

Note

Comparative study of heat shock and pressure shock for triploidy induction in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The study was aimed to explore the effective physical treatment for successful triploidy induction to take the advantage of improving growth in coldwater aquaculture candidate fish, rainbow trout *Oncorhynchus mykiss*. Comparison was made between two common physical treatments viz., heat shock and pressure shock to prevent the extrusion of second polar body in newly fertilised eggs. Heat shock was given on the fertilised eggs at three temperature levels viz., 26, 28 and 30°C with four exposure timings of 5, 8, 10 and 12 min. Pressure shock was applied at three different levels of pressure i.e., 9000, 9500 and 10000 psi for 3 different exposure times of 3, 5 and 7 min. Poidy verification of the treated eggs was done by counting the chromosome number in karyotyping. Results of the field operations reflected 86-90% triploidy induction for pressure shock at 9500 psi for exposure time of 5 min with 72-80% survival from fertilisation to swim-up fry stage and 80% triploidy induction for heat shock at 28°C for 10 min with 60-66% survival from fertilisation to fry stage. Appropriate centigrade temperature minutes (CTM) was calculated as 300-375 min, which is variable at different locations and environmental conditions. Study concluded that pressure shock is more effective over heat shock for triploidy induction in rainbow trout.



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The rainbow trout (*Oncorhynchus mykiss*) is a prominent coldwater fish belonging to the family Salmonidae and recognised as the most widely farmed trout in the world. This is one of the most widely introduced and cultured fish across the globe and its farming is also prominent in the Himalayan region of India. Nevertheless, low productivity due to genetic fatigue and slow growth are major constraints in the expansion of trout aquaculture. A viable strategy that could overcome these farming constraints in large scale operation is the production of triploid fish that are sterile and more heterozygous. Induction of triploidy in fish has been found to be useful for improving growth in juveniles and adult fish as well as for extending survival (Bazaz, *et al.*, 2020). Such chromosomal manipulation and generation of polyploids is not exclusive to

fish. Many plants used in modern agriculture are induced polyploids, selected to increase productivity associated with greater cell size or disease resistance. In triploids, most of the anabolic energy is transferred to somatic growth (Akhmad, *et al.*, 2020). Somatic growth is one of the most fundamental biological processes required for survival and thus has important fitness consequences, and growth rate is frequently used as an indicator of the capacity to acquire food resources (Arnott *et al.*, 2006; Pang *et al.*, 2016). Triploidy induction is characterised with three sets of chromosomes and can be induced in fish by inhibiting the extrusion of the second polar body by shocking eggs shortly after fertilisation. Induction of triploidy may prevent the reduction in post-pubertal somatic growth and carcass quality due to

gonadal maturation and secondary sex differentiation (Poontawee *et al.*, 2007; Fraser *et al.*, 2012; Cleveland *et al.*, 2014). The characteristics of triploid fish are thus known to have a beneficial impact on fish production economics (Berrill *et al.*, 2012). In particular, inducing triploidy using physical shocks such as abrupt changes in pressure and temperature is a safe and reliable method which does not involve potentially harmful chemicals or radiation (Benfey, 1988; 1999). The underlying principle is that the application of thermal or mechanical shock to the zygote at appropriate time of post-fertilisation prevents the extrusion of the second polar body, which results in three chromosome sets in every embryonic cell (Maxime *et al.*, 2008; Benfey, 2016). It has also been proved that mass production of triploid salmonids is feasible through physical shock without major reduction of survival (Quillet *et al.*, 1991; Fraser *et al.*, 2012). It was reported that triploidy rates closer to 100% could be achieved in brown trout through heat shock trials (Arai and Wilkins, 1987; Quillet *et al.*, 1991). Triploidy can be determined by karyotyping and silver staining of nucleolar organising regions (Benfey, 1984; Garcia-Abiado *et al.*, 1999; Piferrer *et al.*, 2009). Karyological studies have provided basic information on the number, size and morphology of chromosomes (Tan *et al.*, 2004) that is important to undertake chromosome manipulations in fish (Khan *et al.*, 2000). The purpose of the present study was to standardise a viable methodology for production of triploid rainbow trout, by physical treatment protocols of fertilised eggs.

