

## Spawning pattern and reproductive strategy of female pouting *Trisopterus luscus* (Gadidae) on the Galician shelf of north-western Spain

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**Abstract** – Pouting, *Trisopterus luscus* is harvested commercially on the Galician shelf by the Spanish inshore artisanal fleet. In spite of a substantial decrease in pouting catches, fishery regulations are limited to size length restrictions. This study provides biological data including length-at-maturity based on histological methods, seasonal maturation, spawning and fecundity. A collection 443 females, from 17 to 42 cm in total length, were sampled from landings (December 2003 to December 2004). Pouting length-at-maturity was estimated as 19.2 cm on average. Pouting females in spawning condition were observed throughout the year and the number of developing oocytes ranged from 20 000 to 1 327 000. Peak spawning was observed between February and April, which correlated well with trends in gonadosomatic index, and inverse to condition factor and hepatosomatic index. Histological examination of the gonads revealed that pouting ovarian development organization is asynchronous, and fecundity is probably determinate.

**Key words:** Length-at-maturity / Fecundity / Spawning pattern / Reproductive strategy / *Trisopterus luscus* / Atlantic Ocean

**Résumé** – **Ponte et stratégie de la reproduction chez les femelles du tacaud *Trisopterus luscus* (Gadidés) du plateau continental de la Galice, nord-ouest de l’Espagne.** Le tacaud, *Trisopterus luscus* est pêché par la flotte artisanale espagnole. En dépit d’une diminution substantielle des captures de tacaud, la pêche est réglementée uniquement à partir d’une taille minimum commerciale. Cette étude fournit des données biologiques comprenant la taille à maturité sexuelle basée sur l’histologie, la maturation saisonnière, la ponte et la fécondité. Un échantillon de 443 femelles, de 17 à 42 cm longueur totale, est examiné à partir des débarquements durant une année (de décembre 2003 à décembre 2004). La taille moyenne à maturité sexuelle est estimée à 19,2 cm. Les femelles de tacaud en condition de ponte sont observées tout au long de l’année et le nombre d’ovocytes s’étend de 20 000 à 1 327 000 par individu. Les pics de ponte sont observés entre février et avril, ce qui est bien corrélé avec l’évolution du rapport gonado-somatique et inverse de celle du facteur de condition et du rapport hépato-somatique. L’étude histologique révèle que le développement ovarien chez le tacaud est asynchrone, et que la fécondité est probablement déterminée.

### Introduction

Knowledge of the reproductive biology of a fish species is essential for effective fishery management (Marshall et al. 2003). There is increasing awareness that the traditional indicators of stock viability are inadequate because the capacity of a population to produce viable eggs and larvae each year is extremely important for stock viability and recovery (Kraus et al. 2002; Murua et al. 2003). Improved estimates of population reproductive potential should thus lead to improved Stock-Recruitment relationships (Marshall et al. 1998).

Fish species may show determinate or indeterminate fecundity (Hunter et al. 1989; Hunter et al. 1992; Murua and Saborido-Rey 2003). In species with determinate fecundity, the number of eggs to be released during the spawning season is fixed before the onset of spawning, and is considered to correspond to potential annual fecundity after correcting for atretic losses. In contrast, species with indeterminate fecundity do not have a fixed annual fecundity because unyolked oocytes continue to mature and be spawned.

Population reproductive potential is not only influenced by spawning stock biomass, but also by age, size and physical condition (Kjesbu et al. 1991; Solemdal 1997; Vallin and Nissling 2000; Marteinsdottir and Begg 2002;

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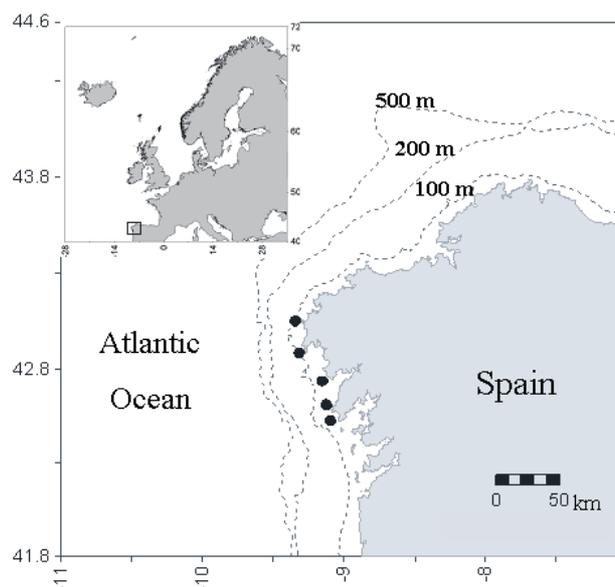
**Table 1.** Estimates of female pouting (*Trisopterus luscus*) mean body length (cm), gutted weight (g) and average relative intensity of follicular atresia (RIA), based on samples collected during the December 2003 through 2004 reproduction assessment study.

Period	Specimens (n)	Number of hauls	Length range (cm)	Gutted weight range (g)	Follicular atresia RIA (%)
2003 December	18	1	23.5–28.2	155–245	0.2
2004					
January	24	1	16.9–40.0	55–914	5.5
February	13	1	19.8–29.5	75–262	0.7
March	56	2	23.0–39.7	126–653	3.3
April	67	2	22.0–33.1	100–368	0.1
May	97	3	16.5–33.7	48–440	19.4
June	35	1	17.9–41.7	65–861	19.4
July	47	2	17.6–33.5	56–467	2.7
October	14	1	21.4–28.5	98–266	26.4
November	40	2	18.9–29.7	69–300	1.1
December	32	1	22.2–35.1	98–442	11.1
TOTAL	443	17			

Marshall et al. 2003). Total egg production may be more greatly influenced by environmental conditions in species with indeterminate fecundity than it is in species with determinate fecundity (Murua and Motos 2006), which depend mainly on energetic reserves. Fecundity studies allow estimation of total egg production (Murua et al. 2003) and recruitment (e.g., Kraus et al. 2002) of a fish stock.

