Reproductive biology of female cardinalfish, *Epigonus crassicaudus* de Buen, 1959

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Summary

The cardinalfish (*Epigonus crassicaudus*) is a long-lived and endemic deep-water fish inhabiting the central and southern coast off Chile. Knowledge about basic biological attributes including maturity aspects is fragmentary in this species. The historical and comprehensive data available are applied to provide a detailed study of the reproductive biology of the female cardinalfish. The gonadosomatic index was computed from 5110 female gonads collected by onboard scientific observers between October 2000 and December 2012. A total of 1467 females gonads collected between March 2007 and December 2009 were subjected to histological analysis of maturation upon which a maturity ogive was estimated. The ovarian development of this specie is asynchronous, characterized by a continuous reproductive cycle in which reproductive activity is found throughout the year with a maximum during the austral autumn and summer (between May and June). Length at 50% maturity was estimated in 23.2 cm of fork length (95% CI: 21.7–23.9 cm). The results presented here are compared with previous sparse estimates available for this species.

Introduction

Cardinalfish *Epigonus crassicaudus* (de Buen, 1959) is a fish characterized by its mesobenthic/pelagic habits. This is an endemic species along the Chilean coast distributed mainly between 29°00′S and San Pedro bay (42°50′S) in depths of between 200 and 400 m (Wiff et al., 2008). Fishing operations targeting cardinalfish ceased after 2010; the most recent stock status labeled this species as depleted (Gálvez et al., 2010; Tascheri et al., 2012). Late introduction of annual quotas in 2003, high uncertainty in stock assessments, as well as caveats regarding the knowledge of demography and population dynamics including that of maturity, have been proposed as the main causes underlying the collapse of this fishery in Chile (Tascheri et al., 2012).

Currently, only basic knowledge for cardinalfish on growth (Gálvez et al., 2000; Cubillos et al., 2009; Ojeda et al., 2010; Contreras-Reyes and Arellano-Valle, 2013), natural mortality (Cubillos et al., 2009; Tascheri et al., 2012) and maturity (Gálvez et al., 2000; Cubillos et al., 2009) is available. Gálvez et al. (2000) and Cubillos et al. (2009) reported on maturity ogives in this species; both reproductive studies showed significant differences between estimates of length at 50% of maturity. These differences can be explained by differences in spatio-temporal coverage of the fish sampled and the maturity scale used in the two studies. These drawbacks in the maturity ogives available for the cardinalfish preclude any firm conclusion concerning reproduction in this species, thus a comprehensive analysis of its reproductive biology is desirable. The main aim of this work therefore was to provide a detailed study of the reproductive biology of cardinalfish using the extensive data available from the fishery-monitoring program collected by the Instituto de Fomento Pesquero (IFOP-Chile).

Materials and methods

A total of 5110 female cardinalfish were collected monthly between October 2000 and December 2012 off central Chile, covering an area between 29°00′S and 42°00′S. Data regarding fork length (FL, cm), total weight (TW, g), gutted weight (GW, g), and ovary weight (OW, g) were collected randomly from each fishing haul by scientific observers onboard. Ovaries were assigned to a macroscopic maturity stage according to the scale defined for cardinalfish: Virginal (Stage 1), Immature (Stage 2), Maturation (Stage 3), Maturation with Recent Spawning (Stage 4), Spawning (Stage 5), and Spent (Stage 6).

A total of 1467 female gonads were collected between March 2007 and December 2009. Samples were fixed in 4% buffered formaldehyde and then processed for histological analysis. Slices of 3 mm gonad tissue were embedded in paraffin; 5 μm thick sections were stained with Harris's haematoxylin and eosin. Gonadal development stage was assessed according to the histological maturity scale for cardinalfish (Table 1). In addition, oocyte sizes were recorded by disaggregating, using water pressure on a sieve (250 μm), and then digitalized using a scanner on a grey scale with a resolution of 800 dpi. Oocytes were then automatically counted and measured using IMAGEJ v.1.34s (http://rsb.info.nih.gov/ij/).