Mature brooders of age 3-4 years were used for the breeding operations during the period from January to March 2019. For heat shock treatments, operation was conducted at three different locations viz., village Skhras, District Anantnag, Kashmir (33.729729°N; 75.149780°E); State Trout Farm, Uttarey, West Sikkim (27.2605976°N; 88.0949539°E) and State Trout Farm, Rabum, North Sikkim (27.35052°N; 88.73811°E). Similarly, pressure shock treatments were performed at State Trout Farm, Talwari, District, Chamoli, Uttarakhand (30.0293115°N; 79.5233704°E). For heat shock, a thermostatic water bath was used which was equipped with aeration system and thermometer. A specific equipment, Aqua Pressure Vessel of the TRC Hydraulics, New Brunswick, Canada was used for applying high pressure on fertilised eggs. The major variables selected for standardisation were timing after fertilisation and intensity and duration of the thermal/pressure shock.

Centigrade temperature minutes (CTMs) is the most important step in the triploidy induction operation. Appropriate CTMs is calculated by multiplication of minutes of extrusion of second polar body and water temperature in °C, which remain slightly different for different locations and environmental conditions. Hence, a study was conducted at different places with different water temperatures. In each breeding operation, milt from the males was mixed with freshly harvested eggs from the healthy females of rainbow trout and 10 live fertilised eggs were observed under compound microscope under 10X objective for noting the timing of extrusion of second polar body at different levels of water temperature.

For heat shock, three breeding operations were conducted at different places. In each breeding operation, the fertilised eggs (2600 eggs) were divided into thirteen batches (200 eggs in each batch), where batch I was kept as control with no thermal shock given to the eggs at 9.5 to 12°C (optimum temperature for breeding), while the remaining batches II to XIII were thermally treated. Batches of eggs II, III, IV and V were given heat shock at 26°C by increasing the

temperature for different duration of 5, 8, 10 and 12 min respectively. At the same time, batches of eggs VI, VII, VIII and IX were given heat shock of 28°C for duration of 5, 8, 10 and 12 min., respectively and batches of eggs X, XI, XII and XIII were given heat shock at 30°C for durations of 5, 8, 10 and 12 min. respectively. After heat shock treatment, each batch of eggs was kept in trays separately for incubation and labeled. For pressure shock, two breeding operations were conducted in different dates at 3 levels of pressure i.e., 9000, 9500 and 10000 psi for 3 different exposure times of 3, 5 and 7 min. In each breeding operation, fertilised eggs were separated into ten batches having 1000 eggs in each batch. First batch was kept as control without treatment. Other nine batches were treated for three pressure levels and three exposure timings. All treated eggs were kept for incubation in trays fitted in troughs having continuous flow of water (0.5 l min⁻¹). The incubation period was calculated as degree days (water temperature °C x no. of days). Trays were properly labeled and were carefully observed for survival and embryonic development. During the experiment, egg size, relative fecundity, fertilisation rate, hatching rate and survival rate were also recorded. Statistical analysis was done by one-way analysis of variance (ANOVA) with 5% significance level.

Direct methods of karyotyping (visualisation and counting of chromosomes) was carried out for triploidy verification in treated and control groups using fry of rainbow trout. The standardised method of Felip *et al.* (2009) was followed which is based on Kligerman and Bloom (1977) for obtaining chromosome spreads.

Metaphase chromosome slide preparation was done with 50 best slides observed and number of chromosomes were counted and triploidy induction rate (TR) was calculated using the formula:

$$\text{Triploidy rate (\%)} = \frac{\text{No. of slides containing triploid plate}}{50} \times 100$$

Triploid yields are strongly influenced by different parameters linked to the magnitude of thermal or pressure shock, duration of the exposure time and time of initiating the shock in the fertilised eggs (Haffray, *et al.*, 2007), species/strain susceptibility (Sacobie *et al.*, 2012), egg quality (Piferrer *et al.* 2009) and many other physiological factors (Maxime *et al.*, 2008). Therefore, it is imperative to know the exact time of initiating the shock in the fertilised eggs. Hence, centigrade temperature minute (CTM) is the most important step in triploidy induction. Studies conducted in different breeding seasons at different geographical locations revealed that appropriate CTMs for rainbow trout is in the range of 300-375 min, which is slightly variable for different locations and environmental conditions. Loopstra and Hansen (2008) also reported CTM of 300 for rainbow trout. Breeding performance of the control group (diploids) is summarised in Table 1, which reflects 62-70% fertilisation rate, 70-78% hatching rate and 72-80% survival up to swim-up fry stage at thermal regime of 9.5-12.5°C. Data of three breeding operations with heat shocks are summarised in Table 2 and revealed that 80% induction can be achieved with heat shock at 28°C for 10 min, with 66% survival. However, 60% success was also achieved with heat shock at 26°C for 12 min with 60% survival. Survival from fertilisation to fry stage for heat shocked eggs was lower than the control (60-66% in triploids and 74-80% in diploids). Breeding operation with heat shocks clearly reflect that low shock temperature (<28°C),

