*Journal of the Marine Biological Association of the United Kingdom*, page 1 of 13. 
doi:10.1017/S0025315416001193

# Reproductive biology of the commercial sea cucumber *Athyonidium chilensis* (Holothuroidea: Dendrochirotida) in southern Chile

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*Reproductive aspects of the sea cucumber* Athyonidium chilensis *were studied over a year in Valdivia, Chile, through gonad index (GI) analysis, macro- and microscopic analysis of the gonads, fecundity and size at first sexual maturity estimations. We also explored the reliability of live size estimators for their use in fisheries.* Athyonidium chilensis *showed continuous gametogenesis and spawning individuals could be found throughout the year. However, spring was the main reproductive time, where an important GI decrease coincided with enhanced spawning activity evidenced through histology. GIs recovered in summer, and new signs of enhanced spawning activity were observed towards autumn (April 2008). GI peaks were observed in August 2007 and March 2008 for females (22.8 and 24.4% respectively) and September 2007 and March 2008 for males (31.9 and 25.9% respectively). Low mean GIs occurred in May and December 2007 for females (15.2 and 11.6% respectively) and May and October 2007 for males (12.7 and 14.1% respectively). Males reached sexual maturity at a smaller size than females (males: 21.2 g, females: 43.7 g eviscerated weight), and mature females showed a high mean absolute fecundity for a species with lecithotrophic larval development ($6.31 \times 10^5 \pm 1.97 \times 10^5$ SD). For fisheries, we recommend a minimum catch size over 237.89 g drained weight to ensure that caught individuals are sexually mature. This study provides relevant information for the conservation and fishery management of* A. chilensis. *Continuous gametogenesis and high fecundity make this species particularly suitable for aquaculture in southern Chile.*

**Keywords:** sea cucumber, Chile, *Athyonidium chilensis*, bêche-de-mer, fisheries, aquaculture, reproductive cycle, fecundity, holothuroid

Submitted 16 June 2015; accepted 2 August 2016

## INTRODUCTION

For centuries, sea cucumbers have been consumed as a luxury food item and also used as medicine in East Asian countries (Conand & Byrne, 1993; Akamine, 2004; Purcell, 2010; Purcell *et al.*, 2013). The constant increase of the market demand led to the overexploitation of traditional fishing grounds and the global expansion of this fishery, which progressively includes new and less valuable species from both tropical and temperate environments (Guzmán *et al.*, 2003; Conand, 2004; Toral-Granda *et al.*, 2008; Anderson *et al.*, 2011; Eriksson & Byrne, 2013). Population declines have generated growing interest in understanding sea cucumber reproductive biology. This knowledge would allow the formulation of effective fisheries management strategies, taking steps towards conservation and to generate aquaculture initiatives and improvements (Purcell, 2010, 2014; Zamora *et al.*, 2016), supporting a global industry estimated at US$130 million in 2001 (Vannuccini, 2004).

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In Chile, two sea cucumber species – *Pattalus mollis* Selenka, 1868 and *Athyonidium chilensis* (Semper, 1868) (Larraín, 1995) – were introduced to the fishery in the early 1990s without management regulation, despite the recommendation by the Food and Agriculture Organization of the United Nations (FAO) of applying a precautionary approach to fisheries of species that lack basic biological information (Toral-Granda *et al.*, 2008). Of these two species *A. chilensis* is the most heavily exploited, particularly in southern Chile. Records indicate that peak catches occurred in 2000 (~1500 t), falling drastically in subsequent years (Sernapesca, 2013). To ensure the supply of this increasingly sought-after product, *A. chilensis* has been proposed by fishers as a target species for the development of a sustainable fishery inside Management and Exploitation Areas for Benthic Resources (MEABRs) (Stotz, 2007), a co-management approach similar to the internationally known territorial user rights for fisheries (TURFs) (Fernández & Castilla, 2005; Gelcich *et al.*, 2008, 2010).

*Athyonidium chilensis* ranges over 3800 km from Perú to southern Chile (18°S–42°S) (Pawson, 1964, 1969). It inhabits exposed rocky intertidal and shallow subtidal areas, especially where the algae *Macrocystis* spp. are present (Pawson, 1964, 1969). *Athyonidium chilensis* is gonochoric and the larvae

have lecithotrophic development (Guisado *et al.*, 2012). Despite the species broad distribution there are few unpublished reproductive studies (Caffi, 1981; Moreno, 2002), an obstacle towards developing good management practices. There is also a special need to extend the *A. chilensis* study distribution, given sea cucumbers can exhibit different reproductive patterns within their latitudinal range (Sewell, 1990; Chao *et al.*, 1995; Ramofafia *et al.*, 2003; Shiell & Uthicke, 2006).

Here we provide information on the reproductive biology of *A. chilensis* in its southern distribution to support initiatives for fisheries management and conservation. We investigated (1) body gravimetric relationships; (2) breeding pattern through gonad index and gonadal histological analysis for both sexes; (3) macroscopic appearance of *A. chilensis* gonad; (4) size at first sexual maturity; and (5) fecundity.

## MATERIALS AND METHODS

### Sample collection and processing

Individuals of *Athyonidium chilensis* were collected on a monthly basis from the rocky intertidal zone of 'La Misión' beach, Valdivia, Chile (39°47′43.73″S 73°23′57.35″W), from May 2007 to April 2008. As *A. chilensis* does not display external sexual dimorphism, an attempt was made to collect 20 large individuals (~10–25 cm total length) monthly to ensure that adults from both sexes were represented in the sample. At the time of the study, size at first maturity was unknown. To estimate this parameter we also collected the smallest individuals encountered in our monthly samplings. Once size at first maturity was estimated, only individuals above that size which showed mature gametes in histological sections were considered for maturity histograms and gonad index (GI) calculations. Sampled specimens were placed in individual plastic bags and transported in a cooler box to laboratories at Universidad Austral de Chile. Each individual was relaxed for 30 min by injecting 15 ml of 10% $MgCl_2$ into the coelomic cavity (Hewatt, 1943). Relaxed total length (Tl) was obtained measuring from the base of the oral tentacles to the anus (±1 mm). A longitudinal incision was made along the dorsal surface of the body, the coelomic fluid was drawn off, and drained body weight (sensu Conand, 1981) (Dw) was determined. Tl and Dw were obtained from half of the sampled individuals during the study period. For all collected individuals, the gonad was removed, drained and weighed (Gw) (±0.05 g). The animals were sexed; female gonads are olive green and male gonads are creamish to orange (Caffi, 1981). Gonads were preserved in buffered 6% formalin for 2 months, rinsed in tap water and transferred to 60% ethanol for further storage until micro and macroscopic analysis. We also recorded the eviscerated body weight (Ew) (±0.05 g), which corresponded to the body wall weight, excluding internal organs.

#### PHYSICAL CHARACTERISTICS AND BODY GRAVIMETRIC RELATIONSHIPS

Ew was used as a reference measure to determine whether Dw or Tl could be accurate size estimators for live individuals of *A. chilensis*. For this we explored possible sex effects on regression slopes and intercepts through an analysis of covariance (ANCOVA) and performed Model II Reduced Major Axis (RMA) linear regression analysis. Homogeneity of variances and normality assumptions were checked using Levene and Shapiro–Wilk tests.

#### GONAD INDEX

Mean monthly GI was calculated for females and males through the year as GI = (Gw/Ew) × 100. Before conducting ANOVA to assess the effect of months and sex over the variation of GI values we checked model assumptions as described above. After arcsine transformation, homogeneity of variance between months in GI was not achieved. However, given the robust nature of ANOVA, we used untransformed data to explore single and combined effects of sex and months over GI (two-way ANOVA) followed by single factor Welch-ANOVA and Games-Howell *post-hoc* test to establish pairwise comparisons of means (Quinn & Keough, 2002).

#### GONAD MICROSCOPIC ANALYSIS

*Athyonidium chilensis* single gonad consists of two bilaterally symmetrical tufts of branched tubules arising from the gonad basis, divided by the dorsal mesentery and attached to the anterior body wall (Caffi, 1981). Holothurian gonad morphology and maturation can differ between species and even populations (Sewell, 1992; Hamel & Mercier, 1996a; Sewell *et al.*, 1997). In general, gonad tubules can be all of similar size and contain gametes at similar stages of maturity or they can be found as tubule cohorts of different size containing gametes at different stages of maturity, the latter known as the tubule recruitment model (TRM) (Smiley, 1988; Sewell, 1992; Hamel & Mercier, 1996a; Sewell *et al.*, 1997). In this study we did not directly assess the dynamics of gonad maturation in *A. chilensis*. However, we haphazardly removed 15 tubules from the central portion of each male and female gonad. The central portion of the gonad contains the biggest-sized gonad tubules, known in holothurians to be involved in the current-year spawning and to contain the most mature generation of gametes (Hamel & Mercier, 1996a; Shiell & Uthicke, 2006). In case the gonad was too small, the entire gonad was processed according to standard histological techniques, sectioned (6 μm thick) and stained with haematoxylin and eosin. Based on previous studies (Sewell, 1992; Ramofafia *et al.*, 2000, 2003), gametogenic stages were assigned to sexually mature individuals based on observations of the germinal epithelium, gonad component cells and their staining properties. Five gametogenic stages were defined: spent (stage I); recovery (stage II); growing (stage III); mature (stage IV); and partly spawned (stage V). The percentage of individuals at each maturity stage was determined for each monthly sample, and monthly spawning frequency was calculated, corresponding to the percentage of individuals with gonads in spawning stages (partly spawned V and spent I). Spawning periods were determined based on months where spawning frequency was over 25% (modified from Chao *et al.*, 1995).

