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Original Article

# An intra-annual analysis of intermediate fecundity, batch fecundity and oocyte size of ripening ovaries of Pacific sardine *Sardinops sagax* in northern Chile

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***ABSTRACT:*** An intra-annual analysis of the intermediate fecundity, batch fecundity and oocyte size of ripening ovaries of Pacific sardine (*Sardinops sagax*) in northern Chile was carried out over an annual cycle from April 1992 to March 1993. Both the shorter and larger oocyte size classes of ripening ovaries were used to estimate the intermediate fecundity and batch fecundity, respectively. The relationship between both fecundates and their respective mean oocyte sizes were studied as well. Clear seasonal variations of the intermediate and batch fecundity were observed, which follows a similar trend reported in previous studies for the reproductive cycle of *S. sagax*. Two peaks were found with two periods of lower production of oocytes between them: the first peak occurred around winter and the second around summer. Mean oocyte diameters of the intermediate mode (ODIM) and mean oocyte diameters of the most advanced mode (ODAM) were found to have the same seasonal traits; that is, bigger oocytes in winter than in summer. The highest decrease in the oocyte diameter occurred during those months with lower reproductive activity. Possible factors influencing the fecundity variations as reproductive season progressed are discussed.

***KEY WORDS:*** **fecundity, northern Chile, oocyte size, *Sardinops sagax*.**

## INTRODUCTION

It is well known that in multiple-spawning fishes spawning frequency and batch fecundity have intra- and interannual fluctuations.[1–4] These variations can be a consequence of different causes, such as available food, size structure of the population, temperature and stock size.[2,5,6] Batch fecundity is highly variable within a species and may vary during a season, between years, and between subpopulations. The controlling variables for theses changes are probably energy supply, egg size and its relation to larval growth and survival, and duration of the production cycle.[3]

Most research regarding the reproductive cycle of Pacific sardine (*Sardinops sagax* Jenyns, 1842) of northern Chile have focused on estimates of spawning frequency and batch fecundity.[7–14] These studies have allowed the identification of one breeding season with two spawning periods: the first period from April to October and the second one from January to March. A period with lower reproductive activity between peaks has also been described.

*Sardinops sagax* has an asynchronic ovary with multiple spawning during the breeding season.[7–10] By analyzing the postovulatory follicles, Herrera and Claramunt found that each female spawns every 6.6 days during winter and every 10.5 days during summer;[11] however, as the reproductive season progresses, spawning intensity unavoidably decreases. Examples of this situation are the numerous reports describing the marked failure of batch fecundity of *S. sagax* at the end of each reproductive period.[7,11,13–16] Although these variations have been well reported, attempts to explain this process as the reproductive season progresses have not been developed in *S. sagax*.

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In northern Chile, the size frequency distributions of ripening ovaries of *S. sagax* have been studied by Herrera and Claramunt.[11,17] They have described two modes of yolked oocytes: (i) the intermediate mode (IM) containing yolked oocytes measuring from 200 μm to 400 μm in diameter; and (ii) the most advanced mode (MAM) containing oocytes in advanced vitellogenesis from 450 μm to 650 μm in diameter. Despite that MAM has been used widely in estimations of batch fecundity, IM has remained largely unexplored. Studying the seasonal fluctuations of IM upon ripening ovaries in the same development stage could provide a measure of the dynamics of oocyte production as a season progresses in species with an extended spawning period. In this context, the purpose of the present study was to investigate the fluctuations of both batch and intermediate fecundity, as well as the seasonal variation of oocyte size of ripening ovaries, on a complete annual cycle of *S. sagax*. The results brought new questions on the reproductive cycle of this species.

