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Triploidy induction in Nile tilapia, *Oreochromis niloticus* L. using pressure, heat and cold shocks

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Summary. The results of a study aimed at the identification of treatment optima for triploidy induction in recently fertilised *Oreochromis niloticus* L. eggs by altering the intensity, duration and timing of application of pressure, heat and cold shocks are reported. Preliminary, but not directly comparable, trials suggested the following treatments to be close to the individual agent optima. Pressure: 8,000 psi 2-min duration applied 9 min after fertilisation (a.f.); heat: 41 °C, 3.5-min duration applied 5 min a.f., cold: 9 °C, 30-min duration applied 7 min a.f. In a directly comparable trial in which the eggs of eight different females were separately exposed to the optimum shocks listed above, individual triploid yields were more variable following cold shocks and mean triploid yields were, therefore, higher following pressure and heat shock. These and other results obtained are presented and the light they shed on the timing of the second meiotic division in this species is discussed.

Key words: *Oreochromis* – Triploidy induction – Pressure shock – Heat shock – Cold shock

Introduction

Several agents, both physical and chemical, that interfere with the normal functioning of spindle apparatus during cell division, thereby leading to the production of polyploid individuals, have been applied to a variety of fish species in recent years (for review, see Chourrout 1987). Triploids are most commonly produced directly, i.e., by the induction of second polar body retention during the second meiotic division of recently fertilised eggs, but they may also be generated indirectly by the crossing of tetraploid and diploid individuals. Tetraploids may be produced by the suppression of the first mitotic division.

Triploid individuals are expected to be sterile because of a failure of homologous chromosomes to synapse correctly during the first meiotic division and therefore to be of benefit in fish culture, since maturation processes often have profound and, ultimately, limiting effects on growth. Because triploid females are expected to be both endocrinologically and functionally sterile, it is commonly suggested that triploid induction be coupled with sex inversion such that all-female, triploid stocks are produced and, in the case of salmonids, this option is being actively pursued (Bye and Lincoln 1986; Johnstone 1989).

Tilapias (*Oreochromis, Sarotherodon* and *Tilapia* spp.) are a group of fishes of major economic importance in tropical and subtropical countries, but their uncontrolled and prolific breeding at a small size in mixed sex culture constitutes a serious constraint on their efficient production. Although interspecific hybridisation of these species leading to all male stocks has been proposed as a possible solution to this problem (Hickling 1960), it has proved difficult to maintain in practice and has now been largely replaced worldwide by direct masculinisation using hormones (Guerrero and Guerrero 1988).

Production of expectedly sterile, triploid *Oreochromis* progeny by genome manipulation techniques using a variety of methods has attracted considerable previous attention (Valenti 1975; Chourrout and Itskovich 1983; Don and Avtalion 1986, 1988; Penman et al. 1987; Pandian and Varadaraj 1988, 1989). The potential of the commercial application of these techniques to replace the use of hormones in tilapia culture is obvious, but the recent nature of this work and the lack of follow-up trials makes this unlikely in the near future. The only published re-

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sults on gonadal maturation in triploid tilapia (Penman et al. 1987) suggest that the overall reduction in gonads in triploids of either sex may not be as great as that observed in salmonids.

In the present study, we aim to compare the characteristics of the various polyploid-inducing agents in a single species. The findings of a search for treatment optima for the production of *Oreochromis niloticus* triploids by exposing newly fertilised eggs to altered intensities, durations and timings of application of pressure, heat and cold shocks are reported. This is the first in a series of trials to identify treatment optima for ploidy manipulation in this species and employs, in this instance, perturbation of the meiotic division of newly fertilized eggs as an experimental model. In subsequent trials we hope to compare the performance of diploids with triploids thus produced, and to investigate the feasibility of interfering with the first mitotic division of *Oreochromis* eggs in order to produce tetraploids and, following fertilization with irradiated milt, gynogenetic individuals.

Materials and methods

Origin of fish stock

The fish used in this study were descended from an electrophoretically tested, pure *O. niloticus* stock originally obtained from a wild population of Lake Manzala, Egypt, in 1979 (McAndrew and Majumdar 1983). Sexually mature fish were maintained under a 12-h photoperiod and were transferred for breeding into a series of 120-l glass aquaria provided with a recirculated, aerated and temperature-controlled (28 ± 1 °C) water supply. A single male and female were accomodated in each tank but were kept seperate by a sheet of perspex. The fish were fed a commercial trout diet (Ewos Baker, Ltd.; 40% protein) at the rate of 2–3% body weight per day.