#### GONAD MACROSCOPIC ANALYSIS

We investigated the effect of sex and maturity stages on variations on the length, diameter and bifurcation of gonad tubules. For this, 15 tubules were removed from the gonad as described above and tubule bifurcation, length and diameter were recorded using an electronic caliper (±0.1 mm). Diameter measurements were taken from the mid-length of the tubule. At stages IV (mature) and V (partially spent), male

gonad tubules acquired a 'beaded' appearance throughout their length. Diameter measurements were taken from the mid-section of the beaded portions. The effect of sex and maturity stages and their interaction over tubule bifurcation, length, diameter and gonad weight were assessed using two- and one-way ANOVA followed by Tukey–Kramer's unplanned multiple comparisons of harmonic means. Model assumptions were checked as previously indicated.

### SIZE AT FIRST MATURITY

To estimate size at first sexual maturity, defined as the eviscerated weight at which 50% of the individuals were gametogenically mature ($Ew_{(50)}$) (Conand, 1981, 1993b; Abdel-Razek *et al.*, 2005; Toral-Granda & Martínez, 2007), the Ew of males and females were plotted against their maturity condition as determined by histology. A binomial code was assigned to each individual (0 = juveniles; 1 = adults), where animals in maturity stages I, II, III, IV and V that had mature gametes on histological sections were considered adults. A logistic regression was fitted to the data and parameters a (intercept) and b (slope) were estimated through $\ln[y/(1-y)] = a + b(x)$. We tested for model significance using Likelihood-ratio tests (Quinn & Keough, 2002).

### FECUNDITY

Fecundity was estimated for 12 mature females (stage IV) using a gravimetric method. For each female, three subsamples of known weight (±0.0001 g) were taken from the middle length of the gonad tubules. Oocytes were squeezed out from the subsamples leaving the tubule lumen empty and vitellogenic oocytes were counted using a dissecting microscope. An average number of oocytes per gram of gonad ($\bar{x}$ oc) was calculated for each female. Absolute fecundity (FA) was calculated according to FA = $\bar{x}$ oc * Gw (g). Calculations on relative fecundities with respect to: drained body weight (as FRDw = FA/Dw), eviscerated body weight (as FREw = FA/Ew) and gonad weight (as FRGw = FA/Gw) were performed (Conand, 1993a; Abdel-Razek *et al.*, 2005; Toral-Granda & Martínez, 2007). Mean fecundities for the 12 measured females are reported ($\bar{x}$ FA, $\bar{x}$ FRDw, $\bar{x}$ FREw, $\bar{x}$ FRGw). For each female, the diameter of 10 vitellogenic oocytes was measured to the nearest micrometer using an eyepiece reticle calibrated to a dissecting microscope; the mean oocyte diameter of the 12 females was calculated.

All statistical analyses were performed using JMP® 10.0.2 statistical software (SAS Institute Inc.) except for the Games–Howell *post hoc* tests for which we used SPSS® 21 statistical software (IBM), and Model II Reduced Major Axis (RMA) linear regression analysis and ANCOVA which were performed in RStudio (RStudio Team, 2015).

## RESULTS

### The sampled population

During the entire year of study (May 2007 – April 2008), a total of 277 individuals of *A. chilensis* were sampled from 'La Misión', including juvenile and sexually mature individuals. Numbers of sexually mature individuals in the samples varied monthly, females ranging from 3 to 13 (mean: 9 ± 2.5 SD) and males ranging from 5 to 13 (mean: 8.7 ± 2.3 SD).

### PHYSICAL CHARACTERISTICS AND BODY GRAVIMETRIC RELATIONSHIPS

*Athyonidium chilensis* physical parameters are presented in Table 1. Males and females had similar mean total length (Tl) (ANOVA, $F_{(1,\ 101)} = 0.58$, $P = 0.449$), eviscerated weight (Ew) (ANOVA, $F_{(1,\ 210)} = 0.02$, $P = 0.894$), drained weight (Dw) (ANOVA, $F_{(1,\ 101)} = 0.84$, $P = 0.363$) and gonad weight (Gw) (ANOVA, $F_{(1,\ 210)} = 1.92$, $P = 0.168$). There were no statistically significant differences in the sex ratio of mature individuals during the study period (Pearson $\chi^2_{(1)} = 0.76$; $P = 0.78$; N = 212).

The slopes and intercepts of male and female regression lines for the relationship between Ew and Dw were not significantly different ($F_{(1,\ 99)} = 0.517$, $P = 0.474$ and $F_{(1,\ 100)} = 0.153$, $P = 0.697$, respectively). The same situation was observed for the relationship between Ew and TL ($F_{(1,\ 99)} = 0.508$, $P = 0.222$ and $F_{(1,\ 100)} = 0.036$, $P = 0.850$, respectively). After fitting a single regression line for each relationship, Dw was a much more reliable estimator of animal size than TL ($r^2 = 0.89$; $P < 0.001$ and $r^2 = 0.29$; $P < 0.001$, respectively) (Figure 1).

### GONAD INDEX (GI)

The annual mean GI was 18.3% (±11% SD) and 20.8% (±10.3% SD) for sexually mature females and males respectively. Throughout the year, monthly mean GI was above 11% for both sexes. However, there were high monthly variations on the GI between individuals, reaching values as low as 1.1% for a sexually mature female at the spent stage (stage I, Gw: 0.63 g, Ew: 57 g, October 2007) and 4.3% for a sexually mature male at the same stage (stage I, Gw: 2.5 g, Ew: 58.5 g, March 2008). GI reached values as high as 64.4% for a female at the mature stage (stage IV, Gw: 59.1 g, Ew: 92 g, September 2007) and 46.21% for a male at the same stage (stage IV, Gw: 44.98 g, Ew: 97.33 g, August 2007). The smallest gonad weights recorded for sexually mature individuals were 0.63 g for females (stage I, GI: 1.1%, Ew: 57 g, October 2007) and 2.5 g (stage I, GI: 4.3% Ew: 58.5 g, March 2007) for males, while the highest gonad weights recorded were 60.9 g (GI: 36.5%, Ew: 167 g, April 2008) for males and 67.1 g (GI: 56.3% Ew: 119 g, July 2007) for females.

**Table 1.** Somatic physical characteristics of mature individuals of *A. chilensis* (females N = 108, males N = 104).

| | Females | | | | Males | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **±SD** | **Min** | **Max** | **Mean** | **±SD** | **Min** | **Max** |
| Total length (cm) | 18.6 | 3.3 | 10 | 26 | 18 | 4.3 | 9 | 25 |
| Eviscerated weight (g) | 92.1 | 28.8 | 46.1 | 166 | 91.6 | 30.6 | 42 | 172.4 |
| Drained weight (g) | 159.5 | 49.7 | 87.2 | 278.2 | 150.4 | 51.6 | 74 | 300.3 |
| Gonad weight (g) | 18 | 12.93 | 0.63 | 67.1 | 20.6 | 14.5 | 2.5 | 60.9 |

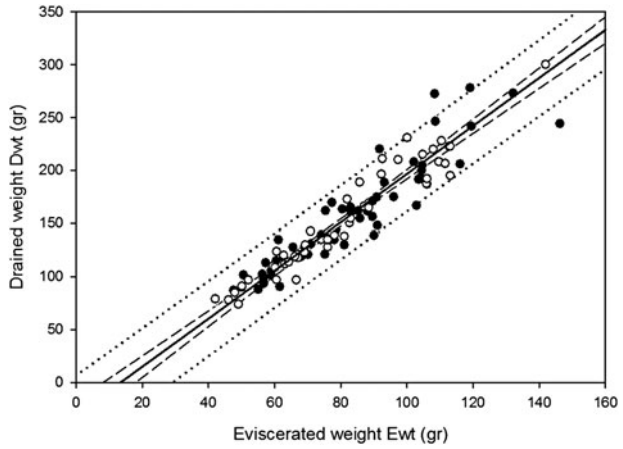


**Fig. 1.** Relationship between drained weight and eviscerated weight of *A. chilensis*. Solid line represents best fit Model II RMA regression $y = -30.576 + (2.273 \times x)$, $r^2 = 0.89$, $P < 0.001$ for males (empty circles) and females (filled circles) combined. Dashed lines represent 95% mean confidence interval and dotted lines represent 95% individual confidence interval.

The GI dynamic through the year was similar between males and females (sex×month, two-way ANOVA, $F_{(11, 188)} = 0.937$, $P = 0.506$) (Figure 2), and there was no statistically significant difference on the yearly mean GI between sexes (Welch's ANOVA, $F_{(1, 209.9)} = 2.72$, $P = 0.101$). However, mean GI varied significantly between months (Welch's ANOVA, $F_{(11, 77.7)} = 3.87$, $P = 0.002$), with the most important difference found between December 2007 and March–April 2008 (Figure 2). In general, GI was low at the end of autumn (May 2007) and increased during austral winter (Figure 2). High GIs occurred in August and September 2007 (females and males respectively), decreasing through spring to reach minimum values in October 2007 for males and December for females. The GI recovered again in summer showing high GI values in March 2008 for both sexes, followed by a slight decline in April 2008 (Figure 2). The variability on monthly GI was generally high; it was higher during months with high mean GIs and was lower during months with low mean GIs (Figure 2).

