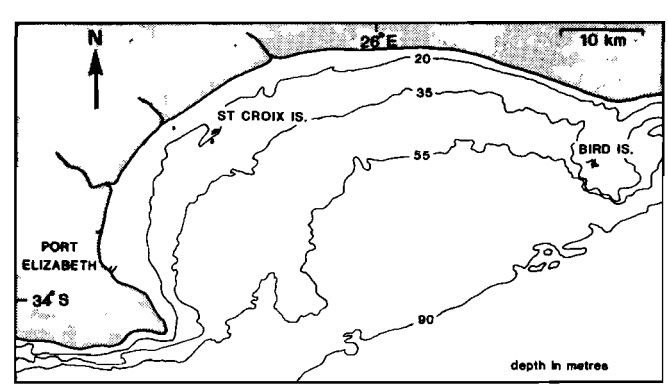

Figure 1 Map of Algoa Bay, South Africa, showing the location of the offshore island, St Croix.

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cycle. Results of the whole sampling period were pooled to present a composite picture of seasonal changes over one year.

The gonads were classified according to macroscopic appearance and preserved in Bouin's solution (Humason 1979). Sections of 5μm to 10μm thickness were stained with Harris's hematoxylin and eosin Y. The oocytes were measured with a calibrated (0,01 mm) graduated eyepiece.

Results

Histology of the gonads and gametogenesis

The ovo-testis (Figures 2 – 6)

The outer layer of the ovo-testis, the tunica albuginea, consists typically of smooth muscle fibres, collagenous fibres and loose connective tissue. Connective tissue and collagenous fibres clearly separate the ovarian and testicular parts, which is the case in most hermaphroditic sparids (Atz 1964; Mehl 1973).

Posteriorly, the ovarian parts of each ovo-testis are fused to form a common oviduct. Lacunae in the connective tissue layer surrounding the oviduct unite posteriorly to form a sperm duct, which encircles the oviduct (Figure 5). The sperm duct has a trabeculate appearance owing to the presence of numerous connective tissue folds. In the middle and anterior section of the ovo-testis, the sperm duct forms an anastomosing network between ovarian and testicular parts. As the fish increases in length, either the ovarian part (Figures 2 & 3) or the testicular part (Figures 4 & 5) becomes dominant. Ultimately a functional female or a functional male develops. Functional females (or males) are considered to act only as females (or males) since the gonads consist dominantly of ovarian (testicular) tissue, although rudimentary tissue of the atrophied latent sex may be present (Figure 6).

True sex reversal is marked by the more frequent appearance of one sex (male or female) in either the smaller or larger size classes. On performing a Student's *t* test, no significant difference ($p > 0,01$) was found to exist between the size frequency distributions of the two sexes (Figure 7), which indicates that true sex reversal may not take place in *C. nufar*.

Transformation of the ovo-testis into a functional female takes place with the volumetric increase of the ovarian part and a subsequent regression of testicular material. Similarly, evolvement of a functional male begins with the volumetric increase of the testicular part. With further development the testicular portion acquires a v-shaped form

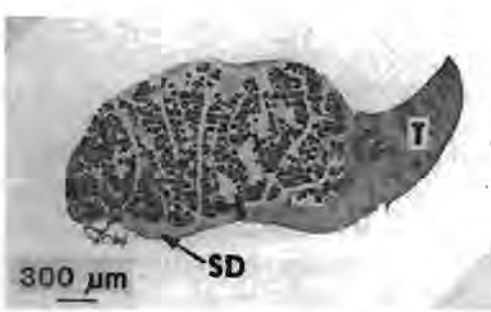

Figure 2 Section of an ovo-testis, showing a transitional stage developing into a functional female. SD = sperm duct, T = testis.

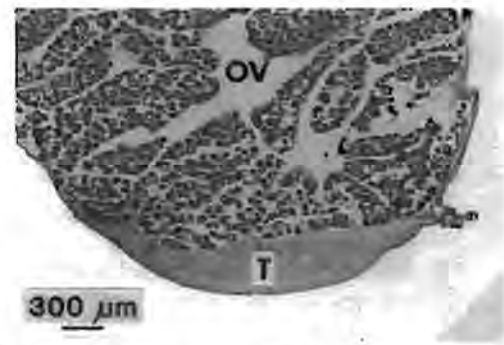

Figure 3 Section of an ovo-testis, showing nearly complete absence of the testicular portion and dominance of the ovarian part. OV = ovary, T = testis.

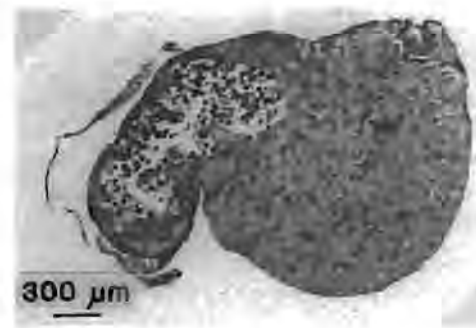

Figure 4 Section of an ovo-testis, showing a transitional stage developing into a functional male.

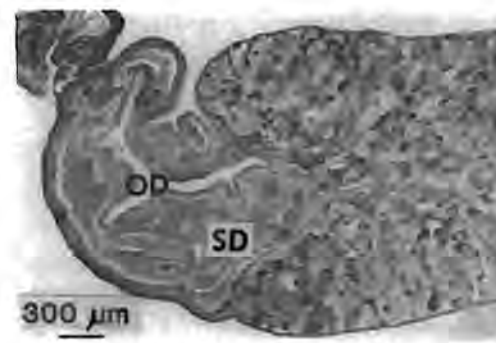

Figure 5 Section of an ovo-testis, functionally male, showing the presence of a rudimentary oviduct. OD = rudimentary oviduct, SD = functional sperm duct.

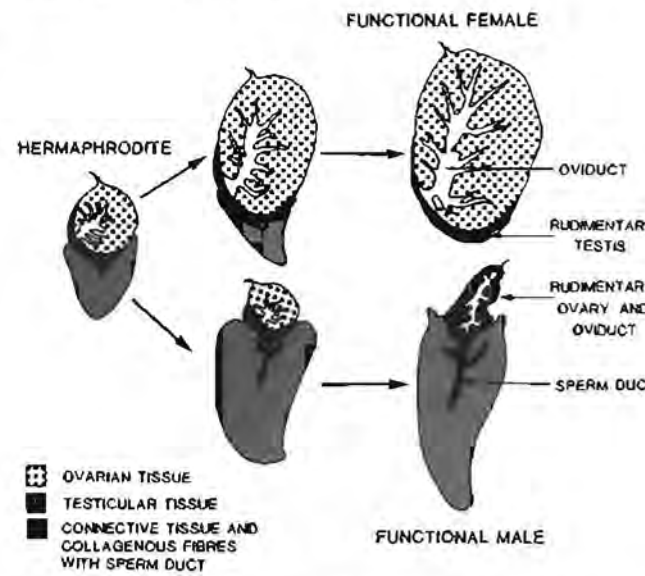

Figure 6 A schematic illustration of the development of a functional female or functional male from a hermaphroditic individual.

