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Gonadosomatic index and seasonal variations of plasma sex steroids in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) from the western Indian ocean

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Abstract – A total of 361 skipjack tuna (*Katsuwonus pelamis*) between 41.5 and 71.5 cm fork length and 333 yellowfin tuna (*Thunnus albacares*) between 48 and 150 cm fork length were collected in the western Indian ocean, between latitude 5° N to 10° S and longitude 45° to 65° E from February 1989 to November 1990. Yellowfin populations have one major reproductive season during the north monsoon while in skipjack the reproduction is almost continuous all year round with two peaks in activity during north and south monsoons. The gonadosomatic indexes (GSI) are correlated with the three climatic situations: the north monsoon, the south monsoon and two inter-monsoon seasons. The steroid hormone variations (oestradiol, oestrone and testosterone) are well correlated with the GSI in females. In males, maximum levels coincided with the two main reproduction periods in skipjack while the maximum values of testosterone and 11-ketotestosterone levels were only found during the north monsoon. Even if both species are continuous spawners, these results indicate the existence of different reproductive traits, especially when considering steroidogenesis and vitellogenesis. These findings suggest that over our fishing zone, the reproductive activity is significantly modulated by climatic changes. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

GSI / reproduction / steroid hormone / tuna

Résumé – Rapports gonado-somatiques et variations saisonnières des stéroïdes sexuels chez le listao (*Katsuwonus pelamis*) et l'albacore (*Thunnus albacares*) dans l'ouest de l'océan Indien. Au total 361 listaos (*Katsuwonus pelamis*) de 41,5 à 71,5 cm, et 333 albacores (*Thunnus albacares*) de 48 et 150 cm ont été pêchés dans l'ouest de l'océan Indien entre la latitude de 5° N et 10° S et la longitude de 45° et 65° E au cours de la période allant de février 1989 à novembre 1990. Les populations de listao présentent une reproduction pratiquement continue avec deux pics d'activité plus marqués pendant les moussons de sud et de nord, alors que chez l'albacore la plus forte activité de reproduction coïncide avec la mousson de nord. Les rapports gonado-somatiques (RGS) sont corrélés avec les trois situations climatiques : les moussons de nord et de sud et les intermoussons. Les variations des hormones stéroïdiennes (estradiol, estrone et testostérone) sont également bien corrélées avec le RGS chez les femelles. Chez les mâles, les valeurs maximales coïncident avec les deux périodes principales de reproduction chez le listao, alors que les niveaux de testostérone et de 11-ketotestostérone n'atteignent leur maximum chez l'albacore que pendant la mousson de nord. Si on peut considérer que ces deux espèces présentent une reproduction pratiquement continue, nos résultats mettent en évidence des différences notables tant au niveau de la stéroïdogenèse que des variations de RGS, et nous permettent de penser que l'activité reproductrice est fortement modulée par les événements climatiques. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

RGS / reproduction / hormone stéroïde / thon

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1. INTRODUCTION

Skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) are distributed throughout tropical oceans. For the past 30 years, they have been subjected to increasing exploitation. Although skipjack is the most important tuna resource for many countries, commercial fisheries usually prefer yellowfin tuna because of its high economic value. Extensive efforts are under way in many research laboratories around the world to study the importance of these tuna populations in order to keep them at the highest possible level of exploitation for traditional and industrial fisheries. To achieve this, biological parameters such as age, growth, reproduction and spawning time need to be determined.

The reproductive condition of skipjack and yellowfin tuna has been investigated by several techniques: macroscopic aspects of ovaries (Schaefer and Orange, 1956; Orange, 1961; Batts, 1972; Alekseyev and Alekseyeva, 1981; Pereira, 1986), comparison of gonadosomatic indexes (Schaefer and Orange, 1956; Kikawa, 1959; Orange, 1961; Stéquert, 1976; Albaret, 1977; Cayré and Farrugio, 1986; Goldberg and Au, 1986; McPherson, 1991; Stéquert and Ramcharrun, 1996) and examination of oocytes (Bunag, 1956; Schaefer and Orange, 1956; Orange, 1961; Batts, 1972; Stéquert, 1976; Albaret, 1977; Cayré and Farrugio, 1986; Stéquert and Ramcharrun, 1996).

