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Atresia in the Chilean Jack Mackerel *Trachurus murphyi* Nichols, 1920: implication for reproductive dynamics

Paola Alejandra Gonzalez Kother

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Par

Paola Alejandra GONZALEZ KOTHER

**Atresia in the Chilean Jack Mackerel *Trachurus murphyi* Nichols, 1920:
implication for reproductive dynamics.**

Thèse présentée et soutenue à l'Université d'Antofagasta du Chili, le 10 octobre 2019

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To my parents, sister and brothers

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1. GENERAL INTRODUCTION

1.1 Fisheries and fish reproduction

Fish populations include individuals of different age, size, condition factor and maturity stages and therefore, growth and reproductive dynamics, must be explored from a population (demographic) perspective (Saborido-Rey and Kjesbu 2005). Applied fisheries reproductive biology has traditionally focused on estimating size and age at sexual maturity and fecundity as important components of the fish life history (Stearns 1992), whereas there is little focus on assessing reproductive traits at the individual level and how they may impact reproductive success (Lowerre-Barbieri 2009; Wright and Trippel 2009). The amount of energy allocated to growth and reproduction will depend on several factors, some of them intrinsic (genetic, physiological) while others are environmentally driven (temperature and feeding). Intrinsic factor, e.g. energy allocated for maintenance, growth and reproduction is partitioned between survival (including survival behaviour), storage of energy reserves, somatic growth, production of gametes and reproductive activity, all which may affect energy acquisition, assimilation and distribution through feedback regulating systems. While, external factors influence the level of energy intake and allocation patterns. Energy invested in growth and reproduction can be reallocated to other locations (by e.g. reduction in reserves, loss of somatic weight, yolk absorption).

A fish stock's ability to produce viable eggs and larvae has been termed Stock Reproductive Potential (SRP) and is a function of maturity, fecundity, egg quality, and spawn timing (Trippel, 1999). Both environmental change and fishing can impact components of SRP in various ways and the effects of these factors can be difficult to distinguish. Fishing can impose levels of mortality on juveniles and adults that far exceeds natural mortality (Mertz and Myers, 1998), leading to a truncation in age and size structure and a reduction in reproductive life span (Murawski et al., 2001) and spawning time (Scott et al., 2006). Variation in spawning time can be critical to reproductive success, because it affects the

early environmental conditions experienced by progeny, and the period they have to complete phases of development (Cushing, 1990). The understanding of the relationship between spawner abundance and subsequent recruitment is the most important issue in fisheries biology and management (Myer, 2001)

1.2 Maturation and reproductive success

Fish reproduction studies depend on recognizable macroscopic and microscopic (histological analysis) features of gonads together with length, somatic weight and occasionally liver and gonad weight. Ovaries for potential fecundity analysis are usually sampled from mature females, when vitellogenesis is in advanced state. The data derived from these collections are used to correlate maturity stages and fecundity with size and condition factor (e.g. Kjesbu et al., 1998; Yoneda and Wright, 2004). The development of the gonads during gametogenesis can be described at a macroscopic level using two basic indices, maturation staging and the Gonadosomatic Index (GSI). Both indices have to be supported by microscopical analysis such as histology of the developing gonads. The basic indices are most valuable when large samples of fish are taken; for example, in surveys to estimate the reproductive potential of a population subjected to fishing (Wootton and Smith, 2015). It is still far from understanding the dynamic processes associated with the egg formation from the time germ cells turn into oogonia, until the release of ova during spawning. Studies on endocrine, cellular and ultra-structural aspects of oogenesis and oocyte growth (Wallace and Selman, 1981, 1990; Le Menn et al., 2007) have been increased strongly in recent years, mainly due the advance of molecular tools, including large scale transcriptome and proteome analyses, but the information is far from complete (Cerdà et al., 2008a,b).

Fisheries remove individuals at various trophic levels in the ecosystem affecting the distribution of energy and hence the amount of energy available for a particular fish (Saborido-Rey and Kjesbu 2005). Species that reproduce only once (semelparous life history), e.g. some salmonids, invest all the energy in reproduction before dying. Species with repeated reproduction (iteroparous life history) save some energy for survival and possible further growth.

Reproductive activity has an energetic cost (Roff, 1983), females are limited in the time and resources that can devote to producing offspring, decreasing future growth, condition, survival, and reproductive output (Figure 1). Optimal energy allocation is dependent on inherited components (energetic thresholds and endogenous rhythms) and environmental conditions (e.g., food, and temperature). The part of ingested energy remaining after allocation to metabolic process is derived to somatic growth and reproductive investments, which are hence in mutual competition (Brosset et al., 2016). Some species spawn and feed in separate areas, during different seasons, by storing energy and drawing on it later for reproduction (i.e. capital breeding). Other species spawn using energy acquired locally, throughout a prolonged spawning season, allocating energy directly to reproduction (i.e. income breeding) (McBride et al., 2015). Spawning is the most metabolically demanding activity whether it occurs at a single spawning event or over multiple-spawning seasons (McBride et al., 2015)

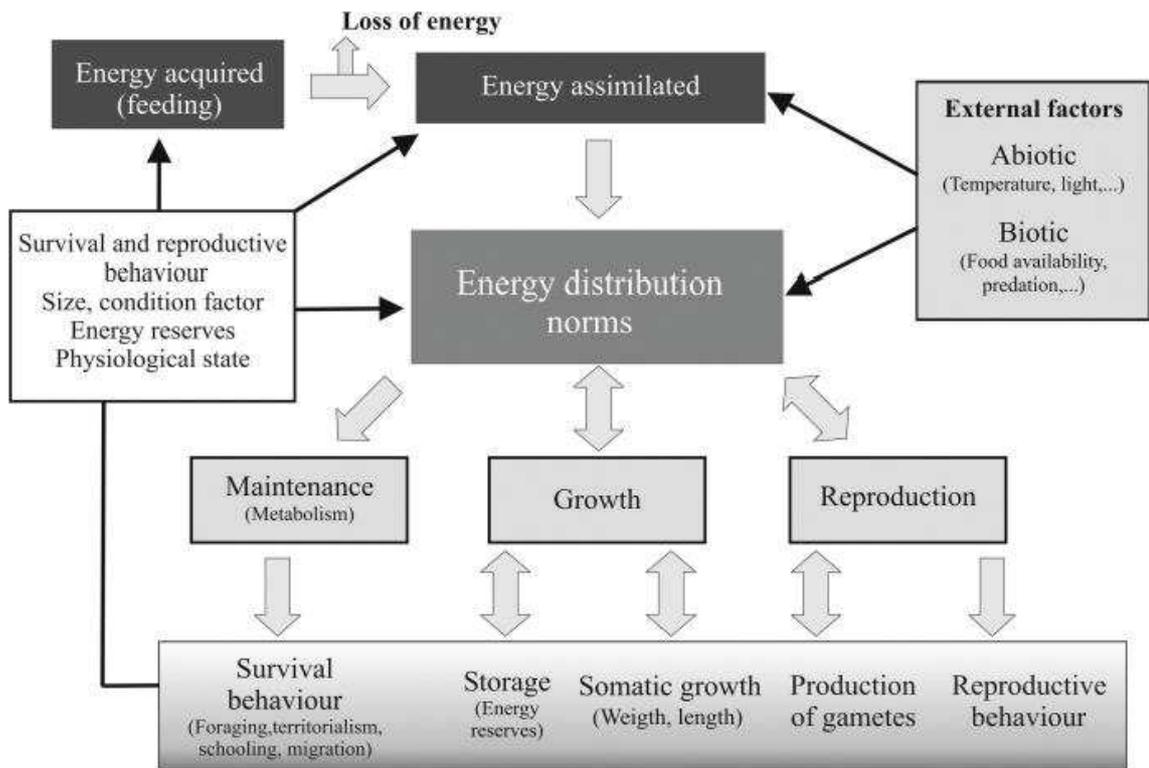


Figure 1: Schematic representation of energy flow allocation. Open arrows represent energy routes while solid arrows represent factors affecting energy allocation. (Source: Saborido-Rey and Kjesbu, 2005)

Larger and older individuals have different reproductive attributes than smaller and younger (Trippel et al., 1997). In many species, the larger (older) females are reported to develop earlier, spawn more frequently, and have an extensive individual spawning seasons than younger, smaller fish (Ganias et al. 2003; Wright and Trippel 2009). In addition, there is a strong relationship between body size and fecundity (Figure 2); thus, older females may disproportionately contribute to the population egg production (Claramunt et al., 2007; Lowerre-Barbieri et al., 2011b). Egg produced by a fish population is a function of the abundance of mature females, their fecundity and the proportion of females that release ova at spawning (Trippel and Harvey 1990)

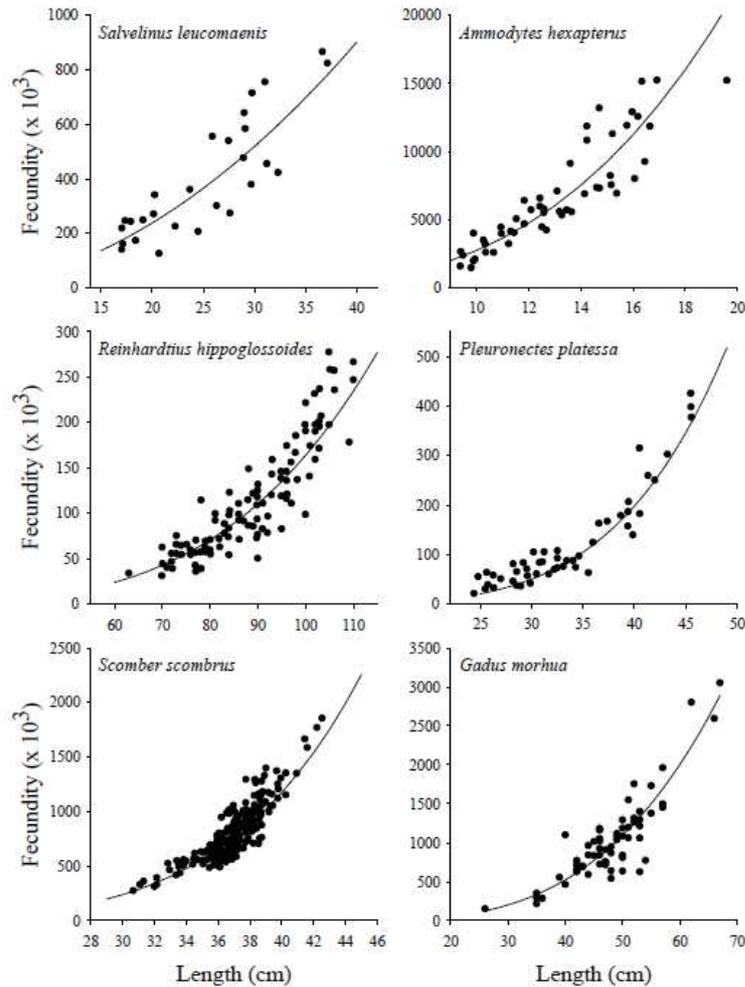


Figure 2. Relationship between potential fecundity and fish length for six species of different environments. (Source: Saborido-Rey and Kjesbu, 2005)

For fishery management and aquaculture, a critical issue is the knowledge of fish reproduction attributes such as the development of gametes, time of spawning, spatial distribution of mature individuals, and gonadosomatic index (Brown-Peterson et al., 2011; Garner et al., 2012). In addition, other indices such as the condition factor and hepatosomatic index, are used together with the gonadosomatic index because these indices facilitate the understanding of energetic dynamics associated with fish reproduction (Jobling, 1995; Santos et al., 1996; Maddock and Burton, 1998). Since knowledge of reproductive biology is a critical component for an adequate fisheries and aquaculture management, further research regarding the physiological mechanisms underlying

reproductive biology are necessary (Gardner et al., 2012). For species with an extensive distribution and are highly migratory, assessment of reproductive processes is especially important because these fish species often occupy a wide range of different habitats (Zhu et al., 2014) and some of the considered reproductive traits may vary, reflecting different life history strategies (Silva et al., 2013)

1.3 Reproductive cycle of female

The reproductive cycle of female involves a period of primary oocyte growth, followed by a yolk accumulation (vitellogenesis), and then the final maturation and ovulation of oocytes prior to spawning (Rideout et al., 2005, Lowerre-Barbieri et al., 2011b). Reproductive cycles represent the gonadal development needed for mature fish to spawn at the appropriate time (Figure 3). In iteroparous species, which multiple reproductive cycles include removal of residual oocytes by atresia and production of a new generation of oocytes for the next spawning season (Rideout et al., 2005; Lowerre-Barbieri et al., 2011b)

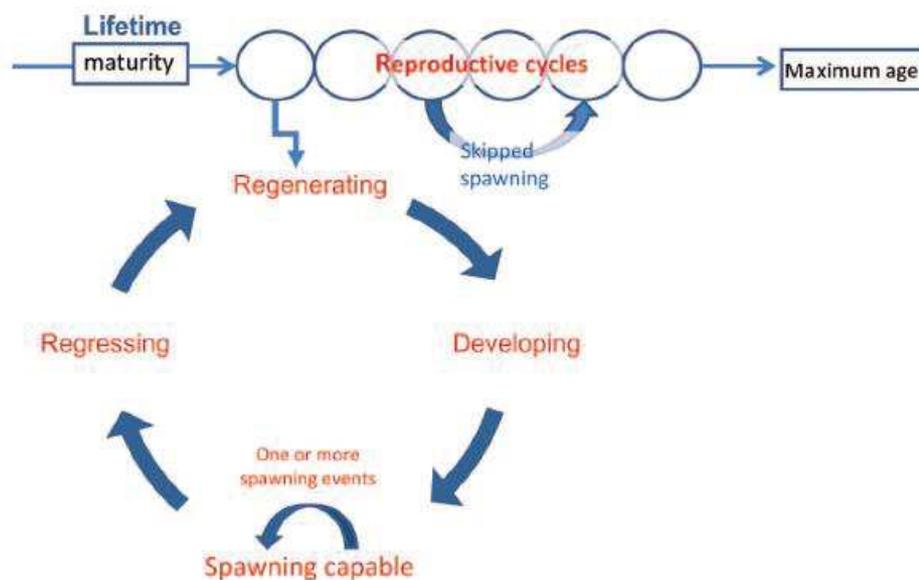


Figure 3. Conceptual model of universal reproductive traits in fishes. (Source: Lowerre-Barbieri et al., 2011)

Although spawning time is in part a heritable trait (Leder et al., 2006; Otterá et al., 2012), reproductive cycles are often influenced by environmental conditions. The maturation is a complex process that must be perfectly timed to ensure that spawning or critical off-spring feeding periods coincide with higher seasonal food availability (Mañanós et al., 2008). Fish have evolved to entrain maturational development with the predictable but constantly changing environmental parameters (e.g. temperature, photoperiod). As stated by Wootton and Smith (2015) reproductive process are conditioned by endogenous cycles and external environmental factors that are critical to finish the process.

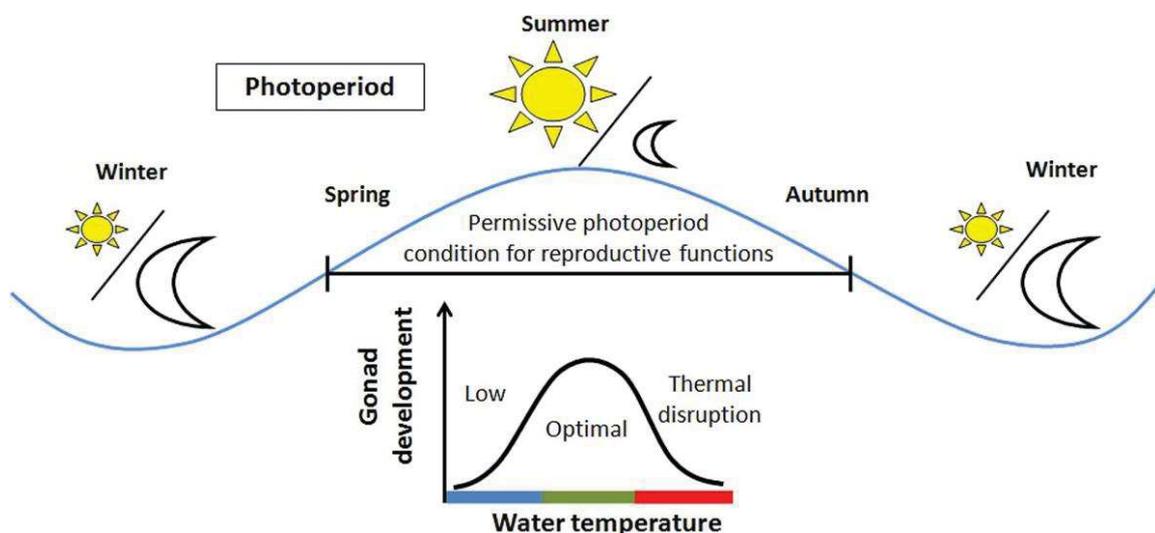


Figure 4: Schematic representation of environmental cues that regulate reproductive cycle in temperate fish species. (Source: Miranda et al., 2013)

Several teleost fish can be exposed to temperatures that are extremely different from their optimal temperature range and gametogenesis can fail and atresia could be induced (Mañanós et al., 2008). Temperature has direct effects on metabolic rates and consequently can modulate the rate at which all physiological processes can occur, including those related to reproduction (Figure 4) (Wang et al., 2010; Miranda et al., 2013). Temperature is considered a key factor that affect reproductive development, e. g. spawning time or expression and activity of regulatory proteins that influence the rate of oocyte growth and

development (Pankhurst and Porter 2003). The reproduction in fish, compared with other physiological processes, only occurs in a bounded temperature range (Pörtner and Farrell, 2008). It is well known that abnormal water temperature conditions could generate a mismatch between reproduction and optimal environmental conditions for progeny development (Durant et al., 2007; Miranda et al., 2013)

1.4 Oogenesis

Oogenesis is defined as the developmental process by an oogonium becomes a fully-grown, mature, and a fertilizable oocyte develop into a viable egg and followed fertilization become an embryo. Embryo viability depends on the accumulation of specific maternal transcripts that are deposited during developing oocytes and successive developmental stages of the embryo (Babin et al., 2007; Lubzens et al., 2017) (Figure 5)

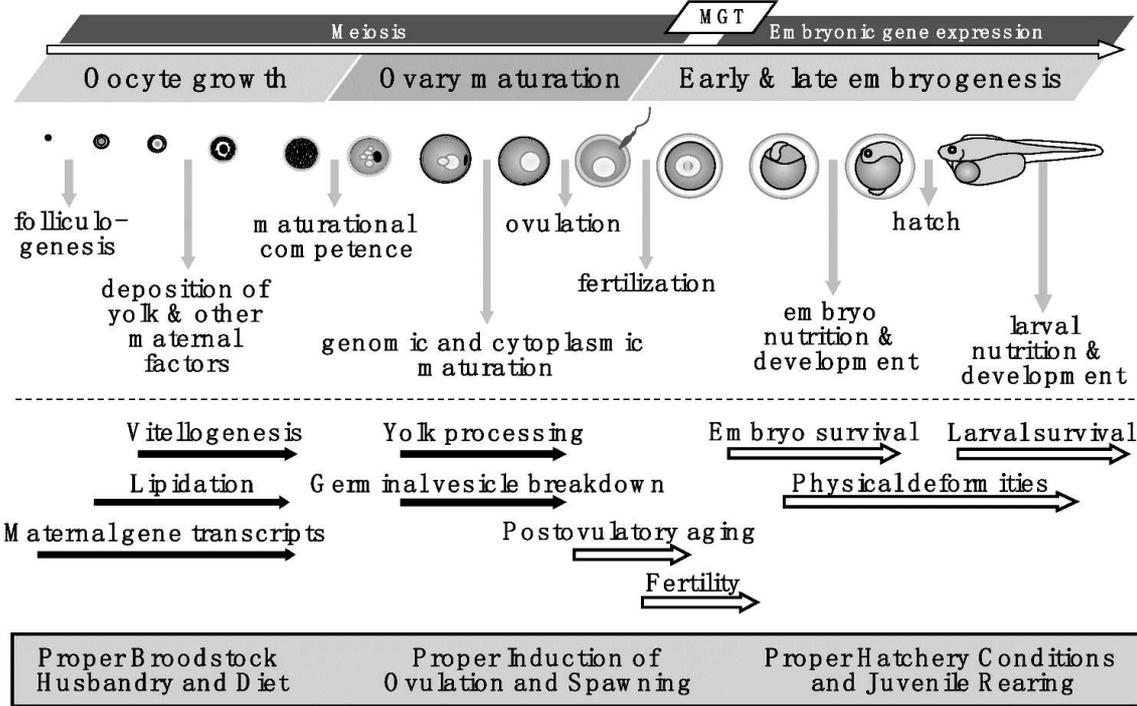


Figure 5. Model depicting the summary of processes and events during oocyte growth, ovary maturation, and early and late embryogenesis in fishes (Source: Reading et al., 2018)

In numerous species of teleost fish, particularly in oviparous species, the process of oogenesis is similar and takes place within the so-called “follicle complex”. This “follicle complex” is composed by an inner surrounding follicular or granulosa cells, outer theca layers (including blood vessels and extracellular matrix), and a basement membrane between granulosa and theca cells (Grier, 2000; Jalabert, 2005; García-López et al., 2011). Progression of oocyte development from primary growth oocytes to ovulation (Figure 6), to secondary growth, which begins with the cortical alveolar stage and then proceeds through vitellogenesis, which can be broken into substages associated with the extent of yolk globules or platelets in the ooplasm. Oocyte maturation occurs after the appropriate trigger and can include germinal vesicle migration, yolk coalescence, germinal vesicle breakdown, and in pelagic spawners, hydration. At ovulation, the follicle ruptures and the oocyte is released. Postovulatory follicles remain in the ovary, where they are resorbed. (Lowerre-Barbieri et al., 2011b) (Figure 6)

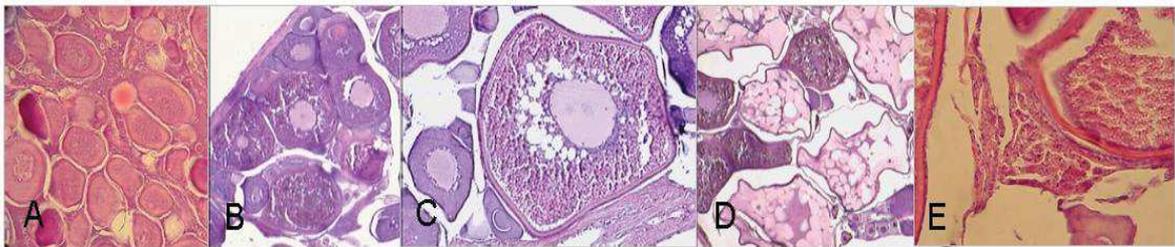


Figure 6. Progression of ovocyte development from primary growth oocyte (A), alveolo cortical oocytes and initial vitellogenic oocytes (B), advance vitellogenic oocyte (C), hydrated oocytes (D) to Ovulation (E) in *Trachurus murphyi*. (Photo: P G-K)

During the third stage, the oocytes increase in size and often acquire oil droplets and cortical alveolar vesicles, indicating the first gonadotropin-dependent stage of development (Lowerre-Barbieri et al., 2011b), then the glycoproteins, digestive enzymes, and mucopolysaccharides are incorporated into cortical alveolar oocytes (Carnevali et al., 2006). In this progression, the meiotic phases occurred similarly to other vertebrates during the

oogenesis (Le Menn et al., 2007; Lubzens et al., 2010). Oocytes in the primary growth and vitellogenic stages are in arrested diplotene step of the first meiotic prophase until the appropriate hormonal stimulus (Grier, 2000; Le Menn et al., 2007; Lowerre-Barbieri et al., 2011a). The oocytes, their follicular cell and different stages of development can produce and respond to different types of molecular signals during the maturation (Jalabert, 2005; Babin et al., 2007; Lubzens et al., 2010). Therefore, different structural and molecular events occurred within oocyte growth, e.g. the accumulation of specific maternal RNA, lipids, vitamins, and hormones (Babin et al., 2007; Agullero et al., 2007; Sullivan et al., 2015). The oocytes undergo dramatic changes during development because they accumulate RNA transcripts, glycoproteins and yolk proteins that are necessary to ensure proper fertilization and embryogenesis (Breton and Berlinsky, 2014).

Three different ovarian follicle developmental phases can be found in fish: synchronous, group-synchronous and asynchronous ovaries. The synchronous ovarian is characterized by a simultaneous development of all oocytes which ovulate at the same time (total spawners) and is typical of semelparous species. In the group-synchronous and asynchronous ovaries, different developmental stages of oocytes can be simultaneously observed inside the ovaries. These ovarian developments are typical of iteroparous species that spawn several times (batch spawner) during the spawning season (Murua and Saborido-Rey, 2003). Three forms of spawning have been proposed based on the development at which the reproductive cycle is interrupted: (1) Retaining, eggs become fully ripened and may be ovulated but are never released due for instance to overcrowding, low dissolved oxygen, high/low salinity and others (2) Reabsorbing, oocytes begin to ripen for the next spawning season but shortly thereafter, gametogenesis is halted and all vitellogenic oocytes are reabsorbed via follicular atresia; and (3) Resting, oldest generation of oocytes remains in a pre-vitellogenic state

throughout the year, i.e. no ripening occurs (Rideout et al., 2005). The two last form can be due to poor nutrition, low temperature and pollution (Figure 7).

Because teleost fish exhibit a large diversity of reproductive strategies (e.g. oocyte and egg development, recruitment of oocytes and spawning pattern), it remains difficult to clearly understanding the molecular and physiological underlying mechanisms (Tingaud-Sequeira et al., 2009; Martyniuk et al., 2013).

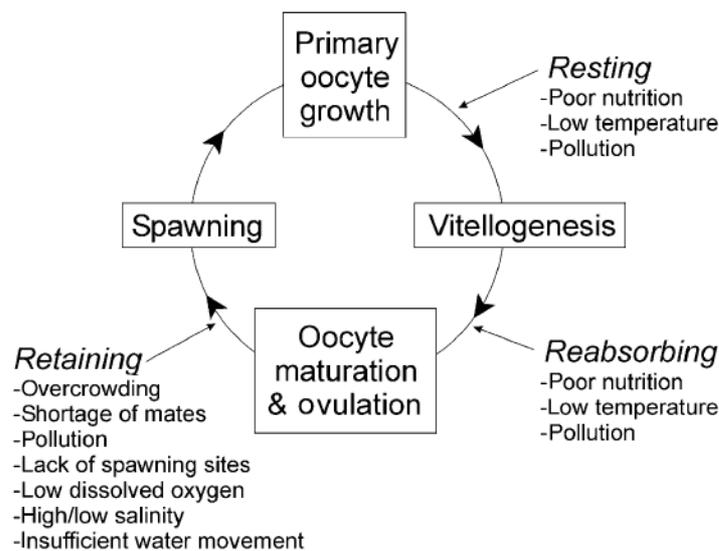


Figure 7. Schematic representation of the female teleost reproductive cycle, including points in development where nutrition and environmental factors can cause spawning omission to occur. (Source: Rideout et al., 2005).

1.5 Follicular atresia

Follicular atresia, or atresia, is a degenerative process common in ovaries of fishes and other vertebrates under natural and experimental farmed conditions (Tyler and Sumpter, 1996). Atresia has been defined as a normal process that takes place inside the ovaries (Figure 8), but is considered to be a reproductive dysfunction when the vitellogenic oocytes cannot complete their development increase of atretic oocytes, a process also known as skipped spawning (Rideout and Tomkiewicz, 2011).

Atresia is observed in all stages of the reproductive cycle, although more frequently in regressing ovaries during the post-spawning period (Miranda et al., 1999; Wootton and Smith, 2015). Quantification of ovarian atresia is important in fisheries studies that aim to assign females to their reproductive state (Hunter and Macewicz, 1985), or to correct estimates of realized annual fecundity in determinate spawners (Hunter et al., 1992; Kjesbu et al., 1991; Kurita et al., 2003).

