

Effect of triploidy on quality traits of shi drum (*Umbrina cirrosa* L.) until the second rearing year

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Abstract

Carcass and flesh morphometric, reological and chemical traits of triploid (3n) and diploid (2n) shi drum (*Umbrina cirrosa* L.) were evaluated through 7 months. Three age groups, 17-, 21- and 24-month-old fish, were investigated. The effects of ploidy were statistically evaluated and the weight of fish was included in the model as a covariate because triploids grew less than diploids. As expected, fish weight was found to be significantly correlated with all the investigated morphometric traits, but showed a negative correlation with some chemical (pH) and colour traits (lightness) of raw fillet. In comparison with controls, triploid shi drums were characterized by different morphological traits that involved a slender body shape. In triploids, a reduction in condition factor, backbone weight, dressing index and an increase in the agility index were also recorded. When the commercial size (i.e. over 300 g) was achieved, triploids exhibited larger coelomatic and fillet (dorsal white muscle) fat deposition than diploids. Among reological traits, colour and texture were affected by ploidy; raw fillet lightness and cooked flesh tenderness were higher among triploids in all the investigated age groups. As fish were fed with a restricted feeding regimen, fillet fat deposition was supposed to be limited. Thus, the effects of ploidy on reological traits may be only partially explained by lipid fillet amount and are supposed to be more related to different fibre muscle architecture.

Keywords: triploidy, morphometric traits, texture, colour, shi drum, *Umbrina cirrosa*

Introduction

The success of intensive aquaculture has improved production performance and quality traits optimizing consumers' preference. Among the biological factors affecting fish quality, sexual maturity seems to have a negative effect on the nutritional properties and palatability of the final products (Felip, Piferrer, Zanuy & Carrillo 2001; Fasolato, Bertotto, Lopparelli, Corato, Francescon, Barbaro & Segato 2003). Triploidization has been proposed in order to produce sterile individuals and, as a consequence, to improve flesh quality in adult fish (Quin, Fast & Ako 1998). Moreover, in finfish, triploidy could increase somatic growth and potentially prevent carcass quality decay due to sexual maturation (Felip *et al.* 2001; Oppedal, Taranger & Hansen 2003; Haffray, Bruant, Facqueur & Fostier 2005). Nevertheless, intramuscular fat deposition and the resulting nutritional value of flesh in chromosomal manipulated fish are still being debated (Johnston, Strugnell, McCracken & Johnstone 1999; Felip *et al.* 2001; Bjørnevik, Epse, Beattie, Nortvedt & Kiessling 2004). In sea bass, Peruzzi, Chatain, Saillant, Haffray, Menu and Falguière (2004) reported that the effect of ploidy on flesh lipid content also depends on age and sex. The flesh lipid amount affected fillet quality traits such as colour and texture (Lopparelli, Segato, Corato, Fasolato & Andrighetto 2004). Furthermore, in marine fish, the effect of ploidy on these parameters is still poorly investigated. In addition, in salmonids, the role of ploidy in muscle structure and its subsequent influence on reological traits are still unclear (Suresh & Sheehan 1998; Bjørnevik *et al.* 2004).

Preliminary studies on juvenile and subadult triploid shi drum (*Umbrina cirrosa* L.) showed significant differences in fat deposition influencing, consequently, flesh nutritional value and texture (Fasolato *et al.* 2003; Segato, Bertotto, Fasolato, Francescon, Barbaro & Corato 2006). As a prosecution of the previous investigations, this study aims to evaluate the effect of ploidy on morphometric and reological traits and nutritional fillet value in shi drum, during a 24-month rearing period, before the first reproductive season.

