



Effects of temperature on the reproductive physiology of female elasmobranchs: The case of the narrownose smooth-hound shark (*Mustelus schmitti*)

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ARTICLE INFO

Keywords:

Sex steroids
Vitellogenesis
Ovulation
Elasmobranchs
Pregnancy
Environmental cues

ABSTRACT

The knowledge of how temperature influences elasmobranchs reproductive physiology allows a better understanding of their reproductive patterns. This study describes the relationship between temperature fluctuations and the plasmatic changes of the sex steroids related to reproduction: testosterone (T), estradiol (E₂) and progesterone (P₄), throughout the female reproductive cycle of the shark *Mustelus schmitti*. A total of 123 adult females were bi-monthly sampled in Buenos Aires, Argentina, coastal waters. Bottom temperatures were recorded at each sampling point and blood samples were taken from each female for plasma sex steroids measurement. Sex steroid plasma levels were analyzed in relation with maximum follicular diameter (MFD), uterosomatic index (USI, as indicator of pregnancy) and temperature using Generalized Additive Models. Plasmatic E₂ and T increased during follicular growth until MFD reached 1.34 and 1.46 cm, respectively. Peak of T occurred at the follicular stage associated with parturition (MFD, 1.4–1.6 cm), just prior to final maturation and ovulation (MFD, 1.6–2.0 cm). Progesterone significantly increased at this last ovarian phase, while T and E₂ decreased. The increase of USI with pregnancy was associated to a decrease in T and mainly E₂ levels, while P₄ remained unaffected. Prior to ovulation, T plasma levels decreased with temperature below to 13 °C and then increased progressively with a pronounced elevation above 17 °C, while E₂ presented an opposite pattern. Progesterone plasma levels changed with temperature showing a similar pattern to that observed for T. Using *M. schmitti* shark as model species, this study shows a clear picture of how seawater temperature variations can affect the reproductive physiology in elasmobranch females. A hypothetical mechanism (based on T elevation driven by temperature increase and its connection by feedback with a P₄ rise and parturition/ovulation induction) is proposed as evidence to support that the increase in temperature can trigger reproductive events in elasmobranchs. In addition to its ecological scope, this work contributes to reinforce the relatively scarce general knowledge of elasmobranchs reproductive physiology.

1. Introduction

Understanding the eco-physiological mechanisms controlling reproductive events in elasmobranchs (sharks, skates and rays) is important to recognize the spatio-temporal dynamics of their populations throughout their life cycle.

As in other aquatic vertebrates, water temperature appears to play a key role in controlling elasmobranch's reproduction, although the physiological mechanisms involved in this control are largely unknown (Maruska and Gelsleichter, 2011; Awruch, 2015). Information gathered in teleost fishes suggests that one of the mechanisms by which water temperature regulates their reproduction is by controlling the

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<https://doi.org/10.1016/j.ygcn.2019.113242>

Received 24 April 2019; Received in revised form 6 August 2019; Accepted 6 August 2019

Available online 07 August 2019

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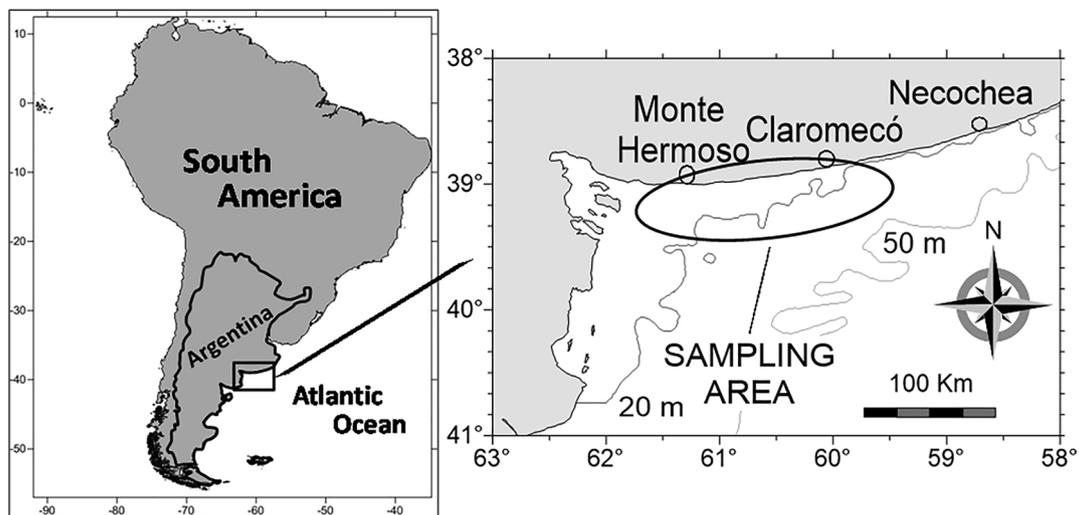


Fig. 1. The study area within *El Rincón* region.

production of gonadal sex steroids, key hormones for the regulation of reproductive functions (Pankhurst and Porter, 2003; Pankhurst and Munday, 2011; Miranda et al., 2013). In elasmobranch females, three major sex steroids, testosterone (T), estradiol (E₂) and progesterone (P₄), are important to regulate ovarian cycle (follicular resting, vitellogenesis and ovulation) as well as other reproductive functions such as pregnancy, in the case of viviparous species (for review see Callard et al., 1993; Callard and Koob, 1993; Lutton et al., 2005; Gelslechter and Evans, 2012; Awruch, 2013). Changes of plasmatic levels of these sex steroids were correlated with water temperature in different elasmobranch species (Sumpter and Dodd, 1979; Heupel et al., 1999; Mull et al., 2008, 2010; Waltrick et al., 2014; Nozu et al., 2018), suggesting a similar situation to that reported for teleosts. Based on evidences gathered in teleosts, it appears that increase in temperature, by influencing T and E₂ levels, may control not only the mechanisms eliciting final maturation and ovulation (Pankhurst and Porter, 2003), but also induce ovary regression and resting (Soria et al., 2008; Elisio et al., 2012, 2014).

In this context, this study explores the effects of temperature in regulating reproductive events in elasmobranch females, by analyzing the relationship between seawater temperature fluctuations and changes in sex steroid plasma levels throughout the reproductive cycle of the shark *Mustelus schmitti* as a model species.

The narrownose smooth-hound, *M. schmitti*, is one of the most abundant and landed elasmobranchs along the coastal waters of the south-western Atlantic Ocean (Massa et al., 2004a,b; Oddone et al., 2005; Menni et al., 2010). It is a small matrotrophic viviparous shark (up to 108.5 cm of total length) displaying a minimal histotrophic strategy for embryos nutrition (Galíndez et al., 2010; Orlando et al., 2015; Quesada, 2018). The species presents an annual reproductive cycle in which follicular growth takes place in parallel with gestation, pregnancy lasts nearly a full year and ovulation-mating occurs soon after parturition (Menni et al., 1986; Oddone et al., 2005; Elisio et al., 2017). The main reproductive grounds of *M. schmitti* comprise different shallow coastal areas within the south-western Atlantic Ocean, being *Río de La Plata* (34–38°S) and *El Rincón* (38–42°S) recognized as the most important ones (Oddone et al., 2007; Colautti et al., 2010; Cortés et al., 2011; Elisio et al., 2017). Adults concentrate in these two main areas during spring-early summer (Jaureguizar et al., 2004; Colautti et al., 2010; Cortés et al., 2011), when the parturition-ovulation and mating events occur associated with the seawater temperature increase above 16–17 °C (Elisio et al., 2017).

In this context, exploring if temperature increase represents a key environmental cue for triggering the reproductive events in *M. schmitti*

by influencing sex steroids plasma levels becomes particularly important in order to understand the reproductive process in this and other elasmobranch species.

