Induction of triploidy in turbot (*Scophthalmus maximus*) does not affect gross body morphology and skeleton characteristics

J. HERNÁNDEZ-URCERA¹, E. TORRES², J. D. BARREIRO³, A. BARREIRO-LOIS³, J.M. ALONSO⁴, R. CAL¹ and J. ROTLLANT^{2*}

¹Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Vigo, 36390-Vigo, Spain

²Aquatic Molecular Pathobiology Lab. Instituto de Investigaciones Marinas (IIM-CSIC). Eduardo Cabello 6, 36208-Vigo, Spain

³Servicio de Diagnóstico por Imagen HVU Rof Codina, Facultade de Veterinaria-Lugo, Spain

⁴CESAM-University of Aveiro, Portugal

*Corresponding Author. Tel. +351 986231930. E-mail: rotllant@iim.csic.es

Triploid turbot (*Scophthalmus maximus*) produced by cold shock showed no differences in their gross body morphology and skeleton characteristics compared with their diploid full-sibs.

Key words: triploidy; morphology; skeleton; turbot; *Scophthalmus maximus*.

The fish farming industry has an interest in the rearing of triploids as a means to prevent sexual maturation in fish before they reach their optimal commercial size (Felip et al., 1999). Additionally, the production of triploid fish ensures that any that escapees from aquacultural facilities are not able to reproduce in the wild. However, the production of triploid fish has often been associated with an increased incidence of morphological malformations, including abnormal jaws and fins (Sutterlin et al., 1987; Jungalwalla, 1991; McGeachy et al., 1996; Sadler et al., 2000; Oppedal et al., 2003), abnormal tail and trunk proportions (Swarup, 1959), facial deformities (Tave et al., 1993) and a reduction in the number of vertebrae (Tiwary and Ray, 2004). Moreover, the nature and the frequency of occurrence of these malformations appear to vary among species. In intensive commercial aquaculture, morphological malformations are undesirable because they reduce the quality and value of the final product (Baeeverfjord et al., 2009) and they may raise concerns about animal welfare (Benfey, 2001).

Turbot (*Scophthalmus maximus*) is an important commercial species in Europe. Although morphological studies of hatchery-reared turbot (Ellis et al., 1997) and the effects of triploidy on growth and gonadal development (Cal et al., 2006) have been performed, there have been no studies on the possible effects of the degree of ploidy and of the different methods used to induce changes in ploidy on the occurrence of morphological alterations. This paper reports common gross body morphological and skeletal characteristics observed among diploid and "cold shock induced" triploid hatchery-reared turbot (*Scophthalmus maximus*).

Four single-pair matings of turbot were produced at the Centro Oceanografico de Vigo (NW Spain), in June 2009. Triploidy was induced in a portion of the eggs from each female by application of a cold-shock shortly after fertilization, as described by Piferrer et al. (2000, 2003).

The triploidy ratio within each progeny group was estimated by flow cytometric analysis of embryos from 120 eggs sampled 48 hours post-fertilization using an FC500 flow cytometer (Beckman Coulter, Ca, U.S.A.), and following a modified version of the method described by Lecommandeur *et al.* (1994). The triploidy ratios achieved in each progeny were 87, 94, 95 and 97%). Only the progeny from the mating producing the highest triploid rate (97%) were examined further. Thereafter, treated (3n) and control (2n) larvae were reared separately in four 1000-l tanks. When they were 2 months old, fish were vaccinated against *Vibrio* sp. by immersion in Gava-3 (100mL/L) (Laboratorios HIPRA, Girona, Spain). Periodic OX-CTA (OX Laboratories, Barcelona, Spain) baths (50mg/L) were administered throughout the experiment to prevent the presence of parasites. At 5 months of age, random samples of diploid and triploid fish were placed into four 3800-l tanks (2 tanks per ploidy group and 50 fish per tank). The tanks were provided with flow-through water (40 l/min), and the fish were reared under natural photoperiod and temperature conditions. The fish were fed by automatic feeders providing dry pellets of increasing size (Skretting, Burgos, Spain) 7 days a week until they reached 12 months of age.