Materials and methods

Fish and rearing conditions

The triploid shi drum stock was obtained in June 2000 and reared in a Venetian fish farm (Pellestrina, Veneto Agricoltura; for details, see Segato *et al.* 2006). During 24 months of the experimental period, a triploid (T) and a diploid (D) fish stock were reared, from juveniles to adults, in separate tanks. The rearing density was about 20 kg m^{-3} . Fish were fed with extruded diets varying in relation to their size. According to age, the feeding rate varied from 5% to 1% of live weight (LW) and season (starvation period from November to February). Formulation and proximate composition [crude protein (CP): 43.4%; ether extract (EE): 19.3% and gross energy (GE): 21.2 MJ kg^{-1} on wet weight] of the diet fed in the last period of the trial is reported in the previous study (Segato *et al.* 2006). During the rearing season of 2001 (from March to October), the proximate composition of diet was on average as follows: CP = 44.4%, EE = 20.7% and GE = 21.4 MJ kg^{-1} on wet weight. In winter, the water temperature was maintained above 10°C in order to avoid sub-lethal temperature, which was estimated to be around 7°C (Segato, unpublished data).

In April 2002, due to different growth between T and D stocks (LW: $234 \pm 60 \text{ g}$ in T vs. $274 \pm 85 \text{ g}$ in D), two groups of similarly sized fish were selected. Therefore, from each stock 196 individuals (LW: 291 g in T vs. 293 g in D) were transferred into two separated 5 m^3 tanks (density: 5.0 kg LW m^{-3}).

Fish samples

During the second year of life, from November 2001 to June 2002, three sample sets were taken to evaluate fish quality. The first set of specimens (average LW: $209 \pm 48 \text{ g}$ in T vs. $263 \pm 64 \text{ g}$ in D) was collected at 17 months of age (November 2001); the second set at

21 months just after the selection (April 2002; LW: $291 \pm 29 \text{ g}$ in T vs. $293 \pm 28 \text{ g}$ in D); and the third set at 24 months (June 2002; LW: $430 \pm 42 \text{ g}$ in T vs. $467 \pm 33 \text{ g}$ in D). For each ploidy, a total of 30 fish were randomly collected and analysed for biometric, chemical and reological traits. Ploidy assessment performed by flow cytometry on all fish samples confirmed that putative triploids were effectively $3n$ and also that the controls were all diploids ($2n$).

Biometric parameters and morphometric indexes

SL and maximum girth and height were measured. After dissection, viscera, liver, perivisceral fat and gonads were weighed. Data were used to calculate condition factor (K), agility index (AI) as well as dressing index (DI). Gutted trunk, trunk fillet and trunk backbone weights were also recorded (for details, see Segato, Lopparelli, Borgoni, Zanella, Corato & Andrighetto 2005).

Fillet proximate composition, colour and texture

The epiaxial white muscle portion of the fillet was freeze dried to determine proximate composition according to AOAC (2000): protein (N-Kjeldahl 6.25), ether extract (EE: Soxhlet, diethyl ether) and ash. Twenty-four hours *post mortem*, pH and colour were performed in raw fillet. The colour was measured after exposure to air (1 h , $2 \pm 1^\circ\text{C}$) on five body sites from the head to the tail by a chromameter (CR100, Minolta Camera Co., Osaka, Japan), and data were expressed using Hunter-Lab (lightness, L; redness, a; yellowness, b) system (Honikel 1998). Gutted trunks were placed in polyurethane bags and heated in a thermostatic water bath (25 min , 75°C) and iced for 30 min in cool running water, in order to determine cooking weight losses. In the cooked fillet colour, and instrumental tenderness were also assessed. Tenderness was measured as the maximum shear force (MSF) using a Warner–Bratzler (Instron 1000, Instron Corp. Canton, MA, USA) blade applied to cylindrical cores ($\varnothing 1.25 \text{ cm}$) cut from the skinned dorsal fillet.

Statistical analysis

After verifying the normality and variance homogeneity, data were submitted to a one-way analysis of

variance (ANOVA; two levels of ploidy: triploids vs. diploids) considering live weight as covariate. Ploidy per live weight interaction never gives significant results and so cannot be included in the statistical model. Analysis of variance was supported by the general linear model procedure (PROC GLM) of SAS (1999).

Results

During the experimental period, diploids always showed a significantly greater weight gain than triploids. For this reason, the statistical model considered live body weight as a covariate. Thus, the effect of ploidy was evaluated within the same fish size, estimating the degree and the significance of the correlation (*b* value) between fish weight and the quality traits.