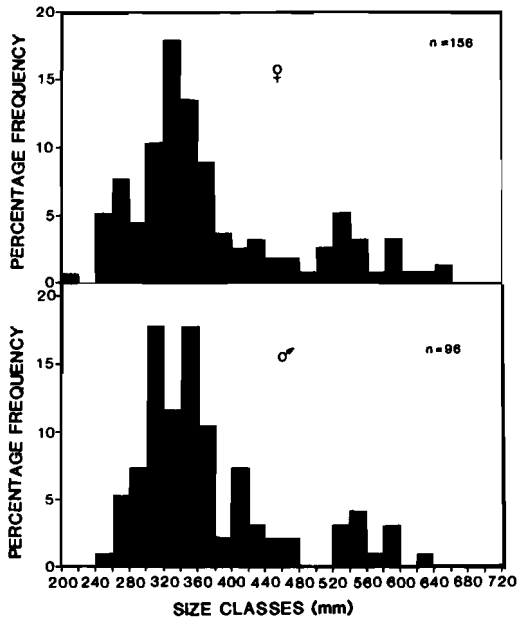

Figure 7 Length-frequency distribution of the functional female and functional male *Cheimerius nufar* (total length).

in transverse section (Figure 6). Advanced sex separation is marked by the presence of a rudimentary ovary (oviduct) and the fish can therefore be regarded as a functional male. Most fish not yet differentiated into functional females or males, were found to be smaller than 320 mm in total length, with few being as large as 360 mm.

The functional sexes (>320 mm) are described in more detail in the following sections.

The ovary and ovogenesis

Ovigerous lamellae, containing oogonia and oocytes in various stages of development, project from the tunica albuginea towards the centre of the ovary. Oogonia and early oocyte stages are found on the periphery of the lamellae and development occurs towards the ovocoel centre. The ovocoel continues as the lumen of the oviduct.

The system used to classify the oocyte stages is based on both nuclear and cytoplasmic changes with special reference to vitellogenesis. A synopsis of criteria used in defining the different reproductive stages is presented in Table 1. Table 2 gives the classification and dimensions of oogonia and oocytes.

Stage 0: Oogonia (residual and mitotic) (Figures 8 & 9). The oogonia of *C. nufar* are elliptical to spherical in shape and are mostly found on the periphery of the lamellae. In hematoxylin and eosin-stained sections, the cytoplasm is very lightly basophilic. The nucleus is large in proportion to the whole cell and although the chromatin material is hardly visible, a single nucleolus is prominent. No attempt was made to distinguish between different types of oogonia.

Stage 1: Chromatin nucleolus oocytes (Figure 9). Various transitional stages during the development of oocytes from oogonia are evident. Following the criteria used by Yamamoto (1956a), the three stages, presynaptic, synaptic and postsynaptic were also found in *C. nufar*; the latter stage being the most prominent. Chromatin nucleolus oocytes in the postsynaptic stage are larger than the oogonia.

Table 1 Classification of oogonia and oocyte stages based on both nuclear and cytoplasmic changes with special reference to vitellogenesis

Stages	Criteria for different stages
0 Residual and mitotic oogonia	Mostly oogonia; subdivisions into different types can be made
1 Chromatin nucleolus oocytes	Basophilic ring of chromatin in periphery of nucleus.
(a) presynaptic	Cytoplasm stains darker.
(b) synaptic	Synaptic 'chromatin network'
(c) postsynaptic	in the nucleus
2 Perinuclear oocytes	Nucleoli in peripheral region of nucleus
(a) pre-perinuclear	
(b) early perinuclear	
(c) late perinuclear	
3 Primary yolk vesicle oocytes	Formation of clear oil vesicles of primary yolk
4 Secondary yolk oocytes	Secondary acidophilic yolk appears (intra- and extravesicular)
(a) early	
(b) late	
5 Tertiary yolk oocytes	Consolidation of secondary yolk to form larger globules and finally a continuous yolk mass
6 Migratory nucleus oocytes	Nucleus migrates from the centre of the cell to periphery. Primary yolk vesicles consolidate
7 Maturing oocytes	Secondary yolk loses granular appearance and becomes homogenous.
(a) early	
(b) late	Lipid droplet formed.
8 Ripe oocytes	Largest oocytes. Homogenous yolk and one or more lipid droplets
Atretic oocytes	
type a	No distinct zoning of cytoplasm; nuclear membrane and zona radiata well defined; nuclear matrix and cytoplasm with increased granular appearance.
type b	Distinct zoning of the cytoplasm; degenerate nuclear membrane.
type c	Degeneration during vitellogenesis; glandular appearance.

The cytoplasm is lightly basophilic, with the nucleus having a well-defined, dark basophilic ring of chromatin on the periphery.

Stage 2: Perinuclear oocytes (Figures 10 & 11). The cytoplasm of these oocytes stain very dark with hematoxylin. Perinuclear oocytes are divided into three distinct stages, based on the size, the position and number of nucleoli, as well as the size of the cells and nuclei.

Pre-perinuclear oocytes are the earliest of the perinuclear oocytes (Van der Horst 1976). The oocytes in *C. nufar* have a spherical nucleus (diameter 20,2μm) which usually contains a large, prominent nucleolus (diameter 8,0μm) situated either off-centre or perinuclear. The gradual decrease in nucleolar size and increase in nucleoli numbers, appear to indicate that the large nucleolus of the pre-perinuclear oocyte divides to give rise to the greater number of smaller nucleoli found in later stages (Table 2).

Table 2 Classification of oogonia and oocyte stages. The mean and range of the largest and the smallest diameters (measured at right angles) are given, as well as the number of oogonia and oocytes measured. Diameter measurements are only representative of fixed material and have not been corrected for shrinkage or swelling caused by histological preparation