## MATERIALS AND METHODS

### Sampling

Fish samples were collected aboard commercial fishing boats from April 1992 to March 1993 throughout an area of exploitation approximately between 19°10′S and 21°30′S, off the Chilean coast (Fig. 1). Total length and ovary weight were measured to the nearest 0.1 cm and 0.001 g, respectively. Gonadosomatic index (GSI) [GSI = Gonad wet weight × (Total bodyweight)$^{-1}$ × 100] was calculated. The present study included only those females with ripening ovaries in the same reproductive stage closest to the hydrated stage in which the vitellogenesis process has finished, without evidence of spawning activity (VT; Fig. 2d). This phase was confirmed in the whole ovaries by histological analysis of the ovarian parenchyma, as described by Herrera and Claramunt elsewhere.[11,20]

Because the influence of the female's size on the reproductive aspects of the Chilean sardine *S. sagax* has already been well documented,[12] specimens were separated into three size classes of total length: A, 24.1–26 cm; B, 26.1–28.0 cm; and C, 28.1 30.0 cm. A total of 843 females were analyzed (Table 1).

To study fecundity, a small transversal gonad piece, measured to the nearest 0.001 g, was submerged in Gilson's solution for at least 1 month. It is necessary to emphasize that Herrera and Claramunt have determined previously by using two-factor ANOVA and a Mann–Whitney's *U*-test that neither the sampling location within the ovaries nor the frequency distribution of oocytes influences the fecundity estimations, respectively.[11] The sample was stirred until the connective tissue and ovary membranes were removed. The oocytes were then passed through a system of sieves ranging from 200 μm to 1000 μm with an interval of 50 μm between the upper and lower sieves. The range and the intervals used have been described by Herrera and Claramunt as adequate for the separation of oocyte modes.[11] The system of sieves was placed over a variable speed shaker connected to a small suction pump. The pump elimi-

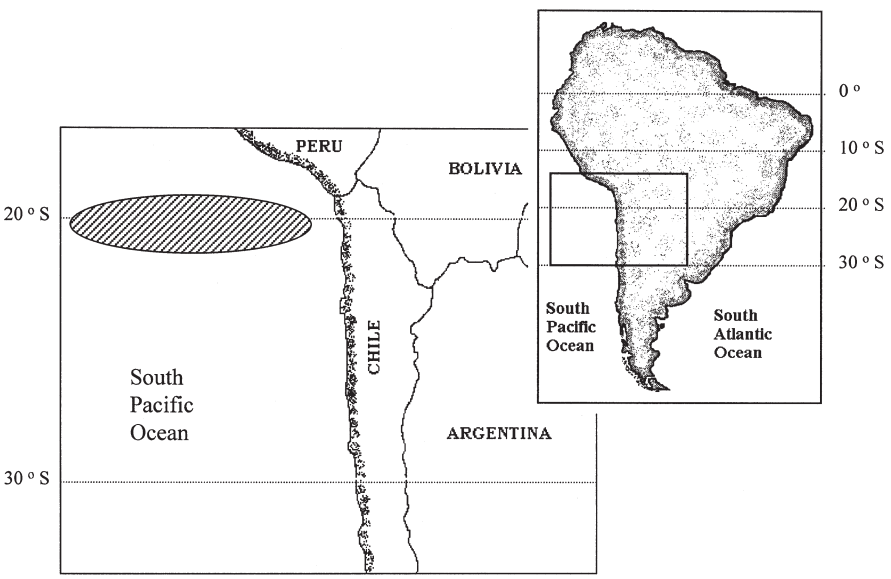


**Fig. 1** Distribution area of the commercial catches (hatched area) of *Sardinops sagax* used in the present study, located off northern Chile approximately from 19°10′S to 21°30′S.