Artificial fertilisation

Sexually mature female *O. niloticus* spawn at approximately 14- to 20-day intervals under the experimental conditions described above. Readiness of females to spawn was ascertained by examining the degree of swelling of the urogenital papilla and by the prespawning behaviour of the fish. Ripe females were removed from the tanks and their eggs were obtained by manual stripping, as far as was possible, immediately after a first batch of eggs had been deposited. Once stripped, the fertilisability of *O. niloticus* falls rapidly, probably due to desiccation. Since, however, some experimental series were time-consuming, it was considered appropriate in some cases that eggs be obtained by sequental stripping over a period of up to 1 h. Preliminary data suggested that this method of obtaining the eggs resulted in uniformly high fertilisation rates. Fertilisation was carried out in vitro by mixing 0.1–0.2 ml of dry sperm per batch of eggs (ca. 100) followed by the addition of 10–20 ml of 28 ± 1 °C water. Control (untreated) eggs were taken frequently and reared separately for comparative purposes. A total of 39 female fishes was used in the optimization trials and some females were used more than once but in different experimental series; the superscripts in Tables 1, 2 and 3 show the number of females and is also equal to the number of replicates used for that particular treatment.

Triploid induction

After fertilisation, and when not being triploidised, eggs were at all times incubated at 28 ± 1 °C. Eggs were exposed to elevated hydrostatic pressure in a 1-l vessel kindly made available by Dr. A. G. MacDonald, Department of Physiology, University of Aberdeen, Scotland. Eggs to be pressurised were held in individual, uncapped vials and, after the vessel had been sealed and purged of air, pressure was applied by a manually operated water pump. The time taken to raise the pressure level from ambient to 8,000 psi was, typically, in the region of 30 s, with the passage from 8,000 to 10,000 psi taking a further 10 s. Pressure release was gradual (typically 30 s 9,000–0 psi). For the induction of heat and cold shocks, batches of eggs were transferred, in individual netting baskets, to a temperature-controlled, 50-l water bath regulated by a heater/stirrer (Gallenkamp, Ltd.).

Egg incubation

Fertilised, treated and control ova were identically incubated in a series of 750-ml, round-bottomed plastic jars connected to the water system referred to above. Additionally, the water was sterilised by exposure to UV light (ca. 62,000 μW s/cm^2) and there was a provision made to control the water flow so as to ensure gentle movement of the eggs at all times (Rana 1986). The survival rate in each group was checked at three development stages, namely, morula: 6–8 h after fertilisation (a.f.); pigmentation: 45–50 h a.f.; and hatching: 80–90 h a.f.. The numbers of normal and deformed fry at hatch were also recorded.

Karyological examination

Karyotypes were prepared from newly hatched or 1-day-old larvae following slight modifications of published methods (Kligerman and Bloom 1977; Chourrout and Itskovich 1983; Don and Avtalion 1986). Embryos were placed in petri dishes containing 0.002–0.005% colchicine solution (Sigma, Ltd.) for 4–6 h at 28 ± 1 °C. The yolk sac and head tissues were removed in chilled 0.7% NaCl, and the remaining embryonic tissues were transferred to distilled water (hypotonic solution) for 8–12 min before being fixed in 4:1 methanol: acetic acid (two changes). Dissociation of epithelial cells was carried out by placing the tissues, after removal of excess fixative by blotting, in a cavity of a perspex slide with 2–3 drops of 60% glacial acetic acid, followed by mincing for 1 min with a glass rod. After 15–20 min, the cell suspension was dropped from a height of 30–40 cm onto a glass slide on a warmed hot plate (44–48 °C) and the excess was withdrawn within 8–12 s, using a single microhaematocrit dropper. Slides were then stained with freshly prepared, 10% Giemsa in 0.01 *M* phosphate buffer (pH 7) for 15–20 min. *O. niloticus* triploids are characterised by the presence of three rather than two large, easily identified, submetacentric chromosomes (Fig. 1). The triploid rate was determined by the karyotypic analysis of several (>3) karyotypes per individual and 15–20 individuals per treatment. The triploid yield of each batch was the product of the survival rate to hatch (relative to the control adjusted to 100%) and the observed triploid rate, and was expressed as a percentage of the eggs originally present.

Experimental design

In the search for treatment optima, it was initially decided that attention should be focussed on performing particular experimental series that would indicate the desired future directions in which to move. Thus, in the case of pressure, having first established the optimum time after fertilisation for shock initiation at one presumed effective intensity (8,000 psi) and duration (2 min), we moved towards establishing the most effective intensity and duration of shock using several females. Since, for