At both 6 and 12 months, 10 fish were sampled from each tank. The fish were euthanized with an overdose of the anaesthetic MS-222 (500 mg/l, Sigma-Aldrich, Madrid, Spain). The ploidy level of each fish was determined by measurement of the individual mean length of the erythrocyte major axis (30 erythrocytes per fish) in a blood sample, stained with Hemacolor (E. Merck. Darmstadt., Germany), as previously described by Piferrer *et al.* (2003). The individual mean length was then compared with the overall mean length for diploids and triploids. The mean erythrocyte major axis length was $11.5 \pm 0.51 \mu\text{m}$ (mean $\pm$ SD) in control diploids and 15.3 ± 0.29

µm in cold-shock treated triploid fish ($P < 0.001$). All fish were confirmed to be of the ploidy level appropriate to their corresponding test group.

Fish were photographed with a digital camera (Nikon coolpix 990) mounted on a tripod (Stitz P230BQ). Photographic analyses were carried out using tpsDig1 image analysis software (F. J. Rohlf, New York University, U.S.A.). The measurements of 8 morphometric parameters were recorded for comparison between diploids and triploids (Hubbs & Lagler, 1947; Bonar *et al.*, 1988; Bhowmick *et al.*, 1981; Tiwary *et al.*, 1999). The morphometric parameters measured were: total length (TL), standard length (SL), body width (BW), head length (HL), upper jaw length (UJL), lower jaw length (LJL), orbital length (OL) and interorbital length (IOL) (Fig. 1). Eight different body proportion ratios were then calculated from the morphometric parameters measured (See Tables I and II for details).

X-ray photographs of diploid and triploid fish were then taken (exposure parameters, 44 kV and 25 mAs) using a mammography computed radiology system (Fujifilm Capsula FCR XLII). All radiographs were analyzed using DICOM analysis software (K-PACS, V. 1.0.1 by IMAGE Information Systems Ltd.). The number of abdominal and caudal vertebrae, dorsal and anal fin rays and the occurrence of spinal deviations were determined in each X-ray picture. SL (standard length) and BW (body width, relative to vertebrae number 12) were also evaluated. Spinal deviation was determined by calculating the angle formed by the line joining the last 8 vertebrae of the spinal cord and the tangent to the curve formed by the spine in the first vertebrae SA) (Fig. 3).

As there were no statistically significant differences in the "morphometric" data between replicate tanks, the data were pooled, thus giving $n=20$ per ploidy group within each of the two sampling times. Results are presented as mean $\pm$ standard deviation and/or range. Student's *t*-tests were performed to analyse ploidy effects. Non-parametric

data were log-transformed when necessary to achieve normality and homogeneity of variance (INSTATm, GraphPad Software V2.04a). The significant level was $P < 0.05$.

Fish weight at 6 and 12 months was not significantly affected by cold-shock induced triploidy. Control fish weighed 59 ± 7.8 g (6 months) and 543 ± 90 g (12 months), and triploid fish 55 ± 10 g ($P=0,1665$) (6 months) and 471 ± 68 g ($P=0,0761$)(12 months). The mortality rates were similar for both experimental groups: 2% and 1,5% at 6 months in the diploid and triploid groups, respectively. No mortality was recorded at 12 months.

This lack of difference in growth and mortality rates among juvenile (<1 year) diploid and triploid turbot is consistent with previous observations of Cal *et al.* (2006). Additionally, these authors observed that triploids were larger than diploids at 2 years, a time before which diploid fish has initiated sexual maturation, unlike the sterile triploids.

None of the morphometric body ratios showed significant differences between ploidy groups sampled at 6 and 12 months (Table I. A,B). Neither were there any significant differences between diploid and triploid fish as regards spinal angle (SA), number of vertebrae and fin rays in the dorsal and anal fins (Table II. A,B).

The incidence of fish with skeletal abnormalities was similar in both experimental groups: 30 ± 14 % and 35 ± 6 % at 6 months in the diploid and triploid groups, respectively (Fig. 3). At 12 months, the incidence was 30 ± 14 % and 25 ± 7 %, respectively.

