Reproductive cycle, size at first maturation and fecundity in the golden ling, *Genypterus blacodes*, in Chile

FELIPE PAREDES

RICARDO BRAVO

Facultad de Ciencias del Mar Universidad de Valparaíso Casilla 5080, Reñaca Viña del Mar, Chile email: fparedesv@hotmal.com

Abstract Reproductive aspects of the golden ling, Genypterus blacodes, from the southern Chilean fishery are presented. Gonad samples were obtained from commercially caught fish between September 1999 and October 2000. Stage of sexual maturity, gonadosomatic index (GSI), and size of females at 50% maturation determined by logistic methods and by plotting the mean GSI by total length, are presented for both years. Batch fecundity was calculated from subsampling mature ovaries containing oocytes in advanced stages of maturity. Changes in GSI and monthly diameters of oocytes showed that ling had a spawning season which extended between August and November. The sizes at first maturity in 1999 and 2000 were 86.6 and 84.8 cm total length (TL), respectively, using the logistic model, and 84 cm for both years using the mean GSI-size plotting method. The mean batch fecundity was 333 330 oocytes per individual for fish sized 85-120 cm TL. Fecundity increased with increasing weight and total length.

Keywords *Genypterus blacodes*; reproductive cycle; fecundity; sexual maturity, South of Chile

INTRODUCTION

The golden ling, *Genypterus blacodes* (Schneider, 1801) (Ophidiidae), is a demersal fish distributed in Chile's coastal zone from Coquimbo (30° S) to Cape Horn (57° S) (Fig. 1). This species has been traditionally exploited along with the southern hake, *Merluccius australis*, in demersal fisheries of southern Chile between latitudes of $41^{\circ}31'$ and 57° S (Fig. 1). This species is normally distributed over a wide depth range of 50–500 m (Young et al. 1984; Beentjes et al. 2002). Outside Chile, *G. blacodes* occurs in Argentina, Uruguay, New Zealand, southern Australia, and Tasmania (Young et al. 1984).

Development of the southern demersal fishery began in 1977 when foreign factory trawlers were authorised to operate south of 37°S. Annual landings of golden ling have fluctuated during the history of the fishery beginning with 1500 t in 1978 reaching a historic maximum in 1988 with c. 15 000 t (http://www.subpesca.cl). In 1998, total landings were only 5721 t and there are concerns that the species has been overfished (Rivas 2004).

Apart from monitoring gonadosomatic index (GSI) cycles, there have been few studies carried out in Chile on ling reproduction. Among studies carried out on G. blacodes are those of taxonomic interest (Chong 1984), stomach contents (Bahamonde & Zavala 1981; Renzi 1986), age and growth parameters (Chong & Aguayo 1990; Horn 1993), macroscopic and microscopic structure of the ovary in samples from its Atlantic range (Louge et al. 1992), description of spawning stages from Argentinean waters (Machinandiarena et al. 1998), regional morphometric variations in New Zealand (Colman 1995), and instantaneous rate of natural mortality (Ojeda et al. 1986). Most of the information extant on this species in Chile (Aguayo et al. 1985, 1986, 1987, 1990, 1991, 1992, 1994; Chong 1993) is related to its fisheries biology, such as knowledge on the cycle of its GSI, size structure, and age groups of catches, catch per unit effort, stock evaluation in the fishing area, and estimations of total

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Fig 1 Distribution of *Genypterus* blacodes along the coast of Chile and study area.

permissible catch carried out by the Institute for Fisheries Development (IFOP).

In a study of fish reproductive dynamics in Chile, Chong (1993) found that total and modal fecundity were relatively low from August to December 1992 compared with the same data from *Merluccius gayi* and *Merluccius australis*. Chong's study also showed that the size of 50% of the females at maturity in *G. blacodes* was c. 90 cm.

Reproductive aspects of fish species such as fecundity, size at first sexual maturity, and reproductive cycle allow estimation of the reproductive potential and resource renewal rate in species that are or have been exploited (Rivas 2004). In this study, we present data of the reproductive dynamics of *G. blacodes* between 1999 and 2000, and compare it to Chong's study (1993).

MATERIALS AND METHODS

Reproductive data for golden ling were obtained from the commercial fishery in the southern hake (M. *australis*)-golden ling (G. *blacodes*) fishery zone (Fig. 1). Female specimens and their respective ovaries were obtained monthly from the catches between September 1999 and October 2000. Ling from the commercial catch were weighed to the nearest 1 g, measured with an ichtyometer for total length (TL) to the nearest 10 mm, and female ling were sampled for ovaries aboard ship. Ovaries were weighed to the nearest 5 g and fixed in 10% formalin in sea water (Hunter & Macewicz 1985).

