TECHNIQUES TO REGULATE SEX RATIO AND BREEDING IN TILAPIA**

T. J. PANDIAN and K. VARADARAJ

School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India.

ABSTRACT

Available information on the advantages and limitations of the techniques of hybridization and hormone treatment for the production of all male tilapias is summarized. The critical minimum dose to ensure 100% sex reversal is determined for *Oreochromis mossambicus*. The possibility of producing triploid and tetraploid *O. mossambicus* through heat shock treatment of fertilized eggs is described.

INTRODUCTION

For a given unit of food energy tilapias are known to produce the maximum protein with high quality of flesh[1]. The other attributes, which make the tilapias more suitable for fish farming, are their general hardiness, resistance to diseases[2], ability to survive at low oxygen tension (e.g. *O. mossambicus*: 1.7 mg/litre[3], *Sarotherodon macrotis* 0.26 mg/litre[4]), a wide range of salinity (e.g. *O. mossambicus* 75‰[5]), temperature (e.g. *O. mossambicus*: 10–42°C [5]) and on a wide range of foods[6]. In fish farming, procuring spawn is one of the difficult tasks but tilapias present no such problem; indeed it is difficult to prevent them from spawning. Such uncontrolled spawning often results in gross overcrowding and many of their offsprings survive to compete with their parents for available food and space[7]. For instance, *O. mossambicus* mature at the age of 2–3 months and then onwards produce 75–1000 offsprings once in every 22–40 days[5]. Elimination of uncontrolled reproduction is most desirable to channel the available energy for the efficient growth, and quick harvest of marketable sized tilapias.

Control of reproduction in tilapias may be achieved by use of predators and monosex culture. In the first, predators are used to consume young tilapia[1]. This method has met with varying degrees of success; the role of the predator has been shown to be inadequate or too strong under different ecological situations[8]. However, the most effective and widely used technique for population control is monosex culture[9]. This is achieved by (a) manual sexing of fingerling; (b) hybridization, (c) sex-reversal by hormone treatment; and (d) chromosomal manipulation to produce all sterile fry.

a) *Manual sexing*

Manual sexing of tilapia has been tested by several workers[7,10,11]. The sexes are identified by examination of the urinogenital papillae. Two orifices are present in the female but only one in the male[12]. Although manual sexing is laborious and requires some skill, individuals (> 50 g) can easily be sexed. One can segregate about 2,000 male tilapias in a working day[13]. The major disadvantages of this method are human error in sexing and the wastage of females[1]. One female inadvertently introduced into a pond of males can undo all the labour involved in sexing.

b) *Hybridization*

Hickling[14] was the first to describe hybridization between *O. mossambicus* (♀) and *O. hornorum* (♂), which resulted in the production of all male progeny. Since then several interspecific and intergeneric hybridizations that resulted in all male progeny have been reported (table 1). Several theories have been proposed to explain the sex-determining

Table 1 *Summary of studies on hybridization in tilapias*

| Species used in hybridization | | F_1 offspring (%) | | Reference |
♂	♀	♂	♀	
Oreochromis aureus	× *O. niloticus*	100	0	Fishelson[34]
O. hornorum	× *O. niloticus*	100	0	Pruginin[35]
O. hornorum	× *O. mossambicus*	100	0	Hickling[14]
O. macrochir	× *O. niloticus*	100	0	Lessent[36]
O. variabilis	× *O. niloticus*	100	0	Welcomme[37]
*O. aurea hornorum**	× *O. niloticus*	100	0	Wohlfarth[38]
O. mossambicus	× *O. spilurus niger*	0	100	Whitehead[39]
O. hornorum	× *O. aureus*	0	100	Lee[40]
O. vulcani	× *O. aureus*	0	100	Pruginin[41]
O. niloticus	× *O. leucostictus*	0	100	Pruginin[35]
O. niloticus	× *O. spilurus niger*	0	100	Pruginin[35]
O. niloticus	× *O. mossambicus*	0	100	Chen[42]

