Seasonal changes in oocyte development, growth and population size distribution of *Percilia gillissi* and *Trichomycterus areolatus* in the Itata basin, Chile

Cambio estacionales en el desarrollo de oocitos, crecimiento y distribución de talla poblacional de *Percilia gillissi* y *Trichomycterus areolatus* en la cuenca del río Itata, Chile

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ABSTRACT

There is scarce basic biological data on native freshwater fish species from Chile and next to zero on growth, gonad development and reproduction, which are critical for the purposes of the conservation of their natural populations. Seasonal analysis of sub-individual (oocyte development) and population (length frequency, growth rates) endpoints in *Trichomycterus areolatus* and *Percilia gillissi* were evaluated from February 2007 to January 2008. Oocyte development has a marked seasonality for *P. gillissi*, with mature eggs in October and declining frequency of mature oocytes into January. For *T. areolatus* we found some mature eggs in July, with highest number of mature eggs in October, coincident with previous data on maximum gonad size. Both species showed a multiple spawning reproductive strategy, with a long spawning season starting in spring to early summer. Increased numbers of juveniles were observed during mid-summer for *P. gillissi*, and during the end of the summer and beginning of autumn for *T. areolatus*. Juveniles had a higher growth coefficient (K) (0.56-0.38 mm month⁻¹) than adults (0.29-0.15 mm month⁻¹), and almost all juveniles in the population reached maturity before the spring (>50 mm for *T. areolatus* and >40 mm for *P. gillissi*) and were incorporated into the population as a new recruitment cohort. Knowledge of the seasonal variability of these individual and population level responses can help better understand the biology of the species, and simultaneously improve the management and conservation of freshwater biota in Chile.

KEYWORDS: Seasonal cycles, native fish Chile, oocyte development, multiple spawning reproductive strategy, population size-structure.

RESUMEN

Existen escasos datos de biología básica sobre las especies de peces nativas chilenas de agua dulce y casi ninguno en sobre el crecimiento, el desarrollo gonadal y reproducción, que son fundamentales para los propósitos de la conservación de sus poblaciones naturales. El análisis estacional de los parámetros sub-individuales (desarrollo de oocitos) y poblacionales (frecuencia de tallas y tasa de crecimiento) en *Trichomycterus areolatus* y *Percilia gillissi* fueron evaluados entre febrero de 2007 y enero de 2008. El desarrollo de los oocitos posee una marcada estacionalidad en *P. gillissi* con oocitos maduros en octubre y una disminución de estos estados maduros hacia enero. En *T. areolatus* encontramos oocitos maduros en Julio, pero con una mayor abundancia durante octubre, coincidente con datos previos de máximo desarrollo de la gónada. Ambas especies mostraron una estrategia reproductiva de desoves múltiples, con una temporada de desove que comienza en primavera hasta principios de verano. Ambas especies evidencian un mayor número de juveniles durante mediados de la estación estival (*P. gillissi*), finales de la estación estival (*T. areolatus*) y principios de otoño, con un número decreciente hacia primavera. Los juveniles tienen un coeficiente de crecimiento (K) mayor (0.56-0.38 mm mes⁻¹) comparados con los adultos (0.29-0.15 mm mes⁻¹), y antes de primavera prácticamente todos los individuos en la población alcanzan la madurez (>50 mm for *T. areolatus* and >40 mm for *P. gillissi*) y los juveniles son incorporados a la población en una nueva

fase de reclutamiento. El conocimiento de la variabilidad estacional de estas respuestas individuales (desarrollo de oocitos) y poblacionales (frecuencia de tallas, tasas de crecimiento) puede ayudarnos a un mejor entendimiento de la biología de las especies, y simultáneamente mejorar el manejo y conservación de la biota dulceacuícola.

PALABRAS CLAVE: ciclos estacionales, peces nativos chilenos, desarrollo de oocitos, estrategia de desoves múltiples, estructura de talla poblacional.

