AMERICAN UNIVERSITY OF BEIRUT

REPRODUCTION OF THE LESSEPSIAN INVADER *FISTULARIA COMMERSONII* OFF THE COAST OF LEBANON

by
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AN ABSTRACT OF THE THESIS OF

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The opening of the Suez Canal allowed the unidirectional introduction of tropical species from the Red Sea into the Mediterranean Sea, a phenomenon termed Lessepsian migration. One of the recent invaders is the bluespotted cornetfish, *Fistularia commersonii*, recorded for the first time in the Mediterranean in the year 2000. Within a few years of its introduction, the species colonized almost the entire Mediterranean. The present study was conducted along the Lebanese coast between 2005 and 2010. It characterized the reproductive biology of *F. commersonii* in an effort to explain some of the causes of its invasive success in the Mediterranean.

In the Eastern Mediterranean, the reproductive season began in April, when seawater temperature was above 20 °C, and extended across nine months. Spawning started in May and extended until December. Somatic condition increased throughout the reproductive season and was low only during the cold months.

Spermatogenesis was cystic within unrestricted lobular testes. *F. commersonii* appeared to have an asynchronous ovarian development and a batch-spawning pattern. The presence of new and old post-ovulatory follicles confirmed repeated spawning events. Three developing batches of oocytes were frequently found at one time in the ovary. The average batch contained 18,000 oocytes. Number of oocytes per batch decreased progressively with the season. Gonad weight is the best predictor of both fecundities. Oocyte development is similar to most fish species, but the perinucleolar stage showed a single large nucleolus as opposed to several peripheral nucleoli. Oocyte atresia was frequent but low in reproductively active gonads, increasing in frequency and amount towards the end of the spawning season.

Macro- and microscopic staging approaches were compared. The macroscopic scheme was considered appropriate, with higher than 75 % concordance. Moreover, *F. commersonii* had a general weight-length relationship of $W_G = 1.066 \times 10^{-4} L_T^{3.4063}$, showing a positive allometric growth. The sex ratio between females and males is 1 : 1, and sexual maturity is reached at 57 cm L_T.

The reproductive features investigated in the current study discussed some of the causes behind *Fistularia commersonii* success and adaptations in the Mediterranean.

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INTRODUCTION

A. The Mediterranean Sea

1. History, Geography, and Geology

Lebanon is one of the many countries that have the Mediterranean Sea as a part of their coastal waters. The Mediterranean itself may be considered one of the main affected locations among aquatic environments worldwide in light of increasing biological invasions and climate change (Lejeusne *et al.*, 2010).

Today's Mediterranean is geologically a relatively new sea. It is a "wedge-shaped" remnant of the old Tethys Ocean (Bianchi & Morri, 2000). During the Messinian age, from 5.96 to 5.33 million years ago, the Mediterranean basin dried out due to the closure of the corridors connecting it to the Atlantic Ocean (Duggen *et al.*, 2003). This period is known as the Messinian Salinity Crisis and might have resulted in the annihilation of most of the Mediterranean biota (Quignard & Tomasini, 2000). After the reestablishment of a marine pathway with the Atlantic Ocean by way of the strait of Gibraltar, the waters of the Atlantic flowed into the now-empty Mediterranean basin, repopulating it with marine organisms of Atlantic origin (Quignard & Tomasini, 2000). However, this has not been the only event to shape the faunal profile of this nearly landlocked sea, a profile that to this day is constantly evolving and changing.

Geographically, the Mediterranean Sea covers nearly 25 million km^2, with a shoreline of around 46,000 kilometers in length. It has an average depth of 1,470 meters and a maximum depth of around 5,267 meters. The Mediterranean occupies merely 0.82

% of the total area of world's oceans and 0.3 % of the total volume (Lejeusne *et al.*, 2010). It has a narrow continental shelf of less than 200 m, and a steep continental slope (Manca *et al.*, 2004). Physical oceanographers consider the Mediterranean to be a "miniature ocean" (Bethoux & Gentili, 1999).

The Mediterranean Sea can be divided into two major basins, Eastern and Western that in turn can be divided into 11 subregions, according to Laubier (2005): the Alboran, North-Western, South-Western, Tyrrhenian, Adriatic, Ionian, Central, Aegean, North-Levantine, South-Levantine, and Marmara sub-basins. Other schemes of division have also been presented (*e.g.* Bianchi, 2007). The shallow Straits of Sicily are traditionally considered to be the border between the Eastern and Western basins, though this might be because of a surface isotherm of 15 °C in February instead of a physical barrier effect (Bianchi, 2007).

2. Hydrology

Hydrologically, the Mediterranean Sea, and especially the Eastern basin, is characterized by marked oligotrophy in comparison to neighboring oceans due to a deficiency of upwelling areas and low contribution of land discharge (Azov, 1991). It is nutrient-poor and thus with low primary productivity, which decreases from the west to the east, with the Western Mediterranean showing three times more productivity than the Eastern (Turley, 1999).

The Mediterranean also exhibits a temperature gradient from west to east. Sea surface temperatures during winter show a range from around 18 °C in the Levantine to 13 °C in the North-Western sub-basin, with the Alboran sub-basin (Gibraltar) showing 15 °C (Millot & Taupier-Letage, 2005). The sub-basin with the lowest temperatures is

the Adriatic, with a winter mean of 10 °C (Millot & Taupier-Letage, 2005). The Mediterranean deep waters, however, are homogeneous in temperature with an average of 12.8 °C all year round below 250 m depth (Laubier, 2005).

Moreover, the Mediterranean shows higher overall levels of salinity as compared to the Atlantic Ocean as evaporation is greater than precipitation (Laubier, 2005). This enforces a salinity gradient on the entire Mediterranean, with incoming Atlantic waters of a mean salinity of 36.2 parts per thousand (ppt) on one end and the Levantine waters that exceed 39 ppt in salinity on the other (Bethoux & Gentili, 1999; Laubier, 2005). As with temperature, the deep Mediterranean waters have a homogeneous salinity of 38.2 ppt (Bethoux & Gentili, 1999; Laubier, 2005). A low-salinity surface water current flows from the Atlantic into the Mediterranean, whereas a high-salinity deep current, denser than the incoming Atlantic water, moves in the opposite direction across the Gibraltar Strait (Laubier, 2005; Millot & Taupier-Letage, 2005). Circulation of surface waters in the Mediterranean occurs in a counterclockwise direction due to the entering Atlantic waters and the sinking Mediterranean waters at their coldest points in the Adriatic and North-Western sub-basins (Millot & Taupier-Letage, 2005; Lejeusne *et al.*, 2010). In the Levantine sub-basin, overall circulation is cyclonic, having a mainly vertical movement across the Lebanese waters, from the south to the north (Özsoy *et al.*, 1989).

3. Hydrological Disturbance

The normal hydrology of the Mediterranean Sea, however, is under increasing danger. Direct and indirect anthropogenic pressures on the Mediterranean waters from population growth to pollution, habitat destruction, overfishing, species invasions, and

climate change have made it one of the most impacted seas on Earth (Laubier, 2005; Lejeusne *et al.*, 2010). This loss of equilibrium has affected the nutrient cycles, temperature gradients, salinity trends, and circulation patterns.

First, pollution has increased the overall levels of nutrients, especially phosphorus and carbon dioxide in the Mediterranean waters (Bethoux *et al.*, 2005). This increase in phosphorus would lead to increased CO_2 uptake by the sea, and the alteration of the ecosystems' planktonic life from diatoms to non-silicious species (Bethoux *et al.*, 2005). This might have unprecedented effects on the ecological functioning of the Mediterranean ecosystems, for example with blooms of toxic non-silicious phytoplankton, eutrophication, and the subsequent oxygen depletion (Turley, 1999).

Second, and according to climatic models, the Mediterranean will be one of the areas that will be very highly affected by the ongoing climatic changes that are producing an overall warming trend (Parry, 2000), with climate change acting synergistically with other anthropogenic influences to exacerbate the pressure on the native Mediterranean biodiversity (Bianchi, 2007; Lejeusne *et al.*, 2010). Overall warming of the waters is thought to occur as a result of the greenhouse effect, and has been documented and confirmed over the years (Bethoux *et al.*, 1990; Bethoux *et al.*, 1999; Turley, 1999; Vargas-Yáñez *et al.*, 2008). A general increase of water temperature was recorded in the deep waters of the Northwestern Mediterranean by 0.12 °C and the shallower depths by as much as 1.4 °C along the Catalan coast (Bethoux *et al.*, 1990; Vargas-Yáñez *et al.*, 2008; Lejeusne *et al.*, 2010). Though data is absent concerning the Levantine sub-basin, there are reports of a nearly 1 °C increase in the Aegean surface water temperatures nearby (Theocharis, 2008).

Third, the overall salinity levels in the Mediterranean have been increasing, especially in the Levantine, as a result of the decrease of freshwater input into the sea. This is mainly due to the construction of the High Aswan Dam over the Nile in 1964 (Bethoux & Gentili, 1999). According to these authors, these salinity changes would have adverse effects on the overall hydrology of the Mediterranean (Bethoux & Gentili, 1999). The warming trend of the waters is also implicated in the observed increase in the salinity across the Mediterranean (Bethoux & Gentili, 1999). Therefore, there is a clear deficit in the hydrological balance in the Mediterranean due to the high evaporation rates, low freshwater input from land, low seasonal precipitation, and damming of the rivers (Turley, 1999).

Finally, due to the above-mentioned climatic anomalies and salinity changes, major disturbances of the Mediterranean circulation were observed and termed the East Mediterranean Transient (EMT), resulting in hydrological changes in the Mediterranean waters (Lejeusne *et al.*, 2010). The EMT resulted in significant changes in the thermohaline circulation of the Eastern Mediterranean, with data showing its spread into the Western basin as well, with further disruptions in the circulation patterns (Schroeder *et al.*, 2008; Lejeusne *et al.*, 2010). The EMT in turn resulted in further changes in the temperature and salinity patterns, in addition to the nutrient cycles, which in turn altered the biology and ecology of the rich Mediterranean biota (Lejeusne *et al.*, 2010). All the above-mentioned hydroclimatic changes in the Mediterranean are modifying its original faunal profile (Quignard & Tomasini, 2000).

4. Biodiversity

The Mediterranean Sea is an important biodiversity hotspot (Bianchi & Morri, 2000; Myers *et al.*, 2000). In the aftermath of the Messinian Salinity Crisis, organisms from the Atlantic Ocean penetrated into the newly reestablished sea, providing the basis for the current fauna and flora of the Mediterranean (Bianchi & Morri, 2000). Due to the hydroclimatic gradients discussed above, the species with temperate origins settled into the northern and western reaches of the Mediterranean whereas the subtropical species settled southwards and eastwards (Quignard & Tomasini, 2000). The vast diversity of Mediterranean biota has been attributed to both these Atlantic species and the extensive evolutionary history of the Sea since the Tertiary period (Bianchi & Morri, 2000). Current estimates of biodiversity in the Mediterranean are held to be around 10,000 – 12,000 species, excluding bacteria, and new species are consistently being described (Boudouresque, 2004; Laubier, 2005). Bianchi and Morri (2000) pen the number of known species at around 8,500. The Mediterranean is estimated to harbor 4 – 18 % of the marine species of the world (Bianchi & Morri, 2000). Laubier (2005) puts the percentage of Mediterranean fauna in relation to the world marine fauna at 7 % and the flora at 18 %. Moreover, the Mediterranean is characterized by having a high rate of endemism, *i.e.* species exclusively native to indigenous in a particular locality (Bianchi & Morri, 2000; Lejeusne *et al.*, 2010). Quignard and Tomasini (2000) consider the Mediterranean unique in its capability to produce new species and sub-species.

Global fish biodiversity is estimated to be close to 30,000 fishes (Nelson, 2006). Fish biodiversity in the Mediterranean, in its broadest sense, was estimated in 1997 to be 664 species (Quignard & Tomasini, 2000). This number, however, includes 127 exotic species of Atlantic or Red Sea origin (Bianchi & Morri, 2000). Native

Levantine species richness was estimated by the same authors to be 350 species (53 % of total fish biodiversity), whereas North-Western Mediterranean (Catalan Sea) fish biodiversity was estimated to be 463 (70 %), showing the gradient of biodiversity to be decreasing from west to east.

The Mediterranean biodiversity, however, is under direct threat from the constantly increasing anthropic pressure (Lejeusne *et al.*, 2010). Turley (1999) anticipated a great imbalance in the Mediterranean as a product of the interaction of the different anthropogenic disturbances, and resulting in disastrous effects. Under the influence of climatic change, biodiversity is adversely affected through three major mechanisms: (1) direct effects of climatic imbalance on marine species, (2) changes in biotic interactions, and (3) indirect effects of hydrological disturbances (Bianchi & Morri, 2000). The direct effects may be in changing survival rates, patterns of reproduction and behavior, the changing biotic interactions in selectively favoring interspecies competition, and the indirect effects in changing the normal patterns of circulation, among others (Morri & Bianchi, 2001). The same authors established a connection between climatic and biodiversity shifts in the Ligurian Sea (North-Western Mediterranean) (Morri & Bianchi, 2001). According to Lejeuse *et al.* (2010), climatic forcing has changed both top-down and bottom-up interactions in the Mediterranean ecosystems. Moreover, and because of the overall increase in the temperatures of Mediterranean waters, species range expansions northwards have been observed, especially in the colder North-Western sub-basin (Bianchi & Morri, 2000; Lejeusne *et al.*, 2010), in addition to range shifts of some species possibly resulting from the climatic anomalies such as the EMT (Bianchi, 2007). And in light of all these disturbances, the crisis of biological invasions and introductions of exotic species rises

to the forefront of biodiversity and conservation issues of the Mediterranean. As such, one of the markers of global change is the increase in biological invasions (Occhipinti-Ambrogi, 2007). Concerning the Mediterranean Sea, this is embodied by the phenomenon of Lessepsian migration.

B. Lessepsian Migration

The Mediterranean Sea has been invaded by nearly 600 alien species (Galil, 2008), around 67 % of which are Lessepsian migrants, species of tropical origin (Lejeusne *et al.*, 2010). Moreover, the Levantine sub-basin has more than 400 alien species, with Lessepsians representing more than 80 % (Galil, 2008). Lessepsian migration is the unidirectional movement of Red Sea biota into the Mediterranean by way of the Suez Canal (Por, 1978). This movement was named "Lessepsian" in reference to the architect of the Suez Canal, the frenchman Ferdinand de Lesseps (Por, 1978). The construction of the Suez Canal in 1869 removed a major biogeographic barrier, and the Mediterranean and Red Seas were artificially connected (Ben Rais Lasram *et al.*, 2008; Mavruk & Avsar, 2008), allowing the influx of Red Sea biota into the Mediterranean (Bianchi & Morri, 2000). This has resulted in the alteration of the natural Levantine marine communities into mixed Red-Mediterranean ones (Fishelson, 2000; Galil, 2000), which will likely lead to a homogenization of the species assemblages at some point between the Red and Mediterranean Seas (Ben Rais Lasram *et al.*, 2008). Ever since the appearance of the first Red Sea species in the Mediterranean, the mollusc *Pinctada radiata* (Leach, 1814) in 1874, the process has been continuous and is ongoing (Spanier & Galil, 1991; Galil, 2008; Mavruk & Avsar, 2008).

After the construction of the Suez Canal, invasion was mostly blocked and very few resilient species were able to cross to the Mediterranean due to the high salinity of the Bitter Lakes located in the Canal (Spanier & Galil, 1991; Ben Rais Lasram *et al.*, 2008). The weight of Lessepsian migration was not very obvious till the 1970's, when the invasions were accelerated due to the decreased salinity of the Bitter Lakes as well as the lowered freshwater input from the Nile into the Mediterranean following the construction of the High Dam in Aswan in 1967 (Spanier & Galil, 1991; Bianchi, 2007). And so, the bulk of alien species introductions into the Mediterranean took place during the last few decades (Bianchi & Morri, 2000). Lessepsian migration has increased dramatically such that in a recent estimate, over the space of 5 years, there was an average of one introduction per month (Streftaris *et al.*, 2005).

Methods of crossing the Suez Canal into the Levantine basin are debated and many mechanisms hypothesized (Shefer *et al.*, 2004). Por (1978) hypothesized a stepwise movement, whereas other authors favored the single-step hypothesis due to the absence of some species in the Canal itself (Safriel & Lipkin, 1972; Agur & Safriel, 1981). Most of the alien species are littoral or sublittoral, due to the route of introduction being the relatively shallow Suez Canal (Galil, 2008).

Though Por (1978) considered Lessepsian invasion to be harmless to the natural Mediterranean and especially Levantine ecosystems if not outright beneficial, increased interest and research into biological invasions is showing it to be harmful, especially concerning biodiversity (Galil, 2007, 2008; Mavruk & Avsar, 2008; Ben Rais Lasram & Mouillot, 2009).. However, it is not easy to disentangle the causes and effects of the ecosystem-level changes that introducted species are bringing about (Galil, 2007; Ben Rais Lasram *et al.*, 2008). What can be expected is a loss of native biodiversity,

especially endemic species, as the eventual result of the spread of introduced species (Ben Rais Lasram *et al.*, 2008).

Introductions of exotic species have led to native species' extinctions worldwide, though evidence of direct competition being the cause of it is rare (Davis, 2009). Accroding to Davis (2009), intertrophic interactions and habitat loss are more likely causes for the insuing extinction events. Other authors have shown that local extinctions of native species may occur as a result of competitive interactions, predation, or the sheer number of invading individuals (Lande, 1993; Roemer *et al.*, 2002; Olden *et al.*, 2006). The end result will be biotic homogenization with decreased biodiversity where native species are replaced by exotics (McKinney & Lockwood, 1999), though Davis (2009) argued that homogenization does not necessary lead to low biodiversity. However, to date not a single marine species has ever been considered extinct. In the Mediterranean, exotic species have displaced some natives and resulted in a decrease in their abundance, even though the causal factors and the ecosystem-level interactions are not well-studied (Galil, 2007).

It seems that the Mediterranean Sea is moving towards "tropicalization" (Bianchi, 2007). In light of the increase of the temperatures of the Mediterranean waters, there is an increase in the abundance of subtropical species as opposed to the more temperate ones (Lejeusne *et al.*, 2010). The same authors, however, did not attribute the dramatic increase in the number of invading species to the warming trend of the Mediterranean (Lejeusne *et al.*, 2010), whereas others argued that warming might increase the chances of new invasions (Ben Rais Lasram & Mouillot, 2009; Walther *et al.*, 2009). The enlargement of the Suez Canal is also hypothesized to be an important factor for increased invasions (Galil, 2007). Regardless as to whether climate change is

affecting the number of species introductions, there is a common consensus that it is leading to the range expansions of established invasive populations through various direct and indirect mechanisms (Galil, 2008; Ben Rais Lasram & Mouillot, 2009; Walther *et al.*, 2009), though Galil (2008) warned against the simplification of the explanations for causality, suggesting synergistic effects with different variables such as pollution, habitat destruction, overexploitation of species, etc.

As with all the biota, the ichthyofaunal profile of the Mediterranean Sea, and especially of the Levantine sub-basin, has been severely affected by Lessepsian migration (Ben Rais Lasram *et al.*, 2008; Mavruk & Avsar, 2008). In the light of the above-mentioned climatic changes, Lessepsian fishes are expected to have an even greater impact as they establish in greater abundance and range at the expense of the local fish assemblages (Ben Rais Lasram *et al.*, 2008). Currently, around 80 species of Lessepsian fishes have been recorded in the Mediterranean waters, and the list is constantly expanding (Golani *et al.*, 2004; Bariche & Saad, 2005; Golani *et al.*, 2008; Galil, 2009; Bariche, 2010a; Bariche, 2010b). The first reported Lessepsian fish was *Atherinomorus lacunosus* (Forster, 1801), recorded 33 years after the construction of the Suez Canal, in 1902 in Alexandria by Tillier (Ben-Tuvia, 1985). The most recent is *Champsodon vorax* Günther, 1867, reported in May 2010 by Bariche from Lebanon (Bariche, 2010b).

Some Lessepsian migrants are becoming important fisheries species in the Eastern Mediterranean (Spanier & Galil, 1991; Golani & Ben-Tuvia, 1995). They have high catchment per unit effort values in the Eastern basin, with as much as 70 % biomass composition in the demersal fisheries of Mersin Bay, Turkey (Gücü & Bingel, 1994). In 1973, Lessepsian migrants consituted 21 % of the Israeli trawl fisheries, with

Sphyraena chrysotaenia Klunzinger, 1884, representing half the barracuda catchment (Ben-Tuvia, 1973). The Lessepsian goatfish *Upeneus moluccensis* (Bleeker, 1855) and lizardfish *Saurida undosquamis* (Richardson, 1848) are also perfect examples in fisheries impact, where the former has been reported to occupy 87 % of the mullet catchment in shallow waters and the latter nealry two-thirds of the fisheries landings in Mersin in the 1980's (Spanier & Galil, 1991; Gücü & Bingel, 1994; Golani & Ben-Tuvia, 1995). Moreover, the Lessepsian siganids *Siganus rivulatus* Forsskål, 1775, and *S. luridus* (Rüppell, 1829), the sweeper *Pempheris vanicolensis* Cuvier, 1831, and the squirrelfish *Sargocentron rubrum* (Forsskål, 1775) have emerged as the most common species on rocky substrata in the Levantine basin in general and Lebanon in particular (Spanier & Galil, 1991; Harmelin-Vivien *et al.*, 2005).

In light of these radical changes in the Levantine ichthyofauna, with Lessepsian species occupying increasingly broad ranges and attaining higher population densities than the natives, the invasion and establishment of the bluespotted cornetfish *Fistularia commersonii* Rüppell, 1835 (Teleostei: Fistulariidae) came as one more stressor in a long chain of bioinvaders, though one that would leave a significant mark on the Mediterranean ecosystem in an astonishingly short time.

C. Reproduction in Teleosts

1. Reproductive Diversity

Fishes are the most diverse vertebrate group in species numbers as well as ways of life, showing amazing variety in reproductive characteristics (Redding & Patiño, 2000a; Moyle & Cech, 2004). In this regard, many different behaviors ranging from monogamy to promiscuity (Moyle & Cech, 2004; Pavlov *et al.*, 2009), anatomies from gonochoristic to hermaphroditic to asexual (Redding & Patiño, 2000a), and strategies from oviparity to various types of viviparity have been observed (McMillan, 2007). And though some fishes show dimorphisms and dichromatisms that enable sex determination externally, it is usually distinguished through visual inspection of the internal anatomy (Redding & Patiño, 2000a; Moyle & Cech, 2004).

In most dioecious species, testes produce the sperm while ovaries generate the ova (Redding & Patiño, 2000a). On the other hand, strategies other than gonochorism are present, such as hermaphroditism, where a single fish may have both testicular and ovarian tissues (Pavlov *et al.*, 2009). Hermaphroditism may be synchronous (both types of tissues are active concurrently) or sequential (sex change occurring at some point during the life cycle) (Redding & Patiño, 2000a; Pavlov *et al.*, 2009). As such, species may be protandrous (a male turning into a female) or protogynous (a female turning into a male) (Moyle & Cech, 2004). There are other strategies as well such as alternative male strategies or unisexuality, both of which are relatively rare (Moyle & Cech, 2004).

Most teleosts are oviparous, with fertilization happening externally in the water or, in some cases, internally after which the eggs are laid (McMillan, 2007). There are also few ovoviviparous and viviparous species that develop their young internally after copulation (McMillan, 2007). In addition to the above-mentioned anatomic differences,

fishes are characterized by a very broad range of breeding behaviors, with species categorized generally as non-guarders, guarders, and bearers (Moyle & Cech, 2004; Pavlov *et al.*, 2009). Teleosts are usually non-guarders, with species being mostly pelagic or benthic spawners (Moyle & Cech, 2004; Pavlov *et al.*, 2009).

2. General Characteristics

Gonads in teleosts are bilaterally paired, extending along the peritoneal cavity and attached to the dorsal peritoneum. They may be fused partly or completely in some species (Redding & Patiño, 2000a). Teleosts usually have separate gonoducts that end at the genital pore independent of the urinary and digestive tracts, knowing that there may be anatomical modifications in case of specialized forms of reproduction (Redding & Patiño, 2000a; Moyle & Cech, 2004). Testes are usually somewhat flattened with a lobular external morphology which may be irregular along their length, and whitish in color due to the accumulating sperm inside the tissues (Redding & Patiño, 2000a). On the other hand, ovaries are sac-like, sometimes seeming granular due to developing oocytes, and range in color from clear to yellow to pink depending on the eggs' pigmentation (Redding & Patiño, 2000a).

3. Ontogeny and Differentiation

The original differentiation of embryonic cells into sex cell precursors, primordial germ cells (PGC), occurs prior to the development of the gonads in the early stages of ontogeny (McMillan, 2007; Pavlov *et al.*, 2009). The gonads themselves "develop from paired masses of mesodermal tissue on either side of the dorsal mesentery in the dorsolateral lining of the peritoneal cavity," and are covered by a

germinal epithelium (McMillan, 2007). These sexually indiscriminate (indifferent) genital ridges expand into the growing body cavity, and the PGC's migrate into them, just under the gonadal epithelium, forming the basis for oogenic and spermatogenic cells later on (McMillan, 2007; Pavlov *et al.*, 2009). Subsequently, the PGC's multiply mitotically into numerous gonia, forming the initial indifferent gonad (McMillan, 2007; Pavlov *et al.*, 2009). Following this proliferation of PGC's, sexual determination for the gonads takes place as anatomical (related to the structure of the gonad) and cytological (related to the characteristics of the developing gonia) differentiation processes change indifferent gonads into ovaries or testes (Pavlov *et al.*, 2009). Eventually, PGC's mitotically develop into oogonia or spermatogonia (Pavlov *et al.*, 2009).

4. Internal Structure

The ovaries of teleosts may be gymnovarian, where oocytes are ovulated into the coelomic cavity, or, more commonly, cystovarian, where there is a definite ovarian lumen (ovocoel) into which ovulation takes place, and which is in most cases connected directly to the ovarian duct system (Redding & Patiño, 2000a; McMillan, 2007). The ovary, covered by an epithelium, is composed of follicles containing the ova packed into a system of vascularized connective tissue, the stroma, which also forms the tunica albuginea under the gonadal envelope (the ovarian epithelium) (McMillan, 2007). The ova are enclosed within follicles of somatic tissue that originate from the gonadal epithelium (Tyler & Sumpter, 1996; McMillan, 2007). The internal structure of the ovaries is usually characterized by intricate ovigerous folds (lamellae) of the visceral epithelium; these folds extend into the ovocoel and contain the developing follicles (McMillan, 2007). A notable exception to this lamellar organization of the ovaries is

found in syngnathids that have one or two germinal ridges instead, with oocytes developing sequentially in a circular orientation towards a mature ridge where they are ovulated (Begovac & Wallace, 1987; Selman *et al.*, 1991; Ishihara & Tachihara, 2009).

The internal organization of the testes in teleosts is cystic, where a typical spermatocyst is formed of germ cells in association with Sertoli cells and is the location of spermatogenesis (Grier *et al.*, 1980; Redding & Patiño, 2000b). However, testes may be of three types based on the organization of these spermatocysts: anastomosing tubular and lobular, which in turn may be restricted or unrestricted (Grier *et al.*, 1980; Grier, 1993; Parenti & Grier, 2004). In the anastomosing tubular testes, which are characteristic of the primitive fish taxa, "the germinal compartments do not terminate at the testis periphery, but form highly branched, anastomosing loops or tubules" (Parenti & Grier, 2004). The lobular testes, which are characteristic of the derived teleosts, extend into the testicular periphery and end blindly, knowing that the lobules may form a proximal anastomosing network (Grier, 1993; Parenti & Grier, 2004). Moreover, the distribution of spermatogonia inside a lobule further classifies it as unrestricted, where spermatogonia are distributed all along the lobule, or restricted, where they are confined to the distal end of the lobule (Grier, 1993; Parenti & Grier, 2004). The restricted lobular testis is characteristic of Atherinomorphs whereas the unrestricted type is characteristic of Neoteleostei (Grier, 1993; Parenti & Grier, 2004).

5. Oogenesis

Oogenesis in teleosts occurs within ovarian follicles that house the ova (Redding & Patiño, 2000b). The follicle is made up of the oocyte, which is the ooplasm encircled by the vitelline envelope and zona radiata, and the follicular layers derived of somatic tissues, namely the granulosa, theca, and follicular epithelium (Tyler & Sumpter, 1996). After the sexual determination of the gonia, the oogonia go through a phase of intense proliferation (McMillan, 2007). This process, unlike other vertebrates (except amphibians), continues even in the adult stages of the life cycle of teleosts (Tyler & Sumpter, 1996). Oogenesis begins when the secondary oogonia divide mitotically to produce primary spermatocytes in which the meiotic cycle is arrested at the first prophase and they are encased in follicular cells, making up primordial follicles (McMillan, 2007). During the different stages of development, there is an increase in the size of the oocyte due to a growth in both the nucleus and cytoplasm (McMillan, 2007). The ovarian follicles undergo different stages of development leading to ovulation that may be classified into four: (1) primary growth, (2) cortical alveolus, (3) vitellogenesis, and (4) maturation (Wallace & Selman, 1981).

The primary growth stage is divided into two phases: the chromatin nucleolus and perinucleolar stages (Wallace & Selman, 1981). During the primary growth stage, the oocyte greatly increases in volume and the nucleus-to-cytoplasm ratio drops, mainly due to the formation of the Balbiani body, an aggregate of cytoplasmic organelles (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007). During the end of this stage, the acellular vitelline envelope develops and the oocytes move out of the oogonial nests and into the follicular phase (Tyler & Sumpter, 1996). As the oocyte moves from the initial chromatin nucleolus stage into the perinucleolar, nucleus

increases in size (becoming the germinal vesicle) and multiple nucleoli appear around its inner periphery, a process that is always observed in teleosts (Wallace & Selman, 1981; McMillan, 2007).