Table 1. Details of the breeding operation of control group (diploids)

Average weight (female, g)	Average weight (male, g)	Average No. of eggs	Average ova diameter (mm)	Water temperature (°C)	Fertilisation rate (%)	Incubation period (days)	Hatching rate (%)	Survival rate (%)
670	540	678	5.5	12.5	62	34	78	72
712	618	854	5.2	11.8	70	36	74	78
640	502	832	4.6	9.5	68	42	70	80

Table 2. Performance of triploidy induction by heat shock in different treatments

Temperature (°C)	Exposure time (min) of heat shock	Incubation period (degree days)	Hatching rate (%)	Survival rate up to swim-up fry stage (%)
State Trout Farm, Uttarey (02.01.2019) (Altitude 2012 m)				
26	5	437	72	88
	8	437	52	84
	10	437	46	67
	12	425	12	60
28	5	400	74	76
	8	425	50	72
	10	425	42	68
	12	425	8	50
30	5	All damaged		
State Trout Farm, Rabum (04.01.2019) (Altitude 956 m)				
26	5	425	68	82
	8	425	62	80
	10	413	48	64
	12	All damaged	All damaged	-
28	5	425	62	75
	8	425	52	70
	10	401	40	57
	12	All damaged	All damaged	-
30	5	All damaged		
Sarkas District, Anantnag, Kashmir (200.2.2019) (Altitude 1600 m)				
26	5	418	72	82
	8	400	60	80
	10	427	52	64
	12	All damaged	All damaged	-
28	5	400	68	74
	8	400	56	72
	10	400	46	66
	12	All damaged	All damaged	-
30	5	All damaged		

less exposure time (<10 min.) and low dissolved oxygen (<8 ppm) results in no retention of the second polar body. Heat shock at 30°C resulted in immediate damage of eggs, which became completely white in colour. It was also observed that 2-14% alevins were deformed after the heat shock in treated group, however 1-2% deformity was observed in control group of diploids also, which could be due to genetic deformity. Deformed alevins were either haploid or diploids. Heat shock is one of the common technique for triploidy induction in fish, but the application of heat shock to induce triploidy is not always 100% effective and can cause a detrimental side-effect and decreased viability (Bazaz *et al.*, 2020). Induction of triploidy in rainbow trout by heat shock has been reported by various authors (Chourrout, 1980; Solar *et al.* 1984; Dillon, 1988; Guner *et al.*, 2005). Dillon (1988) reported that rate of triploidy

induction ranged from 0-100% and all variables (temperature, time after fertilisation when heat shock began and duration of heat shock) significantly affected triploid yield. The 'time window' (a frame of zygote age, heat shock regime and shock duration) period, sensitive to heat shock was found to be very narrow for this species as triploids of this fish were obtained only at the zygote age of 39 min after fertilisation at 9-10°C with the shock duration of 10-12 min. Beyond this 'time window' no triploids were obtained due to change in either of the zygote age or shock exposure time. This window width for shock may be species-specific and in some cases brood stock-specific (Seeb, 1988). Similar impact of temperature level and exposure time was observed by Solar *et al.* (1984) for rainbow trout. Similar result having less than 100% triploidy induction rate has been reported using heat shock in some

