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Anotonysiluvai Jesu *AROCKIARAJ*, Thiruvaimozhi Arulmozhi *VARMA*, Mohammed A. *HANIFFA*

Fish anatomy

OOCYTE GROWTH IN FRESHWATER CATFISH *MYSTUS MONTANUS* (JERDON, 1849) (SILURIFORMES: BAGRIDAE) MATURING IN DIFFERENT SALINITIES

ROZWÓJ OOCYTÓW U SŁODKOWODNEGO SUMIKA, *MYSTUS MONTANUS* (JERDON, 1849) (SILURIFORMES: BAGRIDAE) DOJRZEWAJĄCEGO W WODZIE O RÓŻNYM ZASOLENIU

Centre for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai, Tamil Nandu, India

Profiles of oocyte growth were obtained from female freshwater catfish *Mystus montanus* held in salinity ranging from freshwater (0‰ salinity) to sea water (35‰ salinity) during two consecutive spawning seasons (1999) of south-west monsoon (June–August) and north-east monsoon (October–December). Females underwent vitellogenesis at all salinities 0, 5, 10, 15, 20, 25, 30 and 35‰) tested. Females maturing in 0‰ (freshwater salinity content) and 15‰ (brackish water salinity content) exhibited a slower rate of oocyte growth and a significantly lower incidence of completed vitellogenesis. The test individuals died in the salinity values of 20, 25, 30, and 35‰. The other test individuals were induced to spawn at different salinities (0–15‰). The number of fertilized eggs per spawning was the highest from females maturing in 5‰ and followed by 10‰. More females were able to spawn twice (two monsoon periods) in 5 and 10‰ salinities than 0 and 15‰ salinities. The present results suggest that increasing the salinity values from 0 to 10‰ in freshwater are adequate for ovarian maturation in freshwater catfish *Mystus montanus* females.

INTRODUCTION

Mystus montanus (Jerdon, 1849) (Siluriformes: Bagridae) has been listed as threatened fish species according to the IUCN criteria (CAMP 1998). To maximise the seed production, the broodstock development and induced breeding technique are necessary for conservation. The above-mentioned catfish is a prime candidate for domestication because it can be cultured in neglected water bodies and it feeds on insect larvae, water fleas, and

worms (Thiaeng 1986). Broodstock have been successfully maintained in tanks and ponds for year, but there have been no reports on natural spawning in captivity (Love 1980). Females will reach the completion of vitellogenesis but will not initiate final maturation (Kinne 1960). As a result, several hormone treatment protocols have been developed to circumvents this „block" to spawning (Tamaru et al. 1994).

Although the induction of spawning of *M. montanus* is now routine, little research has been done on the maturation of *M. montanus* broodstock in captivity. Photoperiod and temperature have been found to determine the seasonal reproductive cycle (Kuo et al. 1973; Kelly et al. 1991). Brusle (1981) suggested that while salinity does not appear to be an environmental cue, it may still influence gonadal maturation. This possibility was investigated in this study by examining the effects of salinity on ovarian maturation in captive freshwater catfish *M. montanus*. Such information is critical to the selection process of a hatchery for the artificial propagation of this fish.

MATERIAL AND METHODS

The experiment was carried out during the year 1999 in 3×1×1 m cement tanks at CARE Aquafarm, St. Xavier's College, Palayamkottai. Each tank contained water of different salinity (0, 5, 10, 15, 20, 25, 30, and 35‰). The salinity range was increased by added sodium chloride—NaCl (0‰ salinity refers to distilled water). Each experimental treatment involved 20 female fish (average length 12 ±2 cm and weight 10 ±1 g). They were fed with finely chopped chicken intestine once per day (16.00 h) for the total duration of the experiment (June–August and October–December; two different monsoon season separately). The water salinity content were checked and adjusted once in a week. All experiments were done in triplicate. The induced breeding experiments were carried out according to the methodology of Haniffa and Arockiaraj (1999).

Monitoring of ovarian maturation was carried out by first anaesthetising the females in 300 ppm 2-phenoxy ethanol (Sigma chemical, Saint Louis, Missouri, USA). Ovarian biopsies were obtained by using a polyethylene cannula (L.D. 0.86 mm; O.D. 1.52 mm) and oocytes were fixed in 10% formalin solution. One hundred oocytes were measured in 50 μm increments as described by Shehadeh et al., (1973). Oocyte growth refers here to the change in oocyte diameter. Oocyte growth both between the monsoon and among various salinities was analysed with analysis of co-variance (Sokal and Rohlf 1969).

The experiment was conducted two consecutive spawning season of south-west monsoon (June–August) and north-east monsoon (October–December) periods for comparison. The spawning data (i.e. female body weight, fecundity, egg diameter, percentage of fertilisation, temperature and salinity) were subjected to one-way ANOVA (Sokal and Rohlf 1969).