2. Materials and methods

2.1. Animal sampling and temperature recording

Pregnant and non-pregnant *Mustelus schmitti* adult females were obtained from Buenos Aires (Argentina) coastal waters, between 38.5 and 39.5°S and < 21 m depth (within *El Rincón* region, Fig. 1). Individuals were caught by longline or rod and reel fishing bi-monthly from October 2014 to August 2015. Shortly after capture, blood samples were taken from each female by caudal venipuncture using heparinized syringes (23G). These samples were preserved at 4 °C for no longer than twelve hours and then centrifuged for 15 min at 4800g. Plasma samples were then stored at –80 °C until sex steroid measurements.

After sampling, sharks were euthanized and the following variables were registered: Total length (TL, cm), eviscerated weight (EW, g), maximum follicular diameter (MFD, cm), uteri weight (UtW, g), presence or absence of ova or differentiated embryos in the uteri, number of embryos and average of embryos total length (cm). Also, the presence/absence of a yellow yolky follicular leading clutch was registered in all cases. When a follicular leading clutch was evident in the ovary and follicles were pale light yellow (and not the characteristic yellow color), a portion of ovary was fixed in 4% formalin diluted in seawater and then processed for Hematoxylin-Eosin routine histological procedure in order to evaluate the presence of vitelline platelets within follicles. Adult reproductive stages were identified as already explained in Elisio et al. (2017). A total of 123 adult females were sampled. Thirty-six were non-pregnant adult females (FIII) (TL range: 56–71 cm), 87 were pregnant females (TL range: 55–83 cm), 23 with ova in their uteri (FIV) and 64 with differentiated embryos (FV) with average of embryos total lengths between 8 and 26 cm. Because of the smallest juvenile reported for this species was 25.2 cm (Chiaromonte and Pettovello, 2000), the samples were considered that represented pregnancy cycle until just prior to parturition.

During sampling, bottom water temperatures (°C) were registered in each fishing site every 10 min using a temperature recorder (Starmon mini underwater temperature recorder, STAR-ODDI, Gardabaer, Iceland). Temperature at which each female was caught was estimated as the average of all temperatures recorded while the respective fishing procedure was performed.

2.2. Plasma sex steroid measurements

Plasma levels of T, E₂ and P₄ were measured by radioimmunoassay (RIA), following protocols previously reported by Ravaglia et al. (1997). Briefly, steroids from the plasma samples (500–1000 µl) were extracted twice with diethyl ether in a 1:5 ratio. The mixtures were vortexed for 1 min and the allowed for the two phases (aqueous below and organic above) to separate perfectly. In each extraction, the aqueous phase was frozen at –80 °C and transferred the organic phase onto a 15 ml glass tube, which was placed in an immersion bath at 45 °C, under an extraction fume hood, for solvent evaporation. Once solvent was completely evaporated, the dry steroid extraction of each sample was resuspended with RIA buffer in their initial plasma volume. Extraction efficiency determined by recovery of ³H-steroid added to plasma was > 95%. The antisera for the three steroids were provided by Dr. Niswender (University of Colorado, USA). All samples measurements for each steroid were performed within the same assay. The lower limits of detection were 23.1 pg/ml for T, 36.4 pg/ml for E₂, and 339.7 pg/ml for P₄. The intra-assay coefficients of variance were 4% for T, 13% for E₂ and 10% for P₄.

2.3. Classification of follicular growth throughout the reproductive cycle

To characterize the follicular sizes associated with vitellogenesis, final maturation, ovulation and follicular resting throughout the

reproductive cycle, the MFD size range was assessed in both, pregnant (FIV and FV) and non-pregnant (FIII) adult females. Due to two clearly differentiated groups of MFD values distribution in FIV (Fig. 2), this reproductive stage was further divided into two different sub-stages: ovulating/early pregnant females (FIVov), with large follicles (≥ 1.6 cm diameter), and post-ovulated/early pregnant females (FIVpostov), with small follicles (≤ 0.5 cm diameter). Non-pregnant FIII females with MFD values like those observed in FIVov females were considered to be at final maturation stage.

2.4. Sex steroid plasma levels throughout reproductive cycle

To analyze the sex steroids profile throughout the reproductive cycle, the following three phases were considered: i. Follicular growth/advanced pregnancy phase (FIII and FV females). This period involves from vitellogenesis to final maturation, pregnancy and parturition. ii. Ovulation/early pregnancy phase. This is represented by FIVov involving the stage in which oocytes are being ovulated and at least one of them already moved to the uteri. iii. Post-ovulation/early pregnancy phase, represented by FIVpostov and involving stage in which all oocytes were already ovulated and moved to uteri and the ovary was associated with resting period.

Because of water temperature is expected to affect hormonal regulation during vitellogenesis prior to ovulation, the influence of this environmental factor was investigated throughout the follicular

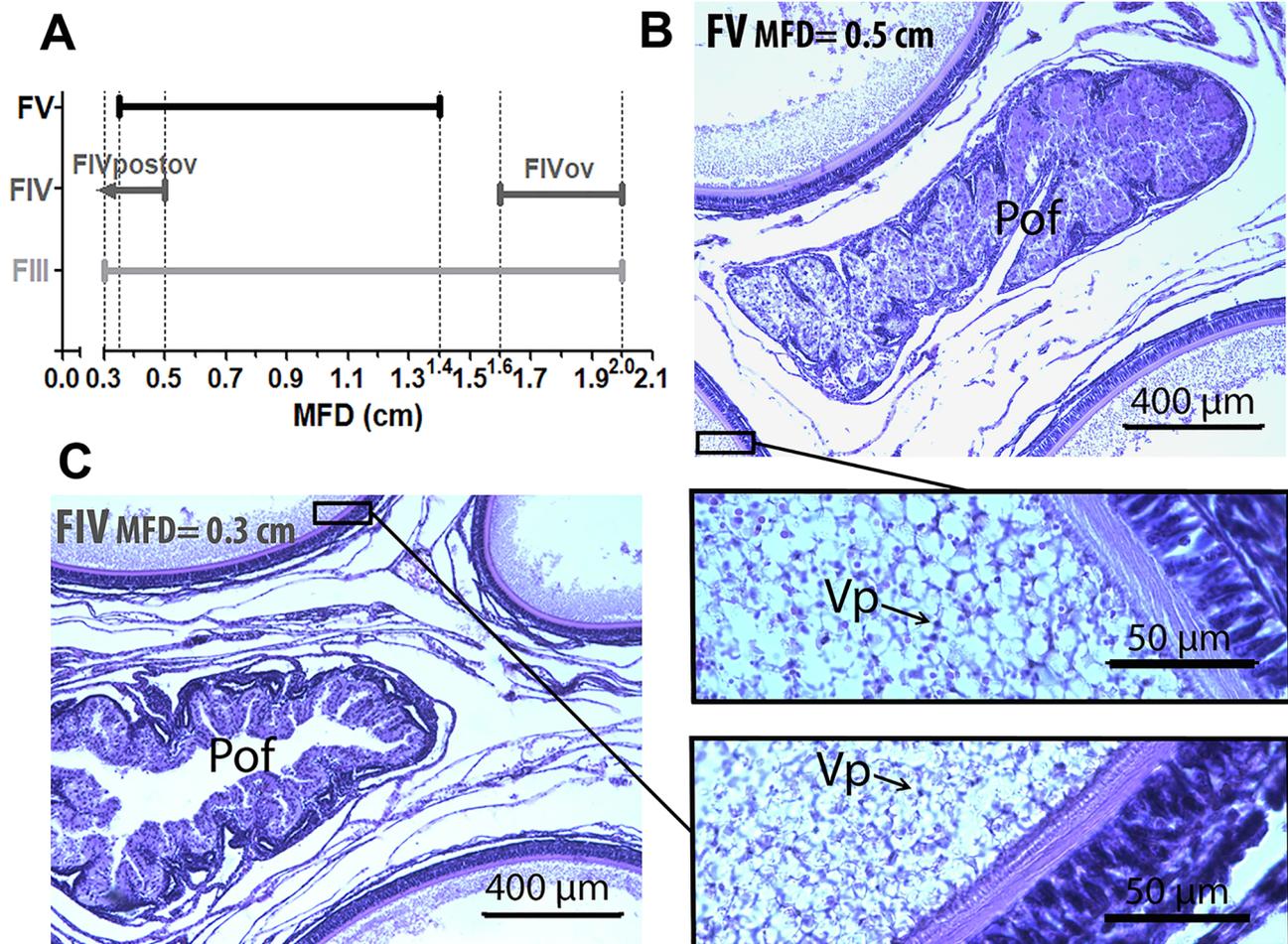


Fig. 2. A: range of maximum follicular diameters (MFD) observed in *Mustelus schmitti* adult females throughout their reproductive cycle. Non-pregnant vitellogenic females (FIII) and pregnant females either with ova (FIV) or differentiated embryos in the uteri (FV) are separately shown. In turn, FIV are divided into ovulating/early pregnant females (FIVov) and post-ovulated/early pregnant females (FIVpostov) in accordance with their respective MFD value. B–C: histological pictures detailing presence of small vitelline plates (Vp) within the oocytes are shown for two different pregnant females. These females showed a leading clutch of small follicles. The histological pictures also show details of a post-ovulatory follicle (Pof). Scale bars are shown for each picture.

growth/advanced pregnancy phase.