Stage	Name	Cell diameter (long axis) µm		Cell diameter (short axis) µm		Nucleus diameter µm		Nucleolus diameter µm		Number of nucleoli		Yolk vesicle diameter µm		Yolk globule diameter µm		Zona radiata thickness µm		Zona granulosa thickness µm		Number of oogonia and oocytes measured
		$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	
0	Residual and mitotic oogonia	12,5	8,5–13,3	8,8	6,8–11,9	7,0	5,9–8,5	–		–		–		–		–		–		20
1	Chromatin nucleolus oocytes	17,5	14,8–21,2	11,2	8,5–13,6	8,6	6,0–10,2	–		1		–		–		–		–		20
2	Perinuclear oocytes a) pre-perinuclear	50,7	23,7–69,5	32,7	17,0–42,5	20,2	13,6–25,4	8,0	3,4–10,0	1	1–2	–		–		–		–		25
	b) early perinuclear	68,8	40,7–84,7	50,0	27,1–67,8	30,0	20,3–38,9	5,0	2,9–6,8	11	6–15	–		–		–		–		30
	c) late perinuclear	87,8	74,5–105,0	60,1	45,7–76,2	43,9	35,6–54,2	3,2	1,7–4,2	10	7–17	–		–		2,2	1,5–3,4	2,8	1,6–3,3	20
3	Primary yolk vesicle oocytes	150,6	98,3–223,9	128,9	84,7–204,3	75,3	59,3–108,7	3,8	2,7–5,1	>15		7,1	3,2–8,1	–		2,4	1,9–3,8	2,8	1,7–4,5	20
4	Secondary yolk oocytes a) early	243,3	187,7–273,9	211,5	187,1–246,8	94,8	85,9–115,4	large nucleoli, 6,7		10 (large)		12,6	8,5–18,6	4,5	2,2–5,1	3,8	2,9–5,8	3,4	3,1–4,2	10
	b) late	332,8	289,8–341,9	271,7	230,7–296,7	113,1	74,1–120,0	small nucleoli, 0,84		>10 (small)		17,7	14,3–20,0	8,9	5,9–10,7	6,2	5,4–9,3	5,9	4,7–7,0	10
5	Tertiary yolk oocytes	410,1	339,1–435,7	355,0	316,1–377,2	93,2	60,9–127,6	large nucleoli, 4,3		13 (large)		60,1	65,2–84,7	coalescing		7,5	6,8–8,7	4,7	3,4–7,9	7
						distorted		small nucleoli, 0,97		>10 (small)										
6	Migratory nucleus oocytes	390,2	333,4–441,2	345,1	322,2–355,6	157,4	116,8–233,4	large nucleoli, 4,9		10 (large)		97,2	88,8–105,6	coalescing		6,3	4,2–7,3	5,9	3,8–6,9	9
								small nucleoli, 0,86		>10 (small)										
7	Maturing oocytes	488,6	444,4–550,56	402,8	333,3–466,7	114,2	99,9–133,4	large nucleoli, 7,2		> 5 (large)		129,2	83,3–166,7	coalesced		6,7	6,2–7,5	4,1	3,3–5,7	8
						distorted		small nucleoli, 1,69		>10 (small)										
8	Ripe oocytes	500?																		

Early perinuclear oocytes are larger in size and have smaller, but more numerous nucleoli (diameter 5,0µm). Figure 10 shows the transformational stages of a pre-perinuclear oocyte forming an early perinuclear oocyte.

Late perinuclear oocytes are characterized by numerous nucleoli (7 to 17) arranged tightly along the nucleus periphery (Figure 11). Small, intensely hematoxylin stained bodies are found in the central network of the nucleus, which could possibly be chromatin nucleoli (Yamamoto 1956b). The cytoplasm of the later perinuclear oocytes has a slight granular ('frothy') appearance which is in accordance with the findings of other authors (Abraham, Blanc & Yashouv 1966; Braekevelt & McMillan 1967; Baird 1974; Van der Horst 1976). A thin follicular layer enclosing a zona radiata is also apparent in the late perinuclear oocytes. In some oocytes a lighter zone is present along the inner side of the zona radiata (Figure 11). Yamamoto (1956a) and Van der Horst (1976) also noted this zone and suggested that

it could possibly be a 'pre-deposit' zone concerned with the formation of the zona radiata.

Stage 3: Primary yolk vesicle oocytes (Figures 12 & 13). Primary yolk vesicle oocytes exhibit a wide size range. This may be attributed to the various stages of yolk vesicle formation. During routine preparation the primary yolk washes out and appears as vacuoles in stained sections (Braekevelt & McMillan 1967).

Two distinct rows of primary yolk vesicles are formed. The first row is formed in the peripheral region of the cell. These vesicles are larger and more defined than those of the second row, situated around the nucleus. The nuclear row is only formed after the peripheral row is established. Further development indicates that the yolk vesicles in the peripheral region of the cell become less visible, but more defined as a 'continuous row'. The yolk vesicles around the nucleus gradually increase in size and number, and the cytoplasm becomes more 'frothy'.

Stage 4: Secondary yolk oocytes (Figures 14 & 15). Early secondary yolk oocytes (Figure 14) are characterized by the appearance of intravesicular and extravesicular secondary yolk globules which are lightly acidophilic. The zona radiata and follicular layer increase in thickness and the former becomes more eosinophilic.

Late secondary yolk oocytes are characterized by cytoplasm completely filled with yolk globules which are slightly larger on the periphery of the cell than around the nucleus (Figure 15). At this stage, very few yolk vesicles of

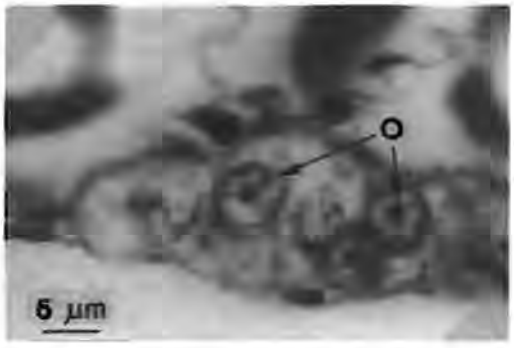

Figure 8 Section of an ovary, showing the oogonia. O = oogonia.

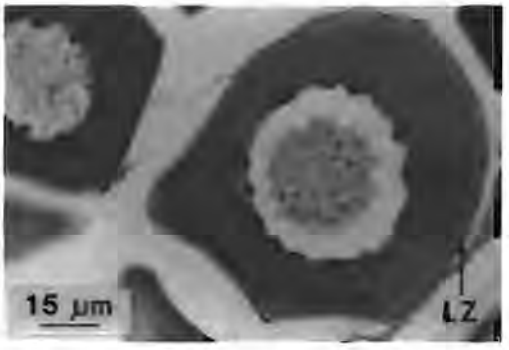

Figure 11 Late perinuclear oocyte, showing a distinct zona radiata and zona granulosa. LZ = lighter zone.

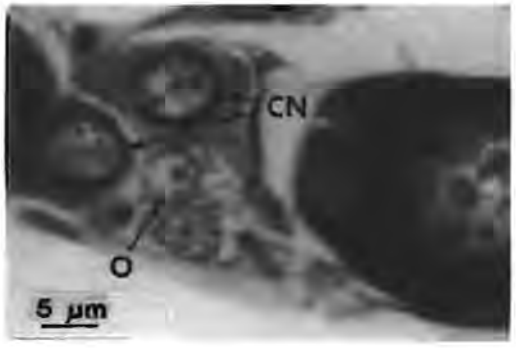

Figure 9 Section of an ovary, showing oogonia and chromatin nucleolus oocytes in the post-synaptic stage. CN = chromatin nucleolus oocyte, O = oogonium.

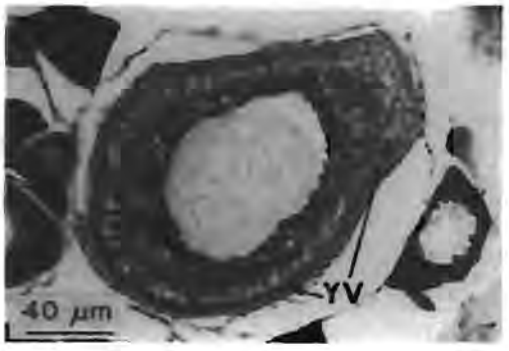

Figure 12 Primary yolk vesicle oocyte, showing yolk vesicles in the periphery and around the nucleus of the cell. YV = yolk vesicles of primary yolk.

