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Standardisation of Colchicines Concentration for the Karyotyping of *Schizothorax richardsonii* Found in Kumaun Hill Region, Uttarakhand, India

Toshibaa[1]*, Bisht H.C.S.[1] and Pandey N.N.[2]

[1]Department of Zoology, D.S.B. Campus Kumaun University Nainital 263002, Uttarakhand, India
[2]ICAR-Directorate of Coldwater Fisheries Research, Bhimtal 263136, India

**Corresponding Author*

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Abstract: To date, various techniques for achieving unambiguous and identifiable metaphase chromosomal spreads in a single species of fish have been devised. However, only few studies have compared the efficacy of these treatments in a variety of fish species. This study investigated the relationship between chromosome preparation parameters such as different colchicine concentrations (0.03, 0.05 and 0.07 %) prepared in 100 ml distilled water and exposure time (4 and 7 h) for the *Schizothorax richardsonii* (Asela). According to the findings of this study, 0.05% colchicine concentration for 7 h results in excellent metaphasic chromosomal spreads ($P<0.05$). This standardised concentration of colchicine is optimal for the karyotypic analysis of the fish *Schizothorax richardsonii.*

Keywords: Chromosome, Colchicine, Metaphasic spreads, Microtubule, *Schizothorax richardsonii*

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Introduction

Cytogenetics include karyotypic analysis which is based on the study of chromosome number, morphology, and size at the metaphase stage. Karyological investigations provide basic chromosome information that can be used for species identification and genetic engineering (Almeida-Toledo *et al*., 2000; Poletto *et al*., 2010; Borba *et al*., 2012).

Certain cytogenetic methods such as karyotypic analysis and work on taxonomy study (Crego-Prieto *et al*., 2013), methods for chromosome manipulation (Christopher *et al*., 2010; Gilna *et al*., 2014; Thresher *et al*., 2014), and commercial fish stocks are being genetically improved which can provide basic information on interspecific hybridization in the fish breeding programme (Gui and Zhu, 2012). Various Karyotyping procedures, such as tissue culture, have been employed to date (Lomax *et al*., 2000), and chromosomal analysis is critical for fish

breeding from the perspectives of genetic control, quick creation of inbred lines, cytotaxonomy, and evolutionary studies (Kirpichnikov, 1981). Karyological research (Tan *et al*., 2004) provided information on the quantity, size, and shape of chromosomes, which is required for chromosome manipulations in fish (Khan *et al*., 2000).

The most frequent microtubular toxin is colchicine ($C_{22}H_{25}NO_6$). Colchicine inhibits spindle microtubules and disperses metaphase chromosomes in the cytoplasm prior to nuclear envelope breakdown (NEB) in metaphase cells (Caperta *et al*., 2006), In *Danio rerio,* both colchicine concentration and incubation period were considerably influenced by larval age; in *Clarias gariepinus*, only the incubation period was significantly influenced by larval age (Karami *et al.*, 2015). The chemical concentration and incubation duration must be carefully chosen to create distinct and detectable metaphase chromosomal spreads (Rieder and Palazzo, 1992). While exposure to insufficient quantities and/or time spindle poisons will not prevent cells from entering metaphase, exposure to extremely high and low concentrations, as well as abnormally long and short durations, may cause chromosomal condensation (Rieder and Palazzo, 1992; Wood *et al*., 2001). Snow trout belongs to the Schizothoracinae subfamily and is listed as vulnerable by the IUCN (2012) in India. Snow trout, also called locally as "Asela," is a cold-water autochthonous riverine and short-distance migratory fish. During the winter, it migrates downstream, and in early June, when the water becomes turbid, it migrates upstream. The migration pattern varies among species to species and also affected by the volume of water in rivers and the temperature of the water.

The purpose of present study was to evaluate the optimal concentration of colchicine for karyotypic analysis of *Schizothorax richardsonii*, an important indigenous coldwater fish, in order to aid in its characterization and genetic improvement through the use of genetic tools such as triploidy induction, monosex production, and transgenic intervention.