The gonadosomatic index (GSI) was computed as the ratio between ovary weight (OW) and gutted weight (GW) as:

Table 1
Maturity stages of *E. crassicaudus* female gonads according to the histological scale in Gálvez et al. (2010)

Stage	Terminology	Histological criteria
1	Virgin	Presence only of previtelogenic oocytes of chromatin nuclear type. Oocyte sizes up to 228 μm
2	Immature	Presence of three types of oocytes: chromatin-nucleolus, perinucleolar and cortical alveoli. Oocyte sizes up to 300 μm
3	Early maturing	Presence of advanced cortical alvioli oocytes, beginning the incorporation of eosinophilic yolk granules partially occupying the cytoplasm. Oocyte sizes up to 500 μm
4	Late maturing	Oocytes with large yolk globules and germinal vesicle (GV) in the cytoplasm surrounded by several coalescent oil droplets. Mature oocytes up to 600 μm
5	Mature	Oocytes completely yolked and migration of GV toward the animal pole. Oocyte sizes up to 700 μm
6	Ripe or hydrated	Mature oocytes with cytoplasm completely fused and homogeneity of yolk and beginning of hydration. Most advanced oocytes above 750 μm
7	Spawning	During ovulation, postovulatory follicles and hydrated oocytes observed
8	Partial post-spawning	Presence of yolked oocytes and postovulatory follicles (PFO) in different reabsorption phases
9	Spent	PFO and yolked oocytes (non-ovulated) undergoing atresia. Parenquime shown with previtelogenic oocytes

$$GSI = \frac{OW}{GW} \times 100$$

To validate the independence between GSI and individual size, we used a log-log scale to linearize the power function between OW and GW (model selected by residual analysis) and then evaluated isometry by testing the slope of this relationship being significantly different than 1 using a *T*-test (Somarakis et al., 2004). We also evaluated homogeneity by testing how similar these slopes were among different maturity stages by using covariance analyses (DeVlaming et al., 1982; Erickson et al., 1985).

The reproductive cycle, GSI, and the proportion of active mature females (PAM) were modelled using Generalized Additive Models (GAM) following Wood (2006). Females achieving stages 3–5 in the macroscopic scale were classified as active mature. The GAMs used a two-dimensional tensor product by month and length strata as a covariate. Model selection for GSI and PAM was performed using generalized cross validation (GCV) and an unbiased risk estimator (UBRE), respectively (Wood, 2006).

Fish that had reached at least stage 3 in the histological scale were classified as mature. Only gonads collected between the beginning and the peak of the main reproductive season were considered for estimation of the proportion of mature female at length, P_L using a logistic function:
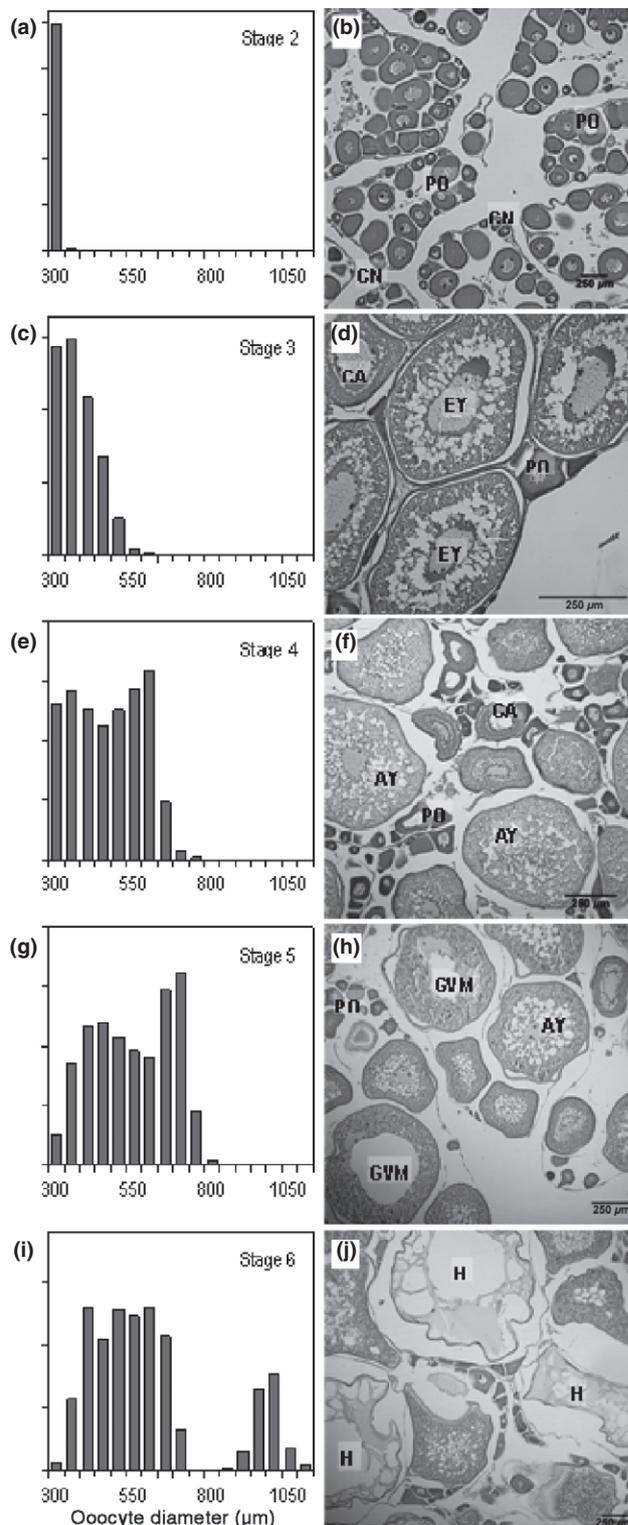