RESULTS

The test animals died in the salinities 20, 25, 30, and 35‰ in the first and second day of the experiment. The animals could not adopt themselves to high salinity. The rate of oocyte growth from females maturing in 5‰ and 10‰ salinities was found to be significantly (p <0.01) higher than the other two salinity groups of 0‰ and 15‰. (Fig. 1, Table 1). The profiles of oocyte growth obtained from female catfish maturing at the various salinities were subjected to regression analysis and models of oocyte growth for each group were determined (Table 1). The rate of oocyte growth from females maturing in 0‰ and 15‰ salinities did not differ significantly.

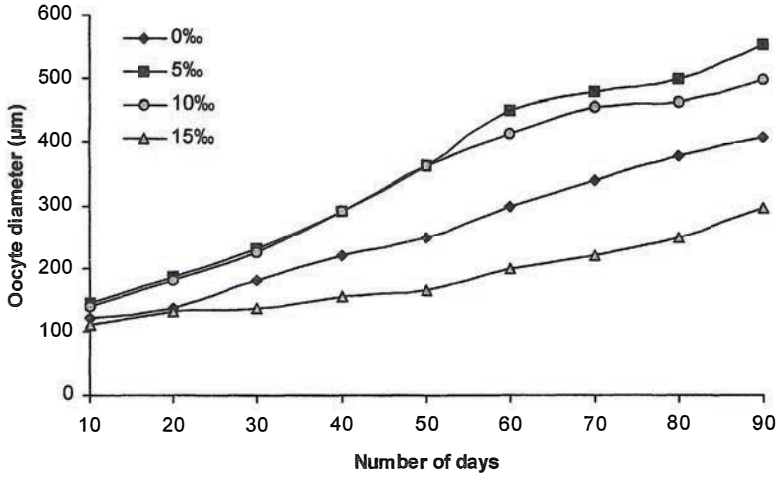

Fig. 1. Profiles of oocyte growth obtained from female *Mystus montanus* maturing at different salinities (0, 5, 10, and 15‰) in south-west and north-east monsoon

The initiation of vitellogenesis, however, in the 15‰ salinity group was significantly (p < 0.01) delayed during both south-west and north-east monsoon. The number of females with oocyte reaching 502–556 µm (as recorded in both monsoon seasons in well suited salinity content) varied significantly (p < 0.01) (Fig. 2) only 50% of the females maintained in 0‰ salinity reached the state of maturing at which spawning could be induced. Approximately 50% of 5‰ and 10‰ salinity females developed oocytes of 500 µm or greater (Fig. 2).

There was no detectable difference in the body weight of females used in the spawning trials (Table 2). Although the average prespawn oocyte diameters for all groups (0–15‰) was 440.5 ±114.7 µm and some variation was detected. In the two monsoon pe-

riod females maturing in 0‰ had a significantly smaller average prespawn oocyte diameter when compared to females maintained in 5 and 10‰. This 0‰ group also produced significantly ($p < 0.01$) smaller eggs and fewer eggs g/body weight when compared to the females maturing in 5 and 10‰ salinity.

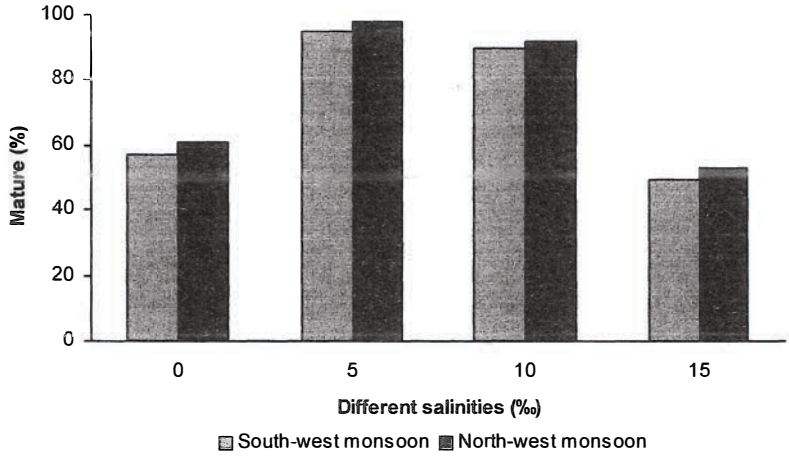

Fig. 2. Percentage of female *Mystus montanus* that reached average oocyte diameter of ≥ 440 μm while being held at different salinities

The number of fertilised eggs per spawn exhibited significant (χ^2 test, $p < 00.1$) variation among maturation salinities. 5 and 10‰ salinity content females produced the greatest number of fertilised eggs per spawn followed by females maturing in 0‰ salinity with the lowest number of fertilised eggs obtained from females maturing in 15‰ salinity. It should be noted that although 20 females were placed in 5 and 10‰ salinity, during the two monsoon period, a total of 15 spawners were reported from this group were induced to spawn twice in one season. Several females in 0‰ salinity group also spawned twice, but none of the females, in 15‰ salinity group spawned more than once.