As yellowfin and skipjack tuna have been shown to be multiple spawning species (Joseph, 1963; Hunter et al., 1986; Stéquert and Ramcharrun, 1995), the spawning periods, their intensity and their duration are not clearly estimated by standard methods. In order to improve our knowledge, we measured blood steroid levels related to reproductive activity. The main objective of the present study was to verify, for skipjack and yellowfin tuna from the western part of the Indian ocean, if the levels of some steroid hormones involved in reproduction were correlated with the gonadosomatic indexes (GSI). We also compared these hormone profiles with variations of gonadosomatic index in relation with environmental conditions such as sea surface temperature, photoperiod and monsoon. The relationship with the monsoon was of importance because our sampling area is under monsoon influence with three climatic situations, the north monsoon, the south monsoon and two inter-monsoon seasons. This is the first report associating endocrinological studies, reproduction and environmental factors in these tuna species.

2. MATERIALS AND METHODS

2.1. Field sampling

A total of 361 skipjack tuna (156 females and 205 males) and 333 yellowfin tuna (169 females and 164 males) were collected in the western Indian ocean, between latitude 5° N to 10° S and longitude 45° to 65° E. Sampling occurred on French purse seiners based in Mahé (Seychelles islands), from February 1989 to November 1990. Fish were caught near the surface in the morning (between 7:00 and 11:00 hours) and water surface temperatures were recorded at each sampling time. Photoperiod was calculated using the 'Bureau des longitudes' web site www.bdl.fr.

Each fish was caught alive in a reduced purse seine net with an 80-cm (skipjack) or 2-m (yellowfin) scoopnet. Blood was collected using a 10-mL heparinised syringe containing a protease inhibitor (phenylmethylsulfonyl fluoride 1 mM), from the ventral aorta, at the connection with the bulbus arterius, and immediately centrifuged (10 min at 6 000 *g*). The plasma was separated and frozen at –30 °C in 2.5-mL aliquots.

All fish were measured to the nearest 0.5 cm from the tip of the snout to the fork of the tail (fork length) and weighted with a spring balance to the nearest 100 g. Skipjack tuna sampled were between 41.5 and 71.5 cm fork length and yellowfin tuna were between 48 and 150 cm fork length. Gonads were removed, placed in a plastic bag with identification labels and frozen, then weighed to establish the gonadosomatic index (GSI):

$$GSI = 10^2 \times (Wg/W)$$

where Wg is the weight of the gonads in grams, and W the total weight of the fish (non-gutted).

2.2. Radio immunoassay (RIA) methodology

The method used for steroid hormone measurement was based on that described by Fostier et al. (1978). Briefly, the plasma samples were extracted twice in a mixture of cyclohexane-ethyl acetate (50:50, v/v). The organic phase was then evaporated and the residue recovered and subjected to chromatography on Sephadex LH 20 (Pharmacia) column (100 × 5 mm) with dichloromethane-methanol (95:5) as the solvent. This chromatography allows the separation of androgens and estrogens. The different fractions were then evaporated and recovered in 500 µL RIA phosphate buffer (0.01 M, pH 7.25; NaCl 9 ‰ and 1 ‰ gelatin). Specific recovery was determined by adding 2 000 dpm of the desired tritiated steroid to the plasma sample before extraction. For each sample, two aliquots of 100 µL were used for the assay. For standard curves (from 5 to 800 pg·tube^{-1}) each point was prepared in triplicate. The specific antibodies were added to each tube (standards and samples) at appropriate dilution and incubated with 100 µL tracer (10 000 dpm·100 µL^{-1}) for 3 h at 4 °C. Precipitation was performed with 25 % polyethylene glycol 6000 (Merck) and centrifugation at 2 500 *g* for 2 × 30 min at 4 °C. Each tube received 2.8 mL scintillation solution (Amersham) and radioactivity was counted in a Beckmann scintillation counter.