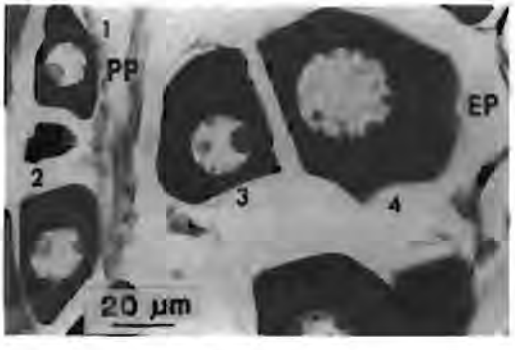

Figure 10 Transition stages in the formation of a pre-perinuclear and early perinuclear oocyte. Progressive oocyte development 1 – 4. EP = early perinuclear oocyte, PP = pre-perinuclear oocyte.

Figure 13 Section of an ovary, illustrating the size difference between primary yolk vesicle oocytes and late perinuclear oocytes. LP = late perinuclear oocyte, PY = primary yolk vesicle oocyte.

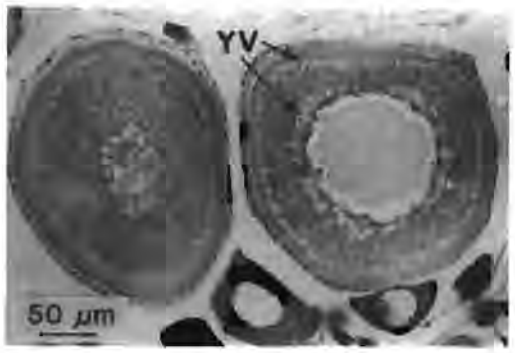

Figure 14 Early secondary yolk oocytes, showing the presence of secondary yolk globules. The two rows of primary yolk vesicles are still distinct. YV = yolk vesicles of primary yolk.

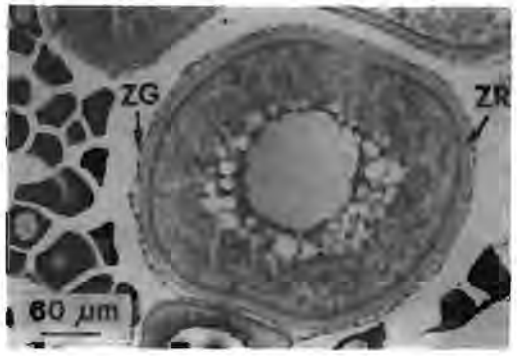

Figure 15 Late secondary yolk oocyte, showing the enlarged central vesicles of primary yolk and the yolk globules. ZG = zona granulosa, ZR = zona radiata.

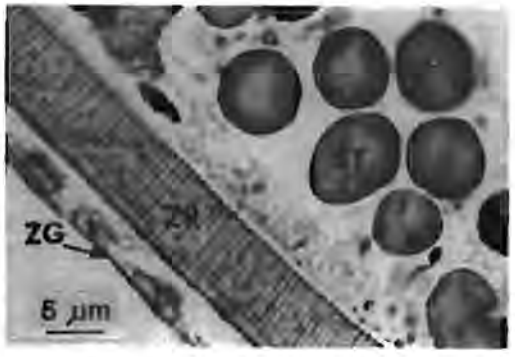

Figure 16 Radial striations in the zona radiata. SY = secondary yolk globules, ZG = zona granulosa, ZR = zona radiata.

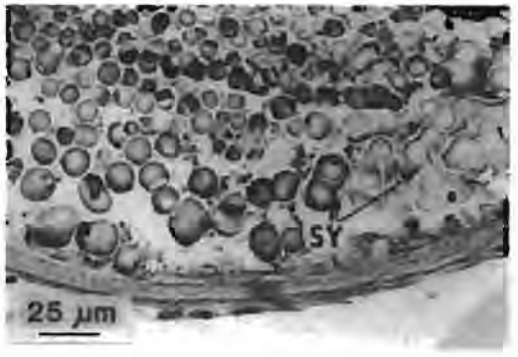

Figure 17 Section of a tertiary yolk oocyte, showing the coalescence of secondary yolk globules. SY = secondary yolk globules.

primary yolk are visible in the periphery of the oocyte, but the yolk vesicles surrounding the nucleus have become greatly enlarged, some showing the presence of intravesicular secondary yolk globules. The eosinophilic zona radiata thickens and pore canals appear as cross striations in the sections (Figure 16). These striations were also reported by Hurley & Fisher (1966), Cala (1970), Van der Horst (1976) and Tesoriero (1977). An increase in the thickness of the follicle granulosa layer is apparent.

Stage 5: Tertiary yolk oocytes (Figure 17). Larger, irregular-shaped yolk globules are formed when the secondary yolk of these oocytes begins to coalesce. This process gives rise to a continuous mass of yolk in later developmental stages (Figure 17). Primary yolk vesicles accumulate around the nucleus, coalesce, and form fewer, but larger lipid droplets. This phenomenon takes place in most teleosts where one or more lipid droplets are formed in the ripe eggs (Yamamoto 1957; Abraham 1963; Abraham *et al.* 1966; Zhitenev, Kalinin & Abayev 1974; Baird 1974; Van der Horst 1976). Formation of the lipid droplet initiates the process of nuclear migration in the next stage, when it displaces the nucleus off-centre (Figure 18).

The coalescing of secondary yolk globules proceeds more rapidly in the anti-nuclear region, as is the case in *Liopsetta obscura* (Yamamoto 1957). This is more pronounced in the migratory nucleus oocytes (Stage 6).

Stage 6: Migratory nucleus oocytes (Figures 18 – 21). In this stage the nucleus has shifted to the periphery of the cell. The centre of the cell is occupied by a single large vesicle of primary yolk, the lipid droplet. Most of the nucleoli are irregular in appearance and retain their peripheral distribution until they finally disappear. Before the nucleoli disappear, the remaining nuclear matrix appears granular, with minute basophilic 'particles' spread throughout (Figure 20).

The apparently viscid substance which forms a narrow zone at the nucleus-lipid vesicle interface (Figures 18 & 19) was also reported for *Liopsetta* (Yamamoto 1956b). Displacement of the cytoplasm by the yolk globules and subsequent compression against the nucleus and lipid droplet, could possibly account for this basophilic zone. The consolidation of secondary yolk globules in the anti-nuclear region of the cell is well illustrated in the late migratory nucleus oocyte, shown in Figure 21. This process of secon-

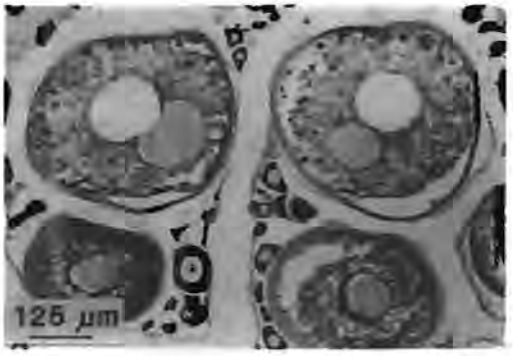

Figure 18 Migratory nucleus oocytes, showing the coalescence of secondary yolk globules in the anti-nuclear region.