*hybrid resulting from the cross between *O. aureus* and *O. hornorum*

mechanism in tilapias. Hickling suggested that at least two kinds of sex-determining mechanisms XX♀-XY♂ and WZ♀-ZZ♂ are present in tilapias. However, not all the sex ratios of tilapia hybrid progeny can be explained by the available theories. Hammerman and Avtalion[15] presented a model, which takes into account the possible sex-determining, effects of autosomes as well as sex chromosomes. Although hybridization techniques to produce all-male tilapia were being developed during the last 20 years, its applicability to monosex culture of tilapia has remained very limited for the following reasons: (i) difficulty in maintaining pure parental stocks that consistently produce 100% male offspring[16], (ii) poor spawning success[17] and (iii) incompatibility of breeders resulting in low fertility[18].

c) *Sex reversal by hormone treatment*

A monosex population can be developed through hormone-induced sex reversal by interfering with the sex-determining mechanism for half the population. For example, if a population of only males is desired, an androgen is administered through diet or water to a normal population of young fish; the genetic females are induced to develop as functional males but the genotypic males are apparently not affected and therefore develop normally as functional males[19]. Such hormo-

nally-induced sex reversal is permanent because the action of sex genes is believed to be restricted to a short period during the early development of gonads and subsequently latent or inactive after the onset of gonadal sex differentiation[20].

In tilapias, gonad is irreversibly differentiated during a specific period in a fry; hence the sex determination can be manipulated during the differentiation period between 10 and 20 days of posthatching in *O. mossambicus*[21,22]. By and large, this is perhaps the most practicable technique for the production of all-male tilapias. However, some authors have failed to obtain 100% sex reversal for one or the other of the following two reasons (table 2): (i) they have failed to synchronize the hormone treatment duration with that of gonadal differeniation[23]; and (ii) they have adopted a feeding regime, in which some fry in the last peck of the hierarchy order did not receive the effective minimum hormone-treated diet to ensure complete sex-reversal; in such individuals the gonadal differentiation proceeded only up to a point resulting in the production of intersex or even female[24].

The treatment of sexually plastic tilapia fry with androgens like methyltestosterone or ethynyltestosterone has repeatedly produced all-male population, but variations in the treatment conditions such as stocking density,

Table 2   *Confusing reports on the induced sex-reversal of O mossambicus with reference to dose and duration*

Dose of 17α-methyl-testosterone		Feeding rate	Duration and timing	Masculini- zation (%)	Remarks	Reference
(μg/g diet)	(μg/g fish/day)	(% of body weight/d)				
10	0.6	6	69 days 7–75th day	100	Abnormal opercle lower jaw & head	Clemens[25]
30	1.8	6	69 days 7–75th day	100	Abnormal opercle lower jaw & head	Clemens[25]
50	–	–	19 days 7–25th day	100	20% has ovarian cavity	Nakamura[22]
50*	1.5	3	30 days 7–36th day	100	–	Guerrero[23]
60	–	–	Fry (9–10 mm) for 42 days	100	–	Anonymous[43]
5*	–	30	10 days 10–20th day	100	–	Varadaraj[21]
20	1.2	6	69 days 7–75th day	95	Female	Clemens[25]
40	2.4	6	69 days 7–75th day	88	Only one sterile	Clemens[25]
50	3.0	6	69 days 7–75th day	81	–	Clemens[25]
1000	–	–	19 days 7–25th day	1.5	51% ♀♀	Nakamura[22]
1000	–	–	44 days 7–50th day		10% intersex	Nakamura[22]
30	0.9	3	14–28 days Fry 9–10 mm treated	69–98	–	Guerrero[23]
50	–	–	42 days Fry 9–19 mm	81–85	–	Anonymous[43]
30	3.0	10 ≈	30 days 11–14 mm fry	47	Promotes growth	Macintosh[24]
30	6.0	20	30 days 7–11 mm fry	79	Promotes growth	Macintosh[24]
500*	–	–	I –	high ratio of ♀♀	Feminization	Hackmann[44]