Towards the end of the primary growth phase, cortical alveoli start to appear around the periphery of the oocyte, signaling the transition into the second stage of development (Wallace & Selman, 1981; Tyler & Sumpter, 1996). These alveoli are synthesized endogenously, and appear as the first cytoplasmic inclusions to be seen clearly with a light microscope, and towards the end of the stage fill up the entire ooplasm (Tyler & Sumpter, 1996). In addition to the alveoli, the zona pellucida (zona radiata) and lipid vesicles appear in the cytoplasm (Selman *et al.*, 1991; McMillan, 2007). During this stage, the zona pellucida thickens and in some cases numerous small lipid droplets accumulate in a perinuclear position, disappearing when the actual yolk starts to appear (McMillan, 2007). Later in oocyte development, the cortical alveoli fuse with the oolemma and release their content into the previtelline space at fertilization as a block to polyspermy (Wallace & Selman, 1981; Tyler & Sumpter, 1996). Due to the above-mentioned developments, the size of the oocyte continues to increase by a large extent (Tyler & Sumpter, 1996).

Following the cortical alveolus stage, the vitellogenic stage continues to increases the size of the oocyte, sometimes by a very large margin, as extraneous yolk is incorporated into the cytoplasm of the oocyte (Wallace & Selman, 1981; Tyler & Sumpter, 1996). The yolk globules are derived from a hepatic precursor protein, vitellogenin, which is appropriated into the oocyte from the blood (McMillan, 2007). Even though cortical alveoli continue to be synthesized by the oocyte, the accumulating yolk globules displace them towards the periphery of the ooplasm as the zona pellucida

continues to thicken (McMillan, 2007). As vitellogenesis advances, the yolk globules may remain as globules or may fuse into a yolk mass giving the oocytes their typical transparent aspect (Wallace & Selman, 1981).

In the final step before the release of the oocyte in ovulation, it goes through a series of maturation events, transforming from an oocyte into an egg (Tyler & Sumpter, 1996). During early maturation, the centrally located germinal vesicle migrates towards the animal pole and breaks down, releasing the nuclear material into the ooplasm (McMillan, 2007). Protein uptake continues in the early phases of maturation but stops after the breakdown of the germinal vesicle (Tyler & Sumpter, 1996; McMillan, 2007). With the germinal vesicle breakdown (GVBD), meiosis resumes, with the condensation of the chromosomes and the release of a polar body (McMillan, 2007). Yolk globules coalesce in most species, and a final hydration step, especially in species with pelagic eggs which may contain up to 92% water, greatly increases the size of the oocyte and makes it even more buoyant (Tyler & Sumpter, 1996). Eventually, the oocyte is ovulated into the ovarian lumen, transforming into a mature egg with a tough chorion derived from the zona pellucida (Wallace & Selman, 1981; McMillan, 2007). With the ovulation of the mature egg, the ruptured follicular layer is left behind and is referred to as a post-ovulatory follicle (POF) that is clearly seen in the ovarian lumen but degenerates quickly (West, 1990; McMillan, 2007). The follicular epithelium undergoes hypertrophy and phagocitosis and ends up a small folded mass (McMillan, 2007).

In teleosts, as with other vertebrates, oocytes may degenerate and be resorbed in what is referred to as atresia (Tyler & Sumpter, 1996; McMillan, 2007). There are many views about the causes and factors affecting atresia, though there is an agreement that it may be present in physiologically healthy individuals and comes as a “mopping

up" event in post-spawning ovaries, and towards the end of the spawning season, especially for asynchronous ovaries and batch-spawning species (see below) (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007). Atresia begins with the disappearance of the nucleus and the thickening of the follicular epithelium, upon which follicular cells break apart the vitelline envelope, invade the ooplasm and phagocitize it, leading to the collapse of the follicle (McMillan, 2007).

Reproduction in teleosts is a cyclic process in the main, with certain exceptions (McMillan, 2007). In light of these general characteristics of oocyte development, ovaries may be classified into three categories based on the pattern of development of the oocytes within the ovary: (1) synchronous, (2) group-synchronous, and (3) asynchronous (Wallace & Selman, 1981). In synchronous ovaries, all the oocytes are at the same stage of development, and are released in one spawning event; in group-synchronous ovaries, there are at least two populations of oocytes within the ovary, with a previtellogenic reserve and one batch of developing oocytes to be spawned in the season at the very least; and in asynchronous ovaries, oocytes of all stages are randomly present, and a dominant population may or may not exist (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007; Pavlov *et al.*, 2009). Species with asynchronous ovaries are in most cases batch spawners, and fresh oocytes continue to be recruited from the previtellogenic reserve, resulting in indeterminate fecundity and a protracted spawning season, knowing that the most advanced batch of oocytes may go through a hydration event separating it from the other populations prior to spawning (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007; Pavlov *et al.*, 2009).

6. Spermatogenesis

Spermatogenesis in the different testicular types happens within the spermatocysts (Grier, 1981). It may be divided into three phases: (1) meiosis, (2) spermiogenesis, and (3) spermiation (Redding & Patiño, 2000b). Before the onset of meiosis, there is a period of intense mitotic division when PGC's proliferate into spermatogonia, and the spermatogonia themselves multiply mitotically but do not undergo complete separation, thus forming a syncytium of clones joined by cytoplasmic bridges (Pavlov *et al.*, 2009). Upon the onset of meiosis, the spermatogonia divide into primary and then secondary spermatocytes, ending with a syncytium of spermatids (Pavlov *et al.*, 2009). Subsequently, the spermatids transform through nuclear and cytoplasmic modifications into spermatozoa in the process termed spermiogenesis (Grier, 1981; Redding & Patiño, 2000b; Pavlov *et al.*, 2009). Following this phase of differentiation, the syncytia and spermatocysts break down, releasing the spermatozoa into the duct system in spermiation (Redding & Patiño, 2000b; Pavlov *et al.*, 2009). This process is referred to as the cystic type of spermatogenesis, where spermatogenesis is observed only in the spermatocysts (Redding & Patiño, 2000b; Pavlov *et al.*, 2009). Another form of spermatogenesis was also observed, the semi-cystic type, where spermatogenesis does not occur solely inside the cysts but spermatids transform into spermatozoa outside the cysts, leading to an asynchronous scheme of spermatogenesis and a protracted spawning phase (Mattei *et al.*, 1993; Pavlov *et al.*, 2009).

The morphology of the spermatozoon is also diverse, ranging from aflagellate to uniflagellate to biflagellate, with primitive spermatozoa having small rounded heads in external spawners to spermatozoa with elongated nuclei in species that fertilize

internally (Grier, 1981). Teleosts do not have spermatozoa with true acrosomes (Redding & Patiño, 2000b).

Moreover, in many teleosts with batch-spawning females, males show a protracted spawning period due to fresh cycles or incessant spermatogenesis, or spatial differences within the same testis where some cysts mature completely while others mature their spermatozoa in a prolonged manner, in addition to the above-mentioned semi-cystic spermatogenesis (Mattei *et al.*, 1993; Pavlov *et al.*, 2009).

D. *Fistularia commersonii*

1. Fistulariidae

Fistularia commersonii Rüppell, 1835, is one of the four species of cornetfishes comprising family Fistulariidae, which is made up of only one genus, *Fistularia* (Fritzsche, 1976; Nelson, 2006). Family Fistulariidae, once considered a part of the now-unused order Syngnathiformes, was transferred recently to order Gasterosteiformes that includes formerly syngnathiform species (Nelson, 2006).

Cornetfishes, are marine species found in the tropical and subtropical shallow waters of all the world's oceans. They are predators with elongate and depressed bodies (Fritzsche, 1976; Nelson, 2006). The elongate tubular snout found in this family is very well adapted for feeding in coral reefs, acting as a pipette, in addition to the open water (Nelson, 2006). *F. corneta* Gilbert & Starks, 1904, is limited to the tropical regions of the Eastern Pacific, *F. tabacaria* Linnaeus, 1758, is distributed mostly in the tropical areas of both sides of the Atlantic Ocean, *F. commersonii* is of a general Indo-Pacific distribution, whereas *F. petimba* Lacepède, 1803, has a similar distribution to *F. tabacaria* in addition to an Indo- West-Pacific range (Fritzsche, 1976).

2. Fistularia commersonii in the Mediterranean

Contrary to most Lessepsian species that show a gradual growth in population size, *Fistularia commersonii* displayed an explosive growth pattern that probably contributed to its rapid expansion westwards (Golani & Ben-Tuvia, 1989; Azzurro *et al.*, 2004; Bariche *et al.*, 2009a). As such, it was placed in the list of the 100 "worst invasive" species, with a profound impact on the native biodiversity (Streftaris & Zenetos, 2006).

The first specimens in the Mediterranean were recorded in January 2000 along the Levantine coast (Golani, 2000). In 2001, *F. commersonii* had expanded its range to the Anatolian coasts of Turkey and the waters of Rhodes Island in the Aegean Sea, where 37 specimens were caught in a period extending from summer to winter (Bilecenoglu *et al.*, 2002; Corsini *et al.*, 2002). A year later, in December 2002, an adult specimen was caught by a trammel net at 12 m on rocky substratum from the island of Lampedusa in the Straits of Sicily in the Central Mediterranean, one of the very few Lessepsian species that have accomplished such a range expansion, and was considered to be sure evidence of the speedy spread of this invader towards the Western Mediterranean (Azzurro *et al.*, 2004). Another year later, in the summer of 2003, another specimen was caught in the northern Aegean Sea at 25 m again with a trammel net, representing then the northernmost point of its new range (Karachle *et al.*, 2004). As a result of this rapid range expansion *F. commersonii* was named the Lessepsian sprinter (Karachle *et al.*, 2004). The species was also recorded off of Tunisia in 2004, one of only eight Lessepsian species present in Tunisian waters (Ben Souissi *et al.*, 2004). In October 2005, a specimen was also caught from the island of Sardinia in the

Tyrrhenian, showing the range expansion of *F. commersonii* into the Western basin of the Mediterranean Sea (Pais *et al.*, 2007). The species further expanded its range to the northern coasts of the Tyrrhenian Sea in December 2006 (Ligas *et al.*, 2007).

In October – November 2007, *F. commersonii* specimens were caught by different catchment methods over sandy, muddy, and *P. oceanica* meadow from the Central Tyrrhenian and by trawlers on muddy substratum at 60 m in the Ligurian Sea, which presented the westernmost record (Garibaldi & Orsi-Relini, 2008). The latter authors hypothesized that the caught specimens were not a part of a settled population, but were the pioneers in the expansion phase, also predicting the expansion of the species into the French and Spanish coasts after a year (Garibaldi & Orsi-Relini, 2008). This forecast was achieved much sooner than expected, when a specimen was caught and believed to be *F. commersonii* from the Iberian peninsula in Granada, Spain (Sanchez-Tocino *et al.*, 2007), thereby marking the range of the species to almost the entire Mediterranean, especially since it was also recorded in the eastern Algerian waters in January – February 2008 over a variety of substrata (Kara & Oudjane, 2008). This latter record is highly significant in presenting the only Lessepsian species to have reached Algeria (Kara & Oudjane, 2008). Finally, in August 2008, the northernmost record of *F. commersonii* was documented in the Northwestern Ligurian Sea, where a school of juveniles was observed at 3-4 m depth (Occhipinti-Ambrogi & Galil, 2008). Garibaldi & Orsi-Relini (2008) anticipated a fast “Conquest of the West” by this Lessepsian invader.

3. Biology of Fistularia commersonii

The different aspects of the biology and ecology of *F. commersonii* are not very well known, except for its dietary habits. Different authors stress on the importance of further investigations into the life history and biological and ecological traits of the species in order to decipher the reasons for its unprecedented success (Kalogirou *et al.*, 2007; Kara & Oudjane, 2008; Psomadakis *et al.*, 2008; Bariche *et al.*, 2009a).

F. commersonii lives either solitarily or in small schools (Golani, 2000). It shows flexibility in terms of habitat requirements. Fritzsche (1976) described it to be more widespread over sandy bottoms and reefs. Golani (2000) also described it to be more common around reefs, in addition to low sandy bottoms. The local Mediterranean records showed that the species was capable of living in many different habitats, ranging from rocky bottoms and reefs to muddy and sandy ones to seagrass meadows to mixed environments (Bilecenoglu *et al.*, 2002; Corsini *et al.*, 2002; Azzurro *et al.*, 2004; Pais *et al.*, 2007; Garibaldi & Orsi-Relini, 2008; Kara & Oudjane, 2008; Psomadakis *et al.*, 2008).

The diet of *F. commersonii* has been studied in its native Indo-Pacific range as well as its invasive range in the Mediterranean Sea. Different authors agree that it is a higher-order piscivore that may supplement its diet with crustaceans or squids (Fritzsche, 1976; Golani, 2000; Takeuchi *et al.*, 2002; Kalogirou *et al.*, 2007; Bariche *et al.*, 2009a). In its natural range, in a study conducted in Southern Japan, the species preyed primarily on pelagic (*e.g.* Carangidae) and reef fishes (*e.g.* Labridae), with the minor presence of several different crustaceans (*e.g.* Mysidacae, Decapoda) (Takeuchi *et al.*, 2002; Takeuchi, 2009). According to the same authors, the prey size increased with an increase in the length of the fish (due to an increase in gape size), with the

larger fish being able to selectively target the type of fish to pursue (Takeuchi *et al.*, 2002; Bariche *et al.*, 2009a; Takeuchi, 2009). Moreover, in their foraging behavior, the species showed a variation in techniques utilized from stalking to chasing, where the smaller fish were mostly restricted to stalking and the larger ones used both methods (Takeuchi, 2009). The same author described the foraging behavior to be solitary or in association with con- or heterospecifics, producing a wide diversity of foraging patterns that greatly improve the cornetfish's success (Takeuchi, 2009).

In its invasive habitat in the Mediterranean, two studies were performed on the diet of this species, showing similar results to the Japanese study (Kalogirou *et al.*, 2007; Bariche *et al.*, 2009a). Moreover, the species preyed upon a large variety of species, mostly natives, and became more of a generalist in its diet upon increase in length (Kalogirou *et al.*, 2007; Bariche *et al.*, 2009a). However, Bariche *et al.* (2009) showed that diet did not vary significantly with length, and that the species preyed more on schooling fishes in the water column, whereas Kalogirou *et al.* (2007) results showed the presence of benthic and supra-benthic fishes. Both studies agree on *Boops boops* (Linnaeus, 1758) and *Spicara smaris* (Linnaeus, 1758) being the most preferred prey (Kalogirou *et al.*, 2007; Bariche *et al.*, 2009a).

Traditionally, the reproductive characteristics of fishes have been studied in order to assess different fisheries or aquaculture management issues, and recently in terms of the climate change scenarios (Tsikliras *et al.*, 2010). Moreover, and within the context of biological invasions, uncovering these characteristics in introduced fishes is necessary to further evaluate the reasons for their invasive potential and success (Bariche *et al.*, 2003; Azzurro *et al.*, 2007; Bariche *et al.*, 2009b). In *F. commersonii* in particular, the authors reporting the first specimens across the Mediterranean have

described a few ovaries from the local records and hypothesized that the reproduction played a crucial role in its establishment and spread (Corsini *et al.*, 2002; Azzurro *et al.*, 2004; Occhipinti-Ambrogi & Galil, 2008; Psomadakis *et al.*, 2008). However, the reproductive cycle has not been conclusively investigated and described, and remains unknown.

4. Fistularia commersonii Reproduction

There is very limited information about the reproductive aspects of *F. commersonii*, mainly restricted to the ovaries of a few specimens described as first records in the Mediterranean Sea. The authors agreed as to the morphology of the ovaries being joined into apparently one elongated slightly depressed organ, with the two lobes divided by a septum of connective tissue continuing with the tunica, with the color ranging from pinkish orange to reddish (Azzurro *et al.*, 2004; Pais *et al.*, 2007; Psomadakis *et al.*, 2008). Testes were describing as being whitish in color (Psomadakis *et al.*, 2008).

In the December 2002 specimen caught off the island of Lampedusa, Azzurro *et al.* (2004) described the ovary to have an asynchronous assortment of previtellogenic and vitellogenic oocytes without any signs of atresia. On the other hand, an October 2005 specimen from Sardinia, Italy, was described by Pais *et al.* (2007) to be a stage VII postspawning ovary based on an eight-point gonadal maturity scale for total spawners (Holden & Raitt, 1974), which contained a synchronous mixture of mostly immature oocytes with some translucent and atretic oocytes. Based on the same scale, Psomadakis *et al.* (2008) described their specimens caught in Italy between October and November 2007 to be stage II to IV maturing or mature individuals (including males).

The latter authors, in view of these observations, hypothesized a protracted spawning season for this species in the Mediterranean, with possibile changes from its original range (Psomadakis *et al.*, 2008).

In light of the absence of a comprehensive understanding of the reproduction of *F. commersonii*, the current study aimed to characterize its various reproductive aspects in an effort to further understand how it was able to quickly expand its range and adapt its reproduction to the new environment of the Mediterranean.

E. Study Aims

This study aimed towards investigating a wide range of characteristics involved in the reproduction of *Fistularia commersonii*, namely:

1. Population structure through weight-length relationships, length-frequencies, and sex ratios
2. Macro- and microscopic descriptions of gonadal morphology
3. Microscopic description of oocyte development
4. Construction and characterization of ovarian and testicular maturity stages through macro- and microscopic description
5. Macro- and microscopic maturity staging, and staging accuracy
6. Length at first sexual maturity
7. Reproductive pattern through oocyte diameters
8. Fecundity (batch and relative), and fecundity-somatic parameter relationships
9. Reproductive indices (Gonadosomatic and condition)
10. Description of oocyte atresia

CHAPTER II

MATERIALS AND METHODS

A. Sampling

Sampling was carried out on the coast of Lebanon during two intervals; 812 samples were collected in the period extending from October 2005 to November 2006 for routine macroscopic work, whereas 206 samples were collected from July to December 2009, as well as 55 additional samples in the months of April and May 2010 for microscopic study. The 2005-06 specimens were collected from all along the northern Lebanese coast, with the major sampling sites being Tripoli, Al Abdeh, Ouzai, Qalamoun, Batroun, and Arida. The 2009-10 specimens were collected from Amchit and Dawra (Fig. 1), and care was taken to sample and process the largest specimens, with a preference for females.

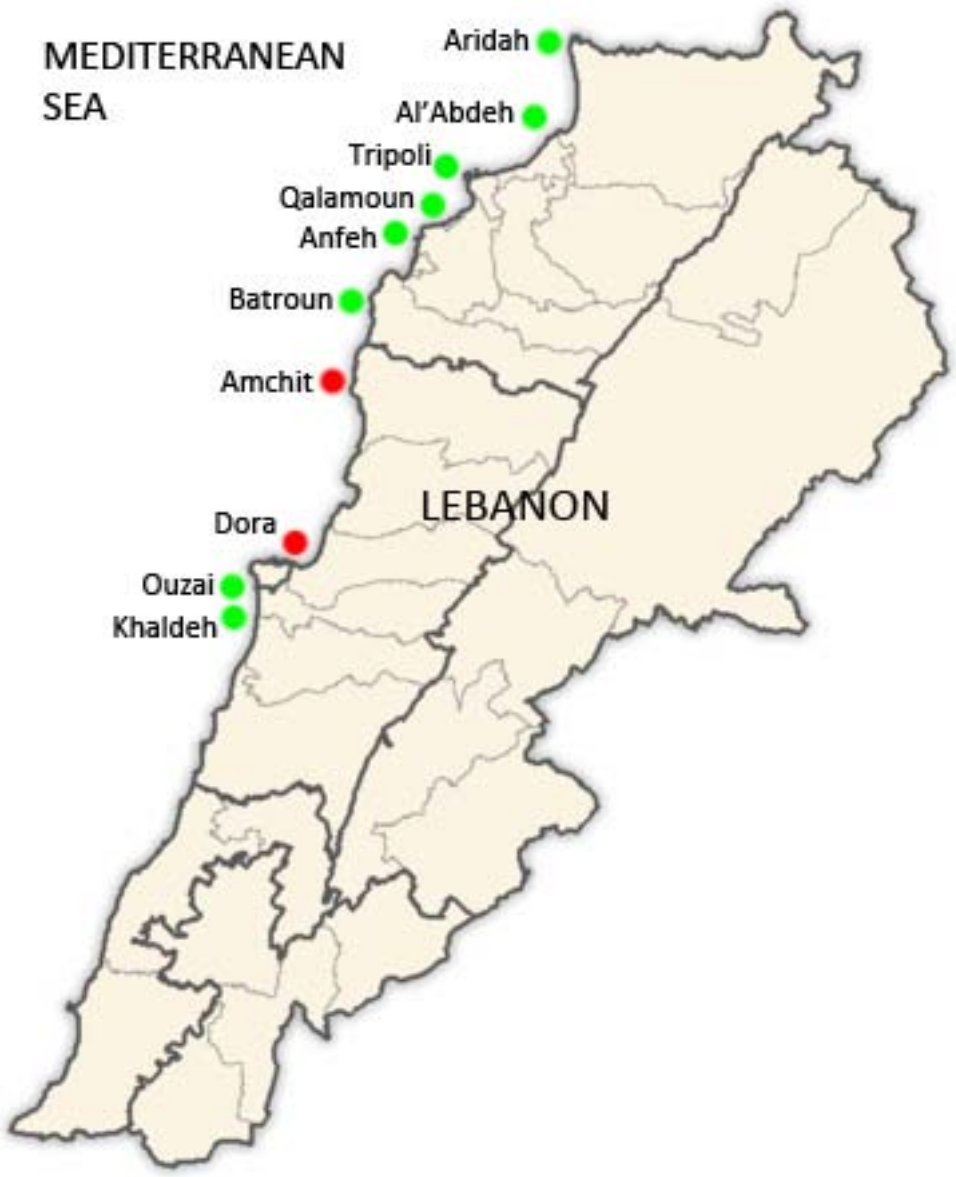


Figure 1: Sampling sites along the Lebanese coast for the 2005-06 (green points) and 2009-10 (red points) periods.

Sampling was performed on a weekly basis. *Fistularia commersonii* specimens were purchased from fishermen who caught them using trammel nets, beach seines, and encircling trammel nets as the species was most susceptible to these catchment methods. The air and surface water temperature (in °C) were obtained through measurements on the Beirut shoreline, whereas the salinity (in ppm) was recorded weekly throughout the

2005-06 period from the Beirut shoreline. The variations of the weather data are presented in Fig. 2.

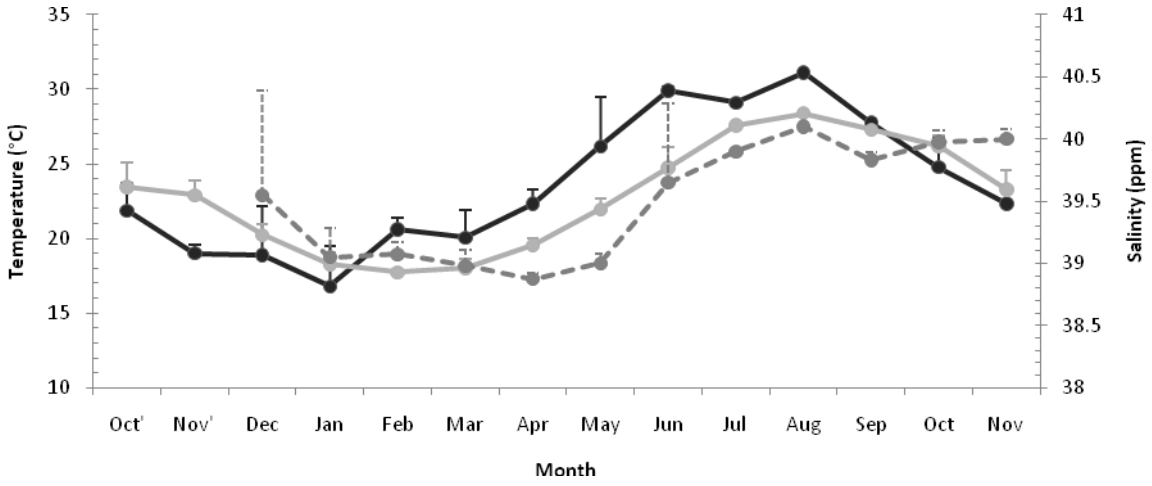


Figure 2: Monthly weather data across the 2005-06 sampling period. Air temperature (mean + s.d.; black line), surface water temperature (mean + s.d.; light grey line), and salinity (mean + s.d.; dashed grey line).

Fresh fish caught within hours were purchased directly from the fishermen, either in the early morning or late afternoon in different localities, transported to the laboratory on crushed ice, and processed immediately.

B. Processing and General Biological Data

Standard biometric measures were taken for each fish before dissection. The total length (length from the tip of the snout to tip of upper caudal lobe; L_T) and standard length (distance from the tip of the snout to the hypural plate; L_S) were measured to the nearest mm (Anderson & Neumann, 1996). The total fish weight (W_T) was recorded to the nearest 0.01 g. The fish was then dissected, the viscera and gonads removed, and the gutted weight (W_G) recorded also to the nearest 0.01 g. The fat

accumulated along the vertebral column was not removed and was included in the measurement for gutted weight.

Subsequently, gonads were weighed to the nearest 0.01 g (W_g), sexed, and photographed for documentation and analysis. Concurrently, they were described in terms of size, firmness, color, vascularization, and presence of visible oocytes or sperm within the gonads, as well as the occurrence of flowing milt or spawned oocytes. The gonads were fixed and stored for subsequent study. Based on these characteristics, the macroscopic gonad maturity table was constructed. The ovarian maturity table was modified from Lowerre-Barbieri *et al.* (1996), whereas the testicular maturity table was constructed based on several sources (Bariche *et al.*, 2003; Claereboudt *et al.*, 2005; Azzurro *et al.*, 2007; Moore *et al.*, 2007).

The gonads obtained in the 2005-06 period were all fixed in 4 % buffered formaldehyde for routine macroscopic analyses while gonads obtained in 2009-10 were either stored in Bouin's fixative (July-December 2009) or in 4 % buffered formaldehyde (April-May 2010). Preserved samples that showed posthumous degeneration were not used in histological analyses.

The gonads from the 2009 months were immersed for 24-48 hours in Bouin's fixative. They were then washed in distilled water and stored in 70 % ethanol (Hunter & Macewicz, 1985a). As for the samples from 2010 stored in formaldehyde, the procedure was slightly modified from Lowerre-Barbieri *et al.* (1996). Gonads were kept immersed for a few days, soaked in distilled water for another 24 hours, transferred to 50 % ethanol and then to 70 % ethanol, where they were stored pending histological processing.

C. Population Structure

The population structure of the sampled populations was analyzed using weight-length relationships, length-frequency analyses, and sex ratios. The overall studies, undifferentiated between the sexes, were carried out for both the 2005-06 and 2009-10 samples. For comparatives in the population parameters between the sexes, in addition to the sex ratios, only the 2005-06 specimens were used.

The weight-length (*W-L*) relationships of the two sampled populations were analyzed by regression analyses separately as well as after pooling the available weight-length data, with a hypothesized power function of the form $W_G = a\, L_T^{\,b}$, where W_G is the gutted weight and L_T is the total length, and *a* and *b* are regression parameters. *W-L* regression analyses were also performed for the sexes separately, with the same power function hypothesized. At all points in the regression analyses, the Analysis of Variance (ANOVA) tables were assessed for the highest *F*-values indicative of the best regression, as well as the r^2 values. In addition, the slopes of the resultant relationships for the two sampling periods, as well as between the sexes, were compared using Student's *t*-tests (Zar, 2009).

Length-frequency histograms were constructed separately on the two sampled populations, with the lengths grouped into 5 cm intervals (20= 15.1-20 cm; 25= 20.1-25 cm; etc). Normality of the sampled populations was tested using the non-parametric Kolmogorov-Smirnoff test (Zar, 2009). The means of the male and female subpopulations were compared using a Student's *t*-test as well as the non-parametric Mann-Whitney test in case of deviations from normality. Monthly frequency histograms and tables describing length-frequencies per month were also constructed.

The sex ratios were analyzed using χ^2 goodness-of-fit tests with a hypothesized ratio of 1 : 1 (females : males). Sex ratios were calculated for the whole duration of sampling as well as for the months separately in order to assess monthly variations in the ratios of the sampled population. Sex ratios across total length were also analyzed, with the data divided into 5 cm intervals.

D. Reproductive Indices

The Gonadosomatic index (*GSI*), was calculated for each sampled population and sex separately. The *GSI* for each specimen was calculated using the formula $GSI = 100 \times W_g / W_G$ (King, 1995). The monthly *GSI* averages were also calculated. Bias was avoided by excluding immature individuals; only fish with L_T above which 99% of the population was mature were included (after calculation of length at first maturity).

Condition indices were calculated. The used indices were Fulton's and Ricker's condition indices, as well as Relative Weight (King, 1995). The indices for the sexes separately were also assessed in the 2005-06 samples.

Fulton's condition index was calculated following the formula $K = 100{,}000 \times (W_G / L_T^3)$ (Anderson & Neumann, 1996) for each specimen, and then monthly averages were calculated and graphed to determine the seasonal patterns of variability of the samples' somatic condition, followed by *K* for the sexes separately. Ricker's condition index was calculated using the formula $K_r = 100{,}000 \times (W_G / L_T^b)$ where *b* refers to the slope of the power function derived from the *W-L* relationships (Bolger & Connolly, 1989). K_r was calculated for the sexes, using the slopes of the power functions derived from the *W-L* relationships of the sexes. Relative Weight (W_r) was calculated from the formula $W_r = 100 \times (W_G / W_s)$ where W_s is the length-specific standard weight

calculated through the power function obtained from the *W-L* relationships (Anderson & Neumann, 1996). W_r was calculated for the sexes similar to Ricker's K_r.

