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Susca V., Bridges C.R.

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Study of first sexual maturity in female bluefin tuna (*Thunnus thynnus*) from the central Mediterranean Sea

V. Susca*, A. Corriero, M. Deflorio**, S. Desantis**, C.R. Bridges* and G. De Metrio****
*Institut für Zoophysiologie, Lehrstuhl für Stoffwechselphysiol, Heinrich-Heine Universität, Düsseldorf D-40225, Germany
**Dept. of Animal Production, University of Bari, Str. Prov. Casamassima km 3, Valenzano, Bari 70010, Italy

SUMMARY – This preliminary study indicates that the minimum size at maturity is 110 cm (L_F) in female bluefin tuna (BFT) from the Mediterranean Sea. It is notable that pre-adult specimens (with L_F ranging from 100 to 110 cm) showed signs of gonadal development, characterized by the appearance of oocytes in the lipid stage. This finding occurred contemporaneously to both the appearance of E_2 and Vtg in the plasma. The evidence of the present study needs to be confirmed in a larger fish sample including sampling at the different phases of the reproductive cycle.

Key words: Tuna, sexual maturity, *Thunnus thynnus*, Vitellogenin, oocyte diameter, fork length.

RESUME – *"Etude de la première maturité sexuelle chez des thons rouges femelles (*Thunnus thynnus*) de la Méditerranée centrale". Cette étude préliminaire indique que la taille minimum à la maturité est de 110 cm (L_F) chez les thons rouges femelles de la mer méditerranée. Il est à remarquer que des spécimens pré-adultes (dont L_F va de 100 à 110 cm) ont montré des signes de développement gonadal, caractérisé par l'apparition d'ovocytes au stade lipidique. Cette découverte a eu lieu en même temps que l'apparition d'E_2 et Vtg dans le plasma. Ces faits concluants de la présente étude devront cependant être confirmés par un échantillonnage de poissons à plus grande échelle, y compris l'échantillonnage à différents stades du cycle reproductif.*

Mots-clés : *Thon, maturité sexuelle,* Thunnus thynnus*, vitellogénine, diamètre de l'ovocyte, longueur à la fourche.*

Introduction

The bluefin tuna BFT (*Thunnus thynnus*) is one of the most important economic fishing resources in the world. This species is subject to an intense fishing effort, which has raised questions concerning the survival of the stock (Sissenwine *et al.*, 1998). In spite of the economic importance of the species, the knowledge of its reproductive biology is limited to research based on: macroscopic classification of the gonads (Rodríguez-Roda, 1964, 1967); seasonal variations of the gonadosomatic index (I_G) (de la Serna and Alot, 1992); and the distribution of eggs and larvae (Piccinetti *et al.*, 1977, 1997; Cavallaro *et al.*, 1997; Nishida *et al.*, 1997). In depth studies, including histological analysis of ovaries, have been carried out in Western Atlantic BFT (Baglin, 1982), whereas histological as well as endocrinological studies are lacking in Eastern Atlantic and Mediterranean BFT stocks.

Since knowledge of reproductive biology is an extremely important tool for determining the correct policy for the management of fish stocks the present study deals with the size at first sexual maturity of female BFT from the Mediterranean Sea, correlating plasma levels of Vitellogenin (Vtg) and 17ß-Estradiol (E_2) and histological maturity stage of the ovaries.

Specimen collection

Blood and gonad samples were obtained, during the months of April and May 1998 and 1999 from female BFT with L_F ranging from 90 and 140 cm, caught commercially by long lines in the North Ionian Sea (Gulf of Taranto) and by traditional traps (Tonnare) operating in Carloforte and Portoscuso (Sardinia, Italy). Males were also sampled and used as controls. Soon after the capture fish L_F was

measured and blood was collected from the heart with heparinized syringes and cannula (longline fish) or directly in 15 ml tube (trap fish) from a cut, made by fishermen, under the pectoral fin to bleed the fish. The blood (with 1 mM PMSF) was kept on ice after sampling at sea, then centrifuged and plasma collected and stored at –20°C (usually < 4 h after capture). Fragments of ovaries were fixed in Bouin's solution for histological analysis and immunohistochemical investigations.