*indicates μg hormone per litre water in which fry were reared

temperature and duration have contributed to inconsistent results[19]. These observations clearly show that the desired hormonal action depends on the efficacy of the steroid as well as the dose, method of administration, and time and duration of treatment, all of which may vary in different species of tilapias[19]. Hitherto most investigators have arbitrarily chosen hormone dose, feeding level and duration (table 2). Available information on the induction of sex reversal through hormone treatment is also confusing; for instance Nakamura[22] claimed to have achieved 100% sex reversal but 20% of his animals—according to his own statement—have developed ovarian cavity. Clemens[25] also

claimed that supplementation of 10 or 30 μg methyltestosterone/g of the diet ensured 100% sex reversal but the diet containing 20 μg failed to induce 100% sex reversal. Thus, the idea of fixing the minimum effective methyltestosterone dose required for 100% sex reversal in tilapias was not even thought of so far.

In *O. mossambicus* an experiment was designed to study the interaction between feeding rate (10, 20 and 30% body weight/day) and hormone dose (5, 10, 20, 30 and 40 μg hormone/g diet). Table 3 shows that the minimum dose required for 100% masculinization shifted to lower levels when feeding rate was increased from 10 to 30% body weight/
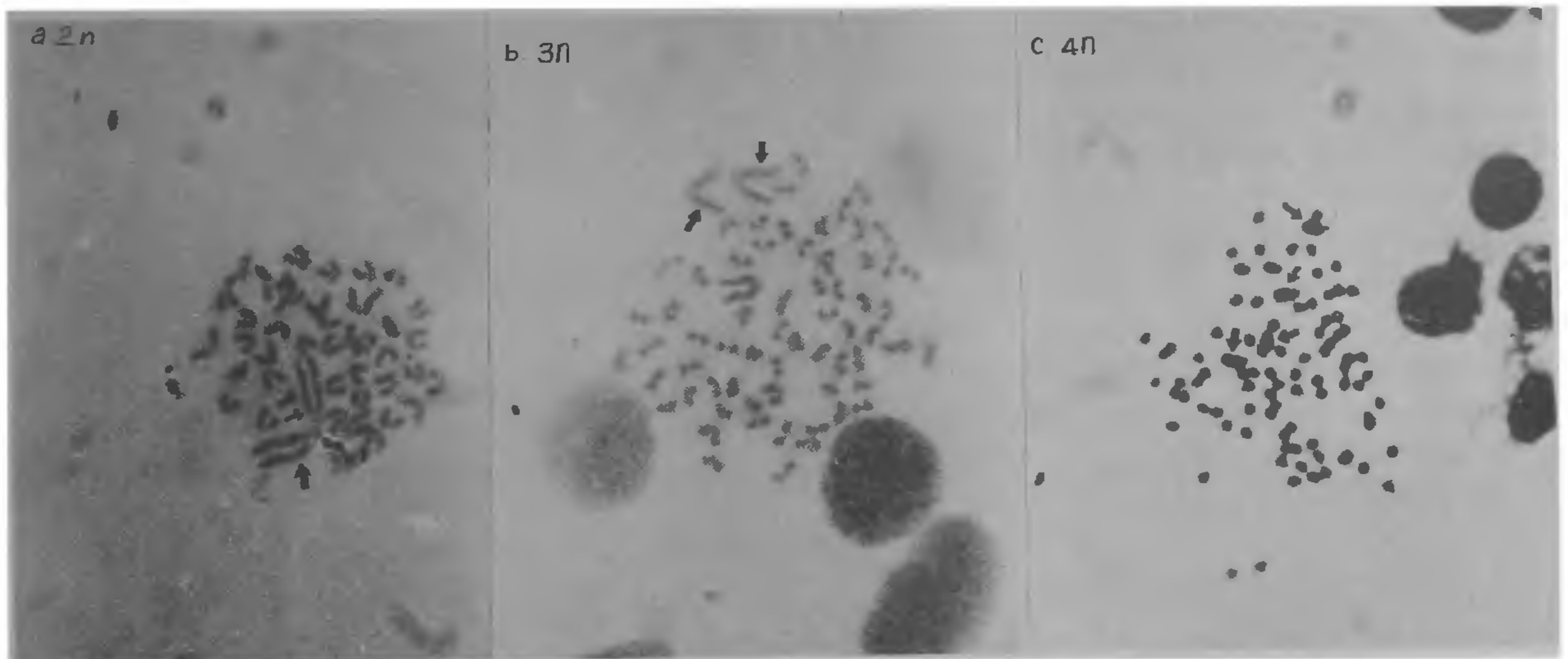
day. A calculation of the hormone uptake for the given food indicated that the feeding regimes. which ensure the uptake of a minimum of 1.5 μg hormone/g fish/day induced 100% masculinization (table 3). Incidentally the uptake of lower dose (< 1.5 μg/g fish/day) at the tested feeding regimes resulted in the production of some females and/or intersex, while the uptake of higher dose (8.0 μg/g fish/day) ensured 100% masculinization, but led to higher mortality (table 3); the observed mortality is due to the abnormal development of mouth. Stunted growth and malformation of liver have been observed in sex reversed tilapias, which were subjected to higher dose of 17α-methyltestosterone. Therefore, the growth of O. mossambicus of all the tested categories were followed for 45 days; the minimum effective dose not only induced 100% sex reversal but also ensured faster growth than the control; however, the group receiving higher dose grew remarkably slower. Evidently, the determination of the minimum effective dose for each tilapia species is an obligate requirement to ensure 100% masculinization and faster growth.