INTRODUCTION

There are many seasonal changes in individual fish and fish populations that might affect their sensitivity to environmental stress, and affect the sustainability of fish populations. Changes in gonad size, for example, are a consequence of physiological changes in sex steroid production and gonad development, which are ultimately, a consequence of previous biochemical and neuroendocrine changes at lower levels of organization. Serious changes in individual endpoints such as gonadal growth, have the potential to cause changes in the reproduction of individuals and population dynamics of the species. Changes at low levels of biological organization due to environmental stressors (Munkittrick *et al.* 1992; McMaster *et al.* 1991; Van der Kraak *et al.* 1992; Parrott *et al.* 2004), can also lead to impaired reproduction and sustainability of natural populations (Yeom & Adams 2007).

At higher levels of organization, seasonal changes in size structure reflect important information about population dynamics, and reflect changes in reproductive performance, growth, and recruitment that will also affect population sustainability over longer temporal scales. Considerations into the relationship between the reproductive output and the resulting growth of the population is crucial to understanding of how fish populations will respond to sustained perturbations (Lowerre-Barbieri *et al.* 2011). Any such consideration depends on a clear understanding of the life-cycle dynamics of the population, from the production of viable eggs, through all processes that affect the probability of survival and maturation (Jakobsen *et al.* 2009). Knowledge of the seasonal variability of these individual responses can help us better understand how effects can occur, and simultaneously to improve the design of aquatic monitoring programs to detect change, with adequate statistical design incorporating this variability and seasonality (Munkittrick *et al.* 2009).

Chilean isolation from the rest of the continent has resulted in the existence of extremely unique species (Ruiz & Berra 1994). This situation is clearly reflected in the low number of freshwater fish species in Chile, represented by 11 families, 17 genera and 44 species, with 40% of these species classified as endangered (Dyer 2000; Habit *et al.* 2006). The latter is particularly relevant when considering that 80% of the species in Chile are endemic, with high

retention of primitive features, low diversity, small body size and adaptation to high gradient streams and fluctuating flows (Campos *et al.* 1993; Ruiz & Berra 1994; Vila *et al.*, 1999; Dyer 2000). The end result is in a unique group of freshwater fish, with high biogeographic and conservation value. However, there are important gaps in knowledge of their systematic, distribution and basic biology (Habit *et al.* 2006). Two of the most abundant fish species, with a larger geographic distribution in central-south Chilean basins are two small bodied fish: *Trichomycterus areolatus* Valenciennes 1846 (Trichomycteridae, common name: Bagre; maximum reported length: 15 cm; Arratia 1983) being found from the Coquimbo (30°S) to the Los Lagos (43°S) region (Arratia 1981; Dyer 2000) and *Percilia gillissi* Girard, 1854 (Percilidae, common name: Carmelita; maximum reported length: 9 cm; Ruiz & Marchant 2004) present from the Aconcagua valley (32°S) to the Los Lagos region (41°S) (Arratia *et al.* 1981; Zunino *et al.* 1999; Dyer 2000). Despite their large distribution, there is still limited knowledge of their basic biology. Both species live mainly in rhithron like zones of the river with shallow riffle and rapid habitats with *T. areolatus* highly associated with the substrates, while *P. gillissi* is a midwater dweller. Both species have a similar diet composed by benthic macroinvertebrates (Duarte *et al.* 1971; Ruiz 1994; Habit *et al.* 1998). Reproductive data for *T. areolatus* provided by Manriquez *et al.* (1988) and Habit *et al.* (2005) are coincident in describing their spawning season (Mid-spring to early-summer), but with differences in the estimation of fertile adults. There is a lack of consistent data on the reproductive biology of the *Percilia* genus, but for the congeneric species (*P. irwini*) spawning is estimated to occur in late winter and early spring, between August and November (Habit & Belk 2007). Recently, we have assessed seasonal changes in metabolic and reproductive parameters such as gonadosomatic indices (GSI) for both species and described the macroscopic development of the gonad, along with identifying the size at first maturity (Chiang *et al.* 2011a). Despite one study describing the different stages of oocyte maturation for *T. areolatus* (Huaquin *et al.* 2002), there is nothing published for *P. gillissi*. There is also no data on seasonal development of oocytes for either species and on the temporal growth of individuals in the population. The main objectives of this work were to 1) study female reproduction by analyzing the seasonal development of the oocyte, 2) describe seasonal population size distribution and 3) estimate population growth rates for both juvenile and adult size classes.