Table 3. Conformity of heat shocked fry of rainbow trout by karyotyping

Temperature (°C)	Exposure time (min) of heat shock	No. of plates observed	No. of triploid plates	% of triploidy induction (TR)
First operation				
26	5	40	0	0
	8	40	0	0
	10	40	0	0
	12	10	6	60
28	5	40	0	0
	8	40	4	10
	10	40	24	60
	12	-	-	-
Second operation				
26	5	40	0	0
	8	40	0	0
	10	40	2	5
	12	-	-	-
28	5	40	0	0
	8	40	0	0
	10	40	22	55
	12	-	-	-
Third operation				
26	5	40	0	0
	8	40	0	0
	10	40	12	30
	12	-	-	-
28	5	40	0	0
	8	40	16	40
	10	40	32	80
	12	-	-	-

of the earlier studies in fishes viz.; 76.5% in *Oncorhynchus mykiss* (Diaz *et al.*, 1993) and 80% in *Cyprinus carpio* (Basavaraju *et al.*, 2002). Lower survival rates have also been reported in triploids of *Salmo salar* (Galbreath and Thorgaard, 1994) and *S. trutta* and its hybrids (Mckay *et al.*, 1992) in comparison to diploids of these species. One of the important factors that could be responsible for the reduced triploid survival may be inbreeding depression resulting from retention of second polar body (Ueda, 1988).

In pressure shock treatments (Table 4), viable eggs were obtained with pressure of 9000 and 9500 psi for exposure time of 3 and 5 min and best result was achieved at pressure of 9500 psi for exposure time of 5 min, with triploidy induction rate of 86-90%. However, 20-26% success was also achieved at pressure of 9000 psi for exposure time of 5 min. Pressure shock at 10000 psi totally resulted in dead eggs (Table 5). Only 1-2% deformity in alevins was observed with pressure shock. Success in inducing triploidy by pressure shock in rainbow trout was also achieved by several workers (Chourrout 1984; Lou and Purdom 1984; Lincoln 1989; Hamor *et al.*, 1996; Yesaki *et al.*, 1996; Wickwire 2000). The amount of pressure, shock duration and timing of shock initiation used to obtain triploid rainbow trout were different for the previous studies (Chourrout, 1984; Lou and Purdom, 1984; Lincoln, 1989; Hamor *et al.*, 1996; Yesaki *et al.*, 1996; Wickwire, 2000). Present comparative study reveals that pressure shock treatments reflect better

TR, survival rate and less deformity over the heat shock. Lower triploidy rate with heat shock might be due to non-uniform temperature treatment on egg biomass. Eggs located near the centre of the egg tray may not receive the same thermal shock treatment as eggs near the perimeter of the tray. Moreover, egg biomass remains with lower temperature and after emerging the egg biomass in treated water, consistency in temperature is difficult to maintain. On the other hand, eggs in the pressure treatment receive the same shock regardless of egg size or location within the pressure chamber. Teskeredzic *et al.* (1993) also reported that variation in egg size affects the triploidisation and egg survival rates. In field trials, better success for triploidy induction was achieved with pressure shock rather than heat shock (Pressure shock - 30/32, heat shock - 24/32). In treated groups, three sets of chromosomes (88-90) were observed in chromosome plates.

The manipulation of chromosome and chromosome sets are major components of aquatic animal genetics improvement. By manipulating chromosomes, we intend to create new genetic constructs that exhibit commercially useful traits. Though, triploidy induction has been successfully achieved in the other leading trout producing countries, findings of this study is the first attempt in India to standardise a systematic triploidy induction protocol for rainbow trout, which would be useful for better production of this fish in Himalayan region and in other temperate regions of the country.

Table 4. Details of triploidy induction by pressure shock

Pressure (psi)	Exposure time for pressure shock (min.)	Nos. of eggs utilised for pressure shock	Nos. of live eggs after pressure shock	Water temperature. (°C)	Fertilisation rate (%)	Incubation period (days)	Hatching rate (%)	Survival rate up to swim-up fry (%)
18.03.2019 (Operation 1)								
Operation 1.1, Weight of female (g): 1195, Weight of male (g): 1375, No. of eggs obtained: 860, Avg. ova diameter (mm): 5.1								
9000	3	290	284	9.5	65	40	72	74
	5	290	282	9.5	65	40	68	72
	7	280	34	9.3	All dead			
Operation 1.2, Weight of female (g): 1275, Weight of male (g): 1380, No. of eggs obtained: 870, Avg. ova diameter (mm): 5.2								
9500	3	290	286	9.4	63	41	70	76
	5	290	282	9.4	63	41	72	80
	7	290	42	9.5	All dead			
Operation 1.3, Weight of female (g):1210, Weight of male (g): 1360, No. of eggs obtained: 810, Avg. ova diameter (mm): 5.2								
10000	3	270	21	9.5	67	All dead		
	5	270	14	9.5	-			
	7	250	9	9.4	-			
19.03.2019 (Operation 2)								
Operation 2.1, Weight of female (g): 1285, Weight of male (g): 1335, No. of eggs obtained: 885, Avg. Ova diameter (mm): 5.3								
9000	3	290	245	9.6	69	40	74	68
	5	290	232	9.6	69	40	70	74
	7	300	24	9.5	All dead			
Operation 2.2, Weight of female (g): 1325, Weight of male (g) : 1460, No. of eggs obtained: 945, Avg. ova diameter (mm): 5.3								
9500	3	315	304	9.5	67	38	74	80
	5	315	302	9.6	67	39	73	79
	7	315	31	9.5	All dead			
Operation 2.3, Weight of female (g): 1170, Weight of male (g): 1345, No. of eggs obtained: 815, Avg. ova diameter (mm): 5.3								
10000	3	270	20	9.6	68	All dead		
	5	270	12	9.7	-			
	7	275	6	9.5	-			