Materials and Methods

Live specimens of *Schizothorax richardsonii* (40 individuals) were collected from Sirodi stream located at Bhowali; district Nainital, Uttarakhand by cast netting. They were transported at the Bhimtal ICAR-Directorate of Coldwater Fisheries Research (DCFR). Before analysis, the specimens were kept alive in a 400 L aquarium at 18 to 20°C for acclimatization. For Karyological preparation the protocol of Kligerman and Bloom (1977) was followed. *S. richardsonii* live specimens were injected with 0.03, 0.05, and 0.07% colchicines intramuscularly into the abdominal cavity at the base of pectoral (subcutical region, upper side of lateral line) at a rate of 1 ml per 100 g body weight of fish for chromosomal arrest at the metaphase stage. The fish were then placed in aquaria at temperatures ranging from 18 to 20 °C for 4 to 7 h. Before sacrificing, fish were anesthetized with clove oil. After anesthesial treatment fish was sacrificed and their cephalic kidney was excised, homogenised, and hypotonized simultaneously in 6.0 ml of potassium chloride 0.56% for 45 min at room temperature for smear preparation. Suspensions were spun at 1000 rpm in 15 ml centrifuge tube for 25 min. Following that, 1 to 2 ml of fresh and chilled Carnoy's fixative (3:1 mixture of methanol and acetic acid) was carefully added to the tube and spun at 1000 rpm for 5 to 8 min. The supernatant was discarded, leaving 1 ml above the cell pellet, and the cells were resuspended in the remaining supernatant, which was increased to 6 ml by adding new, cold fixative. The cells were properly fixed by spinning them 3 to 4 times and resuspending them in cold fixative at least 35 min interval, or until a clear cell suspension was obtained. The chromosomal plates were created by dropping the cell solution from a distance of about 2 to 2.5 feet onto a grease free clean glass slide. Using the flame drying process, the slides were incubated in a hot air oven at 40 °C until completely dry. The dry chromosomal spread slides were stained for 45 min at room temperature with 8% Giemsa in Phosphate buffer

Table 1: Metaphasic spread frequency varies with colchicine concentration at varying exposure times

Exposure time (h)	Concentration of colchicine (%)	Metaphasic spread frequency (%)
4.0 h	0.03%	30%
	0.05%	80%
	0.07%	50%
7.0 h	0.03%	40%
	0.05%	95%
	0.07%	70%

saline (pH 7.0). The slides were washed briefly with distilled water and air dried. The stained slide was viewed with a binocular microscope and snapshots were taken at 40X magnification.

The interactions and effects of colchicine concentration and exposure period in *Schizothorax richardsonii* were studied using a two-way ANOVA. The significance threshold was set at ($P<0.05$). IBM SPSS Statistics was used to make all statistical comparisons.

Results

The Karyological research of teleost fish involves technical challenges not seen in the study of other vertebrates, and these difficulties arise from the small size and large number of chromosomes (Cucchi and Baruffaldi, 1990). Such studies are currently conducted using two methodologies: direct, *in vivo*, and indirect, *in vitro*. The preparation of slides for optical microscopy is simple when using direct procedures. These methods rely on the use of colchicine to inhibit the proliferation of rapidly proliferating cell populations during the metaphase. The number of metaphase chromosomal spreads is affected by colchicine concentration and incubation time. Figure 1(A) shows that a 0.05% colchicine concentration for 4 h resulted in an 80% clear metaphase chromosomal spread frequency, whereas 0.03 and 0.07% colchicine concentrations resulted in 30% and 50% metaphase chromosomal spread frequency, respectively. Colchicine concentration of 0.05% for 7 h results in 95% clear metaphase chromosomal spread frequency (Fig. 1B), whereas 0.03 and 0.07% results in 40% and 70% metaphase chromosomal spread frequency, respectively (Table 1). Figure 1C depicts a comparison of colchicine concentrations over time, with 0.05% colchicine concentration at 4 and 7 h producing clear metaphasic chromosome spread.

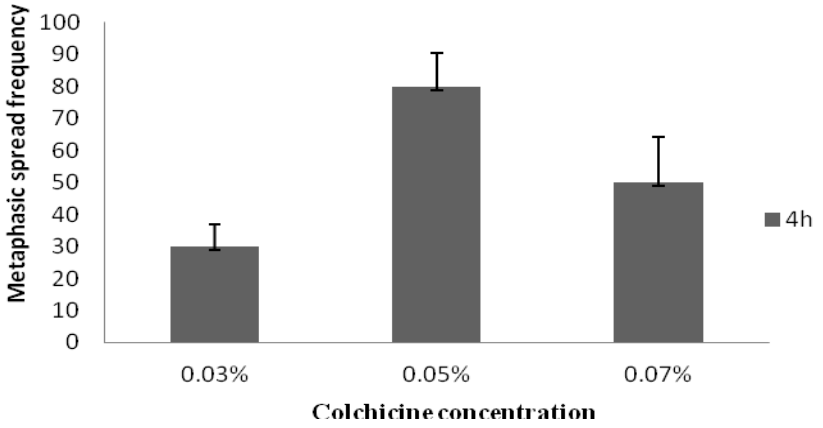
According to the findings of this study, a colchicine concentration of 0.05% for 7 h of incubation produces clear and identifiable metaphasic chromosomal spreads in *Schizothorax richardsonii* for karyotypic analysis (Figs. 2C, D). Whereas, 0.03 and 0.07% Colchicine concentrations did not show visible metaphase chromosomal spreads. The significance ($P = 0.0350$ in row and $p= 0.0089$ in column) was observed throughout the experiment.