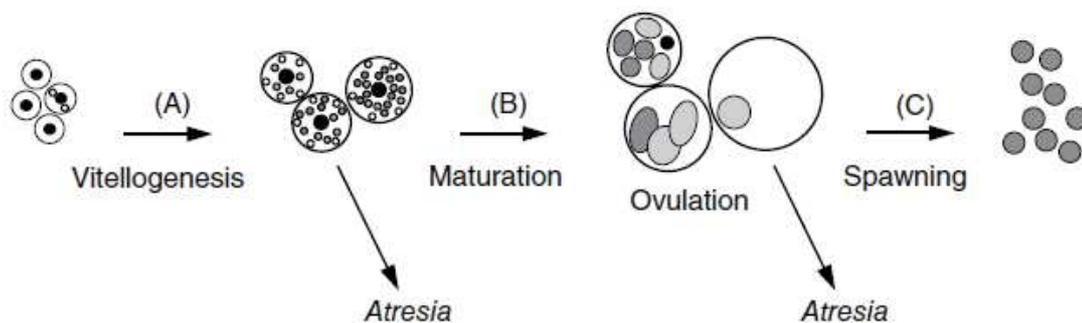


Figure 8. Principal sexual dysfunctions in females under aquaculture condition. (A) The inhibition of vitellogenesis; (B) inhibition of oocyte maturation, resulting in atresia of postvitellogenic oocytes; (C) inhibition of spawning, resulting in retention of ovulated oocytes. (Source: Mañanós et al. 2009).

The increased atretic oocytes can have serious consequences for fish populations because nonspawned vitellogenic oocytes may reduce the reproductive potential due a decrease of the number of viable offspring (Rizzo and Bazzoli, 1995; Miranda et al., 1999; Santos et al., 2005). Atresia is most often observed in vitellogenic oocytes, but it can occur at any phase of development (Johnson et al., 2009) and at any time during the spawning season (Rideout et al., 2000; Morua et al., 2003).

The sequential steps of follicular atresia in teleosts and other oviparous vertebrates can be summarized as: 1) the disorganization of the nucleus and cytoplasm of the oocyte; 2) the distortion of the follicle with the fragmentation of the zona radiata (or zona pellucida), 3) subsequently broken up of the zona radiata into segments of irregular shape; 4) the fusion

and liquefaction of the yolk globules, 5) hypertrophy of the follicle cells which acquired phagocytic features for ingestion and digestion of the yolk, 6) in the advanced stages of atresia process, the ooplasm is invaded by the follicular and thecal cells forming a yellow-brownish pigmented cell (Miranda et al., 1999; Ganas et al., 2003; Lubzens et al., 2010; Morais et al., 2012).

Most studies have focused in the quantification of atresia in the early stages of resorption (i.e. alpha stage) (Murua et al., 2003) because the distinction of later stages is often not easy to identify (Hunter and Macewicz, 1985). Moreover, due to their short duration early atretic stages may provide more reliable measures of atretic rate (Hunter and Macewicz, 1985). Although late follicular atresia is rarely considered and quantified, its study may provide useful information on past reproductive activity (Ganas et al., 2008).

In recent years, the study of atresia has increased due it seems to be the major cause of reduced fecundity for any species (Ganas et al., 2008). Additionally, the recording of atresia allows us to assign real reproductive status for female, indicating the end of the spawning period as well as whether females failed in their spawning cycle, a phenomenon known as skipped spawning (Hunter and Macewicz, 1985; Ganas et al., 2008; Rideout et al., 2000).

The histological characteristics used to identify the stages of atresia are indicated in Table 1 and Figure 9. The alpha- and beta-atresia stages have been independently assessed as a potential mechanism to determine previous spawning events (Ganas et al., 2008). The initial alpha-atresia is taken into account and quantified for the assignment of females to different spawning states (active, inactive/immature) as well as to determine the reproductive variables in fish population studies, such as fecundity and length/age at first maturity (Hunter and Macewicz, 1985; Karlou-Riga and Economidis, 1997; Ganas et al., 2008). Normally, beta-atretic oocytes are not considered because they may be confused with

Post Ovulatory Follicles (POF) in spawning individuals (Witthames et al., 2009; Kjesbu et al., 2010).

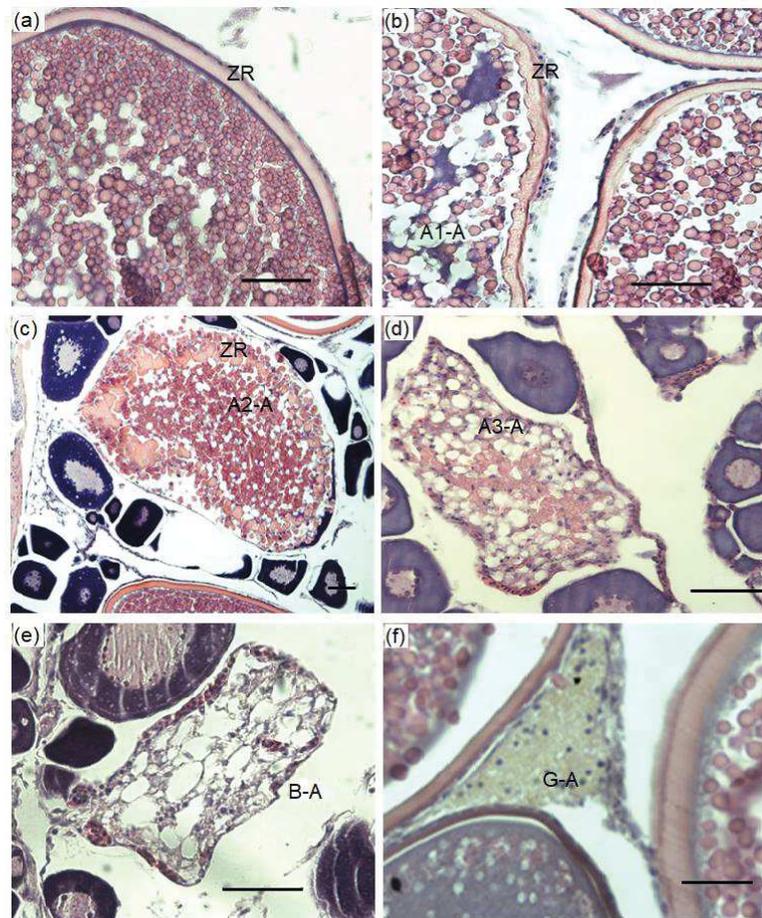


Figure 9. Atretic follicles in *Trachurus murphyi* ovaries. (a) = non atretic oocyte, (b) A1-A = Initial alpha atresia, (c) A2-A = Intermediate atresia oocytes, (d) A3-A = Advance alpha stage atresia, (e) B-A = Beta atresia oocyte, and (f) G-A = Gamma atresia. ZR= Zona radiata. Scale bar = 50 μ m (Photo P G-K)

Understanding the trends and dynamics of atretic oocytes allows to identify any change in the spawning period, such as a potential skipped spawning (Rideout and Tomkiewicz, 2011). Spawning events and atresia are temperature-dependent processes (Hunter and Macewicz, 1985; Witthames and Greed, 1995) and females can spawn or delay during migration if the temperature regime is not suitable for spawning conditions (Ma et al., 1998; Haslob et al., 2013).

Table 1. Phases of atresia and its main characteristics determined in fish, according to Hunter and Macewicz (1985) and Valdebenito et al. (2011)

Phase of Atresia	Main characteristics
Alpha (α)	Characterized by chorion ripples, nuclear disruption, early breakage of yolk globules and hypertrophy of granulosa cells
Beta (β)	The granulosa cells migrate and engulf the yolk. The teak cells are not observed invading the interior of the oocyte. The end of beta atresia is characterized by the complete disintegration of the chorion
Gamma (γ)	The reabsorption of the oocyte content continues during this stage, until the complete reabsorption of the content. Small, irregular-looking follicles appear in yellow-brown staining with H-E staining. The thecal cells of the follicle still surround the remaining oocyte
Delta (δ)	It is characterized by the presence of residual components after the reabsorption of yolk and cytoplasm. Dark yellow-brown pigments and granular pigment are observed in the granulosa cell.
Epsilon (ϵ)	Difficult to evaluate, some authors have postulated that at this stage "cells of the corpus luteum could differentiate into new oogonial cells"

For the highly migratory carangid the yellowtail kingfish *Seriola lalandi* Valenciennes 1833, a temperature exceeding the optimal range can cause an aborted spawning event (Poortenaar et al, 2001; Stuard and Drawbridge, 2013). Similarly, for *Sardina pilchardus*, the high prevalence of atresia was also related to temperature (Ganias, 2009). Additionally, several hydrographic conditions (salinity, pH, winds, currents, day length and chlorophyll-a), or a combination of them may explain the presence and intensity of atresia (number of atretic oocytes), because the indeterminate spawners may respond quickly to changes in environmental condition (Haslob et al., 2013), but the level of energy reserves available at the beginning of spawning season is also an important factor to understand atresia.

Endogenous factors such as age, length or nutritional status may induce the presence of atretic oocytes. In the sardine of Monterrey *Sardinops sagax*, temporal changes in the prevalence of atresia (the number of atretic fish divided by total number of examined fish) have been observed, an increased prevalence is associated when there is a greater proportion of females under the length of first sexual maturity present in the reproduction period (Torres-Villegas et al., 2007). In some species of *Sebastes*, young females may abort

maturation, characterized by the formation of developing oocyst atresia masses (Hannah, 2014). Meanwhile, condition status and energy supply are important factors that regulate fecundity, and it is likely that together they determine the annual fecundity (Rijnsdorp, 1990). In the Atlantic sardine *Clupea harengus*, the development of the oocytes is influenced by the somatic energy content and during a cycle of oocyte maturation the fecundity decreases due the impact of atresia driven by a poor nutritional condition of the female. (Kurita et al., 2003)

1.6 Parasites affect reproduction

Fish serve as hosts to a range of parasites that are taxonomically diverse and that exhibit a wide variety of life cycle strategies (Wootton, 1990). Many fish parasites are associated with a reduction in host reproductive effort, and they appear to achieve this either through the energetic demand they place on their host, the release of hormone analogues that block sexual maturation (Barber et al 2000) and mechanic castration (Chávez and Oliva, 2011). Parasitism may affect life history traits such as condition, reproduction (parasitic castration) and mortality, although the research in this field is rather scarce (Ferrer-Maza et al., 2014). For European hake *Merluccius merluccius* the effect of metazoan parasitism in reproductive capacity (fecundity, egg quality and atresia) was study, but result showed that metazoan parasites not causing severe impairment to physical condition or reproductive capacity for *M. merluccius* females (Ferrer-Maza et al., 2014). Parasites can affect fish reproduction either directly or indirectly, depending on the target organ. Protozoans parasites can show evasion strategies of the host immune response and cause changes in the reproductive process (Sitja-Bobadilla, 2009). Members of the phylum Apicomplexa, mainly Family Eimeriidae are among the most important pathogen parasites in vertebrates and invertebrates, with economic implications (Silva et al., 2019). In *Strombus gigas* (Mollusca

- Gastropoda), the increased number of parasites, induced an attenuated reproduction (Banqueiro et al., 2012. Coccidian of the genus *Goussia* infect several organs (liver, stomach, intestine) (Figure 10), and the impact in the body condition of a wide range of fish species are well known (Abollo, et al., 2001). Gene expression of parasites and host will allow us to understand the effect of the parasite on the host, but also the tradeoff between parasite infection and host response.

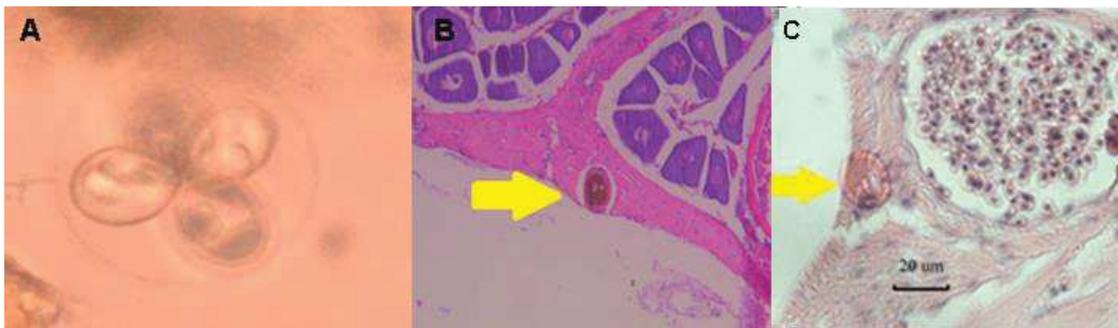


Figure 10. Oocyst and sporocysts of coccidian *Goussia cruciata*: fresh mature oocyst in liver (A), fibrous capsule of connective tissue surrounding oocysts in ovary (arrow) (B) and free oocyst in stroma of ovary (arrow) (C) of *T. murphyi* (Photo: P G-K)

1.7 Molecular approach to study of atresia

The study of molecular mechanisms involved in atresia has begun to be studied from a perspective that allows to determine genes regulating this process (Nicol et al., 2013, Miranda et al., 2013). Studies in transcriptomics have provided valuable information about the molecular events that lead to ovulation (Babin et al., 2007; Tingaud-Sequeira et al., 2009, Martyniuk et al., 2013), and for many species the transcriptional response can be associated with physiological changes and morphological events that are occurring in the ovary (Gardner et al 2012, Martyniuk et al., 2013).

Ovaries can provide transcriptomic specific maturation profile in mature individuals, which can be associated with specific biological processes for each stage of oocyte development, which would allow understanding the dynamics of the process of atresia in natural populations (Villeneuve et al., 2010; Lubzens et al., 2010; Martyniuk et al., 2013).

For some species (The Atlantic bluefin *Thunnus thynnus*, sole *Solea senegalensis*, Japanese medaka *Oryzias latipes* and black bass *Micropterus salmoides*) it has been determined candidate genes that would have a putative role in the process of atresia. Of the described, the *Fst* gene encoding the protein follistatin (FST) is probably a marker of ovarian age (Herpin et al., 2013), genes associated with lipid trafficking, such as the genes of the FABP superfamily (*Fabp1*, *Fabp4* and *Fabp11*) (Agulleiro et al., 2007, Gardner et al., 2012). For *Micropterus salmoides* expression profiles for atresia are quite different to other oocyte development stages, being genes related to oxidative metabolism higher in atretic but lower the genes that participate in gap junction pathways of the cytoskeleton, among others (Martyniuk et al., 2013).

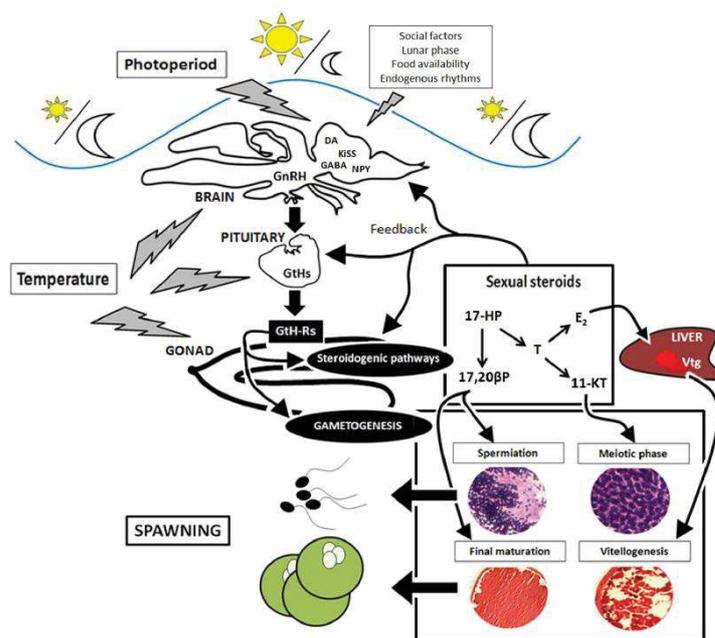


Figure 11. Schematic representation of the environmental influences on the regulation of fish brain–pituitary–gonad axis. Photoperiod information is decoded by different hormonal pathways at brain level and together with other external factors (social interactions, lunar phase, food availability) and endogenous rhythms affect the reproductive functions. (Source: Miranda et al., 2013)

Because atresia can occur at any stage of oocyte development and could be influenced by environmental factors (Figure 11), such as temperature or by the physiological status of

females (Martyniuk et al., 2013); study which genes expressed in an atretic ovary versus non-atretic ovary could account for the existence of a primary endogenous component (physiological) or a secondary exogenous (temperature) that may explain the prevalence and intensity of atresia observed in the species under study, and determine its importance for the stock reproductive potential.

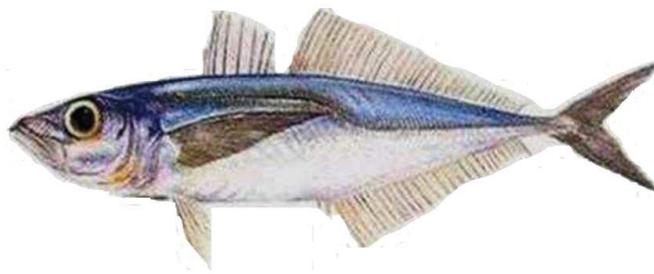


Figure 12. Chilean jack mackerel, *Trachurus murphyi* Nichols 1920

1.8 Biological Model: Chilean Jack mackerel, *Trachurus murphyi* Nichols 1920

The pelagic Chilean jack mackerel *Trachurus murphyi* Nichols 1920 (Figure 12) is one of the most important fishing resources in the South-eastern Pacific Ocean. Due to the overexploitation favoured by the oversizing of all the industrial fleets, they ended up triggering the biggest fishing crisis of the decade for this fishing resource. For the population of *T. murphyi* in Chile, the reduction was 44% of the biomass, with landings of ca. 4,600,000 tons by 1996 dropping dramatically to less than 300,000 tons by 2017 (Figure 13); generating critical changes in the age structure of the populations and all the sustainability of the fishery of this resource (Figures 13) (Arcos et al., 2004; SPRFMO 2018).

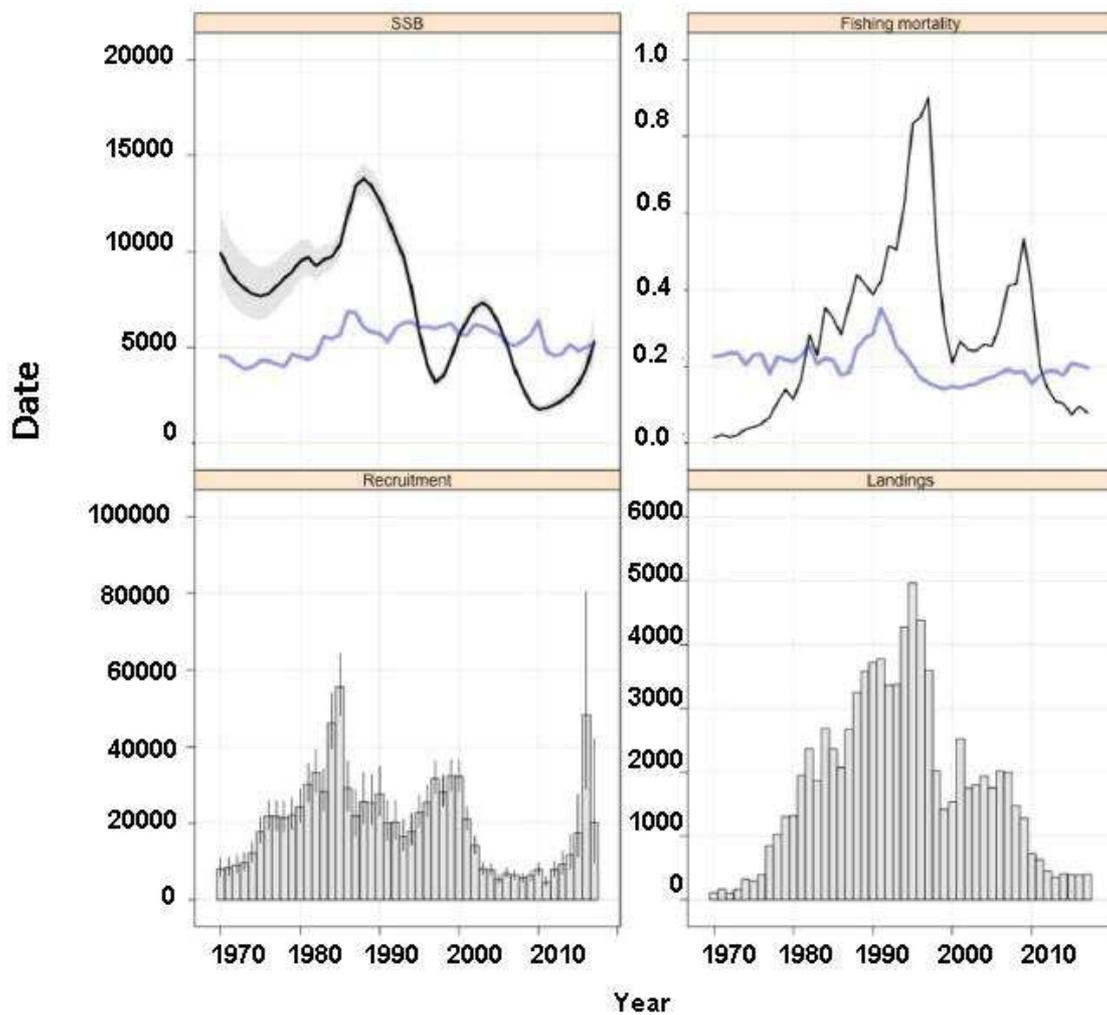


Figure 13. SSB spawning biomass (t), average fishing mortality, recruitments at age 1 (millions) and catches (thousands t). The blue lines represent the annual references of the RMS. (Source: SPRFMO 2018)

The distribution of the Chilean jack mackerel covers from the Galapagos Islands to southern Chile, both in oceanic and coastal waters (Serra, 1991). In international waters it is distributed in large schools up to 160 ° W, and mainly between 33 ° S and 48 ° S (Grechina, 1992). Due to a recent colonization process, it has reached New Zealand and Australia (Grechina, 1998; Cárdenas et al., 2009). Studies using mitochondrial DNA and microsatellites indicate a panmictic population in the Pacific Ocean (Poulin et al., 2004; Cárdenas et al., 2009). Ahsford et al. (2011) compared the chemistry of the nucleus of the otoliths in individuals from different areas of distribution (from northern Perú to southern

Chile as well as New Zealand), main conclusion was that the Pacific Ocean Chilean jack mackerel comes from the same spawning area, which would be located off central Chile.

The spatial distribution of *T. murphyi* consists of three main habitats: 1) a breeding area located off the coast of southern Peru and northern Chile, 2) a feeding area located off the south-central part of Chile 3) an oceanic spawning area in front of central Chile, which has an extension of 1800 km during spring (Figure 15) (Arcos et al., 2001; Cubillos et al., 2008; Vásquez et al., 2013).

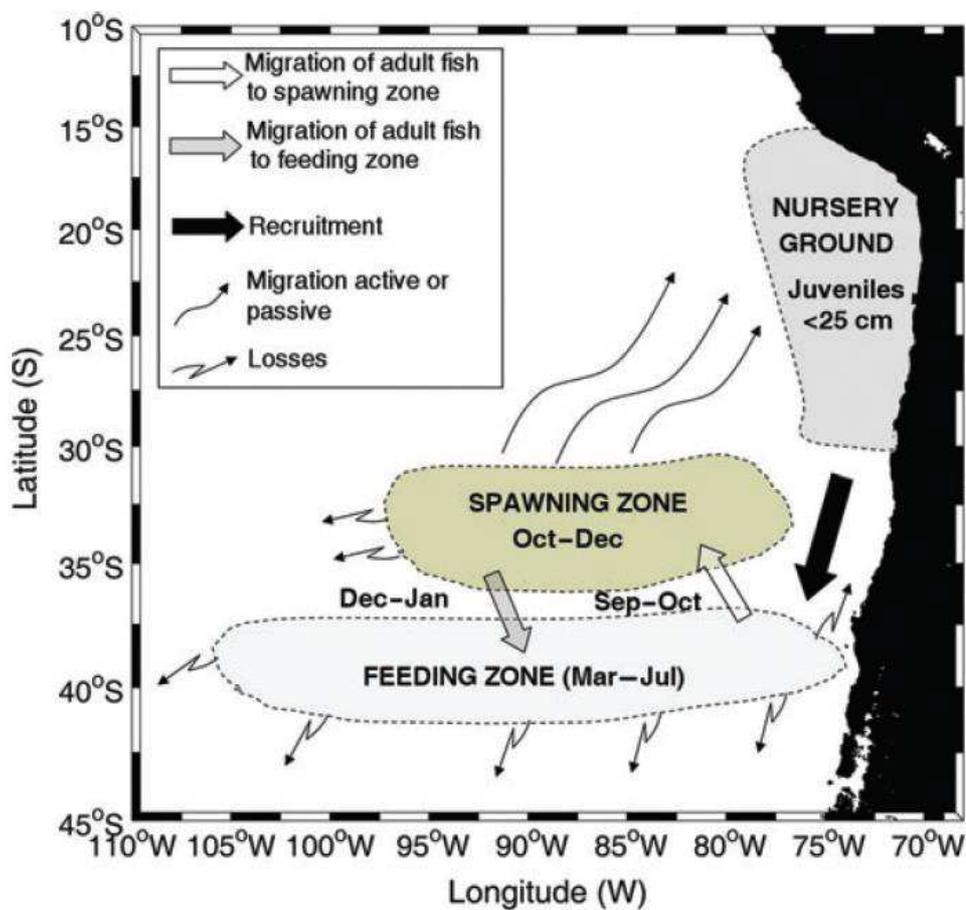


Figure 14. Conceptual spatial model of the jack mackerel population in the southeastern Pacific Ocean (Source: Vasquez et al., 2013)

T. murphyi is a partial, asynchronous spawning that migrates for reproductive purposes off the coast of Chile during spring (Leal et al., 2013). The main spawning area occurs between 80° and 92°W with a maximum at 35°S associated with a sea surface temperature (SST)

ranging from to of 15-16°C but can reach 19°C in some years (Cubillos et al., 2008). Also, *T. murphyi* is considered an indeterminate spawner, then egg production is not fixed at the beginning of the spawning season, and in a similar way that other indeterminate species; continuous recruitment of new batches of mature oocytes can be spawned repeatedly during the reproductively active period (Murua and Saborido-Rey, 2003). During the spawning season, that can last for five months, females of *T. murphyi* can spawn every 3 to 5 days. The almost constant eggs production during an extended period requires a considerable amount of energy resources that can be obtained (1) from energy reserves accumulated prior to spawning, (2) directly from food input during the spawning season, or (3) from both sources (Hunter and Leong, 1981). Species highly migratory like *T. murphyi*, are exposed to several variables (environmental and physiology) that can produce that females delay or fail the reproduction. During non-adequate conditions (environmental and/or physiological) egg production can decrease or fail via atresia. Atresia might be considered an important mechanism for the adjustment of fecundity through the resorption of oocytes (Abaunza et al., 2003).

In this specie, despite their economic importance, reproductive biology is not fully understood but some recent studies have contributed to characterize the reproductive cycle (Leal et al., 2013; Perea et al., 2013). Most of the reproductive background regarding the impact of oceanographic conditions in the oceanic spawning habitat off central Chile has been described (Cubillos et al., 2008) with emphasis in the spatial distribution of egg and larvae (Cubillos et al., 2008; Vásquez et al., 2013). Description of follicular atresia and its interannual variations and potential relationships with reproductive variables (e.g. fecundity) it has not been evaluated for this species. Similarly, the new molecular approaches to the study of reproductive biology in fish, as transcriptional studies, have not been performed in this species either.

