Short Communications

Characteristics of mature oocytes from four species of marine teleosts

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Received 19 February 1988; accepted 9 August 1988

Fresh mature translucent oocytes from four marine teleosts, *Liza richardsonii, Liza dumerilii, Monodactylus falciformis* and *Pomadasys olivaceum* are described. The characteristic features of the mullet oocytes, their diameter and number and size of oil droplets, are compared with those observed in artificially spawned fish. Such descriptions are not only of use in aquaculture, they are also of diagnostic value in zooplankton studies.

Vars volwasse deursigtige oösiete van vier mariene teleoste, *Liza richardsonii, Liza dumerilii, Monodactylus falciformis* en *Pomadasys olivaceum* word beskryf. Die kenmerkende eienskappe van die oösiete, soos hul deursnee en die aantal en grootte van die oliedruppels word vergelyk met dié van vis wat kunsmatige kuitskiet ondergaan het. Sulke beskrywings is nie net van belang in akwakultuur nie maar is ook van diagnostiese waarde in die studie van soöplankton.

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The voluminous literature available on the reproductive biology of teleost fish includes many detailed accounts of gametogenesis. The majority only give descriptions of oocyte development up to the tertiary yolk stage. Relatively few studies outline the final stages of oocyte maturation which involves the formation of oil droplets, fusion of yolk globules and migration of the nucleus to the animal pole. The lack of information on pre-ovulatory stages reflects inadequate sampling of fully ripe females in the wild and scarcity of observations on natural spawning under captive conditions. Descriptions of mature oocytes have, however, been obtained from fish induced to complete gametogenesis and spawn by means of hormone injections (Kuo, Nash & Shehadeh 1974; Zhitenev, Kalinin & Abayev 1974). Whether these oocytes are of comparable quality to those produced in nature has not been established.

The present article describes mature translucent oocytes obtained from ripe-running specimens of four common nearshore teleosts, *Liza richardsonii, Liza dumerilii, Monodactylus falciformis* and *Pomadasys olivaceum* caught in Algoa Bay. The appearance of mature oocytes from the wild-caught mullet is compared with that observed during a recent study on induced spawning in *L. richardsonii* (Bok & Jongbloed 1987).

A few ripe-running fish were caught during seine netting operations off King's Beach, Algoa Bay, Port Elizabeth, within the period September 1979 to February 1980. Release of mature oocytes appeared to be spontaneous in these fish, although the possibility of it being induced by handling during the netting procedure should not be discounted. Further oocytes for examination in the laboratory were obtained by light abdominal massage of the same fish. Oocytes were suspended in ovarian fluid or 0,9% physiological saline on a cavity slide, which allowed them to float with the oil droplets uppermost. The diameters of 30 spherical translucent oocytes from each species were measured using an eyepiece micrometer. The number and size of oil droplets were also noted. Photographs were taken with a Zeiss photomicroscope, employing bright field illumination.

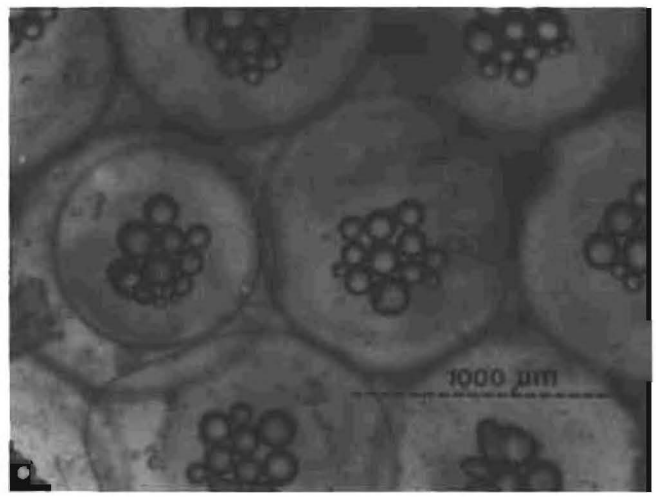

Figure 1 Mature oocytes of *Liza richardsonii* with large numbers of oil droplets visible.

