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FLUCTUATING ASYMMETRY IN DIPLOID FEMALE AND STERILE TRIPLOID RAINBOW TROUT *(Oncorhynchus mykiss)*

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Fluctuating asymmetry in diploid female and sterile triploid rainbow trout (Oncorhynchus mykiss)

Viability of an organism and possibility to survive in natural environment could be judged by the magnitude of fluctuating asymmetry (FA) which is defined as random deviation from perfect symmetry of an organism. In order to estimate if there is the difference in FA between diploid female and sterile triploid rainbow trout *(Oncorhynchus mykiss)* the number of rays in pelvic and pectoral fins was determined on both sides of body in 150 individuals from two populations which were of the same genetic origin and were reared under same farm conditions. Units of asymmetry were determined as the absolute value of difference between counts on both sides of body. Results indicate that diploids exhibit larger FA than triploids in both traits; however the difference between both populations is statistically significant only if the number of units of asymmetry for both traits for each fish is summed up. The need to estimate the viability of these two populations on the basis of other traits is discussed and the necessity to use the metric traits to determine FA is stressed out.

Key words: fish / rainbow trout / *Oncorhynchus mykiss* / viability / fluctuating asymmetry / developmental stability / sterile triploids / diploid females

Fluktuacijska asimetrija pri diploidnih in sterilnih triploidnih samicah kalifornijske postrvi (Oncorhynchus mykiss)

Vitalnost nekega organizma in verjetnost, da preživi v naravi, je mogoče presojati na podlagi velikosti fluktuacijske asimetrije (FA). Ta je določena s tem, koliko telo določenega osebka odstopa od popolne simetrije. Da bi ocenili ali se samice in sterilni triploidni osebki kalifornijske postrvi *(Oncorhynchus mykiss)* med seboj razlikujejo v FA, smo pri 150 osebkih iz obeh populacij določili število plavutnic v prsnih in trebušnih plavutih na obeh straneh telesa. Kot mero za asimetrijo smo uporabili absolutno razliko v številu plavutnic v prsnih in trebušnih plavutih, na vsaki strani telesa. Rezultati kažejo, da je za obe lastnosti FA večja pri diploidnih osebkih; razlika med populacijama je dovolj velika, da jo lahko štejemo kot statistično značilno le, če obe lastnosti obravnavamo združeno. Delo problematizira možnost presojanja sposobnosti preživetja le na osnovi FA in poudarja, da bi bilo nujno za določanje FA uporabiti metrične lastnosti.

Ključne besede: ribe / kalifornijska postrv / *Oncorhynchus mykiss* / vitalnost / fluktuacijska asimetrija / razvojna stabilnost / sterilni triploidni organizmi / diploidne samice

1 INTRODUCTION

Fluctuating asymmetry (FA) is the phenomenon observed in organisms which are bilaterally symmetrical. It occurs when the trait on one side of body differs in a random way from the same trait on the other side. It can be expressed as a difference of the trait observed on the left and right sides; the difference can be expressed as difference in number, size, shape or some other feature. FA is a measurement of developmental stability; developmental homeostasis keeps FA at a low level. Increased level of FA reflects the fact that development of an organism was instable. This instability could be caused either by genetic factors or by environment. The number of studies investigating the impact of miscellaneous environmental stressors on developmental stability is rather high and the variety of organisms studied is wide (Eriksen *et al.,* 2008). Two opposite hypotheses exist regard-

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ing the relationship between genetic factors and FA; the first one claims that more heterozygous individuals have greater developmental stability which results in lower FA, while second one believes that when genes complexes which ensure developmental stability are disrupted (for instance by hybridization which increases heterozygotity) stability decreases and FA is elevated (Wilkins *et al,* 1995).