Methods

Histological and immunohistochemical evaluation of ovary maturity stage

Ovary samples were embedded in paraffin wax. Sections (5 μm thick) were stained with Haematoxylin – Eosin. The immunohistochemical detection of anti BFT-Vtg serum positive cells was performed using the avidin-biotin-peroxidase complex (ABC) procedure. Peroxidase activity was visualised by incubating with Vector DAB Peroxidase Substrate Kit (Vector, Burlingame, CA). Oocyte diameters were measured on histological slides using Quantimet (Leica, Cambridge, UK) image analyser.

Plasma 17ß-Estradiol (E_2) and Vitellogenin (Vtg) measurement

For the measurement of E_2 200 μl of plasma were extracted two times with Dichloromethane, dissolved again in PBS and measured by ELISA as described by Cuisset *et al.* (1994). The assay for BFT Vtg was established combining several different methods for Vtg ELISA as reported in the literature for other fish species.

Results

The results obtained correlating Vtg and E_2 plasma levels and histological analysis are summarised as follows in Fig. 1.

Recrudescence period (April)

(i) Fish with L_F ranging from 90 and 100 cm showed almost undetectable (<0.15 ng/ml; <0.16 mg/ml) E_2 and Vtg plasma levels. The histological observations revealed immature ovaries with oocytes in perinucleolus stage.

(ii) Fish with L_F ranging from 100 and 110 cm showed mean E_2 and Vtg plasma levels of 1.04 ng/ml and 0.93 mg/ml respectively. The histological analysis demonstrated the presence of both perinucleolar and lipid stage oocytes.

(iii) Fish with L_F ranging from 110 and 120 cm showed mean E_2 and Vtg plasma levels of 1.44 ng/ml and 1.74 mg/ml respectively. The ovaries showed no histological difference from the previous size class.

Ripening period (May)

(i) Fish with L_F ranging from 100 and 110 cm showed a slight increase of E_2 and Vtg plasma levels respect to the previous month (2.5 ng/ml and 3.33 mg/ml respectively), but no evolution of ovary maturity stage.

(ii) Fish with L_F ranging from 110 and 120 cm showed a significant increase of E_2 and Vtg plasma levels (12.98 ng/ml and 25.70 mg/ml respectively). The histological analysis demonstrated the presence of oocytes in late vitellogenic stage. The mean oocyte diameter increased significantly, in respect to the previous class (from 55 to 90 μm).

(iii) Fish with L_F > 120 cm showed an increase of Vtg plasma level and mean oocyte diameter

(32.54 mg/ml and 106 μm respectively) in respect to the previous length class and a significant increase of percentage of vitellogenic oocytes (from 5% of the previous class to 11.2%).