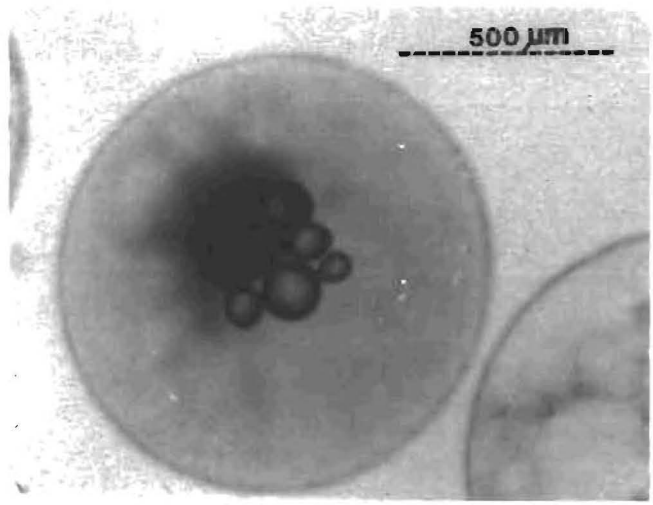

Figure 2 Mature oocyte of *Monodactylus falciformis* with six oil droplets clearly visible.

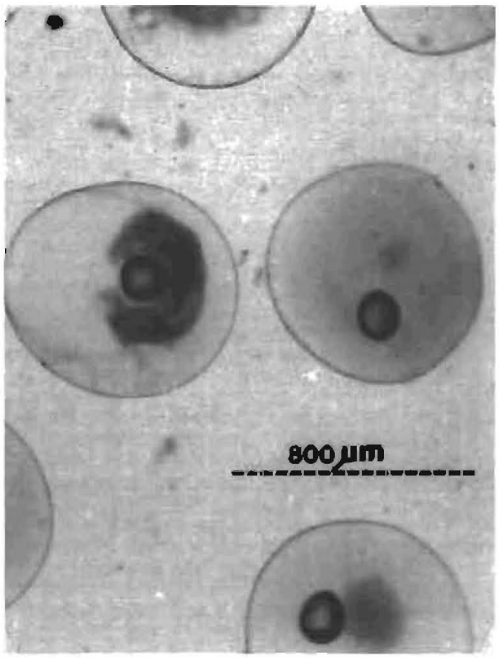

Figure 3 Mature oocytes of *Pomadasys olivaceum* showing one oil droplet per oocyte.

Figures 1 to 3 are photomicrographs showing the mature oocytes obtained from *L. richardsonii*, *M. falciformis* and *P. olivaceum* respectively. No photographs were taken of *L. dumerilii* oocytes. The oocytes appeared to be non-adhesive, they were all spherical and translucent with no apparent surface sculpturing. The characteristic features, mean oocyte diameter and number and size of oil droplets, are listed in Table 1. These appear to be the first descriptions of mature oocytes from teleosts within the genera *Monodactylus* and *Pomadasys*. Comparative data are, however, available for mullet (see Brusle 1981 for review).

Although eggs bearing multiple oil droplets have been reported previously in several mugilid species there is some controversy over this trait. Several authors (Kuo, Shehadeh & Milisen 1973; Nash, Kuo & McConnell 1974) consider it to be an unnatural situation which results in reduced viability of the eggs. Yashouv & Berner-Samsonov (1970), however, found that the small oil droplets they observed in eggs of *Mugil cephalus* and *Mugil capito* eventually coalesced and successful development ensued. The eggs obtained by induced spawning of *L. richardsonii* (Bok & Jongbloed 1987) are of similar diameter (850–1 000 µm) and appearance (up to 16 small oil droplets) to the mature oocytes obtained from wild-caught fish. The good fertilization and hatching rates they obtained with these eggs suggest that doubts on viability associated with the presence of multiple oil droplets may be unfounded. Although the eggs described by Bok & Jongbloed (1987) appear to be morphologically similar to those we describe it is possible that the accelerated development associated with induced spawning affects the biochemical composition of the eggs (Nash & Koningsberger 1981). Comparative studies on subsequent development are therefore needed to determine whether or not the quality of artificially spawned eggs differs from that of naturally spawned eggs.

