fishes

MDPI

Article

Matings Between Individuals with Similar Major Histocompatibility Complex (MHC) Improve Offspring Survival in the Rainbow Trout (*Oncorhynchus mykiss*)

Cinzia Bergamino [1], Mattia Tomasoni [2], Silvia Colussi [1,*], Vittoria Montemurro [1], Ilhan Altinok [3], Marino Prearo [1], Charalampos Kotzamanidis [4], Pier Luigi Acutis [1], Ana Isabel Vela [5], Simona Sciuto [1], Giuseppe Esposito [1], Donatella Volpatti [6], Elena Bozzetta [1], Katia Parati [7], Lucia Aidos [8], Lucio Fariano [2] and Benedetto Sicuro [9]

[1] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, 10154 Turin, Italy; cinzia.bergamino@izsplv.it (C.B.); vittoria.montemurro@izsplv.it (V.M.); marino.prearo@izsplv.it (M.P.); pierluigi.acutis@izsplv.it (P.L.A.); simona.sciuto@izsplv.it (S.S.); giuseppe.esposito@izsplv.it (G.E.); elena.bozzetta@izsplv.it (E.B.)
[2] Azienda Agricola Canali Cavour, 12044 Centallo, Cuneo, Italy; luciof@libero.it (L.F.)
[3] Faculty of Marine Sciences, Karadeniz Technical University, 61530 Sürmene, Trabzon, Turkey; ialtinok@gmail.com
[4] Veterinary Research Institute, ELGO-DIMITRA, 57001 Thessaloniki, Greece; kotzam@vri.gr
[5] Center for Veterinary Health Surveillance, Universidad Complutense de Madrid, 28040 Madrid, Spain; avela@ucm.es
[6] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, 33100 Udine, Udine, Italy; donatella.volpatti@uniud.it
[7] Istituto Sperimentale Italiano L. Spallanzani, 26027 Rivolta d'Adda, Cremona, Italy; katia.parati@istitutospallanzani.it
[8] Department of Veterinary Sciences and Animal Science, University of Milan, 26900 Lodi, Lodi, Italy; lucia.aidos@unimi.it
[9] Department of Veterinary Sciences, University of Turin, 10095 Grugliasco, Torino, Italy; benedetto.sicuro@unito.it
* Correspondence: silvia.colussi@izsplv.it

Abstract: The major histocompatibility complex (MHC) consists of genes involved in immune response and molecular discrimination between self and non-self. MHC genes are the most polymorphic in vertebrates. The origin and maintenance of polymorphism in MHC genes in populations is still unresolved. Mechanisms such as sexual selection and heterozygote advantage have been suggested as explanations for this high variability. In this study, a farmed population of rainbow trout (*Oncorhynchus mykiss*) characterized by the presence of specific MHC class IIB gene haplotypes at a frequency higher (30%) than that expected from random matings was investigated. Therefore, it was hypothesized that disassortative matings occur with an adaptive advantage for females, resulting in improved reproductive performance when mated with individuals with similar MHC haplotypes. Genetic analyses of the breeders were performed to define the MHC haplotypes and to perform specific matings. The effect of mating was evaluated by analyzing the survival rate of the offspring at various stages of incubation until swim-up. The reproductive performance of the offspring derived from specimens with similar haplotypes showed a better survival trend during the first life stages and reduced malformations. The results obtained are in contrast with the heterozygous advantage theory, therefore it was hypothesized, as for other salmonid species, the presence of a positive selection towards locally adapted MHC genes that promotes reproduction between genetically similar individuals.

Keywords: similar haplotype; offspring survival; reproductive performance; larvae deformity

Key Contribution: This study reveals that similar MHC class IIB haplotypes may improve offspring survival and reduce malformations in rainbow trout, indicating positive selection toward local adaptation.

Citation: Bergamino, C.; Tomasoni, M.; Colussi, S.; Montemurro, V.; Altinok, I.; Prearo, M.; Kotzamanidis, C.; Acutis, P.L.; Vela, A.I.; Sciuto, S.; et al. Matings Between Individuals with Similar Major Histocompatibility Complex (MHC) Improve Offspring Survival in the Rainbow Trout (*Oncorhynchus mykiss*). *Fishes* **2024**, *9*, 436. https://doi.org/10.3390/fishes9110436

Academic Editor: Changan Wang

Received: 12 September 2024
Revised: 24 October 2024
Accepted: 25 October 2024
Published: 27 October 2024

1. Introduction

The major histocompatibility complex (MHC) is the most important genetic component of the immune system and the most polymorphic gene found in vertebrates. The MHC is composed of class I and class II genes; the first plays a fundamental role in presenting endogenous antigens to CD8+ T-cells, while MHC class II genes are involved in presenting exogenous antigens to CD4+ T-cells [1,2].

MHC polymorphisms are linked to disease resistance, thus may influence the long-term survival probability of populations [1]. As such, it has become a model study for evaluating how selection could act to maintain significant adaptive genetic diversity in populations [2]. Reproductive mechanisms such as disassortative mating and maternal–fetal interactions have been suggested as mechanisms that maintain MHC diversity [1,3]. The hypothesis that MHC diversity might be linked to sexual behavior and mate choice arises from the capacity of MHC to distinguish self from non-self at the cellular level, and thus may also underpin a mechanism for kin recognition at the organism level [2]. This type of identification may also involve olfactory systems, which have been identified in a variety of species, including fish [3,4].

Numerous adaptive hypotheses have been proposed to explain how MHC might influence mating behavior and reproductive success [2]. Dissimilar MHC mating preferences may act to increase offspring heterozygosity, to avoid inbreeding or genetic incompatibility, or to achieve optimal MHC diversity in offspring [1,2].