At the Ichthyology Laboratory of the Marine Science Faculty at the University of Valparaiso, ovaries were re-weighed to the nearest 0.01 g, labelled, and stored in 10% buffered formalin. The stage of sexual maturity (SSM) was subsequently determined by observing macroscopic and microscopic characteristics of the ovaries. This procedure included dissection of small pieces of gonadal tissue to liberate oocytes from the stroma of the ovary. The size of the largest oocyte in the sample was measured to the nearest 0.06 mm. Because the oocytes were not perfectly spherical, the orientation of oocytes to the reference line was the largest diameter measured. Sexual maturity of females (SSM) was determined using macroscopic and microscopic characteristics and histological samples (Table 1). Histological samples were processed using standard procedures for paraffin embedding and 5 µm sectioning (Mazhirina 1978). Three SSM were adapted from Davis & West (1993) but included macroscopic staging of the ovary, and were: (1) unyolked; (2) yolked; and (3) ripe (Table 1). SSM 1 denominated Unyolked was separated into two substages according to Davis & West (1993); perinucleus stage (SSM 1 PNS) and yolk-vesicle stage (SSM 1 YVS) (Fig. 2).

The following gonadosomatic index was calculated using gonad weight and total body weight:

$$GSI = \frac{Gonad weight}{Body weight - Gonad weight} \times 100$$

The 50% size at first sexual maturity of females was estimated applying the criterion of $L_{50\%}$, which represents the length at which 50% of the females were found to be mature, using the logistic function model:

$$P_j = \frac{100}{1 + \mathrm{e}^{\mathrm{a} + \mathrm{b}Lj}}$$

where P_j is the proportion of mature individuals in the size interval "*j*", and a and b are position coefficients of the above defined logistic function. The size at maturity was also determined by plotting mean GSI ascertained in the spawning season (September–October in 1999 and August– September in 2000) by TL arranged by 4-cm size

Stage	Macroscopic	Whole-oocyte	Histological
Unyolked Perinucleus stage	Small ovary, filiform. In some instances presents a tubular/flattened section	Transparent with visible nucleus, becoming granular and translucent with increasing size (oocyte diam. <0.25 mm). Homogenous and basophilic cytoplasm	Oocytes lamelae in parallel disposition, previtellogenesis reserve oocytes abundant and with central multinucleolar germinative vesicles.
Yolk-vesicle stage	Medium and light pink ovary, covered by a resistant and smooth membrane	Granular, translucent, becoming darker with increasing size, but still translucent (oocyte diam. >0.25 mm)	Simultaneous presence of reserve and vesicles oocytes. In the final stage oocytes with yolk granules
Yolked	Big ovary, turgid, pink or light yellow. Covered by a resistant and smooth membrane	Previous phase to oocyte hydration. Completely opaque except for previteline border (oocyte diam. <0.90 mm). This stage includes the previous spawned ovaries	Yolked ovary, oocytes initially loaded with big vitelum granules surrounded by immature reserve and vesiculosus oocytes
Ripe	Large ovary, turgid, with hydrated oocytes. Covered by a smooth and resistant membrane; contains a great quantity of transparent mucus. Light yellow ovary	Oocytes translucent except for the oil droplet. Distinguished from Yolked when parts of the yolked become translucent (oocyte diam. >0.9 mm)	Terminal maturation indicates presence of occytes with migrant germinative vesicle and hydrated oocytes with postovulatory follicles. Maximum development of the zone radiate

 Table 1
 Macroscopic and microscopic (whole-oocyte and histology) staging scheme used to describe ovarian maturity of golden ling, *Genypterus blacodes*.



Fig. 2 Photomicrography of transverse sections of *Genypterus blacodes* ovaries showing: **A**, unyolked ovary (Yolk-vesicle stage; SMS 1); **B**, yolked ovary (SMS 2); and **C**, ripe ovary (SMS 3). (L, lamellae; t, connective tissue; arrow, basophile oocyte (reserve); op, partial vitellogenesis oocyte; V, vitellogenic oocyte; h, hydrated oocyte; m, mature oocyte; and f, postovulatory follicle.)

classes, as described by Grimes & Huntsman (1980). Only data collected during the 2 months of major reproductive activity of each year were used for this analysis as these had the highest GSI values (Finucane et al. 1986). Here the data were arranged in ascending order of size and grouped into TL size classes of 4 cm. The 4-cm size classes were chosen over smaller size classes, as a few elevated GSI data points within the latter may elevate the mean value of a range, thereby causing a bias in size at first sexual maturity.