In fish it is possible to experimentally manipulate the chromosome sets. By different techniques individuals of the same species with different sets of chromosomes originating from both parents or from one parent only could be produced (Komen and Thorgaard, 2007). In fish crossing of individuals from different species many times results in viable offspring. Fishes and specifically salmonids were therefore used to study the relationship between heterozygosity and developmental stability for types of heterozygotity introduced by manipulating chromosome sets or hybridization which could be found only exceptionally in natural populations (Leary *et al.,* 1985; Wilkins *et al*., 1995; Young *et al,* 1995; V0llestad and Hindar, 2001).

Populations of rainbow trout where all individuals are either females (resulting from fertilizing ova of "normal" females with sperm of individuals which are genetically females but produce sperm) or sterile triploids (resulting from retention of second polar body in such ova induced by thermal or pressure shock) are widely used in aquaculture practice due to apparent advantage of such populations over populations of mixed sexes in production traits. In order to prevent reproduction of introduced species which could endanger native population it is also suggested that only such populations should be used for restocking waters where introduced species is not native (Aprehamian *et al.,* 2001). The question of interest for aquaculturists and fish managers is whether sterile triploids exhibit larger viability than females. According to Leary *et al.* (1984) viability of an organism and possibility to survive in natural environment could be judged by the magnitude of FA. The aim of our study was therefore to compare the difference in FA between two populations of rainbow trout; namely population of all-females and population of sterile triploids.

2 MATERIALS AND METHODS

Number of pectoral and pelvic fin rays on left and right side of 150 females and 150 sterile triploids was determined by counting. After the fish were euthanized by electric shock the pectoral and pelvic fins on both sides were cut off and put for one minute into 50% solution of NaOH. The macerated skin and muscle tissue adjoining rays was removed by tweezers; the fin was put on blotting paper to dry up. The number of rays was established when pulled out from fin base one by one. All the counting was done by same person. For a batch of fish treated at the same day, the counting was consecutively done for each fish first on one side and then on the other side in order to avoid bias which could result from knowing the count on one side of individual fish while counting the number on the other side. For each fish and for each fin asymmetry was determined by subtracting the count on one side from the count on the opposite side.

Both populations from which fish were sampled were of the same age (at the beginning of sampling 240 days after hatching) and reared under same condition at Fish research station Pšata which belongs to Biotechnical faculty of University of Ljubljana. Each day approximately 20 fish from both groups were sampled and treated.

Both groups were of the same genetic origin. Eggs stripped from broodstock kept at Fish research station Pšata were fertilized by sperm obtained from sex-inverted females using the methodology described by Ingram (1986). After fertilization half of eggs were put directly in incubation trays for incubation while half of eggs were firstly exposed to temperature shock and after that treated the same way as the eggs not exposed to temperature treatment. The heat shock was done according to slightly adapted method described by Ingram (1986). By heat shock triploid induction could range from 10 to 100% (Solar *et al.,* 1984). Only those fish from second group were used for FA in which examination of gonads revealed sterility.

3 RESULTS

The average, the lowest and the highest number of rays in pectoral and pelvic fins in both groups is shown in Table 1. The average number of pectoral rays is higher than average number of pelvic rays. The same was observed by Leary *et al.* (1985). The lowest number of pelvic and pectoral rays observed in diploids is lower than in triploids, while highest number does not differ between two groups. The average number of observed pectoral rays and pelvic rays found in our population is similar to numbers given by Leary *et al.* (1985). The difference in the average number of pelvic and pectoral rays between two groups is not statistically significant.