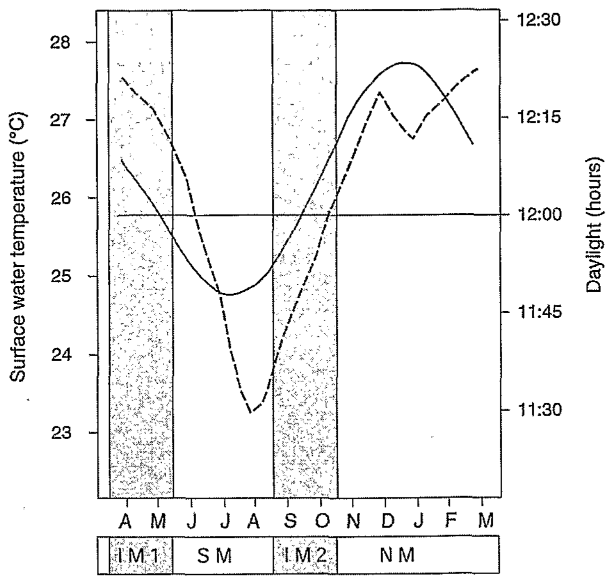

Figure 1. Surface temperatures (dotted line) and day length duration (solid line) in the sampling zone. IM1: inter-monsoon 1, SM: south monsoon, IM2: inter-monsoon 2, NM: north monsoon.

2.3. Statistics

Mean value and standard error of the mean (SEM) were calculated for each month and climate period (inter-monsoons, north and south monsoons). A one-way analysis of variance was conducted and if the F-test was significant ($P < 0.05$), differences between means were tested by Fisher's protected least squares significant differences (PLSD) test.

3. RESULTS

3.1. Environmental conditions in the sampling zone

All over our sampling zone, the surface temperature (*figure 1*) varied from a maximum of 27.5 °C during the north monsoon (November to March) to a minimum of 23.5 °C by the end of the south monsoon (June to August). Minimum and maximum day length were 11:30 and 12:40 hours respectively (*figure 1*). A close correlation was observed between photoperiod and temperature variations. Temperature and day length decreased during the south monsoon period and increased during the north monsoon.

3.2. Gonadosomatic indexes

The mean GSI showed one major peak for yellowfin tuna (*figure 2a*) which suggests that there is one main reproductive season during the north monsoon (November to March). A minor spawning period involving a small number of spawning females was also observed during the south monsoon (June to August).

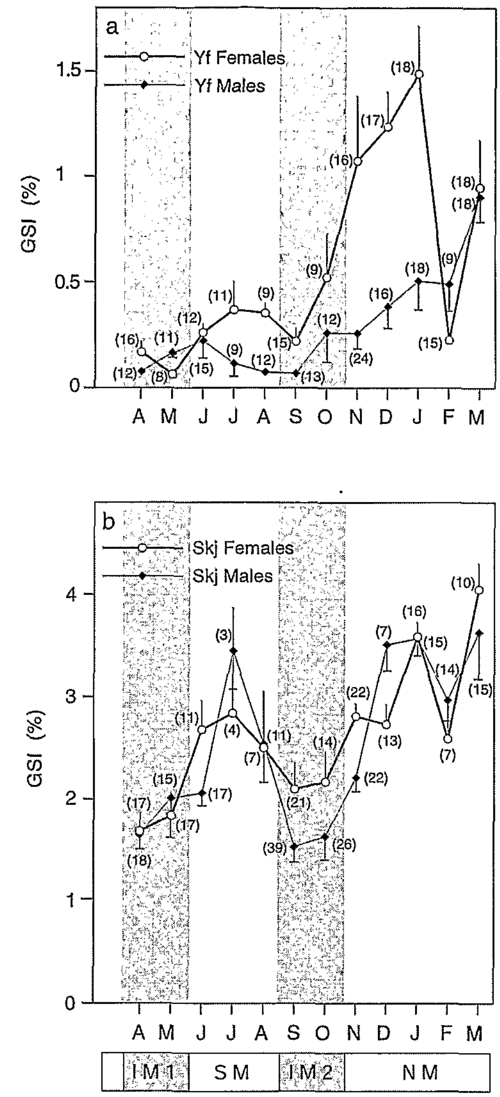

Figure 2. Mean monthly variations of GSI for male and female yellowfin tuna (**a**) and skipjack tuna (**b**). Vertical bars indicate the standard error of the mean. IM1: inter-monsoon 1, SM: south monsoon, IM2: inter-monsoon 2, NM: north monsoon, Yf: yellowfin, Skj: skipjack. Number of fish sampled is in parentheses.