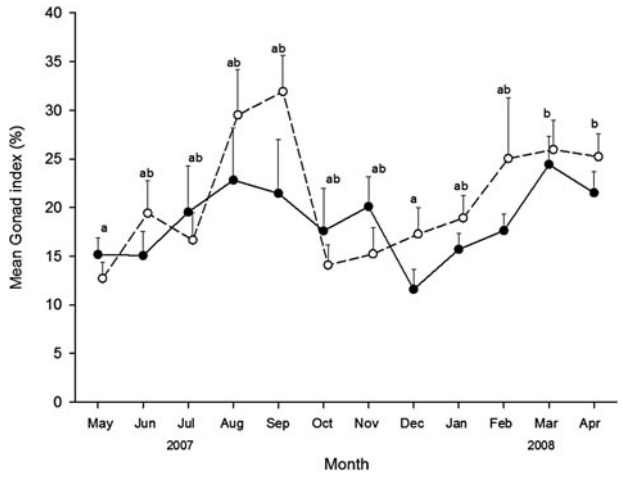


**Fig. 2.** *Athyonidium chilensis* gonad index (GI) through the year. Males (empty circles) and females (filled circles). Letters above bars indicate *post-hoc* test results for the effect of month in two-way ANOVA (month, sex). Points that do not share the same letter indicate significant differences. Bars indicate standard error.

## GONAD MICROSCOPIC ANALYSIS

For males and females at stage I (spent) tubule diameter was the smallest and gonad wall thickness started increasing compared with stage V (partly spawned) (Figures 3A & 4A). Gonad tubules had a fairly empty lumen, except for a few relict gametes and debris, and it was possible to observe signs of increased gametogenic activity; pre-vitellogenic oocytes and spermatogonia appeared lining the germinal epithelium in increasing numbers (Figures 3A & 4A). At stage II the gonad wall was the thickest, reproductive cells continued growing and started migrating to the narrow tubule lumen as pre- and mid-vitellogenic oocytes and spermatocytes, still attached to the folded germinal epithelium and spermatogenic column (Figures 3B & 4B). The maturation process from stage II (recovery) to stage V (partly spawned) was evidenced by the thinning and stretching of the gonad wall and an increase in the lumen space defined by tubule diameter (Tables 2 & 3) (Figures 3B–E & 4B–F). By stage III (growing) and IV (mature) tubule diameter increased and the female tubule lumen got progressively packed with fully grown late vitellogenic oocytes surrounded by and attached to their follicular envelopes (Figure 3C, D). Male tubule lumen got filled with tightly packed spermatozoa (Figure 4C, D). At stage V (partly spawned), spawned, partly spawned and unspawned gonad tubules could be found in the same individual. At this stage the majority of female gonad tubules had some free lumen space and contained few intact oocytes, damaged relict oocytes and remains of follicular envelopes, debris and phagocytes (Figure 3E). Male gonad tubules contained diffusely arranged spermatozoa, empty lumen space and phagocytes (Figure 4F–I).

The frequency distribution of male and female maturity stages changed through the year (Figure 5). However, mature individuals (stage IV) were present all year round. Mature females were more abundant in September, October 2007 and March, April 2008, and mature males were more abundant in July–September 2007 and February–April 2008. Partly spawned females (stage V) were present in similar percentages almost all year round; from May 2007 to Dec 2007 and April 2008. Partly spawned males were restricted to fewer months; from August–September 2007 and April 2008. These two periods were separated by intense gametogenic activity evidenced by high percentages of gonads in stages of recovery (stage II) and growing (stage III). Spent females (stage I) were encountered in May, August, December 2007 and January 2008, while spent males (stage I) were only encountered in August 2007.

For females, spawning periods – months where over 25% of individuals had gonads in spawning stages – progressed from May to December 2007 and April 2008 with higher proportions of spawning females in May, June, August, December 2007 and April 2008. For males, spawning periods took place from August 2007 to September 2007 and then April 2008. Spawning periods for both sexes overlapped from the end of winter towards beginning of spring (August and September 2007) and at beginning of autumn (April 2008).

## GONAD MACROSCOPIC ANALYSIS

Gonad tubules filled the body cavity of adult individuals of *A. chilensis* in advanced stages of maturity (stages IV and V). These entangled with the digestive tract, anterior portion of the longitudinal muscles and respiratory trees. As individuals became ripe, male gonad colour turned from light cream to

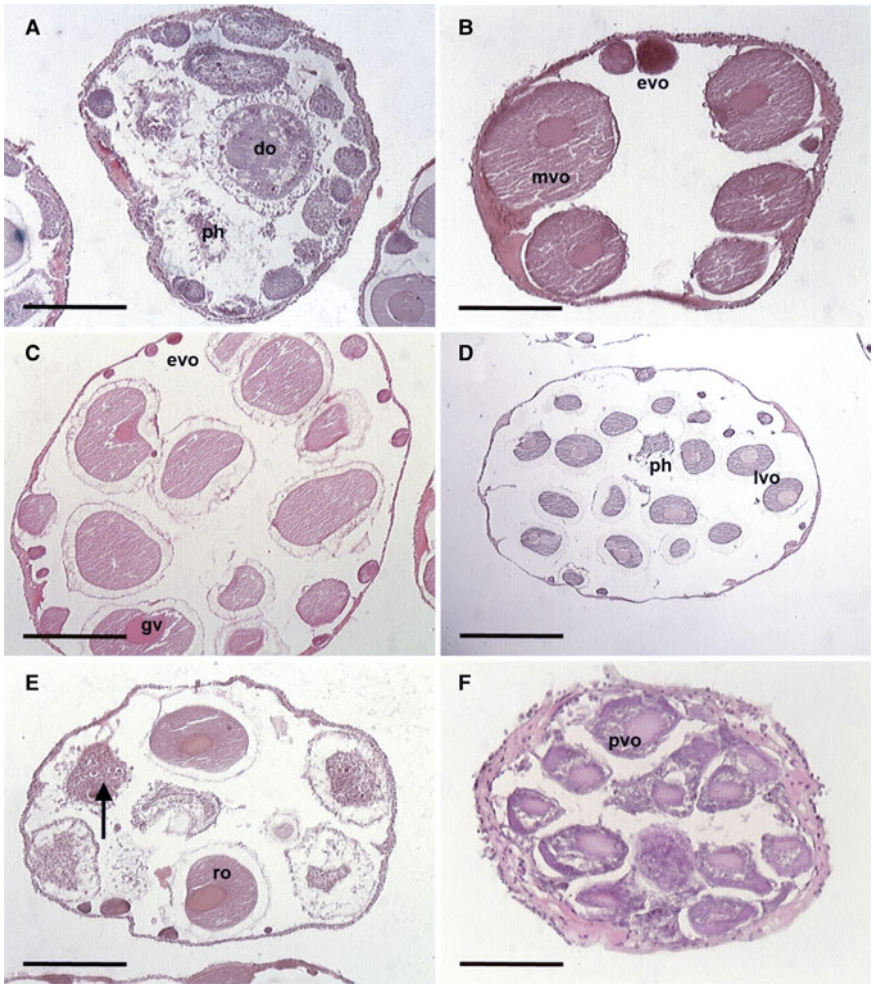


**Fig. 3.** Representative images of oogenesis in *A. chilensis*: (A) stage I, spent ovary; (B) stage II, recovering ovary; (C) stage III, growing ovary; (D) stage IV, mature ovary; (E) stage V, partially spawned ovary; (F) ovary from a juvenile individual. Abbreviations: do, degrading oocyte; ph, phagocytes; pvo, pre-vitellogenic oocyte; evo, early vitellogenic oocyte; mvo, mid-vitellogenic oocyte; lvo, late vitellogenic oocyte; gv, germinal vesicle; ro, relict oocyte. Arrow indicates highly degraded oocyte. Scale bars: A, 292 μm; B, 160 μm; C and E, 320 μm; D, 640 μm; F, 80 μm.

creamy orange and tubules acquired a beaded appearance. The female gonad wall was transparent and oocytes darkened from green to brown as they grew.

When looking at the effect of sex and maturity stages over macroscopic gonad features we observed no combined effects over gonad tubule length (two-way ANOVA, $F_{(4,\ 145)} = 1.02$, $P = 0.399$), diameter (two-way ANOVA, $F_{(4,\ 145)} = 0.79$, $P = 0.535$) or bifurcation (two-way ANOVA, $F_{(4,\ 145)} = 0.87$, $P = 0.483$). Gonad tubule length was similar between sexes (ANOVA, $F_{(1,\ 153)} = 0.58$, $P = 0.447$). However, female gonad tubule diameter was significantly wider (mean: 1.05 mm ± 0.31 SD) (ANOVA, $F_{(1,\ 153)} = 30.81$, $P < 0.0001$) and had significantly fewer bifurcations (mean: 2.34 ± 0.79 SD) (ANOVA, $F_{(1,\ 153)} = 11.65$, $P = 0.0008$) than male gonad tubules (diameter mean: 0.79 mm ± 0.29 SD, bifurcations mean: 2.79 ± 0.85 SD). Gonad tubule length, diameter and bifurcation differed significantly between maturity stages across sexes; gonad tubules became longer from stage I (spent) to IV (mature) and shortened significantly at stage V (partly spawned) (ANOVA, $F_{(4,\ 150)} = 10.03$, $P < 0.0001$) (Figure 6). Gonad tubule diameter increased from stage II (recovery) to IV (mature) (Figure 6). At stage V (partly spawned) the gonad tubules got particularly distended and filled with liquid, where phagocytic activity could be observed by the naked eye as dark clusters of debris. Female gonads at stage V contained loosely arranged oocytes and had a glossy appearance. From stage V (partly spawned) tubule diameter decreased significantly and reached minimum diameter at stage II (recovery) (ANOVA, $F_{(4,\ 150)} = 9.71$, $P < 0.0001$) (Figure 6). Gonad tubule bifurcation decreased from maximum bifurcation at stage II (recovery) to minimum bifurcation at stage I (spent) (ANOVA, $F_{(4,\ 150)} = 3.23$, $P = 0.014$) (Figure 7). There were no combined effects of sex and maturity stages over gonad weight (two-way ANOVA, $F_{(4,\ 162)} = 0.12$, $P = 0.975$), and mean yearly Gw was similar between sexes (ANOVA, $F_{(1,\ 210)} = 1.92$, $P = 0.168$). However, there were significant differences on Gw between maturity stages, gradually increasing from stage I (spent) towards stage IV (mature) and dropping after spawning (ANOVA, $F_{(4,\ 167)} = 32.06$, $P < 0.0001$) (Figure 7).