Student's *t*-tests were used to contrast the condition indices between the sexes in the 2005-06 samples.

E. Size at Maturity

The length at first sexual maturity (L_{50}) denotes the length at which 50 % of the population has reached reproductive maturity. It was calculated for the sexes separately in the 2005-06 samples. Fish within the spawning season were assigned into two categories, either mature or immature, based on the macroscopic staging. Mature fish were considered to be specimens staged as developing and on in the macroscopic gonad maturity table. Binary logistic regression analyses were performed on the reproductive status and L_T of the selected samples, without grouping into size intervals. This produced logistic equations of the form $P = 1 / \{1+e^{[-r\,(LT-\,L50)]}\}$ that expressed the proportion of sexually mature fish in terms of the total length and the L_{50} (King, 1995). Also, in order to corroborate the method, logistic regression based on the Marquardt method of nonlinear regression was performed using the FISHPARM program, with the specimens grouped into 5 cm size intervals, producing similar logistic equations denoting L_{50} (Prager *et al.*, 1989).

F. Oocyte Diameters

In order to assess the spawning pattern of *F. commersonii*, oocyte diameter histograms were constructed. The methodology used was half-automated digital analysis of whole oocytes macroscopically. Eight ovaries in different stages of

development from each sampled population were analyzed. The selected ovaries (n = 8 for each period) were in the redeveloping, developed, gravid, and running-ripe stages.

From the midsection of each of the ovaries, a sample of around 0.5 g, weighed to the nearest 0.001 g, was taken while being careful to sample the entire width of the gonad, in case of spatial differences in the distribution of the oocytes at different developmental stages. The samples were then transferred into water and gently shaken to dissociate the oocytes from one another. Oocytes were poured afterwards into Petri dishes containing a standardized length (for calibration later on), and a high-detail digital photograph on macro mode was taken of the plate, with the plate illuminated from underneath to highlight the oocytes. The pictures were digitally enhanced (using Photoshop® CS4) before being analyzed.

In order to measure the oocytes, the Analysis toolkit integrated into Adobe® Photoshop® CS4 Extended was used. Through this toolkit, a digital scale was calibrated to the standard length included in the plate, and the ruler tool used to measure the individual oocytes manually. A minimum of 500 oocytes per sample were measured, along a random axis of reference (West, 1990; Lowerre-Barbieri *et al.*, 1996), to the nearest 0.001 mm. As a big number of the oocytes encountered were primary growth (previtellogenic) oocytes, six samples from 2009-10 were analyzed without measuring this subpopulation of oocytes in order to have a clearer picture of the more advanced stage oocytes. In the 2005-06 samples, 200 previtellogenic oocytes in addition to the original 500 were measured.

Subsequently, the oocyte diameter measurements were exported into Excel 2007, where they were grouped into length intervals of 0.05 and 0.025 mm and plotted as length-frequency histograms. The frequency data was exported into the Fisheries

Statistical Analysis Tools (FiSAT II) software (Gayanilo *et al.*, 2005), which was used to construct Bhattacharya plots, decomposing the frequency histograms into different Gaussian distributions (Bhattacharya, 1967) in order to identify the different batches of oocytes present at one time in the ovary.

The percentage of shrinkage due to histological processing in the different populations of oocytes was calculated by contrasting the microscopic means obtained through histology with the macroscopic means obtained through Bhattacharya's method on performed.

G. Fecundity

For the fecundity measurements, 53 ovaries were selected from the 2009-10 samples. Ovaries containing advanced yolked to partially hydrated oocytes were considered. The method of preparation of the oocytes before counting is very similar to that of the diameter measurements described above. In this case, three subsamples of each ovary were processed, from the anterior, middle, and posterior sections of the same ovary, each around 0.5 g, weighed to the nearest 0.001 g. A picture was taken and modified, and the count tool was used to manually tally all the oocytes of the most advanced population in each subsample. The batch fecundity for each subsample was calculated afterwards according to the gravimetric method (Murua *et al.*, 2003), using the formula $F_b = n \times W_{sub} / W_g$ where F_b is the batch fecundity estimate, n is the number of oocytes counted, and W_{sub} is the weight of the subsample. The effect of the subsample's position (anterior, mid, or posterior) within the ovarian lobe on the resultant F_b was assessed using a repeated-measures ANOVA test.

The batch fecundity estimates were used to calculate the mean batch fecundity, in addition to the relative fecundities according to the formula $F_r = F_b / W_G$, where F_r is the relative batch fecundity. Then, the monthly averages of the batch and relative fecundities were calculated and plotted to assess the monthly variations. One-way ANOVA tests were used to check for differences in the monthly fecundity averages. Moreover, the batch and relative fecundity estimates were grouped into 5 and 10 cm total length intervals, and one-way ANOVA and non-parametric Kruskall-Wallis tests were used to assess for variations of the fecundities relative to L_T.

Regression analyses were then performed on F_b and F_r in order to test the dependence and level of correlation of these estimates with L_T, W, and W_G. The ANOVA tables obtained were assessed for the highest F-values, and the relevant regression parameters and r^2 values were recorded. As per the existing literature, linear and power functions were given preference (Holden & Raitt, 1974).

H. Histology

The gonads obtained from the 2009-2010 specimens were all processed histologically (except those that showed posthumous changes). The procedures followed were standard techniques of paraffin sectioning and Hematoxylin-Eosin staining (Hunter & Macewicz, 1985a; Murua *et al.*, 2003). The individual steps of the procedure were modified according to need, based on different variables such as subsample size, method and duration of fixation, and the quality of the obtained sections.

The standard methodology of preparation for paraffin sectioning entails three main steps: (1) dehydration, which removes the water present in the tissues, (2)

clearing, which removes the dehydrating agent and mediates the transition to the final step, namely (3) infiltration, which substitutes the clearing agent with liquid paraffin. The tissue is then embedded, ready to be sectioned.

The subsamples to be analyzed were taken from the midsection of the gonads and were generally around 1 cm^3 or less, knowing that thicker subsamples would need additional time in each step of the preparation process. The subsample was then dehydrated in increasing percentages of ethanol, moving from the 70 % ethanol that it was stored in to 95 % and then 100 % ethanol. Thereupon, it was transferred into two changes of 100 % xylene. Following the clearing step, it was infiltrated with molten paraffin (histology grade; melting point 57-58 °C) in two changes, and finally embedded with a fresh change of paraffin into blocks to be sectioned. The blocks were left to dry for at least 12 hours before being sectioned using a rotary microtome, producing paraffin sections of 5-10 µm. The sections were then mounted on a clean slide using an albumin fixative covered by a thin film of water, and placed on a slide warmer. The slides were left on the warmer for at least two hours in order for the water to evaporate and the paraffin sections to be fixed well to the slides.

The fixed sections were placed in xylene first to remove the paraffin from the tissues. Following the removal of paraffin, the slides were rehydrated in decreasing percentages of ethanol, moving from 100 % to 95 % and ending in 70 % ethanol. At this point, they were stained with Harris' Hematoxylin solution, a basophilic dye that stains purple (Humason, 1967). After staining, the slides were washed in running water and placed in Scott's solution that blues the Hematoxylin present in the tissues (Humason, 1967). The slides were washed again, and counterstained with the acidophilic Eosin Y

dye that stains pink. Finally, they were dipped a few times in 70 % ethanol to moderate the color of the Eosin dye, rinsed, and left to dry.

The slides were then analyzed. In order to construct the microscopic gonad maturity table, the oocyte developmental stages needed to be described for this species. The different stages of oocyte development were described on the basis of aspect, color, and position of the nucleus, the position, size, and number of nucleoli, the dye-affinity of the cytoplasm, the features of the ovarian follicles (zona radiata, granulosa layer), and cytoplasmic inclusions (cortical alveoli, lipid vesicles, protein yolk granules, yolk mass, hydration). For each stage of development, measurements of the oocytes were performed, using a modification of the technique used to measure the oocytes macroscopically. For each stage, 40 oocytes sectioned through their nuclei were measured (Mayer *et al.*, 1988; Tomkiewicz *et al.*, 2003). They were chosen from different ovaries and digitally photographed using a camera joined to a microscope. A standard scale was also photographed at the different magnifications, and then the measurements performed in CS4 Extended as before. The measurements were performed to the nearest 0.01 µm, and the means and ranges of each stage calculated and recorded.

Following the description of oocyte development, the ovaries and testes in different stages were described. The ovaries were described according to the presence of different populations of oocytes, from the earlier stages to the most advanced, the presence of post-ovulatory follicles and remnant hydrated oocytes as well as atresia, and general ovarian organization. The testes were described according to the general state and organization of the spermatogenic cysts and lobules, and the presence of spermatids and spermatozoa in the tubules, sperm sinuses, and sperm ducts. On these bases, the

microscopic gonad maturity table was constructed parallel to the macroscopic one. Moreover, the presence and level of atresia in the different oocyte populations was noted. The ovaries were classified microscopically according to the most advanced-stage oocyte present (West, 1990), taking into consideration the presence or not of post-ovulatory or atretic follicles.

Following the penning of the microscopic maturity table, the monthly percentages of the different gonad stages were calculated and plotted to assess their seasonal variations, in parallel to the percentages obtained macroscopically. The data obtained through microscopic staging was also used to evaluate the accuracy of macroscopic staging. Total and stage-specific accuracy percentages were calculated using the formula $\% \ error = 100 \ x \ (1 - m / M)$, where m is the number of macroscopic classifications agreeing with the microscopic ones, M. Concordance, the agreement or reproducibility of results, was assessed using non-parametric Kendall's Tau-b (τ) tests for ordinal data, and reliability was assessed through calculating intraclass correlation coefficients that test relationships between arbitrarily classified paired data (ICC, similar to Pearson's correlation coefficient; r) (Zar, 2009).

I. Statistical Packages and Other Software

Most statistical tests, including curve-fitting, were performed using SPSS for Windows (16.0.0), copyright© SPSS Inc. or Microsoft® Excel 2007. Bhattacharya plots were constructed using the FAO-ICLARM software, FiSAT II (version 1.2.2), copyright© FAO. Nonlinear logistic regression was performed using FISHPARM, 3.0 (Prager *et al.*, 1989). Digital photographs were taken using Sony Cybershot 4.1 MP and 12.1 MP cameras. Micrographs were taken using a Zeiss AxioCam digital camera

connected to an Axiovert 200 fluorescent microscope. Photo manipulations as well as counts and measurements were all performed using Adobe® Photoshop® CS4 Extended.

CHAPTER III

RESULTS

A. Population Structure

All *Fistularia commersonii* specimens collected ranged from 19.2 to 113.1 cm L_T, and 3.17 to 1159.62 g W_G. Specimens obtained in 2005-06 ranged from 19.2 to 112.4 cm L_T, and 3.17 to 730.39 g W_G. The 2009-10 specimens ranged from 48.3 to 113.1 L_T, and 48.59 to 1159.62 g W_G. Descriptive statistics are summarized in Table 1.

Table 1: Descriptive statistics for the total length (L_T, cm) and gutted weight (W_G, g).

Sampling period	*n*	Mean	S.D.	Range
	Total length (L_T)			
2005-06	812	59.05	18.59	19.2 – 112.4
2009-10	261	82.19	9.75	48.3 – 113.1
Pooled data	1073	64.68	19.58	19.2 – 113.1
	Gutted weight (W_G)			
2005-06	812	137.40	124.31	3.17 – 730.39
2009-10	261	383.04	157.27	48.59 – 1159.62
Pooled data	1073	202.48	170.43	3.17 – 1159.62

1. Weight-Length Relationships

Figure 3 represents the weight-length relationships for the *F. commersonii* samples. Regression was highly significant between the two variables in the all cases, and the best fitting function was a power function. The regression coefficients and elevations (slopes and intercepts) of the *W-L* curves in the two sampling periods were compared, and showed no significant difference ($P > 0.05$). The data were then pooled and common regression coefficients calculated (Fig. 3a, Table 2).

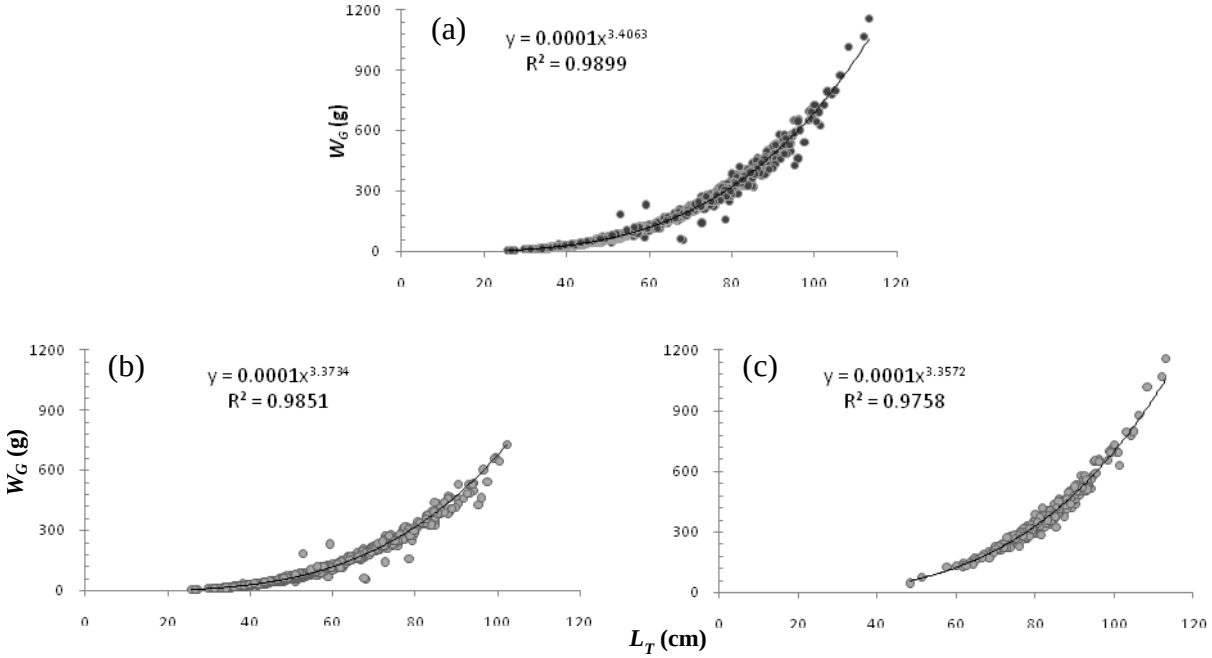


Figure 3: Weight-length relationships for the total (a), 2005-06 (b) and 2009-10 (c) *F. commersonii* samples. W_G: gutted weight; L_T: total length.

Figure 4 shows the weight-length relationships for the sexes separately in the 2005-06 samples. The regression coefficients (slopes) of the *W-L* curves were also compared, and showed a significant difference ($P < 0.05$) between the sexes. Table 2 summarizes the parameters of all the weight-length relationships calculated.

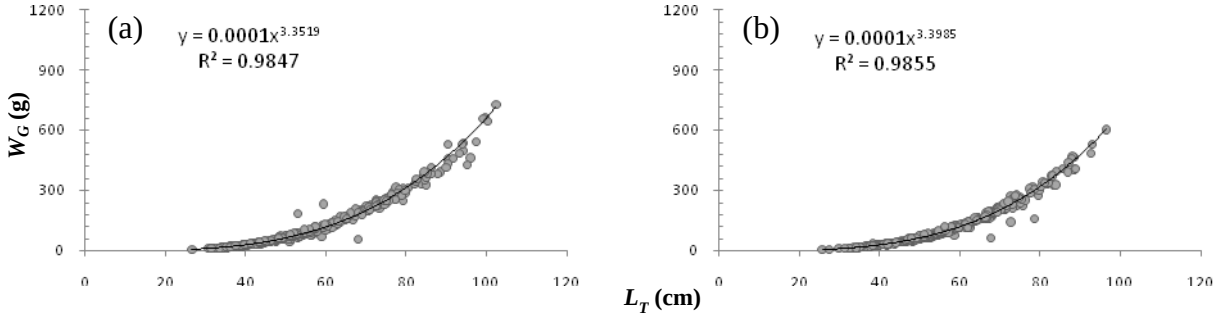


Figure 4: Weight-length relationships in 2005-06 for females (a) and males (b). W_G: gutted weight; L_T: total length.

Table 2: Weight-length regression parameters.

Population	*N*	Constant (*a*)	Slope (*b*)	r^2
	Unsexed			
2005-06	812	1.208×10^{-4}	3.3734	0.9851
2009-10	261	1.350×10^{-4}	3.3572	0.9758
Pooled data	1073	1.066×10^{-4}	3.4063	0.9899
	Sexed (2005-06)			
Female	424	1.314×10^{-4}	3.3519	0.9847
Males	388	1.094×10^{-4}	3.3985	0.9855

2. Length-Frequencies

Figure 5 shows the length-frequency distributions of the *F. commersonii* samples. The Kolmogorov-Smirnoff test for normality showed that the 2005-06 samples were not normally distributed ($P < 0.05$), whereas the 2009-10 samples were normally distributed ($P > 0.05$). Because of the deviation of the former samples from normality, the populations were compared using the non-parametric Mann-Whitney test, which showed a significant difference between the two sampled populations ($P < 0.05$). Similarly, the gutted weights also showed significant differences between the two sample periods ($P < 0.05$).

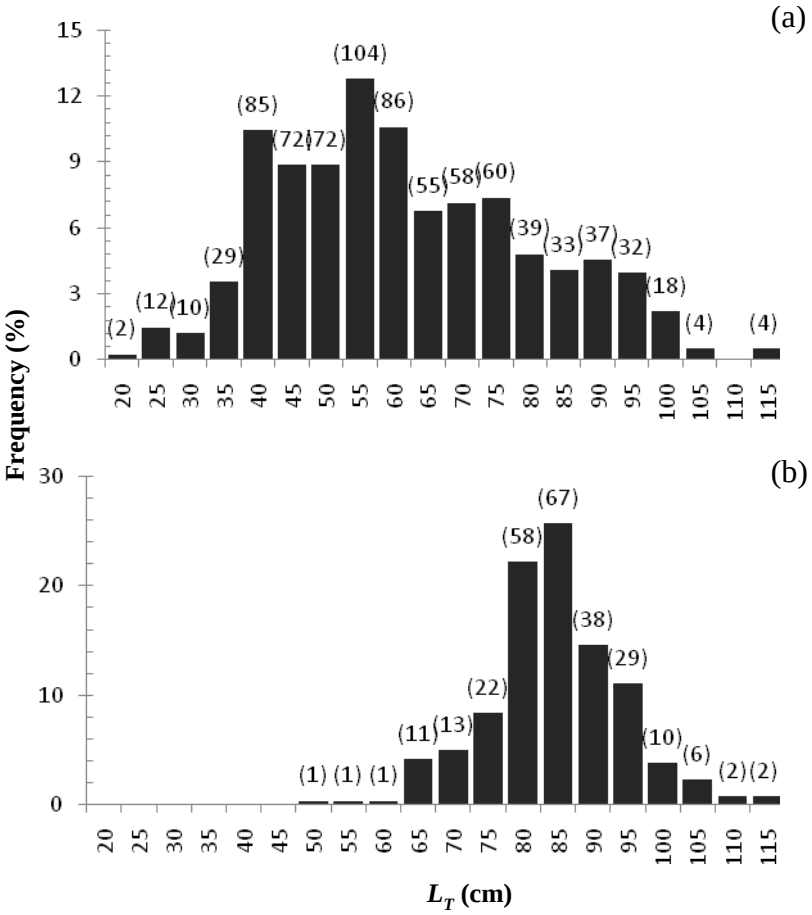


Figure 5: Length-frequency distributions for the 2005-06 (a) and 2009-10 (b) *F. commersonii* samples. L_T: total length; frequencies (*n*) in brackets.

Table 3 shows the monthly variations in the number of *F. commersonii* samples procured from the Lebanese coast for the two sampling periods. Table 4 shows the monthly variations of the length frequencies of the specimens across the two sampling periods.

Table 3: Monthly variations of the frequency of *F. commersonii* samples obtained in 2005-06 (a) and 2009-10 (b). L_T: total length; all values in cm, except for frequencies (*n*; in brackets on graphs).

Month	*n*	Range	Mean	S.D.
2005-06				
Oct'	41	55.7-112.4	87.93	11.76
Nov'	13	19.2-85.4	37.20	26.04
Dec	9	47.5-94.2	73.94	16.42
Jan	7	29.8-77.2	51.49	20.34
Feb	184	25.7-96.5	46.23	13.64
Mar	91	31.4-102.4	57.63	19.28
Apr	62	31.3-100.4	58.78	15.74
May	108	35-97.5	54.96	13.22
Jun	55	34.1-92.7	60.61	10.05
Jul	37	54.6-77.5	66.29	6.13
Aug	48	59.3-85.4	69.68	5.55
Sep	48	61.9-87.1	72.76	5.19
Oct	72	43.2-112.4	72.26	20.59
Nov	37	19.2-85.4	45.33	16.71
2009-10				
Jul	44	64.7-99.9	80.68	7.54
Aug	27	61.8-99.4	80.55	7.46
Sept	9	73.7-90	82.50	6.67
Oct	57	70.3-100.7	83.18	7.13
Nov	40	48.3-108.3	80.89	12.09
Dec	29	76-104.2	86.29	6.70
Apr	31	61.7-113.1	80.83	11.91
May	24	62.5-112	83.34	15.50

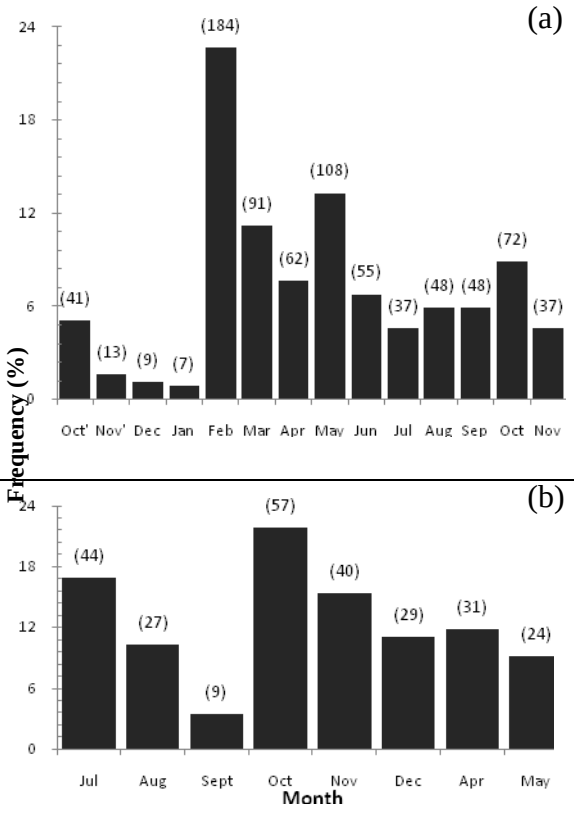


Table 4: Monthly variations in the length-frequencies of the *F. commersonii* samples in 2005-06 (a) and 2009-10 (b). L_T: total length; all values in cm, except for frequencies (*n*).

(a)

Range	Oct'	Nov'	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	*total*
20		1												1	2
25		6												6	12
30		3		1	3									3	10
35				1	21	2	3	1	1						29
40				1	60	16	3	4						1	85
45				1	30	13	5	17					2	4	72
50			2		14	7	6	17	4				14	8	72
55					17	14	14	33	7	1			11	7	104
60	2				11	11	10	17	18	6	3		4	4	86
65	1				12	3	4	4	14	7	7	2	1		55
70	1			1	3	1	2	2	5	12	17	13	1		58
75	1		3	1	3	2	1	1	2	9	14	20	3		60
80	2	1	1	1	4	3	4	3	1	2	6	8	2	1	39
85	4	1			2	8	7	2				4	4	1	33
90	10	1	2		1	4	2	3	1		1	1	10	1	37
95	11		1		2	3		2	2				11		32
100	6				1	3		2					6		18
105	1					1	1						1		4
110															0
115	2												2		4
n	41	13	9	7	184	91	62	108	55	37	48	48	72	37	812

(b)

Range\Month	Jul	Aug	Sep	Oct	Nov	Dec	Apr	May	*total*
50					1				1
55					1				1
60					1				1
65	2	1			1		4	3	11
70	4	1			1		1	6	13
75	2	2	2	8	3		5		22
80	9	9	2	15	11	7	3	2	58
85	17	9		13	9	6	11	2	67
90	7	2	5	8	4	7	2	3	38
95	2	2		10	5	7	1	2	29
100	1	1		2	1	1	2	2	10
105				1	1	1	1	2	6
110					1			1	2
115							1	1	2
n	44	27	9	57	40	29	31	24	261

Figure 6 shows the length frequency distributions in the two sampling periods, with the sexes separated. The descriptive statistics of the two sampled periods are

summarized in Table 5. The Kolmogorov-Smirnoff test showed the male subpopulation in 2005-06 to be normally distributed ($P > 0.05$), unlike the female subpopulation ($P < 0.05$), whereas in the 2009-10 samples both subpopulations were normally distributed ($P > 0.05$). Because of the deviation of the female subpopulation in 2005-06 from normality, the distributions were compared using the non-parametric Mann-Whitney test, which showed no significant difference between the two sampled subpopulations ($P > 0.05$). For the 2009-10 samples, and since the sample numbers differed significantly, the Mann-Whitney test was used again to compare the values, which were significantly different from each other ($P < 0.05$).

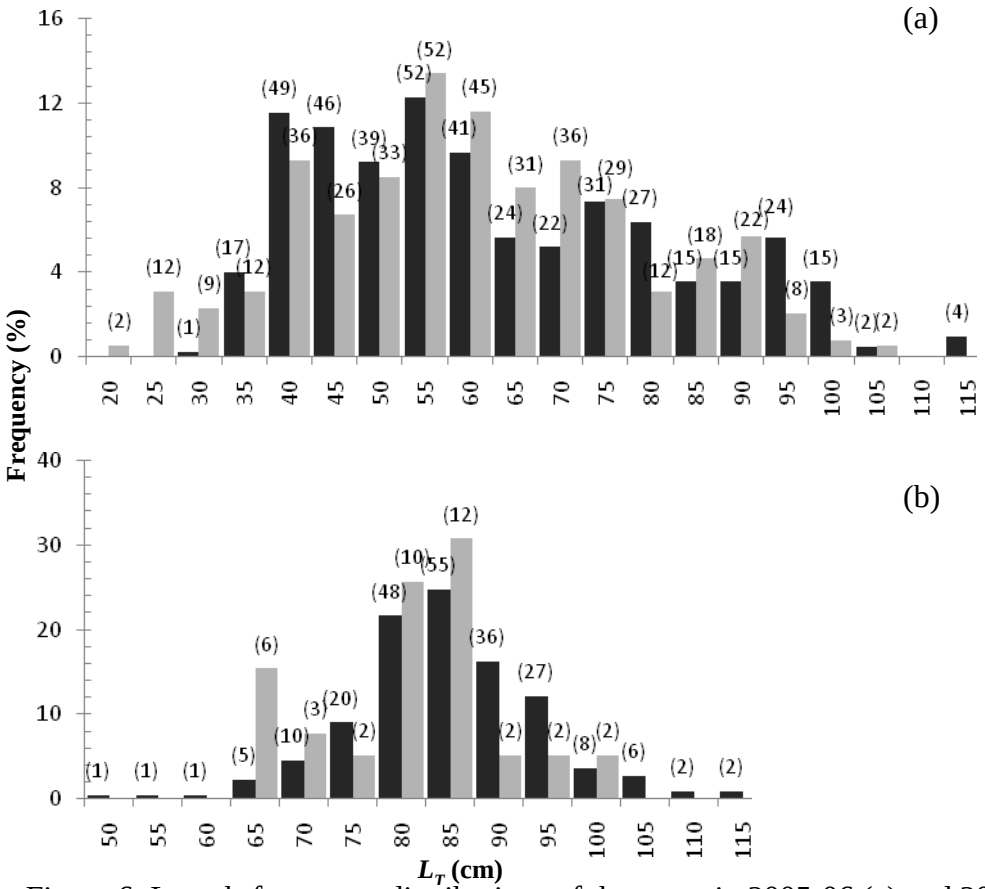


Figure 6: Length-frequency distributions of the sexes in 2005-06 (a) and 2009-10 (b). Females (black) and males (grey). L_T: total length; frequencies (n) in brackets.

Table 5: Descriptive statistics for the total length (L_T, cm) across the sexes. *n* denotes the frequencies.

Sex	*n*	Mean	S.D.	Minimum	Maximum
	2005-06				
Female	424	60.34	19.22	26.6	112.4
Male	388	57.64	17.81	19.2	100.1
	2009-10				
Female	222	82.83	9.66	48.3	113.1
Male	39	78.55	9.58	61.7	99.9

3. Sex Ratio

The total sex ratio for the 2005-06 samples was 1.1 : 1 females to males and did not significantly deviate from the expected 1 : 1 ratio ($P > 0.05$).

The monthly 2005-06 samples showed no deviation from the hypothesized 1 : 1 ratio in all the months except for March ($P < 0.05$; Fig. 7a). The monthly percentages were somewhat balanced between males and females, except for November, December, and January, where there was a skew towards males. The distribution of the females vs. males across the size classes showed significant deviations from the 1 : 1 ratio in seven size classes ($P < 0.05$) , as show in Fig. 7b; the two smallest classes out of the seven showed a skew towards males, whereas the rest of the seven were favored towards females.