2. CONTEXT OF THE THESIS

In mature females, atresia can occur at any time during the reproductive cycle. Sometime, females delay its reproduction, inclusive for one year, increasing the prevalence and intensity of atretic oocytes. In highly migratory species, such as *T. murphyi*, it is expected that during the spawning period, some females will have atretic oocytes due to potential energy loss during annual migration. As atresia can have strong consequences in recruitment, the knowledge of the spatio-temporal dynamics of this process is important to assign the status of female in the spawning stock during spawning season. Atresia can have direct effect on the number of eggs that females can produce. Like other reproductive traits, atresia depends on the female's physiological conditions (e.g. age, length, condition) and environmental factors (e.g. temperature). In the context of climate change, environmental variables such as temperature, can act directly or indirectly at all levels of the reproductive axis (brain-pituitary gland, liver, gonads), allowing regulation of different metabolic pathways that may affect reproductive processes.

Transcriptomic studies provide valuable information about the molecular events that lead to ovulation and atresia. Studies of gene expression in teleost fishes with different breeding strategies have been performed. Due that the transcriptional response may be associated with a physiological and morphological change in the ovary, understanding the cellular and molecular mechanisms involved in the process of atresia, is critical to understand the reproductive dynamics of this important economic species.

3. HYPOTHESIS

3.1 Hypothesis

- Due the extended distribution of the spawning area of *T. murphyi*, Prevalence and intensity of atresia in *Trachurus murphyi* is expected to show spatial and temporal variability correlated with length, gonadosomatic index and condition factor. Alternatively, variations in atresia will be correlated with extrinsic factors (sea surface temperature, year).

-Species highly migratory like *Trachurus murphyi*, are exposed to several variables (environmental and physiology) that can produce that females delay or fail the reproduction, then is expected a differential transcriptomic expression between females showing atresia and not atresia.

- The presence of parasites can be generated change in physiological response in host, including reproductive process, then is expected that females showing protozoan parasites *Goussia cruciata* will have more atretic oocytes than females without this parasite.

4.- OBJETIVES

- Describe and evaluate reproductive atresia processes and establish temporal reproductive variation in the potential spawning area.
- Determine whether the prevalence and/or intensity of atresia are related to endogenous variables (fork length, gonadosomatic index, condition factor) or environmental variables (sea surface temperature)
- Identified through a bibliographic review the potential molecular markers associated with the atresia processes in teleost fishes.
- Identified the transcriptome expression of genes associated a female that showed atresia and not atresia, that could be used as predictor to atresia in this species.
- Determine the potential effect of *Goussia cruciata* (Apicomplexa) in some biological traits (e.g. atresia) of this species.

5. RESULT

CHAPTER 1

Paola González-Kother, Marcelo E. Oliva, Arnaud Tanguy & Dario Moraga. 2019. A review of the Potential Genes Implicated in Follicular Atresia in Teleost Fish. Marine Genomic. <https://doi.org/10.1016/j.margen.2019.100704>

CHAPTER 2

Atresia in the Chilean jack mackerel *Trachurus murphyi* (Teleostei, Carangidae): A first assessment for the South-eastern Pacific Ocean.

Paola González-Kother, M Teresa González and Marcelo E. Oliva

Submitted to: Revista Biología Marina y Oceanografía

CHAPTER 3 First transcriptomic approach to the study of atresia in *Trachurus murphyi* using RNA-Seq analysis: FABPs Family gene role in Atresia.

Paola González-Kother, Marcelo E. Oliva, Arnaud Tanguy & Dario Moraga

To be submitted to Marine Genomic

CHAPTER 4

González-Kother, P & M.T. González. 2014. The first report of liver coccidian *Goussia cruciata* in jack mackerel *Trachurus murphyi* from the South Pacific and its relationship with host variables. Parasitology Research 113:3903-3907.

CHAPTER 1



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Review

A review of the potential genes implicated in follicular atresia in teleost fish

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ABSTRACT

In recent years, numerous studies conducted on teleost fish have highlighted the contribution of transcriptomic studies in elucidating the physiological mechanisms underlying the molecular events of oogenesis and follicular atresia, enabling the identification of potential genes and molecular networks that participate in both the reproductive cycle and the process of follicular atresia. Atresia can affect the reproductive potential of females by reducing the healthy eggs that a female can spawn in both aquaculture and wild populations. The substantial diversity of reproductive strategies exhibited by teleost fish has contributed to the difficulty in identifying common genes between species, but a set of core genes has emerged as potential markers for atresia in relation to apoptosis/autophagy, lipid metabolism, oxidative metabolism and other physiological processes similar to those identified in other vertebrates, even mammals. We review the current status of the genes that have been identified in ovaries with atretic oocytes. Our primary goal is to review the current status regarding gene expression during gonadal development and follicular atresia. This information will enable us to understand the factors and expression patterns involved in the follicular atresia of teleost fish.

1. Introduction

Among the 32,500 known teleost fish species, a wide variety of reproductive traits can be found, reflecting different life history strategies (Nelson et al., 2016). Reproductive characteristics have mainly been studied using anatomical criteria and classification of gonads by histological approaches (Silva et al., 2013). Currently, the molecular approach has enabled the identification of sets of genes that participate in the reproductive cycle of teleost fish, although many gene functions remain poorly understood (Breton and Berlinsky, 2014). Identification of genes regulated during the different stages of gonad development is of major importance in studies for fish species of economic importance for aquaculture and fisheries management, such as Salmoniformes and Pleuronectiformes (Lubzens et al., 2010; Lowerre-Barbieri et al., 2011b; Migaud et al., 2013). Indeed, the control of sexual maturation and spawning to produce high quality eggs is a primary requirement for successful aquaculture production (Migaud et al., 2013).

Several reviews describing the process of reproduction, mainly

oogenesis, have been published for teleost fish (Lubzens et al., 2010; Lowerre-Barbieri et al., 2011a; Lubzens et al., 2017). Like mammals, the reproductive cycle of teleost fish is characterized by a sequential progression of morphological, endocrine and molecular changes during gametogenesis (Jalabert, 2005; Lubzens et al., 2010; Lowerre-Barbieri et al., 2011a). Fishes showed a diversity in the mechanisms involved in sexual plasticity, gametogenesis and reproduction (Kitahashi and Parhar, 2013). This diversity in fish reproductive strategies has led to difficulty in the identification of the key genes participating in the underlying aspects of the molecular and regulatory mechanisms of reproduction, since genes could remain specific to each species of teleost fish (Tingaud-Sequeira et al., 2009; Kitahashi and Parhar, 2013; Breton et al., 2019).

For reasons not understood to date, in natural and cultured fishes, the normal female reproductive cycle can show a disruption in the reproductive process (Habibi and Andreu-Vieyra, 2007). These females fail to complete maturation and ovulation due to the elimination of vitellogenic oocytes prior to ovulation in a process known as follicular

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atresia (Miranda et al., 1999; Morais et al., 2012). Follicular atresia may be an important problem in cultured fishes, when females showing large amounts of vitellogenic follicles failed to mature and ovulate during the spawning season (Rideout and Tomkiewicz, 2011). The process of follicular atresia remains extremely complex, and little is known about genes and other processes in the ovary that either regulate atresia or are the consequences of atresia (Yamamoto et al., 2016).

Regardless of the species studied, follicular atresia is a highly regulated process that is essential for the maintenance of ovarian homeostasis during the reproductive cycle (Krysko et al., 2008; Lowerre-Barbieri et al., 2011a). In most mammals, follicular atresia is primarily induced by the apoptosis of granulosa cells (Thomé et al., 2009; Yan et al., 2018). Instead, studies in freshwater teleost fish have suggested that apoptosis and autophagy act during follicular atresia (Santos et al., 2005; Valdebenito et al., 2011; Morais et al., 2012). In teleost fish, the development and maturation of the gonads are controlled by genetic and environmental factors and are regulated by a network of molecular signals (Chen et al., 2013). Gene expression studies have been performed to identify the molecular mechanisms by which the oocytes can grow and mature during oogenesis, and putative genes involved in oocyte development and follicular atresia have been recently discovered (Villeneuve et al., 2010; Martyniuk et al., 2013). Next-generation sequencing (NGS) technologies have strongly contributed to the identification of genes and physiological networks that participate in oocyte development, including atresia, and more generally to the understanding of the molecular mechanisms involved in the physiology of the reproductive process. In this paper, we aim to review the transcriptomic data associated with reproduction in teleost fish to identify genes that are expressed during atresia.

2. Atresia of teleost fish

Environmental stressors, such as food availability, contaminant exposure, and global warming, can potentially interfere with oogenesis by disrupting the brain-pituitary-gonad axis and inducing atresia of ovarian follicles (Table 1) (Babin et al., 2007; Yamamoto et al., 2011; Molina et al., 2018; Sullivan and Yilmaz, 2018). Atresia has been defined as a normal process that takes place inside the ovaries, but it is considered a reproductive dysfunction because vitellogenic oocytes cannot complete their development, increasing atretic oocytes, a process also known as skipped spawning (Rideout and Tomkiewicz, 2011). The increase in the proportion of atretic oocytes can have serious consequences for fish populations because nonspawn vitellogenic oocytes may reduce the reproductive potential of the species through a decrease in the number of viable offspring produced (Rizzo and Bazzoli, 1995; Miranda et al., 1999; Santos et al., 2005).

In the Argentinian silver side *Odontesthes bonaerensis*, red seabream *Pagrus major* and striped bass *Morone saxatilis*, a high water temperature in the gonads provoked regression by inhibiting the expression of specific genes at the level of the brain-pituitary-gonads, reflected by an increase in atresia (Miranda et al., 2013; Akhavan et al., 2016). Cyclic

ovarian development is synchronized by hormones that are predominantly produced by the brain-pituitary-gonad axis (BPG) (Okuzawa and Gen, 2013; Chen et al., 2013; Pinto et al., 2017). Some mechanisms of this BPG axis in fish are not fully understood because their regulation is complex (Lubzens et al., 2010; Zohar et al., 2010; Miranda et al., 2013). Hormones are key molecules in reproduction control, such as gonadotrophin releasing hormone (GnRH), which has a main role in the regulation of the synthesis and release of gonadotropin hormones (GtHs), follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Habibi and Andreu-Vieyra, 2007; Miranda et al., 2013). GtHs have an endocrine control of reproduction, promoting the synthesis of sex steroids through the stimulation of specific enzymes and regulating gametogenesis (Lubzens et al., 2010; Levavi-Sivan et al., 2010). In mammals, GnRH has been shown to participate in follicular atresia by inducing apoptosis in oocytes, mainly in granulosa cells. However, in teleost fish, the relationship between GnRH and ovarian atresia is not clear (Lubzens et al., 2010; Thomé et al., 2009). For those reasons, the follicular atresia status is mainly determined using morphological characteristics.

2.1. Transcriptomics of oogenesis

Regardless of the specific pattern of ovarian development in fish, all oocytes pass through a series of defined stages, generating complex transcriptomic and proteome expression networks according to the developmental stage of the follicle (García-López et al., 2011; Lubzens et al., 2017; Breton et al., 2019). Currently, transcriptomic analysis strongly contributes to a better understanding of complex biological processes, such as growth, reproduction, and the immune response (Wang et al., 2009; Gallardo-Escárate et al., 2014). The transcriptome contains different species of transcripts, including mRNAs, noncoding RNAs and small RNAs, and gene expression levels during development and under different conditions have been quantified (Wang et al., 2009). miRNAs have been shown to participate in organogenesis by controlling miRNA renewal and the activation and/or inhibition of specific transcripts' expressions at different developmental stages (Bizuyehu and Babiak, 2014). Specific miRNAs have been potentially implicated in the early stages of embryo development (Giraldez, 2005), in the destabilization of maternal transcripts (Wienholds, 2005) and in cellular differentiation (Bartel, 2009).

The involvement of miRNAs in oogenesis has been shown in the regulation of mechanisms involved in follicular development, vitellogenesis, oocyte maturation and sexual differentiation in the rainbow trout *Oncorhynchus mykiss* (Ma et al., 2012; Juanchich et al., 2013), in the zebrafish *Danio rerio* (Presslauer et al., 2017; Wong et al., 2018), and in the medaka *Oryzias latipes* (Bouchareb et al., 2017). Some miRNA have been specifically detected in fish testis, but due to the similar functions of both ovaries and testis, their involvement in the regulation of gene expression in both gonads cannot be excluded, as shown by the expression of *sox9b* in germinal cells present in the basal membrane, which is common to both gonads (Nishimura et al., 2016).

Table 1
Factors involved in reproductive dysfunctions and follicular atresia in teleost fish.

Factor	Species	Effect or Alteration	Reference
Temperature	<i>Acipenser transmontanus</i>	Temperature induced oocyte development arrest and follicular atresia.	Linares-Casenave et al., 2002
	<i>Odontesthes bonariensis</i>	High temperature produced disruption at all levels of reproductive axis and increased atresia.	Elisio et al., 2012; Miranda et al., 2013
Captivity	<i>Anoplopoma fimbria</i>	Reproductive dysfunctions at different levels of the reproductive axis, including atresia.	Guzmán et al., 2013
	<i>Engraulis mordax</i>	Starvation induced atresia.	Hunter and Macewicz, 1985
Endocrine disruption by chemicals	<i>Chalcalburnus tarichi</i>	Increased atresia and fibrotic areas in the ovarian tissue.	Kaptaner and Ünal, 2011
	<i>Danio rerio</i>	High doses of chemical increased follicular atresia.	Molina et al., 2018
Endocrine disruption by nutritional stress	<i>Oncorhynchus kisutch</i>	Nutritional stress induced atresia and changes in ovarian transcripts.	Yamamoto et al., 2011
Photoperiod	<i>Dicentrarchus labrax</i>	Changes in photoperiod induced atretic oocytes	Zanuy et al., 1995

Table 2
Potential molecular marker genes for follicular atresia in teleost fish.

Gene Function	Gene	Sequence description [Gene identification (species)]	Relation with atresia [Species identification]	Reference
Apoptosis	<i>bcl2</i>	Bcl-2 protein family [Several species]	Ovary showed high protein expression during late atresia [<i>Asyanax bimaculatus</i> , <i>Leporinu obtusidens</i> , <i>Prochilodus argenteus</i>]	Morais et al., 2012
	<i>bcl-2</i>	Bcl-2 protein family [Several species]	Ovary showed higher protein expression in early and advanced atresia than during late atresia [<i>Asyanax bimaculatus</i> , <i>Leporinu obtusidens</i> , <i>Prochilodus argenteus</i>]	Morais et al., 2012
	<i>casp3</i>	Caspase3 [<i>Oncorhynchus mykiss</i>]	Gene increased in inhibin network during atresia [<i>Micropterus salmoides</i>]	Martyniuk et al., 2013
	<i>casp8</i>	Caspase 8 [<i>Oncorhynchus kisutch</i>]	Gene showed high expression during late atresia [<i>Asyanax bimaculatus</i> , <i>Leporinu obtusidens</i> , <i>Prochilodus argenteus</i>]	Morais et al., 2012
	<i>casp9</i>	Caspase 9 [<i>Oncorhynchus kisutch</i>]	Gene expression increased in fasted fish with severe atresia [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2011
	<i>pdc4d</i>	Programmed cell death protein 4 [<i>Salmo salar</i>]	Gene increase in fasted fish with severe atresia [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2011
	<i>litaf</i>	Lipopolysaccharide-induced tumor necrosis factor (TNF)- α factor [<i>Salmo salar</i>]	Potential early indicator of ovarian apoptosis/atresia. [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
	<i>klf6</i>	Kruppel-like factor 6 [<i>Salmo salar</i>]	Gene showed high expression in fasted fish ovaries leading to high incidence of atresia [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
	<i>bcl-1</i>	Bcl-1 [Several species]	Gene is a potential early marker of apoptosis/atresia [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
	Autophagy	<i>bcl-1</i>	Ovary showed high protein expression in advanced atresia [<i>Asyanax bimaculatus</i> , <i>Leporinu obtusidens</i> , <i>Prochilodus argenteus</i>]	Morais et al., 2012
Apoptosis/autophagy	<i>cathepsin-D</i>	Cathepsin-D [Several species]	Protein expression increased more in early and advanced than in late atresia [<i>Asyanax bimaculatus</i> , <i>Leporinu obtusidens</i> , <i>Prochilodus argenteus</i>]	Morais et al., 2012
	<i>s100a10</i>	S100A10 calcium binding protein [<i>Epimiphelus coioides</i>]	Gene showed up-regulation in atretic ovaries [<i>Solea senegalensis</i>]	Tingaud-Sequeira et al., 2009
Antiapoptotic function	<i>fat</i>	Follistatin [<i>Oryzias latipes</i>]	Gene is a potential marker of ovarian age [<i>Oryzias latipes</i>]	Herpin et al., 2013
	<i>krt79</i>	Follistatin [<i>Danio rerio</i>]	FST network decreased during atresia [<i>Micropterus salmoides</i>]	Martyniuk et al., 2013
	<i>s8</i>	Type 2 keratin k 8b (S2) [<i>Oncorhynchus mykiss</i>]	Gene is a potential early marker of altered ovarian follicle growth [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
	<i>thbs</i>	Type 1 keratin s8 [<i>Oncorhynchus mykiss</i>]	Gene showed down-regulation in fasted fish ovaries [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
Lipid metabolic process	<i>apoc1</i>	Thrombospondin [<i>Bos taurus</i>]	Gene showed up-regulation in atretic ovaries [<i>Solea senegalensis</i>]	Tingaud-Sequeira et al., 2009
	<i>fabp11</i>	Apolipoprotein C-1 [<i>Hemibarbus myloodon</i>]	Gene expression was up-regulated in atretic ovaries [<i>Solea senegalensis</i>]	Tingaud-Sequeira et al., 2009
	<i>amh</i>	fatty acid-binding protein (FABP) [<i>Solea senegalensis</i>]	Gene was strongly upregulated in somatic cells surrounding atretic follicles [<i>Solea senegalensis</i>]	Agullero et al., 2007
	<i>abv</i>	Anti-Mullerian hormone [<i>Oncorhynchus kisutch</i>]	Gene expression increased in atresia and caused a reduction of the healthy ovarian follicles [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
	<i>hsc2b</i>	Alveolin [<i>Oncorhynchus mykiss</i>]	Gene is expressed in oocytes that fail to develop into vitellogenic oocytes in fasted fish [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016
		hydroxy-delta-5-steroid dehydrogenase 3 beta [<i>Salvelinus alpinus</i>]	Gene expression changed before atresia became evident [<i>Oncorhynchus kisutch</i>]	Yamamoto et al., 2016

In the zebrafish *D. rerio*, a similar trend is observed for miR-22, which plays a major role in spermatogenesis and has potential involvement in maternal development (Zhang et al., 2017). In rainbow trout *O. mykiss*, the expression pattern of miRNAs is also modified according to the postovulatory stage, altering egg quality, and two miRNAs, let-7 and miR-21, have been shown to regulate the development of eggs (Ma et al., 2012). In the Nile tilapia *Oreochromis niloticus*, the embryos of females and males are characterized by different expression patterns of miRNAs (Eshel et al., 2014). In the testes in tilapia at later stages, the most abundantly expressed miRNA families are miR-33a, miR-132, miR-135b, and miR-212 (Xiao et al., 2014). However, no data regarding the involvement and regulation of miRNAs during atresia are available, offering a new area of research.

In fishes of at least six orders (Characiformes, Salmoniformes, Beloniformes, Gadiformes, Perciformes and Pleuronectiformes), recent transcriptomic studies allowed the identification of 20 core genes that play a potential role in atresia (Table 2) that are mainly associated with apoptosis or indicate aging ovaries associated with atresia (Aguilero et al., 2007; Tingaud-Sequeira et al., 2009; Yamamoto et al., 2011; Morais et al., 2012; Herpin et al., 2013; Yamamoto et al., 2016). In wild largemouth bass, *Micropterus salmoides*, 3664 genes have been identified in ovarian development, of which 15 were associated with follicular atresia (Martyniuk et al., 2013). In a culture of coho salmon *Oncorhynchus kisutch* with food restriction, 30 genes were associated with the reproductive process, and eight were closely associated with follicular atresia (Yamamoto et al., 2016). In Senegalese sole, *Solea senegalensis*, an extensive study allowed the identification of 118 ESTs, including 18 transcripts that were differentially regulated when compared to atresia with vitellogenic and mature ovaries (Tingaud-Sequeira et al., 2009).

The identification of genes in the breeding cycle of *Micropterus salmoides* has enabled the identification of several networks playing a role in oocyte maturation and genes exhibiting a strong downregulation in atresia (Martyniuk et al., 2013). During atresia, downregulated pathways include gap junction and actin cytoskeleton regulation, gonadotropin and mast cell activation, and vasopressin receptor signaling, while upregulated pathways include oxidative phosphorylation and reactive oxygen species (ROS) metabolism (Martyniuk et al., 2013).

This review identified potential genes involved in follicular atresia in teleost fish, mainly associated with the expression of apoptosis genes and lipid process genes.

2.2. Apoptosis/autophagy-related genes

Several studies have indicated that both apoptosis and autophagy could act cooperatively during follicular atresia to ensure more efficient ovarian regression after spawning (Thomé et al., 2009; Morais et al., 2012). Apoptosis also involves the sequential activation of a cascade of caspases that have been linked to atresia (Habibi and Andreu-Vieyra, 2007). In the coho salmon *Oncorhynchus kisutch*, the involvement of caspase-8 and caspase-9 has been demonstrated, especially in fasted females (Yamamoto et al., 2016). In the freshwater characiforme fishes, *Astyanax bimaculatus*, *Leporinum obtusidens*, and *Prochilodus argenteus*, Morais et al. (2012) proposed a model that explains the participation of caspase-3, the Bcl-2 protein family (bcl-2 and bax), the lysosomal cathepsin-D and beclin-1 in the regulation of follicular atresia via autophagy and apoptosis in fish ovaries, with those genes being over-expressed in the follicular and thecal cells during early and advanced regression of ovaries. Additional apoptosis related genes, mainly caspase-3, are related to Bcl-2 and Bax genes and regulate the follicular atresia and apoptosis of granulosa cells (Nassauw et al., 1999).

Other genes identified with a potential relationship to apoptosis/atresia are programmed cell death protein 4 (*pdc4*), genes of the Kruppel-like factor family (*klf*) and lipopolysaccharide-induced tumor necrosis factor (TNF)-alpha factor (*litaf*). In fasted *Oncorhynchus kisutch*, the *pdc4* gene may act as an early indicator of ovarian apoptosis/

atresia or as an early marker of impaired follicle development and/or atresia, but the function of the gene remains unknown (Yamamoto et al., 2016). Genes of the *klf* family, such as *klf6* and *klf16*, have been identified in teleost fish (Charlier et al., 2012; Yamamoto et al., 2016). The gene *klf6*, identified in *O. kisutch*, has been proposed as an early marker of apoptosis/atresia (Yamamoto et al., 2016), while the function of *klf16* remains unclear in *O. mykiss*, although it could be involved in oogenesis by playing important roles during intrafollicular competence acquisition (Charlier et al., 2012).

The lipopolysaccharide-induced tumor necrosis factor (TNF)-alpha factor (*litaf*) is a transcription factor that plays an important role in regulating the expression of TNF- α , and this gene may induce the expression of TNF- α , increasing the incidence of atresia, as indicated in *O. kisutch* (Yamamoto et al., 2011; Yamamoto et al., 2016). However, the study of both the expression and biological relevance of *litaf* in fish is still very limited (Lv et al., 2017).

The transcript associated with the prevention of apoptosis, S100-like calcium binding protein (s100a10), was shown to be upregulated in atretic ovaries of *Solea senegalensis* (Tingaud-Sequeira et al., 2009).

Follistatin (FST), a local regulator of gonadal functions, is a powerful inhibitor of follicle stimulating hormone (FSH) secretion (Chong et al., 2015), and FST is a multifunctional regulatory protein involved in numerous physiological activities such as reproduction, development, neural induction, myogenesis and immunoregulation (Armand et al., 2003; Liu et al., 2014). Chong et al. (2015) suggested that follistatin may participate in caspase3-dependent apoptosis through the Bcl2/Bax gene family in bovine granulosa cells. In teleost fish, the *fst* gene is implicated in folliculogenesis and oocyte maturation, and an early and specific expression of *fst* during ovarian differentiation may be the earliest marker of ovarian differentiation. *Fst* may be a marker of developing oocytes in the rainbow trout *Oncorhynchus mykiss* (Nicol et al., 2013). Conversely, in the medaka *Oryzias latipes*, *fst* gene expression was linked to ovary age, because its expression was higher in the ovarian epithelium and interstitium of old ovaries (Herpin et al., 2013). In contrast, in the largemouth bass *M. salmoides*, the *fst* gene is down-regulated in atretic ovaries (Martyniuk et al., 2013).

Thrombospondin (*thbs*), also known as TSP1, was the first anti-angiogenic agent, and apoptosis was shown to be the main mechanism of its anti-angiogenic activity (Mirochnik et al., 2008). In primate ovaries, TSP1 is upregulated and may be involved in the cessation of angiogenesis in follicles undergoing atresia (Thomas et al., 2008). In atretic ovaries of *Solea senegalensis*, *thbs* was upregulated, and this gene was expressed in hypertrophied and vacuolized follicular cells of atretic follicles (Tingaud-Sequeira et al., 2009).

Humans keratin sustains cell architecture by serving as a cytoskeleton, and it is also involved in the regulation of cell metabolism and signaling, thereby influencing cell proliferation, migration and apoptosis (Komine, 2018). In teleost fish, several keratin genes are expressed in the ovarian stroma and somatic follicle cells (Schaffeld et al., 2002). In *Oncorhynchus kisutch*, the keratin genes s8 (type 1 keratin s8) and *krt79* (type 2 keratin k8b (S2)) exhibit a downregulation in fasted fish ovaries that precedes measurable size changes, suggesting that these genes could be early markers of altered ovarian follicle growth (Yamamoto et al., 2016). In *O. mykiss*, higher expression of keratin 18 and keratin 8 was observed in low quality eggs (Aegerter et al., 2005). In *S. senegalensis*, keratin 8 was identified in vitellogenic ovaries, but at lower levels, while type II keratin E3-like protein was upregulated in mature ovaries (Tingaud-Sequeira et al., 2009). Other types of keratins have been identified in this species, such as keratin type I, CK19 and CK19 and keratin 5, but the relationship of these genes to atresia is unknown (Cerdà et al., 2008).

2.3. Lipid metabolic process-related genes

The alveolin gene (*alv*), a member of the astacin metalloprotease family that encodes cortical alveoli components, is specifically

expressed in the oocytes of teleost fish such as *Oryzias latipes* (Shibata et al., 2012), *Micropterus salmoides* (Martyniuk et al., 2013), the Atlantic bluefin tuna, *Thunnus thynnus* (Gardner et al., 2012) and *Oncorhynchus kisutch* (Yamamoto et al., 2016). In *O. kisutch*, *alv* is strongly expressed in previtellogenic oocytes and has decreased expression during vitellogenesis, while being maintained at elevated levels in secondary follicles that have a slow development and fail to initiate vitellogenesis in fasted fish (Yamamoto et al., 2016). This result may indicate the potential of this gene as a marker of atresia in fish with non-normal nutritional aspects, mainly in cultured fish.