Biometric parameters and morphometric indexes

Biometric traits were significantly affected by ploidy level at 21 and 24 months of age (Table 1). Particularly, at the end of the trial, triploids showed a significantly longer standard length than diploids (28.1 vs. 27.2 cm; $P < 0.01$), resulting in a lower *K* (2.04 vs. $2.23 \times 10^2 \text{ g cm}^{-3}$; $P < 0.01$). The AI was also higher in triploid fish (2.49 vs. 2.29; $P < 0.01$). As expected, regression coefficient (*b*) values showed a positive correlation between linear biometrics and LW.

No significant difference was observed in trunk and fillet weight between triploids and diploids (Table 2). Considering the trunk, triploids always showed a significantly lower backbone weight than diploids. Ploidy level did not affect DI in 17-month-old fish; meanwhile, DI was significantly higher in controls than in triploids in spring and summer. Perivisceral fat amount was significantly greater in T fish than in D, especially in 21-month-old specimens (4.7 vs. 2.7 g; $P < 0.01$). After a feeding period, this difference decreased (16.6 vs. 14.1 g; $P < 0.05$).

Fillet proximate composition, colour and texture

Fillet proximate composition was not affected by ploidy in 17-month-old shi-drums. By comparison among older specimens, a significant increase in EE fillet content was observed in triploids (Table 3). After the winter starvation, the average fillet fat amount was half that recorded in November. As indicated by *b* values, the EE content was also found to be influenced by LW only at the end of the trial (0.0031; $P < 0.05$).

Ploidy did not seem to affect the pH values of fillet 24 h *post mortem* (Table 4). The pH was always negatively correlated with LW, especially in specimens smaller than 300 g (*b* ranged from -0.0012 to -0.0014 ; $P < 0.01$). Among flesh colour parameters, raw fillet L (lightness) appeared to be affected by

Table 1 Effect of ploidy and fish weight covariate (*b*) on biometric parameters and morphometric indexes

Age (months) – sample period	Ploidy				
	Triploid	Diploid	<i>P</i>	SEM	<i>b</i>
17 – November	(209 ± 48 g)	(263 ± 64 g)			
Standard length (cm)	22.1	21.8	NS	0.2	0.0305**
Maximum girth (cm)	17.6	17.6	NS	0.1	0.0281**
Condition factor (10^2 g cm^{-3})	2.14	2.24	NS	0.06	0.0003
Agility index	2.51	2.42	NS	0.04	-0.0005
21 – April	(288 ± 24 g)	(298 ± 32 g)			
Standard length (cm)	25.2	24.6	*	0.2	0.0317**
Maximum girth (cm)	17.6	17.4	NS	0.2	0.0263**
Condition factor (10^2 g cm^{-3})	1.83	1.95	*	0.03	-0.0007
Agility index	2.53	2.54	NS	0.05	0.0011
24 – June	(434 ± 42 g)	(472 ± 44 g)			
Standard length (cm)	28.1	27.2	**	0.2	0.0170**
Maximum girth (cm)	20.0	20.7	**	0.1	0.0172
Condition factor (10^2 g cm^{-3})	2.04	2.23	**	0.05	0.0007
Agility index	2.49	2.29	**	0.03	0.0001

Data represent means of 30 fish, and their mean live weight ± SD is reported in parentheses.

* $P < 0.05$; ** $P < 0.01$.

Condition factor (*K*): [total body weight/(standard length³)] × 100. Agility index (AI): (trunk length + peduncle length/maximum height). SEM, is the standard error of the least squares means.

Table 2 Effect of ploidy and fish weight covariate (*b*) on trunk weight and morphometric traits