The pouting, *Trisopterus luscus* (Linnaeus 1758), is distributed throughout the Atlantic Ocean, from the Skagerrak and the British Isles to southern Morocco, and into the western Mediterranean. This species rarely grows to more than 40 cm in length and 4 years of age (Labarta and Ferreiro 1982). The preferred habitat is areas with rocky and sandy sea bottom on the continental shelf, at depths of 30–100 m (Wheeler 1978; Whitehead et al. 1986). Pouting is a member of the Gadoid family and is of major commercial importance for the artisanal fleet of a number of European countries, primarily France, Portugal, and Spain. The highest recorded landings occurred on the Galician shelf during 1979 (27 036 t), a slow but steady decrease in catches was then observed until 1998 (11 118 t). In 2005, the total catches amounted to 12 460 t. Despite this decline, there was only limited size restriction in Galicia (minimum legal size: 20 cm TL). Information about this species was scarce, e.g. growth (Puente 1988; Merayo and Villegas 1994), distribution, fish assemblage and selectivity (França et al. 2004; Fonseca et al. 2005), feeding ecology and parasitology (Tirard et al. 1996; Fowler et al. 1999).

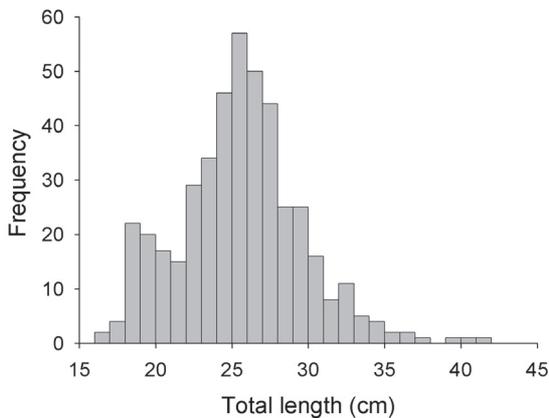
Knowledge of pouting reproductive biology is generally sparse. Previous reproductive studies have been limited in scope (Labarta et al. 1982; Desmarchelier 1985; Merayo 1996a) and assumed that pouting was a species with determinate fecundity, although it presents asynchronous ovarian development organization – a common feature of indeterminate species (Murua et al. 2003). Aspects such as follicular atresia or temporal variation of fecundity were considered for the first time in the present study. Additionally, no evidence for determinate fecundity had been previously demonstrated, and this assumption needed to be verified to provide a proper estimation of total egg production for future fishery management.

**Fig. 1.** Pouting (*Trisopterus luscus*) collection locations (●) for the December 2003 through 2004 reproduction assessment study.

The purpose of this study was to delineate length-at-maturity in female pouting using histological methods, the seasonal cycle of sexual maturation, the extent of follicular atresia, timing of spawning, and fecundity type.

## Materials and methods

Assessment of pouting fecundity type required sample collection to be spread out over time: samples were thus taken from December 2003 to December 2004, but excluded August and September 2004. Pouting samples were collected from landings in Ribeira (Galicia, Spain), at least once a month (Table 1). The pouting had been caught by the artisanal fleet using traps or nets in five different locations (Fig. 1), on the same day as they were sampled. A total of 443 females were



**Fig. 2.** Length-frequency distribution of female pouting (*Trisopterus luscus*) sampled ( $n = 443$ ) during the December 2003 through 2004.

sampled ranging from 17 to 42 cm in total length (Fig. 2). The following information was collected from each female: total length ( $L$  mm), gutted weight ( $W \pm 0.01$ g), maturation stage, gonad weight and liver weight ( $GW$  and  $LW$ ,  $\pm 0.01$ g). For each mature female, the gonadosomatic index (GSI), hepatosomatic index (HSI) and condition factor ( $K$ ) were estimated as follows:

$$GSI = \frac{GW}{W} 100 \quad HSI = \frac{LW}{W} 100 \quad K = \frac{W}{L^3} 100 \quad (1)$$

Ovaries were removed from all specimens ( $n = 443$ ) and fixed immediately in 3.6% buffered formalin. Central portions of the fixed ovaries were extracted, dehydrated, embedded in paraffin, sectioned at  $3 \mu\text{m}$  and stained with haematoxylin-eosin for microscopic analysis using a Leica DM RE (Digital Microscope series RE). For each female, the follicles (oocytes and surrounding follicular layer) were classified into stages of development using histological criteria (West 1990; Tyler and Sumpter 1996; Saborido-Rey and Junquera 1998; Murua and Saborido-Rey 2003). The stages assigned were primary growth, cortical alveoli, vitellogenesis and hydrated. Other ovarian structures, such as atretic oocytes and postovulatory follicles (POF), were identified and their presence was scored for every slide (Table 2). Female maturity status was based on the most advanced oocyte development stage contained within the ovary, the presence of POF and percentage of vitellogenesis atresia (Dominguez-Petit 2007) i.e. the following microscopic description of maturity stages (Table 3).

All females with ovaries in the maturity stages defined above were considered mature. Females were considered immature when only primary-growth-stage oocytes were present and there was no evidence of prior spawning activity, e.g. thick ovary wall.

Macroscopic observations classified females into only two maturity stages (Table 3): immature and mature (mature included stages: ripening, spawning, spawning-hydrated, spent and recovering).

**Table 2.** Description of the different oocyte stages assigned and other structures present in the ovary.

Oocyte Stage	Description
Primary growth	This covers two phases: the chromatin nucleolar phase and the perinucleolar phase. As the oocyte grows, both the cytoplasm and the nucleus increase in size and multiple nucleoli appear in the periphery of the nucleoplasm, which is the perinuclear stage.
Cortical alveoli	The presence of oocytes with cortical alveoli in the periphery of the cytoplasm indicates the onset of the maturation for the following breeding season. After this stage, the vitellogenesis process starts.
Vitellogenesis	Yolk globules begin to be formed.
Hydrated	Oocytes enlarge due to a massive intake of water and become transparent. Cytoplasm is homogeneous and no cell structures can be identified.
Ovarian structure	Description
Postovulatory follicle	Ruptured empty oocyte envelopes are left in the ovary after ovulation of mature oocytes, indicating that the fish have spawned.
Atretic oocytes	Vitellogenic oocytes in different phases of development are lost through degeneration (atresia) in the ovary.

To define female maturity as a function of body length, a logistic equation was fitted to the maturity-at-length data, based on the histological and the macroscopic maturity classification method.

$$\hat{P} = \frac{e^{a+bL}}{1 + e^{a+bL}} \quad (2)$$

and the logit transformation:

$$\ln \frac{\hat{P}}{1 - \hat{P}} = a + bL \quad (3)$$

Where  $\hat{P}$  is the predicted proportion mature,  $a$  and  $b$  the estimated coefficients of the logistic equation and  $L$  the female body length. Length at maturity ( $L_{50}$ ) was considered as the length at which 50% of the females were mature, i.e.  $-a/b$  in Eq. (3). Macroscopic and microscopic determination of size at maturity was compared using the methodology described in Saborido-Rey and Junquera (1998). Statistica 6.0 for Windows software was used to calculate predicted values and coefficients.

