

Fishy business in the Mediterranean – Tuna, tonnara and testosterone

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SUMMARY – New methods based on molecular biology techniques have been used for sex determination and sexual maturity staging in *Thunnus thynnus*, the Bluefin Tuna (BFT). These are based on the determination of steroid hormone concentrations and their ratios and the presence of vitellogenin in plasma and muscle. Sampling techniques for muscle are discussed and the use of such techniques for sex determination in both BFT and swordfish.

Key words: Tuna, sex hormones, vitellogenin, muscle biopsy, keto-testosterone, estradiol, swordfish, testosterone, fisheries management.

RESUME – "Affaire de poisson en Méditerranée – Thon, tonnara et testostérone". De nouvelles méthodes basées sur les techniques de biologie moléculaire ont été utilisées pour la détermination du sexe et du stade de maturité sexuelle chez *Thunnus thynnus*, le thon rouge. Elles sont fondées sur la détermination des concentrations en hormones stéroïdes et leurs ratios ainsi que sur la présence de vitellogénine dans le plasma et le muscle. Les techniques d'échantillonnage du muscle sont discutées ainsi que l'utilisation de ces techniques pour la détermination du sexe chez le thon rouge et l'espardon.

Mots-clés : Thon, hormones sexuelles, vitellogénine, biopsie du muscle, keto-testostérone, estradiol, espardon, testostérone, gestion des pêches.

In many fisheries fish are either landed already gutted or the value of the flesh prohibits ventral opening and the determination of sex and sexual maturation. If sex or sexual maturation is not determined then this can have serious consequences for future management models of stocks and their development. The objectives of our ongoing studies was to provide new molecular techniques to make sexual identification possible in species where no external sexual dimorphisms exists and to further determine the maturation state of the fish this possible and thus assist in stock assessment and management.

To these ends concentrations of Testosterone, 11 Ketotestosterone and Estradiol were determined in Bluefin Tuna (BFT) and Swordfish gonads, muscle and plasma from the Mediterranean. The results are shown in Figs 1 and 2. Using the Sex Steroid Ratio Formula where $[(E2/11-KT)]/[T]$ is compared (Susca *et al.*, 2000) males could be distinguished from female fish during the breeding season. As an alternative the presence of Vitellogenin in muscle or plasma samples can be used as in the Dot-Blot shown in Fig. 3 which can easily distinguish between male and female fish and may be the obvious method of choice for bulk measurements as in fisheries and aquaculture applications.

Further developments include the design and testing of a semi-automatic muscle biopsy sampler where as little as 150 mg of tissue are required for sex determinations and the self-activating punch system can be used either as a "stand alone" hand-held device or mounted on a harpoon or tagging stick. This development is being considered for sampling from live brood stock as well as for use in fish tagging programmes where it is important to have a non-lethal sex determination method (Bridges *et al.*, 2000). In previous fishing seasons both plasma and muscle samples have been taken in BFT together with gonadal samples and full biometric data. Using this approach molecular endocrine techniques have

been calibrated with histological data and are now set for market testing. This will provide a significant handling tool for aquaculture studies of the future and allow monitoring of sex and sexual maturity even at the market place. Similar developments using field trials and sampling are also underway for Swordfish management although due to the possible presence of endocrine disruption in Mediterranean fish this may not be generally applicable in Mediterranean stocks but could be used for Atlantic, Pacific and Indian Ocean stocks when available.

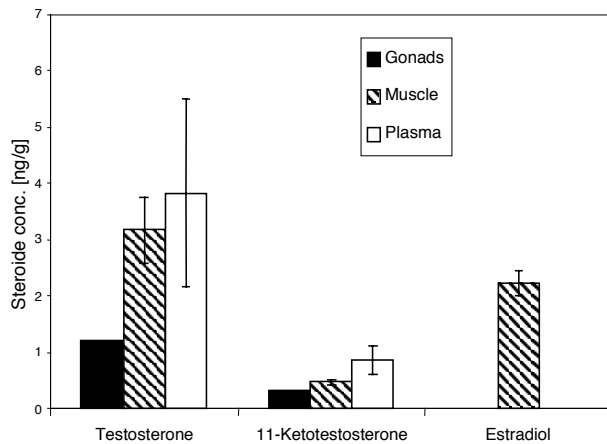


Fig. 1. Steroid hormone concentrations in the various tissues of the Bluefin Tuna measured with standard ELISA techniques (modified from Bridges *et al.*, 2000).

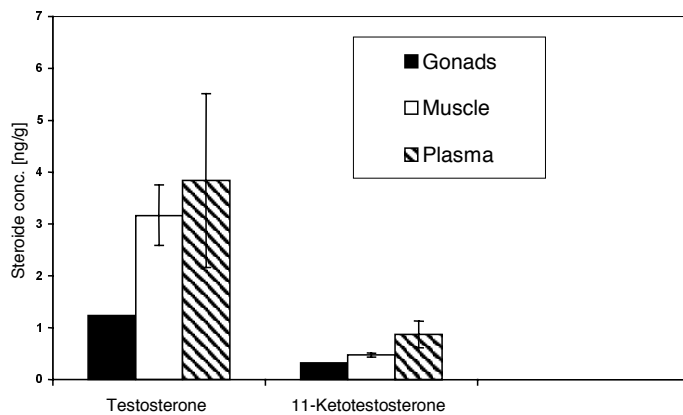


Fig. 2. Steroid hormone concentrations in various tissues of *Xiphias gladius* measured with standard ELISA techniques (modified from Bridges *et al.*, 2000).

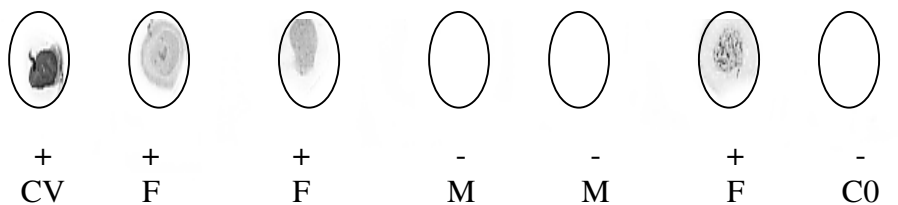


Fig. 3. Use of a dot blot analysis for plasma BFT-Vtg to determine the sex. Positive reaction (+) for Vtg is detected in females in the reproductive status (F). In males (M) the test is negative (-). This simple test can be used for sex determination when the measurement of steroids gives no precise results. CV = positive control, 1 μ g BFT-Vtg; C0 = negative control, 1 μ g bovine serum albumin (BSA). Modified from Susca *et al.* (2000) and based on Susca *et al.* (2001).

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References

- Bridges, C.R., Susca, V., Corriero, A., Deflorio, M. and de Metrio, G. (2000). A new muscle biopsy technique for sex and sexual maturity determination in large pelagic fishes. *ICCAT Collective Volume of Scientific Papers LII, SCRS/2000/192*. ICCAT, Madrid.
- Susca, V., Corriero, A., Deflorio, M., Bridges, C.R. and de Metrio, G. (2000). New results on the reproductive biology of the bluefin tuna (*Thunnus thynnus*) in the Mediterranean. *ICCAT Collective Volume of Scientific Papers LII, SCRS/2000/191*. ICCAT, Madrid.
- Susca, V., Corriero, A., Bridges, C.R. and de Metrio, G. (2001). Study of the sexual maturity of female bluefin tuna: Purification and partial characterization of vitellogenin and its use in an enzyme-linked Immunosorbent assay. *Journal of Fish Biology*, 58: 815-831.