Age (months) – sample period	Ploidy		<i>P</i>	SEM	<i>b</i>
	Triploid	Diploid			
17 – November	(214 ± 39 g)	(273 ± 57 g)			
Trunk (g)	132.3	134.5	NS	1.4	0.5696**
Trunk fillet (g)	95.6	103.0	NS	4.0	0.2428**
Trunk backbone (g)	12.1	13.7	*	0.4	0.0436**
Perivisceral fat (g)	6.6	4.8	**	0.4	0.0550**
Dressing index (%)	93.4	94.1	NS	0.2	– 0.0164**
21 – April	(280 ± 19 g)	(294 ± 33 g)			
Trunk (g)	167.2	169.9	NS	1.7	0.5738**
Trunk fillet (g)	118.3	119.0	NS	3.1	0.4579**
Trunk backbone (g)	13.6	14.9	*	0.5	0.0321**
Perivisceral fat (g)	4.7	2.7	**	0.4	0.0247**
Dressing index (%)	95.5	96.3	*	0.2	– 0.0076*
24 – June	(435 ± 38 g)	(468 ± 46 g)			
Trunk (g)	239.7	243.4	NS	3.0	0.5083**
Trunk fillet (g)	166.4	168.5	NS	3.7	0.3506**
Trunk backbone (g)	19.8	22.8	*	0.7	0.0373**
Perivisceral fat (g)	16.6	14.1	*	0.8	0.0691**
Dressing index (%)	93.6	94.5	*	0.2	– 0.0082**

Data represent means of 18 fish, and their mean live weight ± SD is reported in parentheses.

* $P < 0.05$; ** $P < 0.01$.

Dressing index (DI): (total body weight – (viscera + celomatic fat + liver weight)/total body weight) × 100.

SEM, is the standard error of the least squares means.

Table 3 Effect of ploidy and fish weight covariate (*b*) on dorsal fillet proximate composition (%)

Age (months) – sample period	Ploidy		<i>P</i>	SEM	<i>b</i>
	Triploid	Diploid			
17 – November	(214 ± 39 g)	(273 ± 57 g)			
Moisture	76.0	75.9	NS	0.2	– 0.0039
Protein	21.0	21.1	NS	0.2	0.0038
Ether extract	1.4	1.3	NS	0.1	0.0013
Ash	1.3	1.3	NS	0.1	0.0003
21 – April	(280 ± 19 g)	(294 ± 33 g)			
Moisture	76.1	76.4	NS	0.2	– 0.0016
Protein	21.5	21.5	NS	0.2	– 0.0012
Ether extract	0.7	0.5	*	0.1	0.0022
Ash	1.4	1.4	NS	0.1	0.0002
24 – June	(435 ± 38 g)	(468 ± 46 g)			
Moisture	75.6	75.8	NS	0.2	– 0.0040
Protein	21.6	21.7	NS	0.2	– 0.0014
Ether extract	1.4	1.0	*	0.1	0.0031*
Ash	1.2	1.3	NS	0.1	– 0.0003

Data represent means of 18 fish, and their mean live weight ± SD is reported in parentheses.

* $P < 0.05$.

SEM, is the standard error of the least squares means.

ploidy. Triploids always showed significantly higher L values than diploids. In 24-month-old triploids, significantly lower *b* (yellowness) values than in respective diploids were observed (– 5.6 vs. – 4.6; $P < 0.05$). At 17 and 21 months of age, there was a negative correlation between LW and L. After cooking, differences

in L values between 3n and 2n fish disappeared. Also, a (redness) and *b* colour parameters were similar, except in youngest fishes.

Ploidy seems to have a significant effect on flesh tenderness as indicated by MSF values, while it is ineffective on cooking losses. A highly significant cor-

Table 4 Effect of ploidy and fish weight covariate (*b*) on fillet pH, colour and texture