2.4.1. Changes in sex steroid plasma levels throughout follicular growth/advanced pregnancy phase and effects of temperature

Changes in plasma levels of T, E₂ and P₄ in **FIII** and **FV** females was assessed in relation to the follicular growth stage, presence/stage of pregnancy and bottom temperature using Generalized Additive Models (GAMs). The explanatory variable chosen for assessing the influence of follicular growth stage was the MFD, while for the case of presence/stage of pregnancy was the uterosomatic index (USI = UtW/EW), which reflected significantly the presence or absence of differentiated embryos, their numbers, and their sizes (Fig. S1, supplementary material). Generalized Additive Models were conducted using the *mgcv* package, in R (R core team 2015). Following the general recommendations given by Keele (2008) according to number of samples, a knot of four was selected for assessing smoothers of explanatory variable. A gaussian family was used for all GAMs performed. The explanatory variables of each model were selected by analyzing the generalized cross validation (GCV) scores and partial *F*-statistic and associated *p*-value of each variable. Model validation was carried out by checking normality of residuals and constant variance. The percentages of total deviance explained by the global model (global R² × 100), and partially by each explanatory variable (partial R² × 100) were calculated in each case.

2.4.2. Changes in sex steroid plasma levels throughout ovulation, early pregnancy and follicular resting

In order to associate sex steroid profile changes with ovulation, T, E₂ and P₄ plasma levels during the final maturation phase (**FIII** females, MFD between 1.6 and 2 cm, see Fig. 2 and Table 1) were compared with those levels observed in **FIVov** females. Sex steroid plasma levels during the final maturation phase were those previously estimated by GAMs considering a temperature of 19.85 °C (mean bottom temperature registered where females at the ovulatory phase were caught) and an USI value of 0.01 (mean USI value observed in **FIII** females).

In addition, to evaluate how the endocrine reproductive system regulates early pregnancy and follicular resting after ovulation, changes in sex steroid plasma levels were compared between **FIVov** and **FIVpostov** by a Student *t* test (applying Welch's correction when data lacked homoscedasticity).

2.5. Statistical analyses

Statistical analyses were performed using GraphPad Prism 5.0 and R 3.1.0 Software. The significance levels were set at *p* < 0.05 for all analysis.

3. Results

3.1. Follicular growth throughout the reproductive cycle

The MFD during *Mustelus schmitti* ovarian cycle from initial vitellogenesis until final maturation-ovulation ranged between 0.3 and

Table 1
Relationship between maximum follicular diameter and ovarian and pregnancy stages throughout *Mustelus schmitti* reproductive cycle.

MFD range	Ovarian stage	Uteri
< 0.5	Resting	Ova
0.3–1.4	Vitellogenesis	Ova or differentiated embryos
1.4–1.6		Near-term embryos Parturition
1.6–2.0	Final maturation Ovulation	Empty Ova

Note. MFD = maximum follicular diameter (cm).

2 cm. In all **FIII** and **FV** females with MFD > 0.6 cm, the follicular leading clutch presented the characteristic yolk yellow color of vitellogenic follicles. In contrast, some **FIII** and **FV** with MFD ≤ 0.6 cm values had a follicular leading clutch represented by pale light-yellow follicles. Nevertheless, all these females showed histological evidences of vitellogenin deposition within follicles, indicating that they had initiated vitellogenesis. The MFD in **FV** females ranged from 0.35 to 1.4 cm (Fig. 2).

The MFD range at the ovulation time (**FIVov** females) was between 1.6 and 2.0 cm, while just after ovulation (**FIVpostov** females) follicles showed MFD ≤ 0.5 cm (Fig. 2). Thus, the final maturation stage was considered to begin at a MFD of 1.6 cm. It must be noted that while most **FIVpostov** females showed no evidences of vitellogenic follicles, being thus considered at an ovarian resting stage, others presented a follicular leading clutch of yolky oocytes.

The association between the MFD values and ovarian and pregnancy stages throughout the *M. schmitti* reproductive cycle are summarized in Table 1.

3.2. Changes in sex steroid plasma levels throughout follicular growth/advanced pregnancy phase and effects of temperature

3.2.1. Relationship with MFD

Changes in plasma levels of the three sex steroids assessed were significantly related with MFD, however the degree and type of relationship differed depending on the steroid. With respect to T and E₂, 38 and 52% of their variation were respectively associated to MFD, while only 9% of P₄ variation was related to this variable (Table 2). Estradiol plasma level increased progressively with follicular development until MFD reached around 1.34 cm, and then decreased reaching intermediated values at maximum follicular size (around 2 cm) (Fig. 3). Testosterone plasma levels remained almost constant during the first period of vitellogenesis and began to increase sharply after follicular size surpassed approximately 0.7 cm, reaching their maximum values at MFD of around 1.46 cm. Like E₂, T reached intermediated levels during the maximum follicular size stage. On the other hand, P₄ plasma levels remained stable throughout vitellogenesis and began to significantly increase in the last period of follicular growth, after follicular size surpassed between 1.4 and 1.5 cm (Fig. 3). In average, E₂ reached higher plasma levels during the follicular developmental stage co-occurring with pregnancy, while the higher plasma levels for T and P₄ were observed at a more advanced follicular developmental stage, when females already delivered embryos (Fig. 3).

Table 2

Percentage of sex steroid plasma levels variation explained by maximum follicular diameter, uterosomatic index and temperature throughout *Mustelus schmitti* follicular growth/advanced pregnancy phase.

Explanatory variables	T	E ₂	P ₄
MFD	52 <i>F</i> = 41.0 <i>p</i> = 2 × 10 ⁻¹⁶	38 <i>F</i> = 23.9 <i>p</i> = 7 × 10 ⁻¹¹	9 <i>F</i> = 5.8 <i>p</i> = 9 × 10 ⁻⁴
USI	5 <i>F</i> = 5.90 <i>p</i> = 3 × 10 ⁻³	25 <i>F</i> = 35.7 <i>p</i> = 3 × 10 ⁻¹³	ns
Temperature	15 <i>F</i> = 7.3 <i>p</i> = 1 × 10 ⁻⁴	6 <i>F</i> = 5.8 <i>p</i> = 1 × 10 ⁻³	48 <i>F</i> = 24.5 <i>p</i> = 1 × 10 ⁻¹¹
GLOBAL MODEL	72	69	57

Note. Percentage values of variation explained (% of deviance explained) by each global Generalized Additive Model (GAM) and partially by each explanatory variable are indicated. T = testosterone, E₂ = estradiol, P₄ = progesterone, MFD = maximum follicular diameter, USI = uterosomatic index, ns = no statistically significant relationship (*p* < 0.05). *F*-statistic and associated *p*-value are shown for each case.

