

Rapid Technique of Sex Reversal of Commercial Fish using Various Pulse Waveforms

The present study employs various transient electric fields of square pulses, exponential decay and combination of square pulses with arbitrary mark-space ratio (pulse width: negative half of period) to enhance sex reversal of the commercially important freshwater and marine fish in suspensions for monosex-male. The eggs of Nile tilapia (*Oreochromis niloticus* L.), blue-spotted coral trout (*Plectropomus leopardus*) and cobia (*Rachycentron canadum*) obtained from our parent breeding stocks were carefully selected for electrical inductions in pulsed-electric field strengths of 0.25-87.50 kVm⁻¹ generated between narrow plate electrodes. The prototype of the electrode equipped with the sequential signal pulse-generator (SPG) for on-site inductions was fabricated which could induce 2,000 eggs/SPG unit at one time (to be patent). The electroporation medium (EPM) of tilapia was prepared using HEPES buffer with a minimized concentration of the androgen hormone of 17alpha-methyltestosterone (MT) and the medium for the marine fish contained PBS buffered (1M mannitol added to make the EPM medium isotonic to seawater, pH 7.5 adjusted with NaOH) with the minimized 1,500 µg.l⁻¹ MT (to be patent). We experimentally optimized electroporation by adjusting the number of square wave pulses, pulse durations and the mark-space ratio. For tilapia, we found that 5 square wave pulses, 50 µs pulse durations with 1:1 mark-space ratio were the optimized conditions for sex reversal rate with the maximum values of 87.70%±15.13% (mean ± SD) hatching and 73.04%±6.15% (mean ± SD) survival rate. All male sex reversal rates of tilapia eggs treated with 1,500 µg.l⁻¹ MT were achieved at 81.25%±1.13% with the induced voltage of 375 VDC. For the marine fish, 3 square-wave pulses of 50 µs duration with 2:1 mark-space ratio and 350-420 VDC were the optimized with sex reversal rate of 66.45%-72.51% (n=450), less than 30% egg death. It should be noted that stages of egg development (day post fertilization: dpf) during zygote (1dpf)-cleavage-blastula-segmentation (2dpf)-pharyngula (3dpf) could be employed for electrical sex reversal but pharyngula was the optimized phase. The prototype of the electrode equipped with the controlled SPG for on-site inductions could operate within 15 min for each induction with the rapid time consumption.

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