The numbers obtained by subtracting the count of rays on one side of body from the count on the opposite side is shown in Table 2. These numbers are the measure of asymmetry. If the count for specific trait was the same on both sides, such fish was not considered to be asymmetric for the trait under consideration. As it could

Table 1: *Average, minimum and maximum number of rays for pelvic and pectoral fins in diploid and triploid animals*
Preglednica 1: *Povprečno ter najnižje in najvišje število plavutnic v prsnih in trebušnih plavutih diploidnih in triploidnih živali*

Number of rays	Diploids			Triploids		
	Min	Max	Average	Min	Max	Average
Pectoral fins	24	32	28.56	26	32	28.81
Pelvic fins	16	23	20.94	19	23	21.04

be seen from the numbers in this table, fishes could exhibit the difference of 1, 2, 3 or 4; the latest number is the maximum difference in count for pectoral fin rays as well as pelvic fin rays. For both traits under consideration the majority of fish in both groups were not asymmetric; for pelvic fins around 80% of all fish did not exhibit asymmetry, for pectoral fins more than 60% of the fish were symmetric. It is somehow surprising that pelvic fins which on average have lower number of rays are more asymmetric than pectoral fins. Figures indicate that the percentage of asymmetric individuals is higher in the population of diploids that in the population of triploids. The difference between two populations is 4.7% for pectoral fins and 4.3% for pelvic fins. x^2 - test revealed that difference in numbers of individuals exhibiting 0, 1, 2, 3 or 4 disparity in counts for each trait in two populations is not large enough to be considered statistically significant.

On the basis of the same test done after pooling all individuals which were not symmetric into one group and comparing the numbers of animals in this group with the numbers of animals which were symmetric conclusion was alike even the test was done with lower degrees of freedom. For pectoral fins there were 84 symmetric versus 66 asymmetric individuals in group of diploids and 91 symmetric versus 59 asymmetric individuals in group of triploids. For pelvic fins the numbers of symmetric and asymmetric fish were 111 versus 39 for diploids and 119 versus 31 for triploids. The difference was too small to be statistically significant.

Since we were measuring the magnitude of asymmetry (the number of units by which the left and right side differed) in two traits, we were able to summing the number of units of asymmetry for both traits for each fish. These numbers presented in Table 3 characterize the total magnitude of asymmetry for individual fish.

By such methods the magnitude of asymmetry was increased; there were fishes for which the summed number of units by which the left and right side differed in both traits was as much as 6. x^2 - test revealed that difference in numbers of individuals exhibiting 0, 1, 2, 3, 4, 5 or 6 disparity in pooled counts for both traits in two populations is large enough to be considered statistically significant at P = 0.050. (calculated x^2 value was 12,762).

The asymmetry measured by magnitude defined in such a way was increased; the numbers of symmetric and asymmetric fish were 65 versus 85 for diploids and 69 versus 81 for triploids.

4 CONCLUSIONS

Our results demonstrate that sterile triploid rainbow trout exhibit lower asymmetry than diploid females. The percentage of fish which were asymmetric was larger in the group of diploid females than in the group of sterile triploid for both traits investigated. Even the samples were rather large, these differences were not statistically significant when each trait was considered separately; the difference in pooled counts for both traits exhibited significance. Compared with the samples of similar experiment found in literature, samples used in our experiment were rather large. Wilkins *et al.* (1995) compared FA in diploids and triploids of Atlantic salmon *(Salmo salar)* and hybrids between Atlantic salmon and brown

Table 2: *Number and percentage of diploid and triploid animals exhibiting (a)symmetry for pectoral and pelvic fins*
Preglednica 2: *Število in odstotek diploidnih in triploidnih živali, ki kažejo (a)simetrijo v prsnih in trebušnih plavutih*

Asymmetry measured as L-R ray count (absolute value)	Pectoral fins				Pelvic fins			
	Diploids		Triploids		Diploids		Triploids	
	Number of animals	%	Number of animals	%	Number of animals	%	Number of animals	%
0	84	56.0	91	60.7	111	74.0	119	79.3
1	14	9.3	19	12.7	15	10.0	18	12.0
2	46	30.7	39	26.0	23	15.3	13	8.7
3	1	0.7	0	0.0	0	0.0	0	0.0
4	5	3.3	1	0.7	1	0.7	0	0.0
Total	150	100.0	150	100.0	150	100.0	150	100.0