Oocyte diameter (OD) was measured concurrently with the GSI-size-class plotting method to determine the mean OD for each size class. These data were taken with the size of first maturity in the same manner as for mean GSI values previously presented (Finucane et al. 1986), and the largest oocyte in the sample was measured.

Fecundity was determined by evaluating tissue samples from 28 mature ovaries, excluding the stroma. Tissue samples of ovaries which had been previously weighed were soaked for at least 1 month in Gilson's fluid which caused maceration of the conjunctive tissue and disaggregation of the oocytes (West 1990). The tissue sample was then placed on a set of three nested screens, to sort oocytes by diameter. The top sieve had a mesh opening >0.90 mm, the second 0.25 mm, and the third <0.15 mm. The oocytes were separated using a weak stream of water. The total number of oocytes present in the tissue sample was counted by a subsampling procedure following the method of Fischer & Balbontín (1970). Representative samples of 300-500 oocytes in subsamples were measured using an ocular micrometer accurate to 0.03 mm. The number of oocytes in the tissue samples was calculated, and extrapolated to numbers in the entire gonad assuming that the number of oocytes in the tissue sample were representative of the entire gonad according to the following expression:

$$F = \left(\frac{1}{n}\right) \sum_{i=1}^{n} \left(\frac{Hi \times f}{WO \ i}\right) \times WG$$

where F, batch fecundity, is equal to the number of oocytes of the most advanced modal size presented in the subsample, f, multiplied by the corresponding fraction to the subsample, Hi, and considering the weight of the ovary in Gilson, WG, and the weight of the ovary fixed in formalin, WO *i*. The average of two samples was used in the determinations.

To determine batch fecundity, it was necessary to determine the mode of the most advanced or largest diameter oocytes (Hunter & Macewicz 1985). For this method, we used the size distribution of oocytes from each subsample, and applied the statistical software ELEFAN based on linear progression analysis (Gayanilo et al. 1988).



RESULTS

A total of 2286 ovaries were examined and weighed during the period from September 1999 to October 2000 for SSM analysis. The weights of the ovaries ranged from 2.13 to 852.18 g and the size of female golden ling ranged from 51 to 135 cm TL (Table 2).

Reproductive cycle

Stages of sexual maturity (SSM)

SSM values for the 1999–2000 period (Fig. 3), showed a peak presence of individuals in stages 2 and 3 (collectively considered mature) during September 1999 and August 2000. In September 1999, 40.5% of females were mature, with this percentage decreasing during the following months until December. In January 2000, less than 20% of mature individuals were still present, with stage 3 SSM individuals disappearing in February and reappearing at a low percentage in March and April 2000. Following these months, there were no SSM 3 individuals present, and mature individuals were scarce, reaching only 1.5% of the total number of females in June 2000. With initiation of a new reproductive cycle in August 2000, SSM 3 individuals reappeared, with mature individuals comprising 32.6% of the total population sample. Mature individuals were also observed in September 2000 at a similar level to August 2000 but declined considerably in October (Fig. 3).

Gonadosomatic index (GSI)

The temporal progression in GSI values over the study period showed that *G. blacodes* had a prolonged spawning season ranging from August to

November during the austral spring. Mean GSI maxima, representing spawning peaks, occurred in September 1999 and August 2000 (Fig.4). The highest GSI values generally occurred between August and November, representing the main spawning period.

Individual GSI values showed high variability within the main spawning period. High GSI values representing mature individuals occurred throughout almost all of the study period except from May through July 1999 when GSI values were no greater than two. Minimum GSI values found throughout the

Table 2 Total number of female specimens and ovaries (*n*), range of total length (TL) of fish and range of total weight (TW) of ovaries by month for *Genypterus blacodes*.