Fig. 1. Histological and frequency distributions of oocyte sizes of *E. crassicaudus* in different developmental stages. CN, chromatin-nucleolu; PO, perinucleolar oocyte; CA, cortical alveoli; EY, early yolked; AY, advanced yolked; GVM, germinal vesicle migration; H, hydrated. Scale bar = 250 μm

$$P_L = \frac{1}{1 + e^{(\beta_1 + \beta_2 L)}}$$

where β_1 and β_2 are the parameters estimated using maximum likelihood considering a binomial error distribution (Welch and Foucher, 1988). Uncertainty was incorporated into the model using a parametric bootstrap (Roa et al., 1999). Goodness-of-fit was assessed using the Hosmer-Lemeshow test (HL test), as suggested by Hosmer and Lemeshow (1989).

The length at 50% maturity (L_{50}) was computed as:

$$L_{50} = -\frac{\beta_1}{\beta_2}$$

All statistical analyses were conducted using R statistical software (www.r-project.org).

Results

Histological analysis in Fig. 1 shows the presence of oocytes in different phases of development in a wide range of sizes once the ovaries have reached maturity (stage $\geq$ 3). When the ovary reaches the initial phase of vitellogenesis (stage 3) the oocyte increases in size, displacing the mode of distribution as the yolk continues accumulating in the cytoplasm (see stage 4). Stage 5 was characterized by a mode of oocytes with sizes above 600 μm, although it did not show a clear separation from the rest of the less-developed oocytes. This only occurs during the hydration phase where oocytes reach sizes larger than 750 μm (Fig. 1). This species presented a continuous oocyte development characteristic of fishes with asynchronous ovarian reproductive strategy.

The linear log-log relationship between ovary weight (OW) and gutted weight GW was significant (P < 0.05) in all stages analyzed. The slope of these relationships did not differ significantly from 1 (P > 0.05), except for stage 6 (Table 2). This indicates isometry between OW and GW in most maturity stages. In addition, the slopes across maturity stages were not significantly different ($F = 0.906$, P > 0.05), validat-

Table 2
Descriptive statistics of log-log relationship between ovary weight (OW) and gutted weight (GW) in *E. crassicaudus* samples grouped by maturity stages