The members of the superfamily of lipid-binding proteins (LBP), which are called fatty acid-binding proteins (FABPs) and play a primary role in the regulation of fatty acid uptake and intracellular transport, have been associated with atresia (Chmurzyńska, 2006). During their growth, oocytes accumulate high amounts of neutral lipids (NLs) in ooplasm lipid droplets, and these lipids are later utilized as an energy resource by developing embryos and larvae (Ryu et al., 2013). The FABP-related genes have also been identified in ovaries of a group-synchronous species, the flatfish *Solea senegalensis* (Agullero et al., 2007), in the asynchronous ovarian follicle development of *Thunnus thynnus* (Gardner et al., 2012) and in *Morone salmoides* (Britton et al., 2010; Martyniuk et al., 2013). Different functions have been suggested for FABPs in these species. In *S. senegalensis*, *fabp11* mRNA was found in previtellogenic oocytes and was strongly upregulated in somatic cells surrounding atretic follicles (Agullero et al., 2007), while in *T. thynnus*, *fabp11* seems to be involved in membrane trafficking and the sequestration of lipids in oocytes for normal embryo development (Gardner et al., 2012). Additionally, in *T. thynnus*, nine different *fabps* transcripts have been identified in ovaries, especially the TTC01498 transcript, which shows a high similarity to FABP11 and could play a role in fatty acid trafficking specifically related to oocyte atresia (Gardner et al., 2012). Until now, *fabp11* has been identified only in teleost fish in several organs, including the liver and gonads (Crovetto and Córdoba, 2016). Several FABPs (named FABP1 to FABP-12) have been described in mammals as being potentially involved in pathways regulating cancer development (Amiri et al., 2018). The FABPs proteins could be used as protein markers for the prognosis and diagnosis of cancers. FABP5 has been localized in a specific population of ovarian macrophages in advanced atretic follicles of adult mice (Nourani et al., 2005). Additionally, FABP4, a mediator of lipid trafficking in adipocytes, has a key role in the interaction of cancer cells with adipocytes, which provides fatty acids to the cancer cells and fuels rapid tumor growth (Niemann et al., 2011). The relation of FABPs to the metabolism/pathology of ovaries or follicular atresia in mammals and teleost fish, respectively, indicates that FABPs are potential molecular markers to study for atresia.

In atretic ovaries of *Solea senegalensis*, the gene encoding apolipoprotein A-I (*apoc*) was upregulated. Apolipoproteins have been identified in *O. mykiss*, *G. morhua*, striped bass *Morone saxatilis*, *M. salmoides* and *T. thynnus* and show differential expression patterns throughout oocyte development (Cerdà et al., 2008; Gardner et al., 2012; Martyniuk et al., 2013; Breton and Berlinsky, 2014). The zebrafish *D. rerio* has been proposed as a model for understanding apolipoprotein biology and its relationship to lipid metabolism, especially in larval models. The overexpression of apoA-IV has been associated with a decrease of high-fat food intake in larval zebrafish, indicating that differential expression of apolipoprotein genes could serve as an indicator of health or disease in larvae (Otis et al., 2015). Apolipoproteins can play a role in the transport of cholesterol and act as a precursor for all steroid hormones related to metabolism and vitellogenesis uptake (Babin, 1987; Tingaud-Siqueira et al., 2009), as occurs in *Salmo gairdneri*, where apolipoproteins and the resorption of yolk during follicular atresia were correlated with the presence of egg yolk proteins combined with HDL (Babin, 1987).

The anti-Müllerian hormone (*amh*) gene, a member of the Tgf-beta superfamily, plays an important role in signaling pathways related to

cell growth and differentiation, apoptosis, and cellular homeostasis. *Amh* has been identified in fish ovaries (Yamamoto et al., 2016), and similar to what occurs in mammalian females, the serum levels of *amh* could be considered a good marker of ovarian follicle reserve and fertility potential, since *amh* plays an important role in folliculogenesis (Rodríguez-Mari et al., 2005). In fasted *Oncorhynchus kisutch*, a decrease of *amh* expression may indicate increases in atresia and reduction of the healthy ovarian follicle pool (Yamamoto et al., 2016), while in healthy *O. mykiss*, this gene has been identified only in the testis (Baron et al., 2005). Meanwhile, *amh* in *Gadus morhua* showed various patterns of expression according to the oocyte developmental stage (Breton and Berlinsky, 2014).

In *O. kisutch*, prolonged nutritional stress may disrupt the reproductive system and induce follicular atresia in part via reductions in the downstream effects of steroidogenesis-related genes (*hsd3b*) and E2 production (Yamamoto et al., 2011). The *hsd3b* (hydroxy-delta-5-steroid dehydrogenase 3 beta) gene encodes steroidogenic enzymes involved in estradiol 17 β (E2) and showed clear differences in expression between fed and fasted fish before morphological signs of ovarian atresia were apparent (Yamamoto et al., 2011; Yamamoto et al., 2016).

3. Conclusion

This review enables us to identify potential genes involved in atresia, mainly associated with apoptosis and lipid processing. The identification of genes and pathways involved in atresia could allow the identification of potential genetic biomarkers that could be used both in aquaculture and in breeding studies in wild populations, especially in those subject to high fishing exploitation. The advancement of new sequencing technologies may also be a powerful tool for the discovery of genes and regulatory networks due to the considerable diversities of reproductive strategies shown by teleost fish.

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4.2 CHAPTER 2

Atresia in the Chilean jack mackerel *Trachurus murphyi* (Teleostei, Carangidae): A first assessment for the South-eastern Pacific Ocean

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Abstract

The Chilean jack mackerel *Trachurus murphyi* support an important fishery in the South-eastern Pacific Ocean and has declined considerably over time. *T. murphyi* have asynchronous ovarian development with an oceanic spawning (September to January). Despite the economic importance of this resource critical issue such as characteristics and dynamics of atresia, which affect the reproductive output, are unknown. Females of *T. murphyi* caught in different spawning years were analysed to describe patterns of prevalence and intensity of atresia. General linear models (GLM) were performed by using prevalence and intensity atresia, as response variables and gonadosomatic index, condition factor, sea

surface temperature, postovulatory follicles and month/year as explanatory variables. The batch fecundity and relative fecundity were compared between females showing atresia, postovulatory follicles and non-atretic females. The stages of atresia identified were alpha, beta and gamma. Primary growth and cortical alveolar oocytes atretic were also recorded. The GLM showed that variations in the prevalence and intensity of atresia were explained for all variables, except condition factor for prevalence, while month/year, sea surface temperature, condition factor and incidence of postovulatory follicles were significant variables explaining intensity of atresia. According to our results, the dynamic of alpha and beta-atresia could be considered a potential proxy for any disruption of spawning season. The batch fecundity and relative fecundity were lowest compared with other *Trachurus* spp. This is the first assessment comparing fecundity between atretic and not atretic females for this species.

Key words: Atresia, condition factor, fecundity, spawning season, *Trachurus*

Introduction

In the South-eastern Pacific Ocean (SEP), industrial fisheries for the highly migratory Chilean jack mackerel *Trachurus murphyi* Nichols, 1920, have decreased considerably from 4.4×10^6 ton during the mid-1990s to 355×10^3 ton in 2017 for Chile (SERNAPESCA 2017). The main fishery includes the coast off Peru and Chile, extending from the central coast of Chile to the South-west Pacific Ocean, but a recent colonization process reaching the coast around New Zealand and Tasmania has been described (Serra 1991, Cárdenas *et al.* 2009, Vásquez *et al.* 2013).

In the SEP, Chilean jack mackerel conform a single large population (Poulin *et al.* 2004, Cárdenas *et al.* 2009) that can be found in three different habitats (Arcos *et al.*, 2001)

according to its life history: a) nursery ground area, b) coastal feeding area and c) a large, main oceanic spawning area off central Chile (Bertrand *et al.* 2004, Cubillos *et al.* 2008, Vásquez *et al.* 2013). Interestingly, otolith chemistry indicates that all adult Chilean jack mackerel come from the same oceanic spawning area (Ashford *et al.* 2011). Recently, Parada *et al.* (2017) indicated the presence of a potential new spawning and nursery ground area associated with the seamount region in the Challenger break and the East Pacific ridge (32°S-39°S and 105°W-126°W).

Chilean jack mackerel can spawn throughout their whole distribution (Taylor 2002, Gerlotto *et al.* 2012), and the time extension of the spawning period varied between area and years (Perea *et al.* 2013). For the main oceanic spawning area from central coast of Chile (32°S-38°S, 80°W-92°W) the spawning period extends from September to January when mature fish begin the annual migration from the feeding area to the oceanic spawning area; that extends more than 1800 km off the coast (Cubillos *et al.* 2008, Leal *et al.* 2013).

Like other *Trachurus* species, *T. murphyi* present asynchronous ovarian development (Leal *et al.* 2013); and an indeterminate fecundity considering the size distribution of oocytes, and a long spawning period as well as incidence total of atresia (George 1995). *T. murphyi* is a batch spawner and fecundity estimates should be made using the most advances development oocytes (hydrated oocytes) instead the vitellogenic oocytes (Saborido-Rey & Kjesbu 2005). Atresia is a normal process of oocyte renewal, which increases at the cessation of spawning when all remaining vitellogenic oocytes are resorbed (Saborido-Rey & Kjesbu 2005). As suggested by Ganas *et al.* (2008) the main reason for assessing/quantifying atresia is assign females to their correct reproductive class; especially in fishes with determinate fecundity, because the realised annual fecundity must be corrected for atretic losses, but not for fishes with indeterminate fecundity which showed a generalized prevalence of atresia and resorption of mature oocytes at the end of the

spawning season (West 1990). For *T. murphyi*, is not easy to find females harbouring hydrated oocytes during the spawning season (George 1995, Leal *et al.* 2013), consequently vitellogenic oocytes could be counted, and atresia must be quantified (Saborido-Rey & Kjesbu 2005). Therefore, the main goals were (1) to describe the phases of atresia in *T. murphyi* caught during the post-migration spawning period in order to evaluate the spawning history, (2) to determine the prevalence and intensity of atresia in different spawning periods and (3) to determine the impact of atresia in the fecundity.

Materials and methods

Mature females (n= 3650) of Chilean jack mackerel were sampled from seven cruises using industrial fishing vessels, during the main oceanic spawning area off central Chile (30°S-39°S; 71°W-88°W). Such trawls are designed to evaluate the spawning biomass based on the Daily Egg Production Methods (Hunter & Lo 1997). Our samples included only specific months within the annual spawning cycle during different years, focusing in the period of higher spawning intensity (Table 1). Females were selected at random from the catches and dissected on board, following the methodology described by Sepúlveda *et al.* (2009). Fork length (FL, cm) and body weight (BW, g) were measured, the significance of potential differences in these variables between spawning periods was analysed using Kruskal–Wallis test. Differences between immature and mature females for FL and BW were analysed using Mann-Whitney test. The sea surface temperature (SST) data was provided by Instituto de Investigación Pesquera (INPESCA, Chile

Gonadosomatic index (GSI) and condition factor (*K*) were recorded for each specimen. GSI was calculated as $GSI = (GW/BW) \times 100$; where GW is gonad weight (g). *K* was estimated as $K = (BW/FL^3) \times 100$. The mean values of GSI and *K*, obtained for the different maturity stages during each sampled period, were compared using the Kruskal-Wallis tests. Potential

relationships between GSI and K for the total samples were analysed with Spearman's correlation coefficient.

For histological analysis, the ovaries were fixed in 10% neutral buffered formaldehyde; a cross-section of tissue was sampled from the middle parts of the ovary. Each sample was embedded in paraffin, sectioned transversely with a thickness of 5 μm and stained with haematoxylin and eosin. The most advanced batch of oocytes and the reproductive phases of ovaries were based on the developmental stage of the oocytes and the presence of postovulatory follicles (POF) and atresia. We followed the methodology suggested by Brown-Peterson *et al.* (2011) and applied for Leal *et al.* (2013) to describe and characterize the reproductive maturity phases for this species. The prevalence of atresia (PA) was defined as the number of fish containing ovaries with at least one phase of atretic oocytes relative to the number of all individuals examined, expressed as a percentage (Ganias *et al.* 2008). The presence/absence of atresia was modelled using a generalized linear model (GLM) with a binomial error distribution for the response variable and a logit link function (Ganias *et al.* 2008). Prior to selection of variables to be included in the GLM for presence/absence of atresia, Spearman's correlations were performed to detect collinearity between the quantitative variables (FL, K , GSI and SST). The explored explanatory variables were FL, K , GSI, POF, SST and month/year (MY). Three models were performed. The first model included as response variable all atresia phases identified (alpha, beta, gamma and cortical alveolar atresia) and their combinations, the second model included only A-atresia, and the third model included only B-atresia. The selection of the best models was checked with Akaike's Information Criterion (AIC) and Deviance (D^2). Additionally, the Receiver Operating Characteristic (ROC) curve was used to evaluate the sensitivity and specificity of the models.

For females with alpha-atresia, the intensity of atresia (IA) was calculated as the number of alpha-atretic oocytes divided by the total number of vitellogenic oocytes (alpha-atresia + non-atretic follicles) following Nunes *et al.* (2011). Simple counting from histological sections was used as a proxy of intensity of atresia (Ganias *et al.* 2008, Nunes *et al.* 2011). The IA was evaluated using a generalized linear model (GLM) with a normal distribution for the response variable and a log link function. The explanatory variables included in the model were FL, K , SST and MY.

Batch fecundity (BF) was estimated via gravimetric methods on hydrated females for three female stages in relation to non-atretic oocytes ($n= 71$), atretic oocytes ($n= 41$) and presence of POF ($n= 66$). These females were caught during the spawning period S6 (November and December to 2014). Relative batch fecundity (RF), defined as the number of hydrated oocytes per gram of body weight, was determined by dividing BF by body weight. Significance differences in BF and RF amongst three female stages were evaluated using Kruskal-Wallis tests and the Dunn “*a posteriori* test”. In this case, effects on the explicatory variables FL, K and female stages for BF and RF were tested with a generalized linear model (GLM) with a Poisson distribution for the response variable and a log link function. GLM analysis and ROC curve were performed in R Software (R Core Team 2006)

Results

Reproduction and condition

Fork length (FL) for the whole sample ranged from 23 cm to 64 cm (mean 34.1 cm \pm 5.2), while the body weight (BW) ranged from 115 g to 3090 g (mean 481 \pm 234). Significant differences were found for FL ($H= 946.63$, $P < 0.001$) and BW ($H= 1156.76$, $P < 0.001$) between month/year, with the females caught in January 2013 being the smallest ones

(Table 1). Also, immature females (n= 43) showed differences from matures females (n= 3607) for FL ($U= 15288.5$, $P < 0.0001$) and BW ($U= 18830$, $P < 0.0001$).

Immature females and developing females were observed at low frequency during several seasons spawning (Fig. 1). Conversely, potentially spawning capable females (range: 2%-81%) and actively spawning females (range: 8-18%) were recorded in all samples except January 2013, increasing the proportion of regressing (61%) and regenerating (17%) females (Fig. 1). The absence of females in the actively spawning reproductive phase and the high frequency of advanced maturity phases (regressing and regenerating) indicated that the long spawning period ended by January.

The GSI showed significant differences between the reproductive maturity phases ($H= 1414.91$, $P < 0.05$); high values ($GSI > 5$) were observed in females showing the actively spawning phase. Immature females (n= 43) showed the lowest GSI values ($GSI < 1.0$) when compared with regressing and regenerating females (Fig. 2).

K ranged from 0.43 to 1.92 (mean 1.12 ± 0.13), but three females showed $K > 2.0$ (data not included) in all spawning periods analysed (Fig. 2). Additionally, K varied between the reproductive phases ($H= 47.97$, $P < 0.05$), although K for immature females did not show differences from mature females ($U= 72788.5$, $P = 0.48$). Considering the whole sample, GSI was negative (but marginally) correlated with K ($\rho = -0.053$, $P < 0.001$).

Atresia morphology

Three main sequential stages of atresia were observed: Alpha (A), Beta (B) and Gamma (G) (Fig. 3). Several ovaries were characterized by the presence of primary growth (PG) and Cortical Alveolar (CA) atretic oocytes. For A-atresia three morphological stages were identified: initial (A1), intermediate (A2) and advanced (A3) (Fig. 3b, c, d). During A-atresia, follicular diameter diminished gradually. The first signal for A1 atresia was an internal, non-smooth zona radiata, which subsequently showed a small fragmentation

(Figure 3b). Some oocytes may show thickening of zona radiata before their fragmentation. Additionally, the disintegration of the nucleus is evident from dark basophilic staining. At this stage, the oocyte retains its shape and sometimes looks like a healthy oocyte. In A2-atresia, the disintegration and fragmentation of the zona radiata were evident and broke up into irregularly shaped segments; also, unstained vacuoles were apparent inside the ooplasm (Fig. 3c). For the A3-atresia phase, the follicle had the appearance of beta-atresia, but few yolk globule follicles were evident when compared with the other stages (Figure 3d). A decreased prevalence was evident for the three A-atresia sub stages from the initial stage to the advanced one, 67% in A1-atresia (initial), 52% in A2-atresia (intermediate) and 24% in A3-atresia (advanced). These sub stages of A-atresia can also co-occur, where the combination of sub stages A1-atresia and A2-atresia was the most prevalent (PA= 38%). B-atresia was characterized by the presence of many empty vacuoles and some blood vessels, and in the initial development, its morphology was different at POF (Fig. 3e). The G-atresia was characterized by yellow-brown granular pigments inside the granulose cells (Fig. 3f).

Prevalence of atresia

Overall and during all months/year were identified atretic oocytes, being Alpha (A)-atresia the most frequently stage observed (Figure 4). For A-atresia (n= 1485), the 39% co-occur with a combinations of Beta (B)-atresia, Gamma (G)-atresia and Cortical Alveolar (CA)-atresia, while with B-atresia (n= 107) the 74% of ovaries co-occurs with G-atresia (Figure 4). Females in regenerating reproductive phases not showed atresia. Alpha-atresia was the most prevalent stage in the remaining reproductive phases, including females with hydrated oocytes that showed A-atresia and B-atresia (Fig. 2). Also, CA-atretic oocytes were observed at low prevalence (<3%) in females within the early developing phase and regressing reproductive phases. Females in the regressing reproductive phase showed

prevalence of atresia >80% due to higher co-occurrence of A-atresia and B-atresia (Fig. 2). Also, 27% of mature females recorded post ovulatory follicles (POFs) in all reproductive phases, except the regenerating phase. Thirty-two per cent of females exhibited POFs in co-occurrence with atresia, mainly A-atresia.

The GLM showed that MY, SST and incidence of POF were the variables showing higher effect on PA in the three evaluated models. As FL was significantly correlated with GSI (Spearman's correlations ρ , $P < 0.001$) was excluded from the analysis. GSI were not significant for explain the presence of atresia in model 2 (Alpha-atresia) while K in all evaluated models (Table 2). In the three models, the proportion of explained variance (deviance) was low but significant. Additionally, the prediction of the models was good (ROC=0.8) (Table 2).

Intensity of atresia

Intensity of atresia (IA) was high in all spawning periods (range: 45-77%); January 2013 showed the highest value of IA (92%), confirming that this month corresponded to the end of the spawning period (Figure 4). GLM showed that MY, SST, POF and K were variables that explained the IA, with MY being the most important variable (Table 2).

Fecundity

Fork length was significantly correlated with BF and RF (Spearman's correlations ρ ; $P < 0.001$ for both). K was only correlated with BF (Spearman's correlations $\rho= 0.014$, $P = 0.047$) but not with RF (Spearman's correlations $\rho= 0.06$, $P = 0.37$). POF females showed the lowest batch fecundity. Both fecundities varied between stages of females: non-atretic females and females with atretic oocytes and POF (Fig. 5). Females with POF showed significance difference with non-atresia females for batch fecundity (BF) ($H= 9.61$, $P = 0.008$, Dunn, $P < 0.05$) and relative fecundity (RF) ($H= 7.48$, $P= 0.02$, Dunn, $P < 0.05$).

GLM showed that BF and RF were significantly explained for FL, *K* and stage of females (non-atretic, atretic and POF), being FL the most important explanatory factor (Table 3).

Discussion

T. murphyi showed three main sequential atretic stages (Alpha, Beta and Gamma), like others *Trachurus* species (Macewicz & Hunter 1993, Karlou-Riga & Economidis 1997 Gordo *et al.* 2008); being prevalence and intensity of atresia influenced by months/years of spawning seasons. This fish has an extensive spawning season (5 months), can spawn an estimate of 5-14 batches for each reproductive period (Ruiz *et al.* 2008, Perea *et al.* 2013), and this condition can explain the co-occurrence of atresia in the different months of the spawning season analysed. Prevalence of Beta-atresia increased strongly at the end of spawning season (January); therefore, the increased proportion of this atresia could be a potential “proxy” to determine the length of spawning seasons, and it would allow us to detect any change in the current or future reproductive period. During December 2013, the prevalence of atresia (>80%) was higher than observed for same month in other years, suggests that spawning season began early this year or was shorter. The turnover rate for the atretic oocytes in Chilean jack mackerel is unknown, but atretic follicles in fishes are thought to be short lived (Hunter & Macewicz 1985a, Kjesbu *et al.* 1991, Kurita *et al.* 2003). Turnover rates at environmental temperatures for alpha-atretic oocytes have been estimated as 7-9 days for *E. mordax* Girard, 1854 at 15-16 °C but with differences between stages of atresia (Hunter & Macewicz 1985b); 4 to 7 days in Atlantic herring *Clupea harengus* Linnæus, 1758 for temperatures from 4.2 to 11 °C depending on the month of catch (Kurita *et al.* 2003); 10 days in cod *Gadus morhua* Linnæus, 1758 at 8 °C with a spawning period of 50 days (Kjesbu *et al.* 1991); and 9 days in sole *Solea solea* (Linnæus. 1758) (Witthames & Walker 1995). Understanding trends and dynamics of atretic oocytes in *T. murphyi* allows

us to identify any change in the spawning period, such as a potential skipped spawning (Rideout & Tomkiewicz 2011) or previous spawning events as has been assessed for Mediterranean sardine *Sardina pilchardus* (Ganias *et al.* 2008).

T. murphyi have a reproductive strategy to disperse over their spawning oceanic area associated to SST warmer than 15-16 °C (Cubillos *et al.* 2008). According to Vasquez *et al.* (2013), the reproductive activity of *T. murphyi* is highly correlated with the Subtropical Convergence or Subtropical Front (STF), which separates relatively warm and salty tropical waters from colder and fresher Subantarctic waters (Vasquez *et al.* 2013, Parada *et al.* 2017). The results indicate that SST is an important variable explaining the presence of atresia in *T. murphyi*, and eventually SST higher or lower than the optimal temperature for spawning, could generate a mismatch between reproduction and optimal environmental conditions for progeny development (Miranda *et al.* 2013). As indicated by Hunter & Macewicz (1985a) and Witthames & Walker (1995), spawning events and atresia are temperature-dependent processes, and females can spawn or delay during migration if the temperature regime is not suitable (Ma *et al.* 1998, Haslob *et al.* 2013). For yellowtail kingfish *Seriola lalandi* Valenciennes, 1833 and *Sardina pilchardus* (Walbaum, 1793), a temperature exceeding the optimal range can cause an aborted spawning event or high prevalence of atresia, respectively (Poortenaar *et al.* 2001, Ganias 2009, Stuart & Drawbridge 2013,).

In this study, *K* significantly affect the intensity but not the prevalence of atresia, suggesting that *K* is not a good index for body condition or that the sample size was not enough to determine changes in energy allocation associated with reproductive event. During the austral spring, *T. murphyi* migrate nearly 1,800 km between the coastal feeding area and the extended offshore spawning area (Serra 1991, Arcos *et al.* 2001). Cubillos & Alarcón (2010) suggested that in the coastal and highly productive SEP, *T. murphyi* accumulates energy reserves (autumn-winter) prior to the reproductive process (spring-summer), indicating that

this species employs a strategy of energy accumulation that is drive to reproduction rather than somatic growth. The trends of K could be evidence that *T. murphyi* continues to feed during migration, indicating that this species is income breeding, i.e., the energy for the reproductive period comes from feeding (Bucholtz *et al.* 2013).

Atresia might be considered an important mechanism for the adjustment of fecundity through the resorption of oocytes in unfavourable seasons (Abaunza *et al.* 2003). The batch fecundity (BF) is an important biological variable for indeterminate spawners and is used in the daily egg production method (DEPM) (Hunter & Lo 1997, Zwolinski *et al.* 2001). In this first assessment, the FL, K , presence or absence of atresia and POF affected BF. Fecundity of non-atretic females differed significantly from POF females, coinciding with Macewicz & Hunter (1993), who indicated that for *T. symmetricus*, the batch fecundity was lower for recently spawned females than females without evidence of a previous spawning event (POF).

In species with indeterminate fecundity such as *T. murphyi*, the recruitment of oocytes can continue for a long period into the spawning period if adequate food is available (Murua & Saborido-Rey 2003). Regarding the relative fecundity (RF), the results showed that *T. murphyi* recorded the lowest values (69 oocytes/g in non-atretic females) when compared with other *Trachurus* spp, 172-250 oocytes/g in *T. trachurus* (Karlou-Riga & Economidis 1997, Abaunza *et al.* 2003), 112 oocytes/g *T. symmetricus* (Macewicz & Hunter 1993). The fecundity is known to vary due to nutritional conditions of the spawner (Kurita *et al.* 2003), but during the spawning season analysed (November-December 2014), K did not show a significant relationship with RF, suggesting that K is not a good proxy for RF as indicated for *T. trachurus* due the fecundity does not decline at the end of the spawning season (Van Damme *et al.* 2014); thus, it is important to correlate the trends of atresia with fecundity during the whole spawning season. In *T. murphyi*, the prevalence and intensity of atresia

may vary between spawning periods; it is therefore necessary to examine the seasonal dynamics of atresia through the whole maturation cycle and the relationships with the body condition, environmental variables (*i.e.*, temperature) and fecundity during the whole spawning season.

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Table 1. Cruises performed for the evaluation of spawning biomass (DEPM) in the Chilean jack mackerel, *Trachurus murphyi* from oceanic spawning area off central Chile between 2004 and 2014.

	Cruise					
	2004	2005	2006	2012-2013	2013	2014
S	S1	S2	S3	S4	S5	S6
Months by S	Nov	Nov	Nov	Dec/Jan	Dec	Nov/Dec
Lat (°S)	32°20'-38°00'	34°20'-38°40'	33°14'-38°50'	32°12'-36°30' 34°57'-35°18'	29°53'-35°40'	32°33'-36°30' 32°41'-38°00'
Long (°W)	75°41'-91°51'	76°50'-87°54'	71°01'-91°46'	71°59'-88°40' 73°47'-74°08'	71°50'-76°50'	74°25'-85°48' 72°12'-86°57'
SST (°C)	15.0-19.0	14.5-17.5	13-17	15-17/13-13.5	13-14.4	15.5-16.4/13.9-15.1
FL (cm)	32(23-58)	32(25-49)	36(27-55)	34(23-48)/30(25-36)	36(27-64)	37(29-47)/37(24-49)
BW (g)	115-1850	200-1110	240-1800	165-1270/180-525	250-3090	330-1200/125-1300
n	988	596	727	287/139	431	162/320

S= Spawning season, Lat= latitude, Log= longitude, SST= sea surface temperature, FL= Fork length, BW= Body weight, n= number of females

Table 2. Chi- Square values of the binomial GLM model analysing the effects of sea surface temperature (SST), gonadosomatic index (GSI), condition factor (K), presence/absence of postovulatory follicles (POF) and month/year (MY) on the prevalence (presence-absence data) of atresia and normal GLM for intensity of atresia in Chilean jack mackerel *Trachurus murphyi*. Model 1= all atresia stages and their combinations, model 2= only alpha atresia and model 3= only beta atresia. AIC= Akaike's Information Criterion, D²= deviance of model, AUC= area under ROC curve, n= number total females

EV	Prevalence atresia			Intensity atresia
	Model 1	Model 2	Model 3	
SST	194.67**	43.77**	63.03**	34.25**
GSI	63.29**	0.02 ^{n.s}	25.87**	0.27 ^{n.s}
K	0.31 ^{n.s}	2.58 ^{n.s}	0.37 ^{n.s}	6.79**
POF	3.50*	4.96*	8.54*	10.50**
MY	470.21**	391.94**	81.07**	422.17**
Criteria				
AIC	3509.1	2506.4	423.06	254285
D ²	17.35	15.15	30.95	
AUC	0.75	0.73	0.88	
n	3606	2881	2076	2881

n.s: non-significant; * 0.05>P>0.01, **P< 0.001

Table 3. Chi- Square values of the poisson GLM model analysing the effects of fork length (FL), condition factor (K), and stage of females (non-atresia, atresia and postovulatory follicles, POF) on the batch fecundity and relative fecundity for Chilean jack mackerel *Trachurus murphyi*.