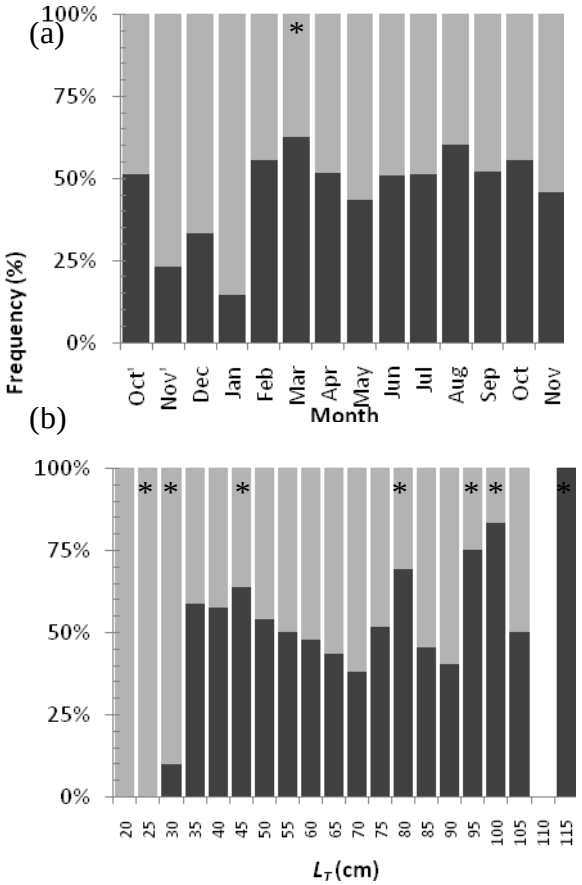


Figure 7: (a) Monthly variations and (b) size-class variations of sex ratios for the 2005-06 samples. Females (dark grey) and males (light grey). Sex ratios significantly different from 1 : 1 ($P < 0.05$) are denoted by (*).

B. Morphological Description of the Gonads

Macro- and microscopic characteristics of *F. commersonii* gonads (n = 1,073) were studied. Gonads sampled during the two periods, 2005-06 and 2009-10, were described and analyzed macroscopically, whereas the microscopic description was based solely on the 2009-10 samples.

1. Ovarian Structure

Macroscopically, the ovaries of *F. commersonii* studied (*n* = 646) appeared as a single structure, long and thin and slightly depressed, located along the abdominal cavity, adjacent or dorsal to the digestive tract. The right lobe was slightly longer than the left one anteriorly. In aspect, it was smooth, and ranged in color from pale yellow to pinkish to red, depending on the different maturational stages. The anterior "head" region was thicker in general, especially in the more mature ovaries, and tapered to a relatively thin posterior "tail" region. The ovaries extended approximately halfway, in case of immature or developing stages, to fully, in case of the later stages of maturity, along the abdominal cavity. Accumulated fat was often found along their length dorsally (Fig. 8a, b).

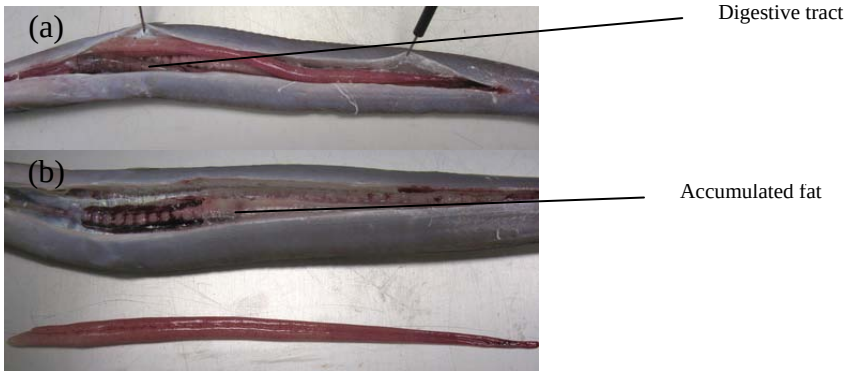


Figure 8: Ventral view of *F. commersonii* specimen showing the ovaries. (a) Ovaries attached dorsally to the body cavity and (b) extracted.

Microscopically, all sectioned ovaries (*n* = 211) also showed as two fused lobes, partitioned by a septum of connective tissue continuous with the ovarian tunica. The two fused lobes showed a slight to significant asymmetry in diameter. Leaf-like ovigerous folds (lamellae), covered by an epithelium, extended into the ovocoel from the ovarian wall, originating from or close to the septum, and extending towards the

distal periphery of the lobe. Thus, the ovarian lumen is located towards the distal periphery of the ovarian lobe and not central in position. Oocytes in various stages of development were found within the ovigerous folds, characteristic of an asynchronous mode of oogenesis. Eventually, the mature oocytes break the lamellar epithelium and moved into the ovarian lumen upon ovulation prior to spawning (Fig. 9). The various developmental stages of the oocytes are described in Table 6. The ovaries were categorized macro- and microscopically into eight stages, as summarized in Table 7.

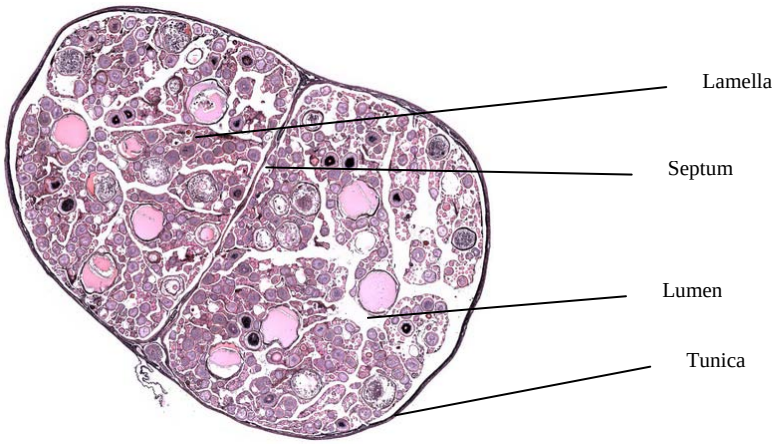


Figure 9: Histological appearance of *F. commersonii* ovaries, transverse cut.

2. Testicular Structure

Macroscopically, the testes (n = 427) appeared as a single structure, long, thin, and flattened dorsoventrally, located along the abdominal cavity, usually more or less dorsal in position to the digestive tract. The right lobe was anteriorly longer than the left one and both significantly thinner and lighter than the ovaries. In aspect, it was swollen and with irregular edges to various degrees, depending on its maturity stage. It ranged in color from pale yellow to pinkish and reddish, depending on the different maturational stages, showing whitish in developing or mature stages (Fig. 10a, b; Table 7). The

anterior “head” region was thicker (dorsoventrally) and with more irregular edges, especially in the more mature testes, and tapered to a relatively thin posterior “tail” region, less irregular in form. Two sperm ducts, located dorsally along the medial section of the testes, were visible in developing and mature testes, appearing white as a result of the contained spermatozoa (Fig. 10a, b).

Figure 10: Ventral view of *F. commersonii* specimen showing the testes. (a) Testes attached dorsally to the body cavity. (b) Extracted testes.

Microscopically, a transverse section of the testes processed (n = 33) also showed two fused lobes, leaf-like in shape, joined ventrally with connective tissue, the sperm ducts also connecting them dorsally. The lobes showed slight to significant asymmetry in size. They were covered by a thin tunica albuginea. Sperm sinuses were observed located proximally, adjacent to the sperm ducts. The spermatogenic compartments extended to the edge of the testes and spermatogenesis occurred all along the lobules. Moreover, the spermatogenic lobules formed an anastomosing network proximally to the sperm ducts (Fig. 11). The characterized the unrestricted lobular type of spermatogenesis. During the course of development, the spermatocysts broke down, releasing the spermatozoa into the anastomosing network. This also characterized the cystic type of spermatogenesis. Moreover, spermatogenesis was more advanced towards

the proximal end of the lobe, and less advanced in the distal end. The spermatozoon was of the uniflagellate type, with a small rounded head and one tail (Fig. 12). The testes are categorized macro- and microscopically into four stages, as summarized in Table 8.

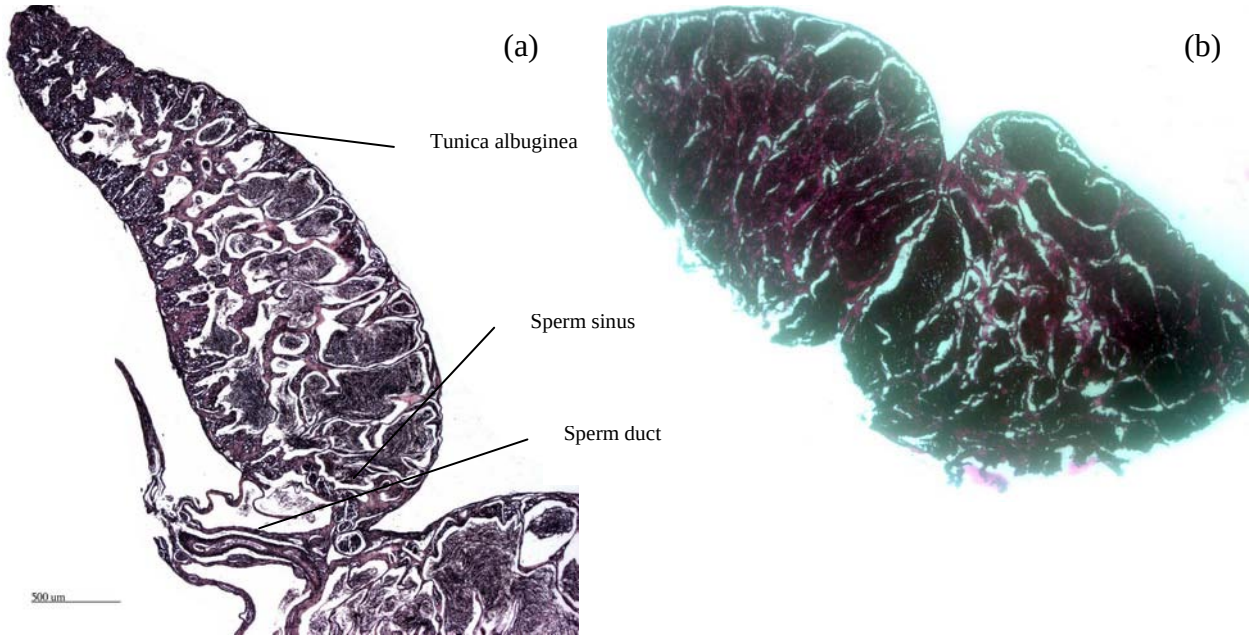


Figure 11: Transverse section of *F. commersonii* testes, one lobe (a) and both lobes (b). Scale bar = 500 µm.

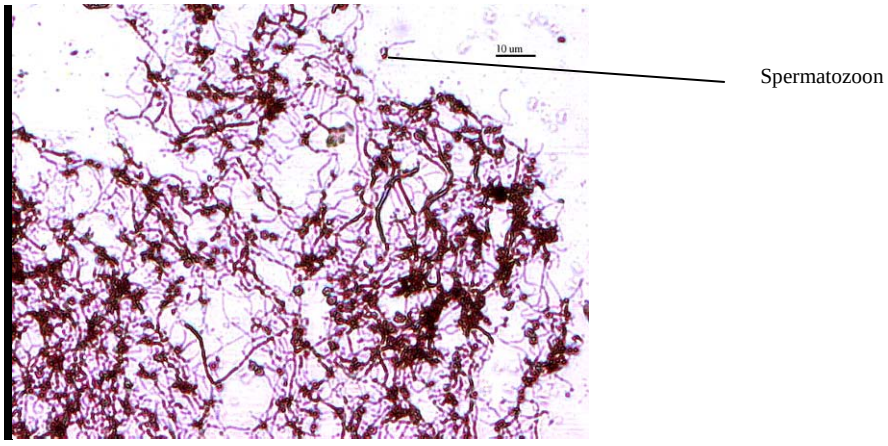
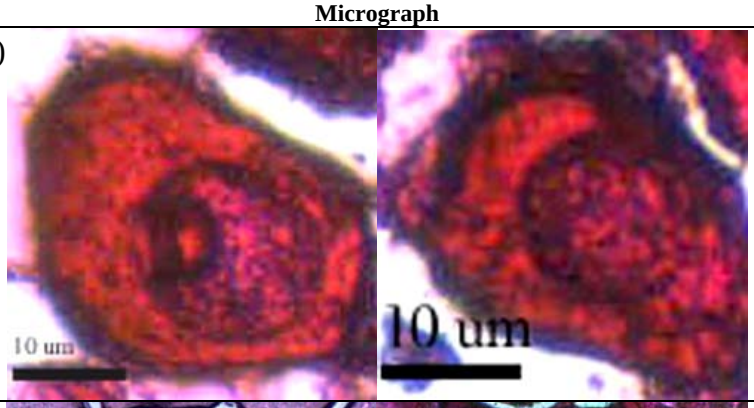
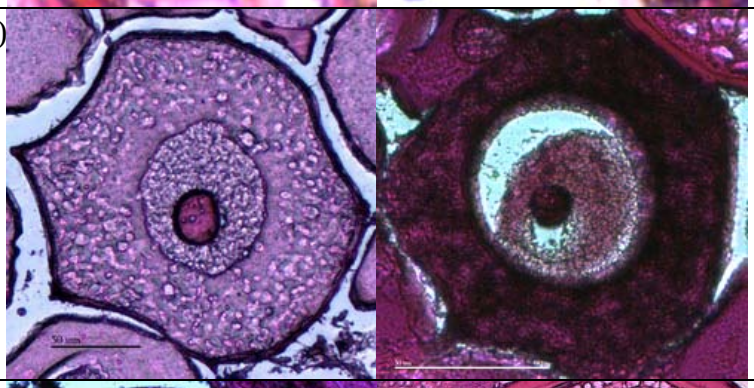
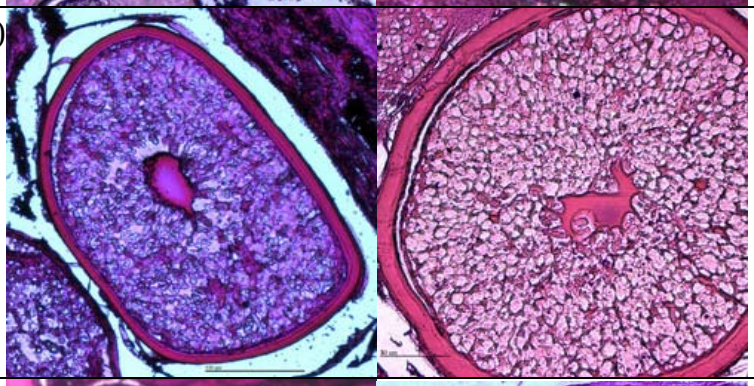
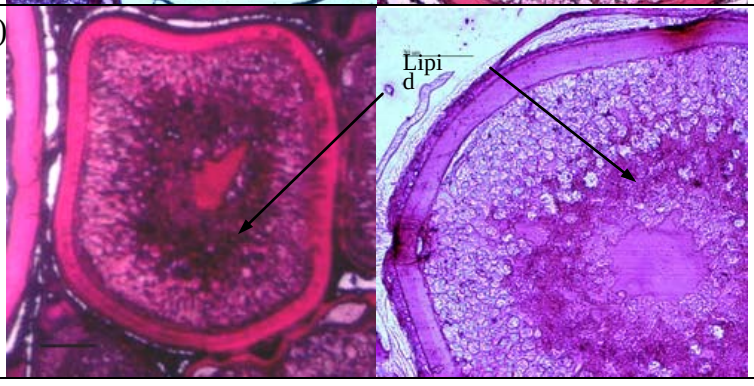
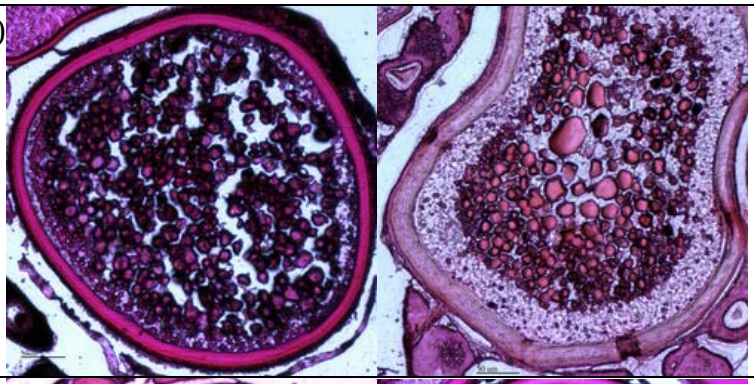
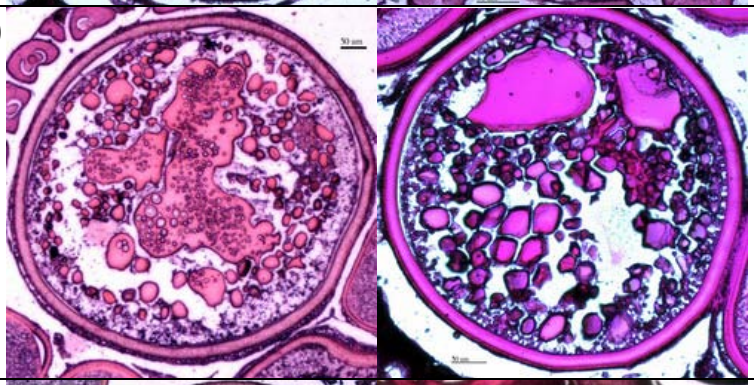
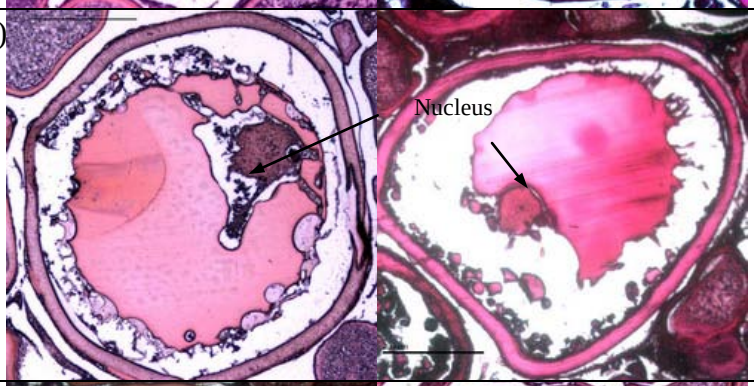
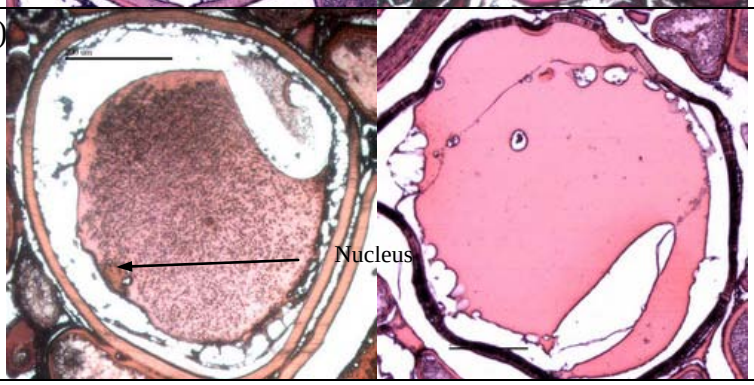


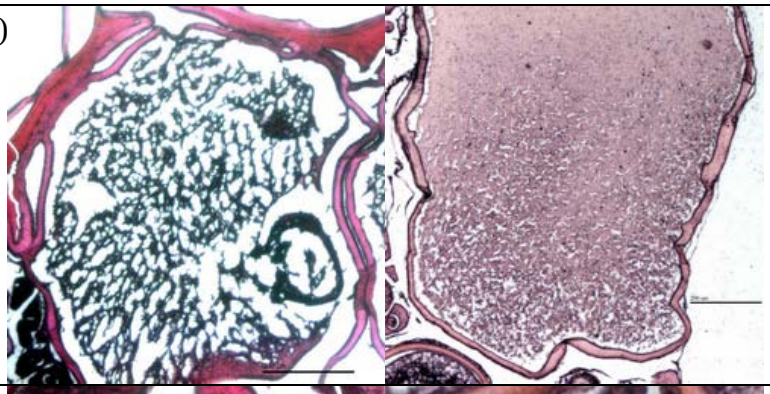
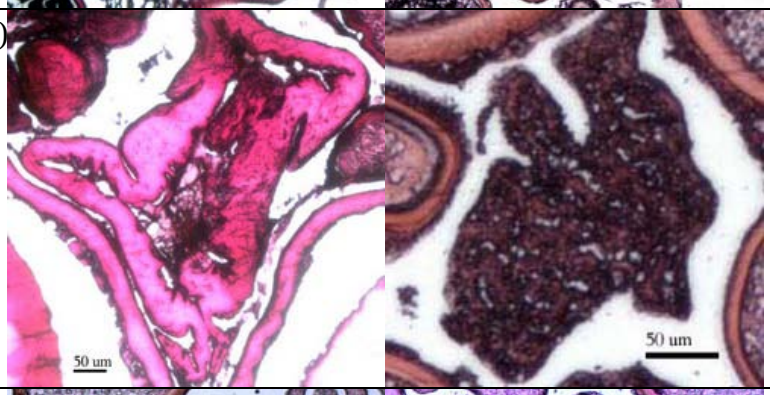
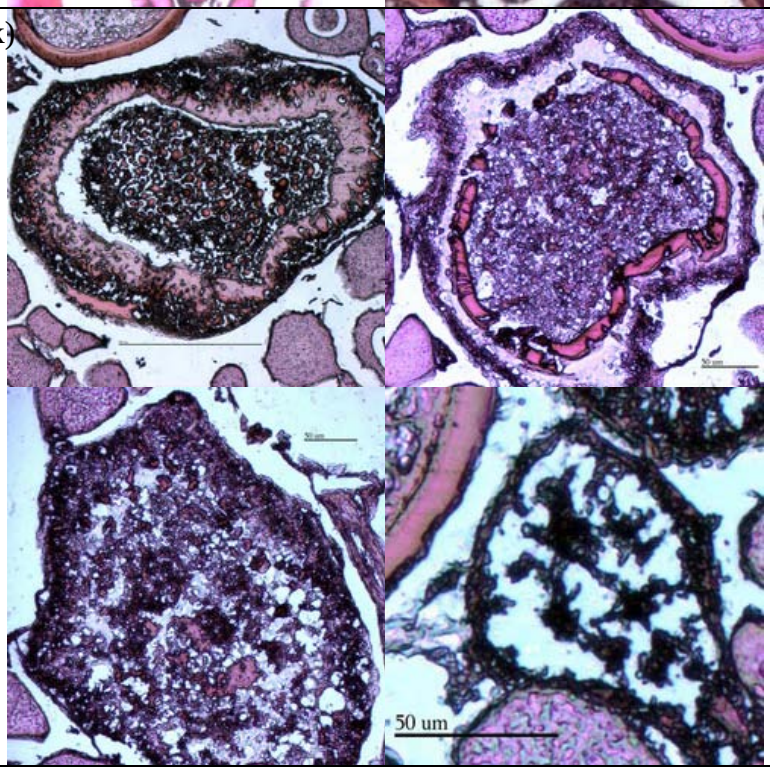
Figure 12: Microscopic appearance of *F. commersonii* spermatozoa. Scale bar = 10 µm.

C. Oocyte Developmental Stages

Table 6: Histological appearance and microscopic characteristics of the oocyte developmental stages, in addition to atretic oocytes and post-ovulatory follicles (POF). All measurements are in micrometers (µm). Scale bar = (a) 10 µm, (b-f) 50 µm, (g-i) 200 µm, (j) 50 µm, (k) 50 µm, except pic1: 200 µm.

Stage	Micrograph	Description
Chromatin nucleolus Mean 49.80 S.D. 9.61 Min. 32.78 Max. 67.34	(a) 	High nucleus-to-cytoplasm ratio. Weakly basophilic cytoplasm, more basophilic nucleus, one big nucleolus observed.
Perinucleolar Mean 104.64 S.D. 20.02 Min. 62.80 Max. 175.14	(b) 	Highly basophilic cytoplasm, less basophilic nucleus. One big nucleolus observed.
Cortical alveolar I Mean 248.89 S.D. 42.45 Min. 168.27 Max. 331.49	(c) 	Cytoplasm increasingly loses basophily and does not stain well. Appearance of cortical alveoli that do not stain, appearing empty. Nucleus increasingly irregular in shape. Appearance of the intensely acidophilic zona radiata.
Cortical alveolar II (lipid vesicle) Mean 313.15 S.D. 24.75 Min. 275.08 Max. 386.70	(d) 	Lipid droplets begin to accumulate around the irregular nucleus. Cortical alveoli still dispersed throughout cytoplasm.

Early vitellogenesis (YI) Mean 403.21 S.D. 51.19 Min. 312.03 Max. 520.88	(e)		Acidophilic (pink) protein yolk globules start to appear, around mid-cytoplasm first, where they grow in size and number. Cortical alveoli displaced towards the periphery of the cytoplasm. Zona radiata thickens and shows striations.
Mid vitellogenesis (YII) Mean 501.85 S.D. 79.96 Min. 375.39 Max. 718.09	(f)		The acidophilic yolk globules grow and coalesce, increasing in size towards the periphery of the nucleus. Zona radiata shows striations.
Late vitellogenesis (YIII) Mean 659.74 S.D. 67.01 Min. 519.30 Max. 770.54	(g)		The acidophilic yolk globules further coalesce, forming a yolk mass in the cytoplasm. Towards the end of the stage, the nucleus starts to migrate towards the animal pole.
Final Oocyte Maturation (FOM) Mean 785.42 S.D. 123.27 Min. 626.42 Max. 1162.0	(h)		Upon the onset of maturation, and yolk globules are continuing to fuse into one homogenous acidophilic yolk mass, the nucleus migrates to the periphery of the oocyte, to the animal pole. Zona radiata prominent, and shows striations. Easily distorted during histological preparation.

Hydration Mean 1028.1 S.D. 96.25 Min. 905.43 Max. 1298.6	(i) 	Germinal vesicle disappears. Oocytes take up water in hydration, progressively losing acidophily. They show a detachment of the follicular layer, and are extremely delicate, thus easily distorted or broken during histological preparations and sectioning.
Post-ovulatory follicle	(j) 	POF's show very acidophilic, the convoluted follicular layers staining very pink immediately after releasing the oocyte. Afterwards, POF degenerates, losing acidophily (pic2).
Atresia (different stages)	(k) 	Nucleus is absorbed. Zona radiata deteriorates – loss of striations and uneven diameter observed. Follicle stains basophilic. Invading granulosa cells inside the cytoplasm degenerate the cytoplasm and remaining yolk. When the yolk has been resorbed, the structure collapses, becoming much smaller.

D. Gonad Maturity Stages

Table 7: Macro- and microscopic description of the ovarian developmental stages in *F. commersonii*. Criteria used to classify females into maturity stages. Gonadosomatic index (*GSI*) ranges for each stage included.

Ovarian stage	Macroscopic description	Microscopic description
1. Immature	Ovaries very small, thin, and fragile. Translucent and very pale yellow in color. No vascularization. (Fig. 13a).	Only primary growth oocytes present (oogonia, chromatin nucleolar, and perinucleolar stages). No atresia. (Fig. 14a)
2. Developing	Ovaries larger than immature, small to medium in size. Light yellow-pink to reddish in color. No oocytes visible through the ovarian membrane. Light vascularization. (Fig. 13b).	Primary growth to cortical alveolar and lipid vesicle-stage oocytes present. Atresia rare. (Fig. 14b)
3. Fully developed	Ovaries medium to large in size. Yellow – light pink in color. Translucent small oocytes visible through the membrane. Light to medium vascularization. No signs of previous spawning present. (Fig. 13c).	Primary growth to tertiary yolked stage oocytes present. Atresia rare. Remnant hydrated oocytes and post-ovulatory follicles (POF) absent. (Fig. 14c).
4. Gravid	Ovaries ranging from medium to huge. Ovary speckled in appearance, maturing translucent oocytes clearly visible. Pinkish early in the season to red in color later on. Light and medium vascularization early in the season to high later on. Ovaries may release some oocytes when handled, though no oocytes are expelled upon pressing the abdomen. (Fig. 13d).	Primary growth to FOM-stage oocytes present. Oocytes partially hydrated and not migrating to the lumen yet. Atresia of yolked oocytes rare. Old degenerating POF's may be present. (Fig. 14d).
5. Running-ripe	Ovarian size medium to large. Fully hydrated and ovulated oocytes very obvious as a clear strip in the ovarian lumen, in the peripheral sides of the joined ovaries. Upon the application of pressure on the abdomen, oocytes are released from the genital pore. Color ranges from light pink early in the season to red at the end. Light to medium vascularization in general. (Fig. 13e).	Primary growth to fully hydrated and ovulated oocytes present, as well as fresh POF's. Hydrated oocytes grouped into ovarian lumen at the distal periphery of the ovary. Atresia of yolked oocytes may be present. Sometimes only hydrated and primary growth to cortical alveolus-stage oocytes present. (Fig. 14e).
3'. Partially spent/ redeveloping	Ovaries somewhat limp, size between medium to large. Reddish in color. Higher vascularization. Remnant hydrated oocytes sometimes visible, interspersed along the lumen or between developing translucent (yolked) oocytes. Sometimes difficult to differentiate macroscopically from stage 3 ovaries. (Fig. 13f).	Primary growth to tertiary yolked oocytes present, as well as remnant hydrated oocytes and POF's. Atresia is common. Similar to stage 3. (Fig. 14f).
6. Regressing	Ovaries medium to small in size, and limp. Red to purplish in color. Highly vascularized. Remnant hydrated oocytes sometimes visible. (Fig. 13g).	Primary growth to cortical alveolar-stage oocytes present. Remnant hydrated oocytes and POF's sometimes present. Characterized by heavy atresia. (Fig. 14g).
7. Resting	Ovaries small, though not as small as immature fish, comparable in size to early developing ovaries. Limp. Reddish and steadily turning pinkish yellow in color and slightly translucent. Low vascularization. (Fig. 13h).	Mostly primary growth-stage oocytes, though cortical alveolar-stage oocytes also present. Atresia may be present. (Fig. 14h).