Histological sections and stereology were used as described by Emerson et al. (1991) to estimate intensity of atresia

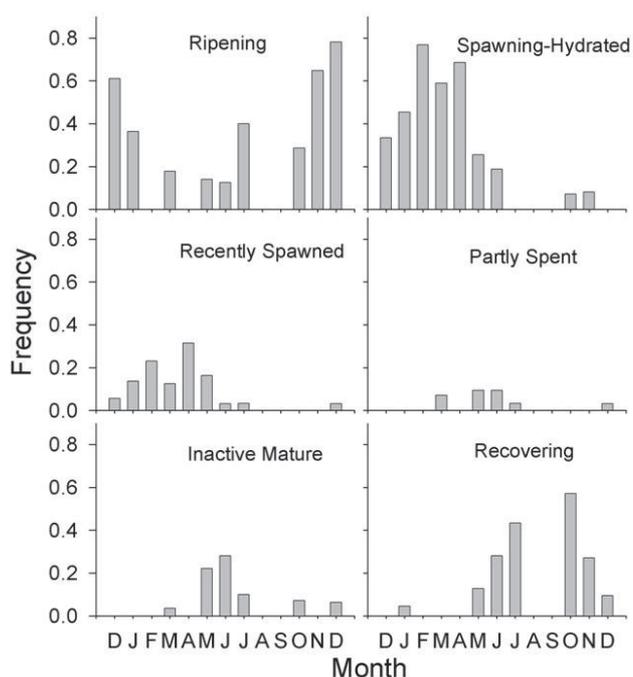
**Table 3.** Description of ovary developmental stages for *Trisopterus luscus*. *Macro- and microscopic maturity classification criteria.*

		Macroscopic description	Microscopic description
<b>Immature stage</b>		Tiny ovaries close to bladder, transparent to orange translucent. No opaque oocytes visible.	Only primary growth staged oocytes were present and there was no evidence of prior spawning activity, e.g. thick ovary wall.
	<b>Mature stages</b>		
	<i>Ripening</i>	Ovaries clearly distinguishable with opaque orange-red colour. Opaque oocytes present.	Oocytes in primary and secondary growth stages: cortical alveoli and vitellogenesis. No hydrated oocytes or postovulatory follicles. Females at this stage are likely to be in pre-spawning condition (a).
	<i>Recently-spawned</i>	Enlarged ovaries; opaque orange to creamy yellow. Larger opaque oocytes.	Presence of POF. No hydrated oocytes. Females at this stage enter the spawning period but are still likely to produce a large number of batches of spawn.
	<i>Spawning-hydrated</i>	As spawning ovaries but with translucent hydrated oocytes present.	Ovary has hydrated oocytes. POF may or may not be present. The presence of hydrated oocytes suggests ovulation would have occurred within a few hours, thus these fish are considered to be in spawning condition.
	<i>Partly spent</i>	Difficult identification	Spawning activity is finishing, with the presence of POF and increasing levels of atresia (>10% vitellogenic oocytes). Residual hydrated-stage oocytes could be present.
	<i>Inactive mature</i>	Ovaries contracted rich in blood vessels. Irregular opaque granules present as sign of atretic oocytes.	Ovaries contain massive levels of atresia (>30% vitellogenic oocytes), occasionally few residual hydrated oocytes. Females are in postspawning condition (a). In some cases this stage could correspond also to skip spawners.
	<i>Recovering</i>	Small as in immature ovaries but with signs of previous spawning, thick wall, reddish-grey and more opaque than immature.	Most of the oocytes are in the primary growth stage. Some residual old atretic oocytes could be present in the ovary. The ovary wall is considerably thicker than in immature females. Occasionally, new recruiting cortical alveoli oocytes are present.

(a) Greer Walker et al. (1994), Murua and Motos (2006).

**Table 4.** Mean and standard error values of the gonado- (GSI), hepatosomatic (HSI) indices and condition factor (K) and average relative intensity of follicular atresia at different maturity stages.

Maturity stage	GSI		HSI		K		Follicular atresia (%)
	Mean	SE	Mean	SE	Mean	SE	
Ripening	1.8	0.1	5.0	0.2	1.10	0.01	1
Spawning-hydrated	5.4	0.2	3.7	0.1	1.08	0.01	0
Recently spawned	3.5	0.2	3.7	0.3	1.03	0.01	0
Partly spent	3.7	0.4	2.0	0.2	1.07	0.02	23
Inactive mature	2.0	0.2	3.9	0.7	1.09	0.02	54
Recovering	0.8	0.01	4.0	0.6	1.05	0.02	2



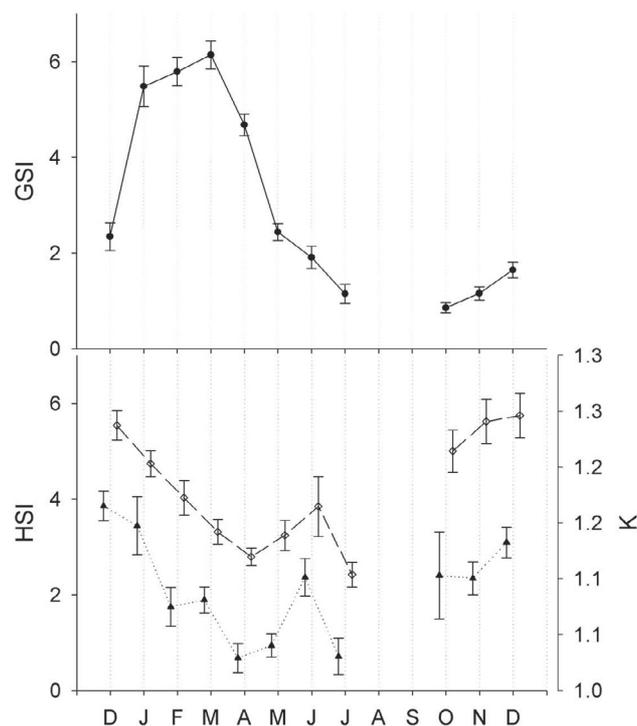
**Fig. 3.** Ovarian maturity stage frequency of pouting (*Trisopterus luscus*), by month of collection during the December 2003 through 2004.

(Kurita et al. 2003) based on the Delesse principle: average relative intensity of atresia (RIA): the geometric mean of relative intensity of follicular atresia among fish with atresia, multiplied by prevalence. This index indicates relative intensity of atresia at the population level.