Detailed descriptions of naturally spawned oocytes are of considerable use. They can be used by aquaculturists to elucidate whether or not the eggs obtained by induced spawning techniques are properly formed and of good quality. Being species-specific characteristics, data on oocyte diameter and number of oil droplets are also of diagnostic value in zooplankton studies (De Chiekomski 1981).

References

BOK, A.H. & JONGBLOED, H. 1987. A preliminary study on induced spawning of southern mullet. *S. Afr. J. Wildl. Res.* 17: 82–85.

BRUSLE, J. 1981. Sexuality and biology of reproduction in grey mullets. In: Aquaculture of Grey Mullets, (ed.) Oren, O.H. International Biological Programme 26. Cambridge University Press, Cambridge.

DE CHIEKOMSKI, J.D. 1981. Ictioplancton. In: Atlas del Zooplancton del Atlantic Ocean Sud-Occidental y metodos de trabajo con el zooplancton marino, (ed.) Boltovsky, D. INIDEP Mar del Plata, Argentina.

KUO, C.M., NASH, C.E. & SHEHADEH, Z.H. 1974. A procedural guide to induce spawning in grey mullet (*Mugil cephalus* L.) females by injection of human chorionic gonadotrophin. *Aquaculture* 3: 1–14.

KUO, C.M., SHEHADEH, Z.H. & MILISEN, K.K. 1973. A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, *Mugil cephalus*. *J. Fish Biol.* 5: 459–470.

Table 1 Characteristic features (oocyte diameter, number and diameter of oil droplets) of mature oocytes from four marine teleosts

	Oocyte diameter (µm) n = 30		Number of oil droplets		Diameter of oil droplets (µm)	
Species	Average	± *SD*	Average	Range	Average	Range
Liza richardsonii	954	33	9	8–13	82	25–150
Liza dumerilii	845	32	16	14–18	–	–
Monodactylus falciformis	1022	35	5	4– 8	104	63–156
Pomadasys olivaceum	784	45	1	1	135	133–155

NASH, C.E., KUO, C.M. & MCCONNELL, S.C. 1974. Operational procedures for rearing larvae of the grey mullet (*Mugil cephalus* L.). *Aquaculture* 3: 15–24.

NASH, C.E. & KONINGSBERGER, R.M. 1981. Artificial Propagation. In: Aquaculture of Grey Mullets, (ed.) Oren, O.H. International Biological Programme 26. Cambridge University Press, Cambridge.

YASHOUV, A. & BERGER-SAMSONOV, E. 1970. Contribution to the knowledge of eggs and early larval stages of mullets (Mugilidae) along the Israel coast. *Bamidgeh* 22: 72–89.

ZHITENEV, A.N., KALININ, D.S. & ABAYEV, Y.I. 1974. The state of the gonads of the striped mullet (*Mugil cephalus*) and the sharpnose mullet (*Mugil saliens*) leaving estuaries to spawn and their reaction to a pituitary injection. *J. Ichthyol.* 14: 232–239.

A rapid, non-sacrificial chromosome preparation technique for freshwater teleosts

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Received 16 September 1987; accepted 23 August 1988

The use of fin epithelium from the tilapia, *Oreochromis mossambicus*, and the grass carp, *Ctenopharyngodon idella*, was investigated to provide a rapid, non-sacrificial procedure for determining ploidy. A combination of colchicine, prolonged hypotonic treatment, dissociation of cells followed by Giemsa staining makes it possible to achieve good quality metaphase chromosome spreads using small fish without the use of sterile conditions, centrifuges or sacrificing the specimen. In situations such as the induction of triploidy or tetraploidy, it is necessary to have a quick, reliable method of assessing the results of experimental design. The technique presented in this report provides numerous, well-spread metaphase chromosomes with a tissue handling time of less than 2 h.