Discussion

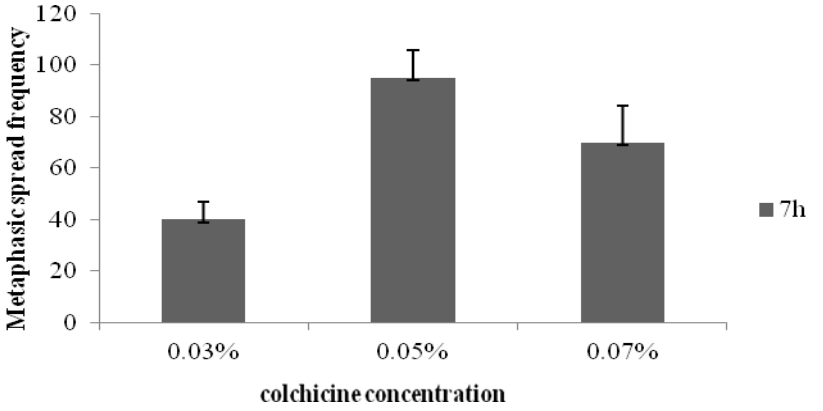
Colchicine is an alkaloid produced from the plant *Colchicum autumnale* L. that binds to tubulin dimers *in vitro*, forming a tubulin-colchicine complex that largely inhibits Microtubule assembly (Panda *et al*., 1995). Colchicine affects the cytoskeleton *in vivo* by inducing Microtubule depolymerization, which inhibits mitotic spindle formation. This characteristic is used to induce mitotic arrest in both animal and plant species (Darlington and LaCour 1963, Planchais *et al*., 2000).

Chromosome measurements are difficult, especially in fish, because their chromosomes are so small and compact in comparison to those of mammals. Another issue is that fish karyotypes

(A)



(B)



(C)

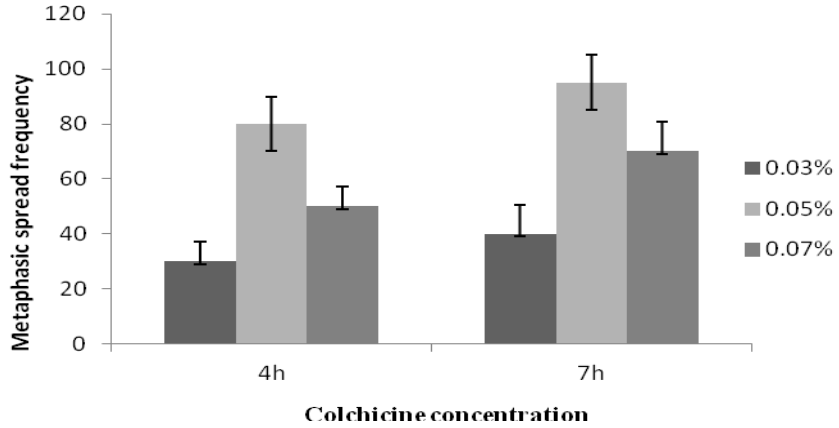


Fig. 1: (A) Metaphasic chromosomal spread at 4 h for all three colchicine concentration; (B) Metaphasic chromosal spread at 7 h for all three colchicine concentration; (C) Comparison of Metaphasic chromosomal spread at 4 h and 7 h.