The mean GSI for skipjack tuna suggests that reproduction is almost continuous year round; however, GSI was lower during the inter-monsoon periods (*figure 2b*) indicating a lower reproductive activity. A comparison between the two species reveals that the maximum GSI values for females was lower in yellowfin (around 1.5) than in skipjack (around 4). The maximum GSI values for males were similar to those of females in skipjack and their variation parallels exactly that of the females. In yellowfin tuna, however, the GSI was lower in males than in females and the values do not exactly parallel each other.

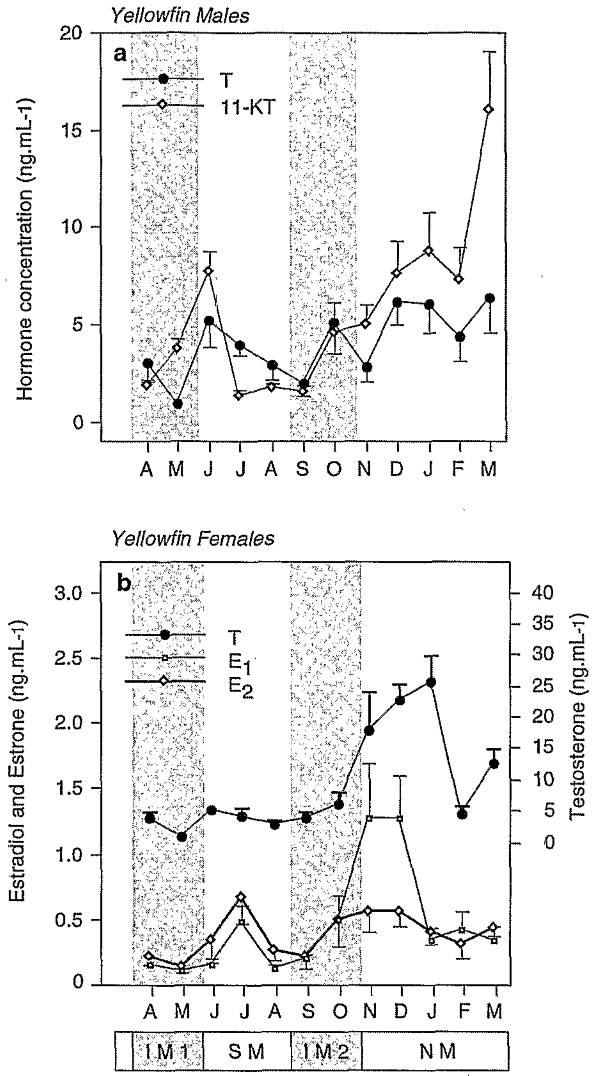

Figure 3. Monthly variations of testosterone (T) and 11-ketotestosterone (11-KT) in yellowfin tuna males (**a**) and T, oestrone (E_1) and oestradiol (E_2) in yellowfin tuna females (**b**). IM1: inter-monsoon 1, SM: south monsoon, IM2: inter-monsoon 2, NM: north monsoon. Vertical bars indicate the standard error of the mean. Number of fish sampled as in *figure 2*.