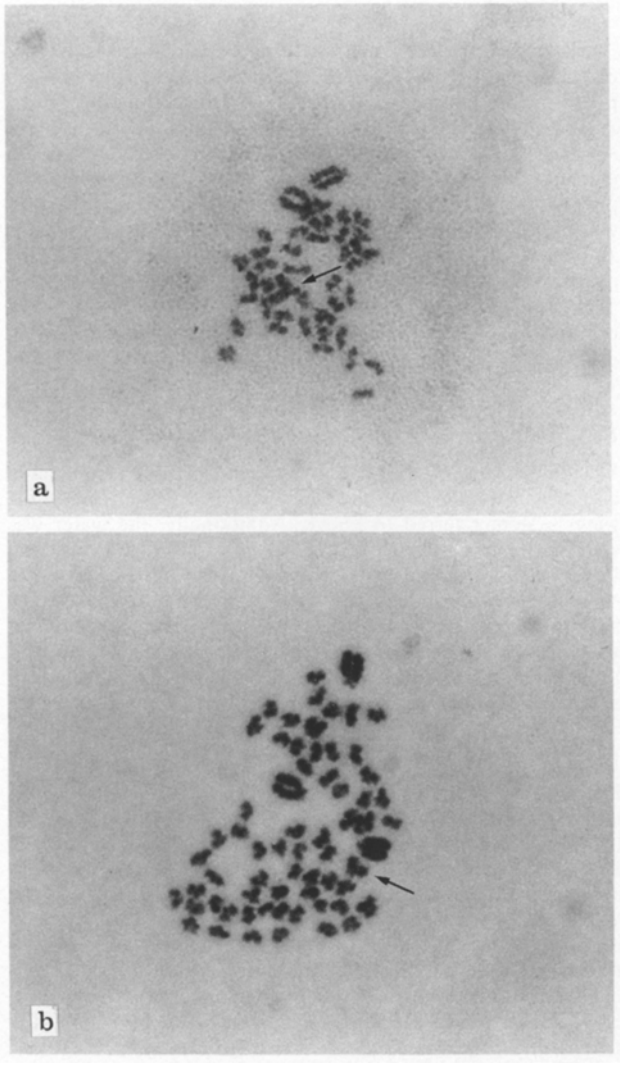

Fig. 1 a–b. Metaphase chromosomes of *O. niloticus*, **a** diploid ($2n=44$) spread, **b** triploid ($3n=66$) spread. Overlapping chromosomes are indicated by *arrows*

pressure, the change from one intensity level to another was not greatly rate-limiting to the performance of the trials, it was possible, in any one working day and without prejudice to the condition of the eggs (whether stripped or not) to test all the variables of importance. This was adjudged not to be the case for the temperature shock experiments, since it took some considerable time to ensure that the temperature was sufficiently stabilised at any new working level. Thus, different temperature trials were conducted on different days using, necessarily, different females. The extent to which trials conducted on different days using different females can be compared rests on the degree of difference in response between different females to each agent and although, in the presentation of our results on the search for treatment optima, triploid rate and triploid yield figures are presented relative to the appropriate control values in order to better facilitate the association of results from different trials involving different fish, we would urge a measure of caution in their detailed comparison. Furthermore, results are presented as means together with their standard errors, primarily in order to indicate the degree of difference between trials involving different females rather than to suggest that data of this sort are normally distributed.

It was subsequently decided that the extent of any interindividual variation in response should be the subject of a separate experiment, once apparent optima had been identified. To do this, the eggs of eight different females were taken, fertilised and then divided into four lots. Three groups were taken and separately exposed to the preferred pressure, heat and cold shocks, as identified in the earlier part of this study, the fourth group being maintained as a control. In this comparative trial essentially similar, but necessarily different, numbers of eggs (80–200), were taken for treatment from the different females. Because of this and because the data were in the form of proportions, the results were analysed by an analysis of variance using a generalised linear model with a logit link function, the survival rates being assumed to have a binomial distribution. Furthermore, because the survival rates were observed to vary according to treatment, they were weighted during the analysis.

Results and discussion

It is convenient to begin by first discussing the results of those experiments conducted using high hydrostatic pressure. This is appropriate since, because no changes in temperature were involved in their execution, they can be considered as being the most informative about the normal timing of the events of the second meiotic division at the temperature used (28 °C). The full list of experiments conducted and the results obtained are given in Table 1. The averaged results, showing the effects on triploid yields of altering pressure level and the timing of application of 2-min duration shocks, are presented in a more convenient format in Fig. 2. All levels of 2-min duration pressure shock were most effective when given in the period 8–10 min after fertilisation at 28 °C; earlier (5–7 min a.f.) administration of 7,000 and 8,000 psi shocks resulted in a few deformed survivors. Later (11–12 min a.f.) applications of shock resulted in lowered triploid rates at all pressures. Lower levels of shock (7,000–7,500 psi) administered at 8–9 min a.f. resulted in generally lowered triploid yields because triploid rates were lower. At higher pressures (9,000–10,000 psi), triploid yields were once again reduced but, in this case, it was a consequence of both lowered rates and fewer animals surviving the treatments. The optimum duration of pressure shock at the effective intermediate pressure (8,000 psi) was 2 min (Table 1); shocks shorter than this were less effective because they produced fewer triploids, and longer shocks were less effective because they allowed fewer survivors.