Stage	α (SE)	β (SE)	r^2	|t|	n	GW mean (SD)	OW mean (SD)
1	−1.84 (0.17)	0.94 (0.07)	0.69	0.9	82	262.0 (178.6)	2.6 (1.9)
2	−1.76 (0.14)	0.93 (0.05)	0.45	1.3	365	495.6 (114.9)	5.8 (2.3)
3	−1.66 (0.42)	0.93 (0.16)	0.33	0.4	74	524.8 (92.1)	7.9 (2.9)
4	−1.25 (0.35)	0.89 (0.13)	0.30	0.9	110	479.3 (111.5)	14.4 (6.2)
5	−1.25 (0.14)	0.92 (0.05)	0.38	1.6	491	478.2 (134.7)	17.3 (7.6)
6	−0.30 (0.47)	0.61 (0.17)	0.13	2.2*	82	500.2 (124.2)	24.2 (11.0)
8	−1.12 (0.22)	0.87 (0.08)	0.39	1.6	187	489.2 (125.9)	17.9 (7.1)
9	−2.25 (0.30)	1.12 (0.11)	0.62	1.0	61	462.5 (130.3)	5.6 (2.2)

α, intercept; β, slope; SE, standard error; SD, standard deviation; r^2, coefficient of determination; n, numbers of individuals; |t|, value T-test. *P < 0.05.
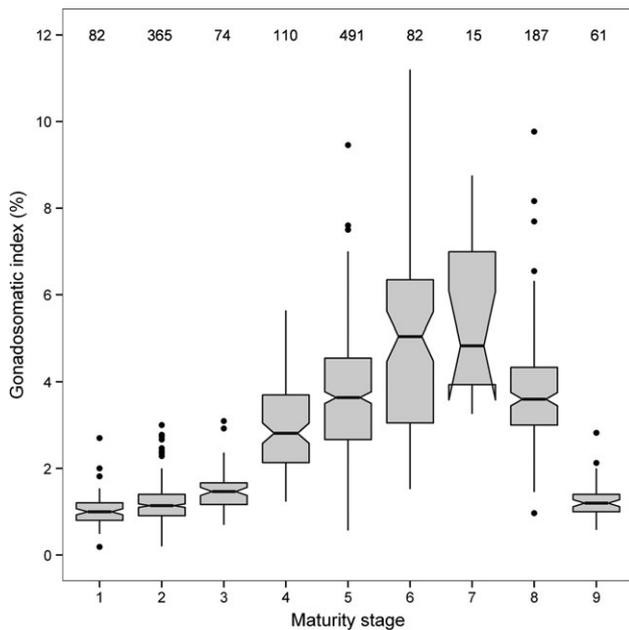

Fig. 2. Boxplot of gonadosomatic index (GSI) in *E. crassicaudus* maturity stages. Numbers at top = sample sizes per stage
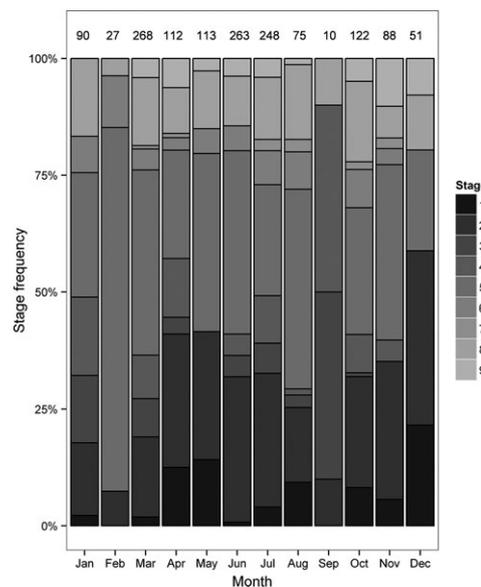