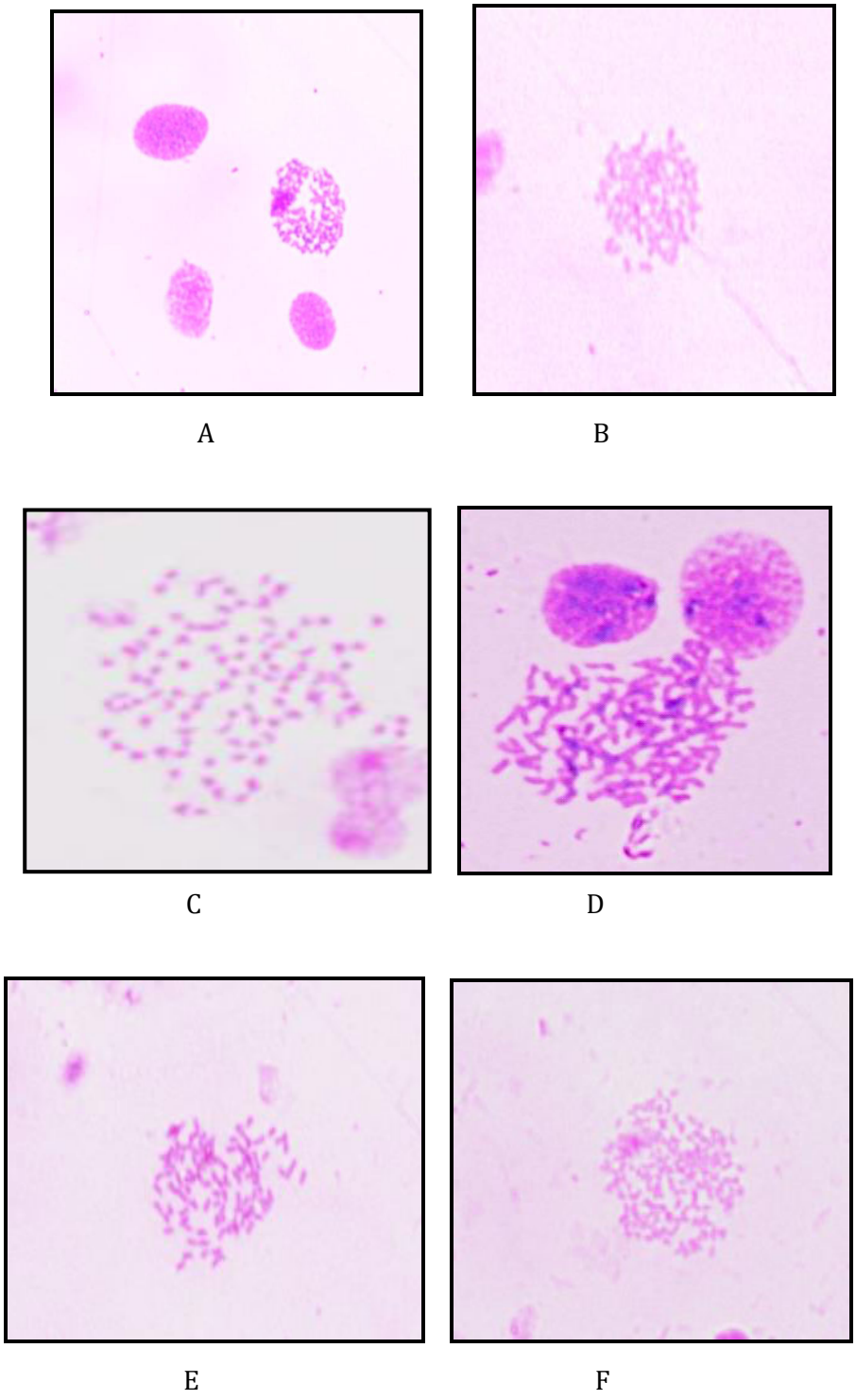


Fig. 2: (A) Metaphase chromosomal spreads *S. richardsonii* at 0.03% colchicine for 4 h; and (B) 7 h; (C) 0.05% for 4 h; (D) 7 h; (E) 0.07% for 4 h; and (F) 7 h.

are not identical, as they are in humans or other animal species, therefore we cannot establish a standard karyotypes for fish because there are variances across species, as well as polymorphism within the same fish species. Karyotype analysis is critical in stock improvement using polyploidy modification, hybridization, and genetic engineering (Tan *et al*., 2004).

The majority of fish chromosomal preparation methods have been developed for a single species.