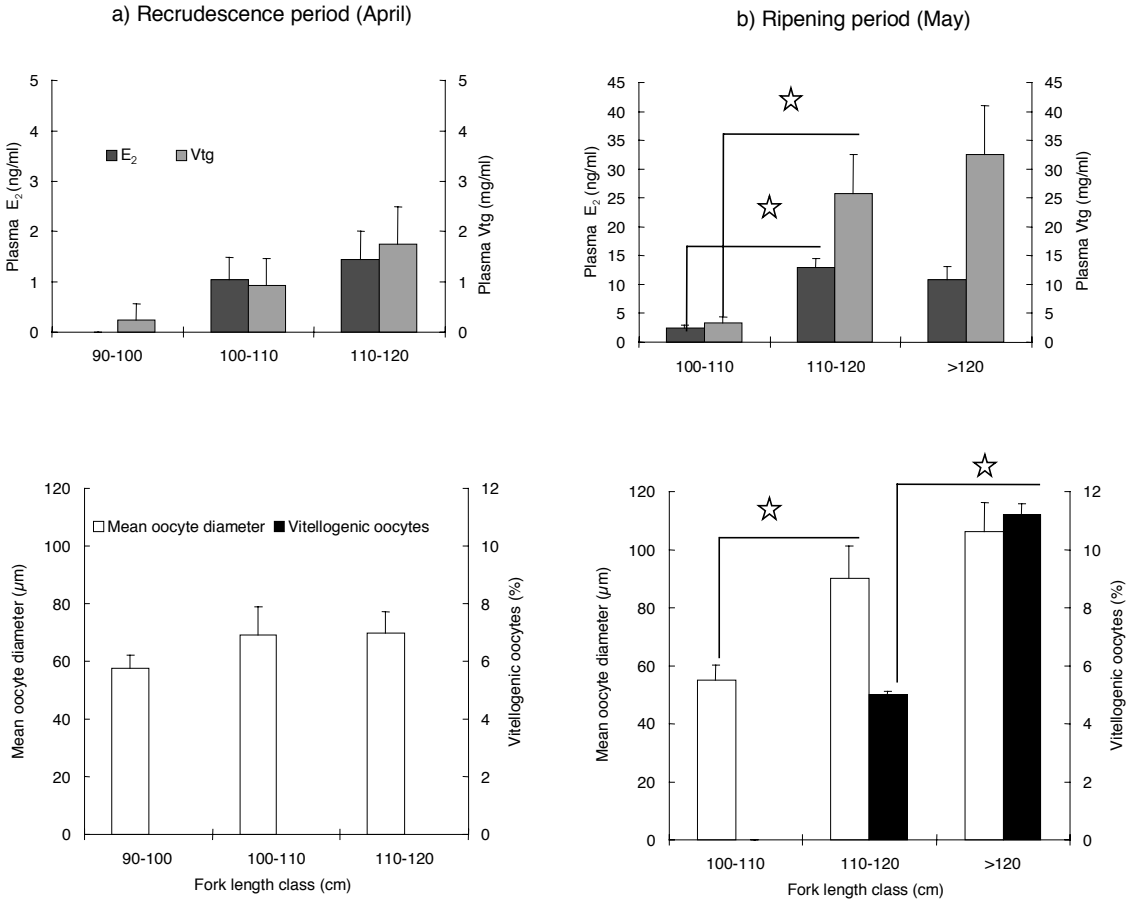

Fig. 1. Changes in plasma levels of 17ß-Estradiol (E_2), Vitellogenin (Vtg), mean oocyte diameter and percentage of vitellogenic oocytes in two different periods of the female BFT reproductive cycle: (a) recrudescence (April) and (b) ripening (May). Stars represent statistical significance $p < 0.05$) assessed by t-Test, N = 7.

The immunohistochemical staining of ovaries with anti BFT-Vtg serum revealed the presence of Vtg-like material in oocyte having a minimum diameter of 220 μm. Immunopositive oocytes were observed only in ovaries of specimens caught in May with $L_F > 110$ cm.

Conclusions

Determination of reproductive status is a key requirement for any fisheries management programme. Vitellogenin (Vtg) has been widespread used in aquaculture species as an indicator of sexual maturity (Mañanos *et al.*, 1994; Bon *et al.*, 1997; Mosconi *et al.*, 1998).

In wild fish populations, such as the large pelagic BFT, the purification of Vtg is particularly difficult due to the problem of obtaining fresh samples from female fish in the correct maturity state. In fact, during sampling the plasma of this fish has often showed high contamination with haemoglobin (our own observation), due to haemolysis from the catch stress, difficulties in blood extraction or the relatively long time between blood sampling and centrifugation.

In this study we established an ELISA for the detection of Vtg and validated the assay correlating the plasma levels of Vtg to those of E_2. Furthermore the immunohistochemical study confirmed the specificity of the anti-Vtg serum.

This preliminary study indicates that the minimum size at maturity is 110 cm (L_F) in female BFT from the Mediterranean Sea. This seems to be in agreement with Rodríguez-Roda (1967) who by macroscopic observation of gonadal maturity stage, found 100% mature females of Eastern Atlantic BFT over 120 cm (L_F).

It is notable that pre-adult specimens (with L_F ranging from 100 to 110 cm) showed a slight gonadal development, characterised by the appearance of oocytes in the lipid stage. This finding occurred contemporaneously to both the appearance of E_2 and Vtg in the plasma. The simulation of gonadal development has been reported by Baglin (1982) in sexual immature Western Atlantic BFT.

The findings of the present study need to be confirmed in a larger fish sample including sampling at the different phases of the reproductive cycle.

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