These results are consistent with the hypothesis that the meiotic spindle apparatus is susceptible to disturbance during a very narrow window of time, with a width of approximately 2 min, between 8–10 min a.f. at 28 °C. Later treatments, though allowing survival, are presumably ineffective in inducing triploidy because second meiotic division has been completed. Treatment optima

Table 1. The effects of different intensities, durations and timings of application of pressure shock on triploid rates and triploid yields in *O. niloticus*

Pressure level (psi)	Time (min a.f.)	Shock dur^n (min)	Survival rate to hatch (% RC$^\#$)	Triploid rate (% ± SE)	Triploid yield (% ± SE)
7,000	5[a]	2	0	0	0
	6[a]	2	11.4 ± 1.8	0	0
	7[a]	2	26.7 ± 4.2	0	0
	8[b]	2	92.8 ± 4.7	41.7 ± 13.0	38.7 ± 13.6
	9[b]	2	83.2 ± 4.5	55.6 ± 4.5	46.2 ± 5.0
	10[a]	2	91.7 ± 4.3	55.0 ± 3.5	50.4 ± 5.6
	11[a]	2	94.4 ± 0.6	12.6 ± 1.0	11.9 ± 1.1
	12[a]	2	75.9 ± 9.1	0	0
7,500	8[b]	2	78.7 ± 7.4	67.6 ± 11.4	53.2 ± 13.2
	9[b]	2	86.9 ± 2.2	80.3 ± 3.3	69.8 ± 4.6
	10[a]	2	81.5 ± 7.0	70.0 ± 7.0	57.0 ± 0.9
	11[a]	2	80.2 ± 6.0	15.1 ± 0.6	12.1 ± 0.4
	12[a]	2	70.6 ± 16.5	5.0 ± 3.5	3.5 ± 2.5
8,000	5[a]	2	0	0	0
	6[a]	2	0	0	0
	7[a]	2	0	0	0
	8[c]	2	87.5 ± 5.5	100	87.5 ± 5.5
	9[b]	1	79.6 ± 8.8	0	0
	9[b]	1.5	77.2 ± 12.0	61.3 ± 5.0	47.3 ± 9.4
	9[c]	2	91.8 ± 2.1	100	91.8 ± 2.1
	9[b]	2.5	64.4 ± 16.9	100	64.4 ± 16.9
	9[b]	3	43.8 ± 10.6	100	43.8 ± 10.6
	9[b]	4	14.0 ± 10.6	66.7 ± 27.2	9.3 ± 10.0
	10[b]	2	78.8 ± 3.5	62.2 ± 5.0	49.0 ± 5.9
	11[b]	2	85.6 ± 3.5	32.6 ± 5.7	27.9 ± 5.7
	12[b]	2	73.6 ± 8.9	4.1 ± 3.4	3.0 ± 2.4
8,500	8[b]	2	78.0 ± 10.3	100	78.0 ± 10.3
	9[b]	2	76.2 ± 3.6	100	76.2 ± 3.6
	10[a]	2	72.6 ± 6.9	62.5 ± 8.8	45.4 ± 7.0
	11[a]	2	73.3 ± 6.7	30.0 ± 3.2	22.0 ± 4.6
	12[a]	2	55.2 ± 1.1	0	0
9,000	8[b]	2	52.0 ± 14.6	100	52.0 ± 14.6
	9[b]	2	53.2 ± 8.8	100	53.2 ± 8.8
	10[a]	2	57.3 ± 2.1	32.8 ± 3.3	18.8 ± 3.9
	11[a]	2	63.3 ± 5.0	25.0 ± 5.8	15.8 ± 5.0
	12[a]	2	50.5 ± 2.0	0	0
10,000	8[a]	2	34.0 ± 6.5	43.3 ± 9.8	14.7 ± 6.0
	9[a]	2	37.8 ± 1.4	27.2 ± 5.5	10.3 ± 2.5
	10[a]	2	55.1 ± 7.8	17.5 ± 5.3	9.6 ± 1.6
	11[a]	2	75.6 ± 2.3	5.5 ± 3.9	4.2 ± 3.0
	12[a]	2	57.4 ± 13.5	0	0

Experiments were conducted using [a] 2, [b] 3 or [c] 4 different females
RC$^\#$ Relative to control after adjustment of the latter to 100%
Mean survival rate in controls was 75.8 ± 2.0% (n = 23)

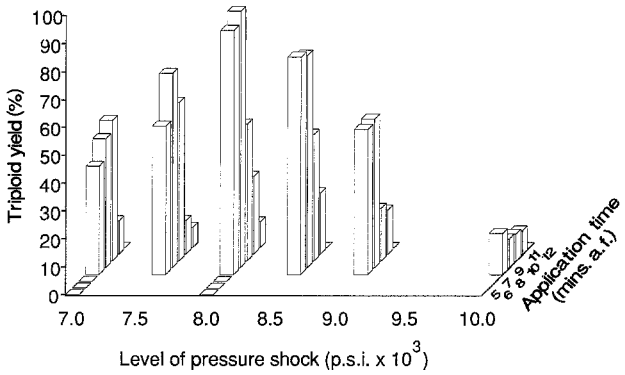

Fig. 2. The effect of different intensities and times of application of 2-min duration pressure shocks on triploid yield in *O. niloticus*

can be considered as being on a plateau, the slopes of which it is necessary to "climb" or to avoid "sliding down" in order to have the most consistent effect. A closer inspection of the triploid yield variance figures suggests that, as might be expected if such a scenario were true, variability was greatest in those trials which, in retrospect, might be considered suboptimal, i.e. in which shocks were given too early or too late or at undesirable durations or intensities.