Table 8: Macro- and microscopic description of the testicular developmental stages in *F. commersonii*. Criteria used to classify males into maturity stages. Gonadosomatic index (*GSI*) ranges for each stage included.

Stage	Macroscopic description	Microscopic description
1. Immature	Testes very small, thin, and threadlike, and extremely fragile. Translucent and very pale yellow in color. Distinguishable from ovaries by being flatter in shape and not as smooth. No vascularization. No milt. (Fig. 15a).	Testes formed of spermatogonia in cysts. No spermatids or spermatozoa visible. (Fig. 16a).
2. Developing	Testes clearly recognizable. Noticeably bigger in size than immature. May be translucent yellow in earlier stages, and then becomes progressively light pinkish-white in color due to the accumulation of milt in the tissues. Light vascularization appears. Shape of the testes increasingly irregular, especially the anterior region. Milt often present in sperm ducts, and some flows upon handling the testes. (Fig. 15b).	Spermatogenic lobules apparent; spermatogenesis advances in the spermatocysts. Spermatids and spermatozoa to varying degrees of abundance clearly visible within the lobules. The lobules open into each other (anastomose) and eventually into the sperm sinuses at the periphery of the sperm ducts, emptying the spermatozoa into the sperm sinuses and sperm ducts towards the end of the stage. (Fig. 16b).
3. Running	Testes similar in size and shape to stage 2. Color increasingly pinkish to reddish. Medium vascularization. Sperm ducts full of milt, and the areas surrounding them appear whitish due to milt accumulated in the tissues. Easily identified as milt flows from the genital pore upon the application of pressure on the abdomen. (Fig. 15c).	Sperm sinuses empty into the sperm ducts which are now full of spermatozoa. Towards the end of the stage, the spermatogenic lobules contain few spermatids or spermatozoa. (Fig. 16c).
4. Spent	Testes still lobed in shape and may continue to be big in size. Identifiable through the reddish color due to high vascularization. No white-appearing milt in the tissues. May have residual milt in sperm ducts that may still flow when handled. Sometimes difficult to differentiate from stage 2 testes. (Fig. 15d).	Spermatogenic lobules regress into cysts as of before the beginning of development, with the lumina occluded. Very few to no residual spermatids or spermatozoa present in the tissues. Residual spermatozoa may persist within the sperm duct. (Fig. 16d).

Table 9: Summary of *GSI* statistics per gonad stage.

Stage	*Mean*	*S.D.*	*Range*	*Mean*	*S.D.*	*Range*
		Female			*Male*	
1	0.38	0.15	0.07-0.89	0.05	0.05	0.01-0.34
2	1.23	0.54	0.52-2.74	0.37	0.18	0.10-1.12
3	4.82	2.59	1.42-9.85	0.56	0.32	0.25-1.73
4	9.76	2.97	4.25-18.20	0.52	0.21	0.14-1.05
5	9.92	3.56	5.83-15.06			
3'	5.53	2.20	1.94-11.76			
7	2.86	1.36	0.71-4.87			
8	1.10	0.46	0.27-1.90			

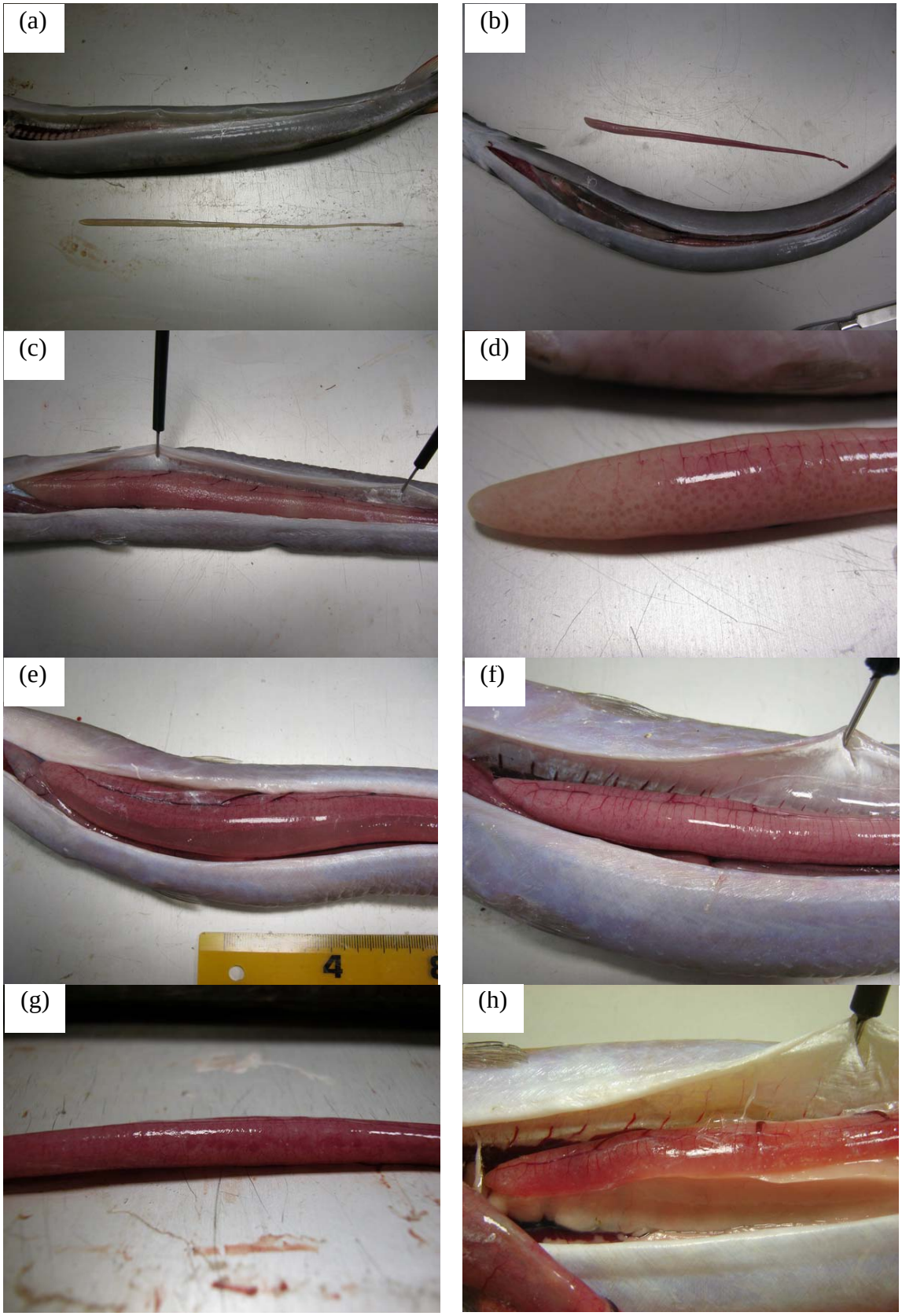


Figure 13: Macroscopic appearance of the ovarian stages. (a) Stage 1, immature; (b) stage 2, developing; (c) stage 3, developed; (d) stage 4, gravid; (e) stage 5, running-ripe; (f) stage 3', redeveloping; (g) stage 6, regressing; (h) stage 7, resting.

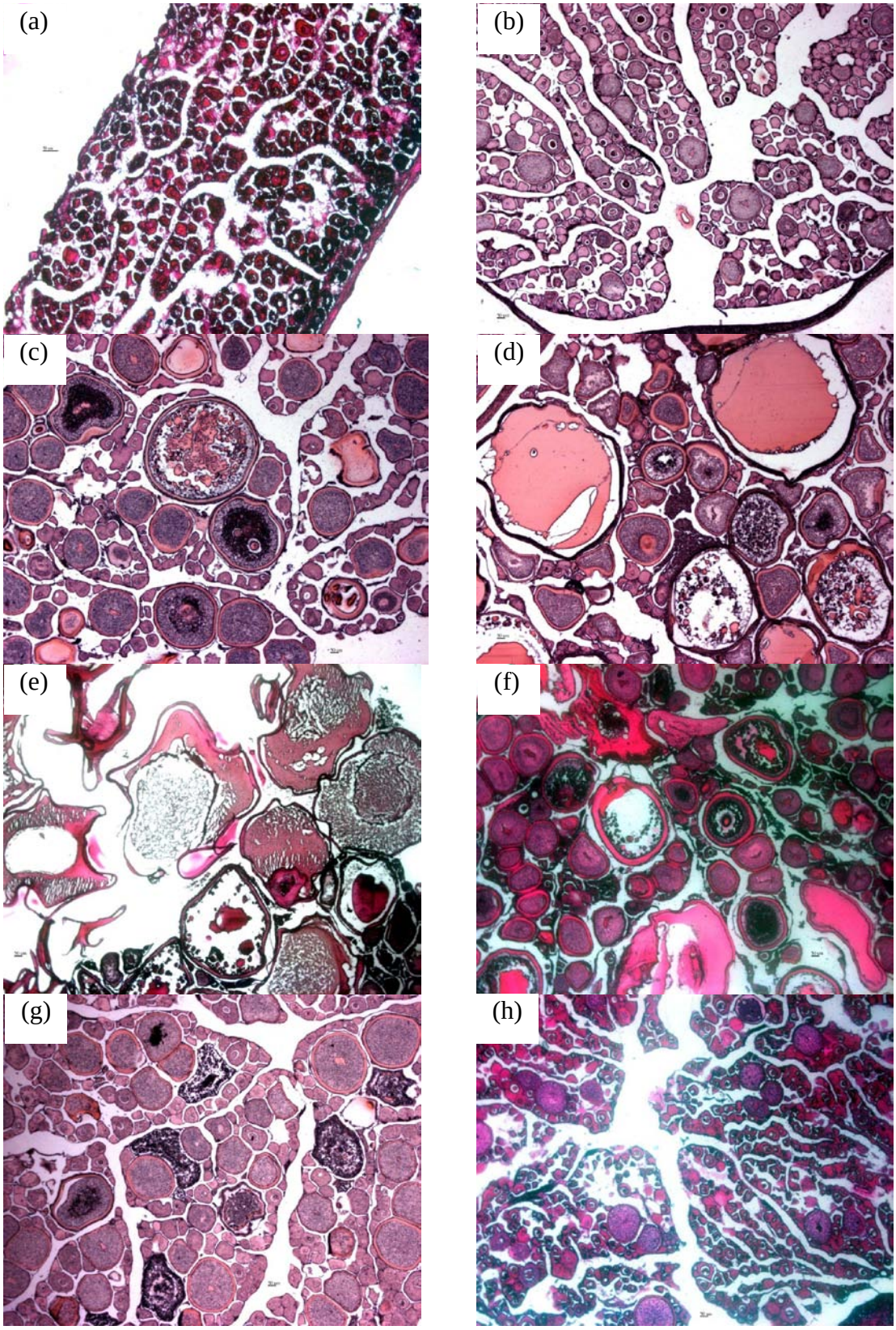


Figure 14: Micrographs of the ovarian stages. All scale bars are 50 µm. (a) Stage 1, immature; (b) stage 2, developing; (c) stage 3, developed; (d) stage 4, gravid; (e) stage 5, running-ripe; (f) stage 3', redeveloping; (g) stage 6, regressing; (h) stage 7, resting.

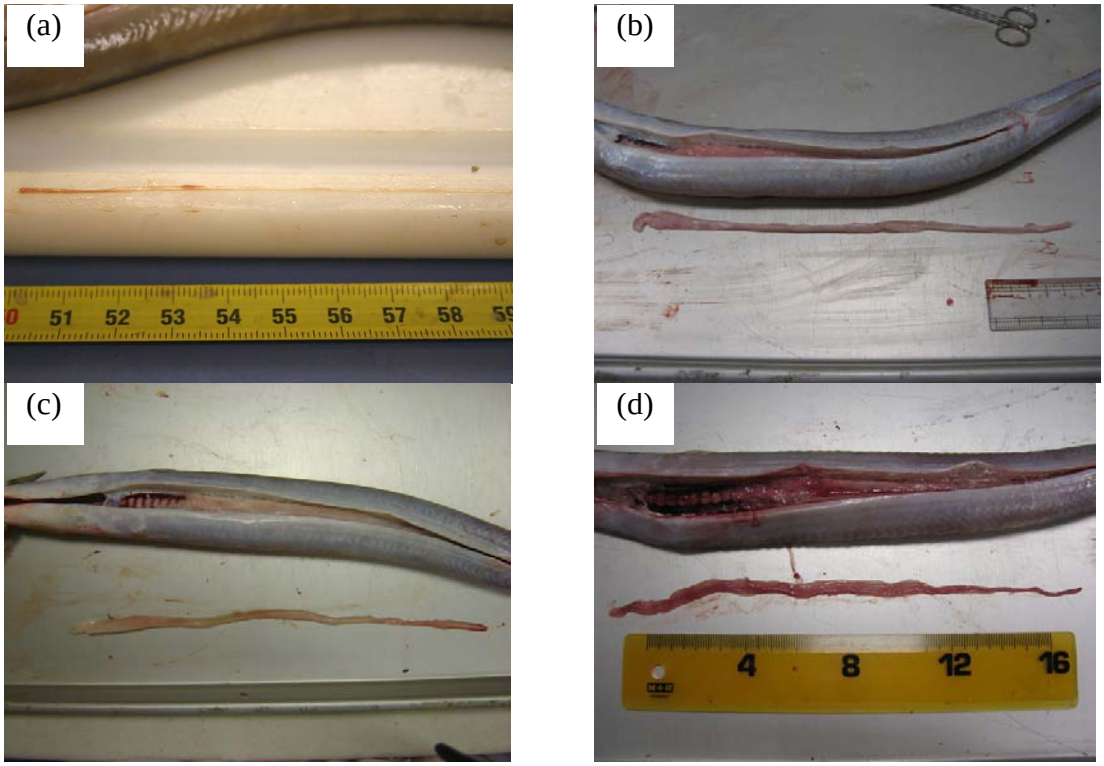


Figure 15: Macroscopic appearance of the testicular stages. All scale bars are 50 µm. (a) Stage 1, immature; (b) stage 2, developing; (c) stage 3, running; (d) stage 4, spent.

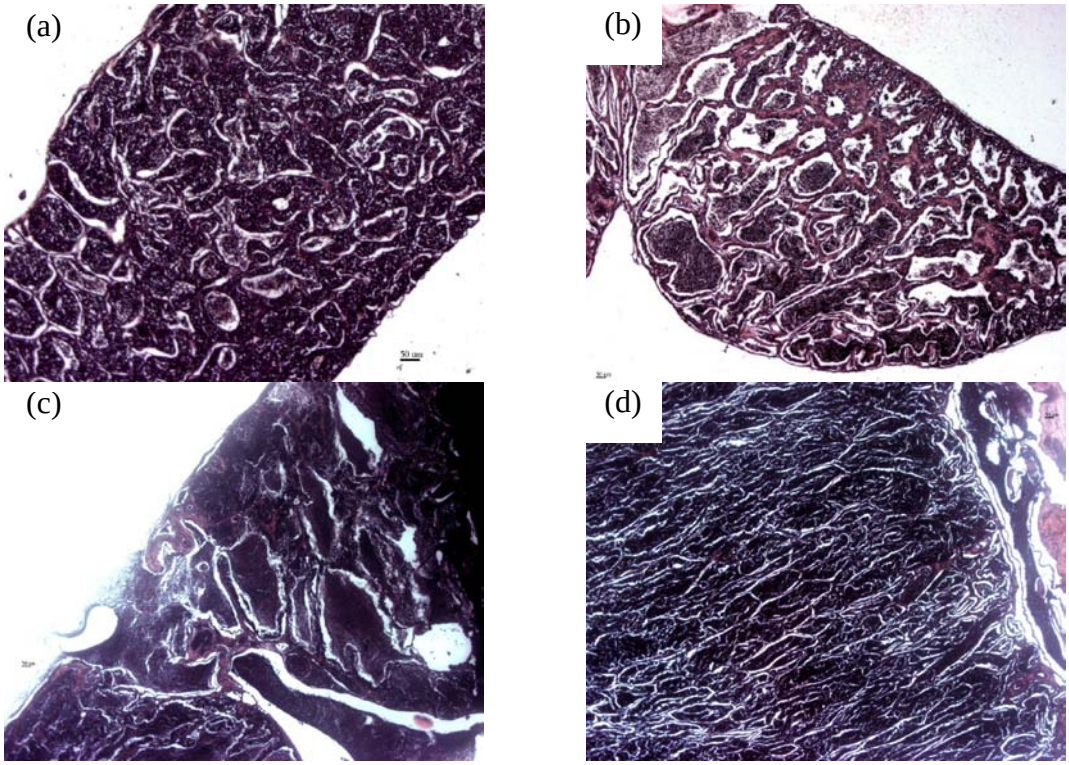


Figure 16: Micrographs of the testicular stages. All scale bars are 50 µm. (a) Stage 1, immature; (b) stage 2, developing; (c) stage 3, running; (d) stage 4, spent.

E. Maturity Staging

All specimens collected in the two sampling periods were staged macroscopically, whereas the 2009-10 samples were staged microscopically in addition to macroscopically.

1. Macroscopic Staging

In the 2005-06 *F. commersonii* samples, the female reproductive period started in April, where developing females (stage 2) were found. A gravid female (stage 4) was found in late May, signifying the start of the spawning period. Fully developed (stage 3) females were found starting June, further confirming the start of the spawning period. Redeveloping females (stage 3') were found in early July, showing evidence of spawning events earlier. Reproductively active females continued till October, where running individuals (stage 5) were still found. In November, redeveloping females (stage 3') were still found, whereas only females in regression (stage 7) were found in December, showing the extent of the spawning and reproductive seasons to be till November. On the other hand, regressing females (stage 7) started to appear since July and resting individuals (stage 8) were found starting October. In the beginning of the reproductive season, no resting females were observed after May. As for the males, developing and spent individuals (stages 2 and 4) were found almost the whole round, with running males (stage 3) were found in the period extending from May to December.

For the 2009-10 females, spawning and redeveloping females (stages 5 and 3') were found since July, showing the spawning season to have started even earlier. During this sampling period, some reproductively active females (gravid; stage 4) were

found in December. Moreover, regressing females (stage 7) were found starting September, with resting (stage 8) individuals appearing starting October 2009. On the other hand, the two sampled months in 2010 showed reproductively active females (gravid; stage 4) since April, with running and redeveloping individuals (stages 5 and 3') present in May. For the males, and similar to the 2005-06 sampling period, newly spent females (stage 4) were found starting July, with running males (stage 3) present till December. Moreover, in the two sampled months of 2006, developing and running males (stages 2 and 3) were found.

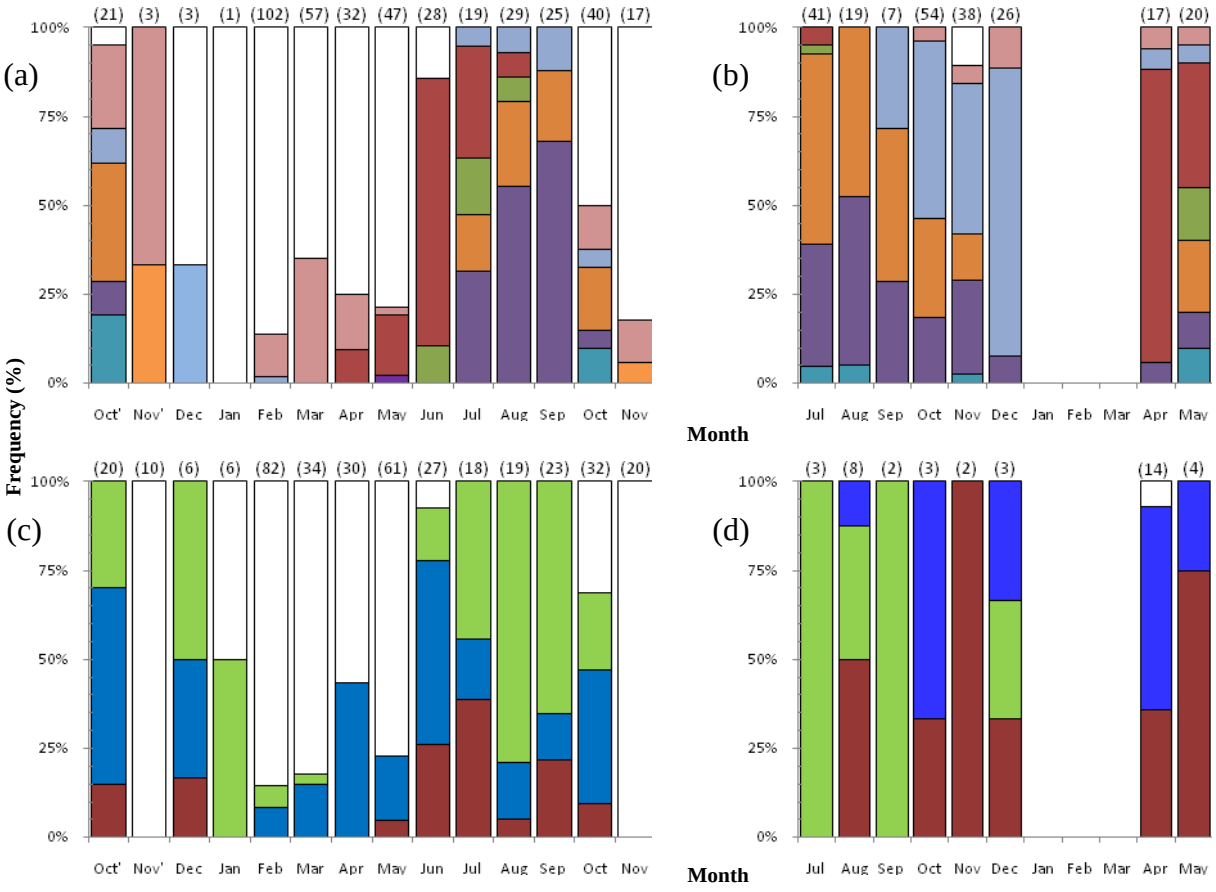


Figure 17: Temporal variations of the gonad maturity stages described macroscopically in the 2005-10 (a, c) and 2009-10 (b, d) *F. commersonii* samples, for females (a, b) and males (c, d). Monthly frequencies (*n*) in brackets. For the females stages (a, b), immature (white), developing (red), developed (green), gravid (purple), running (blue), redeveloping (orange), regressing (light blue), resting (pink). For the male stages (c, d), immature (white), developing (blue), running (red), spent (green).

2. Microscopic Staging

The 2009-10 samples were analyzed microscopically, and staged accordingly to assess the differences between the macro- and microscopic staging systems. Histological analyses showed generally similar results to the macroscopic staging systems, with a few exceptions. In the females, regressing individuals (stage 7) appeared in August instead of September. Moreover, in 2006, April contained a few redeveloping females (stage 3'), signifying the beginning of spawning events before May.

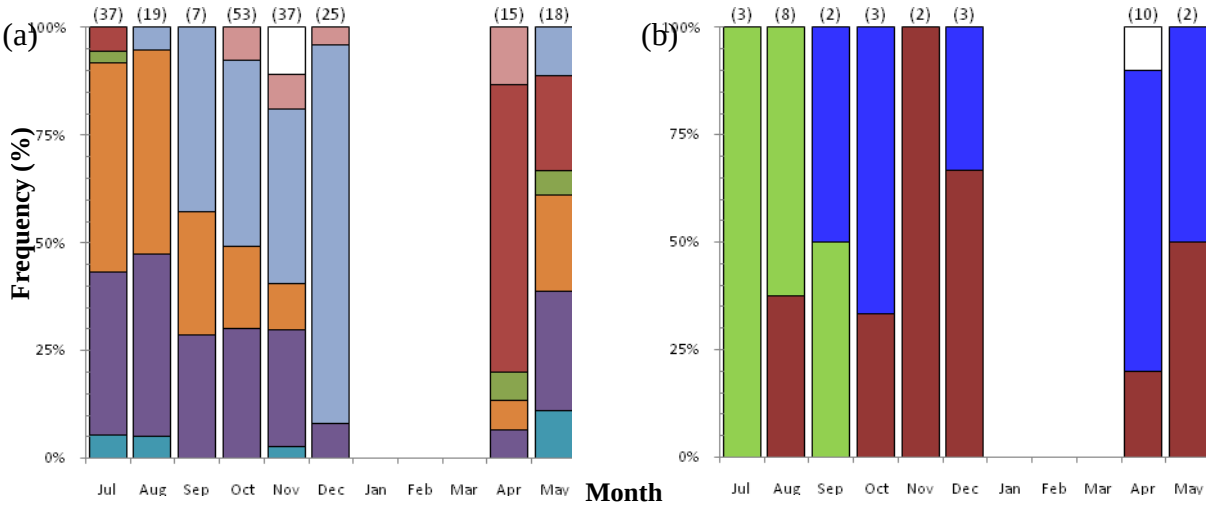


Figure 18: Temporal variations of the gonad maturity stages described microscopically for the 2009-10 *F. commersonii* samples, for females (a) and males (b). Monthly frequencies (*n*) in brackets. For the females stages, immature (white), developing (red), developed (green), gravid (purple), running (blue), redeveloping (orange), regressing (light blue), resting (pink). For the male stages, immature (white), developing (blue), running (red), spent (green).

3. Staging Accuracy

Macroscopic staging showed a total accuracy of 78 % (*n* = 211) in case of the females and 82 % in the males (*n* = 33). In order to assess the concordance between the two staging systems, the non-parametric Kendall's tau-b coefficient test also showed

78.2 % concordance for females (τ = 0.782), whereas the intraclass correlation coefficient (ICC) showed 80.9 % agreement (r = 0.809). For the males, concordance was at 77.4 % (τ = 0.774), whereas ICC showed 81.6 % agreement (r = 0.816). Tables 10 and 11 present the crosstabulation of macro- vs. microscopic staging.

Table 10: Crosstabs of macro- vs. microscopic staging in female *F. commersonii* samples in 2009-10. The labels refer to the maturity stages. The numbers refer to frequencies. Concordant frequencies are highlighted in bold and underlined.

		Microscopic stage								
		1	*2*	*3*	*4*	*5*	*3'*	*7*	*8*	*% Error*
Macroscopic stage	*1*	**4**	0	0	0	0	0	0	0	0
	2	0	**15**	2	0	0	1	0	1	6
	3	0	0	**0**	2	0	1	1	0	100
	4	0	0	0	**37**	0	9	1	0	36
	5	0	0	0	0	**6**	0	0	0	0
	3'	0	0	1	19	0	**35**	1	0	27
	7	0	0	0	0	0	2	**61**	3	8
	8	0	1	0	0	0	0	2	**6**	40
	Total	4	16	3	58	6	48	66	10	

Table 11: Crosstabs of macro- vs. microscopic staging in male *F. commersonii* samples in 2009-10. The labels refer to the maturity stages. The numbers refer to frequencies. Concordant frequencies are highlighted in bold and underlined.

		Microscopic stage				
		1	*2*	*3*	*4*	*% Error*
Macroscopic stage	*1*	**1**	0	0	0	0
	2	0	**10**	1	0	17
	3	0	1	**9**	2	18
	4	0	1	1	**7**	22
	Total	1	12	11	9	

As shown in Table 10, the highest frequencies of errors were recorded in classifying developed (stage 3; 100 %), gravid (stage 4; 36 %), and spent (stage 8; 40 %) females in 2009-10. The developing females (n = 3) were either classified as

developing or redeveloping. The gravid females (n = 58) were misidentified as redeveloping. Most spent females (n = 10) were misidentified as regressing. Moreover, most misidentifications in redeveloping females (stage 3'; n = 48) were due to classifications as gravid. On the other hand, as shown in Table 11, developing males (n = 12) were misidentified as running or spent, running males (n = 11) as developing or spent, and spent males (n = 9) as end-stage running.

F. Size at Maturity

Size at first sexual maturity for the sexes in 2005-06 was calculated using two different methods. The binary logistic function was used on the raw L_T data, not classified into length intervals. The logistic functions obtained were $P = 1 / \{1+e^{[-0.491 (LT-54.61)]}\}$ for the females and $P = 1 / \{1+e^{[-0.583 (LT- 54.72)]}\}$ for the males. It is necessary to note here that the r^2 values were 1 since the regression accounts for all the data points instead of length intervals. The L_{50} estimate was 54.61 cm for females, as opposed to 54.72 cm for males.

On the other hand, the Marquardt method for non-linear logistic regression showed slightly different results. The lengths (L_T), grouped into 5 cm intervals, were analyzed using FISHPARM, and the following functions obtained: $P = 1 / \{1+e^{[-0.403 (LT-57.54)]}\}$ (adjusted r^2 = 0.998) for the females and $P = 1 / \{1+e^{[-0.510 (LT-57.04)]}\}$ (adjusted r^2 = 0.999) for the males. L_{50} estimates were 57.54 cm (S.E. = 0.126) for females and 57.04 cm (S.E. = 0.069) for males.