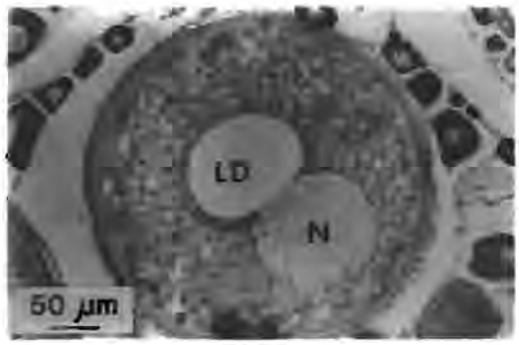

Figure 19 Migratory nucleus oocyte, showing the off-centre nucleus and formation of a lipid droplet. LD = lipid droplet, N = nucleus.

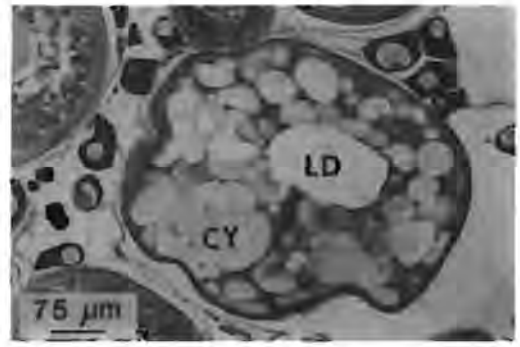

Figure 22 Early maturing oocyte, with coalesced secondary yolk. CY = coalesced secondary yolk, LD = lipid droplet.

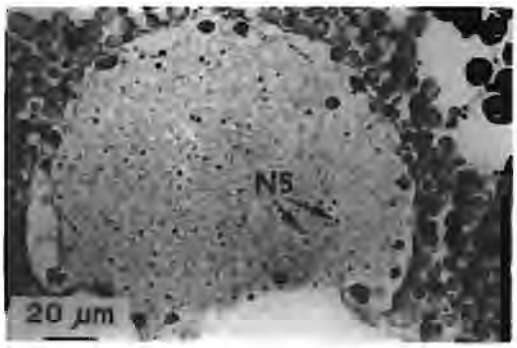

Figure 20 The granular nucleus of a migratory nucleus oocyte, showing the nuclear membrane and numerous nucleoli. NS = nucleoli.

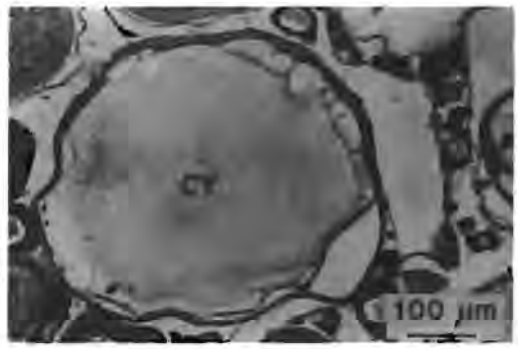

Figure 23 Late maturing oocyte, with homogenous yolk. CY = coalesced secondary yolk.

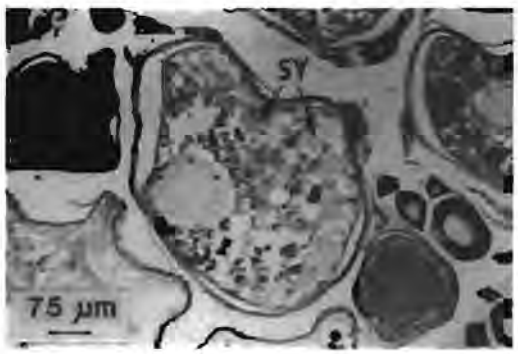

Figure 21 Late migratory nucleus oocyte, changing into an early maturing oocyte. SY = secondary yolk globules.

dary yolk coalescence commences at the periphery of the oocyte and proceeds centripetally until the yolk forms a homogenous mass.

Stage 7: Maturing oocytes (Figures 22 & 23). Early maturing oocytes have large areas of homogenous, light eosinophilic, secondary yolk. A single lipid vesicle is present near the centre of the oocyte (Figure 22). No clear nucleus with basophilic nucleoli could be detected in the early maturing oocytes of *C. nufar*, as was reported for *Liopsetta* (Yamamoto 1956a).

As a result of accumulated lipids, it is difficult to section the oocytes at this stage of development. Late maturing oocytes (Figure 23) have a homogenous yolk and the presence of a lipid droplet is characteristic. Similar oocytes were reported for *Liopsetta obscura* (Yamamoto 1957), *Mugil cephalus* and *M. saliens* (Zhitenev *et al.* 1974).

Stage 8: Ripe oocytes. Ripe oocytes could not be described, as no actively spawning females were sampled for histological analysis. From the work of Gilchrist (1916) and Ranzi (1933, 1969) it would appear that ripe eggs are greater than 750 µm in diameter and that they contain only one large lipid droplet.

Atretic oocytes (Figures 24 – 29). Atretic oocytes were present in 10,8% of the 130 females examined histologically and occurred either during or just after the spawning season. Three types were encountered in *C. nufar*:

(a) Atretic oocytes with no distinct zoning of the cytoplasm, showing a distinct granular appearance (Figures 24 & 25). Judging by the size of the large nucleoli, it would appear that these atretic cells originate from late perinuclear oocytes. The nuclear membrane and zona radiata remain well defined, while the nuclear matrix and cytoplasm show an increase in granular appearance.

(b) Atretic oocytes with distinct zoning of the cytoplasm and degenerate nuclear membrane (Figures 26 & 27). This form of atresia takes place in the later oocyte developmental stages. Figure 26 reveals definite zoning of cytoplasm which, in accordance with Yamamoto (1956a), is 'a preliminary process to the partial reabsorption of the egg'. As the follicle granulosa is still intact, these atretic oocytes could possibly be identified with the non-hypertrophic atresia of *Eucalia* (Braekevelt & McMillan 1967). The late primary yolk vesicle oocytes in Figure 27 also show an increased disorganization of

the cytoplasm, possibly leading to total resorption. Some of the late atretic stages show a radical hypertrophied cytoplasm, although the nuclear membrane appears intact but irregular. Nucleoli are present in the granular nucleus and a slight zoning of the cytoplasm can be distinguished (Figure 28).

(c) The third type of atretic oocyte present in *C. nufar*, is formed by the degeneration of oocytes during the process of vitellogenesis, probably after secondary yolk formation (Figure 29). Numerous yolk globules are visible and the cell has an overall glandular appearance, with the follicular cells forming a hypertrophied layer around the inner cell mass. Similar atretic oocytes were found in *Zoarces viviparus* (Wallace 1903), *Fundulus heteroclitus* (Matthews 1938), *Mugil capito* (Abraham *et al.* 1966), *Mormyrus kannume* (Scott 1974) and *Liza dumerili* (Van der Horst 1976).

The testis and spermatogenesis

The testis of the functional male consists of a mass of elongated lobules which open into minor sperm ducts and finally into the main sperm duct (Figure 30). Each lobule has numerous cysts, within which the spermatogenic cells develop. Spermatogenesis is usually most advanced near the main sperm ducts. In very ripe testes, the main sperm duct area is filled with sperm, whereas the peripheral testicular region contains cysts exhibiting various stages of spermatogenesis.