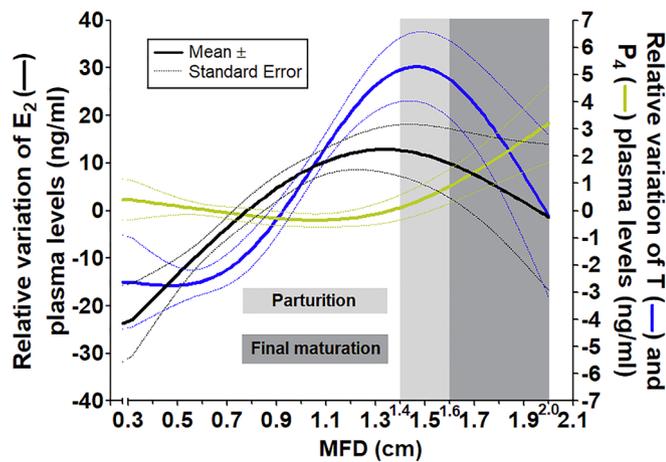


Fig. 3. Relative variation of testosterone (T), estradiol (E_2) and progesterone (P_4) plasma levels with maximum follicular diameter (MFD) throughout *Mustelus schmitti* follicular growth/advanced pregnancy phase. Non-pregnant adult females (FIII) and pregnant females with differentiated embryos (FV) were included in this analysis.

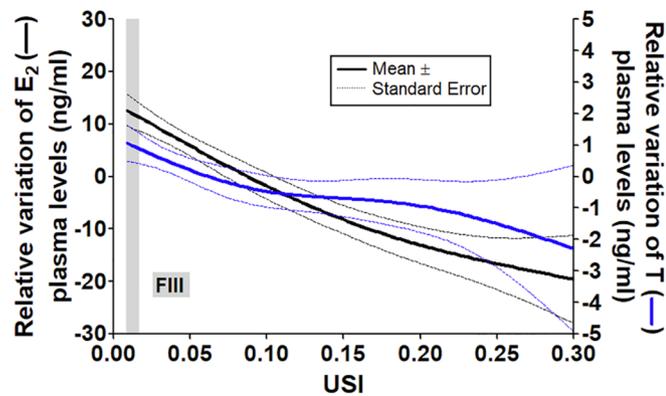


Fig. 4. Relative variation of testosterone (T) and estradiol (E_2) plasma levels with Uterosomal index (USI) throughout *Mustelus schmitti* follicular growth/advanced pregnancy phase. Non-pregnant adult females (FIII) and pregnant females with differentiated embryos (FV) were included in this analysis. Area in shadow indicates the uterosomatic index values observed in FIII. No statistically significant variation of progesterone (P_4) plasma levels was observed with USI (see Table 2).

3.2.2. Relationship with USI

Although to a lesser extent compared to that observed with MFD, plasma level changes of T and E_2 were also significantly related with the USI variation throughout the pregnancy cycle. The degree of this relationship was higher for E_2 (25% of variation) than for T (5% of variation) (Table 2). Compared with FIII (USI ≤ 0.015), FV (USI > 0.015) had lower average T and E_2 plasma levels. This negative effect of pregnancy on plasma sex steroids was progressively rising as USI values increased with embryos length growth, and this pattern was more marked for the case of E_2 than for T (Fig. 4). In contrast to that observed for T and E_2 , no significant relationship between P_4 plasma levels and USI was observed throughout follicular growth/advanced pregnancy phase (Table 2).

3.2.3. Relationship with temperature

Changes in plasma levels of the three sex steroids assessed were significantly related to bottom temperature variation. However, the degree and type of relationship depended on each steroid. Temperature explained a 15 and 6% of T and E_2 plasma level changes respectively, being these percentage values lower than that obtained for the case of P_4 with a 48% of its variation related to this environmental factor

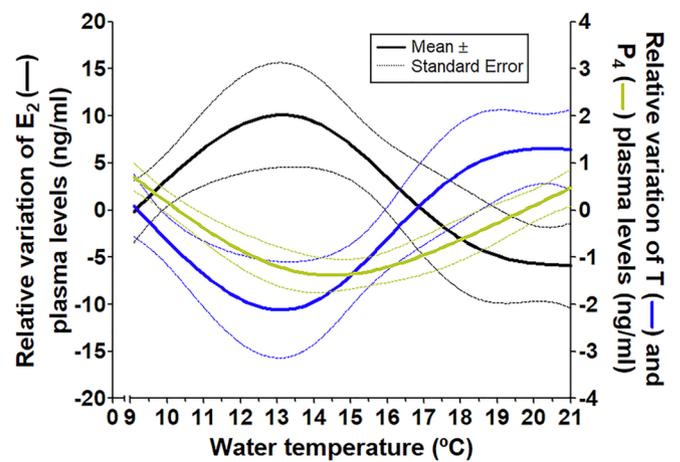


Fig. 5. Relative variation of testosterone (T), estradiol (E_2) and progesterone (P_4) plasma levels with water temperature throughout *Mustelus schmitti* follicular growth/advanced pregnancy phase. Non-pregnant adult females (FIII) and pregnant females with differentiated embryos (FV) were included in this analysis.

(Table 2).

Independently of the influence of follicular developmental stage and pregnancy, T plasma levels decreased with seawater temperatures below 13 °C and then they began to increase progressively. The most pronounced rise in T was observed when seawater temperature surpassed 17 °C, and the highest levels were reached approximately at 19 °C. Conversely, E_2 plasma level variation with temperature showed an exactly opposite pattern to that observed for T (Fig. 5). On the other hand, a progressive decrease in P_4 plasma levels was observed when temperature increased from 9 to 14.5 °C, and then this steroid began to increase again when seawater temperature exceeded this last value (Fig. 5).

3.3. Changes in sex steroid plasma levels throughout ovulation, early pregnancy and follicular resting

Plasma levels of the three sex steroids (T, E_2 and P_4) showed a clear variation pattern throughout ovulatory process. While T and E_2 plasma levels decreased significantly during the ovulation/early pregnancy phase, when oocytes are moving to uteri (FIVov), P_4 showed an opposite pattern. In this sense, most FIVov females had P_4 plasma levels much higher than that observed at the final maturation stage. However, it must be noted that this clear pattern of P_4 increase was not observed for some FIVov females (Fig. 6).

During post-ovulation/early pregnancy phase (FIVpostov), the sole reproductive stage in which ovarian resting evidences were observed, T and E_2 plasma levels remained as low as during ovulation. On the other hand, P_4 plasma levels in all FIVpostov females returned to those values observed at the final maturation stage (Fig. 6).

4. Discussion

The main aim of this study was to evaluate the effects of temperature on the reproductive endocrinology of *Mustelus schmitti* females (as model species) and provide physiological evidence to support the hypothesis that the increase in seawater temperature can trigger ovulation and probably parturition and mating events in elasmobranchs. Further to its contribution to understand the elasmobranchs reproductive ecology, this study contributes to reinforce the relatively scarce general knowledge of their reproductive physiology. It must be noted that this is the first report of the reproductive physiology for *M. schmitti*.

First, the initial vitellogenetic phase was histologically detected when the follicles of the leading clutch appeared as pale light yellow