### SIZE AT FIRST MATURITY

The smallest immature female with gonads was 1.7 g Ew and 6 cm Tl. The smallest immature male with gonads was 7.4 g Ew and 9.5 cm Tl. For these individuals, sex could only be determined by microscopic observation (Figures 3F & 4J),

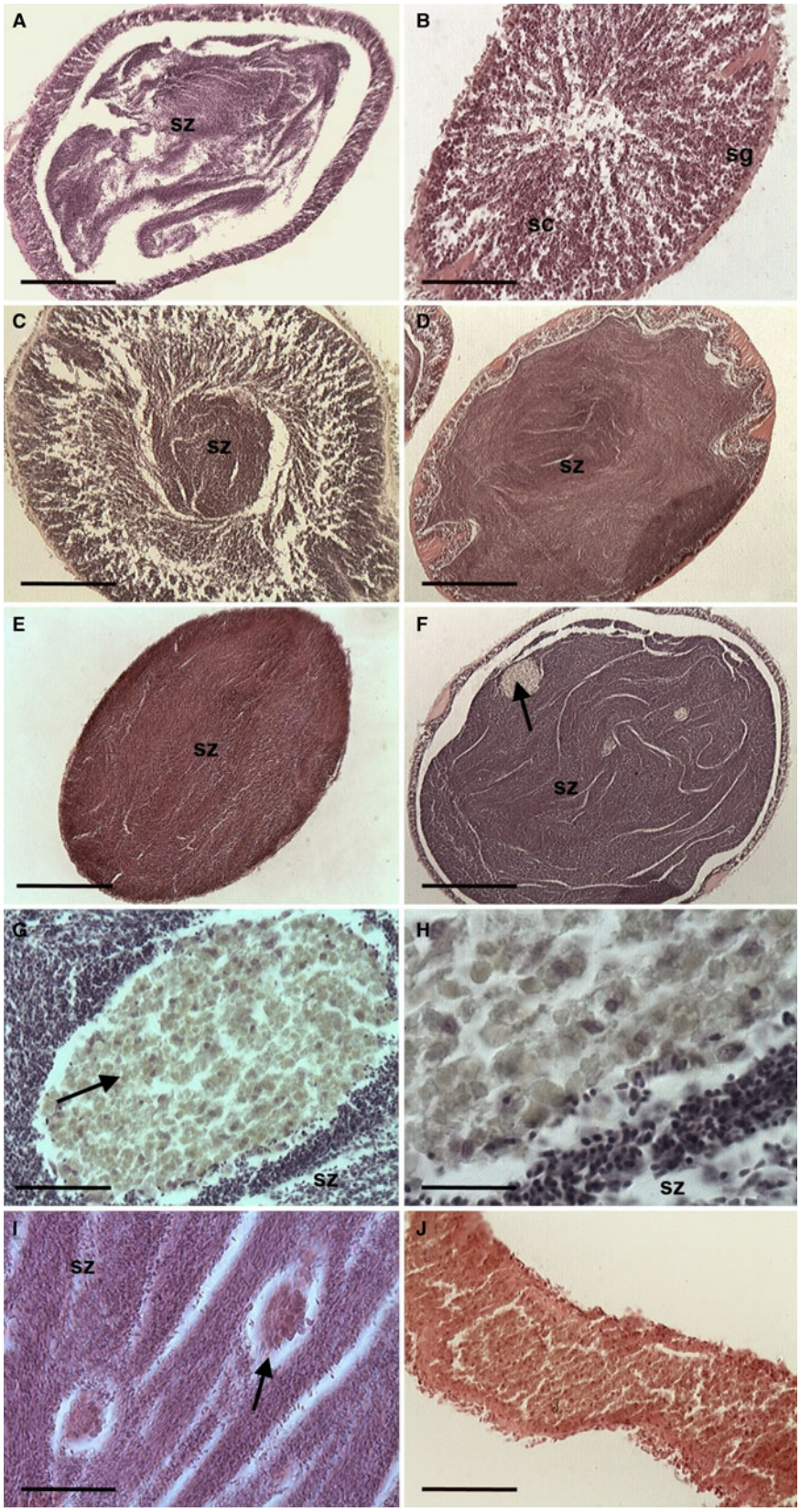


**Fig. 4.** Representative images of spermatogenesis in *A. chilensis*: (A) stage I, spent testis; (B) stage II, recovering testis; (C) stage III, growing testis; (D) stage IV, mature testis (early); (E) stage IV, mature testis (late); (F) stage V, partly spawned testis; (G) phagocytic activity indicated in F; (H) phagocytic activity and spermatozoa indicated in F; (I) another kind of possible phagocytic activity; (J) testis from an immature individual. Abbreviations: sg, spermatogonia; sc, spermatocytes; sz, spermatozoa. Arrows indicates phagocytic activity. Scale bars: A and C, 160 μm; B and J, 80 μm; D–F, 320 μm; G and I, 40 μm; H, 16 μm.

**Table 2.** Description of female *A. chilensis* ovarian maturity stages determined through histology.

| **Oogenesis stages** | **II (*Recovery*)** | **III (*Growing*)** | **IV (*Mature*)** | **V (*Partly spawned*)** | **I (*Spent*)** |
|---|---|---|---|---|---|
| Tubule diameter (μm) | Slightly wider than I | Wider | Maximum | Slightly increased, starts reducing towards spent | Minimum |
| Ovary wall | Maximum thickness, folded | Thinner, less folded | Minimum thickness, stretched | Thin and wrinkled in spawned tubules | Thicker and wrinkled |
| Germinal epithelium | Arising pre and early vitellogenic oocytes | More early and some mid vitellogenic oocytes | Pre and mid vitellogenic oocytes almost absent | Some pre vitellogenic oocytes arising from the germinal epithelium | Some early vitellogenic oocytes |
| Tubule lumen | Narrow lumen with eosinophilic granular material, some pre, early, and few mid vitellogenic oocytes which were bent due to reduced lumen space | Wider lumen, bigger mid vitellogenic oocytes progressively occupy lumen empty spaces | Wide, filled with packed mid and late vitellogenic oocytes. Some phagocytes and lysed oocytes | Wide, with loose relict oocytes. Remains of follicular epithelium, debris and phagocytes, even inside unspawned tubules | Almost empty. Few relict and damaged oocytes, remains of follicular epithelium, debris from lysed cells and phagocytes |
| Gamete properties | Basophilic pre and early vitellogenic oocytes turn into eosinophilic as they grow to mid vitellogenic oocytes | Mid vitellogenic oocytes present a distinct germinal vesicle in central position and are surrounded by follicular epithelium | Vitellogenic oocytes were surrounded by follicular epithelium and had a prominent germinal vesicle sometimes situated at the periphery of the cell | Relict oocytes maintained a packed appearance and started to degrade | Relict oocytes appear significantly degraded |

**Table 3.** Description of male *A. chilensis* testes maturity stages determined through histology.

| **Spermatogenesis stages** | **II (*Recovery*)** | **III (*Growing*)** | **IV (*Mature*)** | **V (*Partly spawned*)** | **I (*Spent*)** |
|---|---|---|---|---|---|
| Tubule diameter (μm) | Slightly wider than I | Wider | Maximum | Slightly increased and starts reducing towards spent | Minimum |
| Testes wall | Maximum thickness, folded | Thinner, less folded | Minimum thickness, stretched | Thin and wrinkled in spawned tubules | Thicker and wrinkled |
| Germinal epithelium | Thick spermatogenic column extend to the tubule lumen containing spermatogonia, spermatocytes and spermatids | Thinner spermatogenic column contained spermatogonia, spermatocytes and spermatids | Very thin spermatogenic column in close contact with lumen contents | Spermatogenic column thicker and separated from lumen contents by a distinct continuous gap. Evident spermatogenesis | Spermatogenic column got wider and contained spermatogonia and spermatocytes. Gap still present |
| Tubule lumen | Narrow central lumen is empty | Spermatozoa and some phagocytes present | Filled with packed or diffusely arranged spermatozoa. Some phagocyte clusters present | Contained diffusely arranged spermatozoa, phagocyte clusters and empty spaces | Almost empty, with few relict spermatozoa, debris and phagocyte clusters |