Explanatory variable	Fecundity	
	Batch	Relative
FL	110166**	286.99**
K	116188**	22.91**
stage of females	35479**	47.3**

**P< 0.001.

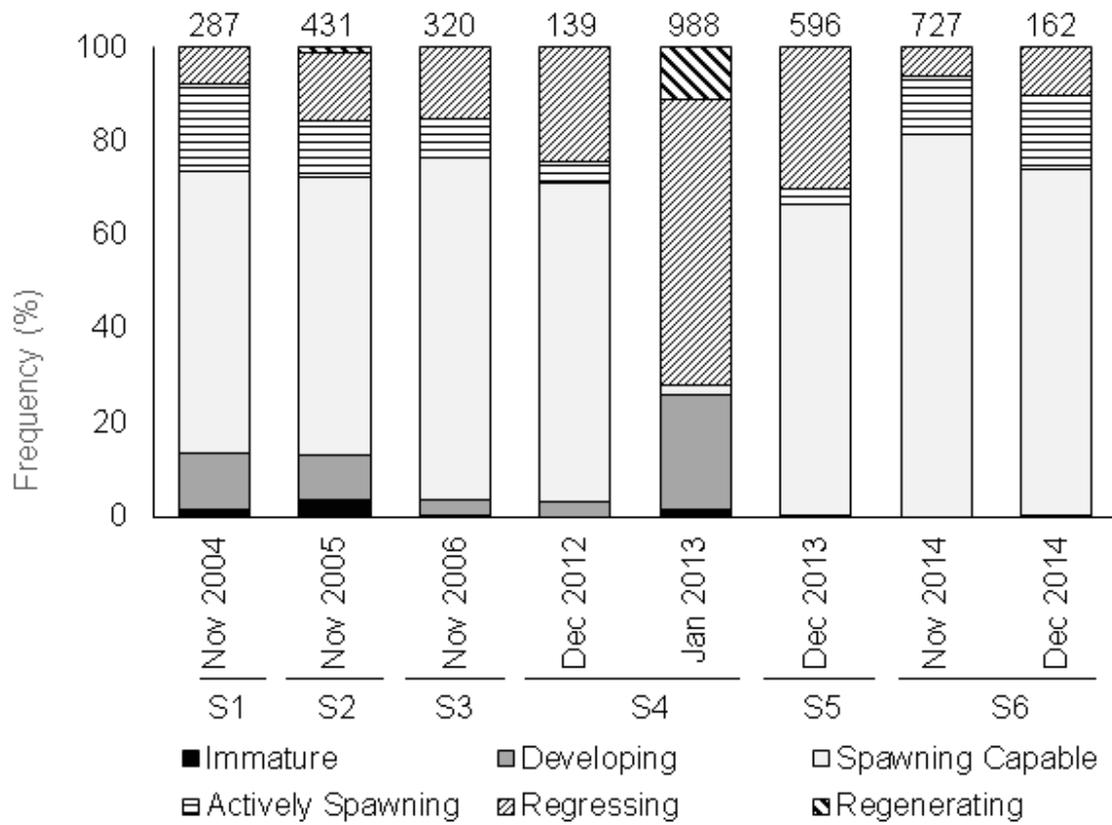


Figure 1. Frequency distribution (%) of reproductive phase of female *Trachurus murphyi* for months and spawning seasons (S). Numbers of specimens examined per month

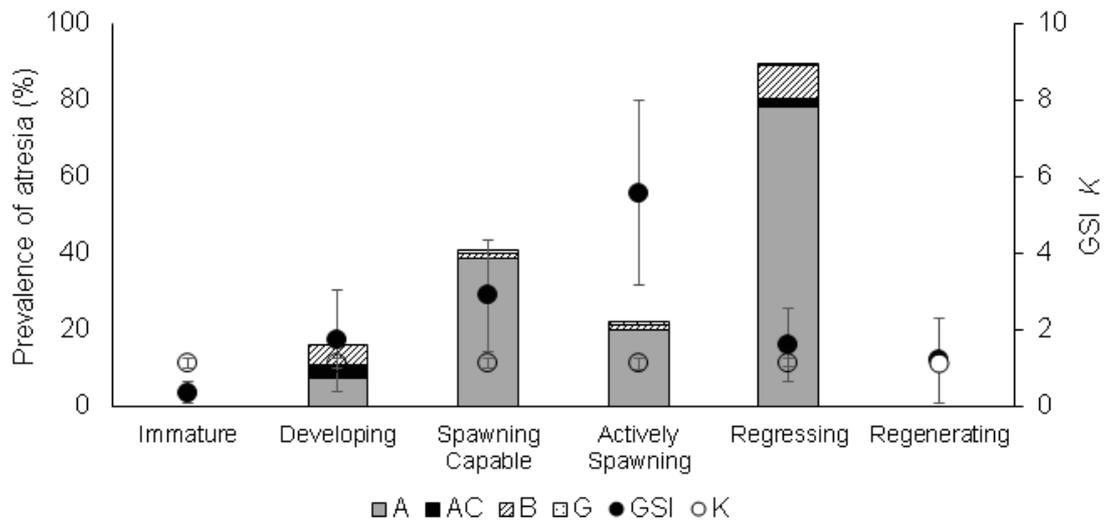


Figure 2. Gonadosomatic Index (GSI), condition factor (K) and prevalence of atresia (%) for reproductive phase. A= Alpha-atresia, AC= Cortical atresia, B= Beta-atresia, G= Gamma-atresia

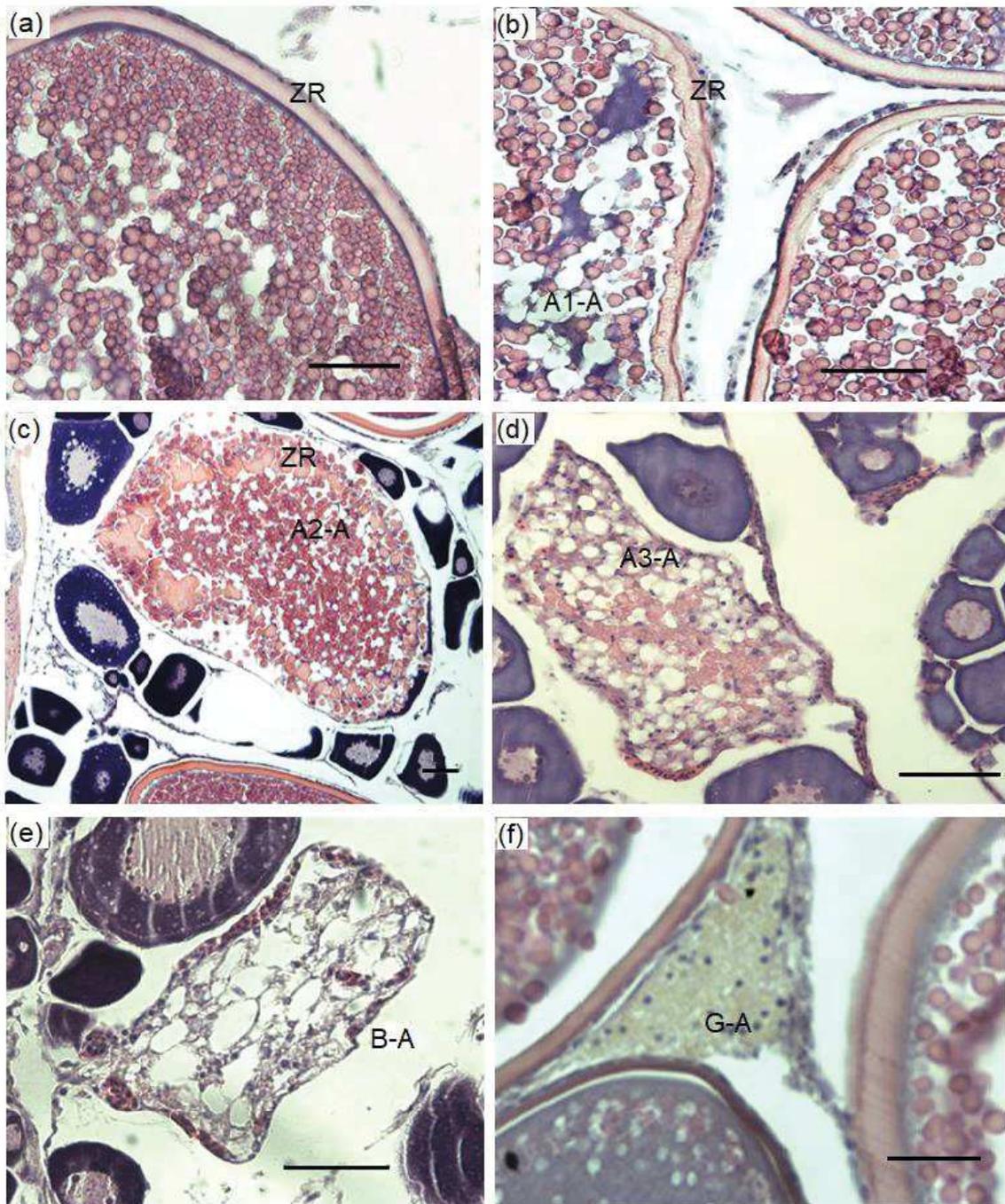


Figure 3. Atretic follicles in *Trachurus murphyi* ovaries. Histological sections were stained with haematoxylin and eosin. (a) = non atretic oocyte, (b) A1-A = initial alpha atresia, (c) A2-A = intermediate atresia oocytes, (d) A3-A = advance alpha stage atresia, (e) B-A = beta atresia oocyte, and (f) G-A = gamma atresia. ZR= Zona radiata. Scale bar = 50 μ m

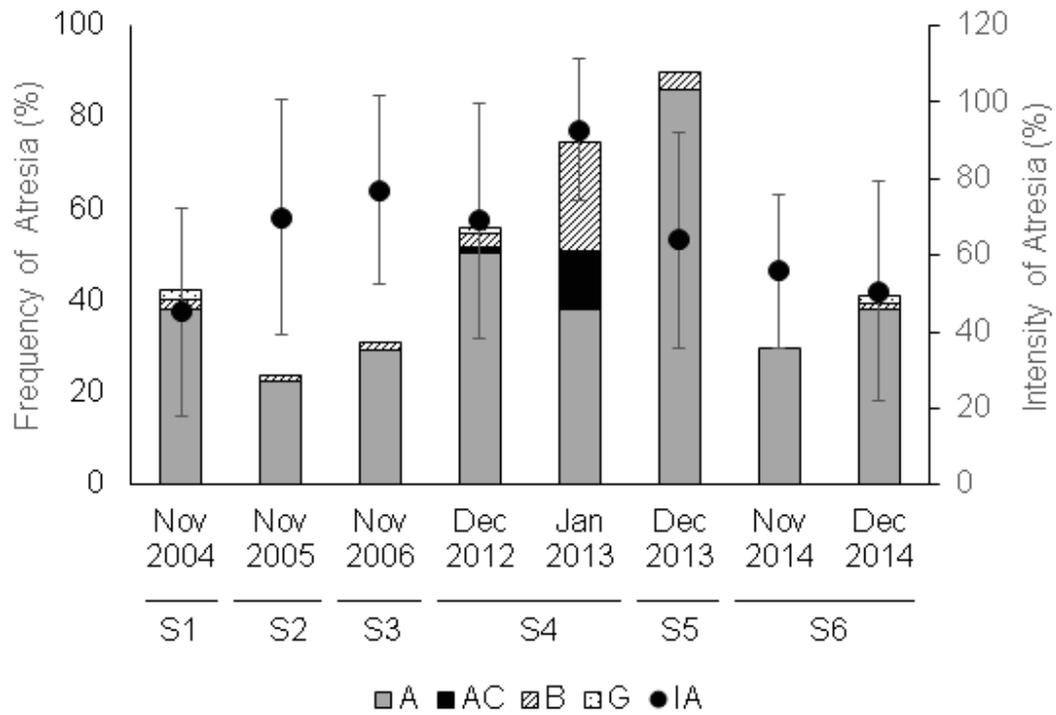


Figure 4. Frequency of atresia (%) and Intensity of atresia (IA) for months and spawning seasons (S). A= Alpha-atresia, AC= Cortical atresia, B= Beta-atresia, G= Gamma-atresia

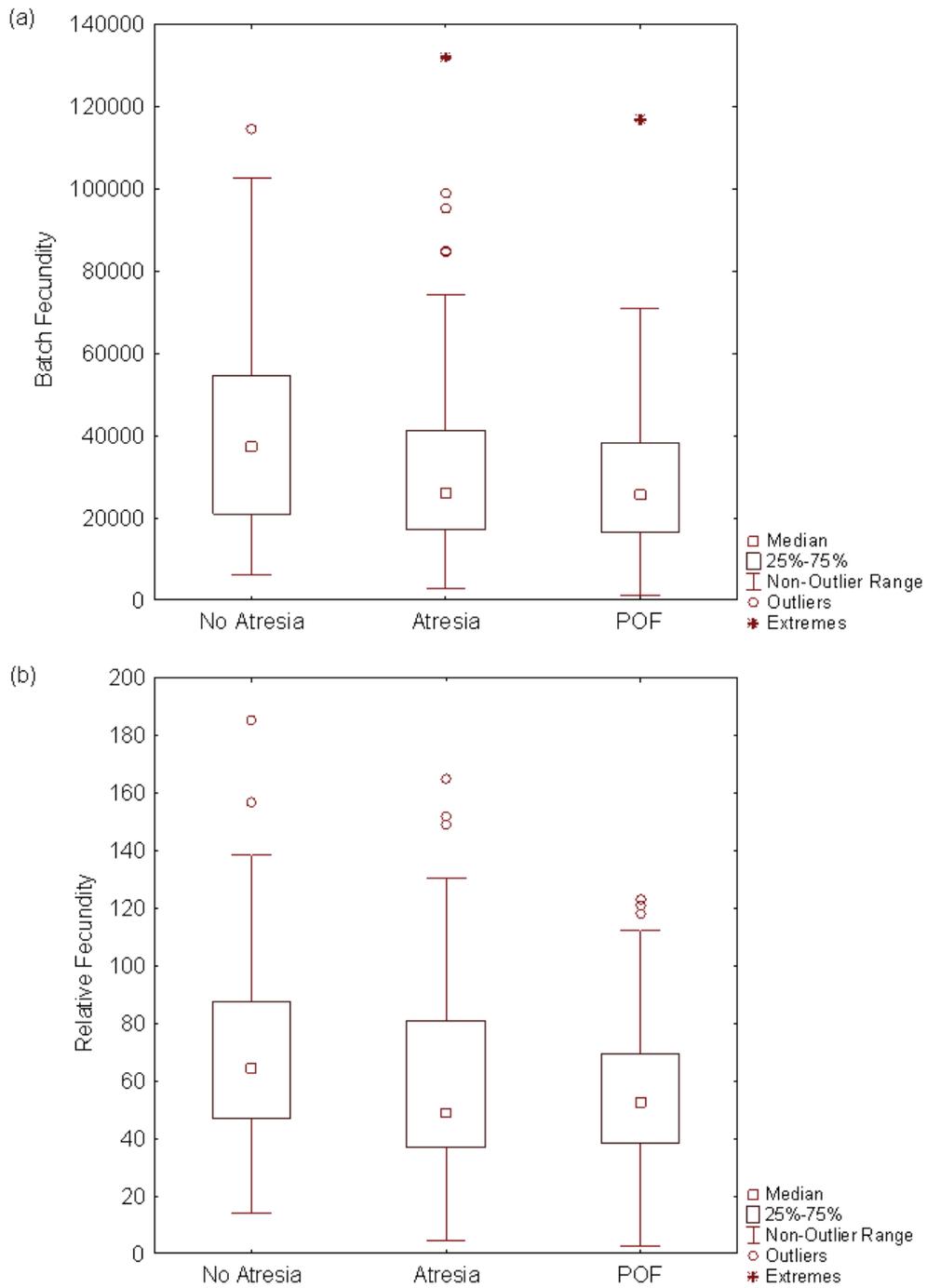


Figure 5.- Relationships of (a) Batch Fecundity and (b) Relative Fecundity in relations of females stages of *Trachurus murphyi* (Non-atresia, atresia and postovulatory follicle (POF))

4.3 CHAPTER 3

First transcriptomic approach to the study of atresia in *Trachurus murphyi* using RNA-Seq analysis: genes associated with the follicular atresia.

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Abstract

Atresia can be considered as a disruption of the reproductive process when observed at high intensities during the spawning season as well as at other stages of the reproductive cycle in organisms. To study atresia, we performed an exploratory transcriptomic study in the Chilean jack mackerel *Trachurus murphyi* to acquire new molecular knowledge about this physiological process associated to the reproductive process. In order to identify transcriptional changes in females exhibiting different atresia levels, the transcriptome of a mix of gonads and livers was analysed using Illumina RNA-seq and de novo assembly. The database was generated for two groups of females; adult females with high atresia intensity and juvenile females with minor or absent atresia. A total of 280 million of reads were obtained, which were assembled a total of 290 473 high quality 47055 transcripts including 15 088 transcripts with an e-value < e-5 and 18704 transcripts with at least one match with deposited sequences. The results show the presence of potential genes associated with the atresia process identified for other species of teleost, such as follistatin (*fst*), thrombospondin (*thbs*) and fatty acid-binding protein (*FABP*) genes. Several apoptosis and caspase genes were identified in the transcriptome. The results of the transcriptomic approach are discussed in terms of potential genes associated with the atresia process.

Introduction

Modern genomics approach has changed our approach to solve and understand fundamental aspects of fisheries resources (Ovenden et al., 2015, Valenzuela-Quiñonez 2016, Souza et al., 2018). Transcriptomics and Next generation RNA-sequencing (RNA-seq) techniques allows us to describe many biological processes at a molecular level (Deng et al., 2018), such as development, adaptive evolution, host immune response, stress (Qian et al., 2014), reproduction (Martyniuk et al., 2013, Gardner et al., 2013), and responses of species to environmental challenges (Wilson et al., 2018). In the studies of focused on fish reproduction, some specific gene networks regulation may be associated with the physiological and morphological changes occurring in the ovary (Martyniuk et al., 2013). The molecular sequence of oocyte maturation, identified through the regulation of genes expression patterns that underlies vitellogenesis, ovulation and atresia has been studied in fisheries species such as Atlantic bluefin tuna, *Thunnus thynnus* and Wild Largemouth Bass *Micropterus salmoides* (Gardner et al., 2012, Martyniuk et al., 2013).

Atresia has direct consequences by decreasing fecundity, with a potential impact on economic activities in aquaculture and fisheries. It can be considered as a disruption of the reproductive process when observed at high intensities during the spawning season as well as at other stages of the reproductive cycle. (Hunter and Macewicz, 1985; Martyniuk et al., 2013). Like occurs in mammalian, in teleost fish atresia seems to be regulated by apoptotic and autophagy processes but it can be induced by changes in nutrition as determinates in wild fish and culture fish populations ((Martyniuk et al., 2013, Yamamoto et al., 2016). Recently, González-Kother et al. (2019) had identified twenty potential genes relationships with atresia process in teleost fish. Those genes have relationship with apoptosis (e.g. Bcl-2 protein family (*bcl-2* and *bax*),

Caspase genes (*casp3*, *casp8* and *casp9*); autophagy (*Beclin-1*); apoptosis/ autophagy (Cathepsin-D), Antiapoptotic function (S100A10 calcium binding protein s100a10) or Apoptosis-related function (e.g. follistatin (*fst*)). Also, lipid metabolic process genes were identified (e.g. Apolipoprotein C-I (*apoc-1*); fatty acid-binding protein 11 (*Fabp 11*)).

The Chilean jack mackerel, *Trachurus murphyi* Nichols 1920 shows a wide distribution in South Pacific coasts (Peru, Chile, New Zealand), conforming a panmictic single large population (Poulin et al., 2004; Cárdenas et al., 2009) that can be found in three different habitats (Arcos et al., 2001) according to its life history: a) nursery ground area, b) coastal feeding area and c) a large, main oceanic spawning area off central Chile (Bertrand et al., 2004; Cubillos et al., 2008; Vásquez et al., 2013). Due at the overexploitation favored by the oversizing of all the industrial fleet, they ended up triggering the biggest fishing crisis of the decade for this fishing resource (Zhu et al., 2014; SENAPESCA, 2017). For population of *T. murphyi* in Chile, the reduction was 44% of the biomasa, landing of ca. 4.600.000 tons by 1996 drop dramatically to less than 300.00 tons by 2017; generating critical changes in the age structure of the populations and all sustainability of the fishery of this resource (Arcos et al., 2004; SPRFMO 2018).

T. murphyi is an asynchronous species that shows an extended spawning period (i.e. five months from September to January next year for the main spawning season) (Leal et al., 2013); and migrates long distances for breeding and metabolic process is main support this migration and the subsequent growth of oocyte and spawning. The spawning occurs is oceanic waters and the fish migrate near 2000 km from the coast (Cubillos et al., 2008). During spawning season, *T. murphyi* showed high levels of prevalence of atresia, and at the end of spawning season increase the intensity of atresia (González-Kother, 2019). Due that the transcriptional response may be associated with a physiological and morphological change in the ovary, understanding the cellular and molecular mechanisms involved in the process of atresia, is critical to understand

the reproductive dynamics of this important economic species. Exploratory transcriptomic study was performed in order to study physiological process associated to the reproductive process in this species and to generate putative molecular markers that could be used as predictors of reproductive disruption in wild populations of fish.

2. Materials and Method

2.1. Samples collection and RNA extraction

Trachurus murphyi individuals were collected from two areas of South-eastern Pacific Ocean. Juveniles females (n=9) corresponding to non-atresia condition were collected in coast of northern Chile (23°S, 70°W) by scuba diving, and mature adult females (n= 20) corresponding to atresia condition were caught by industrial fishing vessels in South Central Chile (33°51'S-72°06'W). Small portions of gonads and liver tissues were transferred to vials with RNAlater® store solution and stored at -20°C. A triplicate of each tissue was collected one each sample. For histological analysis, ovaries were fixed in formaline and stained with hematoxylin-eosin. Described histological criteria (Brown-Peterson et al. (2011) were used to identified reproductive phases. Atretic oocytes were identified following the criteria of Hunter & Macewicz (1985). Total RNA from each tissue was extracted using the ENZA RNA extraction kit (OMEGA) following the manufacturer's instructions. The quantity and quality of RNA extractions were assessed using a nanodrop ND-8000 (Thermo Scientific) and run on an agarose gel to check RNA integrity. Following extraction, total RNA was pooled in equal quantities for the two tissues (gonads and liver) for each atresia and non-atresia samples.

Transcriptome sequencing and assembly:

The RNA-seq libraries were prepared using the Illumina TruSeq Stranded mRNA protocol and sequenced on one lane of an Illumina HiSeq 2500 (High Throughput mode) using a paired-end

read length of 2×125 bp. The reads that did not pass the chastity filter (i.e., internal filtering procedure from Illumina sequencers) were filtered out and the adapters were trimmed automatically at this stage. The sequencing resulted in approximately 148 million 2×125 bp paired-end reads for atresia library and 131 million 2×125 bp paired-end reads for non-atresia library. The library preparation, sequencing and pre-processing automatic filtering steps were performed at the Centre ‘Innovation Génome Québec et Université McGill core facility (Montréal, Canada). The liver and gonad reference transcriptome was built with all reads from both libraries. Raw reads were filtered for adapter removal, minimum length (> 100pb) and minimal quality (Q=30) using Trimmomatic v0.36 (Bolger et al., 2014). The retained PE reads were assembled with Trinity (v2.8.4) (Haas et al., 2013) using default parameters with a minimum transcript length of 200-pb. Open-reading frames (ORFs) for each transcript were predicted using Transdecoder v3.0.1 (Haas et al., 2013). Only the transcripts containing an ORF of at least 100 amino acids were kept. Transcriptome functional annotation was performed using the software suite Trinotate (version3.1.0,(Haas et al.,2013)).Translated sequences from the final assembly were used to perform a blastp search on the Swiss-Prot database and a protein domain search on the Pfam database .Results were then integrated in Trinotate to retrieve functional annotations leveraging egg, GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genome) databases (Huerta-Cepas et al., 2016; Gene Ontology Consortium, 2004; Kanehisa and Goto, 2000).Only highly significant matches were reported in the annotation file (i.e. those with an e-value<10⁻⁵ for the blast hits). Then, clean reads from atresia and non-atresia libraries were mapped against assembled reference transcriptome using Bowtie 2 software and the reads counting was then made using IdxStats software (Peter and Cock, 2013). The RPKM (Reads Per Kilobase of transcript) values were calculated for both libraries (Mortazavi et al., 2008). We used those ratios to identified genes being specifically expressed

in both libraries and genes which RPKM is at least five times higher in one library compared to the other to identified strongly regulated genes.

2.1. Quantitative real-time PCR validation

To validate transcriptome sequencing data, 11 genes potentially involved in reproduction and atresia (González-Kother et al., 2019); were selected for quantitative RT-PCR (qRT-PCR) analysis (Table 2). Expression analysis was performed on the total RNA extracted from gonad and liver of the 29 individuals collected. Two μg of total RNA were reverse transcribed using M-MLV reverse transcriptase (Promega, Madison, WI). For gene expression, a volume of 2 μl of each diluted reverse transcription product (1:200) was subjected to real-time PCR in a final volume of 5 μl containing 40 nM of each specific primer and 2 \times Lightcycler®480 SYBR Green I Master mix (Roche Diagnostics, Mannheim Germany). The amplification was carried out as follows: initial enzyme activation at 95°C for 15 min, then 45 cycles of 95 °C for 10 sec and 60 °C for 30 sec. A dissociation curve was generated and PCR efficiency was estimated for each primer pair. All primer pairs tested generated a single peak in the dissociation curve and a PCR efficiency of 95 to 100%. Gene specific primers were designed using the Primer3software (version 4.0.0, <http://primer3.ut.ee>). The SSU rRNA gene was used as an endogenous control. The relative expression of each gene was calculated according the comparative Ct method using the formula $RQ = \Delta\Delta\text{Ct}$ (with $\Delta\text{Ct}_{\text{gene X}} = \text{Ct}_{\text{gen X}} - \text{Ct}_{\text{RiboL15}}$; $\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{gene X}} - \text{mean Ct}_{\text{gene X}}$).

2.2. Sequences and phylogenetic analyses

For Follistatin-like (FST-like) and Fatty acid-binding proteins (FABPs) identified in our database, phylogenetic trees were generated with the maximum composite likelihood (ML) algorithm in Mega v6 software (Tamura et al., 2013). The Akaike Information Criterion was

used to determine the best evolution model. JTT+ G was used for FST like and LG + G model for FABP proteins. Statistical support for the nodes was estimated used by a bootstrap with 1,000 pseudoreplicates (Felsenstein, 1985). Genetic sequences of FST-like and FABP proteins of other fish species were obtained from the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>).

3. Results

The results of the reproductive development stages of ovaries are shown in Figure 1. All mature females showed atresia, most of them being in an advanced stage, being beta atresia stage the most common identified.

The raw transcriptome assembly contained 290 473 transcripts (Table 1). After filtering for redundancy and functionality, we retained a total of 47 055 transcripts including 15 088 transcripts with an e-value $< e^{-5}$ and 18 704 transcripts with at least one match with deposited sequences. Transcriptome completeness evaluation indicated that 97% of the highly conserved single-copy metazoan genes (n=303) were present in our transcriptome reflecting a relatively good quality of our transcriptome. When considering the number of genes specifically present in the atresia library among the 47055 transcripts, we identified 5266 unique sequences including 2164 annotated with an e-value $< e^{-5}$ and in the non-atresia library, we identified 3552 unique sequences including 1375 annotated with an e-value $< e^{-5}$. The GO ontology distribution for Biological Process, Cellular Component, Molecular Function and Protein class of those genes is presented in the Figure 2. When considering the shared expressed genes between libraries with a RPKM above 5, we identified 3523 genes of those 1683 with an e-value $< e^{-5}$ over-expressed in the atresia library versus 3344 genes with 1496 with an e-value $< e^{-5}$ over-expressed in the non-atresia library.