MHC-dependent matings may favor matings with males with a different MHC profile, as found in the Atlantic salmon (*Salmo salar*) [5,6], although reproductive success is maximized in matings between individuals with an intermediate level of MHC dissimilarity [6]. In the rosy bitterling (*Rhodeus ocellatus*), the females produce a higher number of eggs when mating with MHC-dissimilar males, and offspring survival was increased in the presence of MHC variations [7]. A similar situation occurs in the chinook salmon (*Oncorhynchus tshawytscha*) in which females mate non-randomly based on amino acid divergence, resulting in offspring with MHC class IIB that are more divergent in the amino acid sequence [8].

Several studies have identified an ideal number of MHC alleles because a higher number of different alleles may increase the risk of developing autoimmune responses [9]. This mechanism has been found in the three-spined stickleback (*Gasterosteus aculeatus*), in which females prefer the odor of males with optimal intermediate allelic diversity [3,4,9]. In fact, in this species, MHC peptide ligands have been found to be used as olfactory cues to discriminate between different MHC profiles [4]. Furthermore, in this species, individuals with intermediate MHC diversity have greater reproductive success [10]. Moreover, in the brown trout (*Salmo trutta*), it was observed that individuals with an intermediate MHC dissimilarity mate with high frequencies, and males produce a greater number of offspring, supporting an MHC-dependent model of mate choice optimality [11].

In contrast to the findings that demonstrate the mechanisms promoting the heterozygosity of the MHC complex, selection towards similar MHCs was found in some species [12]. In the Atlantic salmon [12] and the guppy (*Poecilia reticulata*) [13], the males with higher reproductive success have an MHC similar to that of the females. This suggests that the preference for MHC-dissimilar mates is not so unanimous [13].

Furthermore, some studies have not highlighted the presence of MHC-dependent matings, such as in the whitefish (*Coregonus* spp.) [14] and the Atlantic salmon (*S. salar*) [15]. Indeed, studies examining the role of MHC in mate choice have drawn contrasting conclusions, indicating that it is still far from properly understood which processes maintain MHC diversity in the populations [2,9].

In this study, we investigated a farmed population of rainbow trout (*O. mykiss*) characterized by a frequency of a specific MHC IIB haplotype of 30%, a value substantially higher than the mean frequency found in the other haplotypes (1.06 $\pm$ 1.39 %; unpublished data). This haplotype frequency was higher than expected in random mating, indicating the presence of disassortative mating towards specific individuals carrying the "preferred"

haplotype within this population. Therefore, we hypothesize that disassortative mating is adaptive for females, resulting in improved reproductive performance when mated with individuals with similar MHC. To this end, broodstock was genetically analyzed to define MHC class IIB haplotypes and to perform specific matings. Because eggs from a particular female may produce different results when fertilized by different males, we performed a series of mating combinations between the studied specimens. An experimental design was developed to evaluate the reproductive performance of genetically mated rainbow trout. We finally evaluated the effect of mating between individuals with similar MHC through the analysis of the survival rate of the offspring at various stages of incubation until swim-up.

2. Materials and Methods

2.1. Genetic Analysis

Experimental rainbow trout (*O. mykiss*) belonged to a fish farm located in northwest Italy. Buccal swabs have been conducted to collect genomic DNA from broodstock 3–4 years old (50 males and 50 females) at first spawning, univocally identified using implantable microchips.

DNA was manually extracted using the kit ReliaPrep™ (Promega, Madison, WI, USA). PCR and sequencing analyses were carried out on the MHC class II gene. A 257-base-pairs fragment (including primers) of exon 2, encoding for the polymorphic $\beta-1$ domain of the protein (from position 33 to 112), was amplified using the primers B1RA and B1FA, as described by Miller [16]. PCR was carried out in a total volume of 25 µL containing 30–50 ng of genomic DNA using Platinum® qPCR Supermix-UDG (Invitrogen, Carlsbad, CA, USA) and the primers (300 nM each). PCR conditions were the same as reported by Miller [16]. The same primers were also used for sequencing, with BigDye 1.1 chemistry and the ABI3130 genetic analyzer. Sequence alignment was performed using the Lasergene SeqMan software v. 5.0.1 (DNASTAR, Madison, WI, USA).

For each individual, haplotypes were defined based on 23 single nucleotide polymorphisms (SNPs) using PHASE software v. 2.1 following the methodologies described by Stephens [17].

2.2. Mating on a Genetic Base

The broodstock with known haplotypes were periodically monitored for ovulation, in order to avoid post-ovulatory aging of the eggs (over-ripening) and to maintain high egg quality. The abdomens of the females were gently squeezed to release the eggs along with the ovarian fluid, taking care not to contaminate the samples. The eggs were then collected and placed in a sieve to extract the ovarian fluid. After stripping, artificial reproduction was performed according to the methods routinely applied at the fish farm, where the eggs were washed in a saline solution buffered at pH 9.0 and the sperm previously collected and stored in a saline solution enriched with potassium, mixed with the eggs and gently stirred.

A total of 14 females (4 individuals with a homozygous haplotype 2/2, 4 with a heterozygous haplotype 2/x, and 6 with a haplotype x/y, where x and y represent any haplotype other than 2) and 12 males (4 per haplotype) were selected for reproduction. Matings were performed on a genetic basis without the possibility of female choice. Eggs from each female were incubated separately in order to track their reproductive performance. Incubation was performed in a continuous water flow system at a temperature of about 9 °C, in a thin layer tray (10 × 10 × 8 cm).