The only other report on ploidy manipulation using hydrostatic pressure in an *Oreochromis* species is the study on tetraploid induction by Myers (1986). He reported that pressures in the region of 6,500–7,500 psi i.e. lower than those shown to be maximally effective here at inducing triploidy, were effective, when used in combination with low temperatures (7.5 °C), in suppressing the first mitotic division. Although we will later show that this temperature of cold shock is effective when of sufficient duration to induce triploidy by itself, the duration of 7.5 °C shock used by Myers is shorter than that reported by us to be maximally effective. This may mean that the slight difference in pressure ranges reported by these two studies is the result of pressure and cold shock acting synergistically in the earlier study, rather than that the optima for interference with the meiotic event are intrinsically different from those necessary to disturb mitotic division processes.

The results of the heat shock trials are presented in detail in Table 2. Averaged results from all fish showing the effects on yields of alterations in temperature and duration of heat shocks applied 5 min a.f. are presented graphically in Fig. 3. Heat shocks below 40 °C applied at this timing, even those of relatively long duration, were largely without effect on survival rates but were ineffective in inducing triploidy. Increased temperatures of heat shock (40–42 °C) were effective in inducing triploidy but, as the temperature of heat shock was increased, the maximally effective duration of heat shock appeared to become shorter. Thus, at 40 °C the most effective duration of shock, administered 5 min a.f., was between 5 and 6 min, whilst at 41 and 42 °C this duration fell to 3–4 and 3 min, respectively. At the highest temperature, the width of the window in which triploid yields could be produced at high levels was also apparently reduced. Heat shocks of 3.5-min duration at 41 °C were the most effective in inducing high triploid rates at high triploid yields when

Table 2. The effects of different intensities, durations and timings of application of heat shock on triploid rates and triploid yields in *O. niloticus*

Temp. of shock (±0.2°C)	Time (min a.f.)	Shock dur" (min)	Survival rate to hatch (%RC#)	Triploid rate (%±SE)	Triploid yield (%±SE)
37	5[a]	4	99.3±0.3	0	0
	5[a]	5	94.1±1.8	0	0
	5[a]	6	91.5±3.2	0	0
	5[a]	7	90.8±0.2	0	0
	5[a]	8	91.3±1.0	0	0
	5[a]	10	90.2±1.7	0	0
38	5[a]	3	93.4±3.8	0	0
	5[a]	4	94.3±2.7	0	0
	5[a]	5	98.9±0.4	0	0
	5[a]	6	93.0±1.7	0	0
	5[a]	7	81.8±4.3	0	0
	5[a]	8	87.7±2.6	0	0
39	5[a]	3	82.8±1.2	0	0
	5[a]	4	91.6±2.0	0	0
	5[a]	5	91.7±1.4	0	0
	5[a]	6	89.6±1.7	0	0
	5[a]	7	93.1±3.3	0	0
40	5[a]	2	94.9±2.0	0	0
	5[a]	3	83.9±7.6	0	0
	5[a]	4	84.5±1.7	57.8±1.5	48.8±2.3
	5[a]	5	83.8±2.2	70.8±2.9	59.3±4.0
	5[a]	6	81.8±4.2	79.1±2.9	64.7±3.0
41	5[b]	2	36.4±0.4	58.9±3.9	21.4±1.2
	5[b]	2.5	49.0±3.1	72.2±3.2	35.4±0.7
	5[b]	3	72.3±2.2	100	72.3±2.2
	2[b]	3.5	60.0±5.0	54.2±5.8	32.5±5.6
	3[b]	3.5	60.7±5.8	67.1±7.2	40.7±7.4
	4[b]	3.5	69.6±6.4	92.7±2.8	64.5±4.2
	5[b]	3.5	82.1±5.3	100	82.1±5.3
	6[b]	3.5	85.7±4.7	72.6±6.8	62.2±9.2
	7[b]	3.5	83.4±1.8	23.1±5.3	19.3±4.2
	5[b]	4	78.8±0.7	100	78.8±0.7
	5[b]	4.5	61.9±5.5	100	61.9±5.5
	5[b]	5	43.6±5.4	96.7±2.7	42.2±4.9
	5[b]	7	0	0	0
42	5[a]	2	38.6±3.4	13.0±2.1	5.0±1.3
	5[a]	3	57.4±0.4	100	57.4±0.4
	5[a]	4	9.8±2.1	90.0±3.5	8.8±2.7
	5[a]	5	0	0	0

Experiments were conducted using [a]2 or [b]3 different females
RC# Relative to controls after adjustment of the latter to 100%
Mean survival rate in controls was 84.4±1.9% ($n=16$)

delivered 5 min a.f. (Table 2). Earlier timings of administration of this shock caused generally lowered survival and triploid rates and therefore lowered triploid yields. Some of the animals that survived early application of heat shock were aneuploid (hyperdiploid or hypotriploid), and this presumably explains the greater lethality of both early administered heat and pressure shocks. Triploid rate declined sharply with later (6–7 min a.f.) applications of heat shock.