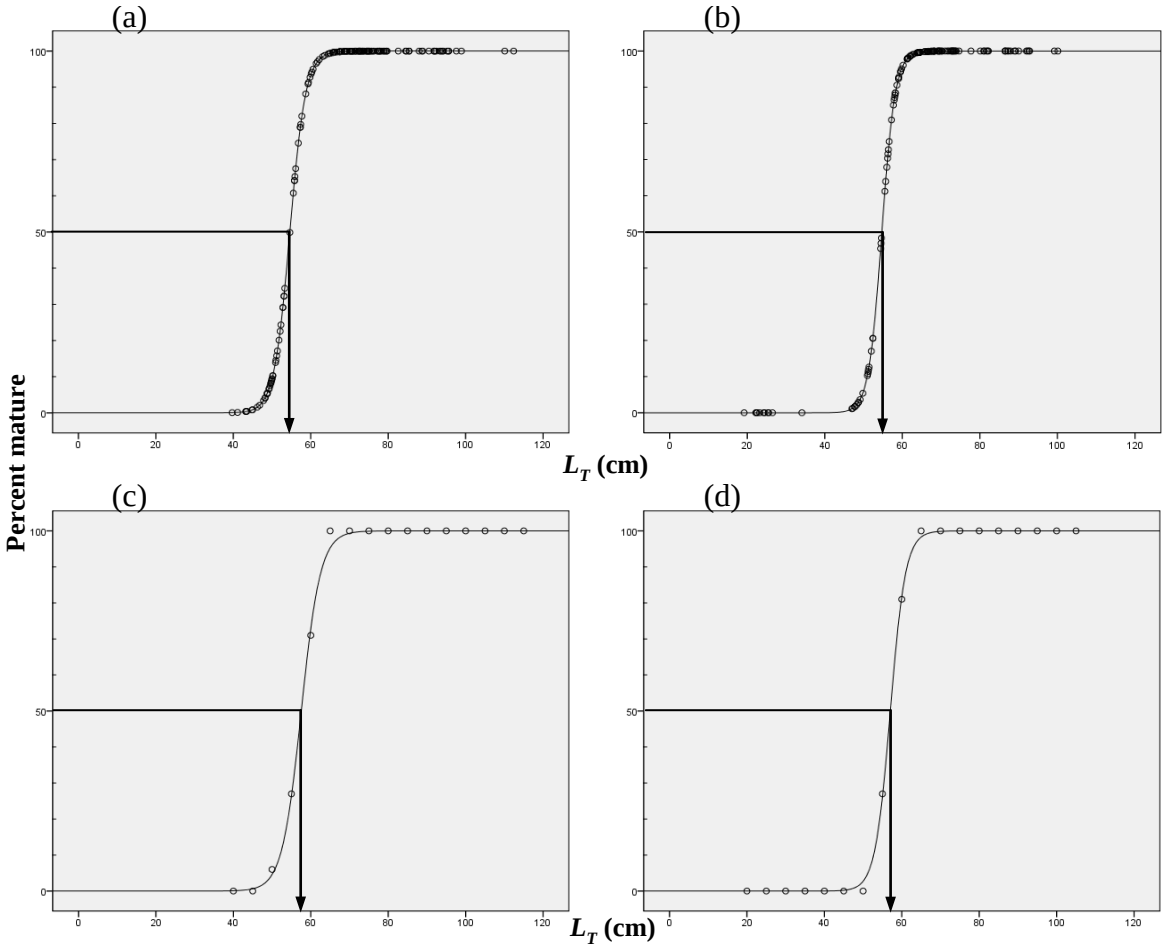
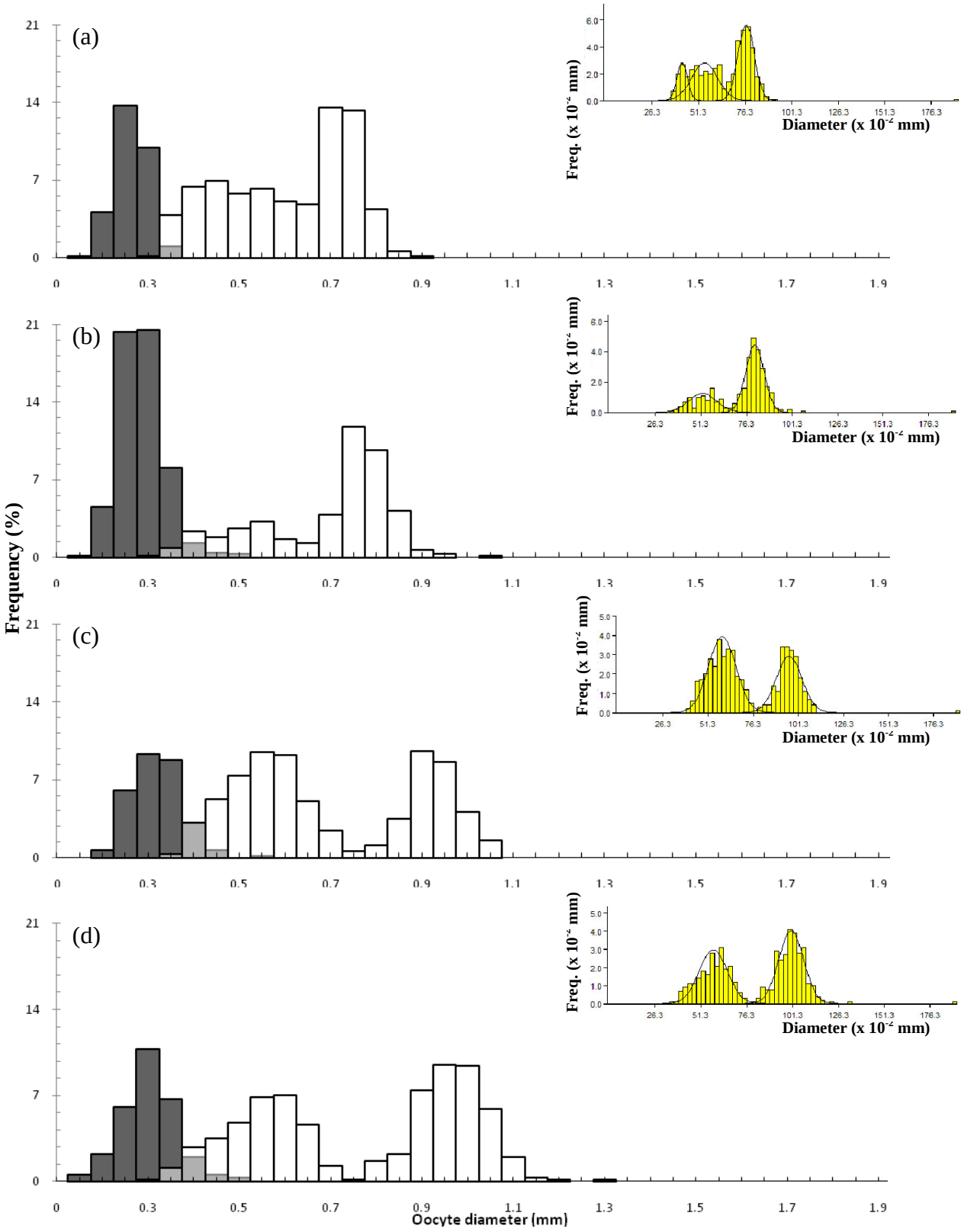


Figure 19: Curves of percentage of mature *F. commersonii* specimens in 2005-06 obtained through a binary logistic function (a, b) vs. Marquardt non-linear method (c, d) in females (a, c) and males (b, d). L_T: total length.

G. Oocyte Diameters

According to Figure 20, the histograms constructed showed oogenesis to be continuous, where there is no gap between the primary and secondary growth stage oocytes. Modes of the subpopulations of oocytes within each ovary are visible based on the comparison with the Bhattacharya plots. These plots showed the presence of two or three batches of developing oocytes within the same ovary (excluding the previtellogenic subpopulation). Moreover, there was consistent overlap between the different subpopulations of oocytes (primary and secondary growth). The degree of overlap gradually decreased as the oocytes were maturing and a clear gap was visible upon hydration. All this clearly shows an asynchronous pattern of oocyte development.


(a)
(b)
(c)
(d)
Frequency (%)
Oocyte diameter (mm)
Freq. (x 10⁻² mm)
Diameter (x 10⁻² mm)

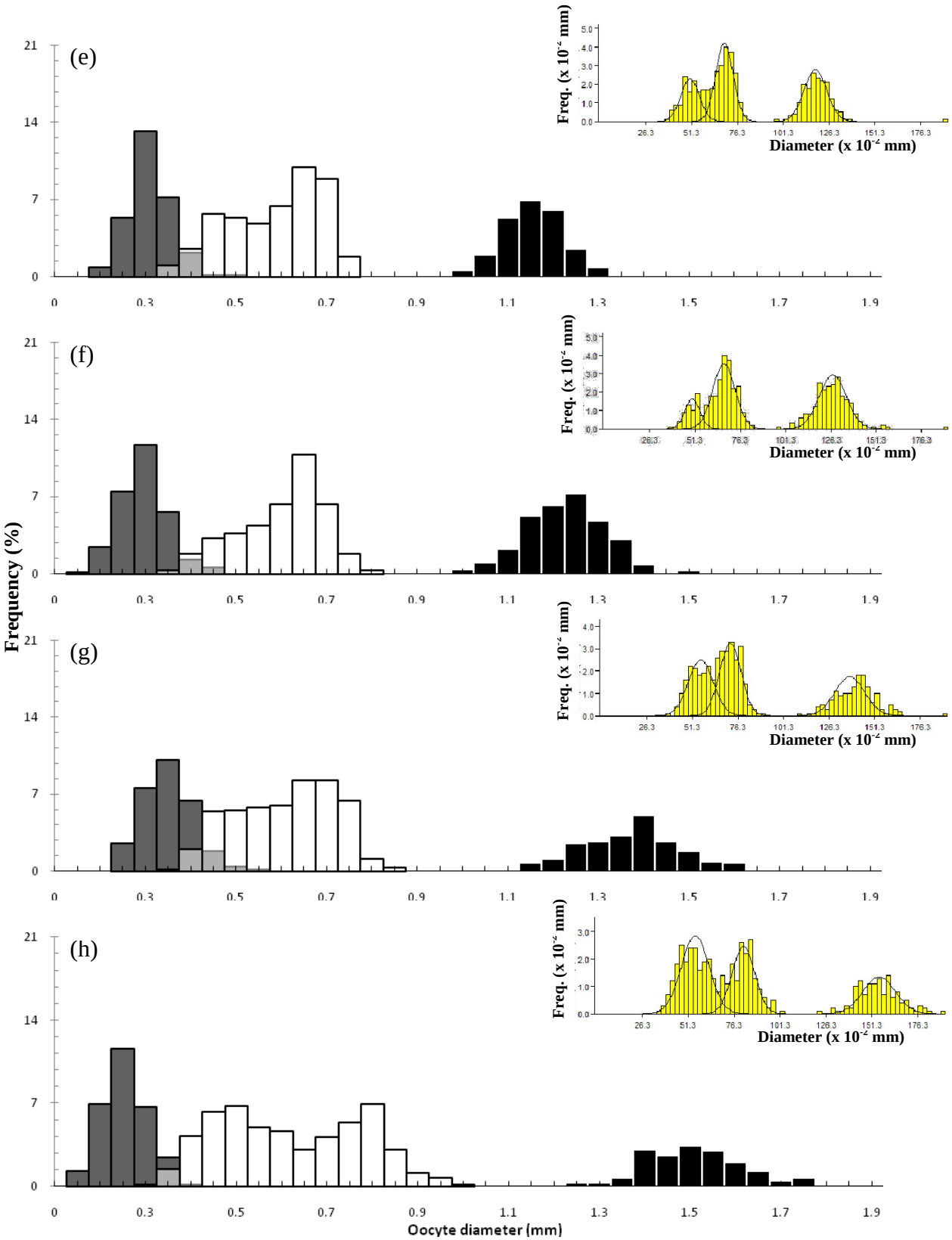
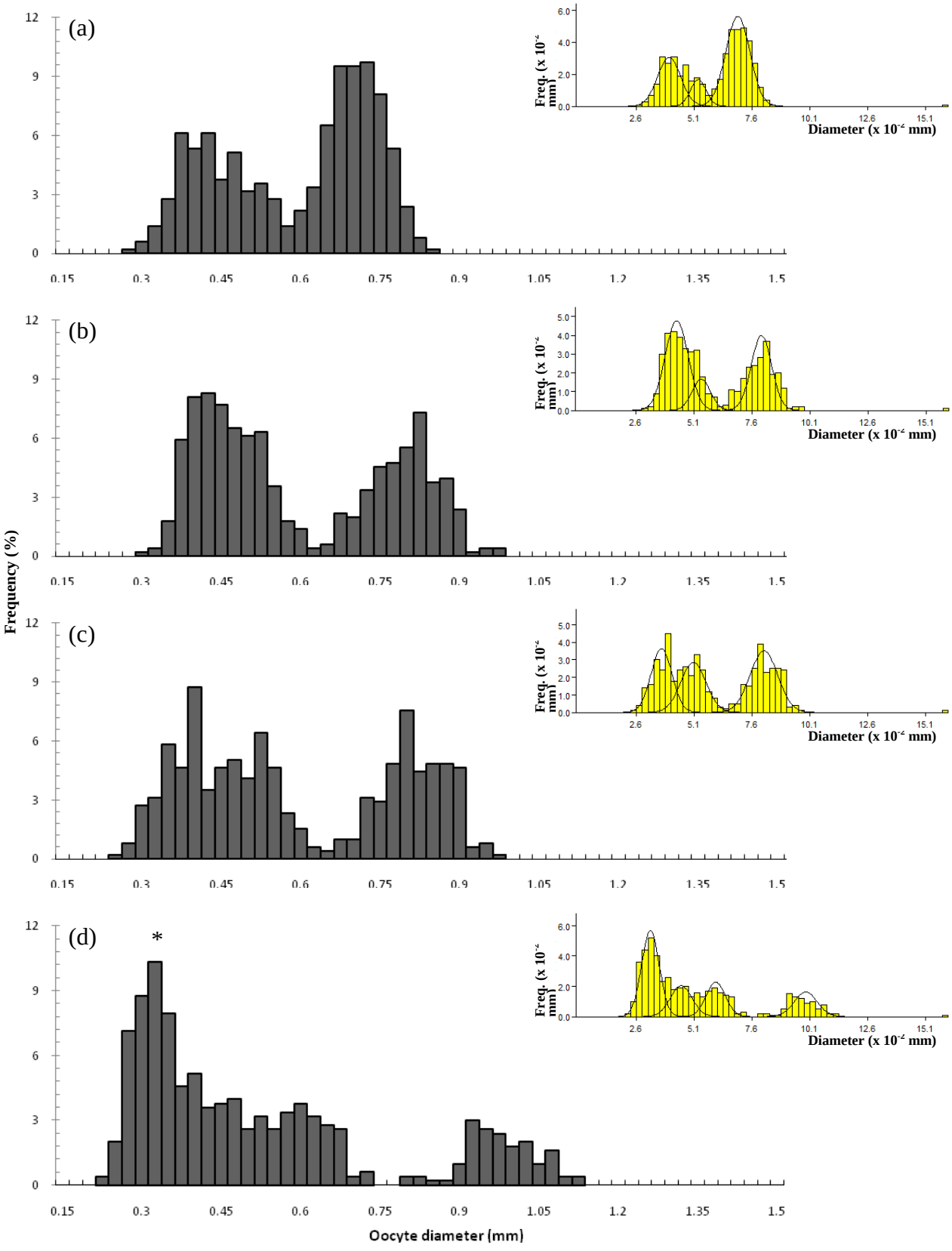


Figure 20: Oocyte diameter distributions for different stages of ovarian development, 2005-06 samples. Primary growth (dark grey bar), secondary growth (white bar), and maturation stage (black bar) oocytes. Light grey denotes the areas of overlap between the dark grey and white bars. Inset: Bhattacharya plots, without primary growth oocytes.

(a) *GSI* = 4.7; (b) *GSI* = 2.5; (c) *GSI* = 4.3; (d) *GSI* = 3.4; (e) *GSI* = 9; (f) *GSI* = 15; (g) *GSI* = 18; (h) *GSI* = 15.

Futhermore, in order to verify the results obtained in 2005-06, additional measurements were performed on another eight ovaries from 2009-10. These measurements were made without taking into consideration the different subpopulations, and excluded the previtellogenic subpopulation in all but two ovaries.

The additional measurements on the eight ovaries from 2009-10 confirmed the spawning pattern observed in 2005-06 (Fig. 21). They also allowed the calculation of the macroscopic means for the different oocyte subpopulations when contrasted with the microscopic descriptions, and the determination of the amount of shrinkage of the oocytes due to histological processing (Table 12).


(a)
(b)
(c)
(d)
*
Frequency (%)
Oocyte diameter (mm)
Freq. (x 10⁻⁴ mm)
Diameter (x 10⁻⁴ mm)

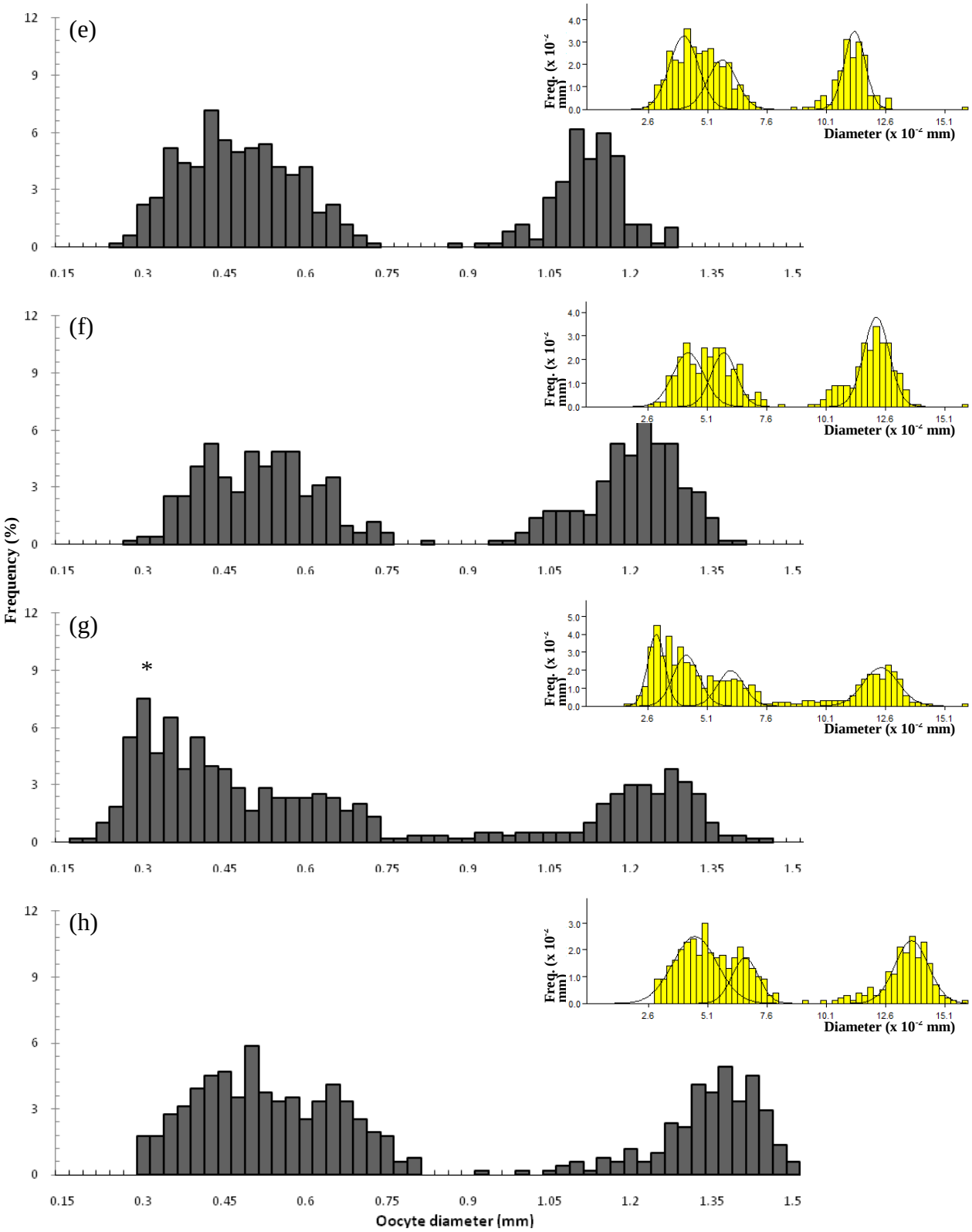


Figure 21: Oocyte diameter distributions for different stages of ovarian development, 2009-10 samples. Inset: Bhattacharya plots. Primary growth oocytes not included, unless denoted by (*). (a) *GSI* = 6.0; (b) *GSI* = 11.3; (c) *GSI* = 6.4; (d) *GSI* = 4.5; (e) *GSI* = 7.2; (f) *GSI* = 16.1; (g) *GSI* = 14.8; (h) *GSI* = 13.1.

Table 12: Amount of oocyte shrinkage for each developmental stage. All measurements are in mm.

Stage	Micro. diameter (S.D.)	Macro. diameter (S.D.)	Difference (%)
Previtellogenic	0.105 (0.020)	0.323 (0.038)	67.5
Cortical alveolar I	0.249 (0.042)	0.371 (0.045)	32.9
Cortical alveolar II	0.313 (0.025)	0.456 (0.047)	31.4
Early vitellogenesis	0.403 (0.051)	0.509 (0.055)	20.8
Mid vitellogenesis	0.502 (0.080)	0.629 (0.075)	20.2
Late vitellogenesis	0.660 (0.067)	0.814 (0.060)	18.9
Final maturation	0.785 (0.123)	0.995 (0.050)	21.1
Hydration	1.028 (0.096)	1.373 (0.071)	25.1

H. Fecundity

The calculated batch (F_b) and relative fecundities (F_r) showed high variability in the 2009-10 samples. The descriptive statistics are summarized in Table 13. The repeated-measures ANOVA test showed no significant effect of the subsample's position (anterior, mid, or posterior) within the ovarian lobe on the resultant F_b ($P > 0.05$).

Table 13: Descriptive statistics of F_b (oocytes/female) and F_r (oocytes/g) in 2009-10 samples.

Fecundity estimate	*n*	Mean	S.D.	Minimum	Maximum
Batch (F_b)	53	17,950	10,919	3,676	61,503
Relative (F_r)	53	50	25	9	107

The monthly averages of F_b and F_r for the sampled ovaries are represented graphically in Figure 22. They showed a decreasing pattern (Fig. 22). One-way ANOVA analyses showed that a significant difference existed between the six sampled months for F_b ($P < 0.05$) and F_r ($P < 0.05$), but no differences between the L_T of the samples per month ($P > 0.05$).

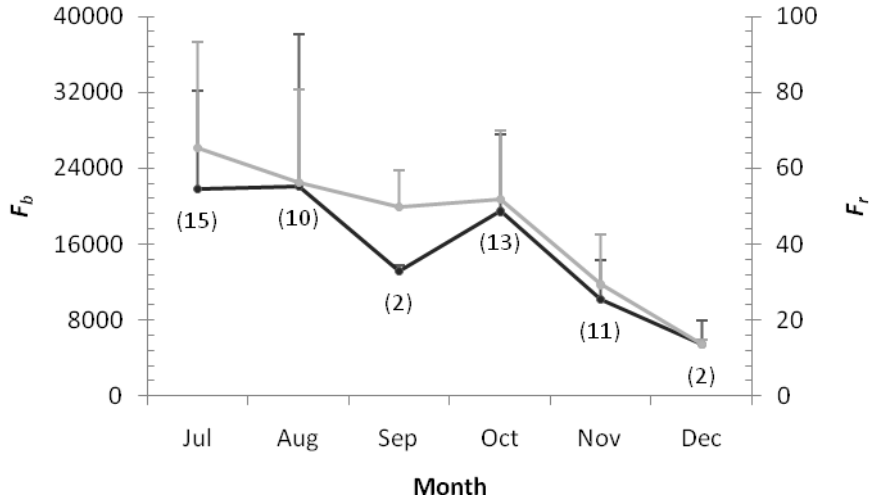


Figure 22: Monthly variations of batch fecundity (F_b; oocytes/female; dark grey line) and relative fecundity (F_r; oocytes/g; light grey line) for the 2009-10 *F. commersonii* samples. Frequencies (*n*) in brackets.

Figure 23 showed the variations of batch and relative fecundity estimates across the total length. The lengths were divided into two intervals, 5 cm and 10 cm, with the former showing greater deviations from parametric conditions in terms of the low sample sizes in some of the intervals. Both ANOVA and Kruskall-Wallis tests showed a significant difference in both intervals for F_b ($P < 0.05$) but no difference in the F_r ($P > 0.05$).

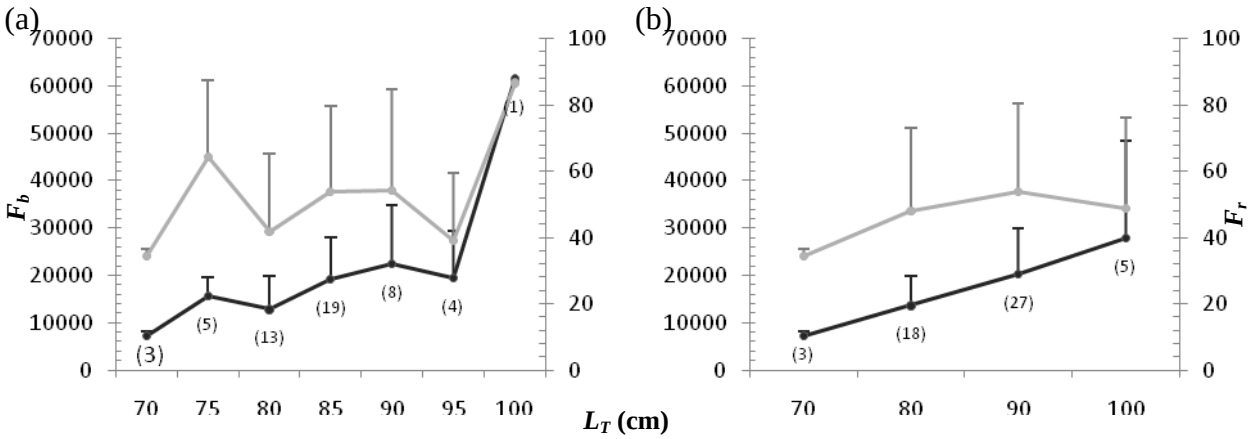


Figure 23: Variations of batch fecundity (F_b; oocytes/female; dark grey line) and relative fecundity (F_r; oocytes/g; light grey line) across lengths for the 2009-10 samples, with L_T grouped into 5 cm (a) and 10 cm intervals (b). L_T: total length; frequencies (*n*) in brackets.

The relationships between batch and relative fecundity and the difference somatic variables are summarized in Figure 24. Gonad weight was the most reliable predictive variable for both fecundities (Fig. 24c, f).

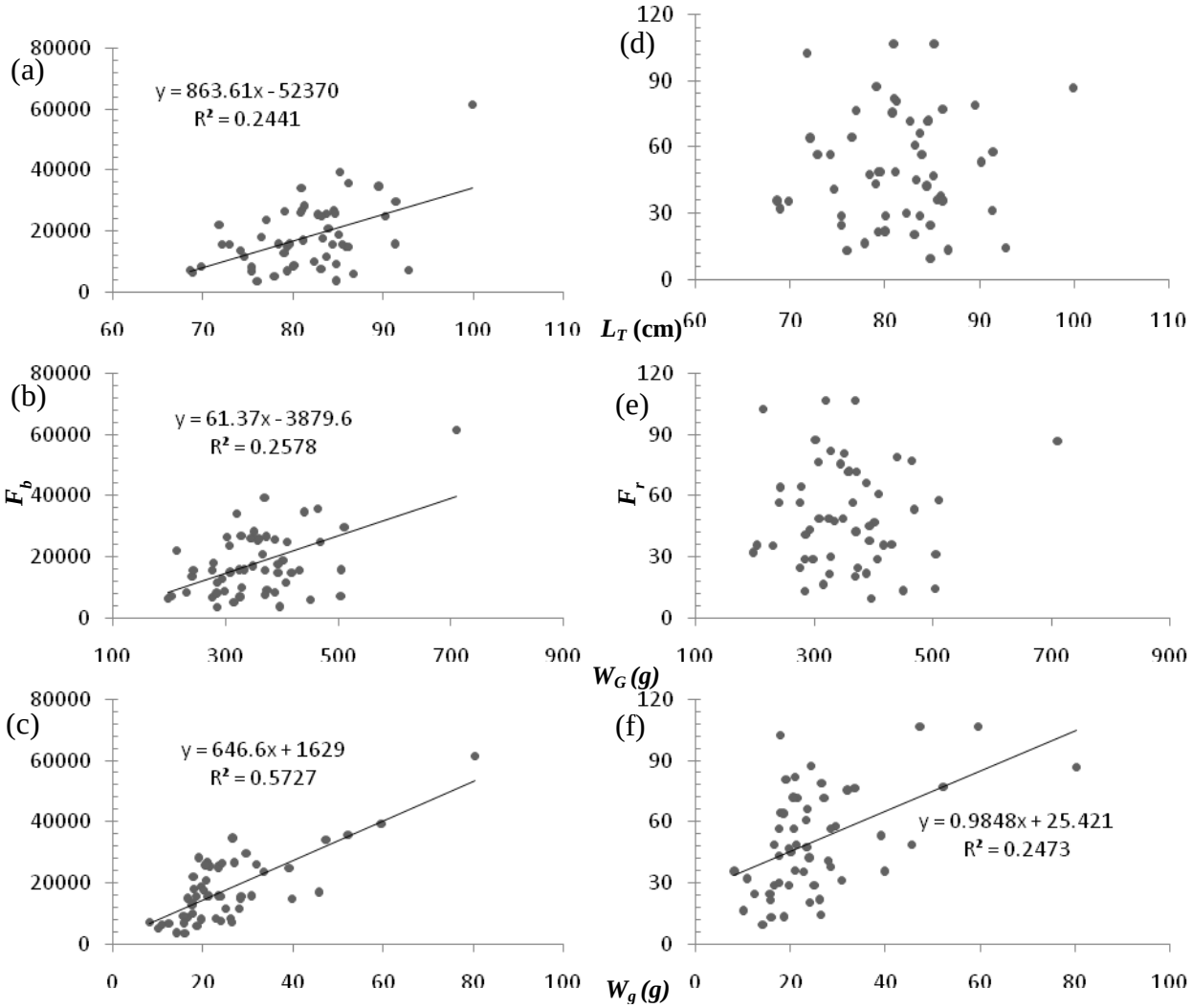


Figure 24: Relationships between batch fecundity (F_b; oocytes/female; a, b, c) and relative fecundity (F_r; oocytes/g; d, e, f) vs. total length (L_T; a, d), gutted weight (W_G; b, e), and gonad weight (W_g; c, f) for the 2009-10 samples.