Salinity ranges were relatively stable during the course of experiment. Temperature, however, varied significantly ($p < 0.01$) among tanks as well as during the course of experiment. A summary of the ranges of these environment parameters observed in each maturation tank is presented in Table 1. The highest temperature was observed in 15‰ salinity tank and decreased in direct proportion to salinity.

Table 1

Summary of observed salinity, average morning and afternoon temperature and linear models of oocyte growth in the various maturation tanks of various salinities; predictated oocyte diameter (ø) in μm

Salinity (‰)	Temperature (°C)		Oocyte growth		Survival (%)	
	South-west monsoon	North-east monsoon	South-west monsoon	North-east monsoon	South-west monsoon	North-east monsoon
0	28.5 ±1	28.0 ±2	Oocyte ø = 2.92 (day) + 147.5 $r^2 = 0.59$; $p < 0.001$	Oocyte ø = 2.96 (day) + 127.0 $r^2 = 0.56$; $p < 0.001$	95	98
5	28.5 ±1	28.0 ±1	Oocyte ø = 4.07 (day) + 190.0 $r^2 = 0.56$; $p < 0.001$	Oocyte ø = 3.39 (day) + 208.0 $r^2 = 0.58$; $p < 0.001$	90	95
10	29.0 ±1	29.0 ±1	Oocyte ø = 3.32 (day) + 204.0 $r^2 = 0.56$; $p < 0.001$	Oocyte ø = 2.92 (day) + 200.0 $r^2 = 0.46$; $p < 0.001$	92	90
15	30.0 ±2	30.0 ±2	Oocyte ø = 2.92 (day) + 128.0 $r^2 = 0.45$; $p < 0.001$	Oocyte ø = 1.84 (day) + 129.0 $r^2 = 0.46$; $p < 0.001$	87	85
20	died	died	—	—	0	0
25	died	died	—	—	0	0
30	died	died	—	—	0	0
35	died	died	—	—	0	0

Table 2

Summary of the spawning data obtained from females maturing at various salinities in 1999 9both south-west and north-east monsoon)

Salinity (‰)	Year 1999 spawning	Spawn	Body weight (g)	Oocyte diameter (μm)	Egg diameter (μm)	Average fecundity	Average fertilisation (%)
0	partial	11 ±2	10 ±1.5	410.3 ±26.4^{bc}	638.6 ±18.4^{bc}	714 ±118^{bc}	50.5 ±22.6^{ac}
5	complete	19 ±1	11 ±1.2	556.3 ±32.8^{bd}	782.7 ±22.5^{bd}	1181 ±238^{bd}	89.0 ±10.5^{bd}
10	complete	18 ±2	11 ±1.5	502.8 ±22.7^{bd}	740.4 ±30.1^{bd}	986 ±198^{bd}	78.0 ±12.0^{bc}
15	partial	10 ±4	12 ±1.5	294.5 ±15.5^{a}	516.0 ±20.6^{a}	662 ±105^{a}	45.6 ±20.0^{a}
20	died	died	—	—	—	—	—
25	died	died	—	—	—	—	—
30	died	died	—	—	—	—	—
35	died	died	—	—	—	—	—

Means with dissimilar letters are significantly different ($p < 0.05$)

DISCUSSION

This study demonstrated that salinities from 5‰ to 10‰ are superior to freshwater for the maturation of *M. montanus* females. Although the rate of oocyte growth did not differ with in this range, the initiation of growth was found to be significantly different between the two monsoon periods for 0‰ salinity. A similar time for the onset of *M. montanus* for maturing in 0‰ salinity (two monsoon periods) was observed in *Mugil cephalus* in 1986, 1989, and 1990 trial and may simply reflect seasonal variations. (Tamaru et al. 1991). However it has been demonstrated that a decrease in both day length and water temperature is responsible for the initiation of vitellogenesis (Kelley et al. 1991). When the monitoring of ovarian stages commenced in August day length was already decreasing. Addition of 0, 5, and 10‰ salinity tanks resulted in a decrease in average water temperature and may explain the similar start of maturation in the 5 and 10‰ salinity tanks in both season as opposed to the variable starting times observed in the 15‰ salinity.