2.3. Female Morpho-Physiological Parameters and Reproductive Performance

Before stripping, each selected female was weighed and measured for length. After stripping, the retrieved eggs were weighed (Table 1). Within 4–6 h from the sampling, pH of the ovarian fluid was measured with the pH meter (Hach sensION™ + pH3). Morpho-physiological parameters (weight, length, egg weight, pH in the ovarian fluid of the females) did not differ significantly among the female groups ($p > 0.05$) (Table 1).

Table 1. Mean ± standard deviation of the morpho-physiological parameters of the females.

	2/2	2/x	x/y	F	*p*-Value
Female weight (g)	1799.00 ± 844.41	2508.00 ± 368.48	2081.00 ± 354.89	1.77	0.22
Female length (cm)	51.00 ± 8.61	57.75 ± 3.59	53.75 ± 5.30	1.27	0.32
pH ovarian fluid	8.20 ± 0.11	8.22 ± 0.21	8.24 ± 0.07	0.12	0.89
Egg weight (mg)	45.01 ± 5.92	51.31 ± 10.13	54.70 ± 2.74	2.76	0.11

For each parameter, the comparison between haplotypes was performed with an ANOVA test.

After fertilization, each pool of eggs, belonging to a single mating, was weighed, manually counted, and placed in a tray. The total fecundity and the relative fecundity were then estimated through Equations (1) and (2) [18]:

$$Total\ fecundity = \frac{number\ of\ eggs}{female} \quad (1)$$

$$Relative\ fecundity = \frac{number\ of\ eggs}{female\ weight} \quad (2)$$

Eyeing, hatching, larval deformities, irregular absorption of the yolk sac, and swim-up rates were evaluated to analyze the percentage of frequencies.

At the eyeing stage (255 degree-day), dead eggs were separated from the eyed ones, and the eyed rate was calculated following Equation (3) [19]:

$$Eyeing\ rate = \frac{(number\ of\ eyed\ eggs \times 100)}{number\ of\ eggs} \quad (3)$$

After the evaluation of the eyeing rate, 250-eyed eggs for each mating were left in their tray. During the hatching phase, eggs were regularly checked, and dead eggs were collected and counted. The hatching rate was calculated according to Equation (4) [19]:

$$Hatching\ rate = \frac{(number\ of\ hatched\ eggs \times 100)}{number\ of\ eyed\ eggs} \quad (4)$$

Subsequently, dead larvae with deformities were collected and counted to estimate the larval deformity rate according to Equation (5) [20]:

$$Larvae\ deformity\ rate = \frac{(number\ of\ larvae\ with\ deformities \times 100)}{number\ of\ alive\ larvae} \quad (5)$$

At the end of the endogenous feeding phase, the number of larvae with an abnormal yolk sac absorption and the number of larvae that survived to the end of incubation were recorded. The corresponding rates were calculated according to Equations (6) [20] and (7) [19]:

$$Irregular\ absorption\ of\ the\ yolk\ sac\ rate = \frac{(number\ of\ irregular\ absorption\ of\ the\ yolk\ sac\ larvae \times 100)}{number\ of\ alive\ larvae} \quad (6)$$

$$Swim\ up\ rate = \frac{(number\ of\ alive\ larvae\ after\ yolk\ sac\ absorption \times 100)}{number\ of\ alive\ larvae} \quad (7)$$

Finally, the trend of the survival rate was evaluated considering the whole incubation period (530 degree-day).

2.4. Characterization of the Larval Deformity

After hatching, a representative sample of the frequency of dead larvae with deformities was collected for each female haplotype (52 samples from genotype 2/2, 287 samples from genotype 2/x, and 136 samples from genotype x/y). Macroscopical analysis focused on the characterization of larval deformities [20]. The larvae were fixed in a 10% buffered

formalin solution and routinely processed. A sub-sample of larvae (20) and all the larvae showing prognathism (10) were histologically analyzed to confirm the initial classification. The samples were routinely embedded in paraffin wax blocks following standard histological techniques and then sectioned at 5 µm thickness through the automated Leica SM2010 R sliding microtome and stained with haematoxylin-eosin (HE). Samples were then observed by pathologists under a light microscope to evaluate the deformities (Zeiss Axio Scope, A1, Jena, 63 Germany) at increasing magnification (10×, 20×, 40×) and digital images were captured by Scanner Axio Scan Z1 Carl Zeiss GmbH.

2.5. Statistical Analyses

Statistical analyses were performed using R software ver. 4.2.3. The presence of differences between haplotypes, for the morpho-physiological parameters of the females, was assessed with an ANOVA and a pairwise *t*-test with Bonferroni correction as a post-hoc test. The other parameters related to the mating on a genetic basis (eggs and larvae), were analyzed with the Kruskal–Wallis test and the Dunn's tests with Bonferroni correction as a post-hoc test.

A generalized linear model with gamma distribution was adopted for analyzing the survival rate, considering the survival rate at each stage as a dependent variable and the mating type and the time as predictors. For different causes, one male with haplotype x/y and two females with haplotype 2/x were excluded from the experiment. Therefore, the mating F 2/x + M x/y was not included in data analyses due to the absence of replicas.

2.6. Ethics Declarations

The procedures were conducted by the farmers following routine external fertilization practices. Observations of deformities were restricted to dead larvae, and no live specimens were subjected to any experimental interventions.

3. Results

3.1. Genetic Analysis

SNPs positions and variability were obtained using GenBank reference sequences U20943 and U20944 in accordance with that published by Colussi [21]; results are reported in Supplementary Materials (Table S1). Samples with missing genotypes were removed from the study and are not reported in Table S1.