For both ploidy groups, posterior abdominal vertebrae fusion (11-12 vertebrae) (Fig. 4A), posterior caudal vertebrae bending/torsion (Fig. 4B) and posterior caudal vertebrae (29-30 vertebrae) fusion (Fig. 4C) were the most frequent skeletal abnormalities recorded. Contrary to observations made concerning Atlantic salmon (Fjelldal and

Hansen, 2010), the prevalence of vertebral deformities in triploid turbot was found to be similar to their diploid full-sibs. Although the prevalence of skeletal deformities recorded in this study was considerable, they were not severe enough to impact marketability or survival of fish

A high incidence of abnormality in morphological characteristics was previously reported in hatchery-reared flatfish compared with wild diploid flatfish (Ellis *et al.*, 1997). This high prevalence of abnormalities may be attributed to different factors within the hatchery environment, such as diet, lighting, population and/or density. However, further research is needed to determine when these morphological malformations start to develop and whether modifying factors within the hatchery environment would help prevent them.

In summary, our results indicate that cold-shock induction of triploidy in turbot does not affect gross body morphology and skeletal characteristics compared with their hatchery-reared diploid full-sibs. As indicated by Ellis *et al.* 1997 and Cal *et al.* 2006, malformations among hatchery-reared diploid turbot are not infrequent, and appear to be related with factors in the hatchery environment. It is possible that such effects are masking a malformation-inducing effect of the cold-shock used to induce polar body retention. Moreover, we have also to state that ploidy group comparison was made among full sibs of one single-pair mating. Nevertheless, under current rearing protocols, the induction of triploidy is not disadvantageous to commercial turbot aquaculture in the form of increased malformation rates, but does offer the distinct advantage of improved survival and growth in fish reared beyond the age of 2 years in order to reach a desired commercial size.

ACKNOWLEDGEMENT

We would like to thank Susana Magadán, María Jesús Lago, Castora Gómez and the staff of the Instituto Español de Oceanografía (IEO) for their help in the flow cytometric analysis and taking care of the fish. We would like also to recognise the helpful input and constructive comments of all the reviewers of Aquaculture journal, which have significantly improved our manuscript. We are grateful to Philip Thomas for assistance with the manuscript. This research was carried out with the financial support of Spanish Ministry of Science and Innovation (AGL2008-00392/ACU) and FPI (BES-2009-016797) and FPI-IEO grants to ET and JHU respectively.

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LEGEND TO THE FIGURES

Figure 1. Weight measurements of diploid turbot (black bars) and triploid turbot (white bars) at 6 and 12 months of age. Data are given as mean $\pm$ st.dev, n = 20 per ploidy and age group. (P < 0.05)

Figure 2. Morphometric external parameters (A) and skeletal parameters (B) measured in each sample of *Scophthalmus maximus* (TL, total length; SL, standard length; BW, body width; HL, head length; UJL, upper jaw length; LJL, lower jaw length; OL, orbital length; IOL, interorbital length; AV, abdominal vertebrae; CV, caudal vertebrae; DS, dorsal fin; AF, anal fin; SA, spinal angle (angle formed by the line joining the last 8 vertebrae of the spinal cord and the tangent to the curve formed by the spine in the first vertebrae). Data are given as percentage $\pm$ st.dev and/or range, n = 20 per ploidy and age group. (P < 0.05)

Figure 3. Frequency (%) of bone deformities based on radiographic examination of diploid turbot (black bars) and triploid turbot (white bars) from 6 and 12 months of age. Each bar represents the total number of deformities, scored as present or absent. Data are given as percentage $\pm$ st.dev, n = 10 per tank (P $\leq$ 0.05) within the same age group, n = 2 tanks per treatment.

Figure 4. Radiographic images of turbot at 12 months from the triploid group. Enlarged picture (bottom to the right) corresponds to the most common malformed areas. Scale bar, 1 cm.

Figure 1

Figure 2

Figure 3

Figure 4