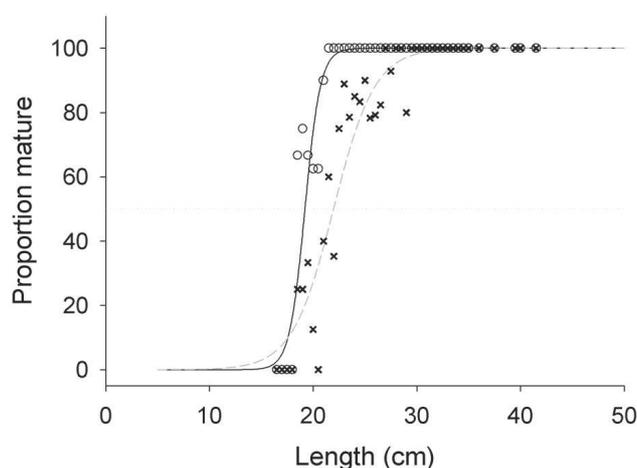
It is possible to determine the number of cells, e.g. atretic oocytes, from histological sections by applying the principles of stereology. In the present work, stereology was applied to estimate the number of atretic oocytes following Emerson’s method (Emerson et al. 1991) based on the Delesse principle, and using the Weibel equation (Weibel et al. 1966) presented by Murua et al. (2003). The Delesse principle states that the fractional volume of a component is proportional to its fractional cross sectional area, in this case the histological ovary section where we applied the Weibel grid to count the points associated with atretic oocytes.

In species with determinate fecundity, the annual fecundity is considered to correspond to potential fecundity after correcting for atretic losses. The annual fecundity, for species with indeterminate fecundity, should be calculated by estimating the number of oocytes spawned per batch, the percentage of females spawning per day (spawning fraction), and the duration of the spawning season (Hunter and Macewicz 1985).

The number of developing oocytes, NDO, was estimated by separating oocytes following the methodology described by Lowerre-Barbieri and Barbieri (1993). The NDO is the standing stock of yolked oocytes (Murua and Motos 2006; Domínguez-Petit 2007). Oocytes ( $n = 952$ ) were previously measured in histological sections to establish their threshold size in the cortical alveoli stage, which was fixed at



**Fig. 4.** Mean monthly variation in the gonadosomatic index (GSI, ●), hepatosomatic index (HSI, ◇) and condition factor (K, ▲) of female pouting (*Trisopterus luscus*), based on the December 2003 through 2004. Standard error of the mean is represented by vertical bars.



**Fig. 5.** Logistic regression model for the estimated percent of sexually mature female pouting (*Trisopterus luscus*) as a function of total length, using macroscopic (X,  $n = 443$ ) and histological (o,  $n = 443$ ) staging methods.

150  $\mu\text{m}$ . The number of oocytes in the ovary ( $n = 99$  ovaries) was estimated by combining the gravimetric method with a computer-aided image analysis system that enumerated and measured oocyte diameters in a subsample of  $\sim 0.050$  g. Measurements (number of oocytes  $> 100$  in each ovary) were performed using QWin software (© Leica Imaging Systems) on a PC (AMD Athlon XP 3000+) connected to a video camera

(Leica IC A) on a stereo microscope (Leica MZ6). The relative number of developing oocytes, RNDO, was assessed by individually dividing the NDO by the W ( $n^{\circ}$  oocytes/g of female). Batch fecundity was estimated from ovaries without POF or with POF older than 24 hours (Domínguez-Petit 2007), using the simple method described by Bagenal and Braum (1978). Hydrated oocytes were counted manually in a 150 mg subsample, and the image analysis system was tested for batch fecundity with a high correlation coefficient between the results of both methods ( $r^2 = 0.908$ ,  $p < 0.001$ ).

Nonparametric Kruskal-Wallis tests (H-tests) were applied looking for difference in GSI, HSI and K indexes throughout the year and between maturity stages. For NDO-Length relationships nonlinear power models were fitted, while for NDO-Weight relationships we used simple linear regression. ANCOVA tests were applied to compare those relationships, after respective linear transformation, between pre-spawning and spawning females.

## Results

### Reproductive cycle

Female pouting maturation was assessed throughout the year (Fig. 3), and at least some of the population was seen to be spawning every month (spawning includes the stages: spawning-hydrated, recently spawned and partly spent). Nearly all females participated in spawning during February (100%), March (75%), and April (100%), indicating that the primary spawning season extends from late winter through early spring. The rate of spawning declined in May (40%) and the proportion of post-spawning females increased (20%). The proportion of females in the recovering stage increased from the end of the spawning season in late spring until early autumn. Similarly, the number of specimens at the ripening stage increased after the primary spawning season. This pattern seems to be corroborated by the seasonal variability in GSI, HSI and K (Fig. 4). The mean GSI values remained low between May and December ( $GSI < 3$ ), then sharply increased between December and January, and peaked between January and March. The peak GSI period coincided with a peak in the proportion of females at the hydrated stage.

HSI and K fluctuated throughout the year almost in parallel (Fig. 4), but followed an opposite pattern to GSI during the spawning season (winter-early spring): the highest values of HSI and K were observed from October to January ( $HSI > 5$ ), prior to peak spawning, and decreasing during the spawning season to reach their minimum in April-July, at the end of main spawning activity. Nevertheless, the three indexes remained at low levels in females at the spent stage (inactive mature) (Table 4). These differences between months were significant for HSI (H-test:  $H_{(10,n=193)} = 70.69$ ,  $p < 0.01$ ) and K ( $H_{(10,n=404)} = 75.81$ ,  $p < 0.01$ ), and changed significantly with maturity stage ( $H_{(5,n=193)} = 33.33$ ,  $p < 0.01$  and  $H_{(5,n=403)} = 23.92$ ,  $p < 0.01$ , respectively). A clear decreasing pattern was observed from the highest values of HSI and K in pre-spawning females to the lowest values at the end of spawning activity (Fig. 4).

**Table 5.** Results of the length-at-maturity ( $L_{50}$ ) analysis and the parameters of the logistic equation.

	$L_{50}$ (cm)	Parameters	
		$a$	$b$
Macroscopic criteria	21.9	-11.00	0.50
Histology criteria	19.2	-25.77	1.34

### Maturity ogives

All specimens sampled during the year were used to estimate maturity ogives and length at 50% maturity ( $L_{50}$ , Fig. 5), based on macroscopic and microscopic (histological) maturity staging methods. There were significant differences between the  $L_{50}$  estimates ( $Z = 7.29$ ,  $p < 0.01$ ) based on the macroscopic, 22 cm, and microscopic, 19.2 cm maturity classification methods (Table 5). Similarly, the maturity ogives differed in shape (Fig. 5). The main differences were observed in larger females that were classified histologically as mature recovering females, but macroscopically as immature. These discrepancies were most prominent among pouting collected during the summer and autumn, after the peak spawning period (February-April).