I. Reproductive Indices

1. Gonadosomatic Index (GSI)

Figure 25 showed the monthly variations of the Gonadosomatic index (*GSI*) for both sexes in the two sampling periods. The *GSI* curve for the 2005-06 sampling period showed one peak. The mean female *GSI* had its lowest values during the months of February and March. Female *GSI* values started to increase starting from April, and peaked in August and September. In the sampled months of 2009-10, the mean female *GSI* peaks in August, and declines till December.

The mean male *GSI* in 2005-06 did not show significant monthly fluctuations and appeared more or less stable. There is a slight increasing trend in the *GSI* values during the months of July to September coinciding with the peak in the female *GSI*. A similar pattern is followed by the males in 2009-10, where the *GSI* showed a peak in July but is more or less stable from August till December. All the monthly GSI values are summarized in Table 14.

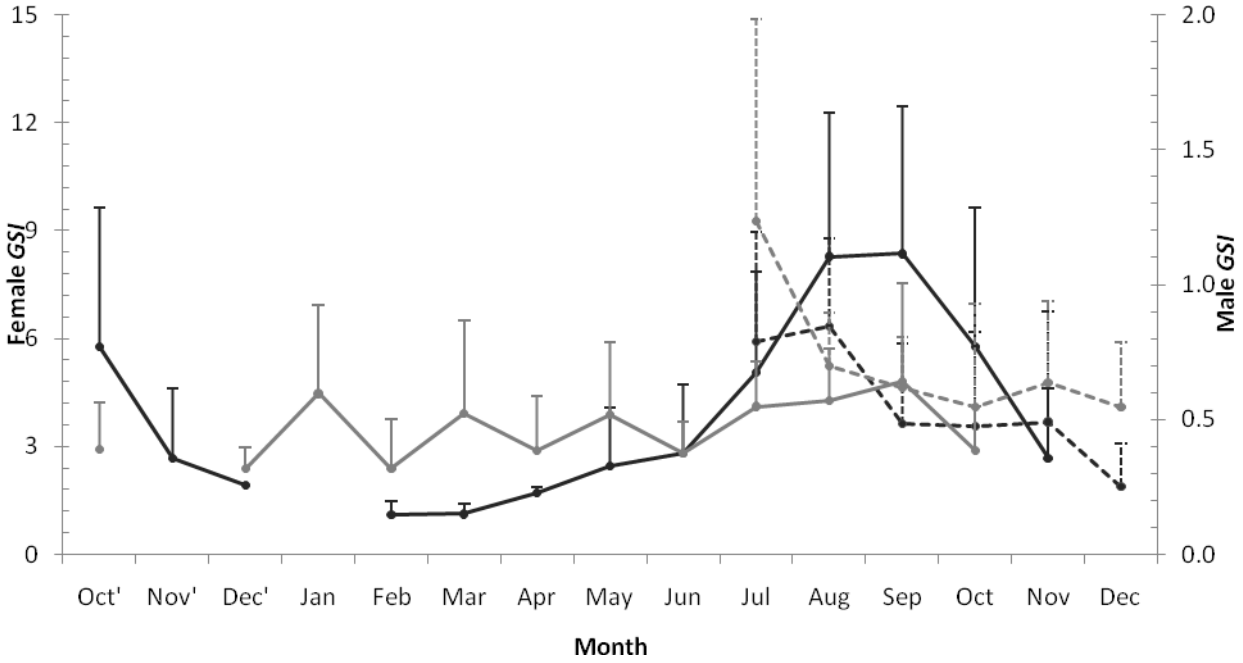


Figure 25: Monthly variations in the Gonadosomatic index (*GSI*) for females (mean + s.d.; black line) and males (mean + s.d.; light grey line) in 2005-06 (solid lines) and 2009-10 (dashed lines) sampling periods.

Table 14: Monthly variations of *GSI* for both sexes in 2005-06 and 2009-10.

Month	Mean *GSI*	S.D.	*n*	Mean *GSI*	S.D.	*n*	Mean *GSI*	S.D.	*n*	Mean *GSI*	S.D.	*n*
	2005-06						*2009-10*					
	Female			*Male*			*Female*			*Male*		
Oct'	5.76	3.87	20	0.39	0.17	19						
Nov'	2.67	1.93	3									
Dec'	1.92	0.00	1	0.32	0.08	6						
Jan				0.60	0.33	3						
Feb	1.11	0.36	5	0.32	0.18	14						
Mar	1.12	0.29	19	0.52	0.35	7						
Apr	1.71	0.15	5	0.38	0.20	15						
May	2.47	1.61	7	0.52	0.27	10						
Jun	2.82	1.90	6	0.37	0.12	12						
Jul	5.07	2.79	15	0.55	0.17	13	5.90	3.05	41	1.24	0.75	3
Aug	8.28	3.98	26	0.57	0.19	17	6.33	2.46	19	0.70	0.20	8
Sep	8.36	4.09	25	0.64	0.36	23	3.62	2.23	7	0.62	0.19	2
Oct	5.76	3.87	20	0.38	0.16	21	3.57	2.62	54	0.55	0.38	3
Nov	2.67	1.93	3				3.69	3.07	34	0.64	0.30	5
Dec							1.89	1.20	26	0.55	0.24	3

2. Condition Indices

Figure 26 represented the monthly variations in the condition indices used (Fulton's *K*, Ricker's K_r, and Relative weight W_r). All the indices in 2005-06 showed a peak in the beginning of winter (November and December) and registered their lowest values in February and March. Following February and March, the condition indices increased till November. A slight dip in this overall increasing pattern was observed during the month of September. The monthly condition values were summarized in Table 15.

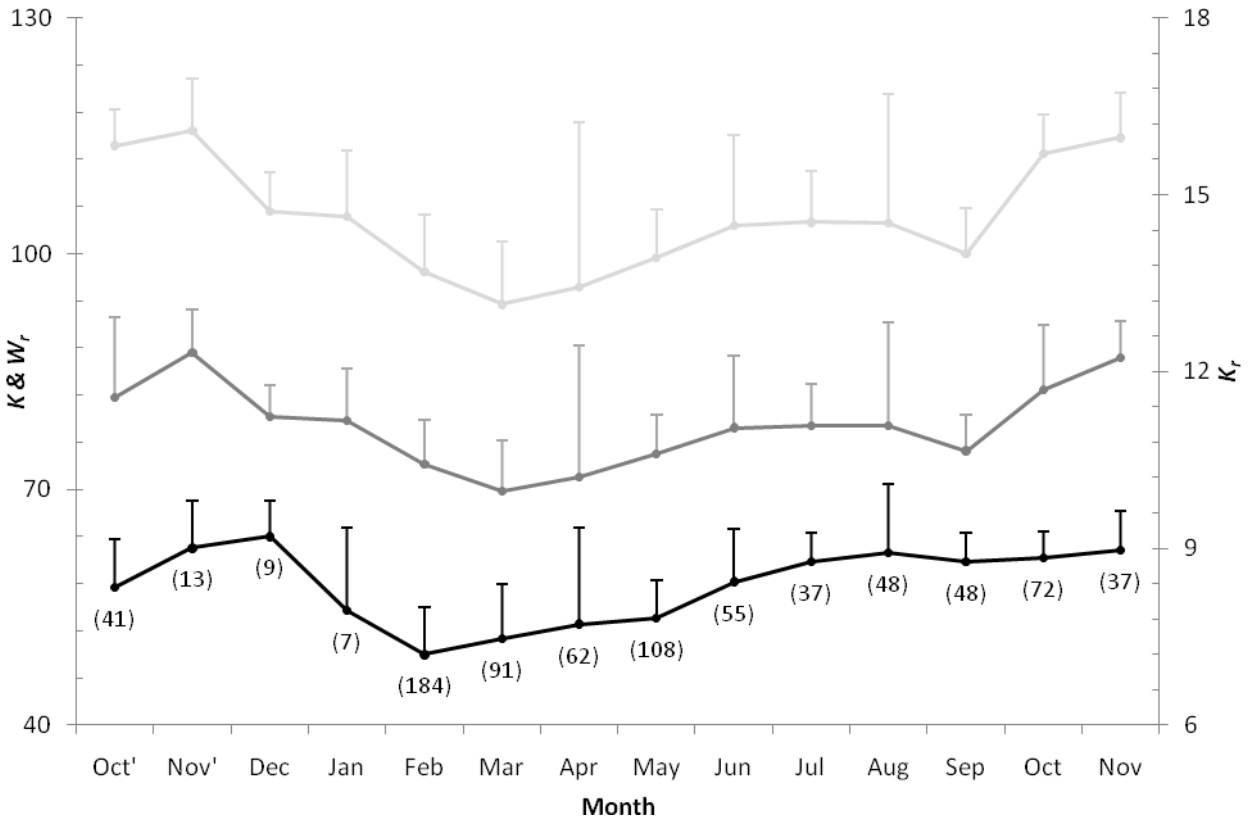


Figure 26: Monthly variations in the condition indices in 2005-06. Fulton's *K* (mean + s.d.; black line), Ricker's K_r (mean + s.d.; dark grey line), and Relative weight W_r (mean + s.d.; light grey line). Frequencies (*n*) in brackets.

Table 15: Monthly variations of the condition indices for 2005-06. Frequencies (*n*) are the same throughout the indices.

Month	Mean	S.D.	Mean	S.D.	Mean	S.D.	n
	Fulton's K		*Ricker's* K_r		*Relative weight* W_r		
Oct'	57.5	6.1	11.6	1.4	113.8	4.6	41
Nov'	62.5	6.0	12.3	0.7	115.6	6.7	13
Dec'	64.0	4.6	11.2	0.5	105.4	5.0	9
Jan	54.6	10.5	11.2	0.9	104.8	8.3	7
Feb	49.0	6.0	10.4	0.8	97.7	7.2	184
Mar	51.0	7.0	10.0	0.9	93.5	8.1	91
Apr	52.9	12.2	10.2	2.2	95.8	21.0	62
May	53.6	4.8	10.6	0.7	99.5	6.2	108
Jun	58.2	6.7	11.0	1.2	103.6	11.5	55
Jul	60.8	3.7	11.1	0.7	104.0	6.6	37
Aug	62.0	8.8	11.1	1.8	103.9	16.5	48
Sep	60.8	3.6	10.7	0.6	100.0	5.8	48
Oct	61.3	3.4	11.7	1.1	112.7	5.0	72
Nov	62.3	4.9	12.2	0.6	114.8	5.7	37

Figure 27 showed the monthly variations of the condition indices for the sexes in 2005-06. The sexes showed similar temporal fluctuations as the whole population of 2005-06, following the same trend across the months of study. The monthly variations of the sexes were contrasted within each condition index used. Student's *t*-tests tests showed no significant difference between the sexes in all the condition indices utilized ($P > 0.05$). The monthly condition values of the sexes in 2005-06 are summarized in Table 16.

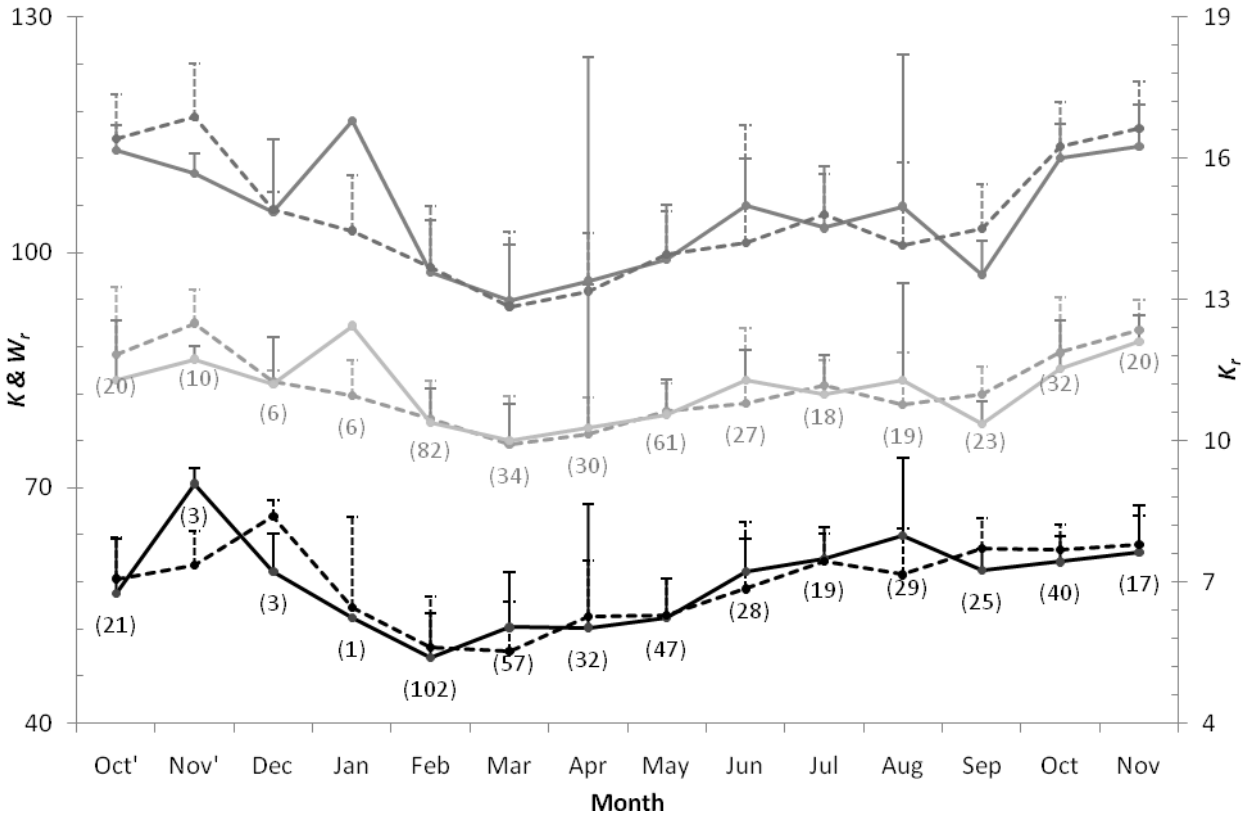


Figure 27: Monthly variations in the condition indices for the sexes in 2005-06, females (solid lines) and males (dashed lines). Fulton's *K* (mean + s.d.; black line), Ricker's K_r (mean + s.d.; dark grey line), and Relative weight W_r (mean + s.d.; light grey line). Frequencies (*n*) in brackets; females (black) and males (grey).

Table 16: Monthly variations of the condition indices for the sexes in 2005-06. Frequencies (*n*) for each sex are the same throughout the indices.

Month	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	*n*	Mean	S.D.	*n*
	Fulton's K				*Ricker's K_r*				*Relative weight W_r*					
	Females		*Males*		*Females*		*Males*		*Females*			*Males*		
Oct'	56.5	6.9	58.4	5.2	11.3	1.3	11.8	1.4	113.1	3.1	21	114.5	5.7	20
Nov'	70.5	2.0	60.1	4.3	11.7	0.3	12.5	0.7	110.1	2.6	3	117.3	6.8	10
Dec	59.3	4.9	66.4	2.0	11.2	1.0	11.3	0.2	105.1	9.4	3	105.5	2.2	6
Jan	53.4	0.0	54.8	11.5	12.4	0.0	11.0	0.7	116.8	0.0	1	102.8	7.0	6
Feb	48.4	5.5	49.6	6.5	10.4	0.7	10.5	0.8	97.4	6.7	102	98.1	7.7	82
Mar	52.2	7.1	49.2	6.2	10.0	0.8	9.9	1.0	93.9	7.2	57	93.1	9.5	34
Apr	52.2	15.7	53.5	7.1	10.3	3.0	10.1	0.8	96.4	28.6	32	95.1	7.3	30
May	53.4	5.0	53.7	4.7	10.6	0.7	10.6	0.6	99.1	7.0	47	99.8	5.4	61
Jun	59.3	4.1	57.1	8.5	11.3	0.6	10.8	1.6	105.9	6.0	28	101.2	15.0	27
Jul	61.0	3.9	60.6	3.6	11.0	0.8	11.2	0.5	103.1	7.8	19	104.9	5.0	18
Aug	64.0	9.8	58.9	5.9	11.3	2.1	10.8	1.1	105.9	19.3	29	100.9	10.5	19
Sep	59.4	2.7	62.2	3.9	10.4	0.5	11.0	0.6	97.2	4.4	25	103.0	5.6	23
Oct	60.6	3.3	62.2	3.2	11.5	1.0	11.9	1.2	112.0	4.4	40	113.6	5.6	32
Nov	61.7	6.0	62.8	3.7	12.1	0.6	12.4	0.6	113.5	5.4	17	115.9	5.9	20

J. Oocyte Atresia

Atresia, the resorption of oocytes, is a common occurrence in *F. commersonii* ovaries. This process was mostly apparent in ovaries that had already spawned and showed POF's. Developing ovaries did not have atretic oocytes. On the other hand, redeveloping, developed, gravid, and running-ripe ovaries often had atretic oocytes in low quantitites. In these ovaries, atresia was prevalent in late vitellogenic oocytes, whereas cortical alveolar- to early vitellogenenic-stage oocytes were intact. Primary growth oocytes were never subjected to atresia.

Atresia occurred irregularly. There was a constant presence of low levels of vitellogenic atresia in many ovaries throughout the spawning season, especially in redeveloping and recently spawned ovaries. Heavy vitellogenic atresia was observed as early as August, and increased in frequency as the season advanced till December. Moreover, towards the end of 2009, and after vitellogenic oocytes were resorbed, there was major atresia in early secondary growth oocytes (cortical alveolar and lipid vesicle stage), where the ovaries were in final regression phase and moving towards the resting state. Atresia was also irregular within the same ovary. This was apparent through the incidence of vitellogenic atresia in ovaries where there were also healthy vitellogenic oocytes. The same was observed in case of atresia in earlier-stage oocytes where healthy and atretic oocytes co-occurred. What was notable was that late-stage atresia of cortical alveolar-stage was a distinct event from vitellogenic atresia, and commenced after all vitellogenic oocytes were resorbed towards the end of the spawning season. This was an indication of the cessation of spawning, and characterized the end of the reproductive season.

CHAPTER IV

DISCUSSION

The reproduction of *Fistularia commersonii* has not been studied extensively, and reproductive data is very scarce in the Mediterranean (Azzurro *et al.*, 2004; Pais *et al.*, 2007; Psomadakis *et al.*, 2008) and nonexistent in its native Indo-Pacific range. As such, any comparatives that can be drawn are limited to the few reports in the Mediterranean, as well as data from previously studied Lessepsian migrants. Overall, the reproductive aspects of this species presented many peculiarities and characteristics. The current study presented a more or less complete picture of the reproduction of this invader and provided insights into its invasive success.

A. Population Structure

1. Descriptive Statistics of the Samples

While the ranges of L_T for 2005-06 and 2009-10 were similar, mean size for 2005-06 (59.1 ± 18.6 cm L_T) was significantly lower ($P < 0.05$) than 2009-10 (82.2 ± 9.8 cm L_T). Similar differences were measured for the gutted weight ($P < 0.05$) (Table 1). This was due to the fact that large specimens were specifically selected to study the microscopic structure of the gonads. This factor comes into play in later analyses as well.

2. Weight-Length Relationships

The *W-L* relationships in all the cases followed the usual power function, with the regression coefficient *b* greater than 3 in both sampling periods, indicating growth to be positively allometric (Fig 3). Therefore, the general shape of the body becomes rounder as length increases, as in most fishes (Anderson & Neumann, 1996).

The slopes of the two relationships were not significantly different, showing a similar sampling methodology ($P > 0.05$; Table 2). The populations were pooled in order to obtain a larger size range and sample size, better describing the *F. commersonii* population in the Eastern Mediterranean. Thus, the *W-L* relationship for *F. commersonii* in the Eastern Mediterranean is $W_G = 1.066 \times 10^{-4} L_T^{3.4063}$.

The regression parameters were comparable to specimens collected from the South-East Aegean (Kalogirou *et al.*, 2007). The slopes of the *W-L* relationships were significantly different between the males and females, the males having a slightly steeper slope ($b = 3.39$ vs. 3.35; Table 2).

3. Length-Frequencies

The length distribution of the 2005-06 samples was not normally distributed as opposed to 2009-10. This is probably due to sampling bias such as the prevalence of certain size classes in some months (Table 4b).

The smallest specimens (20-25 cm L_T; Table 4) were caught in November, which could give an idea about the settlement period of the species in the Mediterranean. Moreover, the monthly means of L_T showing a generally increasing trend from February till October (Table 3) might indicate a very fast growth rate of young individuals. Additional data are needed to verify these two observations.

The length-frequency distributions for the sexes during the two sampling periods showed that all were normally distributed except for the females in 2005-06. The means of L_T for females vs. males were, however, not significantly different ($P > 0.05$; Fig. 6a). The possible causes behind this deviation from normality are probably the monthly discrepancies and sampling biases, and this is certainly the reason behind the deviation from normality for the overall population in 2005-06.

A significant difference in the L_T means for the 2009-10 samples was observed due to the small number of male specimens selected during that period ($P < 0.05$; Fig. 6b).

4. Sex Ratio

The overall sex ratio of *F. commersonii* did not differ from the 1 : 1 ratio. The monthly percentages were more or less balanced between males and females, except for November, December, and January, where there was a skew towards males due to the low sample size (n = 13, 9, and 7 respectively), though sex ratios were not significantly different from 1 : 1 (Fig. 7a). The sex ratio deviated from the overall 1 : 1 only in the month of March, though this might be the result of random sampling errors as no other deviation in the months were recorded.

In the sex ratios across L_T size classes, the males dominated the smallest sizes, whereas the females dominated the larger size classes (Fig. 7b). A possible cause for this deviation might be sampling bias such as the presence of spawning aggregations and therefore increased vulnerability to some catchment methods (Lowerre-Barbieri *et al.*, 1996). This deviation might also be the result of unequal growth rates between

males and females (Fogarty & O'Brien, 2009), and needs to be explored with growth data.

B. Morphological Description

1. Ovarian Structure

The morphological description of the ovaries, macro- and microscopically, has been extensively studied for 22 months. This detailed description complemented the scattered information available in the literature (Azzurro *et al.*, 2004; Pais *et al.*, 2007; Psomadakis *et al.*, 2008). The present study described the macroscopic aspect of the ovaries over a much larger span of time and detail. Special attention was paid to the changes occurring during ovarian development, such as color, shape, vascularization, and size in body cavity. On the basis of these observations, the macroscopic maturity table was created (Table 7).

Information concerning the microscopic description is scarce and incomplete in the literature (Azzurro *et al.*, 2004; Pais *et al.*, 2007; Psomadakis *et al.*, 2008). The present study presents a complete description of the ovarian structure and temporal development (Table 7). A mixture of oocytes at different stages of development was observed in the ovaries, contrary to some previous publications. Pais *et al*. (2007) reported ovaries containing a synchronous batch of oocytes, while another study reported an asynchronous development (Azzurro *et al.*, 2004). Psomadakis *et al.* (2008) also classified *F. commersonii* as total spawners. While the first two studies analyzed one single ovary each, Psomadakis *et al.* (2008) studied seven females. Our observations, which were done on $n = 211$ ovaries in different stages, sampling times,

and size classes, confirmed the asynchronous type of development, as defined by Wallace & Selman (1981).

The order Gasterosteiformes includes the three families Fistulariidae, Syngnathidae, and Gasterosteidae. The first two belong to suborder Syngnathoidei, while the latter belongs to Gasterosteoidei (Nelson, 2006). The ovaries of the studied *Fistularia* showed similarities with Gasterosteidae (*Eucalia inconstans* (Kirtland, 1840)), as opposed to Syngnathidae (*Syngnathus scovelli* (Evermann & Kendall, 1896) and *Hippocampus erectus* (Perry, 1810)), despite the fact that Gasterosteidae belong to a different suborder (Braekevelt & McMillan, 1967; Begovac & Wallace, 1987; Selman *et al.*, 1991).

While the fistularid and gasterosteid showed lamellar ovarian structure with no germinal ridges, the studied syngnathids showed an absence of ovigerous lamellae, having instead germinal ridges that showed a sequential pattern of oocyte development (Braekevelt & McMillan, 1967; Begovac & Wallace, 1987; Selman *et al.*, 1991).

2. Testicular Structure

The morphological description of the testes, macro- and microscopically, has also been extensively studied for 22 months. Psomadakis *et al.* (2008) provided the only morphological data about the testes of *F. commersonii*, limited to macroscopic observations on four individuals. The current study described the testicular development in details particularly color, shape, vascularization, and size in body cavity. On the basis of these observations, the macroscopic maturity table was created (Table 8). Microscopically, no information was available in the literature. This study showed the testicular organization to be unrestricted lobular, and spermatogenesis to be cystic.

Similar to the ovaries, the testicular organization and spermatogenesis of *F. commersonii* were similar to the related gasterosteiform, the stickleback *Pungitius sinensis* (Guichenot, 1869) (Parenti & Grier, 2004) and different from the two syngnathids, *Syngnathus abaster* Risso, 1827 and *S. acus* Linnaeus, 1758 (Carcupino *et al.*, 1999).

C. Oocyte Developmental Stages

Oocyte development in *F. commersonii* showed an atypical stage. In most documented teleosts, multiple nucleoli appear around the periphery of the nucleus at the perinucleolar stage (Wallace & Selman, 1981; McMillan, 2007). This feature was absent in *F. commersonii* oocytes in all 211 sectioned ovaries, where only one large distinctive nucleolus existed, located more or less centrally, that persisted till late cortical alveolar-stage despite the clear presence of the highly basophilic cytoplasm and less basophilic nucleus characterizing the perinucleolar stage (Table 6). To confirm this observation, slight variations of the histological methodology used were performed on samples preserved in different fixatives (4 % buffered formaldehyde and Bouin's fixative). A single nucleolus was always observed. The absence of this very distinctive intracellular event is very unusual and has been reported only in the viviparous *Xiphophorus helleri* Heckel, 1848, which had one or two nucleoli at this stage (Azevedo & Coimbra, 1980). For the purpose of this study, the term "perinucleolar" is still used to designate this previtellogenic stage, though new terminology might need to be developed if it is observed elsewhere.

Other than this peculiarity, oocyte development towards maturation continued as usual (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007), with an

extensive secondary growth stage that incorporated both cortical alveoli and lipid vesicles, in addition to a considerable deposition of yolk into the oocyte later on. Prior to ovulation, the oocyte matured in the FOM step with further increases in diameter and hydrated later, presenting the largest oocyte diameter observed in this species. This significant hydration step is important in species with pelagic eggs, which float due to the water content in the ovulated oocyte (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007), thus *F. commersonii* is most probably a fish with pelagic eggs.

Subsequent to ovulation, post-ovulatory follicles were observed consistently, appearing large at first then deteriorating into smaller bodies, in ovaries showing new developing batches of yolked oocytes, in addition to regressing ovaries. The observed POF's showed the standard morphology described and were easy to identify (Hunter & Macewicz, 1985a; West, 1990; McMillan, 2007). Future studies may explore this aspect especially since fresh POF's and advanced yolked oocytes frequently occurred in the same ovary, signifying that new batches are matured quickly and spawning events may have short separation periods. POF's may be used to estimate the spawning frequency of fish, though it is not a simple process, especially in warm-water spawners (Hunter & Macewicz, 1985a).

Atretic oocytes were also observed in different proportions in different months. The description of atretic oocytes agreed with those found in the literature (Wallace & Selman, 1981; Tyler & Sumpter, 1996; McMillan, 2007). Deeper investigations into the subcellular events and control mechanisms of atresia in this species are beyond the scope of the current study.

D. Gonad Maturity Stages

Due to asynchronous pattern of oocyte development, an eight-point maturity scale was adapted for female *Fistularia commersonii* including both macroscopic and microscopic data. Similarly, a four-point scheme was developed for males. Figs. 28 and 29 depicted the gonadal developmental cycles.