Table 2 lists all the haplotypes found for each subject included in the study. The 23 SNPs that define the haplotype 2 present in the individuals 2/2 and 2/x are reported (Table 3).

Table 2. Haplotypes generated using the software PHASE 2.1 based on Bayesian statistics reported in males and females.

Males ID	Males' Haplotype	Females ID	Females' Haplotype
1	2/34	4	4/42
2	14/51	5	38/47
3	2/24	6	2/42
6	3/45	7	23/42
7	47/56	8	28/49
8 *	2/2	9	18/29
10	1/54	10	41/58
11	42/66	11	39/44
12	60/62	12 *	2/2
13	24/24	13	2/45
15	53/67	14	47/47
17	15/32	15	2/38
18	2/3	16	11/33

Table 2. *Cont.*

Males ID	Males' Haplotype	Females ID	Females' Haplotype
19	35/69	17	27/31
20	7/27	18	27/42
27	13/43	19 *	2/2
31	55/55	20	42/49
32 *	2/2	21	30/52
33	5/19	22	22/27
35	21/47	23	2/42
36 *	2/2	24	25/27
37	8/63	25	48/52
39	26/50	26	59/16
40	46/46	28	2/38
41	33/65	29	47/47
42	24/47	30	9/22
43	1/55	31	17/40
44	20/27	32	17/42
45	57/68	33	2/27
46	24/64	34	2/27
47	2/38	37	2/42
48	2/16	38	6/10
49 *	2/2	40	2/38
-	-	41	27/47
-	-	43	42/42
-	-	44	22/52
-	-	45	36/37
-	-	46 *	2/2
-	-	47	38/41
-	-	48	12/15
-	-	49 *	2/2
-	-	50 *	2/2

* Individuals with a 2/2 haplotype.

Table 3. SNPs composition of the haplotype 2 (23 SNPs in total).

132	133	136	143	145	154	158	163	194	210	211	217	235	272	286	287	296	301	303	305	307	308	310	Haplotype
T	A	G	A	G	A	C	T	T	G	T	C	T	T	T	T	A	G	T	C	A	A	C	2

3.2. Reproductive Performance

Total fecundity did not differ significantly among the female groups (2/2: 5312 ± 3771; 2/x: 3710 ± 1000; x/y: 4006 ± 1046; $F = 0.65$; $p > 0.05$). A significant difference between the 2/2 haplotype and the 2/x haplotype was found for the relative fecundity ($F = 4.34$, $p < 0.05$), while no differences were found for the haplotype x/y. Indeed, females with the 2/2 haplotype produced an average of 2925 eggs per kilogram of live weight, which was higher than the average of females with the 2/x haplotype (1476 eggs kg^{-1}). With regard to the reproductive performance parameters, eyeing rate, hatching, and irregular absorption of the yolk sac did not differ significantly among groups (Table 4). On the other hand, larvae deformity rate showed a significant difference; mating between females and males with a 2/2 haplotype had a lower percentage of larvae deformities than mating between females and males with a 2/x haplotype ($p < 0.05$), while the proportion of malformations in the other mating was moderate. The swim-up rate differed slightly among groups, but the post-hoc test revealed no significant results due to the overlapped variance.

Table 4. Mean ± standard deviation (in percentage) of the reproductive performance parameters at different stages, from the eyeing rate to the swim-up rate, in relation to the matings type.

	F 2/2 + M 2/2	**F 2/2 + M 2/x**	**F 2/2 + M x/y**	**F 2/x + M 2/2**	**F 2/x + M 2/x**	**F x/y + M 2/2**	**F x/y + M 2/x**	**F x/y + M x/y**	**H**	***p*-Value**
Eyeing rate	79.55 ± 20.34	83.66 ± 7.87	90.41 ± 6.41	66.74 ± 16.64	66.50 ± 15.86	81.51 ± 22.06	74.78 ± 31.10	61.24 ± 41.51	6.15	0.52
Hatching rate	99.10 ± 1.05	99.50 ± 0.60	99.36 ± 0.73	98.10 ± 1.24	98.00 ± 1.03	99.28 ± 0.66	99.33 ± 0.83	99.62 ± 0.37	10.57	0.16
Larvae deformity rate	1.62 ± 0.88 ^{b}	2.11 ± 1.07 ^{ab}	2.50 ± 1.62 ^{ab}	10.70 ± 4.14 ^{ab}	13.52 ± 6.33 ^{a}	5.16 ± 3.41 ^{ab}	3.14 ± 3.14 ^{ab}	3.36 ± 0.49 ^{ab}	21.01	<0.01
Irregular absorption of the yolk sac rate	3.53 ± 2.14	2.71 ± 1.10	3.69 ± 3.64	5.03 ± 3.58	4.20 ± 3.36	5.06 ± 5.19	4.29 ± 2.83	3.45 ± 1.48	2.24	0.95
Swim-up rate	94.85 ± 1.68 ^{a}	95.17 ± 1.10 ^{a}	93.81 ± 2.18 ^{a}	84.27 ± 6.51 ^{a}	82.28 ± 8.98 ^{a}	89.79 ± 4.90 ^{a}	92.57 ± 3.03 ^{a}	93.18 ± 1.75 ^{a}	18.16	<0.05

For each parameter, the comparison between haplotypes was performed with the Kruskal–Wallis test. Values with different superscript letters (a, b) indicate significant differences. The mating F 2/x + M x/y has been excluded from the analysis.