Table 5. Conformity of pressure shocked triploid induction by karyotyping

Pressure (psi)	Exposure time (min.) for pressure shock	No. of plates observed	No. of triploid plates	% of triploidy induction (TR)
First operation				
9000	3	30	0	0
	5	30	8	26
9500	3	30	0	0
	5	30	26	86
Second operation				
9000	3	30	0	0
	5	30	6	20
9500	3	30	0	0
	5	30	28	90

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References

- Akhmad, T. M., Odang, C., Alimuddin, A., Muhammad, Z. J. R. and Muhammad, A. S. 2020. Growth performance, survival rate, flesh and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*). *Turk. J. Vet. Ani. Sci.*, 44(2): 290-298. <https://doi.org/10.3906/vet-1905-79>
- Arai, K. and Wilkins, N. P. 1987. Triploidization of brown trout (*Salmo trutta*) by heat shocks. *Aquaculture*, 64(2): 97-103. [https://doi.org/10.1016/0044-8486\(87\)90345-0](https://doi.org/10.1016/0044-8486(87)90345-0).
- Arnott, S. A., Chiba, S. and Conover, D. O. 2006. Evolution of intrinsic growth rate: metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. *Evolution*, 60(6): 1269-1278. <https://doi.org/10.1111/j.0014-3820.2006.tb01204.x>.
- Bazaz, A. I., Ahmad, I., Arab, N., Shah, T. H., Asimi, O. A., Bhat, B. A., Yousuf, Z., Baba, S. H., Razak, N. and Fatima, A. 2020. A review on induction of triploidy in fish using heat, pressure and cold shock treatments. *J. Entomol. Zool. Stud.*, 8(2): 381-385.