Perio	d	n	Range of TL (cm)	Range of ovaries TW (g)
Sep	1999	37	55-120	2.13-680.00
Oct	1999	160	55-113	3.08-510.40
Nov	1999	78	56-105	2.65-415.24
Dec	1999	53	58-111	3.80-369.40
Jan	2000	191	59-124	3.32-238.02
Feb	2000	374	53-127	2.81-160.59
Mar	2000	514	56-135	2.22-306.01
Apr	2000	224	53-127	2.13-238.93
May	2000	115	51-120	2.68-124.35
Jun	2000	67	67-115	3.89-112.31
Jul	2000	50	66-119	3.29-95.90
Aug	2000	46	55-119	4.22-768.48
Sep	2000	183	59-122	2.19-852.18
Oct	2000	194	66-129	2.37-381.23
Total		2286		



Fig. 4 Mean gonadosomatic index (GSI) distribution by month, including minimum and maximum GSI from September 1999 to October 2000.

Fig. 5 Mean oocyte diameter in female ling *Genypterus blacodes* by month (error bars = confidence interval 95%) from September 1999 to October 2000.

study period represented immature individuals (Fig. 4).

Oocyte diameter

Mean monthly oocyte diameter showed a similar temporal pattern to that of mean GSI (Fig. 5). Oocyte diameters increased during the months of maximum reproductive activity resulting from processes of vitellogenesis and hydration, thereby increasing gonadal weights and GSI values.

It was notable that no spent individuals were found during the study period. Immature individuals were found in all months of the study period, particularly during months of low reproductive activity.

Reproductive stage

The relationship between the microscopically determined SSM and mean GSI values for the months of maximum reproductive activity over the study period indicated that ovaries in the process of most active reproduction (SSM 3) had a GSI of over 2.29. The SSM 2 began with GSI values above unity (Table 3).

Size at first sexual maturity

Months of the spawning season considered in the calculation of size at first sexual maturity included September–October in 1999 and August–September in 2000. Thus the central axis may be considered to be September. In general, the GSI increase in individuals reaching first maturity ranged from values <1 to values >1 which were taken into account when interpreting results of the GSI–4 cm size plotting method (Fig.6).

The size at first sexual maturity estimated by plotting the mean GSI by 4-cm size range method provided values within 82.0 and 85.9 cm TL for both years (mean values of 84.0 cm). Size at first sexual maturity estimated in 1999 and 2000 using the former plotting method was confirmed by the oocyte diameter measurements (Fig. 7). In both years the

Fig. 6 Mean gonadosomatic index (error bars = confidence interval 95%) by 4-cm size range of *Genypterus blacodes* for the spawning period during 1999 and 2000.



Table 3 Mean values for gonadosomatic index (GSI, ± 1 SD), mean ovary weight, mean total length (TL), and oocyte diameter by stages of sexual maturity for months of maximum reproductive activity based on grouped data from 1999 and 2000 for female *Genypterus blacodes*. (SSM, stage of sexual maturity; PNS, perinucleus stage; YVS, yolk-vesicle stage.)

SSM	GSI mean	Ovary weight (g)	TL (cm)	Oocyte diam. (mm)
1 PNS	0.44 (0.30)	3.61	60.4	0.15
1 YVS	0.77 (0.16)	15.44	73.3	0.19
2	2.29 (0.51)	67.90	93.3	0.49
3	5.64 (2.96)	358.52	102.8	0.64

maximum increase in oocyte diameter was observed in individuals measuring 82.0–85.9 cm TL. In comparison, estimation of the size of 50% sexual maturity for female *G. blacodes* using the logistic function over the study period produced a value of 86.6 cm TL in 1999 and of 84.8 cm TL in 2000 (Fig. 8)

Fecundity

Microscopic analysis and the frequency distribution of oocyte diameter in mature ovaries, and in those of spawning females, suggested that *G. blacodes* was a partial spawner. The estimation of batch fecundity of ripe or ripening oocytes in this species was carried out on the basis of 28 individuals in August and September 2000 which showed ovaries in a mature and hydrated state (43% and 57%, respectively), which permitted an adequate separation of the group or mode of oocytes to be spawned. The modal fecundity was calculated only for 2000, as the number of mature ovaries observed in 1999 was not sufficient to carry out this analysis.

The batch fecundity estimated for *G. blacodes* varied between 66 167 and 706 658 oocytes for



Fig. 7 Mean oocyte diameter (cm) (error bars = confidence interval 95%) by 4-cm size range of *Genypterus blacodes* for the spawning period during 1999 and 2000.



Fig. 8 Proportion of mature females in relation to size, including size at 50% maturity calculated for *Genypterus blacodes* using the logistic function for 1999 and 2000 data.

specimens weighing between 300 and 10 000 g and 85 and 119 cm TL, respectively. The mean value for all individuals was 333 330 oocytes (\pm 241 642 SD). Within the 28 samples was one unusual result of a 120 cm TL female with a total weight of 12 200 g exhibiting a partial fecundity datum of 1.23 × 10⁶ oocytes, which fell outside (above) the general tendency observed.