Age (months) – sample period	Ploidy		<i>P</i>	SEM	<i>b</i>
	Triploid	Diploid			
17 – November	(207 ± 46 g)	(258 ± 57 g)			
Raw fillet					
pH	6.38	6.39	NS	0.02	– 0.0012**
Lightness (L)	44.3	42.6	*	0.1	– 0.0124**
Redness (a)	0.4	0.2	NS	0.3	– 0.0029
Yellowness (b)	– 3.4	– 3.1	NS	0.3	– 0.0043
Cooked fillet					
Lightness (L)	83.6	82.3	NS	0.8	0.0105
Redness (a)	– 1.8	– 0.5	*	0.3	– 0.0003
Yellowness (b)	7.6	10.0	*	0.9	– 0.0053
Cooking losses (%)	7.0	7.3	NS	0.5	– 0.0085
Maximum shear force (N)	3.8	4.6	*	0.3	0.0098
21 – April	(292 ± 23 g)	(301 ± 36 g)			
Raw fillet					
pH	6.30	6.29	NS	0.02	– 0.0014**
Lightness (L)	45.9	43.4	**	0.3	– 0.0167*
Redness (a)	– 0.7	– 0.9	NS	0.1	– 0.0003
Yellowness (b)	– 4.3	– 4.4	NS	0.1	– 0.0054
Cooked fillet					
Lightness (L)	83.9	83.7	NS	0.6	– 0.0112
Redness (a)	– 1.0	– 0.4	NS	0.3	– 0.0013
Yellowness (b)	7.5	8.4	NS	0.7	– 0.0033
Cooking losses (%)	9.0	7.5	NS	0.6	– 0.0202
Maximum shear force (N)	2.5	3.2	*	0.2	0.0059
24 – June	(430 ± 38 g)	(478 ± 45 g)			
Raw fillet					
pH	6.17	6.19	NS	0.02	– 0.0007*
Lightness (L)	48.1	47.1	*	0.3	– 0.0041
Redness (a)	– 0.1	– 0.1	NS	0.1	– 0.0011
Yellowness (b)	– 5.6	– 4.6	*	0.3	– 0.0075
Cooked fillet					
Lightness (L)	83.9	84.1	NS	0.7	– 0.0073
Redness (a)	– 0.1	– 0.3	NS	0.3	– 0.0084*
Yellowness (b)	7.1	7.6	NS	0.4	– 0.0025
Cooking losses (%)	5.1	5.6	NS	0.5	– 0.0066
Maximum shear force (N)	3.9	5.6	**	0.3	– 0.0104**

Data represent means of 12 fish, and their mean live weight ± SD is reported in parentheses.

P* < 0.05; *P* < 0.01.

SEM, is the standard error of the least squares means.

relation between MSF and LW was observed in the oldest fish samples.

Discussion

As reported for other marine finfish, the deficiency in hormones in maturing triploids may reduce growth, especially for species that does not require a specific fattening period before gonadal development.

Triploid shi-drum showed different morphological traits that resulted in a slender body shape. At 24 months of age, triploids showed a significantly more elongated shape and lower maximum girth than di-

ploids. A lower *K*, both in juvenile and in 50-month-old fish, was previously reported in triploid sea bass (Felip *et al.* 2001; Peruzzi *et al.* 2004). Similar to the present results, a lower *K* was detected in triploid gilt-head sea bream (Haffray *et al.* 2005) and turbot (Cal, Vidal, Gómez, Álvarez-Blázquez, Martínez & Piferrer 2006) just after about 20 months of rearing.

Triploidization seems to affect perivisceral fat deposition and gutted yields (Hussain, Rao, Humayun, Randall, Penman, Kime, Bromage, Myers & McAndrew 1995; Carrasco, Penman & Bromage 1999). In the present study, 3*n* specimens always showed a larger celomatic fat deposition, leading to a significant

decrease in DI, even if such a decrease was not evident before starvation. In gilthead sea bream, triploids showed a better DI due to a lower gonadosomatic index than diploids, but this advantage diminished after spawning by the greater development of their abdominal lipidic reserves and/or liver (Haffray *et al.* 2005). In contrast, both juvenile and mature sea bass triploids showed higher gutted yields than diploids (Peruzzi *et al.* 2004). In shi-drum, a higher perivisceral fat deposition and consequently lower DI were observed in triploids before the first spawning season. Furthermore, a previous research showed a greater celomatic fat deposition in mature triploids than in diploids (about 1 kg of body weight), resulting in a higher whole-body EE content (Fasolato *et al.* 2003).