The evolution of survival rates was analyzed using a generalized linear model and the mating type and time had a substantial impact on the survival rate (Figure 1). Significant effects were seen for the mating types F 2/x + M 2/2, F 2/x + M 2/x, and F x/y + M x/y, which resulted in a decreased survival rate than the F 2/2 + M 2/2 mating.

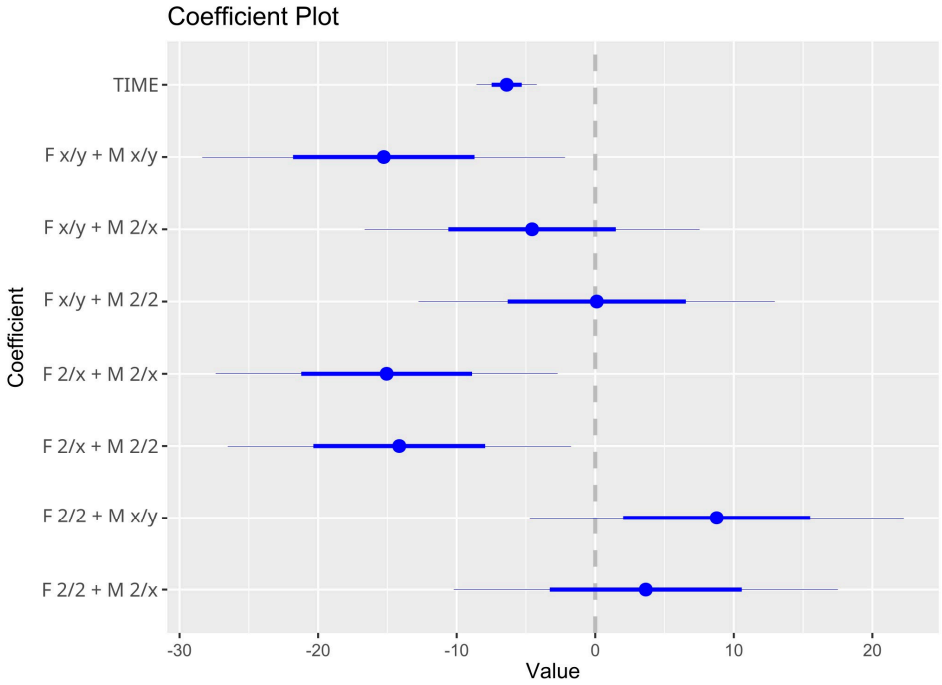

Figure 1. Results of the generalized linear model on the evolution of the survival rate in relation to the mating type. The matings F x/y + M x/y, F 2/x + M 2/x, and F 2/x + M 2/2 showed a significantly lower survival rate than the F 2/2 + M 2/2 mating, which was set as the reference level for the GLM model. The mating F 2/x + M x/y has been excluded from the analysis.

3.3. Characterization of the Larval Deformities

Through macroscopic evaluation of the larvae, the following abnormalities were observed: skeletal torsion, referring to twisting or rotational misalignment of skeletal elements

(Figure 2a); early yolk sac absorption (Figure 2b); cyclopia (Figure 2c); spinal deformation, generally defined as any abnormal shapes of the skeleton (Figure 2d); prognathia (Figure 2e); ocular anomalies and other types of alterations, including minor deformities (Figure 2f). Moreover, histological analyses confirmed the classifications of spinal deformation (Figure 3a) and prognathia (Figure 3b) made through macroscopic evaluations.

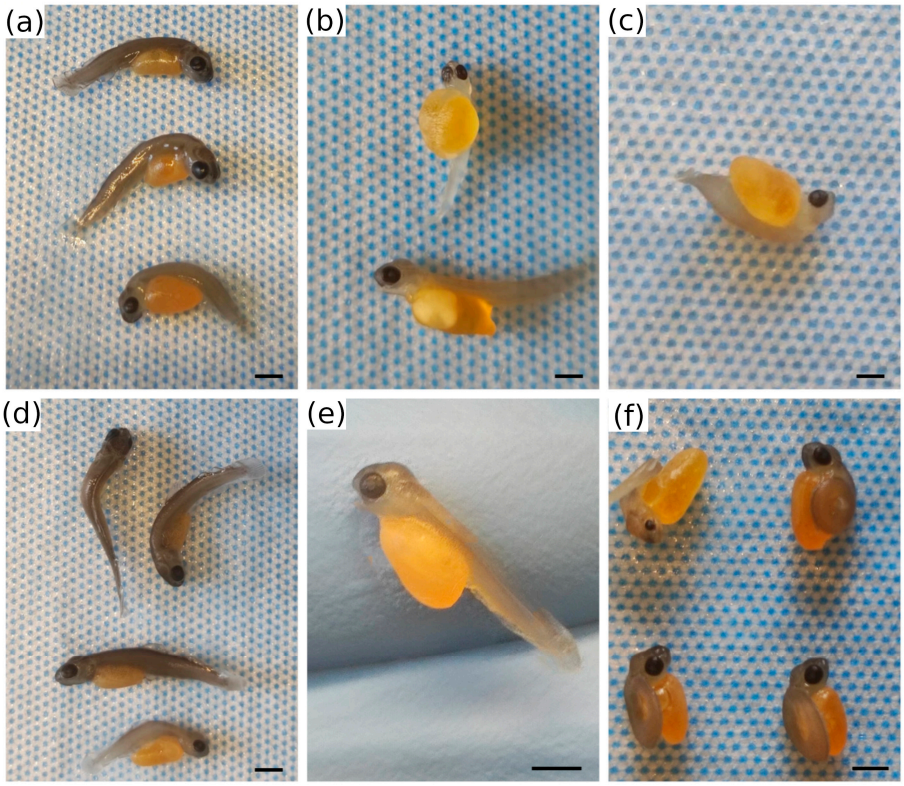

Figure 2. Morphological features of larvae deformities: (**a**) torsion; (**b**) early yolk sac absorption; (**c**) cyclopia; (**d**) spinal deformities; (**e**) prognathia; (**f**) other malformations. Scale bar: 2 mm.