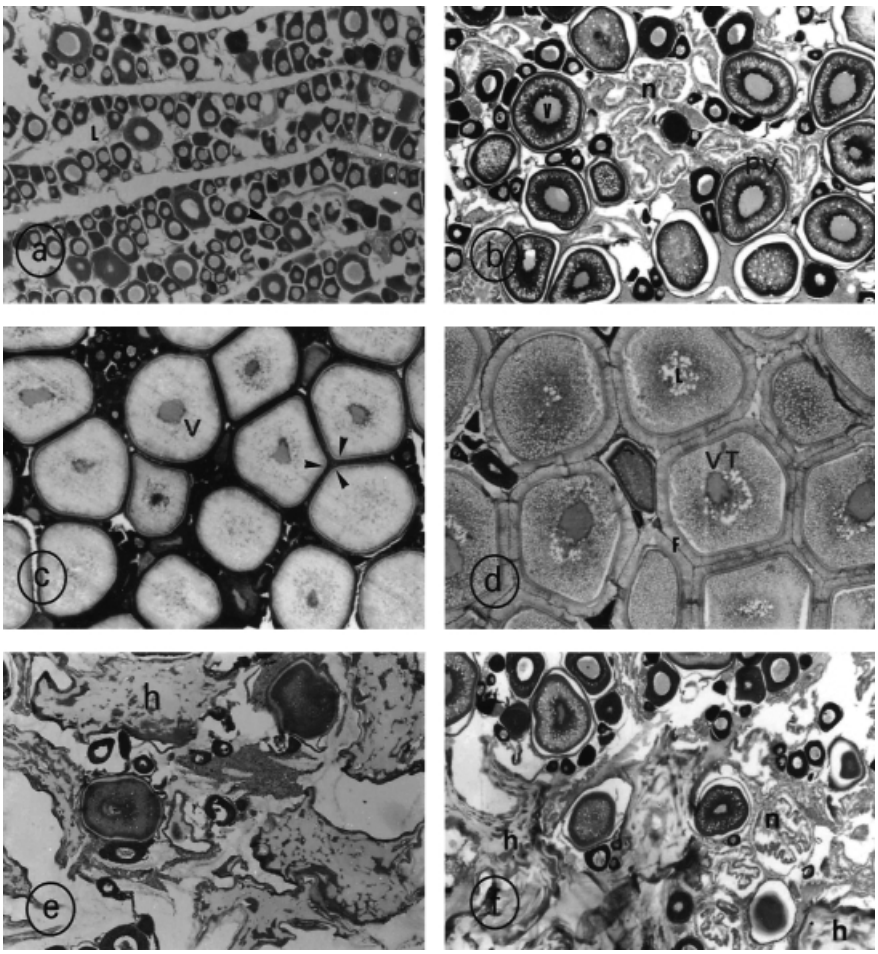


**Fig. 2** Light micrograph ×40 of the ovary stages of the Pacific sardine *Sardinops sagax* in northern Chile. (a) Immature ovary (L, lamella; arrow indicates previtellogenic oocyte). (b) Partially yolked ovaries (PV, partially yolked oocyte; v, germinal vesicle; n, newly postovulatory follicles). (c) Yolked ovary (V, yolked oocyte; arrow indicates radiate zone). (d) Ovary close to hydration stage (VT, ripening oocytes in which vitellogenesis has finished; F, follicular cells; L, fat drops). (e) Hydrated ovary (h, collapsed hydrated oocyte). (f) Partially spent ovary (h, hydrated oocyte; n, newly postovulatory follicle).

**Table 1** Numbers of female by length classes *Sardinops sagax* with ripening ovaries close to hydration stage collected off northern Chile during 1992–1993 spawning season

| Month | Length classes | | |
|---|---|---|---|
| | A | B | C |
| April | ND | 21 | 36 |
| May | 14 | 25 | 20 |
| June | 15 | 39 | 30 |
| July | 17 | 28 | 29 |
| August | 22 | 35 | 36 |
| September | 20 | 33 | 34 |
| October | 13 | 27 | 30 |
| November | ND | 10 | 18 |
| December | ND | 40 | 42 |
| January | 6 | 17 | 32 |
| February | 9 | 35 | 38 |
| March | 12 | 30 | 30 |
| Total | 128 | 340 | 375 |

A, 24.1–26 cm; B, 26.1–28 cm; C, >28 cm of total length; ND, no data available.

nates the cleaning water in order to facilitate the passing of the oocytes. Oocytes retained were counted under a stereomicroscope. Frequency distributions of the diameters of the oocytes were subdivided according to their normal components by using the Modal Progression Analysis (MPA) program of ELEFAN software.[18] The intermediate mode was defined as the oocyte class size just prior to the largest oocyte class (most advanced mode).