The genes used to validate our transcriptome sequencing data of *T. murphyi*, and their expression in ovary and liver, as Adipocyte FABP (*Fabp4*), Head/muscle FABP (*Fabp3*), TAF8 RNA polymerase II, TATA box binding protein (TBP)-associated factor (*Taf8*); TATA-Box Binding Protein Associated Factor 13 (*Taf13*) and pre B cell leukemia homeobox 1 (*Pbx1*), showed a significant differential expression between females (Table 2, Figure 3). These genes indicating that probably many of the genes identified in our transcriptomic database show a tissue regulation. When we analyse their expression in both tissue when considering the status of atresia in samples, we observed that a more contrasted pattern. In liver (Figure 4), all the genes analysed show a global over-expression in samples showing atresia and for follistatin-like (*Fstl*) genes, the Ileal FABP (*Fabp6*), the Thrombospondin 1 (*Thbs1*) and TATA box binding protein (Tbp)-associated factor, RNA polymerase I, B (*Taf13*), the difference are statistically significant. In the gonads (Figure 5), a global over-expression in samples showing atresia is also observed except for *Fabp6*, vitellogenin (*Vtg*) and *Taf13* (even difference are not significant). Those results seem to confirm a putative role of those gene in the reproduction and atresia process for *T. murphyi*. Thrombospondin (*Thbs*) showed similar expression in juveniles and mature females. For Follistatin like 1 (*Fstl1*), Follistatin like 3 (*Fstl3*), Follistatin like 4 (*Fstl4*) not showed clear expression with atresia process, with exception for two juvenile females (data no show). The phylogenetic relationship of *T. murphyi* *Fstl* showed a distinct clade for *Fstl1*, *Fstl3* and *Fstl4*, clustered with their orthologs from other teleost fishes (Figure 6). However due to the low number of samples showing a non-atresia status compare to the individual showing atresia, those results remain preliminary. More, among individual showing atresia, different stage of atresia can be noticed. We cannot exclude that those stages may modulate the gene expression.

Considering the importance of energy metabolism in reproductive process, several annotated transcripts relationships with metabolism as the Fatty acid-binding proteins (FABPs) were recorded. Five FABPs belong to a multigene family of intracellular lipid-binding proteins (iLBPs) were identified: fatty acid binding protein 2, intestinal (*Fabp2*); fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor) (*Fabp3*); fatty acid binding protein 4, adipocyte (*Fabp4*), fatty acid binding protein 6 (*Fabp6*) and Brain-Type Fatty Acid-Binding Protein (*Fabp7*). The qPCR showed an up-expression in ovaries for all *Fabps* analysed (*Fabp3*, *Fabp4* and *Fabp6*) and especially for *Fabp4* that exhibit a very strong tissue expression differential. When considering the atresia status, our expression study in gonads did not show a significant differential expression within the *Fabp* family. But in liver, the three *Fabp* are overexpressed in fish showing atresia especially the *Fabp6* gene. For *T. murphyi* *Fabps* sequences, the phylogenetic analysis provides evidence that the putative *T. murphyi* *Fabp3* and *Fabp4* showed close sequences, with a robust bootstrap value of 92% at the node in this phylogenetic tree for other teleost fishes. For the other hand, *T. murphyi* *Fabp2*, *Fabp4*, *Fabp6* and *Fabp7*, clustered with their orthologs from other teleost fishes (Figure 7).

For determinate the presence of already known genes to participate in ovarian development and atresia, we looked for their presence in the annotated genes database. We identified genes related with ovarian follicle development in both transcriptome, as mutS homolog 4 (*Msh4*); BCL2-associated X protein (*Bax*); uromodulin like 1 (*Umodl1*); DNA meiotic recombinase 1 (*Dmc1*); lysine (K)-specific methyltransferase 2B (*Kmt2b*); proteasome (prosome, macropain) 26S subunit, non-ATPase, 4 (*Smd4*); matrix metalloproteinase 19 (*Mmp19*); bone morphogenetic protein receptor, type 1B (*Bmpr1b*) and delta (*DI*). Follicle stimulating hormone receptor (*Fshr*) were also recorded, this gene participates in primary ovarian follicle growth and cellular response to stimulating hormone stimulus and in positive regulation of luteinizing hormone

secretion. Transcripts of SMAD family of proteins, a well-conserved family of transcriptional factors, relationships with oocytes development were recorded (*Smad2*, *Smad3* and *Smad4*). Genes that showed clear relationships with ovarian follicular atresia in mammalian and other vertebrates were identified, as Neurofilament heavy (*Nefh*), Hyaluronoglucosaminidase family (*Hyal1*, *Hyal2* and *Hyal3*), and Acyl-CoA synthetase bubblegum family member 1 (*Acsbg1*). Apoptosis-related genes were identified in the transcriptome of *T. murphyi* (Table 3). Apoptosis-inducing, TAF9-like domain 1 (*Aptd1*) and THAP domain containing, apoptosis associated protein 3 (*Thap3*) were only identified in juvenile female while BCL2, apoptosis regulator (*Bcl2*) and BOK, BCL2 family apoptosis regulator (*Bok*) were only recorded in mature females (Table 3). The analysis of transcripts identified several caspases with function in inflammatory (e.g. *Casp1*), initiator of apoptosis (e.g. *Casp2*, *Casp8*) and executioner of apoptosis (e.g. *Casp3*, *Casp6*). Also, several caspase activity genes were identified in the transcriptome (Table 4)

4 Discussion

The transcriptome identified in *T. murphyi* showed relationship with ovarian process and specially with atresia in several bonyfish species and mammalian species. In *Micropterus salmoides*, Follistatin a (*fst*) is down-regulated in atretic ovaries (Martinyuk et al., 2013). The role of *Fstl1* in immunity is not completely understood; both anti- and proinflammatory functions for *Fstl1* have been reported (Chaly et al., 2014). The mature females of *T. murphyi* showed overexpression of *Fstl3*, in transgenic mice this overexpression causes defects in gonadal development and function (Xia et al., 2004). We identified overexpression of *Fstl4*, but it is not known that, in mouse and human regulation of this protein has been associated with several health problems (Guo et al., 2012). Like occurs with follistatin (*Fst*) in teleost and other vertebrates (Macqueenand Johnnton, 2008), the phylogenetic analysis showed that *Fstl* (*fstl2*,

fstl3 and *fstl4*) are conserved genes between teleost fish. Thrombospondin (*Thbs*) may be involved in the cessation of angiogenesis in follicles undergoing atresia (Thomas et al., 2008), and in *Solea senegalensis*, *Thbs* is up-regulated in atretic ovaries (Tingaud-Sequeira et al., 2009).

Our result showed the presence of five Fatty acid-binding proteins genes (*Fabp2*, *Fabp3*, *Fabp4*, *Fabp6* and *Fabp7*), coinciding with registered in spotted green pufferfish, *Tetraodon nigroviridis* and zebrafish, *Danio rerio*, where the expression not exclusive to specific organ (Thirumaran and Wright, 2014). In mammalian, *Fabp2* encoded an intracellular fatty acid-binding protein that participates in the uptake, intracellular metabolism, and transport of long-chain fatty acids (Furuhashi and Hotamisligil, 2009) probably have the same function in *T. murphyi*. *Fabp3* had been detected in ovaries from adult mouse, specifically in macrophages of advanced atretic follicles, this protein may be involved in the process of atresia (Nourani et al., 2005). We observed in several ovaries of adult females the presence of macrophages associated mainly to atretic oocytes. *Fabp4* is localized in granulosa cells of atretic follicles and may be involved in apoptosis of those cells potentially through its interaction with peroxisome proliferator-activated receptor γ (Nourani et al., 2005b). Also, *Fabp4* variants are associated with polycystic ovary syndrome (Hu and Qiao, 2011), this gene had not been identified in others teleost fish (Thirumaran and Wright 2014). *Fabp6* results in marked reduction in ovulatory response to super-stimulation (Duggavathi et al., 2015) while *Fabp7* has been associated to tumor cell signalling (Kagawa et al., 2019). Surprisingly *Fabp11* which show a putative role in atresia of teleost, especially in Senegalese sole, *Solea senegalensis* (Agullero et al., 1999) was not identified in our database. Copies of *Fabp11* transcripts (*Fabp11a* and *Fabp11b*) have been identified in other organs different to ovaries (e.g. brain, muscle, testis) in *T. nigroviridis* (Thirumaran and Wright 2014), but in zefrafish ovaries was only identified *Fabp11b* (Karanth

et al., 2008). In a similar way, *Fabp5* that has a function in steroid metabolism as well as in follicular atresia in mammals was not identified in females of *T. murphyi*. Phylogenetic relationship of the *T. murphyi* and other teleost fish for FABPs showed high sequence conservation among teleost (Liu et al., 2007; Thirumaran and Wright 2014).

Several genes recorded in transcriptome of *T. murphyi* showed relationship with oocytes development and follicular atresia in mammals. In *T. murphyi* was identified the SMAD transcripts (*Smad2*, *Smad3* and *Smad4*) and probably these genes have the same function as other vertebrates. In human, several *Smad* genes are differently expressed in granulosa cells, where they may mediate TGF-beta superfamily signaling during folliculogenesis and ovulation (Kuo et al., 2011). In porcine ovary, *Smad2* and *Smad4* may be a key regulator of follicular development and growth of oocytes (Xing et al., 2014). In human ovary, *Smad3* promoted the proliferation and steroidogenesis of ovarian luteinized GCs, and these effects may be mediated by the follicle-stimulating hormone receptor/ luteinizing hormone receptor-protein kinase A proteins (FSHR/LHR-PKA) signaling pathway (Liu et al., 2014).

Other genes identified in *T. murphyi*, as Mammalian Hyaluronidase [hyaluronoglucosaminidase1 (*Hyal1*), Hyaluronoglucosaminidase2 (*Hyal2*) and Hyaluronoglucosaminidase3 (*Hyal3*)] induces ovarian granulosa cell apoptosis and are involved in follicular atresia; in mouse ovarian mRNA levels *Hyal1*, *Hyal2* and *Hyal3* were significantly increased in apoptotic granulosa cells as well as in atretic follicles (Orimoto et al., 2008), while in rat ovaries *Nefh* was expressed in oocytes of follicles from the primary to mature stages, ovulated oocytes, fertilized zygotes, and degenerative oocytes of atretic follicles (Takahashi and Ishizuka, 2012). Both genes, *Nefh* and *Hyal3* were recorded in juveniles and adult females. *Bax* act as signal-transducing factors in mitochondrion-dependent type II apoptosis. In mice, *Bax*

appears to be an important regulator of follicle growth, but is essential for follicle atresia (Greenfeld et al., 2007).

The several genes associated to apoptosis was identified in the transcriptome of *T. murphyi*, mostly different from those described for other teleost species, as zebrafish *D. rerio* and Atlantic salmon *Salmo salar* (Takle and Andersen, 2007). The oocyte apoptosis is the mechanism of ovarian differentiation in zebrafish *D. rerio* (Uchida et al., 2002). For the freshwater Characiformes fish, *Astyanax bimaculatus*, *Leporinus obtusidens*, *Prochilodus argenteus*, Morais et al., (2012) propose a model that explain the participation of caspase-3, the Bcl-2 protein family (*bcl-2* and *bax*), lysosomal Cathepsin-D and beclin-1 in the regulation of follicular atresia via autophagy and apoptosis in fish ovary, those genes showing an up-regulation in the follicular cells and thecal cells during early and advance regression of ovaries (Morais et al., 2012). All these genes were identified in *T. murphyi* but it is necessary evaluate relationships with the atresia in this specie, especially when genes were determined in the transcriptome of juvenile and atretic females The results suggest that atretic expression is not necessarily correlated with a morphological (histological) evidence of atresia meaning that their use as a marker of atresia, at least for the *T. murphyi*, is not adequate. In overall, the gene expression patterns suggest lower changes of transcription activity between juvenile and atretic females.

Conclusion and perspectives for this study.

A specific analysis to detect putative selection in genes will be conducted based on the transcriptomic database available for the different teleost fish species. We will try particularly to identify if some genes involved in the reproduction are under selective pressure linked to the possibility and the intensity for those fish to present atresia. A more complete analysis of

additional gene expression will also have been conducted on a larger set of individuals to improve the data since the level of atresia may strongly influence the gene expression. We will also explore other gene families (e.g. apoptosis, energetic metabolism) expression regulation to improve our understanding of the effect of atresia on the fish physiology. In our expression analysis for *T. murphyi*, we had different stage of atresia but a limited number of samples per stage. To confirm or not our first result and decrease the strong variability observed, we need to increase the number of samples per stage in order to strengthen the statistical analysis.

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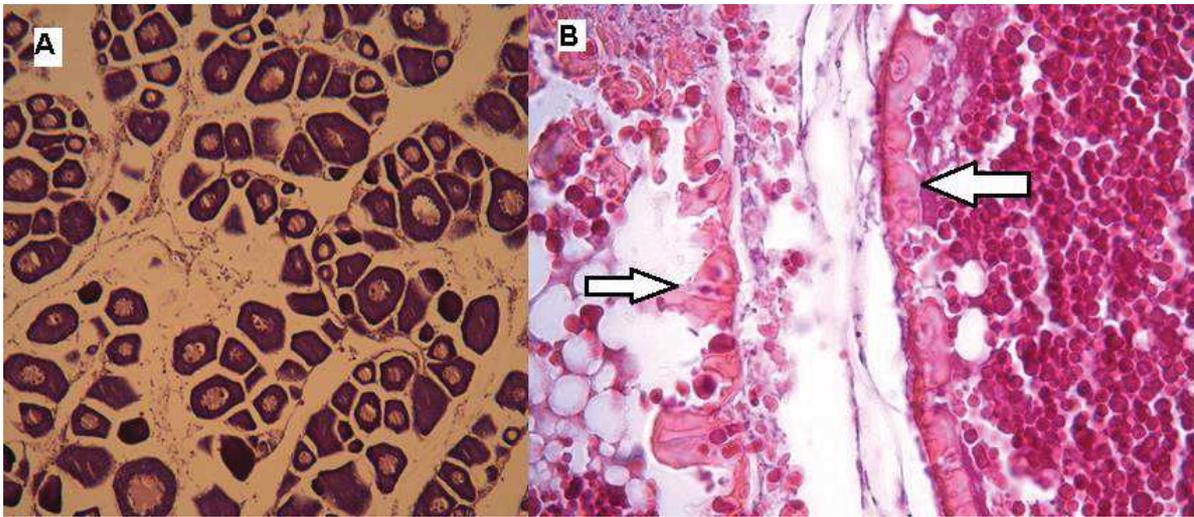


Figure 1. A non-atretic ovary (A) and atretic ovary with alpha atresia (arrow) (B) from *T. murphyi*

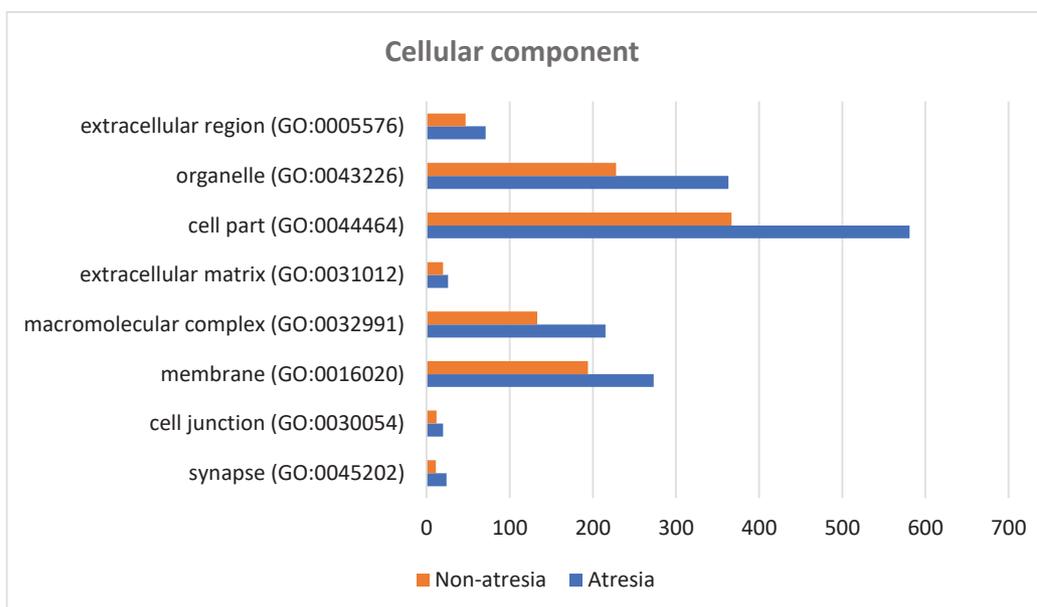
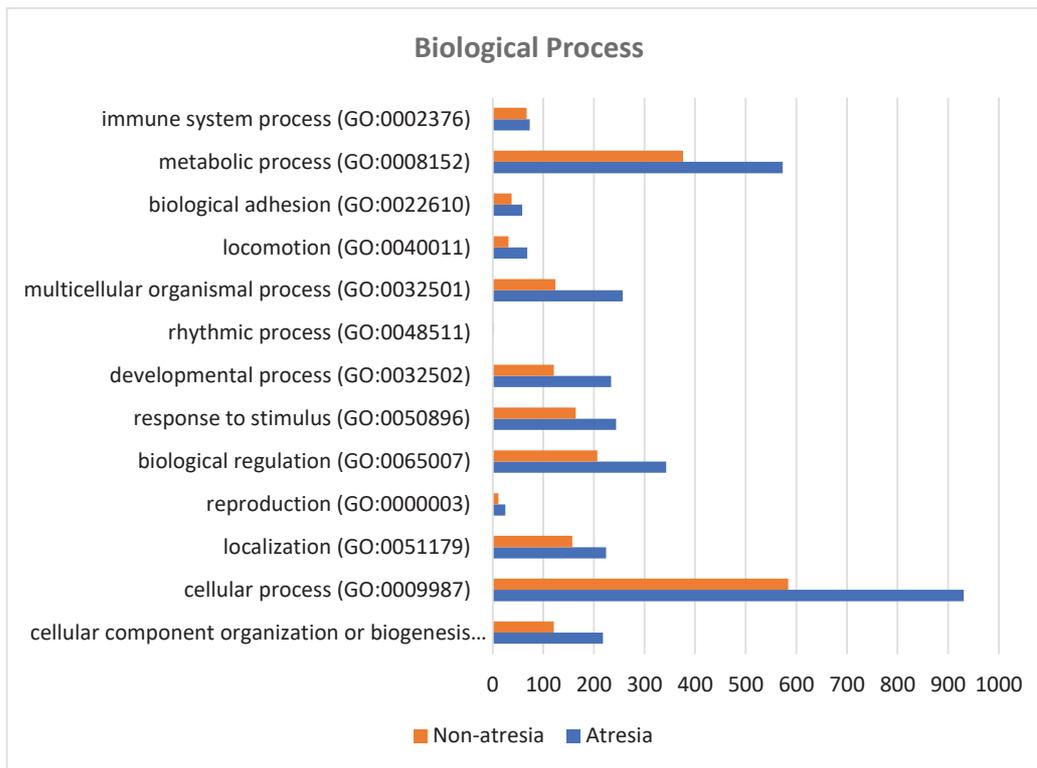


Figure 2. GO ontology of the genes exhibiting a RPKM above 5 between atretic females and non-atretic females libraries of *T. murphyi* for Biological process, Cellular component, Molecular function and Protein class.

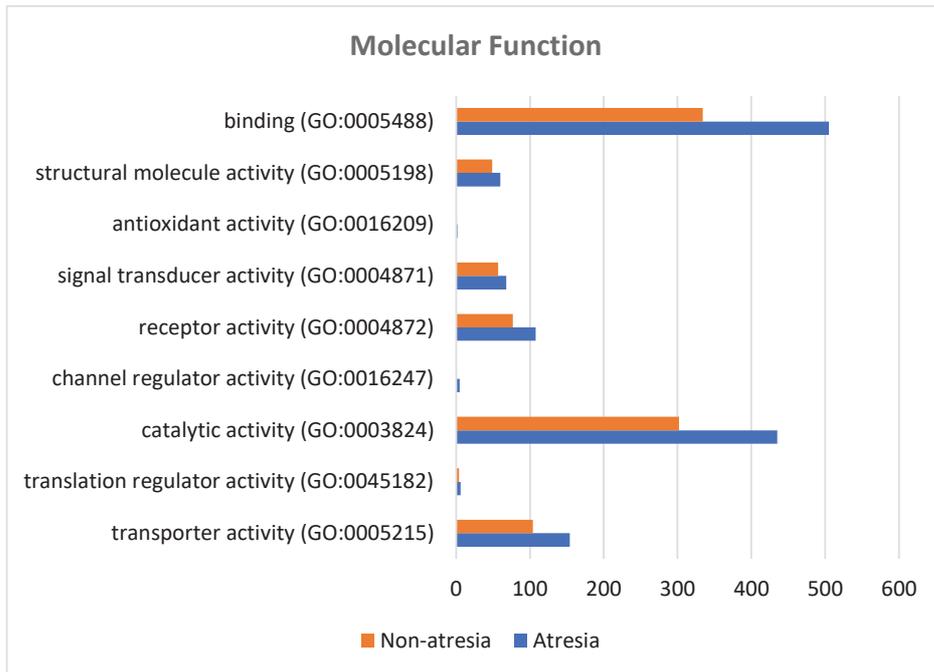


Figure 2. (cont.): GO ontology of the genes exhibiting a RPKM above 5 between atretic females and non-atretic females libraries of *T. murphyi* for Biological process, Cellular component, Molecular function and Protein class

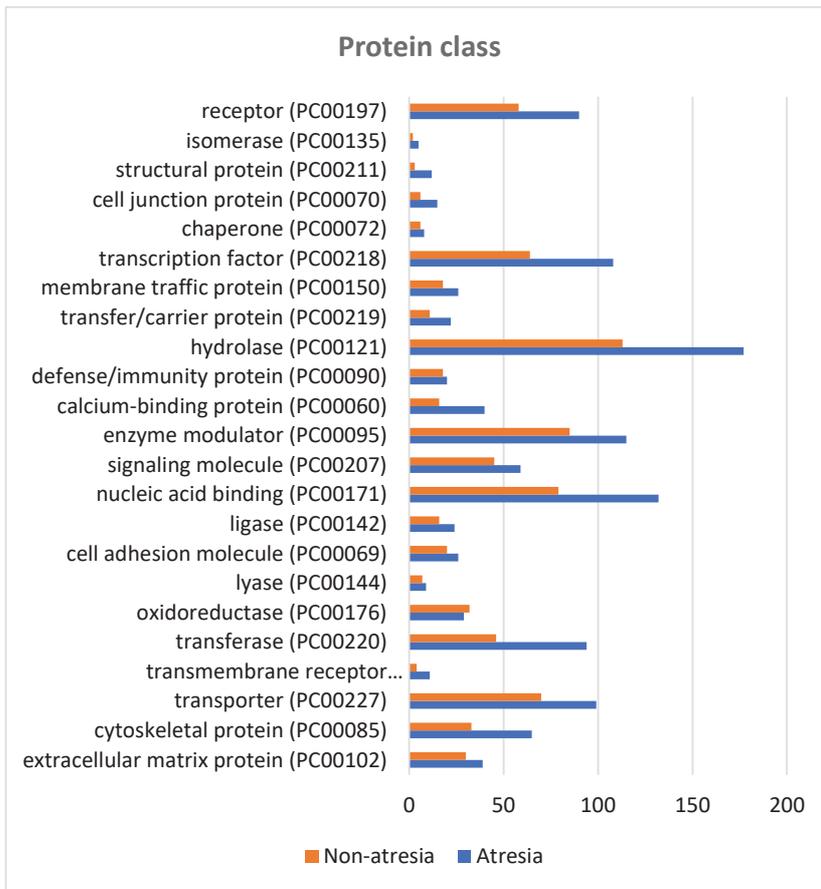


Figure 2. (cont): GO ontology of the genes exhibiting a RPKM above 5 between atretic females and non-atretic females libraries of *T. murphyi* for Biological process, Cellular component, Molecular function and Protein Class

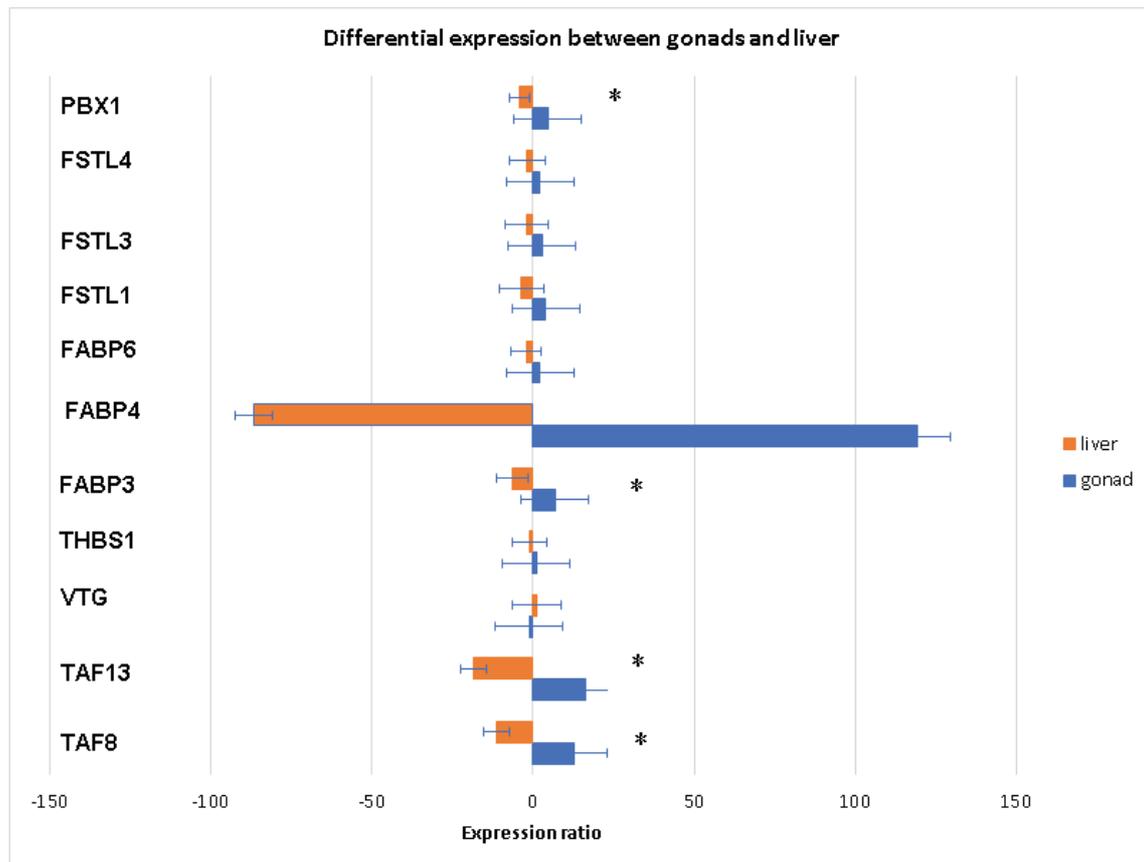


Figure 3. Differential expression between gonads and liver (n=25). Expression ratio are expressed as a relative expression between the two tissues. *: statistical significant value ($p < 0.05$) after t-test.