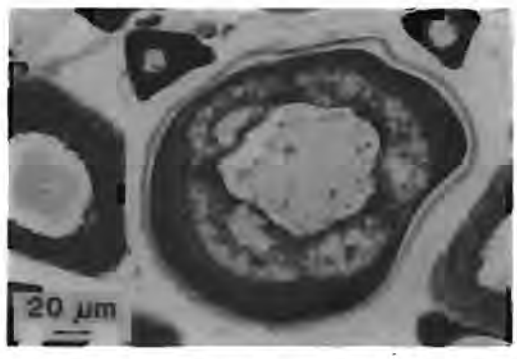

Figure 26 Atretic oocyte (type b), showing the zoning of cytoplasm.

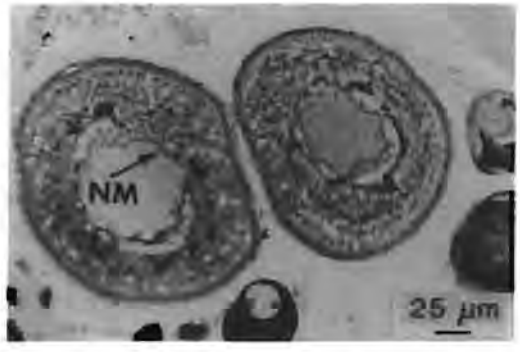

Figure 27 Late primary yolk oocyte, showing disorganization of the cytoplasm. NM = nuclear membrane.

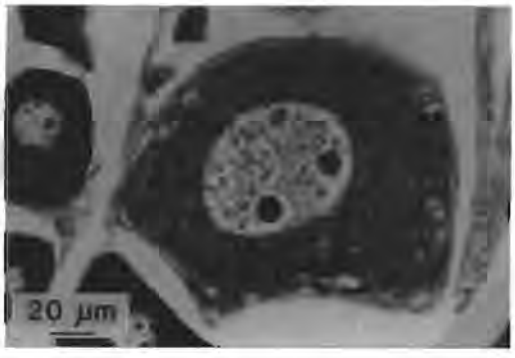

Figure 24 Atretic oocyte (type a), showing the granular appearance of the cytoplasm and the well-defined nucleoli.

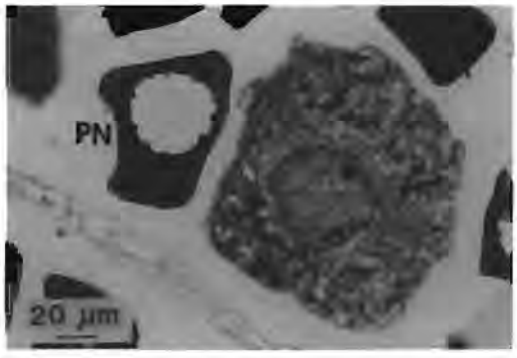

Figure 28 Atretic oocyte (type b) in an advanced state of atresia. Zoning of the cytoplasm is still visible.

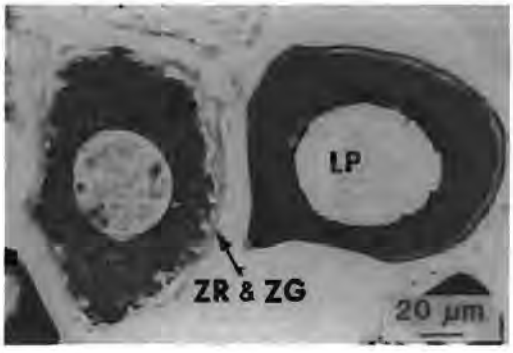

Figure 25 Atretic oocyte (type a) originating from a late perinuclear oocyte. LP = late perinuclear oocyte, ZG = zona granulosa, ZR = zona radiata.

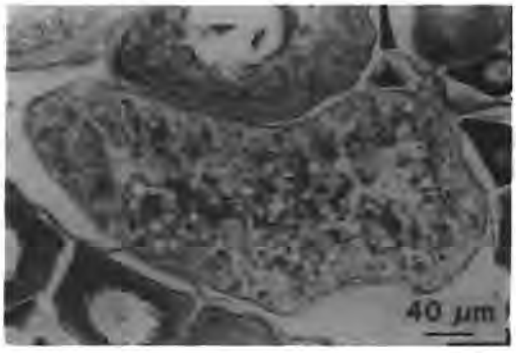

Figure 29 Atretic oocyte (type c), showing the yolk globules and the overall glandular appearance.

Figure 30 Section of a testis, showing the lobules and the sperm ducts, all leading into the main sperm duct.

Billard (1969) found as many as 14 successive generations between spermatogonia and spermatocytes in *Poecilia reticulata*. Although various types of spermatogonia were evident in the testis of *C. nufar*, no attempt was made to distinguish between them. However, two distinct and separable types of spermatogonia were noted. The largest (type a) spermatogonia are closely associated with the tunica albuginea and resemble the primary spermatogonia described by Hyder (1969), except for the presence of a clearly defined nucleolus. The second type of spermatogonia (type b) are more numerous and are smaller, with the chromatin material outlining the nucleus more clearly (Figure 31). These cells seem to be comparable with the secondary spermatogonia found in *Tilapia leucostica* (Hyder 1969) and *Liza dumerili* (Van der Horst 1978).

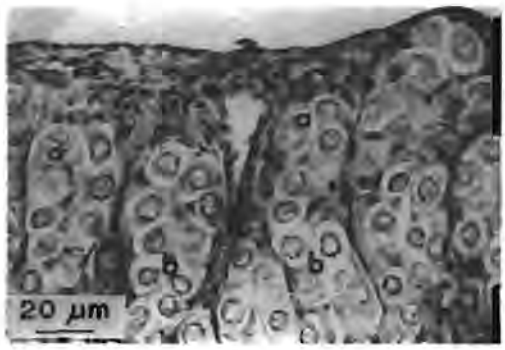

Figure 31 Type a and type b spermatogonia in a section of the testis.

Spermatocytes are distinguished from spermatogonia by their smaller size, both in total cell diameter and nucleus diameter. The primary spermatocytes have a pale-staining cytoplasm, but owing to the dense mass of chromatin material, the nuclei stain dark. The four stages of primary spermatocyte development most distinctly recognizable in *C. nufar* were: pre-leptotene, leptotene, zygotene and pachytene (Figure 32). Similar findings were also reported for *Liza dumerili* (Van der Horst 1978). The secondary spermatocytes are smaller and the nuclei are highly basophilic (Figure 32).

Spermatids have more dense and uniform chromatin in their nuclei than the spermatocytes. Spermatozoa were present in the majority of the testes examined, either in the cysts or in the sperm ducts, or both. The nuclei of the spermatozoa stain dark with hematoxylin and their long flagellar tails are distinct.

The maturation and division of spermatogenic cells take place within a particular cyst, which suggests a high degree of synchronization (Van der Horst 1978). Mature sperm are eventually released from the cysts into the lumen of the lobules from where they are discharged (Figure 33).