- Basavaraju, Y. M. G. C., Mair, G. C., Kumar, H. M., Kumar, S. P., Keshavappa, G. Y. and Penman, D. J. 2002. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp, *Cyprinus carpio*, in Karnataka, India. *Aquaculture*, 204(3-4): 407-418. [https://doi.org/10.1016/s0044-8486\(01\)00827](https://doi.org/10.1016/s0044-8486(01)00827).
- Benfey, T. J. 1988. *The reproductive physiology of triploid Pacific salmonids*, Doctoral dissertation, University of British Columbia, Vancouver, Canada.
- Benfey, T. J. 1999. The physiology and behavior of triploid fishes. *Rev. Fish. Sci.*, 7(1): 39-67. <https://doi.org/10.1080/10641269991319162>.
- Benfey, T. J. 2016. Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. *Rev. Aquac.*, 8(3): 264-282. <https://doi.org/10.1111/raq.12092>.
- Benfey, T. J. and Sutterlin, A. M. 1984. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 36(4): 359-367. [https://doi.org/10.1016/0044-8486\(84\)90328-4](https://doi.org/10.1016/0044-8486(84)90328-4).
- Benfey, T. J., Sutterlin, A. M. and Thompson, R. J. 1984. Use of erythrocyte measurements to identify triploid salmonids. *Canadian J. Fish. Aquat. Sci.*, 41(6): 980-984. <https://doi.org/10.1139/f84-112>.
- Berrill, I. K., MacIntyre, C. M., Noble, C., Kankainen, M. and Turnbull, J. F. 2012. Bio-economic costs and benefits of using triploid rainbow trout in aquaculture: Reduced mortality. *Aquac. Econ. Manage.*, 16(4): 365-383. <https://doi.org/10.1080/13657305.2012.729245>.
- Chourrout, D. 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri* Richardson). *Reprod. Nutr. Dev.*, 20(3A): 727-733. <https://doi.org/10.1051/rnd:19800415>.
- Chourrout, D. 1984. Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids and heterozygous and homozygous diploid gynogenetics. *Aquaculture*, 36(1-2): 111-126. [https://doi.org/10.1016/0044-8486\(84\)90058-9](https://doi.org/10.1016/0044-8486(84)90058-9).
- Cleveland, B. M. and Weber, G. M. 2014. Ploidy effects on genes regulating growth mechanisms during fasting and refeeding in juvenile rainbow trout (*Oncorhynchus mykiss*). *Mol. Cell. Endocrinol.*, 382(1): 139-149. <https://doi.org/10.1016/j.mce.2013.09.024>.
- Diaz, N. F., Iturra, P., Veloso, A., Estay, F. and Colihueque, N. 1993. Physiological factors affecting triploid production in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 114(1-2): 33-40. [https://doi.org/10.1016/0044-8486\(93\)90248-w](https://doi.org/10.1016/0044-8486(93)90248-w).
- Dillon, J. C. 1988. *Production of triploid rainbow trout for evaluation in South Dakota waters*. Thesis of South Dakota State University, South Dakota, USA.
- Felip, A., Carrillo, M., Herraiz, M. P., Zanuy, S. and Basurco, B. 2009. Advances in fish reproduction and their application to broodstock management: A practical manual for sea bass. *Options Mediterraneennes. Serie B, Etudes et Recherches*, (63): 1-95.
- Fraser, T. W., Fjellidal, P. G., Hansen, T. and Mayer, I. 2012. Welfare considerations of triploid fish. *Rev. Fish. Sci.*, 20(4): 192-211. <https://doi.org/10.1080/10641262.2012.704598>.
- Galbreath, P. F. and Thorgaard, G. H. 1994. Viability and freshwater performance of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) triploid hybrids. *Canadian J. Fish. Aquat. Sci.*, 51(S1): 16-24. <https://doi.org/10.1139/f94-290>.
- Garcia-Abiado, M. A. R., Dabrowski, K., Christensen, J. E., Czesny, S. and Bajer, P. 1999. Use of erythrocyte measurements to identify triploid saugeyes. *North American J. Aquac.*, 61(4): 319-325. [https://doi.org/10.1577/1548-8454\(1999\)061%3C0319:uoemti%3E2.0.co;2](https://doi.org/10.1577/1548-8454(1999)061%3C0319:uoemti%3E2.0.co;2).
- Guner, Y., Kayim, M., Kizak, V. and Gullu, K. 2005. Production and performance of triploid rainbow trout in Turkey. *Indian Vet. J.*, 82: 1077-1079.
- Haffray, P., Aubin, J., Houis, V., Labbe, L. and Jalabert, B. 2007. Comparison of pressure or thermal treatments on triploid yields and malformations up to swim up stage in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 272: S265. <https://doi.org/10.1016/j.aquaculture.2007.07.079>.
- Hamor, T., Beck, R. and Stewart, J. 1996. Alteration of ploidy in rainbow trout with heat and hydrostatic pressure. *Proceedings of the 47th Annual Northwest Fish Culture Conference*, American Fisheries Society, Bethesda, Maryland, USA, pp. 174-186.
- Khan, T. A., Bhise, M. P. and Lakra, W. S. 2000. Chromosome manipulation in fish-A review. *Indian J. Ani. Sci.*, 70(2): 213-221.
- Kligerman, A. D. and Bloom, S. E. 1977. Rapid chromosome preparations from solid tissues of fishes. *J. Fish. Board Can.*, 34(2): 266-269. <https://doi.org/10.1139/f77-039>.
- Lincoln, D. 1989. Triploid induction in rainbow trout using hydrostatic pressure. *Trout News*, 8: 8-10.
- Loopstra, D. P. and Hansen, P. A. 2008. *Induction of triploidy in rainbow trout (Oncorhynchus mykiss) using hydrostatic pressure*. Research and Technical Services, Division of Sport Fish, Alaska Department of Fish and Game, Alaska, USA.
- Lou, Y. D. and Purdom, C. E. 1984. Polyploidy induced by hydrostatic pressure in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.*, 25(3): 345-351. <https://doi.org/10.1111/j.1095-8649.1984.tb04881.x>.
- Maxime, V. 2008. The physiology of triploid fish: current knowledge and comparisons with diploid fish. *Fish. Fish.*, 9(1): 67-78. <https://doi.org/10.1111/j.1467-2979.2007.00269.x>.
- McKay, L. R., Ihssen, P. E. and McMillan, I. 1992. Growth and mortality of diploid and triploid tiger trout (*Salmo trutta* × *Salvelinus fontinalis*). *Aquaculture*, 106(3-4): 239-251. [https://doi.org/10.1016/0044-8486\(92\)90256-k](https://doi.org/10.1016/0044-8486(92)90256-k).
- Pang, X., Fu, S. J., Li, X. M. and Zhang, Y. G. 2016. The effects of starvation and re-feeding on growth and swimming performance of juvenile black carp (*Mylopharyngodon piceus*). *Fish. Physiol. Biochem.*, 42: 1203-1212. <https://doi.org/10.1007/s10695-016-0210-x>.
- Piferrer, F., Beaumont, A., Falguiere, J. C., Flajshans, M., Haffray, P. and Colombo, L. 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture*, 293(3-4): 125-156. <https://doi.org/10.1016/j.aquaculture.2009.04.036>.
- Poontawee, K., Werner, C., Muller-Belecke, A., Horstgen-Schwark, G. and Wicke, M. 2007. Flesh qualities and muscle fiber characteristics in triploid and diploid rainbow trout. *J. Appl. Ichthyol.*, 23(3): 273-275. <https://doi.org/10.1111/j.1439-0426.2007.00843.x>.
- Quillet, E., Foisil, L., Chevassus, B., Chourrout, D. and Liu, F. G. 1991. Production of all-triploid and all-female brown trout for aquaculture. *Aquat. Living Resour.*, 4(1): 27-32. <https://doi.org/10.1051/alr:1991002>.
- Solar, I. I., Donaldson, E. M., Hunter, G. A., Benfey, T. J. and Sutterlin, A. M. 1984. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 36(4): 359-367.
- Sacobie, C. F., Glebe, B. D., Barbeau, M. A., Lall, S. P. and Benfey, T. J. 2012. Effect of strain and ploidy on growth performance of Atlantic salmon, *Salmo salar*, following seawater transfer. *Aquaculture*, 334: 58-64. <https://doi.org/10.1016/j.aquaculture.2011.12.014>.
- Seeb, J. E., Thorgaard, G. H. and Utter, F. M. 1988. Survival and allozyme expression in diploid and triploid hybrids between chum, chinook, and coho salmon. *Aquaculture*, 72(1-2): 31-48. [https://doi.org/10.1016/0044-8486\(88\)90144-5](https://doi.org/10.1016/0044-8486(88)90144-5).