Table 3 shows that in all the groups receiving normal or hormone-treated food for a longer or shorter duration, size hierarchy was soon established. This is evident from the wide variations (see SD values) observed in the body weight of individuals belonging to any tested group. In most culture field programmes, it may therefore be difficult to ensure minimum feeding of the effective hormone dose for those, who are last in the social hierarchy; hence in high density culture system, differential consumption of the androgen-treated food, may lead to incomplete masculinization of some individuals 26–29.

To obviate these negative effects one may choose to administer the hormone through the medium; with ethanol added the steroid becomes soluble in water; however, the wrong choice of the steroid, dose and exposure duration may lead paradoxically to feminization (table 2). Noting these discrepancies, Balarin[5] pointed out that research is still needed to determine whether a certain percentage of female is genetically non-responsive to steroid, potency of steroid in water and chemical pathway of steroid activity. He also

Table 3 *Effects of oral administration of 17α-methyltestosterone (5, 10, 20, 30 and 40 μg/g diet) on sex differentiation of O. mossambicus. The experiments were commenced with 50 fry in each aquarium*[31 45]

Treatment dose (μg/g diet)	Feeding rate (% body weight/day)	Hormone consumed (μg/g fish/day)	Hormone consumed for 10 days (μg/fish)	Sex ratio ♀	Inter-sex	♂	Mortality (%)	Weight (mg) X̄ ±	SD
Control	10	0.0	0.00	0.4	0.0	0.6	8	381	092
5	10	0.5	0.08	0.3	0.1	0.6	4	525	116
10	10	1 0	0.18	0.1	0.2	0.7	6	568	097
20	10	2 0	0.39	0.0	0.0	1 0	14	620	102
30	10	3 0	0.50	0 0	0.0	1.0	14	659	115
40	10	4.0	0 66	0.0	0.0	1 0	16	682	120
Control	20	0.0	0.00	0.4	0.0	0.6	6	497	123
5	20	1.0	0.18	0.1	0.1	0.8	6	608	130
10	20	2.0	0.44	0.0	0 0	1.0	8	694	132
20	20	4.0	0 82	0 0	0.0	1.0	14	742	137
30	20	6.0	1.09	0 0	0 0	1.0	18	791	149
40	20	8.0	1.39	0.0	0 0	1.0	26	782	152
Control	30	0.0	0.00	0.4	0.0	0 6	4	586	138
5	30	1.5	0.31	0.0	0 0	1.0	4	849	179
10	30	3.0	0.69	0.0	0 0	1.0	4	1068	201
20	30	6.0	1.30	0 0	0.0	1.0	10	965	203
30	30	9 0	1.70	0 0	0 0	1 0	20	803	162
40	30	12.0	2.08	0.0	0 0	1.0	22	742	120