At all the three age stages studied, no evident difference was found in trunk fillet weight, as previously reported for sea bream (Haffray *et al.* 2005). Conversely, mature sea bass triploid produced a much higher fillet yield but only in females (Peruzzi *et al.* 2004). Triploidization led to a significant reduction in the vertebral column (backbone) weight. This result was probably due to a different morphological development as indicated in the lower maximum girth. In fact, at the end of the experiment, the maximum high was also significantly affected by ploidy (8.3 vs. 8.7 cm; $P < 0.05$ – data not reported in tables). Triploids had a different histological structure from diploids due to larger cells, i.e. a lower ratio between the cell surface and volume (Benfey 1999; Felip *et al.* 2001). This could lead to a minor general bone mineralization as shown by the lower whole-body ash content detected in 3n fishes when compared with the diploid ones (Segato *et al.* 2006).

No significant differences were found in fillet composition, except for EE content in 21 and 24-month-old shi-drums. However, a very low intramuscular fat deposition was detected in comparison with those noticed in other reared marine fish, even though we analysed only white dorsal muscle. This was probably due to the limited food ration offered to the fish during the experiments. Juvenile triploid sea bass showed a leaner fillet than diploids; however, triploids at the age of 45 months had a fatter fillet (Peruzzi *et al.* 2004). A lower level of lipids was recorded in triploid gilthead sea bream during 42 months of rearing, except for the reproductive period (Haffray *et al.* 2005).

Among the most important fish quality traits, flesh colour could influence consumer's preference. In all the three experimental phases, triploidization in-

duced an increase of values in raw fillet lightness (L). Lightness depends on the degree of reflected light according to water-holding capability and lipids' percentage. Thus, the fillet was brighter in triploids than in diploids, probably as a result of a different morphological cell structure and muscle fibre arrangement, and a higher flesh lipid content. A similar correlation between brightness and fillet lipid content was reported for diploid sea bass (Lopparelli *et al.* 2004), although triploid Atlantic salmon showed a darker and redder fillet (Bjørnevik *et al.* 2004). At the end of the present experiment (early summer), triploid shi drum showed a considerable higher negative value of b, corresponding to a bluish colour. As regards 17-month-old fish, the colour of cooked fillet was affected by ploidy. On the other hand, after heating, colour differences disappeared within the second year of rearing. These differences in colour, between raw and cooked fillet, are probably due to denatured muscle fibres inducing a change in physical and chemical properties. A significant correlation between live weight and lightness was observed only before cooking, revealing that heat treatment reduced the influence of biological factors on colour traits.

Ploidy notably affected flesh tenderness [max shear force (MSF)] during the entire experimental period. In particular, triploid fish showed a considerably lower firmness at the end of the trial. The tenderness of triploid muscle may be partially explained by the different fat amount. In the first and second sampled age classes, despite MSF values being significantly lower in triploids than in controls, similar fillet fat amounts were observed. This may suggest that differences in collagen and muscle fibre diameter and density may explain variation in fillet texture. For Atlantic salmon triploids, Johnston *et al.* (1999) reported a muscle architecture characterized by lower fibre density, which seems to reduce fillet chewiness and firmness. Conversely, in rainbow trout, Suresh and Sheehan (1998) showed similar muscle fibre growth dynamics between triploids and diploids. Bjørnevik *et al.* (2004) pointed out that that intraspecies variation, both in texture and gaping, is more related to season and body size rather than to average muscle fibre diameter. Ploidy effect on muscle property traits, i.e. colour and texture, seems to depend on specific features and a complex of biological and rearing conditions (Johnston 2001). In large-sized shi drum, Fasolato *et al.* (2003) evidenced a significant ploidy effect on intramuscular fat deposition, resulting in different fillet quality traits. Mainly, tri-

ploids showed a lower cooking weight loss and firmness, while raw flesh lightness was similar.

Conclusions

Present data, along with those ones reported in a previous study (Segato *et al.* 2006), provide a first framework on the productive performance and quality assessment of triploid shi drum. Before the first reproductive season, the triploid condition seems to negatively influence fish growth and induce a higher celomatic fat deposition, associated with a worse gutted yield. Moreover, triploid fish were characterized by a slender body shape and a more bright and tender fillet, especially when they reach commercial size. Indeed, more experiments are required to assess more precisely the effects of ploidy on quality traits, with particular regard to feeding regimen and stocking density. Finally, taking into account that ploidy was shown to influence considerably marketing quality traits, consumers' preferences should be evaluated before large-scale production of triploid shi drum.

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