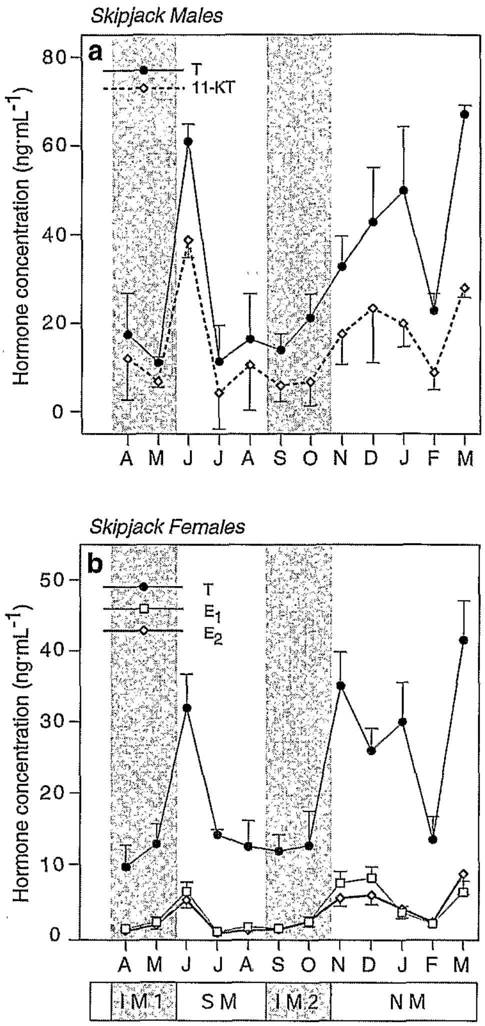

Figure 4. Monthly variations of testosterone (T) and 11-ketotestosterone (11-KT) in skipjack tuna males (**a**) and T, oestrone (E_1) and oestradiol (E_1) in skipjack tuna females (**b**). IM1: inter-monsoon 1, SM: south monsoon, IM2: inter-monsoon 2, NM: north monsoon. Vertical bars indicate the standard error of the mean. Number of fish sampled as in *figure 2*.

3.3. Hormonal variations in males

Plasma testosterone (T) levels in male yellowfin tunas were almost constant year round (*figure 3a*), while plasma 11-ketotestosterone levels (11-KT) peaked in March and were significantly higher ($P < 0.05$) during the north monsoon. The 11-KT levels did not show significant variations during the rest of the year.

In skipjack, plasma T and 11-KT levels increased rapidly in June, declined in July and then increased progressively from November to January (*figure 4a*). The increase of both steroids corresponded to the beginning of the south and north monsoons. Plasma 11-KT and T levels dropped dramatically in February and then increased in March to reach their maximum value. The levels were significantly lower ($P < 0.05$) during the inter-monsoon periods for both steroids.

3.4. Hormonal variations in females

In yellowfin females (*figure 3b*), there was a marked increase in T during the north monsoon which is significantly different from the other periods. The high T values were closely correlated with the GSI values (*figure 2a*). Oestrone (E_1) levels were at their maximum during the same period and are also significantly different ($P < 0.05$) from the other periods. Oestradiol (E_2) levels were relatively low year round, but showed a slight increase, during the monsoons and were low during the inter-monsoons.

Skipjack females had high levels of T during the two monsoons (*figure 4b*) and significantly lower levels ($P < 0.05$) during the inter-monsoons. Plasma E_2 and E_1 levels showed limited variations year round and they had very similar profiles with their minimum values during the inter-monsoons and maximum values during the monsoons. All steroid profiles present a bimodal profile as GSI, with the maximum values during the two monsoons (*figure 2b*).

4. DISCUSSION

The results reported here confirm that hormonal variations are well correlated with GSI variations. Our results allow us to distinguish two important reproductive seasons in skipjack (during the monsoons) with a reproductive activity year round as previously reported in this fishing zone (Timohina and Romanov, 1996). In yellowfin, we observe a marked reproduction period during the north monsoon and a low reproductive activity during the south monsoon period on the basis of the GSI indexes. This situation is probably related to the low sea surface temperatures which have been reported to reduce the spawning activity in yellowfin (Schaefer, 1998; Itano, 2000). These observations are confirmed by the profiles of some steroid hormones involved in the reproduction of teleosts. Plasma T, E_2 and E_1 levels presented a bimodal pattern in skipjack females and only one major peak in yellowfin females since the magnitude of the south monsoon peak in yellowfin is much lower than during the north monsoon season as previously observed with the GSI. This indicates that yellowfin is more sensitive to environmental factors and especially to temperature which is less than 25 °C during the south monsoon. The situation in males is similar; GSI and plasma T and 11-KT profiles are almost parallel with two equivalent peaks in skipjack while two slightly dissymmetrical ones are observed in yellowfin. For both species, the duration of the south monsoon reproductive season is shorter than the north monsoon reproduction period. Nevertheless during the north monsoon, some fluctuations are observed. GSI and plasma steroid hormones present a drop in females as well as in males during February. In females, this situation corresponds to a great proportion of post-spawned fish in the samples with ovaries presenting a great percentage of post-ovulatory follicles as previously mentioned (Stéquert and Ramcharrun, 1996). In males, the GSI variations accompanying T and 11-KT decline are less significant but we do not have any information on the stage of testicular maturation. Since we observed in the following month (March) an increase of GSI and hormonal levels, we also hypothesised the existence of adverse conditions for reproduction during a short period of time during February. The presence of a divergence created by the south equatorial current and south equatorial countercurrent induces a local upwelling of cold water which is responsible for local variations of temperatures. In this situation, the upper warm water layer is reduced to 20–30 m with very steep gradients (around 5 °C per 10 m) leading to water temperatures of 20 °C at 50 m (Stéquert and Marsac, 1986). Finally, as these species have a pronounced migratory behaviour, we cannot exclude the possibility of sampling a population coming from other zones and at different maturation stages.