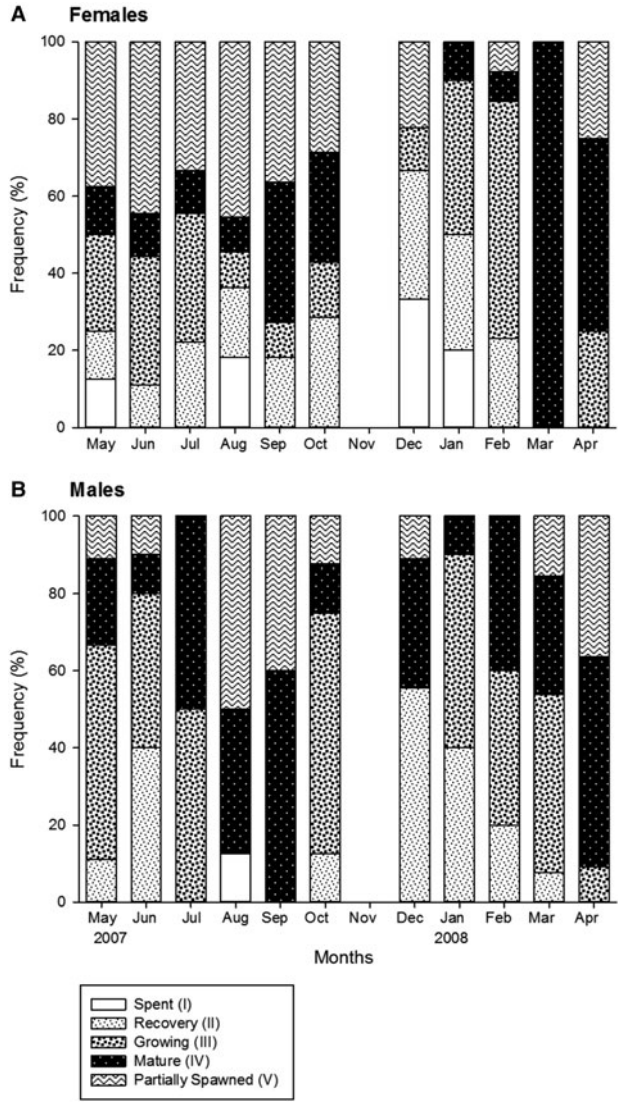


**Fig. 5.** Monthly frequency of maturity stages of *A. chilensis* assessed through histology. (A) females; (B) males. November samples were lost in a fire that destroyed the Faculty of Sciences at Universidad Austral de Chile in December 2007.

while for individuals of sizes over 27 g Ew and 12 cm Tl sex could be determined by the naked eye.

The interaction between Ew and sexual maturity was significant both for females (Logistic-R, $\chi^2 = 78.59$; $P < 0.0001$) and males (Logistic-R, $\chi^2 = 46.60$, $P = 0.0001$). $Ew_{(50)}$ was 43.68 g (95% CI from 33.6 to 50.8 g) for females and 21.17 g (95% CI from 9.5 to 28.53 g) for males, while all individuals were mature at $Ew_{(100)}$ 118.11 g (95% CI from 98.29 to 162.50 g) and 75.01 g (95% CI from 57.20 to 131 g) for females and males respectively (Figure 8). From the linear equation calculated from the relationship between Ew and Dw, $Dw_{(50)}$ was 68.71 g for females and 17.54 g for males. $Dw_{(100)}$ was 237.89 g for females and 139.92 g for males.

### FECUNDITY

GI for the 12 analysed females in stage IV (mature) ranged from 40.61 to 64.4%. Gw, Tl and Ew ranged from Gw: 40.2–67.06 g, Tl: 22–31 cm, and Ew: 100.45–132.11 g. Mean absolute fecundity ($\bar{x}$ FA) was $6.31 \times 10^5$ ($\pm 1.97 \times 10^5$ SD) oocytes per female. Relative fecundities were $1.35 \times 10^4$ ($\pm 2.2 \times 10^3$ SD) oocytes per gram of gonad ($\bar{x}$ FRGw), $2.82 \times 10^3$ ($\pm 1.05 \times 10^3$ SD) oocytes per gram of drained body weight ($\bar{x}$ FRDw) and $5.4 \times 10^3$ ($\pm 2 \times 10^3$ SD) oocytes per gram of body wall weight ($\bar{x}$ FREw). Mean vitellogenic oocyte diameter of adult females in stage IV (mature) was 500 μm (±30.2 μm SD) including the follicular epithelium, and 460 μm (±15.7 μm SD) without the latter.

## DISCUSSION

Continuous gametogenesis and the presence of spawning individuals in our monthly samples revealed that *Athyonidium chilensis* in southern Chile was able to reproduce throughout the year. However, the most important reproductive time was spring, where spawning activity observed through histology was accompanied by an important gonad index (GI) decline and recovery in summer. A slight gonad index (GI) decline and enhanced spawning activity observed at the beginning of autumn 2008 coincided with high percentages of individuals in spawning stages and low GIs observed in autumn 2007. Although a longer study period would be required to confirm an important spawning episode in autumn, this could indicate that *A. chilensis* in southern Chile shows two important reproductive times in the year; one in autumn and another one in spring.

As observed for *Holothuria fuscogilva*, spawning activity in *A. chilensis* increased before any significant drop in the GI could be detected (Ramofafia *et al.*, 2000). This, along with increased GI variability around high GI periods, indicates that spawning in *A. chilensis* commences asynchronously among individuals in the population (Ramofafia *et al.*, 2000; Dissanayake & Stefansson, 2010). This seems to occur as a gradual process involving partial gamete release that gets more coordinated towards major spawning events (Ramofafia *et al.*, 2000). As is the case for most dendrochirotes, *A. chilensis* showed an even male to female sex distribution (Tyler & Gage, 1983; Murdoch, 1984; Costelloe, 1988; Hamel *et al.*, 1993; Chao *et al.*, 1995; Foster & Hodgson, 1995; Hamel & Mercier, 1996a; Singh *et al.*, 2001; Martinez *et al.*, 2011), and the prevalent observation of overlapping generations of oocytes inside spawned gonad tubules suggested that the tubule recruitment model (TRM) for ovarian development in holothurians (Smiley *et al.*, 1991) may not apply for this species (Sewell *et al.*, 1997; Ramofafia *et al.*, 2000).

Reproductive cycles described for other dendrochirote sea cucumbers include single annual reproduction in spring (Costelloe, 1985, 1988; Hamel *et al.*, 1993; Chao *et al.*, 1995; Foster & Hodgson, 1995; Hamel & Mercier, 1996a; Singh *et al.*, 2001; Martinez *et al.*, 2011), summer (Foster & Hodgson, 1995; Martinez *et al.*, 2011) and winter (Rutherford, 1973; Catalan & Yamamoto, 1994), while semi-annual and aseasonal reproduction (Tyler & Gage, 1983; Murdoch, 1984) are less common in temperate free-spawning holothurians (Harriott, 1985; Sewell & Bergquist, 1990; Sewell, 1992; Chao *et al.*, 1995; Navarro *et al.*, 2012). *Athyonidium chilensis* enhanced reproduction in spring and indications of enhanced reproduction at beginnings of autumn suggest that, even for species with lecithotrophic larval development, seasonal environmental factors might play an important role synchronizing population reproductive times. The planktotrophic sea urchin *Loxechinus albus*, another echinoderm

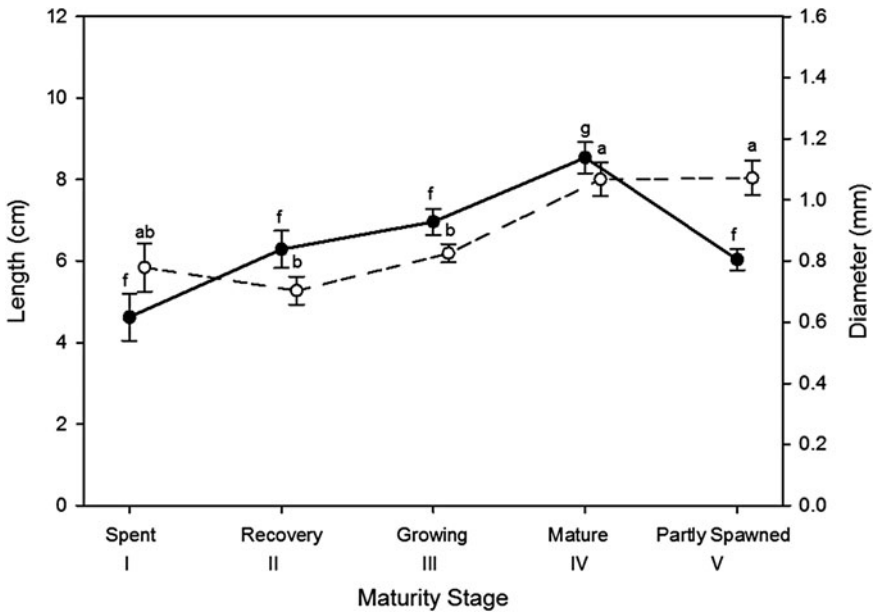


**Fig. 6.** Mean *A. chilensis* gonad tubule length (filled circles) and diameter (empty circles) through maturity stages across sexes. Letters above bars indicate *post-hoc* test results for the effect of maturity stages in two-way ANOVA (maturity stages, sex). Points that do not share the same letter indicate significant differences. Bars indicate standard error.

inhabiting the coasts of Chile, shows an annual reproductive cycle with two episodes of gonadal maturation around spring and autumn (Zamora & Stotz, 1992; Vásquez, 2001); one leads to spawning in spring while the other appears to serve as a source of winter nutrient stores (Buckle *et al.*, 1978). In Valdivia, A. chilensis biggest yearly spawning event in spring coincided with that of L. albus at the same location (Guisado 1995 in Vásquez, 2001). This suggests the importance of high primary productivity periods as drivers of gametogenic processes, which along with temperature, have long been recognized to influence reproduction in holothurians (Costelloe, 1988; Hamel et al., 1993; Chao et al., 1995; Martinez et al., 2011).