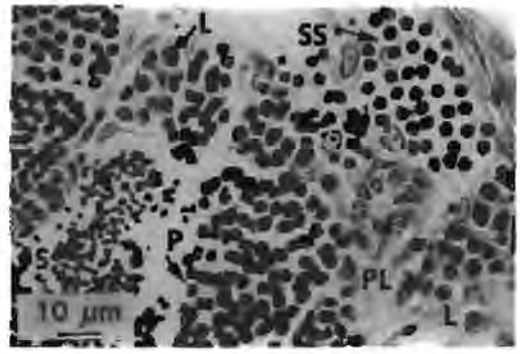

Figure 32 Section of the testis, showing different stages of primary and secondary spermatocyte development, spermatids and spermatozoa. L = leptotene stage, P = pachytene stage, PL = pre-leptotene stage, S = spermatozoa, SS = secondary spermatocytes.

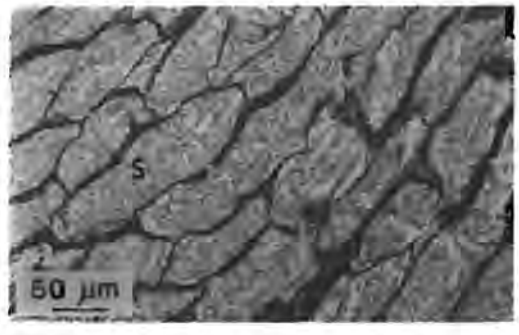

Figure 33 Section of the testis in the spawning phase, showing lobules filled with sperm. Some lobules appear 'patchy' due to partial evacuation of sperm. S = spermatozoa.

Length at maturity

During the breeding season most fish greater than 340 mm in total length had ripe gonads. Differentiation into functional females or males seems to take place at a total length of approximately 320 mm. Coupled with the above evidence, it would appear that *Cheimerius nufar* reaches sexual maturity at 340 mm total length, i.e. in its fourth year of life (Coetzee & Baird 1981b).

Sex ratio

The sex ratio (functional males: functional females) of *C. nufar* was found to be 1:2 and is similar to that found for the sparids, *Chrysoblephus laticeps* (Penrith 1972), *Pterogymnus laniarius* (Hecht 1976) and *Pagrus pagrus* (Manooch 1976).

Breeding cycle

Ovarian tissue of the ovo-testes showed no seasonal activi-

ty until the testicular part had become rudimentary (Figures 2, 3 & 6). Most oocytes were in the perinuclear stage, although earlier oocyte stages were also present. No yolk formation was observed in the oocytes of the ovo-testis.

Cysts of various spermatogenic cells were present in the lobules of the testicular part. Although numerous sperm-filled lobules were present in the ovo-testes no evidence of active spawning could be detected.

The histological and macroscopic appearance of the gonads were used as criteria to establish the seasonal gonadal changes in the functional females and males. The appearance of the ovaries and testes are summarized in Table 3 and Table 4, respectively. Reproductive phases used for macroscopic evaluation are based on those of Van der Horst (1976) and Van der Horst & Erasmus (1978).

Figure 34 shows the monthly percentage composition of 214 females and 109 males in different reproductive phases. Also indicated are daylength and mean monthly sea temperatures in Algoa Bay.

Females were reproductively inactive in winter (June to August). This was followed by an increase in oocyte development resulting in a high incidence of ripe females

Table 3 Comparison of the macroscopic and histological appearance of the ovary

Reproductive phase	Macroscopic appearance	Histological equivalent
1 Inactive	Immature virgin and inactive individuals. Ovaries very small, thin and threadlike. Oval to round and translucent; positioned close to vertebral column.	Oogonia, chromatin nucleolus, pre-perinuclear and early perinuclear oocytes. (Oocyte stages: 0, 1 and 2 a, b)[a]
2 Early recovery	Maturing virgins or recovery of spent ovaries. Ovaries more rounded and large; slight translucent pink colour; eggs not visible with naked eye.	Oogonia and oocyte stages up to (and including) late perinuclear oocytes. (Oocyte stages: 0, 1 and 2 a, b, c)[a]
3 Active recovery	Ovaries opaque and pale yellowish. Eggs very small and hardly visible to the naked eye.	Oogonia and all oocyte stages up to (and including) the early secondary yolk oocytes. (Oocyte stages: 0, 1, 2, 3 and 4 a)[a]
4 Maturing	Ovaries still larger and filling approximately two thirds of the body cavity. Ovaries elongated, opaque and yellowish; oocytes clearly visible, surface appears granular.	Oogonia and all oocyte stages up to (and including) the tertiary yolk oocytes. (Oocyte stages: 0, 1, 2, 3, 4 and 5)[a]
5 Ripe	Mature ovaries, filling most of the body cavity. Dark yellow colour with large visible eggs.	Oogonia and all oocyte stages, plus the migratory, maturation and early ripe oocytes. (Oocyte stages: 0, 1, 2, 3, 4, 5, 6, 7 and 8)[a]
6 Spawning	Ovaries at maximum size and fill body cavity. Ovaries similar to previous stage, but larger eggs present; the roe runs when pressure is applied to ovary; eggs translucent.	Ripe oocytes of maximum size, being released from the follicles. All other oogonia and oocyte stages could be present.
7 Spent	Ovaries small, thin and translucent; appear bloodshot, especially at posterior end. Very similar to phase 2 ovaries.	Oocytes up to late perinuclear stage and going over into early primary yolk vesicle oocytes. Few to many atretic oocytes. (Resorptive ovary)

[a]See Table 1.

Table 4 Comparison of the macroscopic and histological appearance of the testis

Reproductive phase	Macroscopic appearance	Histological equivalent
1 Inactive	Immature virgin and inactive individuals. Testes thin, thread-like and translucent. Sexes not easily distinguishable. Residual sperm present.	Mainly spermatogonia present; sperm in sperm ducts from previous breeding season.
2 Early recovery	Testes actively recovering from spawning. Testes strap-like and thin, beginning to thicken. Milt from previous season may be present.	Spermatogonial proliferation; few cysts with spermato-cytogenic stages; lobules organized; sperm could be present in ducts and few cysts. Marked disorganization around main sperm duct; the latter has vacuated appearance.
3 Active recovery	Testes very prominent in body cavity; opaque and greyish white, with smooth texture.	All stages present; active spermatocytogenesis and spermio-genesis. Start of active preparation for the next spawning period.
4 Pre-spawning	Testes filling about 50% of body cavity, enlarged and opaque white. Main sperm duct clearly visible; milt present.	All stages present; 50% of lobules with sperm; main duct full of sperm.
5 Spawning	Testes lobular and filling most of body cavity. Opaque white with smooth texture. Milt flows freely on handling the fish.	Most of testes and ducts filled with sperm; all stages present in periphery of testes. Confluence of cysts takes place; lobule walls appear hypertrophic.
6 Spent	Testes thin, flaccid, greyish and bloodshot. Similar to phase 2. Residual milt present in the ducts.	Lobules with 'patchy' and 'weblike' appearance; proliferation of spermatogonia and interstitial tissue, testis enters the inactive, recovery phases.