### Oocyte development

Histological examination of the gonads revealed that pouting exhibit an asynchronous ovarian development organization, i.e. oocytes of all stages of development are present without a dominant population. This asynchrony was reflected by the mean oocyte size-frequency distribution (Fig. 6). In pouting, there was a continuous oocyte size-frequency distribution for every maturity stage except ovaries at the hydrated stage, which had a separate mode of very large ( $>800 \mu\text{m}$ ) hydrated oocytes. Throughout all the mature maturity stages, there were several modes within the continuous oocyte size frequency distribution, which indicated the presence of several different batches of oocytes.

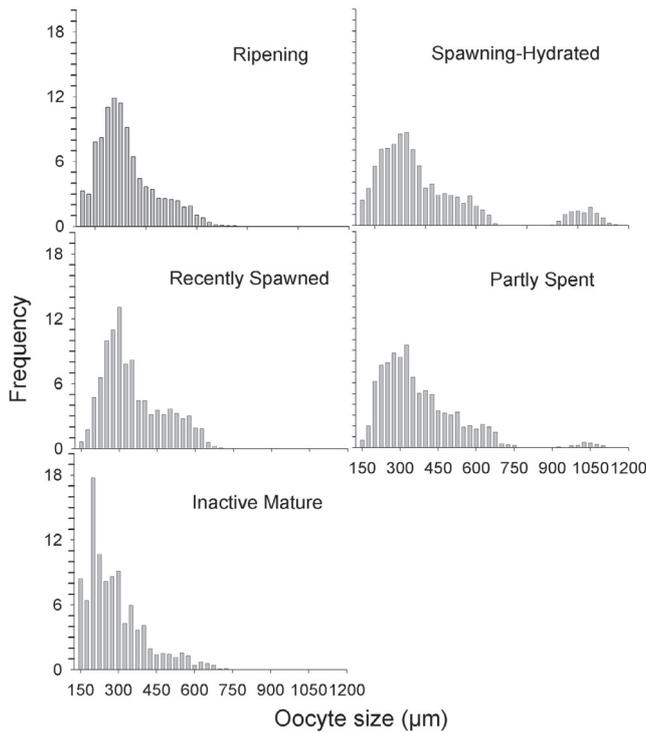
Mean oocyte diameter, excluding hydrated oocytes, showed a slight decrease ( $p = 0.05$ ,  $r^2 = 0.072$ ) as the main spawning season progressed from January to May 2004 (Fig. 7).

### Fecundity

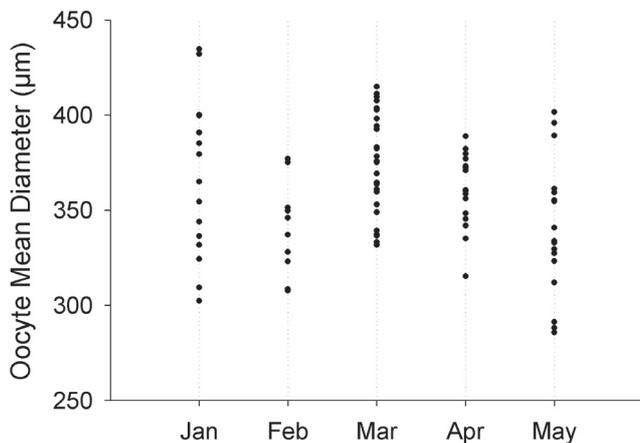
The relative number of developing oocytes (RNDO) per g of gutted body weight, significantly declined ( $r^2 = 0.996$ ;  $p < 0.01$ ) over the main spawning season from January to May (Fig. 8). The linear regression, assessed with the mean monthly (from January to May 2004) values of both fecundity estimators during the spawning season, was:

$$\text{RNDO} = 11362 - 175.325 \times \text{Month} \quad (4)$$

Where 95% confidence interval (CI) of estimated parameter for the linear regression equation (Eq. (4)) was -195 to -156.

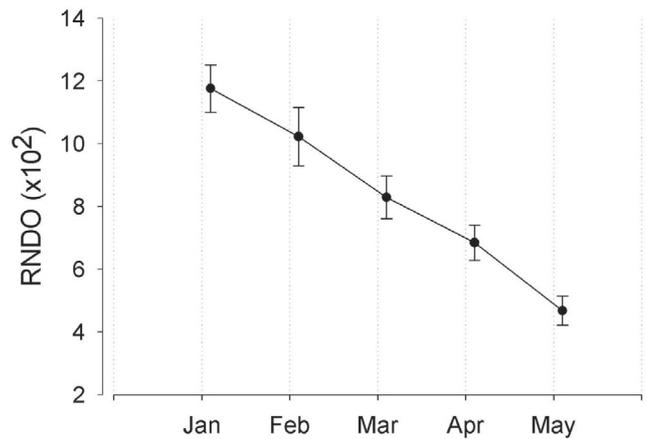


**Fig. 6.** Pouting (*Trisopterus luscus*) mean oocyte size-frequency distribution by maturity stage: ripening ( $n = 9$ ), spawning-hydrated ( $n = 9$ ), recently spawned ( $n = 9$ ), partly spent ( $n = 4$ ) and inactive mature ( $n = 4$ ).



**Fig. 7.** Mean diameter of the developing oocytes by month, during the spawning season for pouting (*Trisopterus luscus*). Dotted line represents fitted curve for linear regression.

NDO for the whole spawning season (January-May) ranged from 20 000 to 1 327 000 oocytes per female (fish length ranged between 19 and 40 cm). Taking into account all the samples taken during the year, NDO-Length and NDO-Weight relationships were significant but both relationships showed a relatively low fit ( $r^2 = 0.649$  and  $r^2 = 0.699$  respectively). However, when only females at stages prior to



**Fig. 8.** Mean relative number of developing oocytes (RNDO) by month of collection during the spawning season for pouting (*Trisopterus luscus*). Standard error of the mean is represented by vertical bars.

spawning were considered, these relationships improved notably (NDO-Length:  $r^2 = 0.869$ ;  $p < 0.01$  and NDO-Weight:  $r^2 = 0.888$ ;  $p < 0.01$ ). Estimates of parameters are listed in Table 6.

The NDO-Weight and NDO-Length (Fig. 9) relationships were significantly different if we compared pre-spawning and spawning females for each of these variables (ANCOVA:  $F = 137.58$ ;  $p < 0.01$  and  $F = 73.95$ ;  $p < 0.01$  respectively).