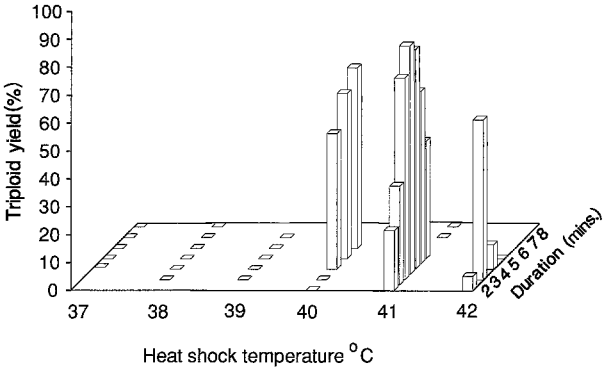

Fig. 3. The effect of different temperatures and durations of heat shock on triploid yield in *O. niloticus* when administered at 5 min after fertilisation

Our results on heat shock administration of triploids are essentially similar to those of Chourrout and Itskovich (1983) and Penman et al. (1987), but are slightly different from those of Don and Avtalion (1986), who advocated the use of a lower temperature (40.5 °C) shock of 3.4–4 min duration initiated earlier (3.5 min a.f.). Heat shocks initiated this early performed poorly in the present trials. In summary, it appears that the most effective heat shocks must be administered earlier in the post fertilisation period than the most effective pressure shocks. This is consistent with the hypothesis advanced above to account for the differential susceptibility to pressure shock treatmets except that, because we conclude that increased levels of heat have the effect of advancing the rate of all biological processes, the timing of effective administration of heat shock is earlier, and the width of the effective window for interference is narrower, than for correspondingly effective pressure shocks. A picture therefore emerges, in the case of heat shock exposure, wherein there are conflicting consequences of treatment. Heat shock tends to accelerate eggs through a window in which it is necessary for them to be retained in order to have maximal effect. Essentially similar results and a similar hypothesis have been advanced to explain the effects of heat shock generation of triploids in Atlantic salmon (Johnstone 1989).

The results of those trials performed using cold shock treatments are detailed in Table 3 and the averaged results of the effects on triploid yield of alteration in temperature and duration of cold shocks administered at 7 min a.f. are presented in a convenient format in Fig. 4. Triploids were produced by all temperatures of cold shock in the range of 7–15 °C, the most effective duration of shock being 20–40 min. At 11 °C the most effective time of administration of a 40-min duration shock was 7 min a.f. (Table 3). Triploid yields were apparently highest after the application of 30-min duration 9 °C shock 7 min a.f. Although triploids were induced by the application of lower temperature shocks (7 °C), lowered

Table 3. The effects of different intensities, durations and timings of application of cod shock on triploid rates and triploid yields in *O. niloticus*

Temp. of shock (±0.2°C)	Time (min a.f.)	Shock dur^n (min)	Survival rate to hatch (% RC$^{\#}$)	Triploid rate (% ± SE)	Triploid yield (% ± SE)
7	7[a]	20	87.7± 8.5	72.5±1.7	63.6± 4.6
	7[a]	30	69.2± 5.8	100	69.2± 5.8
	7[a]	40	76.1±15.8	100	53.8±15.8
	7[a]	50	67.5± 8.0	60.0±7.0	40.5± 0.1
9	7[b]	20	96.0± 1.3	86.5±2.8	83.0± 3.6
	7[b]	30	85.7± 9.7	100	85.7± 9.7
	7[b]	40	65.1± 4.4	100	65.1± 4.4
	7[b]	50	52.1± 5.8	100	52.1± 5.8
11	5[a]	30	91.8± 0.1	25.0±3.5	22.9± 4.0
	5[a]	60	3.4± 0.3	0	0
	6[a]	30	98.5± 0.4	68.3±1.1	67.3± 0.9
	6[a]	40	73.6± 5.0	72.3±4.0	53.2± 6.6
	7[b]	20	87.5± 5.3	23.3±3.6	20.4± 2.7
	7[b]	30	85.0± 0.3	84.2±1.8	71.5± 1.2
	7[b]	40	86.5± 4.8	97.0±2.7	83.9± 4.0
	7[b]	50	51.5± 3.1	83.3±3.6	42.9± 4.1
	7[b]	60	0.5± 0.3	0	0
	8[a]	20	91.6± 5.2	0	0
	8[a]	30	90.2± 0.9	31.3±4.4	28.2± 4.3
	8[a]	40	78.1±12.0	55.0±5.5	42.9± 9.3
	9[a]	30	17.3± 2.3	18.3±1.1	3.2± 0.2
	9[a]	60	3.6± 2.5	0	0
	11[a]	30	45.3± 7.4	0	0
	11[a]	60	17.1±11.2	0	0
15	7[a]	20	82.3± 3.9	0	0
	7[a]	30	56.4± 7.5	16.3±2.6	9.2± 2.7
	7[a]	40	61.6± 1.2	58.3±5.9	35.9± 2.0
	7[a]	50	41.8± 6.6	63.3±2.3	26.5± 5.2