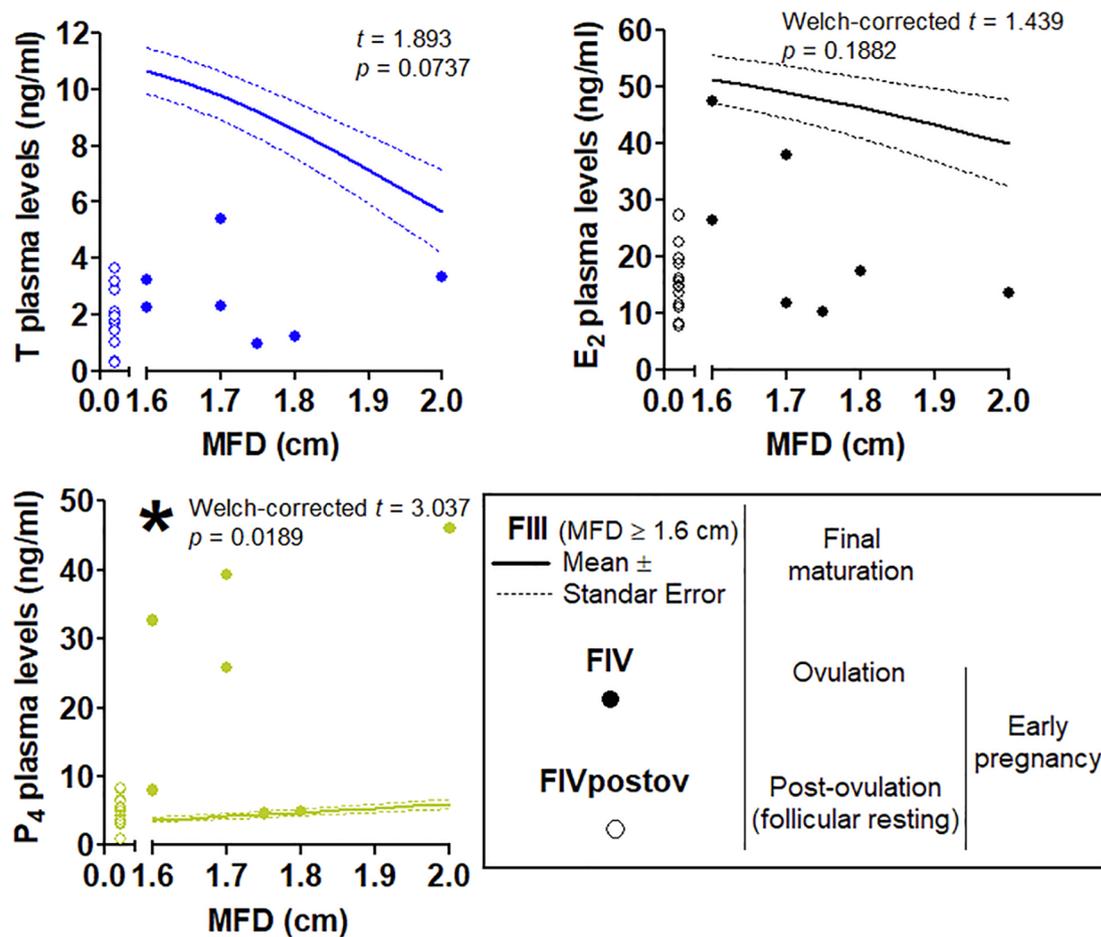


Fig. 6. Change in testosterone (T), estradiol (E_2) and progesterone (P_4) plasma levels throughout final maturation, ovulation, early pregnancy and follicular resting in *Mustelus schmitti*. MFD = Maximum follicular diameter. Final maturation stage was represented by non-pregnant adult females (FIII) with MFD ≥ 1.6 cm, ovulation/early pregnancy stage was represented by ovulating/early pregnant females (FIVov), and follicular resting/early pregnancy stage was represented by post-ovulated/early pregnant females (FIVpostov). Difference in average sex steroid plasma levels between FIVov and FIVpostov was evaluated by Student *t* test (with previous welch's correction for the case of E_2 and P_4), and *t*-statistic and associated *p*-value are shown for each case (asterisk indicates statistically significant difference, $p < 0.05$). Sex steroid plasma levels (mean and standard error) in FIII with MFD ≥ 1.6 cm were estimated by GAMs considering the following conditions: Temperature = 19.85 °C (mean bottom temperature registered where FIII with MFD ≥ 1.6 cm and FIVov were caught), and USI = 0.01 (mean USI value observed in FIII).

with no macroscopically yolk evidences and a follicular size of around 0.3 cm. Similar results were already reported by Galíndez et al. (2014), even at smaller follicular size. The macroscopic evidences of vitellogenic follicles appeared to be consistent from a follicular diameter of 0.6 cm of the leading clutch. On the other hand, oocyte final maturation and ovulation was observed at a follicular size between 1.6 and 2.0 cm. The fact that some FIVpostov females also presented a follicular leading clutch of yolky follicles indicates that, in this species, a new vitellogenic cycle begins during early pregnancy stages, similarly to other species in the genus *Mustelus* (Conrath and Musick, 2002), as well as many other viviparous sharks with continuous ovarian cycles such as *Squatina californica* (Natanson and Cailliet, 1986).

The three steroid hormones assessed in this study (T, E_2 and P_4) are widely recognized as key regulators of reproductive functions in elasmobranchs, as well as in other vertebrates (Callard et al., 1993; Callard and Koob, 1993; Lutton et al., 2005; Maruska and Gelsleichter, 2011; Gelsleichter and Evans, 2012; Awruch, 2013). Particularly, plasma levels of T and E_2 increased progressively with the growth of the leading follicular clutch. However, compared to E_2 , T peaked at a more advanced follicular developmental stage, coincidentally with the moment at which parturition would occur, just prior to the final maturation and ovulation. These plasmatic profiles of T and E_2 would be reflecting changes in the ovarian steroidogenesis during progress of *M. schmitti*

follicular growth, such as was demonstrated in *Squalus acanthias* (Tsang and Callard, 1992). The increase in E_2 plasma levels during follicular development appears to be a common event to all elasmobranch species studied so far, being related to the stimulation of vitellogenesis as in other vertebrate species (Ho et al., 1980; Koob and Callard, 1999; Polzonetti-Magni et al., 2004; Prisco et al., 2008; Awruch, 2013; Maruska and Gelsleichter, 2011). In contrast, although T plasma levels increase throughout vitellogenesis in most viviparous elasmobranchs (e.g., *Negaprion brevirostris*, Rasmussen and Gruber, 1993; *Dasyatis sabina*, Snelson et al., 1997; Tricas et al., 2000; *Rhizoprionodon taylori*, Waltrick et al., 2014), the functional role of this hormone in elasmobranch females remains largely unresolved (Maruska and Gelsleichter, 2011; Awruch, 2015). Noteworthy, a similar event appears to occur also in several teleost fish (see for instance Newman et al., 2008; Muncaster et al., 2010; Pham et al., 2011; Andersson et al., 2013; Elisio et al., 2014); and some evidence suggest that high T plasma levels during late vitellogenesis could promote feedback mechanisms on the brain-pituitary system that elicit the luteinizing hormone (LH) release and thus the ovulation induction (Kobayashi et al., 1989; Trudeau et al., 1991a, 1991b; Pankhurst and Porter, 2003). In this context, the raise of plasma T during late vitellogenesis in elasmobranchs can be related to the physiological mechanisms inducing ovulation.

In several elasmobranch species, the rise of plasmatic P_4 levels was

related to ovulation (reviewed in Maruska and Gelsleichter, 2011). Progesterone plasma levels increased in *M. schmitti* females just after the rise of T levels, together with the decrease of E₂ levels and correlating with the follicular size associated with final maturation and ovulation. Indeed, P₄ reached its maximum plasma levels when oocytes began to move towards the uteri. Similar results were observed also in other viviparous elasmobranchs such as *Sphyrna tiburo* (Manire et al., 1995), *D. sabina* (Snelson et al., 1997; Tricas et al., 2000), and *R. taylori* (Waltrick et al., 2014). Since in continuous breeders, like *M. schmitti*, parturition and ovulation occur almost simultaneously, it would be interesting in the future to evaluate the connection between the endocrine events eliciting ovulation and the mechanisms triggering parturition and mating.

Just after ovulation, P₄ plasma levels fell significantly in *M. schmitti*. It must be noted that the fluctuation pattern of P₄ plasma level during ovulation and the first period of pregnancy in viviparous elasmobranchs appeared to be different among different species (Koob and Callard, 1999). For instance, and in contrast to our species, the highest P₄ plasma levels in *S. tiburo* occurs at post-ovulation (Manire et al., 1995) while, in *S. acanthias*, it occurs during the first half of pregnancy (Koob and Callard, 1999). Similarly, P₄ plasma levels in *Torpedo marmorata* were significantly higher in pregnant follicular resting females than in non-pregnant vitellogenic females (Fasano et al., 1992). Because it was demonstrated that high P₄ levels down-regulate the estrogen-induced vitellogenin synthesis in elasmobranchs inhibiting vitellogenesis (Callard et al., 1991; Perez and Callard, 1993; Prisco et al., 2008), this would explain the relatively short follicular resting period observed in *M. schmitti*, in comparison to the other mentioned species.