MATERIALS AND METHODS

Study area

The Itata basin is located in Chile's Biobío region between 35° and 37°S with a surface area of 11,200 km² and a length of the Itata river about 195 km; the maximum elevation of the basin is at the Nevados del Chillan volcano (3213 m.a.s.l). According to several authors the discharge ranges from 240-750 m³/s in winter months to <20 m³/s during the summer (DGA 2004; Dussaillant 2009). The watershed is comprised of three sub-basins: the Ñuble, Diguillín and Itata, containing the Cholguán, Ñuble, Chillan, Diguillín, Cato, Lonquén and Itata rivers (Dussaillant 2009). The two selected fish species, *T. areolatus* and *P. gillissi* are the most abundant fish species through the entire basin (Habit & Ortiz 2009), especially dominant in the middle zone of the watershed where these studies were conducted. Three sites were selected for the collection of individuals; all sites were upstream the confluence of the Ñuble and Itata

ivers. The sites included two locations on the Itata River (S1, 36°42'17, 21''S 72°26'47,04''W; S2, 36°41'40,13''S 72°26'47,04''W), and one site on the Ñuble River (S3, 36°38'30,00'' S, 72°27'12,64''W) (Fig 1).

Fish capture and sampling

The fish were captured monthly between February 2007 and January 2008 (except for September) using a backpack electrofishing unit (Halltech Electrofisher, Canada) and a block seine (6 mm mesh) in riffles (0.2-0.3 m/s, 0.2-0.4 m depth) with boulder-cobble bedrock (approximately 15 cm diameter) and/or shallow riffles (0.1-0.2 m/s, 0.1-0.4 m depth) with cobble (15 cm diameter) and stone bedrock. Most of the fishes were measured for length (± 0.1 mm) and weight (± 0.01 g), and released back to the river. According to previous unpublished data we had, we monthly sacrificed by spinal severance fish above 35 mm total length to obtain gonad samples, determine sex and for female histology.

FIGURE 1. Seasonal reference sampling sites in Itata basin for recollection of *T. areolatus* and *P. gillissi* (S1-S3).

FIGURA 1. Sitios de muestreo estacional en la Cuenca del Itata durante la recolección de *T. areolatus* and *P. gillissi* (S1-S3).

A total of 1848 individuals (909 for *P. gillissi* and 938 for *T. areolatus*) were collected and seasonal population size structure was assessed according to Pauly & Morgan (1987). All length measurements were put into 2mm size class intervals and analyzed using the FiSAT software package (FAO-ICLARM Fish Stock Assessment Tool). The ELEFAN I K-value scan routine was used with fixed starting and maximum length values for the whole length range. Juveniles were sorted from adults, and the "best" curve was fit to the set of length-frequency data, allowing calculation of K (von Bertalanffy growth model's growth rate coefficient) for each group.

Ovaries were collected from sampled fish and set in Bouin solution (48 h), then washed in 70% alcohol, three times for 15 min. The tissue was subsequently dehydrated with a series of ethanol solutions (70–99%) and chloroform, then twice infiltrated in liquid paraffin at 58 °C for 2 h, then embedded in paraffin at room temperature (16°C) for 24 h. The embedded tissue was sectioned (thickness 7 µm) and stained with a solution of hematoxylin and eosin (0.5%). A total of 524 ovaries were sampled for *P. gillissi* and 568 for *T. areolatus*. The proportion of oocytes of the various stages for the different months of sampling were analyzed using a minimum of 3 slides per ovary and the distinct maturation stages assigned according to a modified scale for *Trichomycterus areolatus* (Huaquin *et al.* 2002) and *Percilia gillissi* (modified from Quiroz 2006; Ancalaf 2008) (Table 1). Gonad Somatic Index (GSI) for sexually mature fish was modified from Chiang *et al.* (2010).

Statistical analysis

Due to this study's seasonal design, the analyses examined data for sex and species separately between sites during the period of gonad maturation. Due to the absence significant differences between the three reference sites, data were pooled and presented here for seasonal comparisons. Changes between months were assessed using an analysis of variance (ANOVA, $p < 0.05$; Systat ©11.0).