Intermediate and batch fecundates were estimated from the number of oocytes in the shorter and larger modal group, respectively, following the expression:

$$F_m = \left(\frac{E_t}{W_t}\right) \times W_s, \quad (1)$$

where $E_t$ is the total number of eggs counted, $W_t$ is the total sample weight (g), and $W_s$ is the weight of both ovaries in grams. The mean diameter of intermediate mode (ODIM) and mean diameter of

most advanced mode (ODAM) were determined as well.

To determine the seasonal pattern associated with the size between both fecundates, the following rate was used:

$$F_{ib} = \frac{IF}{BF}, \quad (2)$$

where *IF* is the intermediate fecundity and *BF* is the batch fecundity. This rate was also used as an indicator of individual production of oocytes as the breeding season progressed. It should be noted that the $F_{ib}$ rate resulted from individual estimations of each analyzed fish.

The seasonal variations of all these variables were analyzed on a monthly basis using one-way ANOVA because a two-way ANOVA (length class and season as factors) could not be applied to an incomplete design. Before ANOVA the data sets were analyzed for normal distribution and uniformity of variance with Kolmogorov–Smirnov and Barlett tests, respectively. When normal distribution failed the Kruskal–Wallis test was applied. Parametric and non-parametric multiple comparisons were carried out by using Tukey's Honest Significant Difference for unequal N and Nemenyi tests, respectively.[19] A result of $P < 0.05$ was considered significant. It is necessary to note that during the present study the intermediate fecundity and batch fecundity were studied by using ANOVA procedures; hence, neither the function between ovary weight on fecundity nor the ANCOVA procedures were considered.

Because Herrera and Claramunt have pointed out previously that the spawning period 1992/93 of *S. sagax* extended from June to October and from December to March,[11] and also to reduce the high number of multiple comparisons, only the maximum values of each period were compared. Furthermore, April and November have been historically described to have a lower reproductive activity. Thus, these months (defined for the present study as weakened months) were compared between themselves and with the maximum values obtained for the winter and summer months.

One-way ANOVA was applied to compare the differences among length classes. To determine normality and uniformity of variance, procedures already described elsewhere were applied. In April, November and December when there were no data for A length class (Table 1), the Dunn method was applied effecting parented comparisons.[19,21] The relationship of mean oocyte diameter between IM and MAM was studied using monthly linear regression of ODIM on ODAM. Estimated parameters were compared using ANCOVA, Student's *t*-test, and Tukey's multiple comparison test.

## RESULTS

### Intermediate fecundity and batch fecundity

Intermediate fecundity (*IF*) showed a clear seasonal trend, whereby two periods are defined clearly (Fig. 3a). The first period extended from April to September and the second from December to February. Maximum values were found during summer in all size classes, except the smaller ones, which showed significant differences ($P < 0.05$) between its highest value in June (53 525 oocytes) and its lowest values in March (28 162 oocytes) and January (38 089 oocytes). Both B and C length classes had their maximum values in February (i.e. 86 292 and 102 373 oocytes, respectively), in which the *IF* in both cases was significantly different from the *IF* of the weakened months. November had minimum values for both B (28 157) and C (39 018) length classes. Whole size classes showed a decrease in *IF* during July and August, but it was not significant ($P > 0.05$). Likewise, there were no significant differences in *IF* among winter and weakened months. The summer period was homo-