Batch fecundity (number of hydrated eggs) was slightly related to female length and gutted weight:

$$\text{Batch Fecundity} = -2979 + 52.042 \times W \quad \text{with } r^2 = 0.312; p < 0.01 \quad (5)$$

$$\text{Batch Fecundity} = 0.028 \times L^{3.802} \quad \text{with } r^2 = 0.293; p < 0.01 \quad (6)$$

Batch fecundity ranged between 50 and 57 500 hydrated oocytes (fish length ranged between 19 and 40 cm) throughout the spawning season.

RNDO and relative batch fecundity yielded a mean of 803 developing oocytes and 46 hydrated oocytes respectively per gram of gutted female throughout the year. If we take into account only pre-spawning females, RNDO yields a mean of 1176. Thus, a female of 300 g weight (30 cm total length approximately) in pre-spawning condition would have 352 800 developing oocytes on average and could spawn a total of 13 800 eggs per batch.

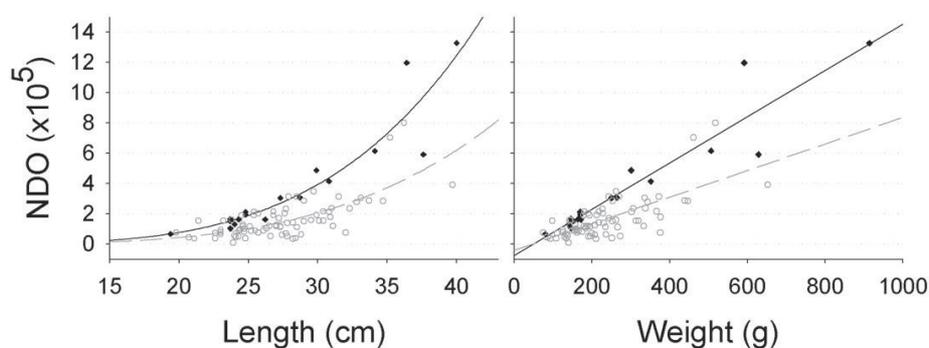
The ratio NDO/batch fecundity gives an approximation of the total mean number of batches that will be released; in this case this relationship was assessed using the mean NDO (210 560 oocytes) divided by the mean batch fecundity (10 805 hydrated oocytes). The result gave an estimate of 20 batches per female during the spawning season.

**Table 6.** Estimates of parameters for numbers of developing oocytes (NDO): NDO-Length and NDO-Weight relationships in female pouting prior to spawning (pre-spawning), spawning and both together (total).

NDO relationship		<i>p</i> value	<i>r</i> <sup>2</sup>	<i>a</i>	CI ( <i>a</i> )	<i>b</i>	CI ( <i>b</i> )
Length <sup>1</sup>	Total	<0.01	0.649	0.027	−0.038; 0.092	4.721	4.042; 5.340
	Pre-Spawning	<0.01	0.869	0.505	−1.375; 2.385	3.993	2.954; 5.032
	Spawning	<0.01	0.539	0.519	−0.816; 1.854	3.808	3.063; 4.552
Weight <sup>2</sup>	Total	<0.01	0.699	−106 436	−156 163; −56 709	1305	1127; 1483
	Pre-Spawning	<0.01	0.888	−73 388	−186 146; 39 370	1537	1236; 1837
	Spawning	<0.01	0.539	−41 379	−92 510; 9 751	921	718; 1123

<sup>1</sup> Power equation:  $NDO = a L^b$

<sup>2</sup> Regression equation:  $NDO = a + b W$

**Fig. 9.** Fitted curves of number of developing oocytes (NDO)-Length and NDO-Weight relationships. Black diamonds correspond to females prior to spawning. Grey circles represent spawning females.

### Follicular atresia

Follicular atresia was observed throughout the year, but the levels of prevalence and intensity varied considerably (Table 1). The lowest average relative intensity of follicular atresia was observed from December 2003 to April 2004 (0 to 5%), i.e. just prior to or during the main spawning season. Conversely, the highest atresia levels, ranging from 0 to 19%, were observed in May, and thus coincided with the beginning of the post-spawning period.

### Discussion

Pouting, *Trisopterus luscus*, is one of the most abundant and commercially important demersal fish on the coast of Galicia. In spite of decreasing commercial catch rates, the only restriction on harvesting is a minimum fish size of 20 cm. There is no scientific stock assessment for pouting and thus no biological reference points to prevent overfishing.

This study determined, based on histological methods, that length at maturity ( $L_{50}$ ) was 19.2 cm. A result that differed from previous estimates of 18.2 cm (Labarta et al. 1982) and 22 cm (Merayo 1996a), which were based on macroscopic assessment. The macroscopic estimate in the present study, 22 cm, was nevertheless similar to that obtained by

Merayo (1996a). Differences between histological and macroscopic ogives normally arise from the difficulty of distinguishing immature from spent fish. Histological staging gives less biased estimates of maturity (Hunter et al. 1992; Stark 2007). However, size at maturity is known to be a highly plastic parameter that changes under external pressure, and so the observed differences may thus be the consequence of changes in the stock (Trippel 1995; Domínguez-Petit et al. 2008).

Histological examination of the gonads revealed that pouting possesses asynchronous ovarian development, i.e. oocytes of all stages of development are present without dominant cohorts (Wallace and Selman 1981; Murua and Saborido-Rey 2003). Pouting has a very protracted spawning season, indicated by the occurrence of some females in spawning condition throughout the year. Most females began spawning during the first part of the year (January to May), and peak spawning occurred between February and April. Similar results were reported by Labarta et al. (1982) and Merayo (1996a). The period of peak spawning was tracked by the GSI results in this study. GSI was being rebuilt in October through December, after the peak spawning period. Most species showing asynchronous oocyte development have indeterminate fecundity, but some may have determinate fecundity (Hunter and Macewicz 1985; Greer Walker et al. 1994; Murua and Saborido-Rey 2003). However, in these species egg release is concurrent with oocyte recruitment, and hence

ovary weight changes only slightly during most of the spawning season: giving a dome-shape GSI curve, or simply no trend at all. However in pouting, the GSI peak was followed by a sharp decrease over the next two months, which indicates that there was a rapid loss of eggs and no replacement. The lack of oocyte replacement over the spawning season implies determinate fecundity.

Several methods were used to ascertain fecundity type. The first method compared variation in the stage-specific oocyte size-frequency distribution during the annual reproductive cycle. The results showed that oocyte size distribution did not present a well-developed hiatus between primary growth oocyte stock and the standing stock of developing oocytes except hydrated ones, which should not be considered for this type of analyses.