Experiments were conducted using [a]2 or [b]3 different females
RC$^{\#}$ Relative to control after adjustment of the latter to 100%
Mean survival rate in controls was 78.4±2.3% (n=13)

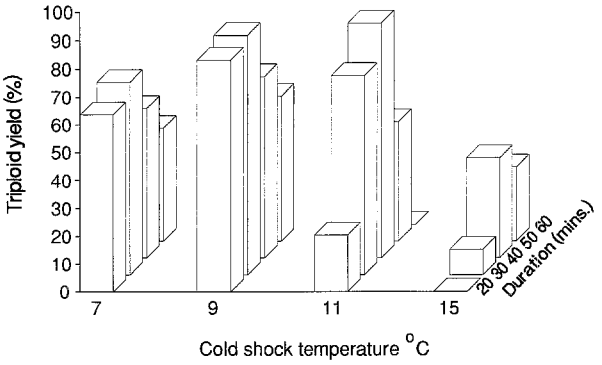

Fig. 4. The effect of different temperatures and durations of cold shock on triploid in *O. niloticus* when adminstered at 7 min after fertilisation

triploid yields were associated with increased numbers of deformed animals at this temperature. Higher temperature cold shocks (15°C) were generally less effective in inducing triploidy.

Previous work on cold shock induction of *Oreochromis* species (Valenti 1975; Don and Avtalion 1988) has been less comprehensive than the present study, using only one temperature (11°C), duration (60 min), and timing of application of shock (5 min a.f.). This particular shock performed poorly in the present trials.

We conclude that cold shocks, because they cause a slowing down of the rate of the meiotic division events, have necessarily to be applied for a longer period than is necessary for either heat or pressure, but must be initiated at approximately similar timing as pressure treatments in order to be effective. Closer inspection of the results suggests that the timing of initiation of cold shock should be slightly earlier than an equivalent pressure shock. This may, if true, indicate that these agents have different lag times before exerting their effects.

Comprehensive though we believe the above trials to be, it is yet possible to argue that far more extensive trials are required to properly identify the nature of response to the various agents under discussion. Thus, we have concluded that for all agents, not only is duration but also the intensity and timing of exposure of importance in maximising yields. At elevated intensities of treatment we believe the present results show that shorter durations of exposure and alternative timings are indicated. It appears likely, therefore, that rather than there being one treatment optimum for a given agent, there are more likely to be families of treatment optima comprising exposures of different intensities and duration, each having a particular timing for best effect, but perhaps not differing substantially in yield. Thus, in the context of the present study we might argue, although the experiment was not performed, that a pressure shock of shorter duration (e.g. 1 min) of 10,000 psi administered at 8 min a.f. might have resulted in similar triploid yields as the apparently preferred 8,000 psi shock of 2-min duration that we have reported. Similarly, a lower intensity of heat shock (e.g. 40°C of 6-min duration) might need to be administered earlier for maximal effect (e.g. 4 min a.f.) than was actually performed.

Clearly, however, there is a point at which it is counterproductive to continue this search for treatment optima. It is arguably more important to outline the general nature of response to different agents and to contrive, as we have attempted to do, to reduce them to a common understanding which might be of use in the identification of preferred options. It is also arguably more important to identify any interindividual difference in response to particular treatments, and this was the rationale behind the second part of this study, where the apparently preferred agent optima were applied in a directly comparable manner to the eggs of eight different females (Table 4). As expected, all animals analysed (ten per treatment

Table 4. The control survival rates and comparative triploid yields following the exposure of eggs from eight different *O. niloticus* females to pressure, heat and cold shocks[a]

Fish no.	Control survival rate (%)	Triploid yield (% RC#)		
		Pressure	Heat	Cold
1	94.0	91.5	68.6	90.6
2	91.5	67.0	67.1	13.6
3	76.3	85.8	90.9	3.9
4	93.3	74.2	69.4	20.7
5	85.8	83.9	75.7	81.6
6	82.0	93.3	86.3	61.6
7	91.7	84.0	69.0	21.6
8	76.4	98.9	35.5	4.0
Mean ± SE	86.4 ± 2.4	84.8 ± 3.4	70.3 ± 5.5	37.2 ± 11.6