Fig. 3. Monthly variation in *E. crassicaudus* histological maturity stages. Numbers at top = sample sizes per month
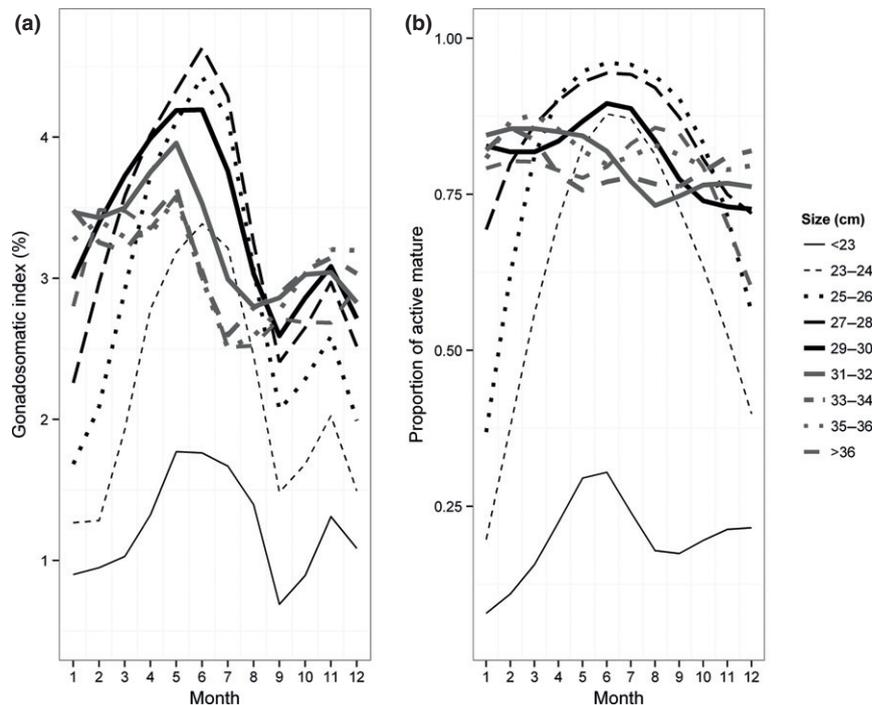
Fig. 4. Generalised additive model of (a) gonadosomatic index (GSI), and (b) proportion of active maturity (PAM) across months and fork lengths (FL) in *E. crassicaudus*

ing the homogeneity assumption. The intercepts were different among stages ($F = 416.125$, $P < 0.0001$), except between stages 2 and 9 ($F = 0.194$, $P > 0.05$) and stages 5 and 8 ($F = 0.693$, $P > 0.05$).

GSI values showed significant differences among maturity stages (Kruskal–Wallis, $P < 0.05$) (Fig. 2). In addition, we observed a high frequency of females in advanced reproductive activity (stages 4–6) throughout the year (Fig. 3). GAM models for GSI and PAM were significant ($P < 0.05$), although both models explained only 13 and 10% of the total deviance, respectively. Estimated GSI and PAM showed a seasonal pattern across size strata. Individuals larger than 28 cm FL showed the highest GSI values between May and June, whereas the maximum in smaller individuals was reached in June (Fig. 4a). Similarly, females larger than 28 cm FL showed the highest continuous PAM throughout the year, with a maximum during the southern autumn and winter (Fig. 4b). According to these analyses, we determined that cardinalfish have a continuous reproductive cycle with no sign of gonadal resting. Most of the reproductive activity is concentrated between the southern summer and winter. Roughly, four phases can be distinguished: (i) an increase beginning in January, (ii) maxima between May and June, (iii) decline between July and August, and (iv) low reproductive activity between September and December.

Parameters estimated for the maturity ogives were significant ($P < 0.05$) for the period analyzed (Fig. 5) and corresponded to $\beta_1 = 17.069$ (SE = 2.351) and $\beta_2 = 0.737$ (SE = 0.095). L_{50} was estimated in 23.2 cm FL (95% CI: 21.7–23.9 cm). In addition, the HL test indicated an adequate goodness-of-fit ($P = 0.47$).
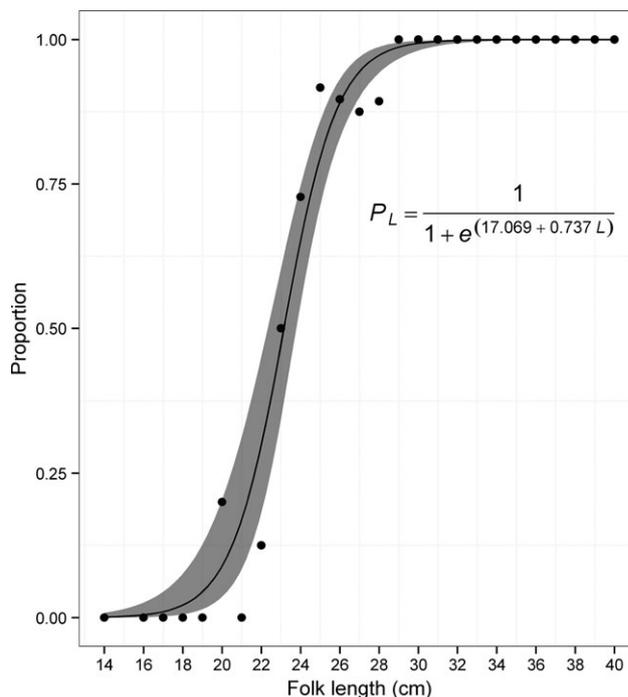