Figures a–c. Karyotype of *O. mossambicus.* a Diploid $2n = 44$; b triploid $3n = 66$; and c tetraploid $4n = 88$. Arrows indicate marker chromosomes ($\times 1000$).

indicated that it is necessary to look for an alternative technique to ensure 100% masculinization or to produce 100% sterile tilapia, as was originally aimed by Hickling[14]

d) *Chromosomal manipulation*

In several fish species, thermal shock is known to produce triploidy and tetraploidy[30], but it is not known whether these polyploids are produced due to the inhibition of second meiotic division or due to the retention of second polar body. However to achieve triploidy or tetraploidy, one has precisely to fix the time and duration as well as the desired temperature to effect cold or heat shock; these characteristics appear to vary from species to species. Pandian and Varadaraj[31] made a series of tests and determined that a heat shock at 42°C on 2.3 min-old (postinsemination) eggs for a period of 3 min induced triploidy; likewise a heat shock at 40°C on 40 min-old (postinsemination) eggs for a period of 10 min induced tetraploidy; thus it has been possible to produce triploid and the tetraploid *O. mossambicus.* To confirm that these heat shocks have resulted in triploidy or tetraploidy, they have adduced karyotypical evidence (figure 1).

Among tilapias triploidy has so far been reported in a single species[32]; when 4 min-old (postinsemination) eggs of *O. niloticus* were exposed to 40–41.5°C for 2.7 min, the shock produced triploids. Our observation on *O. mossambicus* shows that there is good scope for inducing triploidy in other tilapia species also. Valenti[33] claimed that it has been possible to obtain polyploidy (tetraploidy) in *Oreochromis aureus* by exposing the freshly inseminated eggs to 4°C for 15 min, 11°C for 60 min or 38°C for 60 min. He adduced increase in the nuclear volume of erythrocyte as a single evidence to confirm that the chosen thermal shocks have led to the production of polyploidy. The findings of Valenti are however questionable; at best, they indicate that the tilapias are amenable to thermal shock for the production of polypoids. At 29°C, freshly inseminated *O. mossambicus* eggs complete the first cleavage in a period of 65 min. *O. aureus* requires a period of 30 min to complete first cleavage[33] at 32°C; presumably the chosen cold (11 and 4°C) and heat (38°C) shock treatments of Valenti might have suppressed the first cleavage and led to the development of tetraploidic situation.

By mating tetraploid *O. mossambicus* with the diploid partner it is also possible to produce

triploidy. The technique adopted for the production of triploidy by the suppression of the second polar body extrusion or inhibition of second meiotic division cannot be practised by fish farmers, and this does not lead to consistently 100% sterile triploidy production. It appears that it may be easier to produce consistently 100% sterile triploidy by the fish farmers crossing the tetraploid and diploid *O. mossambicus*. Further work is under progress to perfect this technique and to observe the growth efficiency of triploid *O. mossambicus*.