The steroid hormone variations reported here correlate well with GSI in females. Oestradiol which has been reported to be the main steroid involved in the stimulation of vitellogenin synthesis in teleosts (Emmersen and Petersen, 1976; Le Menn, 1979a, 1979b) peaked during the most favourable reproduction periods in both species. The E_2 levels are parallel with E_1 levels which are in the same order of magnitude. The evolution of T levels also parallels that of oestrogens but with higher values. Peaks of these three steroids are found before the maximum spawning activity and they drop by the end of the monsoons.

Hormonal variations in males are in agreement with the results reported in teleosts (see Fostier et al., 1983). The maximum levels coincided with the two main reproduction periods in skipjack while in yellowfin the variations of T are significantly higher during the two monsoons while 11-KT is significantly different only during the north monsoon. It is generally admitted in teleosts that 11-KT is related to spermiogenesis, T being more implicated in the initiation and maintenance of the spermatogenetic process.

In skipjack both T and 11-KT peaked almost at the same time showing a close parallel pattern, indicating that spermiogenesis activity is stimulated during south and north monsoons. These observations allow us to hypothesise that skipjack tuna is a continuous spawner even if two preferential reproduction periods are observed. This implies a continuous stimulation of spermatogenesis and spermiation, and is in agreement with the concomitance of T and 11-KT peaks. Another difference between these species is the difference of maximum hormone levels: in skipjack, T is the major steroid with levels ranging from 12 to 65 $ng \cdot mL^{-1}$ while 11-KT levels varied from 5 to 49 $ng \cdot mL^{-1}$. In contrast, 11-KT is the major steroid in yellowfin with levels ranging from 2 to 17 $ng \cdot mL^{-1}$ while T levels vary only from 1 to 6 $ng \cdot mL^{-1}$. These hormonal differences might be related to the different reproduction strategies previously mentioned.

Even if both species are continuous spawners, these results indicate the existence of different reproductive traits, with yellowfin being more sensitive to unfavourable conditions.

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References

Albaret, J.J., 1977. La reproduction de l'albacore (*Thunnus albacares*) dans le Golfe de Guinée. Cah. ORSTOM. Sér. Océanogr. 15, 389–4194.

Alekseyev, F.E., Alekseyeva, E.I., 1981. Some aspects of the reproductive biology of oceanic and neretic tunas (Scombridae) in the tropical ocean. J. Ichthyol. 21, 52–64.

Batts, B.S., 1972. Sexual maturity, fecundity and sex ratios of the skipjack tuna *Katsuwonus pelamis* (Linnaeus), in North Carolina waters. Trans. Am. Fish. Soc. 101, 626–637.

Bunag, D.M., 1956. Spawning habits of some Philippine tuna based on diameter measurements of the ovarian ova. Philipp. J. Fish. 4, 145–177.

Cayré, P., Farrugio, H., 1986. Biologie de la reproduction du listao (*Katsuwonus pelamis*) de l'océan Atlantique. In: Symmons, P.E.K., Miyake, P.M., Sahagawa, G.T. (Eds.), Proc. ICCAT Conf. Int. Skipjack Year Program, Int. Comm. Conser. Atl. Tunas, Madrid, Spain, pp. 252–272.

Emmersen, B.K., Petersen, I.M., 1976. Natural occurrence and experimental induction by estradiol-17 β, of a lipoprotein (vitellogenin) in flounder (*Platichthys flesus* L.). Comp. Biochem. Physiol. 54B, 443–446.

Fostier, A., Weil, C., Terqui, M., Breton, B., Jalabert, B., 1978. Plasma estradiol-17 β and gonadotropin during ovulation in rainbow trout (*Salmo gairdnieri* R.). Ann. Biol. Anim. Biochem. Biophys. 18, 929–936.