[a] The shocks used were as follows:
Pressure: 8,000 psi, 2-min duration applied 9 min a.f.
Heat: 41 °C, 3.5-min duration applied 5 min a.f.
Cold: 9 °C, 30 min duration applied 7 min a.f.
RC# Relative to control after adjustment of survival rates in the latter to 100%. Triploid rates following all treatments were 100% ($n = 10$)

group) were made triploid by these optimised shocks and the difference between agents related, therefore, to variability in the survival rates. Mean triploid yields following pressure shock were significantly greater ($P < 0.05$) than those seen after cold shock, since interindividual differences in response were marked following exposure to the latter agent. Although, in most instances, individual yield values were greater after exposure to pressure than after exposure to heat treatments, mean yields following heat shock were not significantly different from either pressure or cold at this level ($P < 0.05$).

A somewhat similar situation following temperature shock for the induction of triplods in the use of heat to produce triploid Atlantic salmon has been reported where, because of extreme variability in response to the same heat shock treatment of eggs from different females, pressure shock is the preferred method of triploidisation (Johnstone 1989). These species differences in response to temperature may be the consequences of specific temperature adaptations or may relate to temperature equilibration differences.

It might be presumed that the preferred method of ploidy manipulation in any species would be that which was most specific in it's action on the spindle apparatus, as well as that which conformed most closely to having a "square wave" effect at exposure. Since heat shocks, by accelerating development, have the effect of narrowing the window of opportunity for triploidisation, making timing of shock application more critical and because interindiviual response to cold shocks is apparently greater, we conclude that the present results demonstrate that pressure treatments come closest to this ideal for the generation of *O. niloticus* triploids.

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References

Bye VJ, Lincoln RF (1986) Commercial methods for the control of sexual maturation in rainbow trout (*Salmo gairdneri* R.). Aquaculture 57:299–309

Chourrout D (1987) Genetic manipulation in fish: reviews and methods. In: Tiews L (ed) Proc World Symp on Selection, Hybridization and Genetic Engineering in Aquaculture, Bordeaux, 27–30 May 1986, II:111–126

Chourrout D, Itskovich J (1983) Three manipulations permitted by artificial insemination in tilapia: induced diploid gynogenesis, production of all-triploid populations and intergeneric hybridization. In: Fishelson L, Yaron Z (eds) Int Symp on Tilapia in Aquaculture, Nazareth, Israel, pp 246–255

Don J, Avtalion RR (1986) The induction of triploidy in *Oreochromis aureus* by heat shock. Theor Appl Genet 72:186–192

Don J, Avtalion RR (1988) Comparative study on the induction of triploidy in tilapias using cold and heat shock techniques. J Fish Biol 32:665–672

Guerrero RD III, Guerrero LA (1988) Feasibility of commercial production of sex reversed Nile tilapia fingerlings in The Philippines. In: Pullin RSV, Bhukasawan T, Tonguthai K, Maclean JL (eds) 2nd Int Symp on Tilapia in Aquaculture, ICLARM Conf Proc 15, Dept of Fisheries, Bangkok, and ICLARM, Manila, pp 183–186

Hickling CF (1960) The malacca hybrids. J Genet 57:1–10

Johnstone R (1985) Induction of triploidy in Atlantic salmon by heat shock. Aquaculture 49:133–139

Johnstone R (1989) Maturity control in Atlantic salmon – a review of the current status of research in Scotland. In: Symp on Applications of Endocrinology in Aquaculture, Malaga, Spain

Kligerman AD, Bloom SE (1977) Rapid chromosome preparations from solid tissues of fishes. J Fish Res Board Can 34:249–261

McAndrew BJ, Majumdar KC (1983) Tilapia stock identification using electrophoretic markers. Aquaculture 30:249–261

Myers JM (1986) Tetraploid induction in *Oreochromis* spp. Aquaculture 57:281–287

Pandian TJ, Varadaraj K (1988) Techniques for producing all male and all triploid *Oreochromis mossambicus*. In: Pullin RSV, Bhukasawan T, Tonguthai K, Maclean JL (eds) 2nd Int Symp on Tilapia in Aquaculture, ICLARM Conf Proc 15, Dept of Fisheries, Bangkok, and ICLARM, Manila, pp 243–249

Pandian TJ, Varadaraj K (1989) Sterile female triploid in *Oreochromis mossambicus*. In: Proc Aquaculture Int Congress, Vancouver, Canada

Penman DJ, Skibinski DOF, Beardmore JA (1987) Survival, growth and maturity in triploid tilapia. In: Tiews K (ed) Proc World Symp on Selection, hybridization and Genetic Engineering in Aquaculture, Bordeaux, 27–30 May 1986, II:277–287

Rana KJ (1986) An evaluation of two types of containers for the artificial incubation of *Oreochromis* eggs. Aquaculture Fish Manage 17:139–145

Valenti RJ (1975) Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatments. J Fish Biol 7:519–528