ACKNOWLEDGEMENT

1. Bardach, J. E., (ed.) In: *Aquaculture*, Wiley-Interscience, New York, 1972, p. 352.
2. Roberts, R. J. and Sommerville, C., *The biology and culture of tilapias*, (eds) R. S. V. Pullin and R. H. Lowe-McCornell, ICLARM, Manila, 1982, p. 247.
3. Perez, J. E. and MaClean, N., *J. Fish Biol.*, 1975, **9**, 447.
4. Dusart, J., *Hydrobiologia.*, 1963, **21**, 328.
5. Balarin, J. D. and Haller, R. D. (eds), *Recent advances in aquaculture*, Croom Helm Publishers, Westwisen, 1983, p. 265.
6. Bowen, S. H., *The biology and culture of tilapias*, (eds) R. S. V. Pullin and R. H. Lowe-McConnell, ICLARM, Manila, 1982, p. 141.
7. Hickling, C. F., *Sci. Am.*, 1963, **208**, 143.
8. Huet, M., *Breeding and cultivation of fish*, Fishing New Books Ltd., London, 1970.
9. Mires, D., *Bamidgeh*, 1977, **29**, 94.
10. Meschkat, A., *FAO Fish. Rep.*, 1967, **44**, 411.
11. Guerrero, R. D. and Guerrero, L. A., *J. Biol.*, 1975, **4**, 129.
12. Vaas, K. F. and Hofsteda, A. E., *Inland Fish Res. Stn.*, 1952, **1**, 1.
13. Lovshin, L. L. and Da Silva, A. B., *FAO/CIFA Tech Pap.*, 1975, **4**, 548.
14. Hickling, C. F., *J. Genet.*, 1960, **57**, 1.
15. Hammerman, I. S. and Avtalion, R. R., *Theor. Appl. Genet.*, 1979, **55**, 177.
16. Pruginin, Y., Rothbard, S., Wohlfaith, G., Haberg, A., Moav, R. and Hulata, G., *Aquaculture*, 1975, **6**, 11.
17. Lee, J. C., *Ph.D. dissertation*, Auburn University, 1979, p. 84.
18. Lovshin, L. L., *Res. Dev. Ser.*, *Int. Cent. Aquaculture*, 1977, **14**, 1.
19. Shelton, W. L., Hopkins, K. D. and Jeryen, G. D., In: *Culture of exotic fishes symposium proceedings*, (eds) R. O. Smitherman, W. L. Shelton and J. H. Grover, Fish Culture Section, American Fisheries Society, Auburn, 1978, p. 10.
20. Yamamoto, T., *Fish physiology*, (eds) W. S. Hoar and D. J. Randall, Academic Press, New York, 1969, Vol. 3, p. 117.
21. Varadaraj, K. and Pandian, T. J., *Curr. Sci.*, 1986.
22. Nakamura, M., *Bull. Fac. Fish.*, *Hokkaido Univ.*, 1975, **26**, 99.
23. Guerrero, R. D. and Guerrero, L. A., *Inland Fisheries Project*, *Philippines*, *Tech. Rep.* 1976, **9**, 119.
24. Macintosh, D. J., Varghese, T. J. and Sathyanarayana Rao, G. P., *J. Fish Biol.*, 1985, **26**, 87.
25. Clemens, H. P. and Inslee, T., *Trans. Am. Fish. Soc.*, 1968, **97**, 18.
26. Meriwether, F. H. and Torrans, E. L., *The first Asian fisheries forum*, 25–31 May, 1986, Manila.
27. Goetz, F. W., Donaldson, E. M., Hunter, G. A. and Dye, H. M., *Aquaculture*, 1979, **17**, 167.
28. Van den Hurk, R. and Slof, G. H., *Cell Tissue Res.*, 1981, **218**, 487.
29. Shelton, W. L. and Jensen, G. L., *WRRI Bull.*, 1979, **39**, 1.
30. Chourrout, D., *Reprod. Nutr. Dev.*, 1980, **20**, 727.
31. Pandian, T. J. and Varadaraj, K., *Second International Symposium on Tilapia in Aquaculture*, Bangkok, 1987.
32. Chourrout, D. and Itskovich, J., *Proc. International Symposium on Tilapia in Aquaculture*, Nazareth, 1983, p. 246.
33. Valenti, R. J., *J. Fish Biol.*, 1975, **7**, 519.
34. Fishelson, L., *Fisherman's Bull.*, 1962, **4**, 14.
35. Pruginin, Y., *Rep. FAO/UNDP* (TA)., 1967, p. 16.
36. Lessent, P., *FAO Fish Rep.*, 1968, **44**, 148.
37. Welcomme, R. L., *Rep. E. Afr. Freshwat. Fish. Res. Org.* 1962/63–1964, p. 36.
38. Wohlfarth, G., *Proceeding International Sym-*