The development of the uteri with pregnancy was associated to a decrease in T and particularly E₂ plasma levels. Although the implications of these results are unknown, these steroids could be in part sequestered by uteri or developing embryos, and they probably play a role in regulating embryonic development. It is interesting to note that changes in yolk sex steroid concentrations during the embryonic development in *S. tiburo* were reported (Manire et al., 2004); and also E₂ has been related to the stimulation of matrotrophic embryos nutrition (Manire et al., 1995; Snelson et al., 1997; Tricas et al., 2000). This situation could also happen in *M. schmitti* which presents a matrotrophic viviparous strategy, which begins during early stages of embryonic development (Quesada, 2018). It is also important to mention that T and E₂ could be playing important regulatory roles on other oviductal tract functions (Callard et al., 1993; Awruch et al., 2013).

In relation to the effects of temperature on the reproductive physiology of *M. schmitti* females, a clear non-linear association was observed between changes in sex steroid plasma levels throughout follicular development and water temperature variations. Some other studies have already evaluated linear correlations between temperature and sex steroid plasma levels in elasmobranch females (Heupel et al., 1999; Mull et al., 2010; Waltrick et al., 2014; Nozu et al., 2018). For instance, while no significant correlations between E₂ plasma levels and temperature were observed in females of *Hemiscyllium ocellatum* (Heupel et al., 1999) and *Urobatis halleri* (Mull et al., 2010), positive and negative correlations were reported in females of *R. taylori* (Waltrick et al., 2014) and *Stegostoma fasciatum* (Nozu et al., 2018), respectively. On the other hand, P₄ plasma levels were positively correlated with temperature in females of *U. halleri* (Mull et al., 2010), while no significant correlations were reported for the case of *H. ocellatum* (Heupel et al., 1999) and *R. taylori* (Waltrick et al., 2014). These discrepancies found between different studies could be in part explained by differences in data analysis methodologies, and/or by the non-linear relationship between sex steroids and temperature when considering the whole range of seawater temperatures. It must be noted that plasmatic T levels decreased with temperature below 13 °C and then began to progressively increase reaching a pronounced rise when seawater temperature surpassed 17 °C. Interestingly, E₂ presented an exactly opposite fluctuation pattern to that described for T, appearing

to be in accordance with the activity of gonadal aromatase as already demonstrated in several teleost fish species (Watts et al., 2004; Miranda et al., 2013). Therefore, similarly to teleosts (Pankhurst and Porter, 2003), the increase in temperature above certain species-specific values could induce an inhibition of aromatase activity causing a decline of E₂ and a concomitant rise of T plasma levels. Because of the increase in T could elicit mechanisms promoting LH release (see discussion above), the pronounced rise of T observed in *M. schmitti* under bottom temperatures above 17 °C could trigger the endocrine mechanisms inducing their ovulation. This cause-effect physiological mechanism supports in part the significant correlation observed between the reproductive events occurrence and the increase of seawater temperature for this species (Elisio et al., 2017) and for many other elasmobranchs reproducing during warm season. Finally, as reported in teleosts (Soria et al., 2008; Elisio et al., 2012, 2014; Pankhurst and Munday, 2011; Miranda et al., 2013), it would be interesting to assess if high temperatures could induce (by E₂ decrease, and probably P₄ rise) the impairment of vitellogenesis, the ovary regression and resting period in elasmobranchs.

As far as is known in fish, other environmental variables such as photoperiod and social factors, could be also playing an important role as reproductive cues in elasmobranchs. How these variables, together with temperature, influence on elasmobranchs reproductive physiology probably will depend on region and species. Nonetheless, if temperature was a determinant environmental cue for reproduction in elasmobranchs, variations in ocean thermal regimens driven by climatic phenomena should be particularly considered to understand and predict changes in the spatial and temporal occurrence patterns of their reproductive events.

5. Conclusions

Using *M. schmitti* shark as model species, this study shows a clear picture of how seawater temperature variations would affect the reproductive physiology in elasmobranch females. It is particularly interesting the relationship found between the increase in temperature and the elevation of T plasma levels, associated in turn with P₄ rise and the parturition/ovulation induction. These physiological evidences support that the increase in temperature would be an important environmental cue for triggering the reproductive events in elasmobranchs. In addition to its ecological scope, this work contributes to reinforce the relatively scarce general knowledge of elasmobranchs reproductive endocrinology.

Acknowledgements

This study was supported by grant to Elisio, M. (Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Argentina): PICT2014-2743) and by Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP, Argentina) funding. The authors are grateful to Marta Estrada for technical support in sex steroid measurements and histological procedures and to Eduardo Flores and family and Carlos Bancur for providing the fishing gear and sampling help. This is INIDEP contribution N° 2181.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygcen.2019.113242>.

References

- Andersson, E., Schulz, R.W., Male, R., Bogerd, J., Patiña, D., Benedet, S., Norberg, B., Taranger, G.L., 2013. Pituitary gonadotropin and ovarian gonadotropin receptor transcript levels: seasonal and photoperiod-induced changes in the reproductive physiology of female Atlantic salmon (*Salmo salar*). Gen. Comp. Endocrinol. 191,