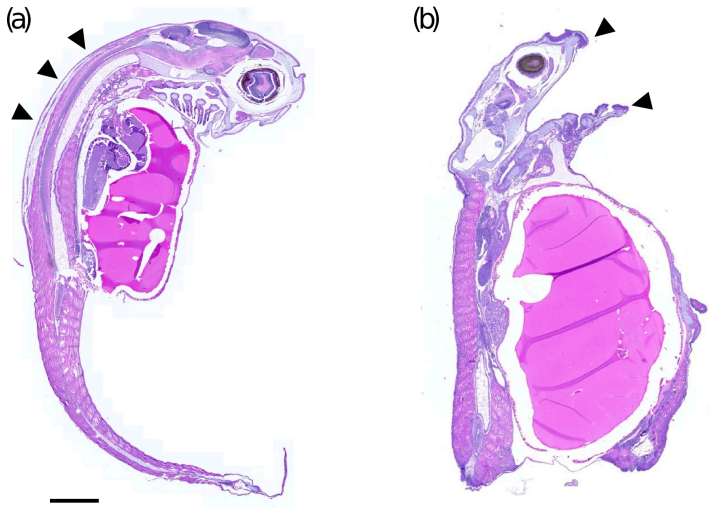

Figure 3. Histological details of specific larvae deformities: (**a**) spinal deformation; (**b**) prognathia. Scale bar: 400 µm.

The type and percentage of larval deformities found for each haplotype are shown in Table 5. The classification of larval deformities highlighted that spinal deformations were the most frequent malformations, accounting for about 50% of the deformities found

regardless of the maternal haplotype. Larvae derived from females with a 2/2 haplotype showed a slightly higher frequency of spinal deformities, torsions, and altered yolk sac resorption, but did not show other types of malformations. In parallel, the larvae derived from females with 2/x and x/y haplotypes also showed prognathia, ocular alterations, and other malformations. Moreover, the larvae with a 2/x haplotype had a higher frequency of ocular abnormalities, while those with the x/y haplotype exhibited a higher frequency (10%) of other malformations.

Table 5. Types of deformities and frequencies for each haplotype, expressed in percentages.

Type of Larval Deformity	2/2	2/x	x/y
Torsion	38.46	36.76	30.31
Early yolk sac resorption	5.77	2.21	1.39
Cyclopia	1.92	0.00	1.39
Spinal deformities	53.85	53.68	50.87
Prognathia	0.00	4.41	4.88
Ocular alterations	0.00	1.47	0.35
Other malformations	0.00	1.47	10.80

4. Discussion

In this study, a farmed population of rainbow trout (*O. mykiss*) was investigated in order to study whether the high frequency of the 2/2 MHC IIB haplotype was attributable to disassortative mating by females. It was then assessed whether mating determined a benefit for females that corresponded to an increase in fitness. This was performed by evaluating the effect of the MHC haplotype on reproductive performance. The development of eggs and larvae resulting from matings of rainbow trout individuals with similar MHC was assessed during the incubation period, in order to evaluate whether MHC similarity determined a higher performance of the offspring.

Since the reproductive performance parameters are correlated with egg quality [22], the morpho-physiological parameters of the females (weights, lengths, pH of the ovarian fluid, and eggs weight) were analyzed. The females selected for mating did not differ among haplotypes in terms of weight and length. Indeed, it was shown that there was a positive correlation between length and fecundity, between length and weight, and between weight and fecundity [23]. In this regard, the female individuals were chosen with homogeneous dimensions, so that the size factor did not influence the quality and number of the eggs.

Egg quality can also be estimated using indirect measurements such as the physicochemical parameters of the ovarian fluid [24]. Indeed, eggs of poor quality are often characterized by low pH values in the ovarian fluid, which may indicate the loss of fecundity and the inability to develop into a normal embryo [22,24]. The ovarian fluid within salmonids differs in pH, and in the case of rainbow trout, it is characterized by a mean value of 8.4 ± 0.1 [25,26]. In the present study, the pH of the ovarian fluid showed no difference between the haplotypes, presenting a mean value of 8.2 ± 0.1. This result, together with the direct observation of ovulation, suggests that the eggs used in this study were of high quality.

The size and appearance of unfertilized eggs can be used to assess the overall development potential of the eggs after fertilization [24]. Therefore, the weight of the egg was recorded, and no difference was noted between the haplotypes.

Although overall fecundity did not differ between haplotypes, the relative fecundity varied. Indeed, the females with the 2/2 haplotype produced a higher number of eggs (2925 eggs kg^{-1}) than the females with the 2/x haplotype (1476 eggs kg^{-1}), while females with the x/y haplotype produced an intermediate number of eggs (1951 eggs kg^{-1}). The differences in relative fecundity ($p < 0.05$) can be related to a slight variation in egg weight and total fecundity.

The embryonic and larval survival rates were investigated to evaluate the quality of the gametes [24]. In addition, the assessment of embryonic malformations or larval deformities was studied for a more detailed evaluation of gamete quality [22,24]. In the present study, the eyeing rate did not differ between matings, and our findings are similar to those recorded in previous studies on rainbow trout [22,27]. Moreover, the hatching rate resulted in values comparable to those reported in previous studies on this species [19,22,27].