The relationship between total fecundity and TL showed a weak correlation; the best fit was a linear relation with a R^2 value of 0.334 (Fig. 9). The parameters of this relationship were as follows:

 $Fp = 16\ 003 \times TL - 1\ 321\ 311$

The relationship between modal fecundity and total weight (TW) in females showed a better fit to a linear relation, with a R^2 value of 0.449 of low significance level (Fig. 10). In this instance, the parameters were as follows:

 $Fp = 73.94 \times TW - 139356$



DISCUSSION

The reproductive cycle of golden ling in the southern fisheries zone of Chile showed a prolonged spawning period during the latter months of winter and to the beginning of summer. Variations in GSI and in oocyte diameters related to the initiation of the reproductive period, and to the stages of sexual maturity over the same period observed by IFOP and other authors (e.g., Young et al. 1984; Chong 1993), both in Chile and further north (41°28′S–47°00′S) (Aguayo et al. 1992, 1994).

New Zealand ling spawn in August through October and Australian ling spawn from May through October (Kailola et al. 2001; see http:// www.fishbase.org). According to Chatterton in Horn (1993), *G. blacodes* spawns at least from August until December. In *Genypterus capensis*, a species from the South African coast closely related to *G. blacodes* (Japp 1990), mature females are seen only in August, September, and October (Payne 1977). Collections of eggs and larvae of this species suggested, however, that the spawning season extended from March to November (Brownell 1979).

The only previous estimation of size at first sexual maturity for G. blacodes in Chile is that of Chong (1993) who estimated using the logistic and linear regression GSI progression methods, sizes at sexual maturity of 90.2 and 90.7 cm, respectively. These values are considerably larger than the ones presented in our study. Our study showed that between 1999 and 2000, the results for size at sexual maturity were similar for the logistic method and for GSI plotting, showing a decrease in size at first maturity. This phenomenon, characteristic of overfished stocks, has been registered in several fish stock worldwide (Beacham 1983; Jorgensen 1990; Bowering & Brodie 1991; Trippel et al. 1997) and maybe caused by a decrease in the abundance of larger, mature specimens.

When comparing the values for size at first sexual maturity between the logistic and GSI-size plotting methods for 1999 and 2000, values are slightly higher using the logistic method (probably because the logistic method estimates when 50% of the individuals are mature), and lower for the GSI-size plotting method which registers the first indications of the maturation process. The logistic method is more accurate, and is a good tool when reliable data are available for SSM. Conversely, although the GSI-size plotting method may be less accurate, it is useful when gonad weights are available, which are more easily obtained data. This method is recommended for determining size of first sexual maturity in other, unstudied species.

The estimation of fecundity was limited by the small numbers of sexually mature ovaries available for the analysis. The only previous determination of fecundity in *G. blacodes* in Chile was that of Chong (1993) who obtained results similar to those of the present study. The relationships between modal fecundity and TL, and between modal fecundity and TW that fit the linear regression model were similar to the estimations of Chong (1993).

Even though the suitability of the Gilson's fluid for fecundity estimations based on hydrated oocytes has been questioned (West 1990), some studies have obtained similar results with Gilson's fluid and other methods such as gravimetric methods, with oocytes fixed in formalin. For example, for *Merluccius gavi* gavi, on the Chilean coast, the mean fecundity estimated by Alarcon & Arancibia (1993) was 143 397 oocytes (using the gravimetric method with oocytes fixed in formalin), whereas the estimation of Cerna & Oyarzún (1998) was 142 030 oocytes (using Gilson's fluid). On the other hand, Balbontín & Bravo (2001) obtained mean values of 148 040 oocytes for the northern area and 139 000 for the southern area distribution of the same species, using Gilson's fluid. With G. blacodes, 57% of the sample was hydrated, with the remaining 43% in advanced vitellogenic stage, but is difficult to estimate the effect of the Gilson's fluid over the total number of hydrated oocytes.

Since *G. blacodes* species has relatively low levels of fecundity and matures at a larger size than, for example, the two Chilean hake species with fecundities of 500 000 oocytes approximately, *G. blacodes* may be more susceptible to overexploitation than the hake.

Finally, considering the differences in the presence of mature specimens between months in 1999 and 2000, we conclude that the spawning season in 1999 was longer than that of 2000, suggesting variability in the length of the spawning season of this species.

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