- 247–258. <https://doi.org/10.1016/j.ygcen.2013.07.001>.
- Awruch, C.A., 2013. Reproductive endocrinology in chondrichthyans: the present and the future. *Gen. Comp. Endocrinol.* 192, 60–70. <https://doi.org/10.1016/j.ygcen.2013.05.021>.
- Awruch, C.A., 2015. Reproduction strategies. In: In: Shadwick, R., Farrell, A., Brauner, C. (Eds.), *Physiology of Elasmobranch Fishes: Structure and Interaction with Environment*, vol. 34. A Academic Press, London, pp. 255–310.
- Callard, I.P., Etheridge, K., Giannoukos, G., Lamb, T., Perez, L., 1991. The role of steroids in reproduction in female elasmobranchs and reptiles. *J. Steroid Biochem. Mol. Biol.* 40, 571–575. [https://doi.org/10.1016/0960-0760\(91\)90278-D](https://doi.org/10.1016/0960-0760(91)90278-D).
- Callard, G.V., Fileti, L.A., Koob, T.J., 1993. Ovarian steroid synthesis and the hormonal control of the elasmobranch reproductive tract. *Environ. Biol. Fishes* 38, 175–185. <https://doi.org/10.1007/BF00842914>.
- Callard, I.P., Koob, T.J., 1993. Endocrine regulation of the elasmobranch reproductive tract. *J. Exp. Zool.* 266, 368–377. <https://doi.org/10.1002/jez.1402660505>.
- Chiaramonte, G.E., Pettovello, A.D., 2000. The biology of *Mustelus schmitti* in southern Patagonia Argentina. *J. Fish. Biol.* 57, 930–942. <https://doi.org/10.1006/jfbi.2000.1358>.
- Colautti, D., Baigun, C., Lopez Cazorla, A., Llompard, F., Molina, J.M., Suquale, P., Calvo, S., 2010. Population biology and fishery characteristics of the smooth-hound *Mustelus schmitti* in Anegada Bay, Argentina. *Fish. Res.* 106, 351–357. <https://doi.org/10.1016/j.fishres.2010.09.004>.
- Conrath, C.L., Musick, J.A., 2002. Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. *Environ. Biol. Fishes* 64, 367–377. <https://doi.org/10.1023/A:1016117415855>.
- Cortés, F., Jaureguizar, A.J., Menni, R.C., Guerrero, R.A., 2011. Ontogenetic habitat preferences of the narrownose smooth-hound shark, *Mustelus schmitti*, in two Southwestern Atlantic coastal areas. *Hydrobiologia* 661, 445–456. <https://doi.org/10.1007/s10750-010-0559-2>.
- Elisio, M., Chalde, T., Miranda, L.A., 2012. Effects of short periods of warm water fluctuations on reproductive endocrine axis of the pejerrey (*Odontesthes bonariensis*) spawning. *Comp. Biochem. Physiol. A* 163, 47–55.
- Elisio, M., Chalde, T., Miranda, L.A., 2014. Seasonal changes and endocrine regulation of pejerrey (*Odontesthes bonariensis*) oogenesis in the wild. *Comp. Biochem. Physiol. A* 175, 102–109. <https://doi.org/10.1016/j.cbpa.2014.05.020>.
- Elisio, M., Colonello, J.H., Cortés, F., Jaureguizar, A.J., Somoza, G.M., Macchi, G.J., 2017. Aggregations and reproductive events of the narrownose smooth-hound shark, *Mustelus schmitti*, in relation to temperature and depth in coastal waters of the southwestern Atlantic Ocean (38–42° S). *Mar. Freshwater Res.* 68, 732–742. <https://doi.org/10.1071/MF15253>.
- Fasano, S., D'Antonio, M., Pierantoni, R., Chieffi, G., 1992. Plasma and follicular tissue steroid levels in the elasmobranch fish, *Torpedo marmorata*. *Gen. Comp. Endocrinol.* 85, 327–333. [https://doi.org/10.1016/0016-6480\(92\)90017-E](https://doi.org/10.1016/0016-6480(92)90017-E).
- Galíndez, E.J., Díaz Andrade, M.C., Moya, A.C., Estecondo, S., 2010. Morphological changes in the pregnant uterus of the smooth hound dogfish *Mustelus schmitti* Springer, 1939 (Gatuzo) (Chondrichthyes, Triakidae). Microscopic study and phylogenetic reproductive implications. *Int. J. Morphol.* 28, 1003–1010. <https://doi.org/10.4067/S0717-95022010000400004>.
- Galíndez, E.J., Díaz Andrade, M.C., Estecondo, S., 2014. Morphological indicators of initial reproductive commitment in *Mustelus schmitti* (Springer 1939) (Chondrichthyes, Triakidae): folliculogenesis and ovarian structure over the life cycle. *Braz. J. Biol.* 74, S154–S163. <https://doi.org/10.1590/1519-6984.23312>.
- Gelsleichter, J., Evans, A.N., 2012. Hormonal regulation of elasmobranch physiology. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (Eds.), *Biology of Sharks and Their Relatives*. CRC Press, Boca Raton, pp. 313–348.
- Heupel, M.R., Whittier, J.M., Bennett, M.B., 1999. Plasma steroid hormone profiles and reproductive biology of the Epaulette shark, *Hemiscyllium ocellatum*. *J. Exp. Zool.* 284, 586–594. [https://doi.org/10.1002/\(SICI\)1097-010X\(19991001\)284:5<586::AID-JEZ14>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1097-010X(19991001)284:5<586::AID-JEZ14>3.0.CO;2-B).
- Ho, S., Wulczyn, F.G., Callard, I.P., 1980. Induction of vitellogenin synthesis in the spiny dogfish, *Squalus acanthias*. *Bull. Mt. Desert Isl. Biol. Lab.* 19, 37–38.
- Jaureguizar, A., Menni, R., Guerrero, R., Lasta, C., 2004. Environmental factors structuring fish communities of the Rio de la Plata estuary. *Fish. Res.* 66, 195–211. [https://doi.org/10.1016/S0165-7836\(03\)00200-5](https://doi.org/10.1016/S0165-7836(03)00200-5).
- Keele, L., 2008. *Semiparametric Regression for the Social Sciences*. Wiley, Chichester.
- Kobayashi, M., Aida, K., Hanyu, I., 1989. Involvement of steroid hormones in the pre-ovulatory gonadotropin surge in female goldfish. *Fish Physiol. Biochem.* 7, 141–146. <https://doi.org/10.1007/BF00004700>.
- Koob, T.J., Callard, I.P., 1999. Reproductive endocrinology of female elasmobranchs: lessons from the little skate (*Raja erinacea*) and spiny dogfish (*Squalus acanthias*). *J. Exp. Zool.* 284, 557–574. [https://doi.org/10.1002/\(SICI\)1097-010X\(19991001\)284:5<557::AID-JEZ12>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1097-010X(19991001)284:5<557::AID-JEZ12>3.0.CO;2-P).
- Lutton, B.V., George, J.S., Murrin, C.R., Fileti, L.A., Callard, I.P., 2005. The elasmobranch ovary. In: In: Hamlett, W. (Ed.), *Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids, and Chimaeras*, vol. 3. Science Publishers Inc., Enfield, pp. 237–281.
- Manire, C.A., Rasmussen, L.E.L., Gelsleichter, J., Hess, D.L., 2004. Maternal serum and yolk hormone concentrations in the placental viviparous bonnethead shark, *Sphyrna tiburo*. *Gen. Comp. Endocrinol.* 136, 241–247. <https://doi.org/10.1016/j.ygcen.2003.12.018>.
- Manire, C.A., Rasmussen, L.E.L., Hess, D.L., Hueter, R.E., 1995. Serum steroid hormones and the reproductive cycle of the female bonnethead shark, *Sphyrna tiburo*. *Gen. Comp. Endocrinol.* 97, 366–376. <https://doi.org/10.1006/gcen.1995.1036>.
- Maruska, K.P., Gelsleichter, J., 2011. Hormones and reproduction in chondrichthyan fishes. In: In: Norris, D.O., Lopez, K.H. (Eds.), *Hormones and Reproduction of Vertebrates*, vol. 1. Academic Press, London, pp. 209–237.
- Massa, A.M., Lucifora, L.O., Hozbor, N.M., 2004a. Condrictios de la región costera bonaerense y uruguayana. In: Boschi, E.E. (Ed.), *El Mar Argentino y sus recursos pesqueros. Los peces marinos de interés pesquero Caracterización biológica y evaluación del estado del estado de explotación*. INIDEP, Mar del Plata, pp. 85–99.
- Massa, A.M., Lasta, C., Carozza, C., 2004b. Estado actual y explotación del gatuzo (*Mustelus schmitti*). In: Boschi, E.E. (Ed.), *El Mar Argentino y sus recursos pesqueros. Los peces marinos de interés pesquero Caracterización biológica y evaluación del estado del estado de explotación*. INIDEP, Mar del Plata, pp. 67–83.
- Menni, C.R., Cousseau, M.B., Gosztonyi, A.E., 1986. Sobre la biología de los tiburones costeros de la Provincia de Buenos Aires. *Anales Soc. Ci. Argent.* 213, 3–26.
- Menni, C.R., Jaureguizar, A.J., Stehmann, M.F.W., Lucifora, L.O., 2010. Marine biodiversity at the community level: zoogeography of sharks, skates, rays and chimaeras in the southwestern Atlantic. *Biodivers. Conserv.* 19, 775–796. <https://doi.org/10.1007/s10531-009-9734-z>.
- Miranda, L.A., Chalde, T., Elisio, M., Strüssmann, C.A., 2013. Effects of global warming on fish reproductive endocrine axis, with special emphasis in pejerrey *Odontesthes bonariensis*. *Gen. Comp. Endocrinol.* 192, 45–54. <https://doi.org/10.1016/j.ygcen.2013.02.034>.
- Mull, C.G., Lowe, C.G., Young, K.A., 2008. Photoperiod and water temperature regulation of seasonal reproduction in male round stingrays (*Urobatis halleri*). *Comp. Biochem. Physiol. A* 151, 717–725. <https://doi.org/10.1016/j.cbpa.2008.08.029>.
- Mull, C.G., Lowe, C.G., Young, K.A., 2010. Seasonal reproduction of female round stingrays (*Urobatis halleri*): steroid hormone profiles and assessing reproductive state. *Gen. Comp. Endocrinol.* 166, 379–387. <https://doi.org/10.1016/j.ygcen.2009.12.009>.
- Muncaster, S., Andersson, E., Kjesbu, O.S., Taranger, G.L., Skiftesvik, A.B., Norberg, B., 2010. The reproductive cycle of female ballan wrasse *Labrus bergylta* in high latitude, temperate waters. *J. Fish Biol.* 77, 494–511. <https://doi.org/10.1111/j.1095-8649.2010.02691.x>.
- Natanson, L.J., Cailliet, G.M., 1986. Reproduction and development of the Pacific angel shark, *Squatina californica*, off Santa Barbara, California. *Copeia* 4, 987–994. <https://doi.org/10.2307/1445296>.
- Newman, D.M., Jones, P.L., Ingram, B.A., 2008. Age-related changes in ovarian characteristics, plasma sex steroids and fertility during pubertal development in captive female Murray cod *Maccullochella peelii peelii*. *Comp. Biochem. Physiol. A* 150, 444–451. <https://doi.org/10.1016/j.cbpa.2008.05.005>.
- Nozu, R., Murakumo, K., Yano, N., Furuyama, R., Matsumoto, R., Yanagisawa, M., Sato, K., 2018. Changes in sex steroid hormone levels reflect the reproductive status of captive female zebra sharks (*Stegostoma fasciatum*). *Gen. Comp. Endocrinol.* 265, 174–179. <https://doi.org/10.1016/j.ygcen.2018.03.006>.
- Oddone, M.C., Paesch, L., Norbis, W., 2005. Reproductive biology and seasonal distribution of *Mustelus schmitti* (Elasmobranchii: Triakidae) in the Rio de la Plata oceanic front, south-western Atlantic. *J. Mar. Biol. Assoc. U.K.* 85, 1193–1198. <https://doi.org/10.1017/S0025315405012294>.
- Oddone, M.C., Paesch, L., Norbis, W., Velasco, G., 2007. Population structure, distribution and abundance patterns of the Patagonian smoothhound *Mustelus schmitti* Springer, 1939. (Chondrichthyes, Elasmobranchii, Triakidae) in the Rio de la Plata and inner continental shelf, SWAtlantic ocean (34°30'–39°30'S). *Braz. J. Oceanogr.* 55, 167–177. <https://doi.org/10.1590/S1679-87592007000300001>.
- Orlando, L., Pereyra, I., Silveira, S., Paesch, L., Oddone, M.C., Norbis, W., 2015. Determination of limited histotrophy as the reproductive mode in *Mustelus schmitti* Springer, 1939 (Chondrichthyes: Triakidae): analysis of intrauterine growth of embryos. *Neotrop. Ichthyol.* 13, 699–706. <https://doi.org/10.1590/1982-0224-20150001>.
- Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. *Mar. Fresh. Res.* 62, 1015–1026. <https://doi.org/10.1071/MF10269>.
- Pankhurst, N.W., Porter, M.J.R., 2003. Cold and dark or warm and light: variations on the theme of environmental control of reproduction. *Fish Physiol. Biochem.* 28, 385–389. <https://doi.org/10.1023/B:FISH.0000030602.51939.50>.
- Perez, L.E., Callard, I.P., 1993. Regulation of hepatic vitellogenin synthesis in the little skate (*Raja erinacea*): use of a homologous enzyme-linked immunosorbent assay. *J. Exp. Biol.* 266, 31–39. <https://doi.org/10.1002/jez.1402660105>.
- Pham, H.Q., Nguyen, A.T., Kjorsvik, E., Nguyen, M.D., Arukwe, A., 2011. Seasonal reproductive cycle of Waigieu seaperch (*Psammoperca waigiensis*). *Aquac. Res.* 43, 815–830. <https://doi.org/10.1111/j.1365-2109.2011.02894.x>.
- Polzonetti-Magni, A.M., Mosconi, G., Soverchia, L., Kikuyama, S., Carnevali, O., 2004. Multihormonal control of vitellogenesis in lower vertebrates. *Int. Rev. Cytol.* 239, 1e46. [https://doi.org/10.1016/S0074-7696\(04\)39001-7](https://doi.org/10.1016/S0074-7696(04)39001-7).
- Prisco, M., Valiante, S., Maddalena Di Fiore, M., Raucci, F., Del Giudice, G., Romano, M., Laforgia, V., Limatola, E., Andreuccetti, P., 2008. Effect of 17 β -estradiol and progesterone on vitellogenesis in the spotted ray *Torpedo marmorata* Risso 1810 (Elasmobranchii: Torpediniformes): Studies on females and on estrogen-treated males. *Gen. Comp. Endocrinol.* 157, 125–132. <https://doi.org/10.1016/j.ygcen.2008.04.011>.
- Quesada, G.C., 2018. *Dinámica energética reproductiva en el gatuzo (Mustelus schmitti). Implicancias sobre la variación anual en el rendimiento de carne*. Bachelor diss. Universidad Nacional de Mar del Plata, Mar del Plata.
- Rasmussen, L.E.L., Gruber, S.H., 1993. Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, *Negaprion brevirostris*. *Environ. Biol. Fishes* 38, 167–174. <https://doi.org/10.1007/BF00842913>.
- Ravaglia, M.A., Lo Nostro, F.L., Maggese, M.C., Guerrero, G.A., Somoza, G.M., 1997. Characterization of molecular variants of GnRH, induction of spermiation and sex reversal using salmon GnRH-A and domperidone in the protogynous diandric fish, *Synbranchius marmoratus* Bloch, (Teleostei, Synbranchidae). *Fish Physiol. Biochem.* 16, 425–436. <https://doi.org/10.1023/A:1007772416539>.