Although several years of observation would be necessary to predict the spawning season of any species at any geographic location (Sewell & Bergquist, 1990), the results from the present one-year study are similar to results obtained in previous unpublished studies on this species. Caffi (1981) and Moreno (2002) studied the reproductive biology of intertidal *A. chilensis* in the central coast of Chile; Concepcion (36°35.9′S 72°58.4′W) and Coquimbo (30°0.5′S 71°26′W). In these studies, *A. chilensis* also showed aseasonal

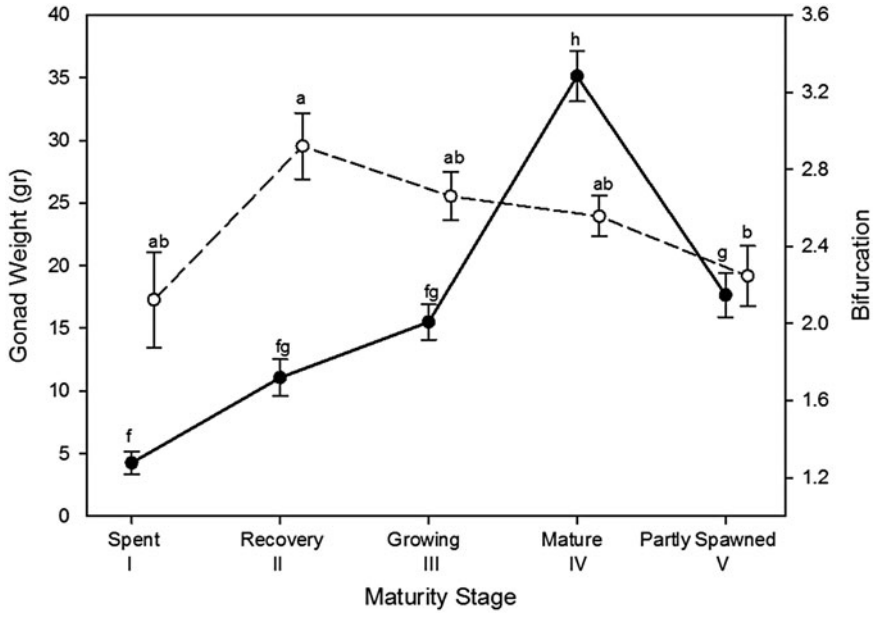


**Fig. 7.** Mean *A. chilensis* gonad weight (filled circles) and mean gonad tubule bifurcation (empty circles) through maturity stages across sexes. Letters above bars indicate *post-hoc* test results for the effect of maturity stages in two-way ANOVA (maturity stages, sex). Points that do not share the same letter indicate significant differences. Bars indicate standard error.

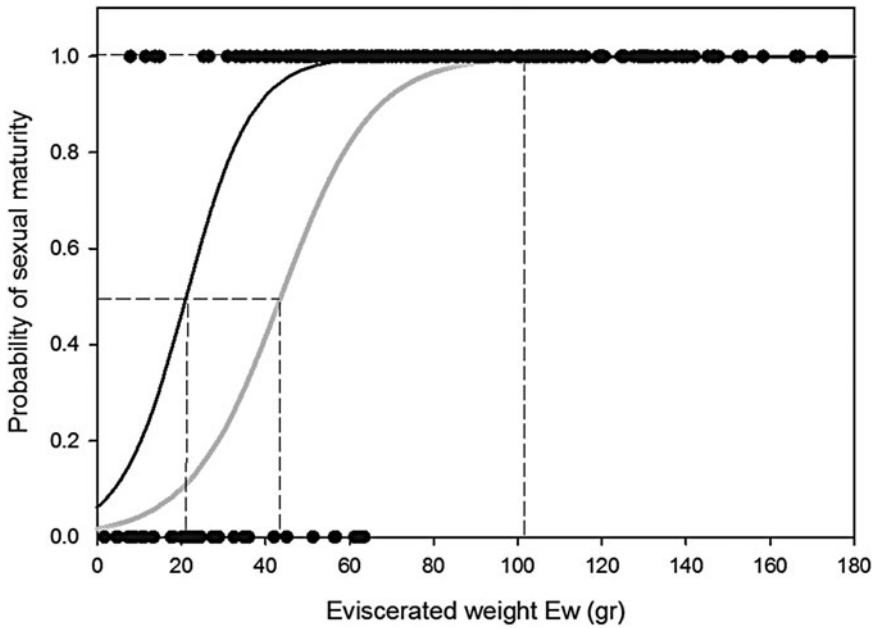


**Fig. 8.** *Athyonidium chilensis* size at first maturity for females (grey line) and males (black line). Female logistic regression parameters A: −4.053 (95% CI from −6.381 to −2.34, $\chi^2 = 16.11$, $P < 0.0001$) and B: 0.093 (95% CI from 0.062–0.136, $\chi^2 = 25.14$, $P < 0.0001$). Male logistic regression parameters A: −2.715 (95% CI from −5.112 to −0.925, $\chi^2 = 6.92$, $P = 0.009$) and B: 0.128 (95% CI from 0.073–0.213, $\chi^2 = 13.84$, $P = 0.0002$). Spaced lines indicate eviscerated weight at which 50 and 100% of individuals are mature.

asynchronous reproduction with partial spawning through the year. However, Caffi (1981) observed that enhanced spawning periods occurred in spring and summer, while Moreno (2002) observed enhanced spawning periods in winter and summer. In both studies, autumn GI declines were not clearly associated with increases in the monthly percentage of individuals in spawning stages. Oocyte sizes and GIs of *A. chilensis* in Valdivia almost doubled those reported for northern populations (Caffi, 1981; Moreno, 2002; Guisado *et al.*, 2012). With a yearly mean GI of 20% and two yearly peaks of 25.22% (±15.93 SD) in August–September 2007 and 25.68 (±9.92 SD) in March 2008, *A. chilensis* GIs in Valdivia were particularly high compared with those found in northern populations and those of other sea cucumber species (Costelloe, 1985, 1988; Hamel *et al.*, 1993; Chao *et al.*, 1995; Muthiga, 2006; Shiell & Uthicke, 2006; Toral-Granda & Martínez, 2007; Asha & Muthiah, 2008; Herrero-Pérezrul & Reyes-Bonilla, 2008; Muthiga *et al.*, 2009; Dissanayake & Stefansson, 2010; Martinez *et al.*, 2011; Morgan & Neal, 2012; Navarro *et al.*, 2012). It would be interesting to study if these high GIs are of common occurrence in southern Chile, or if they might have resulted from a year with particularly favourable conditions for *A. chilensis* reproduction. Male mean GI surpassed female mean GI, which has been previously observed for the dendrochirotes *Psolus patagonicus* Ekman, 1925 (Martinez *et al.*, 2011), *Psolus fabricii* (Düben & Koren, 1846) (Hamel *et al.*, 1993) and *Cucumaria frondosa* (Gunnerus, 1767) (Murdoch, 1984; Hamel & Mercier, 1996a). Also, male gonads were on average bigger than female gonads, which could indicate similar energy investment in gamete synthesis between sexes, because less energy is required for males to synthesize an equivalent amount of gametes (Hamel & Mercier, 1996a). Overall, latitudinal and small-scale reproductive differences may indicate important geographic trade-offs in populations of *A. chilensis*, a common occurrence in holothuroids at the intraspecific level (Sewell, 1992; Ramofafia *et al.*, 2003; Shiell & Uthicke, 2006; Toral-Granda & Martínez, 2007; Muthiga *et al.*, 2009; Navarro *et al.*, 2012). Given reproductive cycles in echinoderms have also been shown to vary with depth (Leahy *et al.*, 1981), studies on subtidal *A. chilensis* populations are strongly encouraged. Subtidal individuals reach bigger sizes than intertidal individuals (authors' personal observation) and could potentially show even higher GIs than those reported in the present study.

Mature females in this study showed very high fecundity for a species with big egg size. Higher fecundities in marine invertebrates are usually associated with smaller egg sizes and planktotrophic larval development (Thorson, 1950; Catalan & Yamamoto, 1994). Absolute fecundity in *Cucumaria frondosa*, another temperate broadcast-spawning dendrochirote with lecithotrophic larval development and similar body size as *A. chilensis*, has been reported to range between 60–400 thousand (Murdoch, 1984) and 8–10 thousand (Hamel & Mercier, 1996a), although this species shows bigger egg diameter (800 μm). For *Eupentacta chronhjelmi*, another temperate species with lecithotrophic larval development, 300 μm egg diameter and one third of the body size of *A. chilensis*, absolute fecundity is 1500 (Catalan & Yamamoto, 1994). In Valdivia, *A. chilensis* shows the highest fecundity among holothuroid species with lecithotrophic larval development, fecundity that seems to be in the lower range of that observed for planktotrophic species (Catalan & Yamamoto, 1994; Toral-Granda & Martínez, 2007; Asha & Muthiah, 2008; Muthiga *et al.*, 2009; Dissanayake & Stefansson, 2010). Intertidal *A. chilensis* might require higher fecundity energy investment given the highly exposed areas it inhabits, where chances of successful fertilization are likely to be very low. Overall, high fecundity, continuous gametogenesis, the presence of spawning individuals year-round with one and possibly two spawning peaks during spring and autumn,

and lecithotrophic larval development lasting less than a week (personal observation; Pérez, 2005; Guisado *et al.*, 2012) make *A. chilensis* a very interesting candidate for aquaculture in southern Chile.

*Athyonidium chilensis* gonad colours fit patterns described for other dendrochirotes; creamy white to orange male and green to brown female gonad (Costelloe, 1985; Hamel *et al.*, 1993; Catalan & Yamamoto, 1994; Foster & Hodgson, 1995). As in other holothuroids, the process of gonad maturation can be seen as an increase in tubule length and diameter towards stage IV (mature) (Hamel *et al.*, 1993; Catalan & Yamamoto, 1994; Foster & Hodgson, 1995; Hamel & Mercier, 1996a; Singh *et al.*, 2001; Asha & Muthiah, 2008; Fajardo-León *et al.*, 2008; Navarro *et al.*, 2012) and decreased length toward stage V (Catalan & Yamamoto, 1994; Ramofafia *et al.*, 2001; Toral-Granda & Martínez, 2007; Dissanayake & Stefansson, 2010; Navarro *et al.*, 2012). Female tubule diameter was wider than males (Asha & Muthiah, 2008; Fajardo-León *et al.*, 2008), while males had more tubule bifurcations than females. This difference could indicate a higher need for sperm production surface area in males and a need for wider tubule lumen space for oocyte storage in females. At the same time, gonad tubules had more bifurcations during the recovery stage, a likely source of increased surface area for gametes arising from the germinal epithelium to be later transferred to an increasing lumen space. The discrete characteristics of gonad tubules and the way they reflect reproductive condition, especially in terms of diameter and bifurcation, facilitates the assessment of the population's reproductive condition.