Die gebruik van die vinepiteel van die bloukurper, *Oreochromis mossambicus*, en die graskarp, *Ctenopharyngodon idella*, is geëvalueer as 'n vinnige metode om chromosoomgetalle te bepaal sonder dat die vis doodgemaak word. 'n Kombinasie van colchicine, verlengde hipotoniese behandeling en die dissosiasie van selle gevolg deur Giemsakleuring, lewer hoë-kwaliteit metafase-chromosome. Die tegniek leen hom daartoe dat klein vissies gebruik kan word wat nie gedood hoef te word nie en dat geen gesofistikeerde apparaat of steriele toestande benodig word nie. In situasies soos die induksie van triploïede of tetraploïede is dit wenslik om op 'n baie vroeë ouderdom te kan bepaal of die induksie 'n sukses was ten einde die eksperimentele prosedure te evalueer. Die tegniek wat in hierdie studie gebruik is, lewer verskeie goedverspreide metafase-chromosome binne slegs 2 h.

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There are many uses to which the chromosomal information of fish can be put, particularly in the areas of cytotaxonomy, mutagenesis and aquaculture (Kligerman & Bloom 1977). Chromosome numbers have been described for a wide range of fish species (eg. Beamish & Miller 1977; Bertollo, Takahashi & Filno 1983; Beck & Biggers 1980; Blaxhall 1983: Hinegardner 1976; Vervoort 1980).

Roberts (1967) suggested that fish are likely to have more intraspecific chromosomal polymorphism than other vertebrates and that karyotypic differences could be used as racial markers while interspecific karyotypic differences are criteria for separating morphologically similar species (Boothroyd 1959; Fukuoka 1972).

It is possible, however, that some of the observed chromosome variations reflect an inadequacy of the technique used, particularly the squash method which often results in poor morphological detail and overlapping of chromosomes (Blaxhall 1975; Hartley & Horne 1985). Until techniques are refined and 'normal' karyotypes are accurately known, only extreme variations can be detected.

Genetic mechanisms of sex determination have been described for a number of species with the complete range from synchronous hermaphroditism through primitive polygenic sex determination to distinct sex chromosomes being exhibited in various fish species (Atz 1964; Avtalion & Hammerman 1978; Harrington 1963; Uyens & Miller 1971; Ohno 1967). A knowledge of the sex-determining mechanisms has great implications in fish breeding, for example in the monosex culture of the tilapias employing the techniques of sex reversal and hybridization.

In reviews of current fish chromosome techniques (Blaxhall 1975; Hartley & Horne 1985; Ojima 1982) criticism has been made of the consistency of results, the sophistication and length of techniques, the frequent necessity of sacrificing the specimen and the specimen size required. It was the purpose of this study to modify the solid tissue techniques of Denton & Howell (1969) and Kligerman & Bloom (1977), to provide a rapid, technically unsophisticated means of deriving accurate chromosomal information from young freshwater fish without sacrificing the specimen. The value of this technique would be both in field studies and in the laboratory where a quick accurate result is desired but without the use of centrifuges, grinders, digestive enzymes or tissue culture and when the specimen must be kept alive.

The fish used in this study were maintained in 300 l, aerated, glass aquaria kept at 28°C by submersible, thermostatically controlled heaters. The two species investigated were *Oreochromis mossambicus* and *Ctenopharyngodon idella*.

The most suitable method for obtaining a high mitotic index is described below.

Trim the edges of the caudal fin two to three days prior to processing to stimulate regeneration of the epithelium. Place fish in aerated 0,005% colchicine solution at 28°C for 3 h. Remove specimen and rinse under running water. Trim caudal fin margins which will