RESULTS

Female gonad histology

Trichomycterus areolatus

Only individuals > 54mm total length (range 29–97.3mm) contained mature gonads in female *T. areolatus*. There were four states of maturity in the oocytes from the early winter period (June) to the early summer (January) (Fig. 2A and 2B). Seasonally, this species showed immature oocytes (Stage I and II), throughout their gonadal maturation cycle. Immature oocytes peaked in abundance in February and were minimal between June to October ($F=8.208_{(10)}$ $p < 0.001$). Oocytes in intermediate stages of maturity (Stage III) were

present at abundances less than 5% in February, increased between July–October with levels often exceeding 25%, then declined in January ($F=11.753_{(10)}$, $p < 0.001$). While the appearance of vitellogenic oocytes (Stage IV) occurs in June, the maximum number of vitellogenic oocytes occurs between October and November ($F=7.851_{(6)}$, $p < 0.001$), declining rapidly thereafter (Fig. 2B).

Percilia gillissi

Over a range of total length (23.1–91.6 mm), females of *P. gillissi* > 43mm total length had ovaries with developing oocytes in all stages of maturity. There were no differences between sites in the development or the occurrence of oocytes in the ovaries of *P. gillissi* (Stage I and II $F=0.146_{(2)}$, $p=0.869$; Stage III $F=0.690_{(2)}$, $p=0.503$, Stage IV $F=0.983_{(2)}$, $p=0.377$, Stage V $F=0.625_{(2)}$, $p=0.539$). Immature oocytes were observed during every month of collection along with a marked seasonality in the frequency of oocytes in all stages of maturity ($F=6.604_{(10)}$, $p < 0.001$, Fig 3A). Seasonally, there is a decrease in the frequency of oocytes in primary states of development (Stage I and II) from March (> 95%) to October (<25%), and then increase in abundance within the ovary towards the summer period (Fig 3A and 3B). The previtellogenic (Stage III) and vitellogenic (Stage IV) oocytes are present throughout the year, with abundances that increased gradually from autumn to spring, whereas mature oocytes (Stage V) were visible since October, with highest frequencies between October and November ($F=5.862_{(3)}$, $p = 0.002$) reduced numbers in January and mature oocytes were not observed after this month (Fig. 3A).

Seasonal Population Size Structure

Trichomycterus areolatus

The size frequency distributions for the populations of catfish did not differ between sites throughout the study ($F=1.593_{(2)}$, $p = 0.476$), and all sites were pooled within month. Seasonally, there was an increase in the frequency of smaller sized fish from early summer (December), with two cohorts of individuals with different size distributions observed from December to early autumn (April). After this date, the size distribution becomes more homogeneous, with larger individuals in November compared to the months of December through to April ($F=14.899_{(10)}$, $p < 0.001$) (Fig. 4A). By separating the population into juveniles (<54mm) and adults (> 54mm), no differences in the total length of adults in any month was observed ($F=1.422_{(10)}$, $p=0.212$), but juveniles showed a significant increase in size from late summer to the spring period ($F=4.00_{(10)}$, $p > 0.001$). Growth coefficient values (K mm month⁻¹) for the whole population size distribution is 0.29 mm month⁻¹, but individuals <54mm reach values >1.3 times higher (0.38 mm month⁻¹), while individuals sexually active demonstrate a decrease in K values (0.15 mm month⁻¹) (Table 2).

TABLE 1. Oocyte development table for *Percilia gillissi* and *Trichomycterus areolatus*.TABLA 1. Tabla de Desarrollo de oocitos para *Percilia gillissi* y *Trichomycterus areolatus*.