A second method was therefore used to define pouting fecundity, by monitoring changes in mean diameters of developing oocytes over the spawning season. The evolution of the mean oocyte diameter showed a low but significant decrease over the spawning season, which could mean that there was recruitment of new oocytes to the stock of developing oocytes over the spawning season, characteristic of species with indeterminate fecundity (Hunter et al. 1989). However, the observed decrease may also be the consequence of the asynchronous development of oocytes. Nevertheless, when mean oocyte diameter was analyzed in the successive ovary developmental stages instead of through the season, a slight increase was observed that suggests there is no *de novo* vitellogenesis after the onset of ripening, i.e. that fecundity is determinate.

This conclusion was supported by the results from another method monitoring the relative number of developing oocytes (RNDO) within the ovaries during the spawning season (Murua and Saborido-Rey 2003). Pouting RNDO decreased during the spawning season, indicating that there was no replacement of the standing stock of oocytes after every spawned batch and thus determinate fecundity (Hunter et al. 1989). Similarly, total fecundity was one million oocytes for a 40 cm female on average, a level characteristic of determinate fecundity. Additionally, NDO-Length and NDO-Weight relationships showed higher numbers of developing oocytes in females prior to spawning. Under the premise that pouting are determinate spawners, NDO in pre-spawning females can be assumed to be an index of potential fecundity.

The relative batch fecundity range of pouting in this study was from 5 to 67 eggs  $g^{-1}$  (fish weight range: 108 to 366 g). This range contrasts with other indeterminate spawning species, which produced larger batches, such as *Merluccius merluccius*, *M. capensis* and *M. paradoxus* with 123, 160 and 306 eggs  $g^{-1}$  of relative batch fecundity respectively, or clupeids like *Sardina pilchardus* with close to 350 eggs  $g^{-1}$  (Osborne et al. 1999; Ganas et al. 2004; Murua et al. 2006). The ratio between the number of developing oocytes and batch fecundity had an average value of 20. Therefore, assuming a spawning season of 4–5 months, pouting could produce a batch every 6–7 days. This means that if pouting is a determinate spawner, a female will spawn an average 20 batches during the spawning season: a figure very close to that for other determinate species (Kjesbu 1989; Kjesbu et al. 1996).

Levels of atresia during the year varied considerably, this criterion should therefore be used with caution. Highest levels of atresia were found at the end of spawning season, May and June, but a smaller peak of atresia was found just before the next spawning season (December 2004). This second peak could be associated with the fecundity regulation process typical in determinate fecundity species like Atlantic herring (Kurita et al. 2003).

The spawning activity of female pouting was reflected in the GSI. GSI development was correlated with the HSI and K, which had a clear inverse seasonal pattern to GSI. The correlation suggests that there was a mobilization of reserves for gonad development and high energy investment in reproduction (Merayo 1996b; Murua et al. 2006). The condition index could be used to forecast potential energy content and nutritional state in the stock, as it is done for other determinate species like cod (Lambert and Dutil 1997). The condition factor index could also be used to predict the species fecundity and reproductive success (Kjesbu et al. 1991; Marshall et al. 2003).

Knowledge of spawning pattern and reproductive strategy is a basic requirement for the improvement of fish stock management (Marshall et al. 2003). This study shows that pouting on the Galician shelf mature at a greater length than was previously estimated, have asynchronous oocyte development, and a protracted spawning season from January to May with a spawning peak between February and April. However, one of the most important findings in this study is that pouting exhibit determinate fecundity. This is a key aspect to understanding oocyte recruitment and reproductive strategy, estimates can be made of individual potential fecundity that will facilitate estimation of total egg production and future studies on stock-recruitment relationships.

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## References

- Ashton W.D., 1972, The logit transformation with special reference to its uses in bioassay. Haffner Publishing Co., New York.
- Bagenal T.B., Braum E., 1978, Eggs and early life history. Methods for Assessment of Fish Production in Fresh Waters. Blackwell Scientific Publications, pp. 165–201.
- Desmarchelier M., 1985, Growth and reproduction of the whiting-pout (*Trisopterus luscus* L., 1758) in the English Channel and in the south of the North Sea. ICES CM1985/G, 29.
- Domínguez-Petit R., 2007, Study of reproductive potential of *Merluccius merluccius* in the Galician Shelf. Doctoral thesis, University of Vigo <http://hdl.handle.net/10261/4377>.
- Domínguez-Petit R., Korta M., Saborido-Rey F., Murua H., Saínza M., Piñeiro C., 2008, Changes in size at maturity of European hake Atlantic populations in relation with stock structure and environmental regimes. J. Mar. Syst. 71, 260–278.