- Tan, X., Qin, J. G., Chen, B., Chen, L. and Li, X. 2004. Karyological analyses on red claw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture*, 234(1-4): 65-76. <https://doi.org/10.1016/j.aquaculture.2003.12.020>.
- Teskeredzic, E., Donaldson, E. M., Teskeredzic, Z., Solar, I. I. and McLean, E. 1993. Comparison of hydrostatic pressure and thermal shocks to induce triploidy in coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 117(1-2): 47-55. [https://doi.org/10.1016/0044-8486\(93\)90122-f](https://doi.org/10.1016/0044-8486(93)90122-f).
- Ueda, T., Sato, R. and Kobayashi, J. 1988: The origin of the genome of haploid masu salmon and rainbow trout recognized in abnormal embryos. *Bull. Jap. Soc. Sci. Fish.*, 54: 619-625. <https://doi.org/10.2331/suisan.54.619>.
- Wickwire, R. 2000. *Salmonid triploidizing experiment*. Alaska Department of Fish and Game, Alaska, USA. www.kokanepower.org/articles/triploid.Pdf.
- Yesaki, T. Y., Scheer, K. W. and Greiner, D. L. 1996. Production-scale pressure shocking of rainbow trout (*Oncorhynchus mykiss*) in British Columbia. *Proceedings of the 47th Annual North-west Fish Culture Conference*, 170: 173.