<i>Percilia gillissi</i> ^a					
Stage		Characteristics of oocyte surface envelope	General appearance	Yolk	Nucleoli
Immature with Primary Growth	I y II	A simple prefollicular cell envelope	Spherical cell. Ooplasma highly basophilic. Cortical alveoli present.	N.O.	Arranged on the periphery of the nucleus and associated with the inner face of the nuclear envelope.
Previtelogenic	III	A simple follicular cells envelope	Ovoid cell. Ooplasm of irregular aspect due to the onset of yolk incorporation.	Starting yolk micropinocytosis.	Arranged on the periphery of the nucleus and associated with the inner face of the nuclear envelope. Comparatively higher proportion.
Vitelogenic	IV	Oocyte thicker cover. Composed by two layers (zona radiata). 10 - 38 μ m thick.	Ovoid cell. Ooplasm irregular and sponge-like due to the high incorporation of yolk. Nucleus shifted to the periphery and irregularly shaped.	Active yolk micropinocytosis. Numerous granules begin to coalescence.	N.O.
Mature	V	Thicker zona radiata. Theca cells differentiated. 20 – 88 μ m thick	Ovoid cell of maximum growth and development	Homogeneous, large flowing yolk mass	N.O.
<i>Trichomycterus areolatus</i> ^b					
Immature and Previtelogenic	I y II	A simple follicular cell envelope.	Spherical cell. Ooplasma highly basophilic. Nucleus is central with mild PAS positive reaction.	N.O.	Arranged on the periphery of the nucleus and associated with the inner face of the nuclear envelope
Vitellogenic	III	Zona radiata composed by two layers, with a PAS positive reaction.	Increase in size. The nucleus moves to the periphery and the cell boundary becomes irregular.	Active yolk accumulation.	Large numbers of nucleoli arranged in the inner edge of the karyotheca.
Mature	IV	Thiner Zona radiata with a prismatic follicular epithelium.	Spherical cell. Nucleus is in the process of disgregation, is finally no longer observed	Globular aspect of the yolk, with some zones PAS positive and negative reaction	N.O.

^amodified from Quiroz (2006); and Ancalaf (2008)^bmodified from Huaquin *et al.* (2002).

N.O. Not Observed

FIGURE 2. A) Seasonal oocyte maturation states for *Trichomycterus areolatus*. EI: oocyte stage I, EII: oocyte stage II, EIV: oocyte stage IV (according to Huaquin *et al.* 2002); Black line represents mean GSI for each month (modified from Chiang *et al.* (2011a). B) Photomicrographs of transverse sections of ovaries from *T. areolatus*; EI & EII: primary oocytes, EIII: previtelogenic oocytes, EIV: mature oocytes, GV: yolk granules, N: nucleus, NC: nucleolus.

FIGURA 2. A) Estados de maduración estacional de los oocitos para *Trichomycterus areolatus*. EI: oocito en estado I, EII: oocito en estado II, EIV: oocito en estado IV (modificado de Huaquin *et al.* 2002); Línea negra representa el promedio de IGS para cada mes (modificado de Chiang *et al.* 2011a). B) Fotomicrografías de secciones transversales de los ovarios de *T. areolatus*; EI & EII: oocitos primarios, EIII: oocitos previtelogénicos, EIV: oocitos maduros, GV: gránulos de vitelo, N: núcleo, NC: nucléolo.

FIGURE 3. A) Seasonal oocyte maturation states for *Percilia gillissi*. EI: oocyte stage I, EII: oocyte stage II, EIV: oocyte stage IV, EV: oocyte stage V (modified from Quiroz 2006; Ancalaf 2008); Black line represents mean GSI for each month modified (from Chiang *et al.* (2011a) B) Photomicrographs of transverse sections of female gonads of *P. gillissi*; EI & EII: primary oocytes, EIII: previtellogenic oocytes, EIV: oocytes in vitellogenic state; EV: mature oocytes; PV: yolk platelets, ZR: zona radiata, EN: nuclear envelope, N: nucleus, NC: nucleolus, AC: cortical alveolus.

FIGURA 3. A) Estados de maduración estacional de los oocitos para *Percilia gillissi*. EI: oocito en estado I, EII: oocito en estado II, EIV: oocito en estado IV, EV: oocito en estado V (modificado de Quiroz 2006; Ancalaf 2008); Línea negra representa el promedio de IGS para cada mes (modificado de Chiang *et al.* 2011a). B) Fotomicrografías de secciones transversales de las gónadas femeninas de *P. gillissi*; EI & EII: oocito primario, EIII: oocitos secundario, EIV: oocito en estado vitelogénico; EV: oocoto maduro; PV: plaquetas de vitelo, ZR: zona radiata, EN: envoltura nuclear, N: núcleo, NC: nucléolo, AC: alveolo cortical.