*Athyonidium chilensis* size at first maturity was slightly lower than that of the dendrochirote *C. frondosa* (55 g Ew) (Hamel & Mercier, 1996a), similar to that of the aspidochirote *Isostichopus fuscus* (161.0 and 170.9 g Dw) (Toral-Granda & Martínez, 2007), and considerably lower than that of the aspidochirotes *Parastichopus parvimensis* (120 to 140 g Ew) (Fajardo-León *et al.*, 2008) and *Holothuria sanctori* (101 to 110 g Ew) (Navarro *et al.*, 2012), all holothuroids of similar total length (Tl) to *A. chilensis*. When comparing the relationship between Tl and drained weight (Dw) against eviscerated weight (Ew) in *A. chilensis*, Dw was a much more reliable predictor of live animal weight, as observed for other sea cucumber species (Herrero-Pérezrul & Reyes-Bonilla, 2008; Dissanayake & Stefansson, 2010; Kazanidis *et al.*, 2010). Estimated size at first maturity based on the relationship between Ew and Dw was 17.54 g $Dw_{(50)}$ for males and 68.71 g $Dw_{(50)}$ for females. Based on this estimation we recommend the use of a minimum harvesting size where 100% of females are mature; 237.89 g $Dw_{(100)}$ for the live animal, which corresponds to 118.11 g $Ew_{(100)}$. This is a precautionary approach because females mature at a bigger size than males and it is not possible to externally predict the sex of *A. chilensis*. At the same time, Dw could be easily over-estimated due to water remaining in the cloaca and respiratory trees or excessive food in the digestive tract.

*Athyonidium chilensis* shows fast larval development, thus limited larval dispersal which is likely to limit the genetic connectivity between populations, making this species particularly susceptible to fishing over-pressure. The information presented in this study could be utilized to reduce fishing pressure during *A. chilensis* peak reproductive times to allow aggregation of individuals and successful reproduction (Hamel & Mercier, 1996b). This study could also be used as a guideline to establish minimum capture sizes for fisheries. Inside MEABRs and through small-scale aquaculture, fishers could assess enhancing sea cucumber stocks through the mass-release of cultured juveniles to restricted areas (Purcell & Agudo, 2013) or by establishing local management regulations to protect the reproductive potential of populations (Bell *et al.*, 2008a, b).

## ACKNOWLEDGEMENTS

We would like to thank people who assisted with sea cucumber sampling, especially Carola Martinez, Juan Pablo Fuentes, Marcelo Flores, Fabián Aros and Cristián Manque. Thanks to Roger Sepulveda and Mary Sewell for valuable input during manuscript preparation. Special thanks to Raúl Arriagada for his help with histology procedures and for providing valuable support after the fire that affected the Faculty of Sciences at Universidad Austral de Chile.

## FINANCIAL SUPPORT

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

## REFERENCES

**Abdel-Razek F.A., Abdel-Rahman S.H., El-Shimy N.A. and Omar H.A.** (2005) Reproductive biology of the tropical sea cucumber *Holothuria atra* (Echinodermata: Holothuroidea) in the Red Sea coast of Egypt. *Egyptian Journal of Aquatic Research* 31, 383–402.

**Akamine J.** (2004) The status of the sea cucumber fisheries and trade in Japan: past and present. In Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.F. and Mercier A. (eds) *Advances in sea cucumber aquaculture and management. FAO Fisheries and Aquaculture Technical Paper*. Volume 463. Rome: FAO, pp. 39–47.

**Anderson S.C., Flemming J.M., Watson R. and Lotze H.K.** (2011) Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries* 12, 317–339.

**Asha P.S. and Muthiah P.** (2008) Reproductive biology of the commercial sea cucumber *Holothuria spinifera* (Echinodermata: Holothuroidea) from Tuticorin, Tamil Nadu, India. *Aquaculture International* 16, 231–242.

**Bell J.D., Leber K.M., Blankenship H.L., Loneragan N.R. and Masuda R.** (2008a) A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. *Reviews in Fisheries Science* 16, 1–9.

**Bell J.D., Purcell S.W. and Nash W.J.** (2008b) Restoring small-scale fisheries for tropical sea cucumbers. *Ocean and Coastal Management* 51, 589–593.

**Buckle F., Guisado C., Tarifeño E., Zuleta A., Córdova L. and Serrano C.** (1978) Biological studies on the Chilean sea urchin *Loxechinus albus* (Molina) (Echinodermata: Echinoidea). IV. Maturation cycle and seasonal changes in the gonad. *Ciencias Marinas Mexico* 5, 1–15.

**Caffi M.** (1981) *Aspectos del ciclo reproductivo de* Athyonidium chilensis *(Semper, 1868) en caleta Colchogüe, Bahía de Concepción, Chile (Echinodermata: Holothuroidea)*. Masters equivalent thesis. Universidad de Concepción, Concepción, Chile.

**Catalan M.A.B. and Yamamoto M.** (1994) Annual reproductive cycle of the Japanese holothurian *Eupentacta chronhjelmi*. *Canadian Journal of Zoology* 72, 387–396.

**Chao S.-M., Chen C.-P. and Alexander P.S.** (1995) Reproductive cycles of tropical sea cucumbers (Echinodermata: Holothuroidea) in southern Taiwan. *Marine Biology* 122, 289–295.

**Conand C.** (1981) Sexual cycle of three commercially important holothurian species (Echinodermata) from the lagoon of New Caledonia. *Bulletin of Marine Science* 31, 523–543.

**Conand C.** (1993a) Ecology and reproductive biology of *Stichopus variegatus* an Indo- Pacific coral reef sea cucumber (Echinodermata: Holothuroidea). *Bulletin of Marine Science* 52, 970–981.

**Conand C.** (1993b) Reproductive biology of the holothurians from the major communities for the New Caledonia lagoon. *Marine Biology* 116, 439–450.

**Conand C.** (2004) Present status of world sea cucumber resources and utilization: an international overview. In Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.-F. and Mercier A. (eds) *Advances in sea cucumber aquaculture and management. FAO Fisheries and Aquaculture Technical Paper*. Volume 463. Rome: FAO, pp. 13–24.

**Conand C. and Byrne M.** (1993) A review of recent developments in world sea cucumber fisheries. *Marine Fisheries Review* 55, 1–13.

**Costelloe J.** (1985) The annual reproductive cycle of the holothurian *Aslia lefevrei* (Dendrochirota: Echinodermata). *Marine Biology* 88, 155–165.

**Costelloe J.** (1988) Reproductive cycle, development and recruitment of two geographically separated populations of the dendrochirote holothurian *Aslia lefevrei*. *Marine Biology* 99, 535–545.

**Dissanayake D.C.T. and Stefansson G.** (2010) Reproductive biology of the commercial sea cucumber *Holothuria atra* (Holothuroidea: Aspidochirotida) in the northwestern coastal waters of Sri Lanka. *Invertebrate Reproduction and Development* 54, 65–76.

**Eriksson H. and Byrne M.** (2013) The sea cucumber fishery in Australia's Great Barrier Reef Marine Park follows global patterns of serial exploitation. *Fish and Fisheries* 16, 329–341.

**Fajardo-León M.C., Suárez-Higuera M.C.L., del Valle-Manríquez A. and Hernández-López A.** (2008) Reproductive biology of the sea cucumber *Parastichopus parvimensis* (Echinodermata: Holothuroidea) at Isla Natividad and Bahía Tortugas, Baja California Sur, Mexico. *Ciencias Marinas* 34, 165–177.

**Fernández M. and Castilla J.C.** (2005) Marine conservation in Chile: historical perspective, lessons, and challenges. *Conservation Biology* 19, 1752–1762.

**Foster G.G. and Hodgson A.N.** (1995) Annual reproductive cycles of three sympatric species of intertidal holothurians (Echinodermata) from the coast of the Eastern Cape Province of South Africa. *Invertebrate Reproduction and Development* 27, 49–59.

**Gelcich S., Godoy N., Prado L. and Castilla J.C.** (2008) Add-on conservation benefits of marine territorial user rights fishery policies in central Chile. *Ecological Applications* 18, 273–281.

**Gelcich S., Hughes T.P., Olsson P., Folke C., Defeo O., Fernández M., Foale S., Gunderson L.H., Rodríguez-Sickert C., Scheffer M., Steneck R.S. and Castilla J.C.** (2010) Navigating transformations in governance of Chilean marine coastal resources. *Proceedings of the National Academy of Sciences USA* 107, 16794–16799.

**Guisado C., Carrasco S.A., Díaz-Guisado D., Maltrain R. and Rojas H.** (2012) Embryonic development, larval morphology and juvenile growth of the sea cucumber *Athyonidium chilensis* (Holothuroidea: Dendrochirotida). *Revista de Biología Marina y Oceanografía* 47, 65–73.

**Guzmán H.M., Guevara C.A. and Hernández I.C.** (2003) Reproductive cycle of two commercial species of sea cucumber (Echinodermata: Holothuroidea) from Caribbean Panama. *Marine Biology* 142, 271–279.

**Hamel J.F., Himmelman J.H. and Dufresne L.** (1993) Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Duben and Koren). *Biological Bulletin* 184, 125–143.