The importance of altering colchicine concentration and incubation period in the fish *Schizothorax richardsonii* was revealed for the first time in this work. The number of cells undergoing mitosis division is directly proportional to the number of mitotic chromosomes dispersed. In contrast, mitotic rate is influenced by species and environmental conditions (Shelton *et al*., 1997). By comparing the number of metaphase chromosomes and mitotic rates in different *Cynoglossus semilaevis* life stages, Shao *et al*. (2010) discovered a greater mitotic rate in larvae compared to juveniles and adults. Wakahara (1972) discovered that the ventral tail-fin epidermis of the larval African clawed frog (*Xenopus laevis*) had a higher mitotic rate at night than it did during the day, demonstrating the importance of environmental factors in obtaining a reliable number of chromosome spreads. In the current study, unambiguous metaphase chromosomal spread was found in *Schizothorax richardsonii* kidney tissue at varying levels of colchicine concentration and incubation period. Colchicine is commonly hazardous in animal cells, even at the lowest doses required to disrupt mitosis (10-7 M) (Eigsti and Dustin, 1955; Rieder and Palazzo, 1992); nevertheless, at mm or greater concentrations, it is an efficient Microtubule depolymerizing agent in plants (Morejohn and Fosket, 1991). The quality of the slides is determined by the staining solution concentration and incubation duration. This study also revealed that soaking fish chromosomes in 8% Giemsa solution for 45 minutes results in higher quality stained chromosomes. Previously, 11% dosages were employed in previous research, although with a shorter incubation period (Hussain and McAndrew, 1994; Shao *et al*., 2010; Pradeep *et al*., 2011). Other studies used lower concentrations, such as 4 and 5% (Kligerman and Bloom, 1977; Inokuchi *et al*., 1994; Fopp- Bayat and Woznicki, 2006), or higher concentrations, such as 14% (Kligerman and Bloom 1977; Inokuchi *et al*., 1994; Fopp- Bayat and Woznicki, 2006; Karami *et al*., 2010). This study discovered that concentrations greater than 8% and incubation durations less than 45 min result in ambiguous chromosomes. To acquire clear metaphase chromosomal spread, the concentration of colchicine and the period of treatment were varied in this study. Karami *et al*. (2010) discovered that incubating *Clarias gariepinus* with 0.05% colchicine for 3 h resulted in a significantly increased number of chromosomal spreads. Whereas 0.03% colchicine concentration results in extremely poor metaphasic spread and 0.07% colchicine concentration results in moderate metaphasic spread and 0.07% colchicine concentration producing moderately metaphasic chromosome spread. Pradeep *et al.* (2011) found that incubating *Oreochromis mossambicus* for 4 to 6 h with 0.01% colchicine resulted in a considerably increased number of chromosome spreads.

Conclusion

The current study reflected that an acceptable concentration and incubation period of colchicine results in distinct metaphase chromosomes spread, which are ideal for karyotypic analysis of an important indigenous coldwater species, snow trout, and would be useful for its characterization and genetic diversity in natural fish population, which is imperative in the conservation and stock management, ploidy determination, hybrid identification and sex characterization.

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References

Almeida-Toledo LF, Foresti F and Toledo-Filho SA. (2000) Karyotypic evolution of Neotropical freshwater fish. In: Chromosomes Today, (eds.) Olmo E. and Redi C.A., Birkhäuser Basel. https://doi.org/10.1007/978-3-0348-8484-6_13.

Borba RS, Silva EL, Pacheco ACS, Parisi-Maltempi PP and Alves AL. (2012) Trends in the karyotypic evolution of the Neotropical catfish family *Heptapteridae Bockmann* 1998 (Teleostei: Siluriformes). Rev Fish Biol Fish. 22(2): 509-518.

Caperta A, Delgado M, Ressurreicao F, Meister A, Jones R, Viegas W and Houben A. (2006) Colchicine-induced polyploidization depends on tubulin polymerization in c-metaphase cells. Protoplasma 227: 147-153.

Christopher JSG, Murugesan AG and Sukumaran N. (2010) Induction of meiotic gynogenesis in the stinging catfish *Heteropneustes fossilis* (Bloch) and evidence for female homogamety. Aqua Res. 42: 129-138.

Crego-Prieto V, Danancher D, Campo D, Perez J, Garcia-Vazquez E and Roca A. (2013) Interspecific introgression and changes in population structure in a flatfish species complex after the Prestige accident. Mar Pollut Bull. 74: 42-49.

Cucchi C and Baruffaldi A. (1990) A new method for karyological studies in teleost fishes. J Fish Biol. 37(1): 71-75.

Darlington CD and LaCour LF. (1963) The handling of chromosomes. George Allen Unwin, London.

Eigsti OJ and Dustin P. (1955) Colchicine in agriculture, medicine, biology and chemistry. The Iowa State College Press, Ames, Iowa.

Fopp-Bayat D and Woznicki P. (2006) Verification of ploidy level in sturgeon larvae. Aqua Res. 37: 1671-1675.