The larval deformity rate was lower than that previously reported on rainbow trout [20] and significant variations between matings were observed ($p < 0.01$), particularly between F 2/2 + M 2/2 and F 2/x + M 2/x, while the other matings presented an intermediate larval deformity rate. The rate of irregular absorption of the yolk sac did not differ between matings and the swim-up rate was higher than the rates reported in previous studies on this species [19,22,27].

The analysis of larval deformities revealed that the offspring of females with a 2/2 haplotype had a lower incidence of deformities, which were limited to torsion, irregular absorption of the yolk sac, cyclopia, and spinal deformation. The lower larvae deformity rate found in the F 2/2 + M 2/2 mating implies a lower number of larval deformity types, supporting the hypothesis of additive factors resulting from the mating of individuals with similar MHC.

The analysis of the survival rate demonstrated a significant effect of the matings: when compared to the mating F 2/2 + M 2/2, the matings F 2/x + M 2/2, F 2/x + M 2/x, and F x/y + M x/y were characterized by lower survival values. These results corroborate the hypothesis of this study; however, the absence of statistically significant results for the other types of matings makes the effects of the matings between individuals with haplotype 2/2 unclear.

MHC are the most polymorphic genes known in mammals and teleost fish [2,21]. Variation in exon II is responsible for most of the observed polymorphisms and this variability is maintained by a number of mechanisms including mating [1,2]. Indeed, increased resistance in offspring may be linked to the acquisition of rare alleles or increased heterozygosity [1,2]. However, matings between individuals with an intermediate level of MHC dissimilarity could provide greater reproductive success [6,10], and in some species selection towards similar MHC has been observed [13,14].

The origin and maintenance of polymorphism in MHC genes in populations is still unresolved [9]. Mechanisms such as sexual selection, frequency-dependent selection by pathogens, and heterozygote advantage have been suggested to explain the maintenance of high allelic diversity in MHC genes [9]. However, in our study it seems that females present a better reproductive performance, i.e., higher survival of the offspring, when the parents present similar MHC profiles, contrasting with the heterozygote advantage hypothesis [1].

The high frequency of the 2/2 haplotype in the population (30%) could be due to selective pressures that drive the evolution of adaptations [8]. We therefore hypothesize, as has occurred in other studies [12], the presence of a positive selection towards locally adapted MHC genes which promotes reproduction between genetically more similar individuals.

5. Conclusions

In this study, we investigated the effect of mating on offspring survival in the early larval stages of rainbow trout (*O. mykiss*). Our results indicate a reproductive advantage of females, in terms of fitness improvement, when mated with individuals with a similar MHC profile. The frequency of haplotypes was higher than expected in random matings, indicating the presence of disassortative mating towards individuals with similar MHC haplotypes. Our results are in contrast with the theory according to which the high MHC polymorphism is maintained by mating between heterozygous individuals. A similar situation has been found in previous studies on salmonids, in which it was hypothesized that this characteristic could be explained by a local adaptation. Given the high commercial weight of rainbow trout, further studies are needed to evaluate whether local adaptation to specific MHC haplotypes may determine a higher survival of offspring.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9110436/s1, Table S1: Polymorphisms MHC.

Author Contributions: Conceptualization, S.C., M.P., L.F. and B.S.; methodology, C.B., M.T., S.C., V.M., I.A., M.P., C.K., P.L.A., A.I.V., S.S., G.E., D.V., E.B., K.P., L.A., L.F. and B.S.; formal analysis, C.B. and V.M.; investigation, C.B. and V.M.; data curation, C.B.; writing—original draft preparation, C.B.; writing—review and editing, C.B., M.T., S.C., V.M., I.A., M.P., C.K., P.L.A., A.I.V., S.S., G.E., D.V., E.B., K.P., L.A., L.F. and B.S.; supervision, S.C., L.F. and B.S.; project administration, S.C and P.L.A.; funding acquisition, S.C., E.B., P.L.A. and L.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the SUPERTROUT PRIMA project, Improving Sustainability and Performance of Aquaculture Farming Systems: Breeding for Lactococcosis Resistance in Rainbow Trout, grant number 1433.