343

posium on Tilapias in Aquaculture, Nazaret, 1983, p. 559.

39. Whitehead, P. J. P., Nature (London), 1960, 183, 878.

40. Lee, J. C., Ph.D. Dissertation, Auburn University, 1979, p. 84.

41. Pruginin, Y., Rothbard, S. Wohlfarth, G., Haberg, A., Moav, R. and Hulata, G., Aquaculture, 1975, 6, 11.

42. Chen, T. P., Aquaculture practice in Taiwan, Fishing News Book Farnham, Surrey, England, 1976.

43. Anonymous, Chin. J. Zool., 1979, 1, 1.

44. Hackmann, E., Chin. J. Zool., 1974, 24, 44.

45. Varadaraj, K. and Pandian, T. J., Second International Symposium on Tilapias in Aquaculture, Bangkok, 1987.

ANNOUNCEMENTS

THE FIRST INDIAN FISHERIES FORUM

The Indian Branch of the Asian Fisheries Society has programmed to hold the First Indian Fisheries Forum at Mangalore from 6th to 10th December, 1987 to bring together scientists engaged in research, extension, education, development and industry in the fisheries sector from all over the country. The primary objective of the Forum is to take stock of the existing situation in different fisheries sectors and to formulate recommendations for follow-up action by the various concerned agencies.

Several technical sessions will be held. The topics covered are: Aquaculture; Fishery Biology and Population Dynamics; Fishery Hydrography (Oceanography, Marine Biology and Limnology); Fish Processing Technology, Fishery Engineering, Fishery Economics and Statistics, Fisheries Education and Training, Aquatic Pollution, Fisheries Extension and information Service.

Original research papers on the above subjects are invited. Each paper shall normally be limited to 8 to 10 typed pages of double spacing on quarto size bond paper in duplicate. References cited must follow the pattern adopted by the journal 'Aquaculture'.

Authors are requested to send abstracts of their papers in duplicate by 15th July 1987, along with the participation intimation form duly filled in. Each abstract should not exceed 250 words.

Ten awards will be given to young scientists of below 35 years of age as on 1.12.1987, on the basis of the merit of their papers and the method of presentation in the Forum. The intending competitors for these awards are required to intimate their desire to that effect while sending the abstracts of their papers. Only the sole author or the first author presenting the paper will be eligible for the award. Each awardee will receive a citation and a cash award.

Paper presented during the Forum will be published after necessary review by about the middle of 1988.

For details please contact: The Secretary, Asian Fisheries Society, Indian Branch c/o College of Fisheries, Mathsyanagar, Mangalore 575 002.

S S BHATNAGAR PRIZE FOR SCIENCE AND TECHNOLOGY FOR 1985-86

The above prize has been awarded to Dr Dilip Kumar Ganguly, Scientist, Head of the Division of Pharmacology and Experimental Therapeutics, Indian Institute of Chemical Biology, Calcutta. The prize has been awarded to Dr Ganguly's work on a chemical model of Parkinson disease. He has recently established that there is a "spinal involvement in the genesis of Parkinson tremor". Dr Ganguly is a founder-fellow of the Indian Academy of Neurosciences and has been the Vice-President of the same Academy.