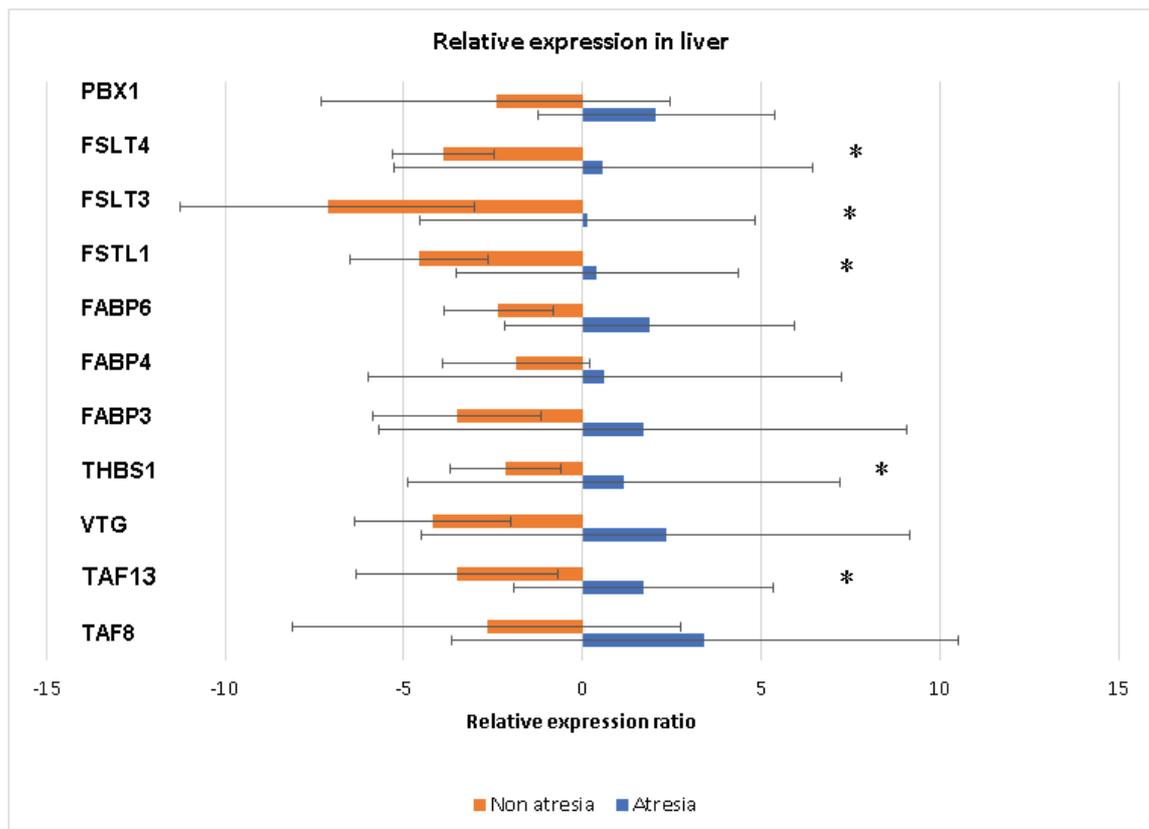


Figure 4. Differential expression between individual with atresia (n=16) and without atresia (n=5) in liver. Expression ratio are expressed as a relative expression between the two tissues. *: statistical significant value ($p < 0.05$) after t-test.

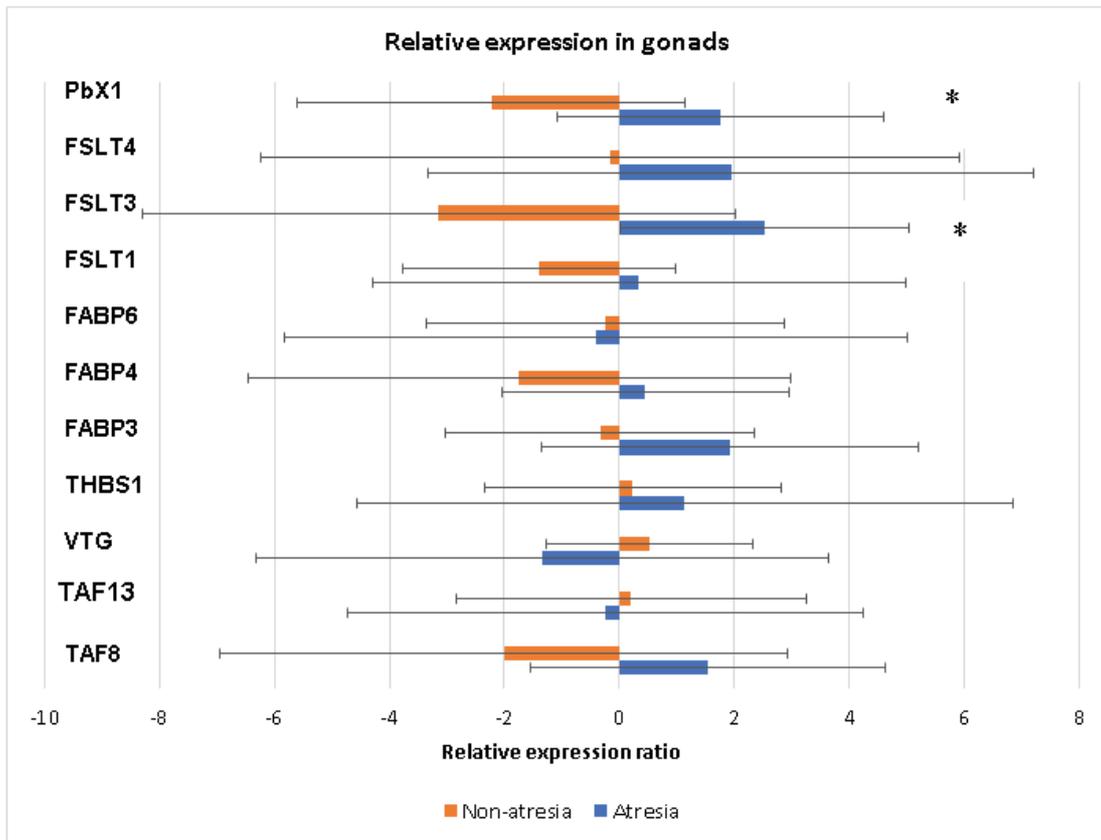


Figure 5. Differential expression between individual with atresia (n=16) and without atresia (n=5) in gonads. Expression ratio are expressed as a relative expression between the two tissues. *: statistical significant value ($p < 0.05$) after t-test.

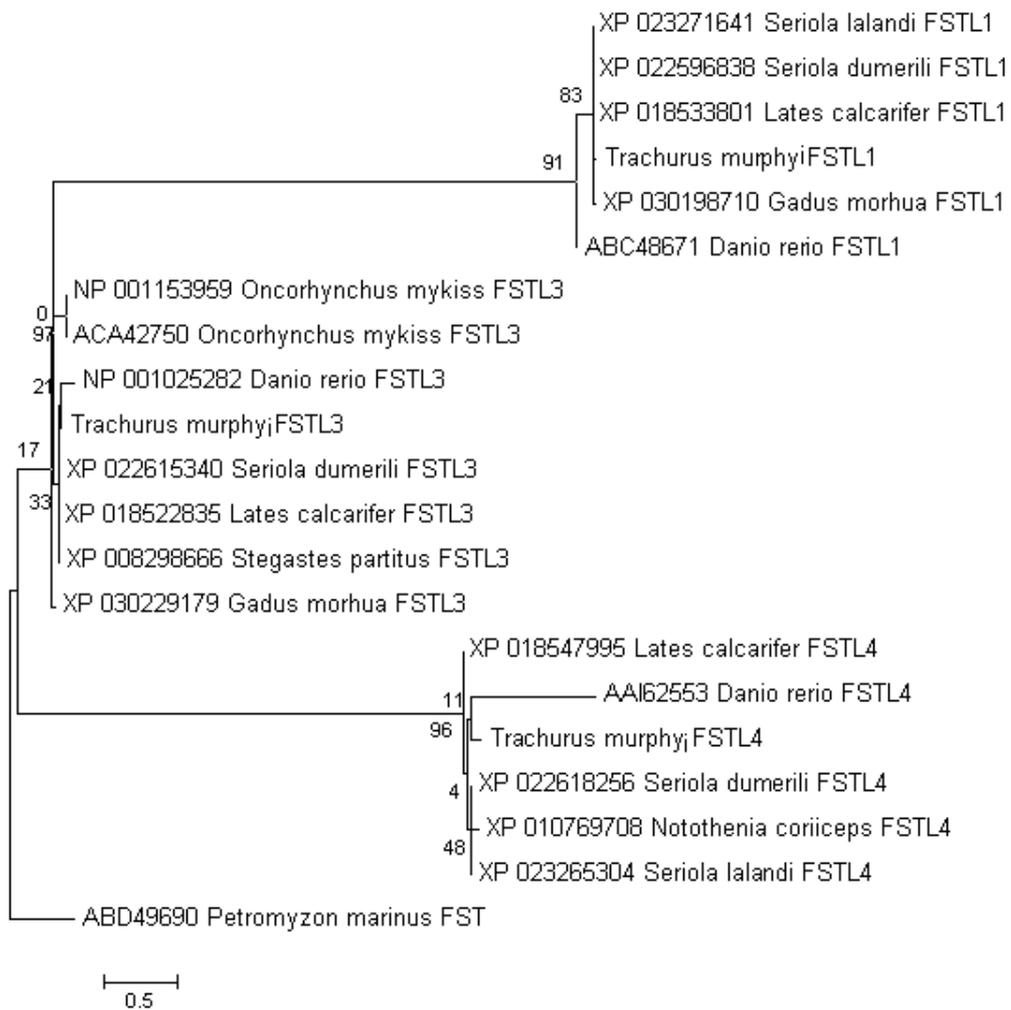


Figure 6. Phylogenetic relationship of the *T. murphyi* and other teleost fish Follistatin-like genes.

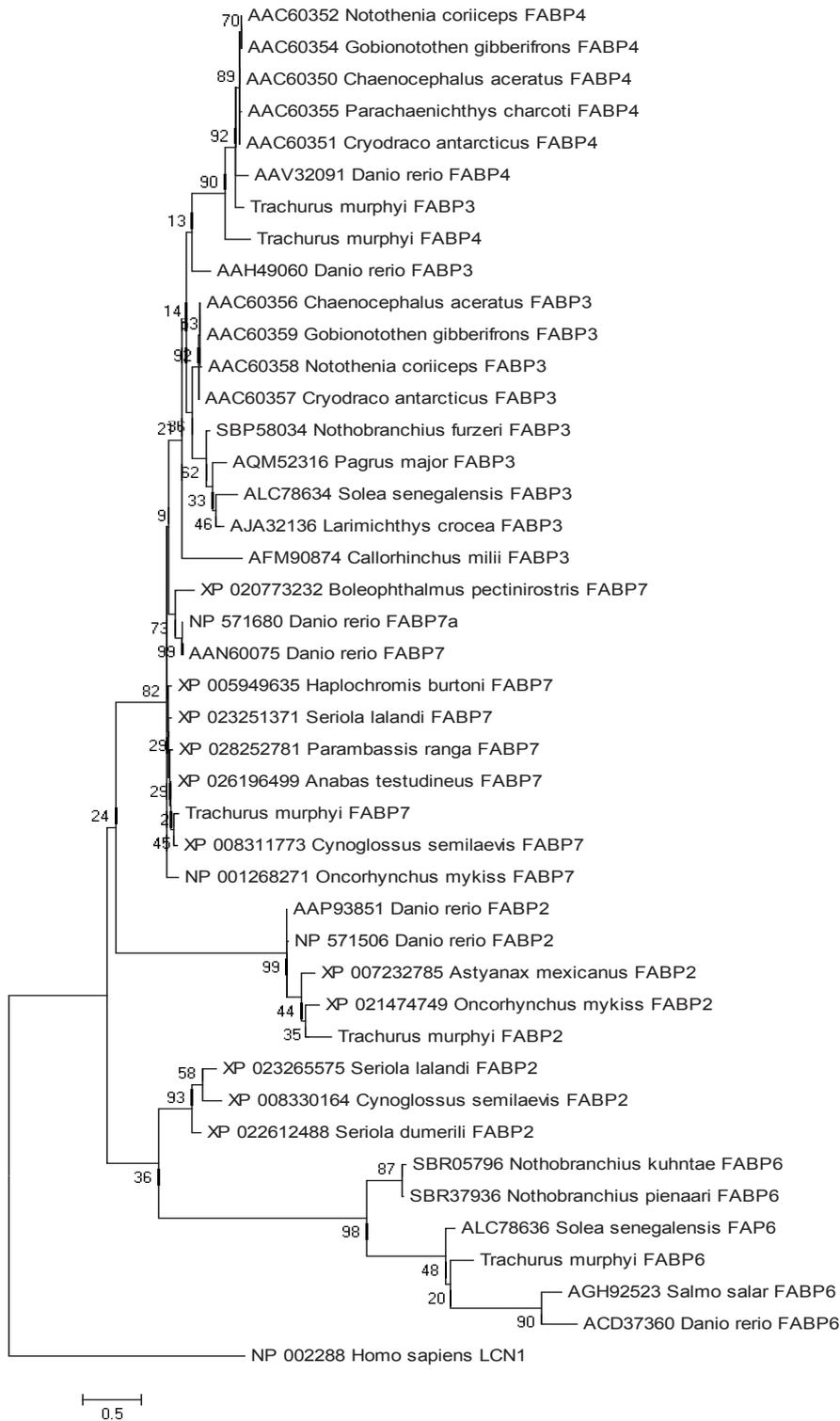


Figure 7. Phylogenetic relationship of the *T. murphyi* and other teleost fish in the FABP family.

Table 1. Data descriptions for assembly and annotation of *T. murphyi* transcriptome

Data	Descriptions
Raw number of contigs	290 473
Total number of contigs	109405
Percent GC	49.23
Contigs N50 (bp)	1621
Median contig length	478
Average contig	904.5
Total assembled bases	262 729 349

1 Table 2. Primers for quantitative PCR (qPCR) amplification of cDNAs in *T. murphyi*. (F = Forward, R = Reverse)

2

Name Gene	Gene	Primer	
Ribosomal protein l8 (rpl8)	<i>Rpl8</i>	F	ACACGTCCCATCGTGTACTAACT
		R	TATTTGACTACAACCTCCCATGAGC
RNA polymerase II, TATA box binding protein	<i>Taf8</i>	F	GTGACTCCTAAAGCTCTGTCAGC
		R	GTGTTTTGATGTAGGTGTGTGGAT
TATA box binding protein (Tbp)-associated factor, RNA polymerase I, B	<i>Taf13</i>	F	ACCAGTACAAGGACCAAGAAAAAG
		R	ACCAGTACAAGGACCAAGAAAAAG
Vitellogenin	<i>Vtg</i>	F	GTGACAATCTGTTTCTTGTGGTTC
		R	TCACTCTTCAGTGCTAGCTCAAGT
Thrombospondin 1	<i>Thbs1</i>	F	CACCCAACAAGCATAGTTATGAAG
		R	GCTCTCCTTGTTTCTTCTAGTGCT
Fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)	<i>Fabp3</i>	F	TAAGGAGAGCAGAACACACCATTA
		R	AATATCGAGAGGGAGATCACAGAT
Fatty acid binding protein 4, adipocyte	<i>Fabp4</i>	F	TTCCATCTTGAATCTCTCTCTCC
		R	GAGTACTTTCAAGACCACCGAAAT
Fatty acid binding protein 6, ileal (gastrotropin)	<i>Fabp6</i>	F	ACTGCTTGCTCTTCATTGTAGTTG
		R	AAAGGATCTAAAACCTGCGTGACTC
Follistatin like 1	<i>Fstl1</i>	F	AATAAGGACACAACGAAGAAGAGG
		R	TCTTTGATAGCCCCTGAATCTAAC
Follistatin like 3	<i>Fstl3</i>	F	GCTGGATTACTCCCAATTTGATAC
		R	CTCTATTTGCCATCACCTCTTCTT
Follistatin like 4	<i>Fstl4</i>	F	CTCTCTTTGTCCTGAACGGTAAAC
		R	TCTTTCCCTAATCCTAGGTCTCCT
Pre B cell leukemia homeobox 1	<i>Pbx1</i>	F	GTGGATGAGGTGTAGAGATGGATA
		R	AGACCGGAGGATACAGTGACAG

3

Table 3. Apoptosis encoding genes in liver and ovary in transcriptome of *T. murphyi*.

Juveniles Females	Matures Females Atresia	Both females
<i>Apitd1</i>	<i>Bcl2</i>	<i>Aifm2</i>
<i>Aven</i>	<i>Bok</i>	<i>Aifm3</i>
<i>Thap3</i>		<i>Aatk</i>
		<i>Aatf</i>
		<i>Bax</i>
		<i>Bfar</i>
		<i>Caap1</i>
		<i>Casp1</i>
		<i>Casp3</i>
		<i>Ccar1</i>
		<i>Cflar</i>
		<i>Ddias</i>
		<i>Mcl1</i>
		<i>Nai1</i>
		<i>Perp</i>
		<i>Siva1</i>
		<i>Thap2</i>
		<i>Tigarb</i>
		<i>Tigara</i>
		<i>Triap1</i>
		<i>Xiap</i>

Aatf, apoptosis antagonizing transcription factor; *Aatk*, apoptosis-associated tyrosine kinase, *Aifm2*, apoptosis-inducing factor, mitochondrion-associated, 2; *Aifm3*, apoptosis inducing factor, mitochondria associated 3; *Apitd1*, apoptosis-inducing, TAF9-like domain 1; *Aven*, apoptosis, caspase activation inhibitor; *Bax*, BCL2 associated X, apoptosis regulator; *Bcl2*, BCL2, apoptosis regulator; *Bfar*, bifunctional apoptosis regulator; *Bok*, BOK, BCL2 family apoptosis regulator; *Caap1*, caspase activity and apoptosis inhibitor 1; *Casp1*, caspase 1, apoptosis-related cysteine peptidase; *Casp3*, caspase 3, apoptosis-related cysteine peptidase; *Ccar1*, cell division cycle and apoptosis regulator 1; *Cflar*, CASP8 and FADD like apoptosis regulator; *Ddias*, DNA damage induced apoptosis suppressor; *Mcl1*, BCL2 family apoptosis regulator; *Nai1*, nuclear apoptosis inducing factor 1; *Perp*, PERP, TP53 apoptosis effector; *Siva1*, SIVA1 apoptosis inducing factor; *Thap2*, THAP domain containing, apoptosis associated protein 2; *Thap3*, THAP domain containing, apoptosis associated protein 3; *Tigara*, tp53-induced glycolysis and apoptosis regulator a; *Tigarb*, tp53-induced glycolysis and apoptosis regulator b; *Triap1*, TP53 regulated inhibitor of apoptosis 1; *Xiap*, X-linked inhibitor of apoptosis.

Table 4. Caspase and caspase activity encoding genes in liver and ovary transcriptome of *T. murphyi*.

Genes	Description
Juveniles Females	
<i>Aven</i>	apoptosis, caspase activation inhibitor
<i>Card6</i>	caspase recruitment domain family member 6
Juveniles and atretic females	
<i>Malt1</i>	Mucosa-associated lymphoid tissue lymphoma translocation protein 1, paracaspase
<i>Caap1</i>	caspase activity and apoptosis inhibitor 1
<i>Card8</i>	caspase recruitment domain family member 8
<i>Card9</i>	caspase recruitment domain family member 9
<i>Card10</i>	caspase recruitment domain family member 10
<i>Card11</i>	caspase recruitment domain family member 11
<i>Card14</i>	caspase recruitment domain family member 14
<i>Card17</i>	caspase recruitment domain family member 17
<i>Card19</i>	caspase recruitment domain family member 19
<i>Casp1</i>	caspase 1
<i>Casp1.L</i>	caspase 1 L homeolog
<i>Casp2</i>	caspase 2
<i>Casp3</i>	caspase 3
<i>Casp6</i>	caspase 6
<i>Casp7</i>	caspase 7
<i>Casp8</i>	caspase 8
<i>Casp8ap2</i>	caspase 8 associated protein 2
<i>Casp9</i>	caspase 9
<i>Casp10</i>	caspase 10

4.4 CHAPTER 4.

The first report of liver coccidian *Goussia cruciata* in jack mackerel, *Trachurus murphyi*, from the South Pacific and its relationship with host variables

P. González-Kother · M. T. González

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Abstract The liver coccidian *Goussia cruciata* is considered as a specific parasite of the genus *Trachurus*, but to date, this parasite has only been reported for Atlantic species (*Trachurus picturatus*, *Trachurus lathami*, *Trachurus trachurus* and *Trachurus mediterraneus*). Therefore, this is the first report of this parasite in a species of *Trachurus* from the South Pacific. The prevalence and abundance of this coccidian in jack mackerel, *T. murphyi*, was determined, and its relationships with host variables such as total body length, condition factor and hepatosomatic index were evaluated. A total of 49 individuals were sampled from a commercial vessel of the central Chilean coast (36° 41' S, 73° 06' W) in November 2013 and February and May 2014. The parasite was identified by means of liver smears using light microscopy. The relationship between the abundance of the parasites and the host total length, condition factor and hepatosomatic index was analysed with Spearman's correlations. The sporogonic stages exhibited sporocysts that were morphologically concordant with coccidian *G. cruciata*. All hosts were parasitised with this coccidian, and the abundance varied between 2 and 224 oocysts per host. The parasite abundance was negatively correlated with the host total length. Infection levels of *G. cruciata* in *T. murphyi* apparently do not produce negative effects on fish condition.

Keywords *Goussia cruciata* · Body condition indices · *Trachurus murphyi* · Southeast Pacific Ocean

Introduction

Coccidians of the genus *Goussia* Labbé, 1896 infect teleost fishes; this parasite mainly invades the digestive tract, but some develop in other organs, such as the liver (Davies and Ball 1993). Coccidian parasites can replace the liver tissue, producing hepatic lesions when infection loads are heavy (Lom and Dyková 1992; Abollo et al. 2001). It has been suggested that liver coccidians affect host body condition, decreasing the hepatosomatic index and condition factor with the degree of infection, as evidenced for the gadoid *Micromesistius poutassou* (Risso, 1827) (Abollo et al. 2001).

Goussia cruciata (Thélohan, 1892) Labbe, 1896 and *Goussia clupearum* (Thélohan, 1894) Labbe, 1896 have been recorded to heavily infect liver and pyloric caeca (Lom and Dyková 1992). *G. cruciata* is a species that is morphologically similar to *G. clupearum*, though *G. cruciata* has been cited as a liver-specific parasite of marine fish species of the genus *Trachurus* Rafinesque, 1810 (Lom and Dyková 1992; Abollo et al. 2001; Braicovich et al. 2012; Costa et al. 2013).

The genus *Trachurus* comprises 14 nominal species distributed in Atlantic, Indian, Mediterranean and Pacific waters (Cárdenas et al. 2009). The coccidian *G. cruciata* (Lom and Dyková 1992; Abollo et al. 2001; Braicovich et al. 2012; Costa et al. 2013) has been reported to infect species inhabiting the Atlantic Ocean, such as *Trachurus picturatus* (Bowditch), *Trachurus lathami* Nichols and *Trachurus trachurus* (Linnaeus), and the Mediterranean Sea, such as *Trachurus mediterraneus* (Steindachner). *G. clupearum* has also been described as parasitising the liver of *T. trachurus* (Gestal and Azevedo 2006). However, there are no previous records of coccidians for the following five species distributed in the Pacific Ocean: *Trachurus japonicus*

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(Temminck and Schlegel), *Trachurus novaezelandiae* (Richardson), *Trachurus declivis* (Jenyns), *Trachurus symmetricus* (Ayres) and *Trachurus murphyi* (Nichols) (Cárdenas et al. 2005).

The jack mackerel, *T. murphyi*, is a species that supports important fisheries along South Pacific, mainly in coasts of Peru, Chile and New Zealand and in international waters outside 200 nautical miles of exclusive economic zone (EEZ) of Chile (Ashford et al. 2011). Previous parasitological studies for this fish species have only considered metazoan parasite fauna (George-Nascimento and Arancibia 1992; Aldana et al. 1995; Oliva 1999).

In this study, the presence of liver coccidians in *T. murphyi* was determined, and its relationships with host variables including total body length, condition factor and hepatosomatic index were evaluated.

Material and methods

A total of 49 specimens of the jack mackerel *T. murphyi* were sampled from a commercial vessel of the central Chilean coast (36° 41' S, 73° 06' W) in November 2013 and February and May 2014. The total length (TL) and total weight (TW) of each specimen was registered prior to liver removal. The liver weight (LW) was determined, and the samples were kept deep frozen in individual plastic bags at -18 °C until parasite analysis. Parasites were determined via the observation of liver smears using light microscopy and were photographed with an Olympus camera. Coccidians were identified at the genus level according to Lom and Dyková (1992) and at the species level according to Gestal and Azevedo (2005) and Abollo et al. (2001). The recorded coccidians presented sporocysts arranged as a cross and as a triradiate star and were identified as *G. cruciata* according to Abollo et al. (2001). A total of 20 oocysts and sporocysts from different hosts were measured using MicroMetric software (Micrometrics Inc., 2009). A Mann–Whitney *U* test was used to evaluate potential sporocyst morphometric differences (length and width).

Parasitological descriptors such as prevalence and abundance were calculated according to Bush et al. (1997). To count the parasites, the liver of each fish was cut in the middle part and two similar pieces of liver were used to make two smears. Each smear was completely examined at ×40 magnification, and all recorded oocysts were counted. The distribution of parasites was calculated using the coefficient of distribution (CD) and index of aggregation (*K*) (Poulin 2007). The condition factor (CF) and hepatosomatic index (HSI) were calculated as $CF = (TW \times LT^{-3}) \times 100$ and $HIS = (LW \times TW^{-1}) \times 100$. Correlations between fish length, condition factor, hepatosomatic index and abundance of parasites were analysed with Spearman's correlations (Zar 1996).

Results

All examined specimens of the jack mackerel *T. murphyi* were parasitised with liver coccidians. However, the livers did not show any change in colour or macroscopic lesions associated with parasite infections. Cysts with surrounding connective tissue and other unknown parasite-like organisms were also observed, but only coccidians were included in the analysis. All coccidians found corresponded to the sporogonic stage. In this stage, the oocysts were spherical, with a mean diameter of 20.2 µm (range 18.5–21.5). Each oocyst showed four sporocysts, without a Stieda body. The sporocysts were arranged as a symmetrical cross shape or a triradiate star, concordant with the coccidian *G. cruciata*. The mean length of the sporocysts was 9.1 µm (range 7–12.5), and the mean width was 6.8 µm (range 5.7–8.2 µm). The length ($U=407$, $P=0.54$) and width ($U=386$, $P=0.36$) of the sporocyst did not differ significantly between the sporocyst shapes.