- Emerson L.S., Greer Walker M., Witthames P.R., 1991, A stereological method for estimating fish fecundity. *J. Fish Biol.* 36, 721–730.
- Fonseca P., Martins R., Campos A., Sobral P., 2005, Gill-net selectivity off the Portuguese western coast. *Fish. Res.* 73, 323–339.
- Fowler A.J., Jensen A.C., Collins K.J., Smith P., 1999, Age structure and dial activity of pouting on the Poole Bay artificial reef. *J. Fish Biol.* 54, 944–954.
- França C., Costa M.J., Cabral H.N., 2004, Use of the coastal areas adjacent to the Douro estuary as a nursery area for pouting, *Trisopterus luscus* Linnaeus, 1758. *J. Appl. Ichthyol.* 20, 99–104.
- Ganias K., Somarakis S., Machias A., Theodorou A., 2004, Pattern of oocyte development and batch fecundity in the Mediterranean sardine. *Fish. Res.* 67, 13–23.
- Greer Walker M., Witthames P.R., Bautista de los Santos I., 1994, Is the fecundity of the Atlantic mackerel (*Scomber scombrus*, Scombridae) determinate? *Sarsia* 79, 13–26.
- Hunter J.R., Macewicz B.J., 1985, Measurement of spawning frequency in multiple spawning fishes. In: R. Lasker (Ed.). An egg production method for estimating spawning biomass of pelagic fish, Application to the northern anchovy, *Engraulis mordax*. NOAA Tech. Rep. NMFS 36, 79–94.
- Hunter J.R., Macewicz B.J., Kimbrell C.A., 1989, Fecundity and other aspects of the reproduction of sablefish, *Anoplopoma fimbria*, in central California waters. *CalCOFI Rep.* 30, 61–72.
- Hunter J.R., Macewicz B.J., Lo N.C., Kimbrell C.A., 1992, Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fish. Bull.* 90, 101–128.
- Kjesbu O.S., 1989, The spawning activity of cod, *Gadus morhua* L. *J. Fish Biol.* 34, 195–206.
- Kjesbu O.S., Klungsoyr J., Kryvi H., Witthames P.R., Greer Walker M., 1991, Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. *Can. J. Fish. Aquat. Sci.* 48, 2333–2343.
- Kjesbu O.S., Solemdal P., Bratland P., Fonn M., 1996, Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 53, 610–620.
- Kraus G., Tomkiewicz J., Köster F.W., 2002, Egg production of Baltic cod (*Gadus morhua*) in relation to variable sex ratio, maturity, and fecundity. *Can. J. Fish. Aquat. Sci.* 59, 1908–1920.
- Kurita Y., Meier S., Kjesbu O.S., 2003, Oocyte growth and fecundity regulation by atresia of Atlantic herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle. *J. Sea Res.* 49, 203–219.
- Labarta E., Ferreiro M.J., 1982, Age and growth of the Galician coast pouting (*Trisopterus luscus* L.). Preliminary data. *ICES CM 1982/G: 65*.
- Labarta E., Ferreiro M.J., Fenandez M.T., Martinez F., 1982, Spawning season and first maturity size and age of the Galician coast pouting (*Trisopterus luscus* L.). *ICES CM1982/G: 64*.
- Lambert Y., Dutil J.D., 1997, Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? *Can. J. Fish. Aquat. Sci.* 54 (Suppl. 1), 104–112.
- Lowerre-Barbieri S.K., Barbieri L.R., 1993, A new method of oocyte separation and preservation for fish reproduction studies. *Fish. Bull.* 91, 165–170.
- Marshall C.T., Kjesbu O.S., Yaragina N.A., Solemdal P., Ulltang, O., 1998, Is spawner biomass a sensitive measure of the reproductive and recruitment potential of northeast Arctic cod? *Can. J. Fish. Aquat. Sci.* 55, 1766–1783.
- Marshall C.T., O'Brien L., Tomkiewicz J., Köster F.W., Kraus G., Marteinsdottir G., Morgan M.J., Saborido-Rey F., Blanchard J.L., Secor D.H., Wright P.J., Mukhina N.V., Bjornsson H., 2003, Developing alternative indices of reproductive potential for use in fisheries management, case studies for stocks spawning, an information gradient. *J. Northw. Atl. Fish. Sci.* 33, 161–190.
- Marteinsdottir G., Begg G.A., 2002, Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.* 235, 235–256.
- Merayo C.R., Villegas M.L., 1994, Age and growth of *Trisopterus luscus* (Linnaeus, 1758) (Pisces, Gadidae) off the coast of Asturias. *Hydrobiologia* 281, 115–122.
- Merayo C.R., 1996a, Reproduccion y fecundidad de la faneca *Trisopterus luscus* (Linnaeus, 1758) (Pisces, Gadidae) en la region central del mar Cantabrico. *Bol. Inst. Esp. Oceanogr.* 12, 17–29.
- Merayo C.R., 1996b, Seasonal changes in the biochemical composition of the muscle and liver of bib (*Trisopterus luscus* L.) (Pisces, Gadidae) from the Cantabrian Sea (N Spain). *Sci. Mar.* 60, 489–495.
- Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera S., 2003, Procedures to estimate fecundity of marine fish species relation to their reproductive strategy. *J. Northw. Atl. Fish. Sci.* 33, 33–54.
- Murua H., Saborido-Rey F., 2003, Female reproductive strategies of marine fish species of the north Atlantic. *J. Northw. Atl. Fish. Sci.* 33, 23–31.
- Murua H., Lucio P., Santurtun M., Motos L., 2006, Seasonal variation in egg production and batch fecundity of European hake *Merluccius merluccius* (L.) in the Bay of Biscay. *J. Fish Biol.* 69, 1304–1316.
- Murua H., Motos L., 2006, Reproductive strategy and spawning activity of the European hake *Merluccius merluccius* (L.) in the Bay of Biscay. *J. Fish Biol.* 69, 1288–1303.
- Osborne R.F., Melo Y.C., Hofmeyr M.D., Japp D.W., 1999, Serial spawning and batch fecundity of *Merluccius capensis* and *M. paradoxus*. *S. Afr. J. Mar. Sci.* 21, 211–216.
- Puente E., 1988, Edad y crecimiento de la Faneca, *Trisopterus luscus* (L. 1758) (Pisces, Gadidae) en la costa atlantica francesa. *Bol. Inst. Esp. Oceanogr.* 5, 37–55.
- Saborido-Rey F., Junquera S., 1998, Histological assessment of variations in sexual maturity of cod (*Gadus morhua* L.) at the Flemish Cap (north-west Atlantic). *ICES J. Mar. Sci.* 55, 515–521.
- Solemdal P., 1997, Maternal effects – A link between the past and the future. *J. Sea Res.* 37, 213–227.
- Stark J.W., 2007, Geographic and seasonal variations in maturation and growth of female Pacific cod (*Gadus macrocephalus*) in the Gulf of Alaska and Bering Sea. *Fish. Bull.* 105, 396–407.
- Tirard C., Thomas F., Raibaut A., Renaud F., 1996, The distribution and abundance of *Lernaeocera lusci* (Copepoda) on hake

- (*Merluccius merluccius*) and bib (*Trisopterus luscus*) (Teleostei). *Int. J. Parasitol.* 26, 1387–1392.
- Trippel E.A., 1995, Age at maturity as a stress indicator in fisheries. *Bioscience* 45, 759–771.
- Tyler C.R., Sumpter J.P., 1996, Oocyte growth and development in teleosts. *Rev. Fish Biol. Fish.* 6, 287–318.
- Vallin L., Nissling A., 2000, Maternal effects on egg size and egg buoyancy of Baltic cod, *Gadus morhua*. Implications for stock structure effects on recruitment. *Fish. Res.* 49, 21–37.
- Wallace R., Selman K., 1981, Cellular and dynamic aspects of oocyte growth in Teleosts. *Am. Zool.* 21, 325–343.
- West G., 1990, Methods of assessing ovarian development in fishes, A review. *Aust. J. Mar. Freshwater Res.* 41, 199–222.
- Weibel E.R., Kistler G.S., Scherle W.F., 1966, Practical stereological methods for morphometric cytology. *J. Cell Biol.* 30, 23–38.
- Wheeler A., 1978, Key to the fishes of Northern Europe, a guide to the identification of more than 350 species. Frederick Warne, London.
- Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielsen J., Tortonese E., 1986, *Fishes of the North-eastern Atlantic and the Mediterranean*. UNESCO, Paris.