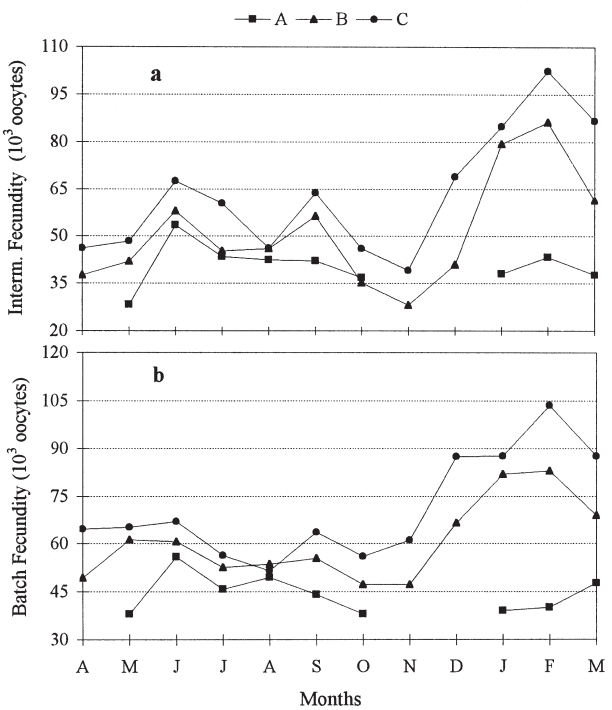


**Fig. 3** Mean values of (a) intermediate fecundity and (b) batch fecundity by length classes. A, 24–26 cm; B, 24–26 cm; and C, > 28 cm of total length.

geneous except for smaller fishes, which had significantly lower values of *IF* than bigger fishes. Generally, only a difference between the winter and summer months was observed (Fig. 3a). Mean analysis by size class showed significant differences only between smaller and bigger length classes.

Batch fecundity (*BF*) showed the same trend as *IF*, while multicomparison analyses throughout a season and among size classes showed similar results as well. The minimum and maximum mean values of the smaller length class were 37 964 and 55 842 oocytes in May and June, respectively. February shows maximum values for both B (82 239) and C (103 665) length classes. In contrast, minimum values occurred in August and November for B (47 254) and C length classes (51 543 oocytes), respectively (Fig. 3b).

### $F_{ib}$ rate

$F_{ib}$ rate also had a clear seasonal tendency to decline around weakened months, and also showed the same decrease in July ($P > 0.05$) in all length ranges. The maximum value of $F_{ib}$ for the smaller length class was found in February (1.21) and the minimum $F_{ib}$ occurred in May (0.73). Both B and C size classes had their maximum values in August (1.16) and January (1.06). The minimum values of $F_{ib}$ were found in November with 0.55 and 0.64 for B and C length classes, respectively (Fig. 4).

No significant difference ($P > 0.05$) in $F_{ib}$ rate was observed either within a period or between periods. In all length ranges, the major differences in $F_{ib}$ were found among maximum values of each period with those of weakened months ($P < 0.05$; Fig. 4). The influence of female size was not detected by the $F_{ib}$ rate ($P > 0.05$).