$$P_L = \frac{1}{1 + e^{(17.069 + 0.737\,L)}}$$

Fig. 5. Proportion of maturity-at-length in histological stages of *E. crassicaudus*. Black dots: observations; line = fitted logistic model; shaded area = the 95% confidence interval

Discussion

Deep-water fishes have been characterized as having a continuous or seasonal reproductive strategy as shown in species

of macruridae (D'Onghia et al., 1999), gadidae (Rotllant et al., 2002) and bericiformes (Minto and Nolan, 2006). However, knowledge of basic biological processes including maturity in deep-water species is still limited. In our study, we found an asynchronous ovary development in *Epigonus crassicaudus* characterized by the presence of oocytes in different stages of development at the same time and with reproductive activity throughout the year, with a maximum reached between May and June. This weak seasonal pattern was found using GAM for GSI and PAM, although a low explained deviance was reported in both models. However, we showed here that GSI is an adequate indicator for the reproductive condition because this metric is independent of the female length (Table 2), showing also a similar pattern with the PAM (Fig. 4). In other species belonging to the genus *Epigonus*, such as *E. telescopus* in New Zealand, spawning occurs during southern autumn and winter (Field et al., 1997), whereas for the same species in the north Atlantic spawning takes place during northern spring and summer (Pshenichny et al., 1986). These intra and inter-specific differences in the reproductive cycle revealed that both the intensity of the period and the duration of the spawning is related to the latitudinal and bathymetrical distribution, as reported in other deep-water species (Rotllant et al., 2002; González et al., 2003).

The presence of cortical alveoli and the beginning of vitellogenesis are the two criteria used to classify mature fish using the ovary development. In fish species with seasonal maturity, the presence of oocytes with cortical alveoli is an indication that maturity has been reached (Brown-Peterson et al., 2011). On the other hand, in fish species with low oocyte development and continuous reproduction throughout the year, vitellogenesis is a more appropriate indicator to classify maturity, as has been shown in other deep-water fish species (Lowerre-Barbieri et al., 2011). Therefore we used the vitellogenesis as an indicator for maturity in cardinalfish because seasonality in reproduction for this species is weak, with no clear signal of gonadal resting. In addition, we found a low frequency of spent females throughout the year, indicating that ovary development of this species is likely to be low. This agrees with other life history characteristics typical of deep-water species as described also in cardinalfish, such as long life span, low growth rate and high vulnerability to fishing exploitation (Gálvez et al., 2010; Ojeda et al., 2010; Tascheri et al., 2012).

The maturity ogives estimated here indicates that L_{50} calculated as 23.2 cm FL (95% CI: 21.7–23.9 cm) is significantly smaller than the L_{50}–value of 26.0 cm FL (95% CI: 25.3–26.7 cm) reported by Gálvez et al. (2000) and the 32.1 cm FL (95% CI: 22.2–45.7 cm) reported by Cubillos et al. (2009), whose estimates were from fragmentary data including a narrow sampling season in a particular area of cardinalfish distribution. The maturity ogives estimated in this study include historical data available from monitored fishing of *E. crassicaudus* and thus are the most comprehensive and probably the most accurate and unbiased estimates available. In addition, this study is the first to define a reproductive season for this species, information crucial to compute an unbiased estimate of maturity ogives.

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