Conflicting results were noticed in the present study as to whether ovarian maturation in *M. montanus* can occur in 5 and 10‰ salinity. A similar report exists that the ovarian maturation in striped mullet *Mugil cephalus* (brackish water fish) can occur in freshwater (Tamaru et al. 1994). Gonado somatic index (GSI) values in *Mugil cephalus* remain at an estimate of 0.7 throughout the year (Brusle 1981) up to the stage of they maturity at which they are hormonally induced to spawn (Yashouv 1969). It is clear that the fish can undergo vitellogenesis in 15‰ salinity and also reach the state of maturity at which spawning can be induced. However, the slower rate of oocyte growth, the limited number of females. Completed vitellogenesis and the lower percentage of fertilised eggs per spawning suggest that this salinity is not ideal. No differences were observed in the rate of oocyte growth between 5 and 10‰. 0‰ salinity from both seasons which suggests that the slower rate observed in 15‰ group is aberrant. It may be argued that the slower rate of oocyte growth and lower number of females to complete vitellogenesis is the result of high temperature observed in the 15‰ tank (Kuo et al. 1974; Nash and Shehadeh 1980). In *M. cephalus* the lower temperature stimulated the vitellogenesis (Tamaru et al. 1974). The similar trend was noticed in the present study.

An alternative explanation is that being held in 15‰ salinity increased the metabolic demand required to counter balance the osmotic stress. The osmotic pressure of mullet serum was reported to be 349 mo^{1}_{sm}/kg (Watanabe 1982). A salinity between 12–13‰ is calculated to be isotonic to mullet serum (Lee et al. 1991). No reference was available on this studies for *M. montanus*. Increasing the salt content from 5‰ to 10‰ during the spawning season represents a major obstruction to the natural progression of events leading up to

spawning in the wild. The substandard maturation results, from females confined to salt water one consistent with their reproductive biology.

Females kept 0 and 15‰ for two consecutive seasons had a significantly lower relative fecundity in comparison to the other treatment groups such as 5 and 10‰. *Mugil cephalus* matured at 13–20‰ salinity (Nash et al. 1974), for *M. montanus* 5–10‰ salinity is advisable.

In summary, female *M. montanus* can attain maturity in 5–10‰ salinity range, which spawning can be hormonally induced. From the perspective of managing female *M. montanus* broodstock, the maturation of females maintained in freshwater appears to be unsatisfactory, because of the slower rate of oocyte growth and lower number of females can attain full sexual maturity. The recommended salinity range for maturation of captive female *M. montanus* is from 5 to 10‰.

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Anotonysiluvai Jesu *AROCKIARAJ*, Thiruvaimozhi Arulmozhi *VARMA*,
Mohammed A. *HANIFFA*

ROZWÓJ OOCYTÓW U SŁODKOWODNEGO SUMIKA, *MYSTUS MONTANUS* (JERDON, 1849) (SILURIFORMES: BAGRIDAE) DOJRZEWAJĄCEGO W WODZIE O RÓŻNYM ZASOLENIU

STRESZCZENIE

Ustalono charakterystyki wzrostu oocytów uzyskanych od samic słodkowodnych sumików indyjskich (*Mystus montanus*) utrzymywanych w zbiornikach wodnych o różnym zasoleniu (0–35‰). Badania prowadzono w ciągu dwóch kolejnych okresów tarłowych (1999) związanych z monsunem południowo-zachodnim (czerwiec–sierpień) oraz monsunem północno-wschodnim (październik–grudzień). Witellogenezę obserwowano u samic we wszystkich badanych wariantach zasolenia (0, 5, 10, 15, 20, 25, 30 i 35‰). Samice dojrzewające w słodkiej (0‰) i słonawej (15‰) wodzie wykazywały wolniejsze tempo wzrostu oocytów oraz istotnie niższy odsetek przypadków gdzie witellogeneza została zakończona. Badane ryby nie przeżyły przetrzymywania w wodzie o następujących poziomach zasolenia: 20, 25, 30, 35‰. Pozostałe osobniki doświadczalne były gotowe do tarła w różnych warunkach zasolenia (0–15‰). Liczba zapłodnionych ziaren ikry w trakcie jednego tarła była najwyższa w przypadku samic dojrzewających w wodzie o zasoleniu 5‰ a nieco niższa u tych, które były przetrzymywane w wodzie o zasoleniu 10‰. Więcej samic podjęło tarło dwukrotnie (obydwa sezony monsunowe) przy 5‰ i 10‰ zasoleniu niż te przebywające w zasoleniach 0‰ i 15‰. Wyniki niniejszych badań wskazują że zwiększenie zasolenia z 5‰ na 10‰ ma pozytywny efekt na dojrzewanie jajników samic słodkowodnego sumika *Mystus montanus*.

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Author's address:

Anotonysiluvai Jesu Arockiaraj
Centre for Aquaculture Research and Extension (CARE),
St. Xavier's College (Autonomous),
Palayamkottai – 672 002, Tamil Nandu, India