Percilia gillissi

The populations of *P. gillissi* contained smaller individuals from December to February, intermediate sizes between March and August and increased total length in October and November ($F=12.098_{(10)}$, $p < 0.001$, Fig 4B). Smaller size frequencies were observed in December with an increase in total length of the population towards spring (Oct-Nov), but two cohorts were not clearly seen throughout the study period (Fig. 4B). Juveniles (individuals $< 43\text{mm}$)

showed a significant increase in length starting in June, while adults ($> 43\text{mm}$) showed smaller sizes between January and February ($F=12.098_{(10)}$, $p < 0.001$, Fig 4B). Growth was faster in juveniles with K values almost 1.6 fold higher than individuals with gonads sexually mature ($0.56 > 0.33 \text{ mm month}^{-1}$). The K values of juveniles was > 1.9 times higher than those calculated for the whole population size distribution throughout the year ($0.29 \text{ mm month}^{-1}$) (Table 2).

FIGURE 4. Seasonal population size distribution structure (total length) for A) *Trichomycterus areolatus* and B) *Percilia gillissi* (February 2007-January 2008). Dark area indicates sexually active individuals, light area indicates juveniles.

FIGURA 4. Distribución estacional de tallas de la población (largo total) para A) *Trichomycterus areolatus* y B) *Percilia gillissi* (Febrero 2007-Enero 2008). Área oscura indica individuos sexualmente activos, área clara indica juveniles.

TABLE 2. Comparative growth coefficients ($K \text{ mm month}^{-1}$) calculated by ELEFAN I K-value scan routine for *Percilia gillissi* and *Trichomycterus areolatus* in the Itata river.

TABLA 2. Comparación de coeficientes de crecimiento ($K \text{ mm mes}^{-1}$) calculados a partir de la rutina de exploración de valores-K ELEFAN I para *Percilia gillissi* y *Trichomycterus areolatus* en el río Itata.

SPECIE	STARTING LENGTH (mm)	L_{∞} (mm)	ELEFAN I K (mm month^{-1})	SCORE
<i>Trichomycterus areolatus</i>	33	54	0.38	0.124
	54	99	0.15	0.152
	33	99	0.29	0.159
<i>Percilia gillissi</i>	25	43	0.56	0.108
	43	81	0.29	0.169
	25	81	0.33	0.188

DISCUSSION

Basic biological data from freshwater fish species in Chile is scarce, with one of the major gaps in knowledge, information about spawning seasons, fecundity, reproduction strategies and growth (Habit *et al.* 2006). Incorporating histological data will help to understand gonadal development and when spawning events occur, and integrating this reproductive data with growth information will help us to understand population dynamics to make better decisions about fisheries management and conservation (Lowerre-Barbieri *et al.* 2011). This knowledge may also help to plan environmental management by separating natural variability from anthropogenic impacts (Chiang *et al.* 2011a, 2011b).

The presence of mature oocytes in the gonad of both species simultaneously with oocytes at all other different developmental stages indicates a multiple spawning reproductive strategy with continuous preparation of the gonad for development and maturation of oocytes in groups (batches) (Galloway & Munkittrick 2006), that are spawned over a period of time in a few or many events as described for other species (Barrett & Munkittrick 2010). The highest observed abundance of oocytes in advanced stages of maturity, were consistent with data of highest gonadosomatic indices (GSI) during spring and early summer, previously reported for both species (Chiang *et al.* 2011a) and to the presence of mature female (external examination) between September and December (*T. areolatus*) (Montoya *et al.* 2012). The histological analysis of the oocytes in *T. areolatus* is protracted from what has been reported by Manríquez *et al.* (1988) using macroscopic observations, which describes this species with a synchronous development of batches of oocytes in the gonad between spring and early summer. Histology allows us to clearly differentiate the stages of maturity of the gonad (Galloway & Munkittrick 2006), as we identified mature eggs from July to January, demonstrating a long spawning season and supported by Montoya *et al.* (2012) that found spawned eggs of *P. gillissi* between November and January; while for *T. areolatus* they found no eggs (maybe because, siluriform eggs are mainly demersal, Huaquin *et al.* 2002) they described larvae and postlarvae/juveniles stages along late spring to early autumn in other river systems.