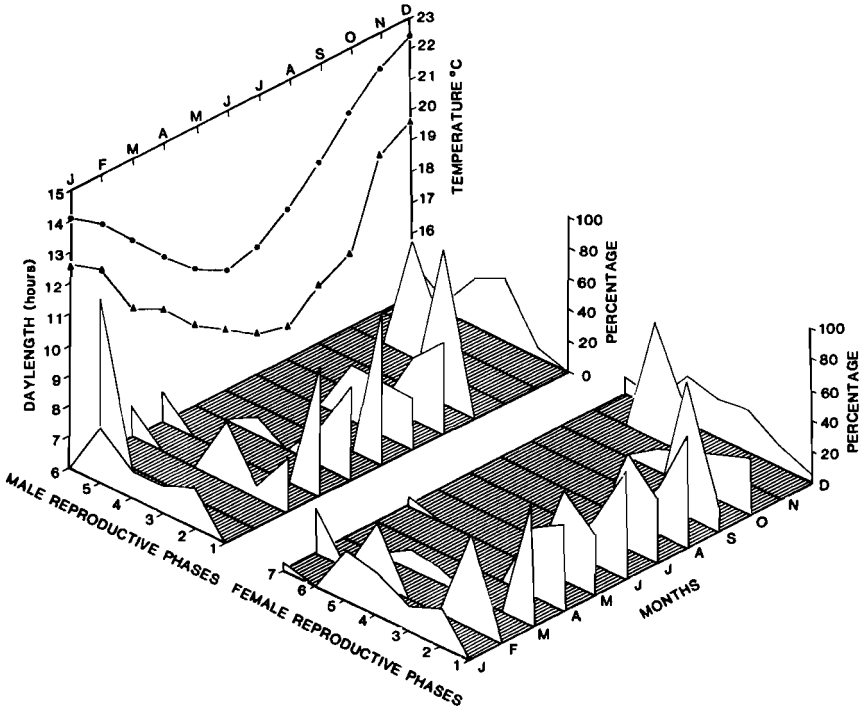

Figure 34 Monthly composition of males and females, respectively, in different reproductive phases (refer Tables 3 & 4). ●—● mean monthly daylength, ▲—▲ mean monthly sea temperatures for the period 1972 to 1977.

in November to January. Spent females were found from December to February, with the onset of the recovering phases being evident. The apparent lack of actively spawning fish could possibly be attributed to the fact that final ripening and subsequent spawning proceeds very rapidly (Yamamoto 1956a; Van der Horst 1976).

Sperm, either residual and/or newly formed, were present in the testes of the males through much of the year. However, a definite increase in sperm production could be observed towards December. Notwithstanding the lack of observations in November, the presence of pre-spawn testes in October and spent testes in March, suggests that spawning starts around November and extends to March/April (Figure 34). The peak spawning period of males ranges from early December through late January, as is the case with females. The fact that the spent phase was recurrent in the months of March and April, suggests that males remain reproductively active for a slightly longer period than females.

Maximum sea temperatures and daylength in November to January, correlate well with the spawning period of *C. nufar* (Figure 34). Conversely, reduced sea temperatures and daylength in winter correspond with the reproductively inactive and recovery period of the fish.

Discussion

Several sparid species exhibit sex reversal (Van Oordt 1929; Reinboth 1962, 1970; Atz 1964; Penrith 1972; Hecht 1976; Robinson 1976). In *Cheimerius nufar* the hermaphroditic fish undergoes sex separation, to give rise to either a functional male or a functional female. Kinoshita (1936) observed similar developments in the sparid, *Sparus longispinis*. He concluded that it is a special form of protandric hermaphroditism, because of the presence of large quantities of spermatozoa in the testicular part of the ovotestis, prior to the appearance of the first oocytes. *Sparus latus* revealed active spermatogenesis and a subsequent volumetric increase in the testicular parts of the ovo-testes, whereas the ovarian parts showed little seasonal activity (Kinoshita 1939).

Active spermatogenesis could also be detected in the testicular part of the ovo-testes of *Cheimerius nufar*. No seasonal volumetric increase or active sperm discharge was apparent. The ovarian part of the ovo-testes remained inactive until sex separation was complete. On reaching reproductive maturity, the pattern of gonadal cell development of functional females and functional males is similar to that which is generally accepted for most gonochoristic species.

In the past, the stages of oocyte development have been classified by a variety of systems, often causing great confusion. Although most authors agree on the basic characteristics incorporated into such a system, schemes used are often too brief (Kuo, Nash & Shehadeh 1974; Hilge 1975, 1977), or incomplete (Abraham 1963; Abraham *et al.* 1966), or exhibit too much detail (Yamamoto 1956a; Braekevelt & McMillan 1967; Van der Horst 1976). The classification system implemented in this study is based on the oogenetic and vitellogenetic changes as described by Yamamoto (1956a, b, c, 1957), Braekevelt & McMillan (1967) and Van der Horst (1976). It reflects the nine major phases in oocyte development, each of which can be subdivided (i.e. pre-, early or late stages) if greater detail is required. The most important changes made to the previous systems include the renaming of the vitellogenetic stages. These are briefly outlined: (i) yolk vesicle oocytes are the first to show distinctive yolk formation, and are therefore named primary yolk vesicle oocytes, (ii) Matthews (1938),

Braekevelt & McMillan (1967) and Van der Horst (1976) reported on the appearance of acidophilic intravesicular secondary yolk formation between the oil vesicles. Yamamoto (1956a) and Van der Horst (1976) described this stage as primary yolk, but in the system presented here these are renamed early secondary yolk oocytes, (iii) the late secondary yolk oocyte stage follows and is analogous to the secondary yolk stage (stage 6) of Yamamoto (1956a), Braekevelt & McMillan (1967) and Van der Horst (1967), (iv) the tertiary yolk oocyte stage is directly comparable to the tertiary yolk stage of Yamamoto (1956a), Braekevelt & McMillan (1967) and Van der Horst (1967). This stage does not imply a third type of yolk, but rather the coalescence of the secondary yolk which represents a major stage in oocyte development.

Atretic oocytes are generally considered to be degenerating oocytes which are subsequently lost from the ovary other than by ovulation (Ingram 1962; Lewis & McMillan 1965; Cala 1970). Atresia in *C. nufar* occurred either during or just after the spawning season. The cause of atresia is not yet well understood. Further, the process seems to differ in the various stages of oocyte development, hence the three types described here.

The breeding cycle of fish is greatly influenced by environmental factors, with temperature and daylength (photo-period) playing major roles (Oslund 1928; Bissonnette 1936; Bullough 1939; Merriman & Schedl 1941; Egami & Hosokawa 1973; Sadleir 1973; De Vlaming & Pacquette 1977; De Vlaming & Shing 1977; Van der Horst & Erasmus 1981). This pattern is also evident in *C. nufar*. The warmer climate of more tropical regions is probably instrumental in initiating spawning of some sparid species during the late winter (Sadleir 1973; Druzhinin 1975; Wallace 1975a, b; Brownell 1979). In this study *C. nufar* was found to spawn during summer (increased sea temperatures and daylength) with a peak in activity during November, December and January. This compares well with the majority of sparids in southern waters which spawn in summer (Brownell 1979).

Acknowledgements

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