Gilna B, Kuzma J and Otts SS. (2014) Governance of genetic biocontrol technologies for invasive fish. Biol Invasions. 16: 1299-1312.

Gui J and Zhu Z. (2012) Molecular basis and genetic improvement of economically important traits in aquaculture animals. Chin Sci Bull. 57: 1751-1760.

Hussain M and McAndrew B. (1994) An improved technique for chromosome karyotyping from embryonic and soft tissues of tilapia and salmonids. Asian Fish Sci. 7: 187-190.

Inokuchi T, Abe S, Yamaha E, Yamazaki F and Yoshida C. (1994) BrdU replication banding studies on the chromosomes in early embryos of salmonid fishes. Hereditas 121: 255-265.

Karami A, Araghi PE, Syed MA and Wilson SP. (2015) Chromosome preparation in fish: effects of fish species and larval age. Int Aquat Res. 7(3): 201-210.

Karami A, Christianus A, Ishak Z, Courtenay S, Syed MA, Noor A and Noorshinah H. (2010) Effect of triploidization on juvenile African catfish (*Clarias gariepinus*). Aquac Int. 18: 851-858.

Khan TA, Bhise MP, Lakra WS. (2000). Chromosome manipulation in fish: a review. Indian J Anim Sci. 70: 213-221.

Kirpichnikov VS. (1981) Genetic Bases of Fish Selection. Springer Verlag, Berlin, Heidelberg, New York, pp. 342.

Kligerman AD and Bloom SE. (1977) Rapid chromosome preparations from solid tissues of fishes. J Fish Res Board Can. 34: 266-269.

Lomax B, Tang S, Separovic E, Phillips D, Hillard E, Thomson T and Kalousek DK. (2000) Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. Am J Hum Genet. 66: 1516-1521.

Morejohn LC and Fosket DE. (1991) The biochemistry of compounds with anti-microtubule activity in plant cells. Pharmacol Ther. 51: 217-230.

Panda D, Goode BL, Feinstein SC and Wilson L. (1995) Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. Biochemistry (Washington) 34: 11117–11127.

Planchais S, Glab N, Inze D and Bergounioux C. (2000) Chemical inhibitors: a tool for plant cell cycle studies. FEBS Lett. 476: 78-83.

Poletto AB, Ferreira IA, Cabral-de-Mello DC, Nakajima RT, Mazzuchelli J, Ribeiro HB, Venere PC, Nirchio M, Kocher TD and Martins C. (2010) Chromosome differentiation patterns during cichlid fish evolution. BMC Genet. 11(1): 50.

Pradeep PJ, Srijaya TC, Zain RBM, Papini A and Chatterji AK. (2011) A simple technique for chromosome preparation from embryonic tissues of teleosts for ploidy verification. Caryologia 64: 235-241.

Rieder CL and Palazzo RE. (1992) Colcemid and the mitotic cycle. J Cell Sci. 102: 387-392.

Shao CW, Wu PF, Wang XL, Tian YS and Chen SL. (2010) Comparison of chromosome preparation methods for the different developmental stages of the half-smooth tongue sole, *Cynoglossus semilaevis*. Micron 41: 47-50.

Shelton WL, Mims SD, Clark JA, Hiott AE and Wang C. (1997) A temperature-dependent index of mitotic interval (s0) for chromosome manipulation in paddlefish and shovelnose sturgeon. Prog Fish Cult. 59: 229-234.

Tan X, Jian GQ, Chen B, Chen L and Li X. (2004) Karyological analyses on redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). Aquac. 234: 65-76.

Thresher RE, Hayes K, Bax NJ, Teem J, Benfey TJ and Gould F. (2014) Genetic control of invasive fish: technological options and its role in integrated pest management. Biol Invasion 16: 1201-1216.

Vasave S, Saxena A and Srivastava SK. (2016) Karyotypic diversity between Rainbow trout (*Oncorynchus*

mykiss, Walbum) and Snow trout (*Schizothorax richardsonii*, Gray). J Plant Res. 2(1): 1-5.

Wakahara M. (1972) Daily variation in mitotic rate in tail-fin epidermis of larval *Xenopus laevis* and its modification by pineal organ-subcommissural organ system and photoperiods. Neuroendocrinol. 9: 267-277.

Wood KW, Cornwell WD and Jackson JR. (2001) Past and future of the mitotic spindle as an oncology target. Curr Op Pharmacol.1: 370-377.