The long spawning seasons for both species; a period from October to January for *Percilia gillissi* and from June to January for *Trichomycterus areolatus*, may explain the long period of recruitment of juveniles for both species. The size limit distinction between juvenile and adult fishes was determined using the initiation of reproductive development as the presence of mature oocytes (Nikolsky 1963, fide Manríquez *et al.* 1988; Jackobsen *et al.* 2009) and for *T. areolatus* only fishes ≥ 50 mm were found with

maturing gonads (Manríquez *et al.*, 1988; Chiang *et al.*, 2011a), while mature oocytes in *P. gillissi* started at size ≥ 43 mm, as described in previous studies by external gonadal examination (Chiang *et al.* 2011a) and confirmed in this study by histological analysis. During our collections, no *T. areolatus* < 29 mm total length or < 26 mm total length for *P. gillissi* were captured. The absence of smaller individuals throughout the study may be due to the collections being focused on a single habitat or the use of nets of mesh size 6 mm, for which smaller juveniles could escape. Manríquez *et al.* (1988) and Arratia (1983) described juveniles sized under 10 mm, with juveniles carrying yolk sacs up to 7.6 mm in total length (Arratia 1983). Despite the absence of juvenile *T. areolatus* below 29 mm, the presence of juveniles throughout the sampling period is consistent with the results of Manríquez *et al.* (1988), Arratia (1983) and Habit *et al.* (2003). They described a greater abundance of juveniles in March-April, together with the presence of two cohorts during this period, oocytes at different stages of maturity indicating a long spawning period for *T. areolatus*, and a recruitment period that clearly begins in late summer.

There were no reproductive or growth data for *P. gillissi* and only limited data for the closely related *P. irwinii* (Habit & Belk 2007). The populations of *P. gillissi* consisted of smaller size classes during the summer period, followed by increased growth in terms of length, to a maximum length in the spring. Juvenile fish were sporadically observed throughout the study period, with greater abundances occurring following the completion of spawning (February-March). Through our observations of increased growth in the size of populations of *P. gillissi*, the increased abundance of young following spawning and the presence of mature oocytes for a reduced period of time, we estimate that the spawning season for this species is shorter than that observed in *T. areolatus*. Populations of both species demonstrated a temporally diverse size structure, which is indicative of a healthy environment (Aedo *et al.* 2009). The calculated K values could be showing an energy investment in fast growth within the first year of life to the size of sexual maturity and a shift of energy investment to gonad development and reproduction. This life strategy ensures successful reproductive investment and maintenance of fish populations.

This study showed that *P. gillissi* and *T. areolatus* have an extended spawning season with a multiple spawning reproductive strategy (spring to early summer and winter for *P. gillissi* late summer to early winter for *T. areolatus*), as well as shifts in energy investment from growth to gonad development during the first year of life. The results of this research confirmed the seasonality and variability of reproductive parameters evaluated previously (Chiang *et al.* 2011a). Multiple spawning fish species such as the two

studied here should be collected prior to the initiation of the first spawning event (spring for *P. gillissi*), in order to estimate reproductive status of wild populations, before any oocytes have been released, otherwise fecundity will be underestimated (Murua *et al.* 2003). Histological data helped us to clarify the higher variability in GSI for *T. areolatus* identified in previous study, during the gonadal development season (October-November) due to the protracted spawning season of the larger adults (Chiang *et al.* 2011a). These larger fish have mature eggs and presumably start spawning in the middle of the winter prior to smaller adults. For monitoring purposes, collections should be made prior to spring for these species as the large adults initiated spawning early, increasing variability in GSI during earlier proposed monitoring seasons (Chiang *et al.* 2011a). This information is key to designing effective biological monitoring programs, which allows us to assess threats and understand the possible causes of deterioration of the populations exposed to stressors of different origins and help in the restoration and/or protection of these species (Galloway & Munkittrick 2006).

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