Table 3: *Number and percentage of diploid and triploid animals exhibiting (a)symmetry for pectoral and pelvic fins*
Preglednica 3: *Število in odstotek diploidnih in triploidnih živali, ki kažejo (a)simetrijo v prsnih in trebušnih plavutih*

Units of asymmetry after summing L-R absolute difference for both traits	Diploids		Triploids	
	Number of animals	%	Number of animals	%
0	65	43.3	69	46.0
1	18	12.0	30	20.0
2	45	30.0	43	28.7
3	9	6.0	3	2.0
4	9	6.0	5	3.3
5	1	0.7	0	0.0
6	3	2.0	0	0.0
Total	150	100.0	150	100.0

trout *(Salmo trutta)*. They collected data from 40 diploid salmon, 19 triploidised salmon, 41 hybrids and 41 triploidised hybrids. Their results indicate that triploidised salmon had FA values which were very similar to those of diploid salmon. There were 21% of triploids and 18% of diploids asymmetric in pectoral fin rays. The percentage of asymmetric individuals in pelvic fins was 11% and 21% respectively. None of differences between two groups was statistically significant. In our experiment the percentage of asymmetric individuals for both traits under consideration was higher.

The direct comparison of our results with these results is not possible. Species examined by Wilkins *et al.* (1995) and species examined in our research belong two different groups of salmonids. Our results can also not to be directly compared to results of Leary *et al.* (1985) even they studied the same species as we did since the main goal of their research was to compare developmental stability of gynogenetic diploid and triploid rainbow trout. Nevertheless, to some extend their results could be used for a comparison with ours as in addition to data collected on experimental population of triploids they also presented data on population of diploids collected in fish from commercial farms. These data show that triploids and diploids do not differ in absolute numbers of rays in pectoral and pelvic fins. For them this is one of the indicators that morphology of the triploid is similar to that of their diploid counterparts. The same can be concluded from our results shown in Table 1. The difference between diploids and triploids regarding these traits and the absolute numbers which were found for both traits in our research are similar to the results presented by them. Their conclusion about FA is the same as the one which can be done on the basis of our results: triploids had less FA than diploids. The mean magnitude of asymmetry which is the sum of the absolute values of the left minus right counts for all traits per individual were 1.67 and 1.76 for two strains of triploids compared to 2.08 and 1.90 for diploids of same strains. In our research these values were 0.97 for triploids and 1.29 for diploids. The difference in absolute numbers resulted from the fact that in our research two meristic traits (pelvic and pectoral fins) were used to calculate this value while Leary *et al.* (1985) used five meristic counts. In research done on salmon and trout (Wilkins *et al.* 1995) values of mean magnitude of asymmetry were 0.90 for diploid and 0.79 for triploid salmon. Three meristic traits were used: number of gill rackers, number of rays in pelvic fins and number of rays in pectoral fins.

In the focus of the question of interest for aquaculturists and fish managers whether sterile triploids exhibit larger viability than females if they exhibit lower FA it is worth to mention the view of Leary *et al.* (1984) that lower FA in triploids does not indicate that triploidity has no deleterious effects on the development of rainbow trout. Therefore the comparisons of viability of two populations should be done on the basis of other traits. The most appropriate would be to use traits which are important when fish is grown for human consumption or for restocking, like growth rate, survival, resistance to diseases.

Majority of studies investigating FA in salmonids were based on meristic traits. (Young *et al.,* 1995; Sanchez-Galan *et al.* 1998; Young *et al.,* 2009; Skog Eriksen *et al.,* 2008). The metric traits were used only in few studies. V0llestad *et al.* (1998) for example measured the diameter of left and right eye as well as upper and lower left and right jaw length in grayling *(Thymallus thymallus)*.

General conclusion about methods used either for performing counts of meristic traits or measuring metric traits is that they are characterized as tedious tasks with low precision. Therefore it would be of great benefit for further research in this area to find method which could be used to count or measure traits with higher precision and speed.

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