- Snelson, J.F.F., Johnson, M.R., Rasmussen, L.E.L., Hess, D.L., 1997. Serum concentrations of steroids hormones during reproduction in the Atlantic stingray, *Dasyatis sabina*. *Gen. Comp. Endocrinol.* 108, 67–79. <https://doi.org/10.1006/gcen.1997.6949>.
- Soria, F.N., Strüssmann, C.A., Miranda, L.A., 2008. High water temperatures impair the reproductive ability of the pejerrey fish *Odontesthes bonariensis*: effects on the hypothyseal-gonadal axis. *Physiol. Biochem. Zool.* 81, 898–905. <https://doi.org/10.1086/588178>.
- Sumpter, J.P., Dodd, J.M., 1979. The annual reproductive cycle of the female lesser spotted dogfish, *Scyliorhinus canicula* L., and its endocrine control. *J. Fish Biol.* 15, 687–695. <https://doi.org/10.1111/j.1095-8649.1979.tb03678.x>.
- Tricas, T.C., Maruska, K.P., Rasmussen, L.E.L., 2000. Annual cycles of steroid hormone production, gonad development, and reproductive behavior in the Atlantic stingray. *Gen. Comp. Endocrinol.* 118, 209–225. <https://doi.org/10.1006/gcen.2000.7466>.
- Trudeau, V.L., Lin, H.R., Peter, R.E., 1991a. Testosterone potentiates the serum gonadotropin response to gonadotropin-releasing hormone in the common carp (*Cyprinus carpio*) and chinese loach (*Paramisgurnus dabryanus*). *Can. J. Zool.* 69, 2480–2484. <https://doi.org/10.1139/z91-349>.
- Trudeau, V.L., Peter, R.E., Sloley, B.D., 1991b. Testosterone and estradiol potentiate the serum gonadotropin response to gonadotropin-releasing hormone in goldfish. *Biol. Reprod.* 44, 951–960. <https://doi.org/10.1095/biolreprod44.6.951>.
- Tsang, P., Callard, I.P., 1992. Regulation of *in vitro* ovarian steroidogenesis in the viviparous dogfish, *Squalus acanthias*. *J. Exp. Zool.* 261, 97–104. <https://doi.org/10.1002/jez.1402610111>.
- Waltrick, D., Jones, S.M., Simpfendorfer, C.A., Awruch, C.A., 2014. Endocrine control of embryonic diapause in the Australian sharpnose shark *Rhizoprionodon taylori*. *PLoS One* 9, e101234. <https://doi.org/10.1371/journal.pone.0101234>.
- Watts, M., Pankhurst, N.W., King, H.R., 2004. Maintenance of Atlantic salmon (*Salmosalar*) at elevated temperature inhibits cytochrome P450 aromatase activity in isolated ovarian follicles. *Gen. Comp. Endocrinol.* 135, 381–390. <https://doi.org/10.1016/j.ygcen.2003.11.004>.