

An efficient strategy for blocking the 1st mitotic cleavage of fish zygote using combined thermal treatment, exemplified by mud loach (*Misgurnus mizolepis*)

Yoon Kwon Nam^{a, *}, Geyong Cheol Choi^b and Dong Soo Kim^a

^a*Department of Aquaculture, Pukyong National University, Busan 608-737*

^b*Chung-Buk Inland Fisheries Research Institute, Chung-ju 380-250*

Blocking the first mitotic cleavage of the zygote is a key tool for chromosome-set manipulations in fish. We developed an improved method for inducing tetraploidy by blocking the mitosis with a combination of heat shock at 40.5°C for 1, 2 or 3 min followed by cold shock at 1.5°C for 30, 45 or 60 min. When applied during the first cleavage metaphase of mud loach (*Misgurnus mizolepis*) zygotes, the optimal combination was heat for 2 min followed by cold for 45 min. At 1 month, the frequency of 4N survivors and the yield from total eggs fertilized was 55.7% and 14.4%, respectively, compared to heat shock alone with 20.0% efficiency and 3.6% yield. The effectiveness of the procedure was confirmed by diploid mitotic gynogenesis using transgenic markers. The overall yield of homozygous diploids, 34.0%, was better than that for single heat shock, 17.3%. The tetraploids and homozygous diploids had higher early mortality than normal diploid controls. However at 1 month, the viability of the tetraploids was the same as normal diploids. For gynogenetic diploids, the survival was similar to normal diploids after 3 months. The high efficiency of this new protocol extends the opportunity to study polyploidy in basic and applied research.

*Corresponding author: yoonknam@pknu.ac.kr