Fostier, A., Jalabert, B., Billard, R., Breton, B., 1983. The gonadal steroids. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology. Academic Press, New York, pp. 277–372.

Goldberg, S.R., Au, D.W.K., 1986. The spawning of skipjack tuna from the southern Brazil as determined from histological examination of ovaries. In: Symmons, P.E.K., Miyake, P.M., Sahagawa, G.T. (Eds.), Proc. ICCAT Conf. Int. Skipjack Year Program, Int. Comm. Conser. Atl. Tunas, Madrid, Spain, pp. 277–284.

Hunter, J.R., Macewicz, B.J., Sibert, J.R., 1986. The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the south Pacific. Fish. Bull. 84, 895–903.

Itano, D.G., 2000. The Reproductive Biology of Yellowfin Tuna (*Thunnus albacares*) in Hawaiian Waters and the Western Tropical Pacific Ocean: Project Summary. Joint Inst. Mar. Atmos. Res., Hawaii Contrib., No. 00-328.

Joseph, J., 1963. Fecundity of yellowfin tuna (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*) from the Pacific Ocean. Inter-Am. Trop. Tuna Comm. Bull. 7, 257–292.

Kikawa, S., 1959. Notes on the regional differences of spawning season of Pacific yellowfin tuna. Rep. Nankai Reg. Fish. Res. Lab. 11, 59–76.

Le Menn, F., 1979a. Induction de la vitellogénine par l'oestradiol et par des androgènes chez un téléostéen marin *Gobius niger* L. C.-R. Acad. Sci. Paris 289, 413–415.

Le Menn, F., 1979b. Some aspects of vitellogenesis in a teleostean fish: *Gobius niger* L. Comp. Biochem. Physiol. 289, 413–415.

McPherson, G.R., 1991. Reproductive biology of yellowfin tuna in the eastern Australian fishing zone, with special reference to the north-western Coral Sea. Aust. J. Mar. Freshw. Res. 42, 465–477.

Orange, C.J., 1961. Spawning of yellowfin tuna and skipjack in the eastern tropical Pacific, as inferred from studies of gonad development. Inter-Am. Trop. Tuna Comm. Bull. 2, 459–526.

Pereira, J., 1986. Maturité sexuelle et sex-ratio du listao (*Katsuwonus pelamis*) capturé aux Açores de 1980 à 1982. In: Symmons, P.E.K., Miyake, P.M., Sahagawa, G.T. (Eds.), Proc. ICCAT Conf. Int. Skipjack Year Program, Int. Comm. Conser. Atl. Tunas, Madrid, Spain, pp. 273–275.

Schaefer, K.M., 1998. Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. Inter-Am. Trop. Tuna Comm. Bull. 21, 1–272.

Schaefer, M.B., Orange, C.J., 1956. Studies of the sexual development and spawning of yellowfin tuna (*Neothunnus macropterus*) and skipjack (*Katsuwonus pelamis*) in three areas of the eastern Pacific Ocean, by examination of gonads. Inter-Am. Trop. Tuna Comm. Bull. 1, 283–349.

Stéquert, B., 1976. Étude de la maturité sexuelle, de la ponte et de la fécondité du listao (*Katsuwonus pelamis*) de la côte nord-ouest de Madagascar. Cah. ORSTOM, Sér. Océanogr. 14, 227–247.

Stéquert, B., Marsac, F., 1986. La pêche de surface des thonidés tropicaux dans l'océan Indien. FAO Doc. Tech. Pêches 282, 1–213.

Stéquert, B., Ramcharrun, B., 1995. La fécondité du listao (*Katsuwonus pelamis*) de l'ouest de l'océan Indien. Aquat. Living Resour. 8, 79–89.

Stéquert, B., Ramcharrun, B., 1996. La reproduction du listao (*Katsuwonus pelamis*) dans le bassin ouest de l'océan Indien. Aquat. Living Resour. 9, 235–247.

Timohina, O.I., Romanov, E.V., 1996, Characteristics of ovogenesis and some data on maturation and spawning of skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758), from the western part of the Equatorial Zone of the Indian Ocean. IPTP Coll., Colombo, Sri Lanka, vol. 9, pp. 247–257.