**Hamel J.-F. and Mercier A.** (1996a) Studies on the reproductive biology of the Atlantic sea cucumber *Cucumaria frondosa*. *SPC bêche-de-mer Information Bulletin* 8, 22–33.

**Hamel J.F. and Mercier A.** (1996b) Evidence of chemical communication during the gametogenesis of holothuroids. *Ecology* 77, 1600–1616.

**Harriott V.J.** (1985) Reproductive biology of three congeneric sea cucumber species, *Holothuria atra, H. impatiens* and *H. edulis*, at Heron Reef, Great Barrier Reef. *Marine and Freshwater Research* 36, 51–57.

**Herrero-Pérezrul M.D. and Reyes-Bonilla H.** (2008) Weight-length relationship and relative condition of the holothurian *Isostichopus fuscus* at Espíritu Santo Island, Gulf of California, México. *Revista de Biologia Tropical* 56, 273–280.

**Hewatt W.G.** (1943) A method for narcotizing holothurians. *Science* 97, 588.

**Kazanidis G., Antoniadou C., Lolas A.P., Neofitou N., Vafidis D., Chintiroglou C. and Neofitou C.** (2010) Population dynamics and reproduction of *Holothuria tubulosa* (Holothuroidea: Echinodermata) in the Aegean Sea. *Journal of the Marine Biological Association of the United Kingdom* 90, 895–901.

**Larraín A.** (1995) Biodiversidad de equinodermos Chilenos: Estado actual del conocimiento y sinopsis biosistemática. *Gayana Zoología* 59, 73–96.

**Leahy P.S., Hough-Evans B.R., Britten R.J. and Davidson E.H.** (1981) Synchrony of oogenesis in laboratory-maintained and wild populations of the purple sea urchin (*Stronglyocentrotus purpuratus*). *Journal of Experimental Zoology* 215, 7–22.

**Martinez M.I., Giménez J. and Penchaszadeh P.E.** (2011) Reproductive cycle of the sea cucumber *Psolus patagonicus* Ekman 1925, off Mar del Plata, Buenos Aires, Argentina. *Invertebrate Reproduction and Development* 55, 124–130.

**Moreno C.** (2002) *Aspectos reproductivos de* Athyonidium chilensis *(Semper, 1868) (Echinodermata: Holothuroidea) en la playa El Francés, IV Región, Chile*. Masters equivalent thesis. Universidad Católica del Norte, Coquimbo, Chile.

**Morgan A. and Neal L.** (2012) Aspects of reproductive ecology and benthic-pelagic coupling in the sub-antarctic sea cucumber *Pseudostichopus mollis* (Theel). *Continental Shelf Research* 43, 36–42.

**Murdoch J.H.** (1984) *Breeding patterns of two species of North Western Atlantic sea cucumbers (Echinodermata: Holothuroidea)*. Honours thesis. Mount Allison University, New Brunswick, Canada.

**Muthiga N.A.** (2006) The reproductive biology of a new species of sea cucumber, *Holothuria* (*Mertensiothuria*) *arenacava* in a Kenyan marine protected area: the possible role of light and temperature on gametogenesis and spawning. *Marine Biology* 149, 585–593.

**Muthiga N.A., Kawaka J.A. and Ndirangu S.** (2009) The timing and reproductive output of the commercial sea cucumber *Holothuria scabra* on the Kenyan coast. *Estuarine, Coastal and Shelf Science* 84, 353–360.

**Navarro P.G., García-Sanz S. and Tuya F.** (2012) Reproductive biology of the sea cucumber *Holothuria sanctori* (Echinodermata: Holothuroidea). *Scientia Marina* 76, 741–752.

**Pawson D.L.** (1964) The Holothuroidea collected by the Royal Society Expedition to Southern Chile, 1958–1959. *Pacific Science* 18, 435–470.

**Pawson D.L.** (1969) Holothuroidea from Chile – Report No. 46 of the Lund University Chile Expedition 1948–1949. *Sarsia* 38, 121–145.

**Pérez L.V.** (2005) *Desarrollo embrionario y larval del pepino de mar Athyonidium chilensis (Semper, 1868) (Echinodermata: Holothuroidea)*. Masters equivalent thesis. Universidad Católica del Norte, Coquimbo, Chile.

**Purcell S.W.** (2010) Managing sea cucumber fisheries with an ecosystem approach. In Lovatelli A., Vasconcellos M. and Yimin Y. (eds) *FAO Fisheries and Aquaculture Technical Paper*. Volume 520. Rome: FAO.

**Purcell S.W.** (2014) Value, market preferences and trade of bêche-de-mer from Pacific Island Sea cucumbers. *PLoS ONE* 9, 1–8.

**Purcell S.W. and Agudo N.S.** (2013) Optimisation of mesh enclosures for nursery rearing of juvenile sea cucumbers. *PLoS ONE* 8, 1–10.

**Purcell S.W., Mercier A., Conand C., Hamel J.-F., Toral-Granda M.V., Lovatelli A. and Uthicke S.** (2013) Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries* 14, 34–59.

**Quinn G.P. and Keough M.J.** (2002) *Experimental design and data analysis for biologists*. New York, NY: Cambridge University Press.

**Ramofafia C., Battaglene S.C., Bell J.D. and Byrne M.** (2000) Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. *Marine Biology* 136, 1045–1056.

**Ramofafia C., Byrne M. and Battaglene C.S.** (2003) Reproduction of the commercial sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) in the Solomon Islands. *Marine Biology* 142, 281–288.

**Ramofafia C., Byrne M. and Battaglene S.** (2001) Reproductive biology of the intertidal sea cucumber *Actinopyga mauritiana* in the Solomon Islands. *Journal of the Marine Biological Association of the United Kingdom* 81, 523–531.

**RStudio Team** (2015) *RStudio: integrated development for R*. Boston, MA: RStudio, Inc.

**Rutherford J.C.** (1973) Reproduction, growth and mortality of the holothurian *Cucumaria pseudocurata*. *Marine Biology* 22, 167–176.

**Sernapesca** (2013) Anuario estadístico de pesca (1998–2013). Available at http://www.sernapesca.cl.

**Sewell M.A.** (1990) Aspects of the ecology of *Stichopus mollis* (Echinodermata: Holothuroidea) in northeastern New Zealand. *New Zealand Journal of Marine and Freshwater Research* 24, 97–103.

**Sewell M.A.** (1992) Reproduction of the temperate Aspidochirote *Stichopus mollis* (Echinodermata: Holothuroidea) in New Zealand. *Ophelia* 25, 103–121.

**Sewell M.A. and Bergquist P.R.** (1990) Variability in the reproductive cycle of *Stichopus mollis* (Echinodermata: Holothuroidea). *Invertebrate Reproduction and Development* 17, 1–7.

**Sewell M.A., Tyler P.A., Young C.M. and Conand C.** (1997) Ovarian development in the class Holothuroidea: a reassessment of the "Tubule Recruitment Model". *Biological Bulletin* 192, 17–26.

**Shiell G.R. and Uthicke S.** (2006) Reproduction of the commercial sea cucumber *Holothuria whitmaei* [Holothuroidea: Aspidochirotida] in the Indian and Pacific Ocean regions of Australia. *Marine Biology* 148, 973–986.

**Singh R., MacDonald B.A., Lawton P. and Thomas M.L.H.** (2001) The reproductive biology of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuriodea) using new quantitative methods. *Invertebrate Reproduction and Development* 40, 125–141.

**Smiley S.** (1988) The dynamics of oogenesis and the annual ovarian cycle of *Stichopus californicus* (Echinodermata: Holothuroidea). *Biological Bulletin* 175, 79–93.

**Smiley S., McEwen F.S., Chafee C. and Krishnan S.** (1991) Echinodermata: Holothuroidea. In Giese A.C., Pearse J.S. and Pearse V.B. (eds) *Reproduction of marine invertebrates*. Volume VI. Pacific Grove, CA: Boxwood Press, pp. 663–750.

**Stotz W.** (2007) Criterios de explotación de recursos bentónicos Secundarios en Áreas de Manejo. *Informe final proyecto FIP* 2005-42. Chile: Universidad Católica del Norte, 424 pp.

**Thorson G.** (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews* 25, 1–45.

**Toral-Granda M.V., Lovatelli A. and Vasconcellos M.** (2008) *Sea cucumbers: a global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper no. 516*. Rome: FAO.

**Toral-Granda M.V. and Martínez P.C.** (2007) Reproductive biology and population structure of the sea cucumber *Isostichopus fuscus* (Ludwig, 1875) (holothuroidea) in Caamaño, Galápagos Islands, Ecuador. *Marine Biology* 151, 2091–2098.

**Tyler P.A. and Gage J.D.** (1983) The reproductive biology of *Ypsilothuria talismani* (Holothuroidea: Dendrochirota) from the NE Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 63, 609–616.

**Vannuccini S.** (2004) *Sea cucumbers: a compendium of fishery statistics*. In Lovatelli A., Conand C., Purcell S., Uthicke S., Hamel J.-F. and Mercier A. (eds) *Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper*. Volume 463. Rome: FAO, pp. 399–412.

**Vásquez J.A.** (2001) Ecology of *Loxechinus albus*. *Developments in Aquaculture and Fisheries Science* 32, 161–175.

**Zamora L.N., Yuan X., Carton A.G. and Slater M.J.** (2016) Role of deposit-feeding sea cucumbers in integrated multitrophic aquaculture: progress, problems, potential and future challenges. *Reviews in Aquaculture*.

and

**Zamora S. and Stotz W.** (1992) Ciclo reproductivo de *Loxechinus albus* (Molina 1782) (Echinodermata: Echinoidea) en Punta Lagunillas, IV Región, Coquimbo, Chile. *Revista Chilena de Historia Natural* 65, 121–133.

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