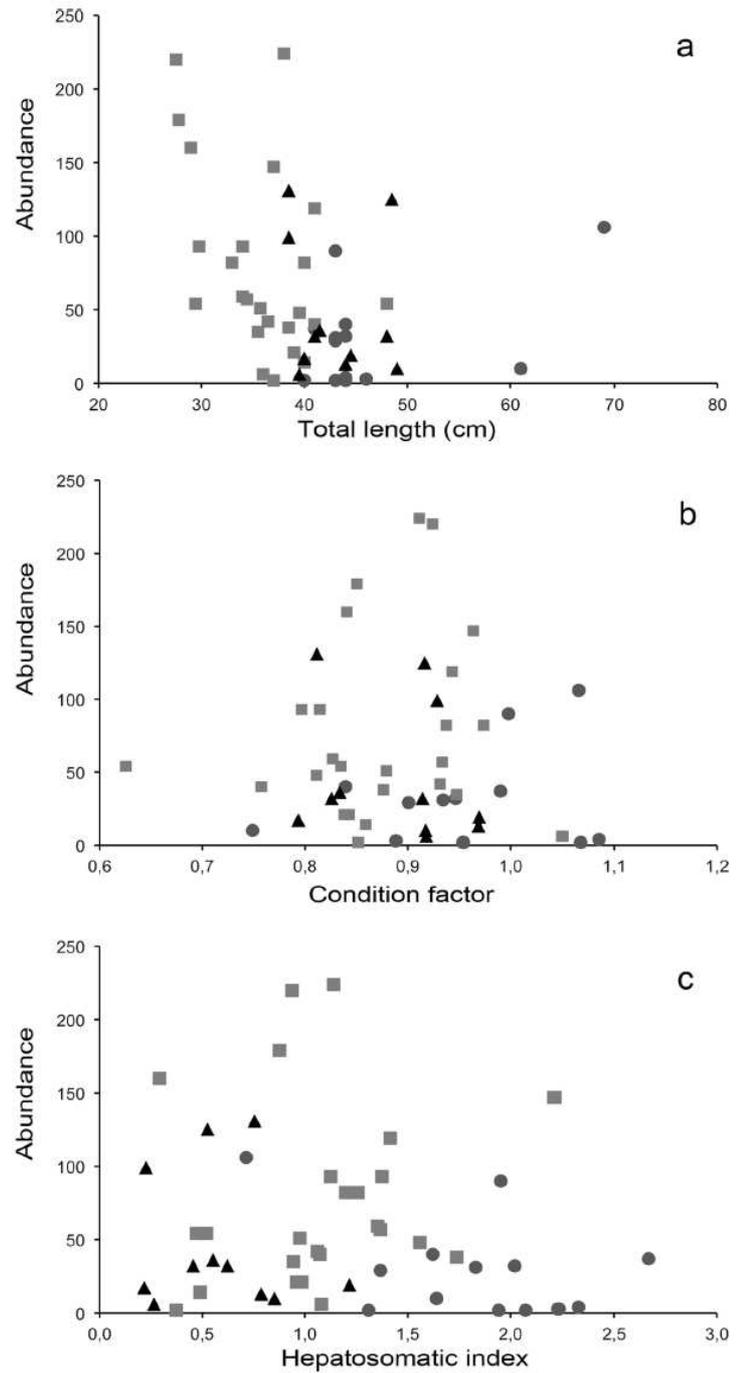
The oocyst abundances ranged between 2 and 224 according to liver smears. The coccidian abundance was negatively correlated with the host total length ($r_s=-0.43$; $P=0.001$), but there were no significant correlations between the parasite abundance and condition factor ($r_s=-0.15$; $P=0.27$) or hepatosomatic index ($r_s=-0.12$; $P=0.38$) (Fig. 1).

Discussion

Sporocyst morphology is one of the most important criteria for assigning coccidians to the appropriate genera (Azevedo 2001; Chapman et al. 2013). For *G. cruciata*, the arrangement of sporocysts in a cross shape has been the principal criterion for differentiating this species (Lom and Dyková 1992; Gestal and Azevedo 2005); however, Abollo et al. (2001) recognised two sporocyst arrangements (cross and triradiate) for *G. cruciata*. In the present study, both sporocyst arrangements were recorded, and the oocyst and sporocyst sizes were concordant with *G. cruciata* (Lom and Dyková 1992; Davies and Ball 1993; Abollo et al. 2001; Gestal and Azevedo 2005).

For all known species of the genus *Goussia* parasitising fishes, only ten develop in the liver (Davies and Ball 1993). Some of these species present low specificity, such as *G. clupearum*, which has been recorded in at least nine fish species of different families [e.g. *Belone belone* (Linnaeus), *M. poutassou* (Risso), *Sardina pilchardus* (Walbaum), *Scomber scombrus* Linnaeus, *T. trachurus*] (Dyková and Lom 1981; Lom and Dyková 1992; Azevedo 2001; Gestal and Azevedo 2005), whereas others, such as *G. cruciata*, have only been found in fish species of the genus *Trachurus* of Atlantic waters. Therefore, this is the first record of the genus *Goussia* in a *Trachurus* species in the Pacific Ocean, and *T. murphyi* constitutes a new host record for the liver coccidian *G. cruciata*, confirming the host specificity reported for this

Fig. 1 Relationship between the abundance (number of coccidians by liver smears) of *G. cruciata* and host body indices of *T. murphyi*. **a** Fish total length, **b** fish condition factor and **c** hepatosomatic index. (Circle November, square February, triangle May)



parasite species (Lom and Dyková 1992; Gestal and Azevedo 2005; Braicovich et al. 2012; Costa et al. 2013). Nevertheless, it is necessary to confirm specific identifications using

molecular markers (Chapman et al. 2013) because it is possible that *G. cruciata* could be a complex species, as has been demonstrated for other parasite groups showing

cosmopolitan distributions (Cantacessi et al. 2008; Sepúlveda and González 2014).

Some species of *Goussia* infecting the liver may affect the fish condition, and heavy infections can cause mortality (Gestal and Azevedo 2006). In *T. murphyi*, *G. cruciata* shows a binomial distribution, which is concordant with the predominant model in wild parasite–host systems (Poulin 2007). However, the observed negative relationship between coccidian abundance and host body length suggests that the parasites do not accumulate over time. Regardless, this relationship does not follow a clear pattern, as the abundance of *G. cruciata* was positively correlated with the body length of *T. trachurus* but negatively correlated with the body length of *T. picturatus* (Abollo et al. 2001; Costa et al. 2013). Additionally, the absence of correlations between coccidian abundance and host body condition indices suggests that infection levels apparently do not produce negative effects on the condition of *T. murphyi*.

Eumetazoan parasites (ecto- and endoparasites) of *T. murphyi* have been studied as possible indicators of stock in the populations of Chile (George-Nascimento and Arancibia 1992; Aldana et al. 1995; Oliva 1999). The coccidian *G. cruciata* was considered useful as a biological tag for the identification of populations of *T. picturatus* of Madeira (prevalence 87–100 %) and the Canary Islands (prevalence 0–16 %) (Costa et al. 2013). A similar result was determined for *G. cruciata* in *T. trachurus* considering its distributional geographical range, with variability in the prevalence of infection between 0 and 98 % in Atlantic and Mediterranean waters (MacKenzie et al. 2008). *T. murphyi* is a highly migratory fish species supporting important fisheries along South Pacific coasts. Molecular studies (Cárdenas et al. 2009) and larval distribution (Vásquez et al. 2013) support the hypothesis that *T. murphyi* is only one population across the South Pacific Ocean. The 100 % prevalence of *G. cruciata* found in *T. murphyi* could be concordant with the above studies. However, only one portion of its distributional geographical range was analysed. Therefore, future studies are necessary to obtain information on the life cycle and ecology of this parasite and to determine whether the prevalence of *G. cruciata* in *T. murphyi* changes spatially or temporally. These aspects are important for determining whether this parasite can be a good candidate as a biological tag for *T. murphyi* in the Pacific Ocean (Catalano et al. 2013).

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6. GENERAL DISCUSSION AND CONCLUSIONS

6.1 General discussion

For fisheries management and aquaculture, understanding the trends and developmental dynamics of oocytes must include the study of atretic oocytes during a reproductive cycle allowing identifying any change in the spawning period, or identified potential disruptor in the studied spawning season or the next (e.g. length of spawning season, fecundity, quality of egg). Species highly migratory like *Trachurus murphyi*, are exposed to several variables (environmental and physiology) that can produce that females delay or fail the reproduction. In this specie, despite their economic importance, reproductive biology is not fully understood but some recent studies have contributed to characterize the reproductive cycle (Leal et al., 2013; Perea et al., 2013). Most of the reproductive background regarding the impact of oceanographic conditions in the oceanic spawning habitat off central Chile has been described (Cubillos et al., 2008) with emphasis in the spatial distribution of egg and larvae (Cubillos et al., 2008; Vasquez et al., 2013).

Trachurus murphyi in an indeterminate spawner, and the production of egg it is not fixed at the beginning of the spawning season, and in a similar way that other indeterminate species continuous recruitment of new batches of mature oocytes can be spawned repeatedly during the reproductively active period (Murua and Saborido-Rey, 2003). During the spawning season, that can last for five months, females of *T. murphyi* can spawn every 3 to 5 days. The almost constant eggs production during an extended period requires a considerable amount of energy resources that can be obtained (1) from energy reserves accumulated prior to spawning (capital breeding), (2) directly from food input during the spawning season (income breeding), or (3) from both sources (Hunter and Leong, 1981).

During the spawning season, females can decrease or delays the production of egg. Fecundity is an important component of total egg production, and changes in fecundity have

a notable impact on reproductive potential and consequently, implications for fisheries management (Kjesbu et al., 1991; Murua and Saborido-Rey, 2003). Know how many eggs is produced by a female in a single spawning season, enable us to estimate spawning biomass with the Daily Egg Production Method (DEPM) (Hunter and Lo, 1997).

In indeterminate spawner, the batch fecundity (BF) is an important biological trait for the spawned biomass estimation with the DEPM and only hydrated oocytes are included in the method. During non-adequate conditions (environmental and/or physiological) egg production can decrease or fail via atresia. Atresia might be considered as an important mechanism for the adjustment of fecundity through the resorption of oocytes (Abaunza et al., 2003). Hydrated oocytes not always are the most abundant when sampling for DEPM; when hydrated oocytes are scarce, other developmental stage such vitellogenic oocytes are considered. This is especially true for *T. murphyi* due a reproductive strategy covering a large area in oceanic waters off central Chile (Barbiere et al., 1999).

This study summarizes what is known about the process of atresia in teleost fish. Atresia has been studied mainly using histological criteria, but recently molecular approach has been incorporated allowing us to identify genes that could be involved in the modulation of the atresia, especially in highly migratory species. In a fisheries context, Hunter and Macewicz (1985) described the rate of atresia in wild and captive northern anchovy *Engraulis mordax*, and this study is considered as a classic in reproductive studies due that indicates the importance of integrating atresia in the determination of reproductive state of female, identified the sequential morphological stages of atresia and its relationships with nutritional condition of wild and culture females. The histological characteristics allow to identify four atretic stages: alpha, beta, gamma and delta (Hunter and Macewicz 1985; Ganas et al., 2008). Atresia has been studies in term of prevalence and intensity, mainly for alpha and beta stage. In the Pacific sardine *Sardinops sagax* the analysis of alpha atresia allowed

estimating when alteration on extension of spawning season occur (Torres-Villegas et al., 2007). For Iberian sardine *Sardina pilchardus*, beta atresia can be used for describing the spawning history (Ganias et al., 2008). In yellowtail flounder *Limanda ferruginea* the downregulation of potential annual fecundity via atresia was analyzed (McElroy et al., 2016). In culture fish, atresia is used for identified disruption of developmental oocytes in artificial induced reproduction (Imanaga et al., 2014). Miranda et al. (1999) compare the duration of the atresia process between total spawning and partial spawning fish, suggesting that the extension of process is shorter in total spawning females. Independent of the study, it is necessary known the morphological characteristics of different stages of atretic stages of oocytes for any given species.

This study identified and characterize the three stages of atresia in *T. murphyi* during spawning season (Chapter 2). Three sequential atretic stages were identified: alpha, beta and gamma, as described for almost all teleost fish, included the horse mackerel *Trachurus trachurus* (Abaunza et al., 2003) and jack mackerel *Trachurus symmetricus* (Macewicz and Hunter 1993). For jack mackerel *Trachurus japonicus*, an induced atresia has been described according the fishing gear possibly due stress of fishing. Consequently, the expression of genes stress was considered as an adequate tool to study atresia in this species (Imanaga et al., 2014). In this study, alpha atretic oocytes could be identified and characterize early in the process (Chapter 2). Beta atresia could be used as proxy of the end of the spawning season, because it increases when the females showed and advanced developmental ovary stage (regenerating phase). Moreover, intensity of atresia in previous bath could be estimate according advanced atretic stage, as in *S. pilchardus* (Ganias et al., 2008). Hunter and Macewicz (1985) in the pioneer paper searching for driver of atresia, the starvation condition measured as food intake was evaluated. Ganias et al (2008) analyzed the relationship between atresia and biological traits (total length, Gonadosomatic index and presence of

postovulatory follicles) and temporal variability (year). Food availability and condition of fish was also considered by Gunnardsson et al. (2017). Among environmental factors affecting atresia, Miranda et al. (2013) evaluate the impact of temperature on atresia. To the best of our knowledge, a multivariate analysis including simultaneously biological traits (Fork length, Condition factor, Gonadosomatic index, presence of postovulatory follicles) and environmental variables (sea surface temperature and sample season) as drivers of atresia, has not been done. In *T. murphyi* the variations of prevalence and intensity of atresia were explained for all variables analysed (Chapter 2), except condition factor for prevalence, while month/year, sea surface temperature, condition factor and presence of postovulatory follicles were significant variables explaining intensity of atresia. The dynamic of alpha and beta-atresia could be considered a potential proxy for any disruption of spawning season. In addition, batch fecundity and relative fecundity were compared between females with atresia and not atresia as a determinant of the lower fecundity of *T. murphyi* when compared with other *Trachurus* spp.

Oogenesis is a complex process requiring the coordinated sequential expression of specific genes and ultimately leading to the release of gamete from the ovary (Juanchich et al., 2013). Mechanisms at molecular level that initiate and regulate oocyte atresia in teleost fish are poorly known (Lubzens et al., 2010) (Chapter 1). Transcriptome techniques are powerful tools and provide novel insights into physiological processes (e.g. reproduction), due that oocytes during its development pass through a series of defined stages, generating complex transcriptomic and proteome expression networks (García-López et al., 2011; Lubzens et al., 2017; Breton et al., 2019). In the rainbow trout *Oncorhynchus mykiss*, zebrafish *Danio rerio*, and medaka *Oryzias latipes*, molecular mechanisms involved in follicular development, vitellogenesis, oocyte maturation and sexual differentiation have been studied (Ma et al., 2012; Juanchich et al., 2013; Presslauer et al., 2017; Wong et al., 2018; Bouchareb et al.,

2017). For *D. rerio* have been demonstrate that several miRNA abundances are consistently higher in all gonadal stages, whereas others show specific expression during sexual stage development (Presslauer et al., 2017). Also, vitellogenesis and final maturation (oocyte mature) are regulated by a cascade of hormones along the brain–pituitary–gonad (BPG) axis (Mylonas et al., 2010). Some mechanisms of this axis in fish are not fully understood because their regulation is complex (Lubzens et al., 2010; Miranda et al., 2013). It is well established in mammals that ovarian follicular degeneration is a hormonally controlled apoptotic process and is an event of programmed cell death (Agullero et al., 2007). The molecular mechanisms of atresia are not well understood. Atresia can be induced by several factors in any parts of the reproductive cycle, especially during development oocytes, among others environmental variables (photoperiod, temperature), nutritional condition (inanition or low energy somatic) and endocrine disruption (contaminant). In mammalian apoptosis in the main mechanism that can involve in atresia, but also have been observed in some fishes species (Morais et al., 2012).

For species of economic importance as well as farmed species studies related to identified genes associated with atresia are increased (Gardner et al., 2012; Martyniuk et al., 20013) Fatty Acid-Binding Protein 11 (*Fabp11*) has been identified as main important gene with putative role in atresia of teleost such as the flatfish *Solea senegalensis* and Atlantic blue tuna *Thunnus thynnus* (Agullero et al., 2007; Garner et al., 2012). Surprisingly, *Fabp11* was not identified in transcriptomic of atretic females of *T. murphyi*, indicate that this gene is probably not an adequate marker for atresia in this species. Several genes associated to reproductive process in mammalian, included atresia were identified in transcriptome of this specie (Chapter 3). The liver transcriptome of *T. murphyi* we identified a global over-expression in samples showing atresia and for follistatin- like (*Fstl*) genes, the Ileal FABP (*Fabp6*), the Thrombospondin 1 (*Thbs1*) and TATA box binding protein (*Tbp*)-associated

factor, RNA polymerase I, B (Taf13), the difference are statistically significant. In ovaries transcriptome of *T. murphyi* a global over-expression in samples showing atresia is also observed except for Fabp6, vitellogenin (Vtg) and Taf13 (even difference are not significant). Those results seem to confirm a putative role of those gene in the reproduction and atresia process for *T. murphyi*. Also, the presence of several genes related with apoptosis and lipid metabolism as Fabp protein, may be indicated the importance of process in atresia of *T. murphyi*. For all genes identified in *T. murphyi* it is necessary evaluate relationships with the atresia, especially for genes that showed differential expression in the transcriptome of juvenile and atretic females (Chapter 3).

It is important to indicate that several genes associated with infection of protozoan parasites has been identified in transcriptome of *T. murphyi*. Genes as the Immunity-related GTPase (IRG) gene family as Immunity Related GTPase cinema (*IRGC*) but also arginase 1 (*ARG*), B cell CLL/lymphoma 3 (*BCL3*), V-set domain containing T cell activation inhibitor 1(*VTCN1*), Interleukin 12B (*Il12b*) could be related with infection with *Goussia cruciata* in *T. murphyi* (Chapter 4). Expression of *BCL3* and *Il12b*, seems to be differential between atretic females (*IRG*, *BCL3* and *Il12b*) whereas *VTCN1* and *ARG* are expressed in both, atretic and non-atretic females. The role of *G. cruciata* in atresia cannot be established, because all fish analyzed were parasitized. Infection levels of *G. cruciata* in *T. murphyi* apparently do not produce negative effects on fish condition (Chapter 4). This transcriptome provides an important database from which functional studies can be performed, and future studies allow us to understand the gene expression associated to reproductive process, atresia, parasitic infection and metabolism.

The results obtained in this thesis, allows to propose a conceptual model for endogenous variables (size females, gonadosomatic index (GSI) and condition factor (K)) and exogenous variables (sea superficial temperature (SST), year-month (Y-M)) that would be modulating

follicular atresia in Chilena jack mackerel *Trachurus murphyi*. Also, the presence of parasites and the feeding are important to understand the follicular atresia in this species (Figure 1).

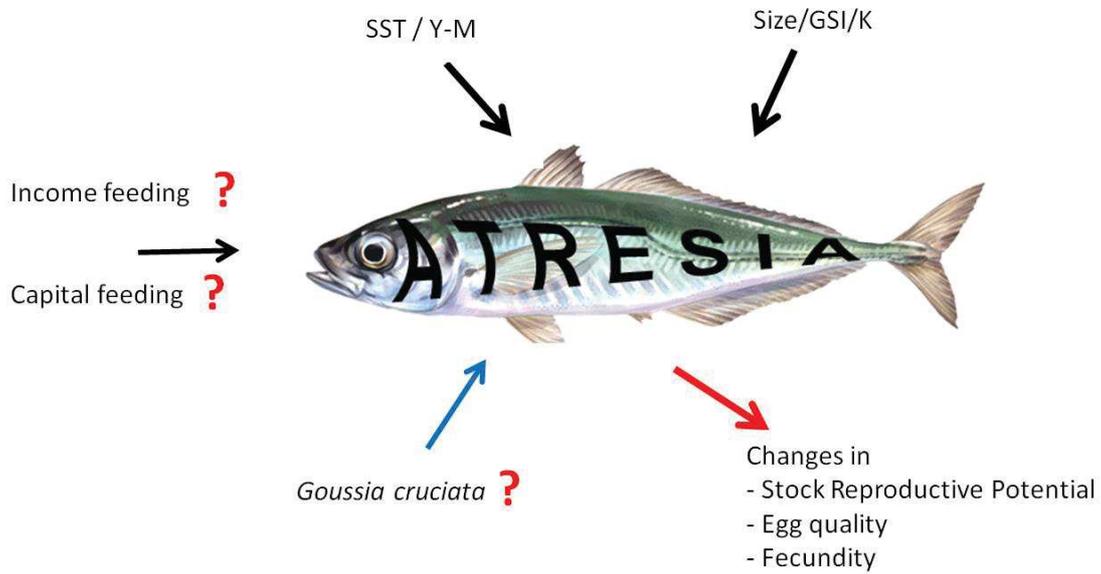


Figure 1. Conceptual model for variables that modeling the follicular atresia in Chilena jack mackerel, *Trachurus murphyi*.

The black arrows indicate direct effect this variables in atresia, the blue arrow potential effect in atresia and red arrow indicate effect of atresia in reproduction. Question mark indicate mechanics not known. SST: See superficial temperature, Y-M: Year-Month, GSI: Gonadosomatic index, K: Condition factor.

6.2 Conclusions

- In this study, three main atresia stages were identified: alpha, beta and gamma. The alpha atresia showed three sub-stages that allowed identified the early beginning of the atretic process in ovaries for this species. In *T. murphyi*, the dynamic of alpha and beta-atresia could be considered as a potential proxy for any disruption of spawning season and the end of spawning season, respectively, because increase its prevalence and intensity at the end of the spawning season. To understand the dynamics of the atresia process, it is necessary to examine the seasonal dynamics of atresia through the whole maturation cycle.
- The binomial GLM models tested shows that the endogenous variables as gonadosomatic index (GSI) and postovulatory follicles determined the prevalence of atresia in *T. murphyi*. The condition factor was not important variable that explain the prevalence of atresia in this species. For model 2 (only alpha atresia) the GSI was not significative for prevalence of atresia. The sea surface temperature (SST) is an important environmental variable for explain the prevalence of atresia observed in all models. All variables with exception GSI explain the intensity of atresia. These results allow us to accept the hypothesis proposed in this thesis.
- In this study, the review of genes that can have a putative rol in atresia of teleost fish, allowed to be identified 20 potential genes associated with atresia in relation to apoptosis/autophagia, lipid metabolism, oxidative metabolism and other physiological process similar to identified in other vertebrates, even mammalian. For transcriptome of atretic females and non-atretic females of *T. murphyi*, showed that the presence of potential genes associated with atresia in this species, such as

follistatin (Fst) and Fatty acid-binding protein (Fabp), and several genes relationship with apoptosis (e.g. caspase) and lipid metabolism. To understand the expression pattern of these genes in ovary and liver of *T. murphyi*, it is necessary to examine several females that showed the whole development oocytes, including females without atretic oocytes.

- For all *T. murphyi* analysed for coccidian, the abundance of *Goussia cruciata* was not relationship with condition factor and hepatosomatic index. Conversely, the abundance of *G. cruciata* was related negatively with fork length. The role of the parasite *G. cruciata* in atresia in this species require the analysis of non-infected females, not found in this study. The transcriptome database showed several genes associate with protozoan infection, therefore it is important to analyse the effect of this type of parasites in this species, especially in the entire reproductive process.

7. FORTHCOMING

- For all atresia stages identified in *T. murphyi*, it is therefore necessary to examine the seasonal dynamics of atresia through the whole maturation cycle and the relationships with the body condition, environmental variables (i.e., temperature) and fecundity during the whole spawning season.
- Estimate if beta atresia can be used as a proxy of reproductive disruption in *T. murphyi*, what would allow us identified for example, extension of spawning period, change in the fecundity and evaluate direct effect in future recruitment in this species.
- In *T. murphyi*, the prevalence and intensity of atresia may vary between spawning period, although the contribution of eggs spawned by atretic females is lower, when compared with non-atretic females, it is important to correlate the trends of atresia with fecundity during the whole spawning season, and the exclusion of atretic females in the Daily Eggs Production Method (DEPM) should be re-evaluated, otherwise spawning biomass estimate from DEPM could be underestimate
- Additional study regarding gene expression in this species, must be done in order to identified those with a differential expression between the different maturity stages, not covered in this study. Besides, the atresia related-genes identified in this this species (e.g. Fabps, apoptosis, caspases), should be evaluated for identified candidate gene for follicular atresia for *T. murphyi*.

- The relationship of parasite *Goussia cruciate* with follicular atresia should be evaluated, it necessary compared non parasitized females with different levels of parasitism intensity and thus be able to understand the effect of this parasites in the reproduction of *T. murphyi*. Also, the genes associate with protozoan infection must be evaluated in the entire reproductive process.

8. REFERENCES

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Título: Atresia en el jurel *Trachurus murphyi* Nichols, 1920: implicaciones para la dinámica reproductiva

Palabras clave: Atresia, transcriptómica, *Trachurus murphyi*

Resumen: El jurel *Trachurus murphyi* soporta una importante pesquería en el Océano Pacífico Sudeste, la que ha disminuido considerablemente con el tiempo. En esta tesis, se describe la morfología de los ovocitos atrésicos en *T. murphyi*, y la prevalencia e intensidad de la atresia. Se identificaron tres estados de atresia: alfa, beta y gamma. Modelos lineales generales indican que el mes/año, temperatura superficial del mar e incidencia de folículos postovulatorios explican la prevalencia e intensidad de atresia. La dinámica de las etapas de atresia alfa y beta podría considerarse como un indicador potencial de cualquier perturbación en la temporada de desove de esta especie. Este estudio constituye una primera evaluación comparativa de la fecundidad entre hembras atrésicas y no atrésicas para esta especie.

La fecundidad parcial y la fecundidad relativa fueron menores en comparación con otras especies de *Trachurus*. Para *T. murphyi* se generó una base de datos transcriptómicos (hígado y ovario). Los resultados muestran un set de genes potenciales como marcadores de atresia, como fatty acid-binding protein (FABP) genes y follistatin. El análisis transcriptómico mostró la expresión de genes asociados a una infección de protozoos. Todos los hígados de *T. murphyi* están parasitados con *Goussia cruciata* (Apicomplexa). La abundancia del parásito se correlaciona negativamente con la longitud total del hospedador. No se puede establecer una relación potencial entre la infección por protozoos y la atresia.

Titre : Atrésie chez le chinchard *Trachurus murphyi* Nichols, 1920: implications dans la dynamique de la reproduction

Mots clés : Atrésie, transcriptomique, *Trachurus murphyi*

Résumé : Le chinchard, *Trachurus murphyi* constitue une espèce importante pour la pêche dans le sud-est de l'océan Pacifique. Dans cette thèse, la morphologie des ovocytes atrétiques, prévalence et l'intensité de l'atrésie ont été décrits. Les stades d'atrésie identifiés sont les stades alpha, bêta et gamma. Les modèles linéaires généraux ont montré que l'incidence des follicules post-ovulatoires, la température de surface de la mer et le facteur mois/année, expliquent la prévalence et l'intensité. La dynamique des stades alpha et bêta-atrésie pourrait être considérée comme un indicateur potentiel de toute perturbation de la saison de frai de cette espèce. Cette étude constitue une première évaluation comparative de la fécondité entre les femelles atrétiques et non atrétiques pour cette espèce.

La fécondité partielle et la fécondité relative sont inférieures à celles observées chez d'autres espèces de *Trachurus*. *T. murphyi*, la base de données transcriptomique (tissus du foie et des ovaires) a été générée. Cette étude a notamment permis d'identifier quelques marqueurs plus spécifiques de l'atrésie comme des protéines de liaison aux acides gras (FABP) et de la follistatine. L'analyse transcriptomique a démontré l'expression de gènes potentiellement associés à une infection par des protozoaires. L'ensemble du foie de *T. murphyi* est parasité par *Goussia cruciata* (Apicomplexa). L'abondance du parasite était corrélée négativement à la longueur totale de l'hôte. Une relation potentielle entre l'infection par les protozoaires et l'atrésie ne peut être établie.

Title : Atresia in the Chilean Jack Mackerel *Trachurus murphyi* Nichols, 1920: implication for reproductive dynamics

Keywords : Atresia, Transcriptomic, *Trachurus murphyi*

Abstract: The Chilean jack mackerel *Trachurus murphyi* support an important fishery in the South-eastern Pacific Ocean which has declined considerably over time. In this thesis, morphology of atretic oocytes in *T. murphyi* as well, patterns of prevalence and intensity of atresia were described. The stages of atresia identified were alpha, beta and gamma. General linear models showed that incidence of postovulatory follicles, sea surface temperature and month/year, explain the prevalence and intensity of atresia. Dynamics of alpha and beta-atresia could be considered a potential proxy for any disruption of spawning season and the end of spawning season. This is the first assessment comparing fecundity between atretic and not atretic females.

The batch fecundity and relative fecundity were lowest compared with other species of *Trachurus*. In *T. murphyi*, the transcriptomic database (liver and ovary tissue) was generated. The results show a set of core potential genes as markers for atresia as fatty acid-binding protein (FABP) genes and follistatin. The transcriptomic analysis demonstrated the expression of genes associated to protozoan infection. All liver of *T. murphyi* were parasitized by *Goussia cruciata* (Apicomplexa), the abundance of the parasite was negative correlated with the host total length. A potential relationship between protozoan infection and atresia cannot be established.