Institutional Review Board Statement: The procedures were conducted by the farmers following routine external fertilization practices. Observations of deformities were restricted to dead larvae, and no live specimens were subjected to any experimental interventions.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sommer, S. The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Front. Zool.* **2005**, *2*, 16. [CrossRef] [PubMed]
2. Piertney, S.B.; Oliver, M.K. The evolutionary ecology of the major histocompatibility complex. *Hered.* **2006**, *96*, 7–21. [CrossRef] [PubMed]
3. Milinski, M. The Function of Mate Choice in Sticklebacks: Optimizing Mhc Genetics. *J. Fish Biol.* **2003**, *63*, 1–16. [CrossRef]
4. Milinski, M.; Griffiths, S.; Wegner, K.M.; Reusch, T.B.H.; Haas-Assenbaum, A.; Boehm, T. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proc. Natl. Acid. Sci. USA* **2005**, *102*, 4414–4418. [CrossRef]
5. Landry, C.; Garant, D.; Duchesne, P.; Bernatchez, L. 'Good genes as heterozygosity': The major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proc. R. Soc. Lond. B Biol. Sci.* **2001**, *268*, 1279–1285. [CrossRef]
6. Evans, M.L.; Dionne, M.; Miller, K.M.; Bernatchez, L. Mate choice for major histocompatibility complex genetic divergence as a bet-hedging strategy in the Atlantic salmon (*Salmo salar*). *Proc. R. Soc. Lond. B Biol. Sci.* **2011**, *279*, 379–386. [CrossRef]
7. Reichard, M.; Spence, R.; Bryjová, A.; Bryja, J.; Smith, C. Female rose bitterling prefer MHC-dissimilar males: Experimental evidence. *PLoS ONE* **2012**, *7*, e40780. [CrossRef] [PubMed]
8. Neff, B.D.; Garner, S.R.; Heath, J.W.; Heath, D.D. The MHC and non-random mating in a captive population of chinook salmon. *Hered.* **2008**, *101*, 175–185. [CrossRef]
9. Reusch, T.B.H.; Häberli, M.A.; Aeschlimann, P.B.; Milinski, M. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* **2001**, *414*, 300–302. [CrossRef]
10. Kalbe, M.; Eizaguirre, C.; Dankert, I.; Reusch, T.B.H.; Sommerfeld, R.D.; Wegner, K.M.; Milinski, M. Lifetime Reproductive success is maximized with optimal major histocompatibility complex diversity. *Proc. R. Soc. Lond. B Biol. Sci.* **2008**, *276*, 925–934. [CrossRef]
11. Forsberg, L.A.; Dannewitz, J.; Petersson, E.; Grahn, M. Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout—Females fishing for optimal MHC dissimilarity. *J. Evol. Biol.* **2007**, *20*, 1859–1869. [CrossRef] [PubMed]
12. Yeates, S.E.; Einum, S.; Fleming, I.A.; Megens, H.-J.; Stet, R.J.M.; Hindar, K.; Holt, W.V.; Van Look, K.J.W.; Gage, M.J.G. Atlantic salmon eggs favour sperm in competition that have similar major histocompatibility alleles. *Proc. R. Soc. Lond. B Biol. Sci.* **2008**, *276*, 559–566. [CrossRef] [PubMed]
13. Gasparini, C.; Congiu, L.; Pilastro, A. Major histocompatibility complex similarity and sexual selection: Different does not always mean attractive. *Mol. Ecol.* **2015**, *24*, 4286–4295. [CrossRef] [PubMed]
14. Wedekind, C.; Walker, M.; Portmann, J.; Cenni, B.; Müller, R.; Binz, T. MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. *J. Evol. Biol.* **2004**, *17*, 11–18. [CrossRef]
15. Promerová, M.; Alavioon, G.; Tusso, S.; Burri, R.; Immler, S. No evidence for MHC class II-based non-random mating at the gametic haplotype in Atlantic salmon. *Heredity* **2017**, *118*, 563–567. [CrossRef]
16. Miller, K.M.; Withler, R.E.; Beacham, T.D. Molecular evolution at MHC genes in two populations of chinook salmon *Oncorhynchus tshawytscha*. *Mol. Ecol.* **1997**, *6*, 937–954. [CrossRef]
17. Stephens, M.; Smith, N.J.; Donnelly, P. A new statistical method for haplotype reconstruction from population data. *AJHG* **2001**, *68*, 978–989. [CrossRef]

18. Akbari Nargesi, E.; Falahatkar, B.; Sajjadi, M.M. Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. *Aquacult. Nutr.* **2020**, *26*, 98–108. [CrossRef]
19. Kazemi, E.; Sourinejad, I.; Ghaedi, A.; Johari, S.A.; Ghasemi, Z. Effect of different dietary zinc sources (mineral, nanoparticulate, and organic) on quantitative and qualitative semen attributes of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2020**, *515*, 734529. [CrossRef]
20. Bonnet, E.; Fostier, A.; Bobe, J. Characterization of rainbow trout egg quality: A case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. *Theriogenology* **2007**, *67*, 786–794. [CrossRef]
21. Colussi, S.; Prearo, M.; Bertuzzi, S.A.; Scanzio, T.; Peletto, S.; Favaro, L.; Modesto, P.; Maniaci, M.G.; Ru, G.; Desiato, R.; et al. Association of a specific major histocompatibility complex class IIβ single nucleotide polymorphism with resistance to lactococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **2015**, *38*, 27–35. [CrossRef] [PubMed]
22. Aegerter, S.; Jalabert, B. Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **2004**, *231*, 59–71. [CrossRef]
23. Bazaz, A.I.; Ahmad, I.; Shah, T.H.; Bhat, F.A.; Asimi, O.A.; Bhat, B.A.; Yousuf, Z.; Razak, N. Study on spawning fecundity and its relation with body size of rainbow trout (*Oncorhynchus mykiss*) from hatchery of Kashmir Himalayas. *JBGSR* **2022**, *11*, 1–6. [CrossRef]
24. Bobe, J.; Labbé, C. Egg and sperm quality in fish. Gen. *Comp. Endocrinol.* **2010**, *165*, 535–548. [CrossRef] [PubMed]
25. Wojtczak, M.; Dietrich, G.J.; Słowińska, M.; Dobosz, S.; Kuźmiński, H.; Ciereszko, A. Ovarian fluid pH enhances motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. *Aquaculture* **2007**, *270*, 259–264. [CrossRef]
26. Zadmajid, V.; Myers, J.N.; Sørensen, S.R.; Ernest Butts, I.A. Ovarian fluid and its impacts on spermatozoa performance in fish: A review. *Theriogenology* **2019**, *132*, 144–152. [CrossRef]
27. Kayam, S. The effect of mating different age groups of broodstocks on the reproductive performance, sex ratio, growth, and survival bate of rainbow trout (*Oncorhynchus mykiss*). *J. Freshw. Ecol.* **2004**, *19*, 695–699. [CrossRef]