1. Ovarian Cycle

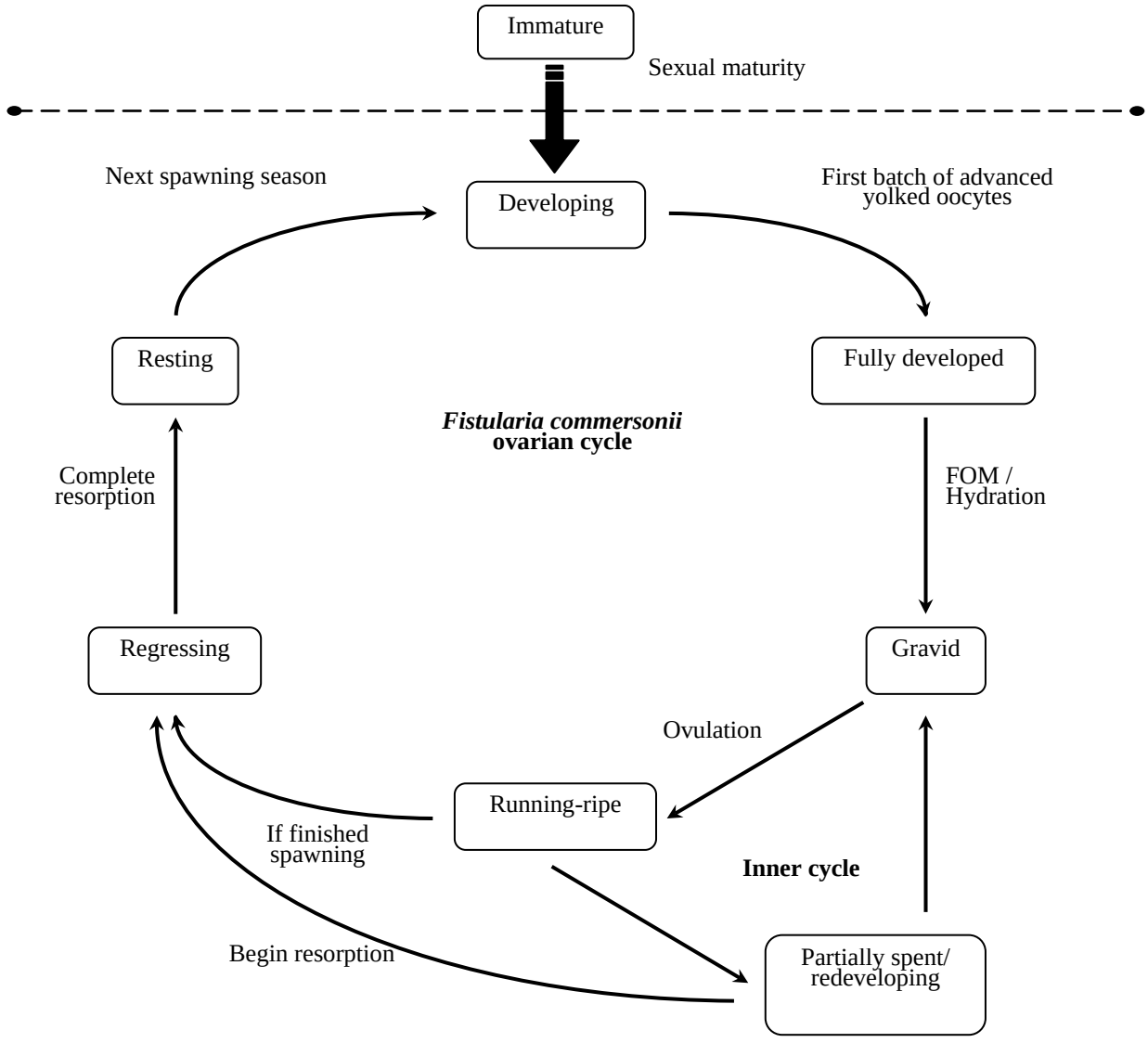


Figure 28: Asynchronous batch-spawning ovarian cycle in *F. commersonii*.

The ovarian cycle of *F. commersonii* in the Mediterranean was similar to that observed in the weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) (Lowerre-Barbieri *et al.*, 1996), in which there was a second series of spawning and redevelopment of fresh batches from the existing secondary growth oocytes within the total cycle of ovarian development (Fig. 28 and Table 7). Based on both microscopic and macroscopic features, it seems that most ovaries undergo the inner cycle of redevelopment and spawning before reaching the spent stage (48 redeveloping out of 211 ovaries in 2009-10, in addition to gravid ovaries with old POF's). It is also possible that smaller fish that commence oocyte development late in the season do not undergo more than one cycle of oocyte development and spawning, or even none at all if they resorb their vitellogenic oocytes, depending on the environmental conditions. This has also been observed in the northern anchovy, in which smaller females have a much shorter spawning season (Hunter & Macewicz, 1985b).

In 2005-06, reproductively active females (gravid and redeveloping stages; Table 7, Fig. 17) were observed from May to November 2006, signifying a spawning season extending over six months. In the 2009-10 period, the season extended till December (first half), where a few specimens were still reproductively active. Moreover, spawning and redeveloping females were caught in April and May 2010, showing that the species is capable of beginning its spawning activity sooner. Although sampling was not performed beyond May 2010, it seems likely that the spawning season in *F. commersonii* is very flexible, probably affected by environmental or other conditions. The spawning season might fluctuate and thus adapt to the prevailing conditions in the new environment.

The *F. commersonii* spawning season presented sharp departures from most studied Mediterranean species, which are summer spawners, with most seasons extending within April to August (Tsikliras *et al.*, 2010). Moreover, while only one-third of Mediterranean species spawn over a long period (more than four months, Tsikliras *et al.* (2010)), the relatively extended spawning period in *F. commersonii* is certainly a characteristic of a warm-water species from the Indo-Pacific. Long spawning periods are common in tropical species (Moyle & Cech, 2004). This has been observed, for example, in siganids living in the Red Sea (Popper & Gundermann, 1975; Amin, 1985b, a; Golani, 1990) and lutjanids in the Gulf of California (Piñón *et al.*, 2009). The spawning periods of 75 East African reef fishes showed all-year-round spawning (Nzioka, 1979).

In 2005-06, specimens collected in April had a mixture of resting and developing ovaries, showing the start of the reproductive season and in May the first gravid female (stage 4) was collected, showing the start of the spawning season (Fig. 17). As time progresses, gonads gradually developed and the percentage of gravid females peaked in September (Fig. 17). It is noteworthy to mention that June was characterized by the lack of gravid females, the largest (90.5 cm L_T) being at the developed stage (stage 3) (Fig. 17a). This is probably due to sampling where large females were not sampled in that specific month. Another explanation might be that larger females started their spawning activity earlier than the smaller ones, as in the queen croaker (*Seriphus politus* Ayres, 1860) that also has an asynchronous pattern of oocyte development and the group-synchronous *Maurolicus muelleri* (Gmelin, 1789) (Demartini & Fountain, 1981; Goodson *et al.*, 1995). A third explanation would be that the start of ovarian maturation is not synchronous in the entire population for the

species. This has also been observed in some multiple spawners with asynchronous oocyte development such as the hermaphrodite *Diplodus puntazzo* (Walbaum, 1792), in which the start of vitellogenesis is not synchronous (Pajuelo *et al.*, 2008), as well as some group-synchronous species such as *Leptocottus armatus* Girard, 1854, *Gasterosteus aculeatus* Linnaeus, 1758, and *Apeltes quadracus* (Mitchill, 1815) (Wallace & Selman, 1979; DeVlaming, 1983).

On the other hand, a few females with regressing ovaries were observed in late July-August, while most of the samples were still reproductively active. This showed that the cessation of spawning is also asynchronous across the population. This also showed that all individuals were not actively spawning during the six-month spawning period, with shorter spawning periods per individual due to physiological restraints rather than environmental ones. The absence of population synchrony might result in an extended spawning season (DeVlaming, 1983).

Fresh post-ovulatory follicles (POF) were observed in July in gravid ovaries also having final maturation-stage oocytes (FOM), signifying that the maturation of fresh batches of oocytes to be spawned may be extremely quick.

Oocyte atresia was a constant but irregular presence in *F. commersonii* ovaries. Consistently low amounts of atresia were observed during the spawning season in reproductively active females, similar to some other species in which low levels of atresia are a common occurrence (Hunter & Macewicz, 1985a; Hunter *et al.*, 1986; Lowerre-Barbieri *et al.*, 1996; Azzurro *et al.*, 2007). Low levels of atresia do not have a large role in the oocyte dynamics of reproductively active females (Wallace & Selman, 1981; Tyler & Sumpter, 1996), though other authors disagree (West, 1990; Pavlov *et al.*, 2009). However, high levels of atresia were observed in *F. commersonii* starting

August. This indicated the end of the spawning season for the species, as suggested by Wallace & Selman (1981), Hunter & Macewicz (1985), and Hunter *et al.* (1986).

2. Testicular Cycle

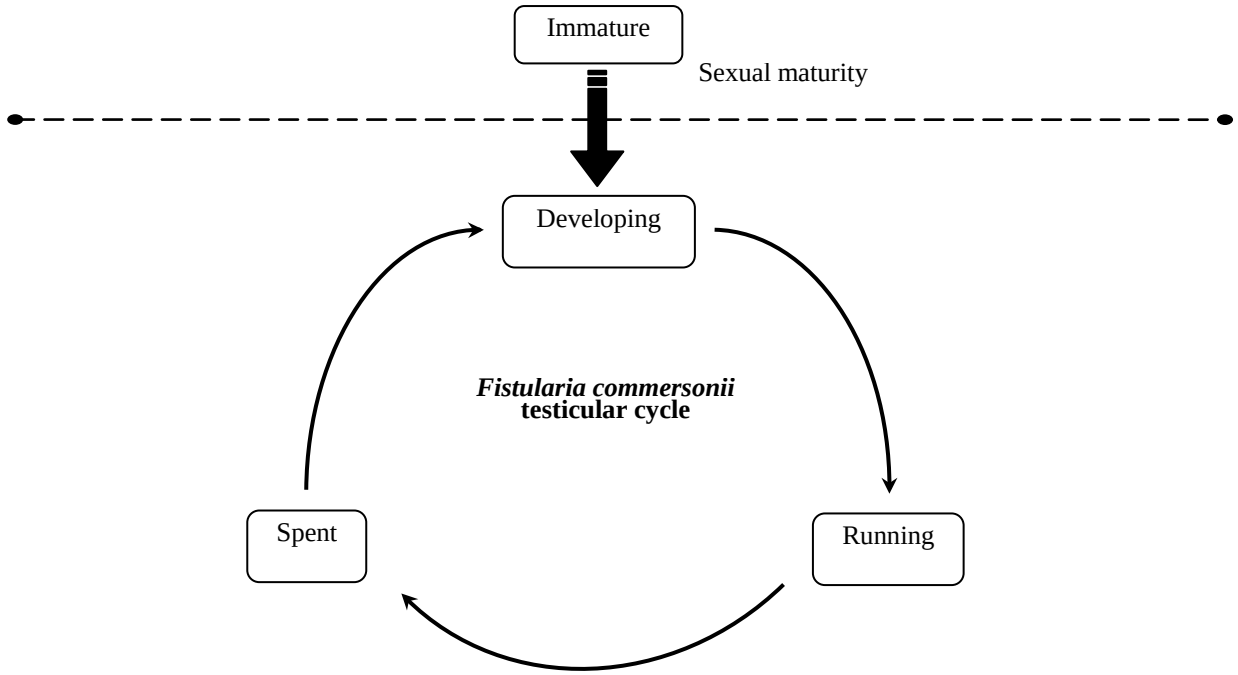


Figure 29: Testicular cycle in *F. commersonii*.

In contrast to the ovarian cycle, and in relation to the adopted four-point scheme, the development of the testes did not show a complicated pattern and was relatively straightforward. Upon the onset of maturity, the testes have an ongoing cycle of development from developing to spent and back to developing after a short resting stage of about a month.

Developing males were observed all year round in 2005-06, with the exception of November, where all caught specimens were immature, and January, where only three sexually mature fish were caught, all in the spent stage. This might be the result of the protracted female spawning season, due to which the male population as a whole has

a very short spent period. Nevertheless, a pattern was observed in terms of an increase in the percentage of developing testes from February to May, in a preparation period preceding the beginning of the spawning period of female *F. commersonii*. Starting from June 2006, spent males began to appear in the samples, showing that the running period for the males is shorter than females as we see males begin running at the same time as females in May. From June and on, the percentage of spent males increased, showing either an asynchronous cessation of the running period or temporary pauses before fresh rounds of spermatogenesis as developing males were also observed in this period. The available data is not enough to present definitive conclusions regarding spermatogenesis in *F. commersonii*.

In 2009-10, very few male specimens were sampled, and though the staging data is presented, it did not allow for clear conclusions. Nevertheless, what was observed in this period is in tune with the conclusions drawn from the 2005-06 individuals. Spent males were observed in July 2009, and developing and running males persisted till December. In 2010, April had both developing and running males, coinciding with the beginning of spawning in 2010 for female *F. commersonii*. This constantly active pattern of spermatogenesis in *F. commersonii* has also been observed in other warm-water species (Miura, 1999; Fishelson *et al.*, 2006), as opposed to cold-water species (Parenti & Grier, 2004).

3. Staging Accuracy

To assess agreement between the two staging schemes, concordance and intraclass correlation were used. The level of staging accuracy was high (above 75 % in Kendall's and 80 % in ICC) for both females and males considered separately,

confirming that the macroscopic staging scheme can be considered highly reliable. Moreover, since the staging data used were in the ordinal scale, Kendall's Tau-b test seems to be more appropriate than ICC as it is specific for ordinal data.

Total staging error for *F. commersonii* females was at 22 % based on histologically assessed specimens (n = 211) as opposed to 18 % in males (n = 33). Concordance values of 0.782 and 0.774 were obtained for females and males respectively, while the ICC values were slightly above 0.8 for both sexes. These results are acceptable in terms of the staging accuracy, as opposed to other studies that obtained error values they considered to be high at 35 % for *Serranus cabrilla* (Linnaeus, 1758), 39 % for *Lutjanus vittus* (Quoy & Gaimard ,1824), and up to 40 % in some months for *Gadus morhua* Linnaeus, 1758 (West, 1990; García-Díaz *et al.*, 1997; Tomkiewicz *et al.*, 2003). García-Díaz *et al.* (1997) found greater complexity in staging characteristic with microscopic observations, while Tomkiewicz *et al.* (2003) improved their staging accuracy be decreasing the macroscopic stages, a means also recommended by Hunter & Macewicz (2003). Macroscopic staging is preferred in studies of fish reproduction because it is inexpensive and fast in obtaining maturity results, even though various microscopic techniques provide better accuracy (West, 1990; Hunter & Macewicz, 2003).

The 18 % error in male *F. commersonii* in 2009-10 could be related to the relatively small sample size (n = 33) rather than any problems with the macroscopic methodology. In females, the high percentage of error in developed and spent females (Table M) was probably due to their low sample size as well (stage 3, n = 3; stage 8, n = 10). Other errors were misclassifications due to the difficulty in macroscopically

identifying yolked oocytes. Similar errors were also reported in *Lutjanus vittus* and *Gadus morhua* (West, 1990; Tomkiewicz *et al.*, 2003).

E. Size at Maturity

To assess size at first maturity in the current study, specimens were selected throughout the spawning period (June to November) as suggested by King (1995). Other authors recommend the use of females in the prespawning period (Hunter & Macewicz, 2003; Murua *et al.*, 2003). No prespawning females were used in the size at maturity assessment because some individuals might start maturing during the spawning period.

The two methods for L_{50} estimation, the binary logistic function vs. the Marquardt method, showed slightly different results. The binary logistic function calculated the L_{50} estimates for both females and males at around 54.5 cm, whereas the Marquardt method estimated it at around 57 cm for both sexes (Fig. 19). Further study is needed to verify which method is more accurate. In the meantime, the established Marquardt method will be followed. Moreover, it was noticable that L_{50} for both sexes was almost the same in *F. commersonii*.

F. Oocyte Diameters and Spawning Pattern

The oocyte diameter distributions and mode of development are indicative of the spawning pattern in fishes (West, 1990). In *F. commersonii*, they showed again a typical asynchronous development in addition to a batch spawning pattern.

The absence of a gap between two consecutive developing batches of oocytes in *F. commersonii* (Fig. 20) is indicative of an asynchronous development as opposed to

a group synchronous pattern (Holden & Raitt, 1974; West, 1990; Murua *et al.*, 2003). Furthermore, the presence of sometimes two and often three developing batches at one time in a single ovary, in addition to previtellogenic oocytes, is typical of a batch-spawning species (Holden & Raitt, 1974; West, 1990). However, the existing number of batches in an ovary is not indicative of the number of batches that may be spawned by the same fish (West, 1990). It was shown that oocytes in some developing batches might not be spawned entirely or even a large number of small oocytes resorbed through atresia towards the end of the season (West, 1990; Murua & Motos, 2006). Furthermore, fresh oocytes may be recruited from the previtellogenic pool, forming additional batches (*de novo* vitellogenesis) (Hunter & Goldberg, 1980; DeVlaming, 1983; West, 1990).

1. Effect of Fixation and Histology on Oocyte Size

It is known that preservation of the ovary causes shrinkage, depending on the used fixative (West, 1990). Previous studies have shown that preservation in Gilson's fluid caused up to 24 % shrinkage in oocyte size (West, 1990). Additional shrinkage occurs as a result of histological processing (dehydration, clearing, infiltration), accounting for up to 13 % discrepancy (West, 1990). Bouin's solution has been reported to cause very minor to significant changes in oocyte diameter. Previous studies reported oocyte shrinkage of 1 to 5 %, ranging to 30 to 48 % as a result of fixation and processing (Kuo *et al.*, 1974; Kraus *et al.*, 2008), and one study reported 9 % more shrinkage in Bouin's fixative as compared to Gilson's fluid (Ntiba & Jaccarini, 1992). In the current study, shrinkage ranged from 18.9 to 25.1 % (21.2 ± 2.3 %) in vitellogenic to mature oocytes, while it was higher in cortical alveolar oocytes (around

32 %) (Table H). This is due to preservation effects in Bouin's fixative as well as histological procedures. The question of how much Bouin's fixative affects oocyte size previous to and after histological processing (*i.e.* the summation of effects) needs to be addressed by future studies.

G. Fecundity

Because of the asynchronous batch-spawning pattern of oocyte development with no gap in the early development oocytes as well as *de novo* vitellogenesis, *F. commersonii* has indeterminate fecundity where the potential annual fecundity is not fixed (Hunter & Goldberg, 1980; Hunter *et al.*, 1985; Hunter & Macewicz, 1985a; Hunter *et al.*, 1992). Therefore, in the present study, the fecundity estimate used is the batch fecundity. In order to obtain a viable annual fecundity estimate, further information is required, in the form of the number of spawning events, female spawning frequency, and the length of the spawning season in addition to the batch fecundity (Holden & Raitt, 1974; Hunter *et al.*, 1985), and is a complicated procedure (Murua *et al.*, 2003).

Batch spawning species have many advantages over total spawners, such as increased overall fecundity as less eggs are spawned but over many instances, lowering risk of predation and food shortage for the eggs and larvae, and lowering risk of encountering unfavorable environmental conditions for the eggs and larvae (McEvoy & McEvoy , 1992). It has been suggested that in fishes there's a strong genetic component in the determination of fecundity (Tyler & Sumpter, 1996). Moreover, within the limitations of the genetic component, growth rates, nutrition, and enviromental conditions, in addition to atresia in some cases, may affect the fecundity of fishes

(McEvoy & McEvoy , 1992; Tyler & Sumpter, 1996; Moyle & Cech, 2004). In this regard, we expect the fecundity estimates of *F. commersonii* to vary across its protracted spawning period.

In this study, a modified form of the traditional gravimetric method was used, which is time consuming but commonly used and considered more reliable (Murua *et al.*, 2003). The ovarian samples used were obtained from a total cross-section of the ovary, as it has been shown that there might be spatial differences in the distribution of the oocytes (West, 1990). This was also observed in the histological sections for *F. commersonii*. Though most studies showed no effect in the position of the subsample on the the fecundity estimates obtained, it has been suggested to have two to three (and up to five) subsamples per ovary for at least 50 ovaries (Hunter *et al.*, 1985; Hunter *et al.*, 1992; Hunter & Macewicz, 2003; Murua *et al.*, 2003). The current study used three subsamples per ovary for 53 females, and no significant difference existed within the subsamples regardless of the position in the ovary.

Moreover, it has been observed that batch fecundity may decrease seasonally (Conover, 1985; Kjesbu, 1989; Macewicz & Hunter, 1993). This was observed in *F. commersonii*, where the sampled ovaries in the beginning of the season showed higher fecundity estimates than those sampled later, and there was a significant difference between the months sampled (Fig. 22). The sizes of the females sampled across the spawning months did not show a significant difference, so the observed monthly decline in fecundities (Fig. 22) is due to the time the sample was caught (beginning vs. end of season). In the earlier months, females were probably spawning their initial batches as opposed to later in the season where the batches spawned were the final batches.

It is well known that larger fish have a higher number of eggs compared to the smallers ones within the same species (Bagenal, 1971; Demartini & Fountain, 1981; Alheit, 1989; McEvoy & McEvoy , 1992; Moyle & Cech, 2004). This was also observed in *F. commersonii* (Fig. 23).

Fecundity-length relationships are usually power functions of the from $F = a\ L^{b}$ (Holden & Raitt, 1974). However, as in many batch spawners, the best fit becomes a linear function due to increased variability (Yoneda *et al.*, 1998; Murua *et al.*, 2006), or no fit at all in others (Hernandez-Herrera *et al.*, 2000; Bushnell *et al.*, 2010). In *F. commersonii*, and due to the causes of variability resulting from an asynchronous development, and considering that the fecundities decreased across the season (Fig. 22), the batch fecundity regression was a linear function with a low regression coefficient ($r^2 = 0.244$; Fig. 24a). The batch fecundity – gutted weight relationship followed the same linear trend as in most other species (Fig. 24b) (Holden & Raitt, 1974). However, there was no correlation between the relative fecundity and total length, as well as with gutted weight (Fig. 24d, e). The best predictor for both F_b and F_r, was gonad weight (Fig. 24c, f).

H. Reproductive Indices

1. Gonadosomatic Index (GSI)

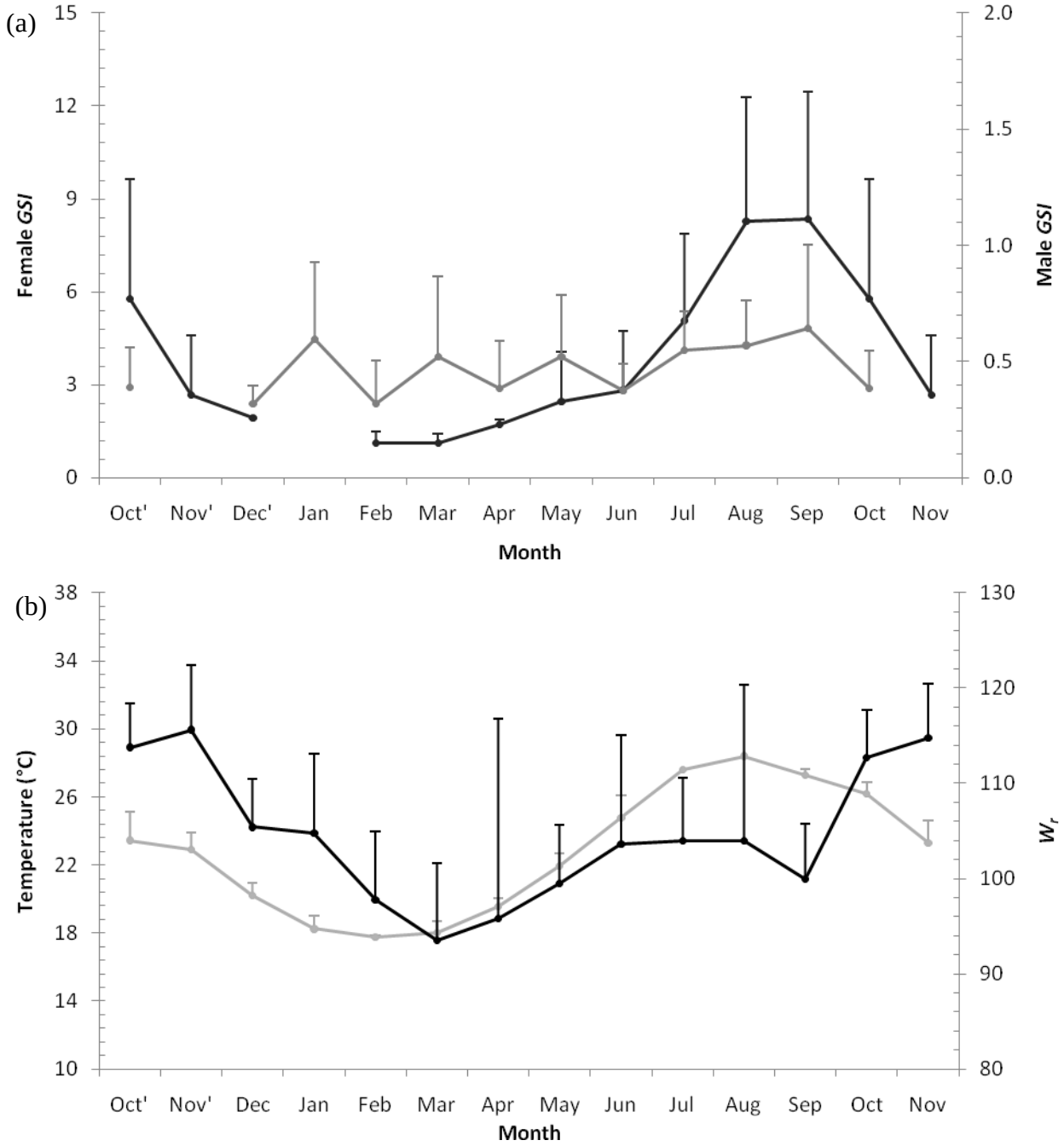


Figure 30: Monthly variations in (a) the Gonadosomatic index (*GSI*) for females (mean + s.d.; black line) and males (mean + s.d.; light grey line), as well as (b) relative weight (W_r; mean + s.d.; black line) and surface water temperature (mean + s.d.; light grey line).

The protracted spawning season discussed above was also observed through the Gonadosomatic index. In 2005-06, females had the lowest mean *GSI* values during the months of February and March (Fig. 25, Table 14). This coincided with the lowest water temperatures registered during that period (Fig. 2). The trend of increase of the female *GSI* closely followed the temporal fluctuations of the water temperature. The beginning of the spawning season in May coincided with the increase in female *GSI* values. Both water temperatures and *GSI* values peaked in August. A similar pattern was observed for the sampled months in 2009, where the observed peak was also during August (Fig. 25, Table 14). The relative drop of female *GSI* observed in September was probably due to the low sample size (n = 7) or to another factor during that month in 2009 (Table 14).

It was noticed that spawning started when surface water temperature was around 22 °C. When water temperature decreased to below 20 °C in January, spawning had ceased by the end of December (Fig. 2, 23). Spawning in *F. commersonii* in the Eastern Mediterranean might be regulated by a water temperature within this range. This observation is in line with the fact that a warm-water species from the Red Sea would find high water temperatures in the Eastern Mediterranean suitable for their reproduction.

In males, *GSI* fluctuated from December to May, and this is probably due to low sample numbers in January and March (Fig. 25, Table 14). However, from May and on there was a slight but constant increase in the monthly means, peaking in September, which coincided with a similar peak in the female *GSI*. While the *GSI* values decreased in October, the lack of mature males sampled in November did not allow us to specify the end of the running period. However, it is relatively well-documented that males are

ready to reproduce, i.e. mature their testes, prior to females and remain available for some time till after the end of the female spawning season (Htun-Han, 1978; Marino *et al.*, 2001; Hardie *et al.*, 2007).

2. Condition Indices

Monthly condition factor variations can be used to analyze the general well-being of fishes over the year, knowing that they result from environmental conditions and physiological differences like feeding abundance and reproductive status (Bolger & Connolly, 1989; King, 1995; Anderson & Neumann, 1996). In this study, the three condition factors calculated showed similar temporal fluctuations (Fig. 26). Consistently low values of all the indices used were recorded from December 2005 to March 2006, where the water temperatures are the lowest within the year. This cold period coincided with the postspawning months of *F. commersonii* (Fig. 26). It seems that the reproduction of *F. commersonii* in the Eastern Mediterranean is limited by the low sea water temperature from December to March.

During these months, the preferred preys of *F. commersonii* along the coast of Lebanon are not available, which makes this fish get closer to the shore and feed on a wider variety of available forage fish (Bariche *et al.*, 2009a). Therefore, the scarcity of prey could be another reason for the drop in somatic condition during the coldest months. Low condition values similar to the current study were also obtained during the winter months in *F. commersonii* in the Aegean (Kalogirou *et al.*, 2007).

Condition started increasing steadily starting April till November 2006 (Fig. 26). This parallels the gonadal development and spawning (May to December) of the species, as well as the availability of its preferred preys, namely *Boops boops*

(Linnaeus, 1758) and *Spicara smaris* (Linnaeus, 1758), along the Lebanese coastal waters (Bariche *et al.*, 2009a).

This steady increase in somatic condition for most of the year in parallel with *GSI* is remarkable and might be one of the reasons for the success of this Lessepsian invader since condition values usually decrease during the spawning season as energy reserves are redirected towards reproductive activity (Zwolinski *et al.*, 2001; Yoneda & Wright, 2004; Murua *et al.*, 2006; Sinovcic *et al.*, 2008). This increase has also been observed in other multiple spawning species that showed high condition values during the spawning months as a result of continued feeding (Galloway & Munkittrick, 2006).

The slight drop in condition in September is probably due to sampling bias. Another explanation might be that peak spawning activity could have slightly affected the somatic condition in that month. Further studies into the hepatic condition and comparatives with the standard condition factors might need to be made to further assess the somatic well-being of *F. commersonii* throughout the year. Finally, there was no difference in the monthly means between the sexes, except for the months discussed above, probably due to synchronous growth and development.

I. Conclusion: Reproduction of *Fistularia commersonii* in the Eastern Mediterranean

The current study was conducted over more than twelve months in 2005-06 and completed by additional sampling in 2009-10. From this work, the following conclusions regarding the reproductive cycle and characteristics in the Eastern Mediterranean could be drawn.

Fistularia commersonii is a Lessepsian invader that appeared to have an asynchronous gonadal development and a batch spawning pattern. The population was constituted of equal number of females and males, and sexual maturity was reached at around 57 cm L_T. The reproductive season is extended over nine months, starting in April. Spawning started in May and extended till December. The onset and cessation of the reproductive activity seemed to be determined by a surface water temperature of above 20 °C. During this period, ovaries contained up to three developing batches at one time. On average, each batch contained around 18,000 oocytes, and this number decreased as the season progressed. Somatic condition in the Mediterranean increased throughout the reproductive season, and decreased only in the coldest months.

The current study shed light on some important aspects of the reproduction of a recent invader that could be considered as one of the most successful Lessepsian species since the opening of the Suez Canal. Further studies are needed to answer the multiple questions raised during the work, such as the periodicity and frequency of spawning, spawning fraction, the actual number of batches spawned in the season per individual, the effect of different environmental factors (temperature, salinity, photoperiod, lunar cycle…), annual fecundity, the role of atresia in affecting fecundity, and the cytological changes in oocyte development (especially concerning the perinucleolar stage). In the future, other parts of the life cycle need to be investigated, such as larval dispersal, settlement patterns, and nurseries, in order to deepen our knowledge of this invader and provide further clues as to its invasive success.

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