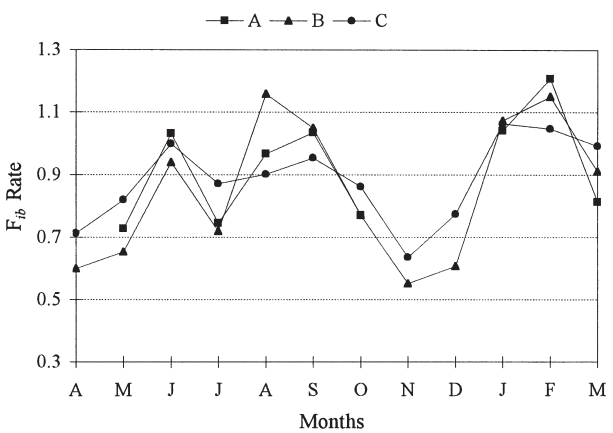


**Fig. 4** Mean values of the $F_{ib}$ rate by length classes. A, 24–26 cm; B, 24–26 cm; and C, > 28 cm of total length.

### Oocyte diameter

The seasonal traits described for the intermediate and batch fecundity were also found in the oocyte diameter. Mean ODIM showed maximum values in July with 277 µm, 289 µm and 293 µm for A, B and C length classes, respectively; whereas the minimum values were found in January (208 µm, A size class) and November for B (216 µm) and C (212 µm) size classes (Fig. 5a). Mean ODIM were significantly different ($P < 0.05$) between the winter and summer periods over whole size ranges; likewise, significant differences were found among maximum values in each peak with those ODIM values of weakened months ($P < 0.05$). Only the summer months showed significant differences ($P < 0.05$) in ODIM by length class (Fig. 5a).

The ODAM seasonal tendency resembled that of ODIM showing the maximum values in July in all length classes with 541 µm, 554 µm and 568 µm for A, B and C classes, respectively. The minimum values occurred in January (481 µm; A), December (480 µm; B) and November (479 µm; C). Multicomparison analyses throughout seasons as well as

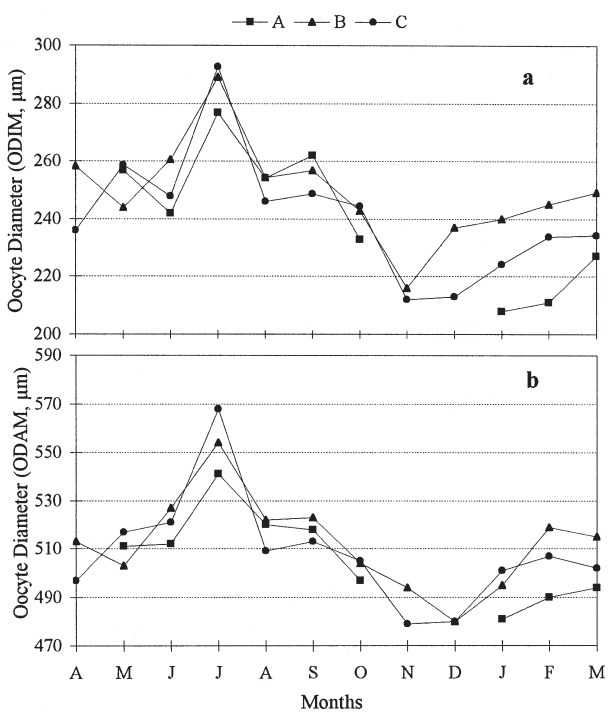


**Fig. 5** Mean values of (a) mean oocyte diameter of intermediate mode (ODIM) and (b) mean oocyte diameter of most advanced mode (ODAM) by length classes. A, 24–26 cm; B, 24–26 cm; and C, > 28 cm of total length.

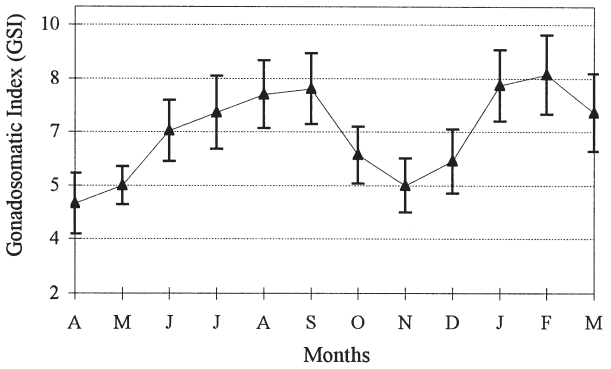


**Fig. 6** Mean gonadosomatic index (GSI) of ripening ovaries of *Sardinops sagax* during 1992–1993 reproductive period in northern Chile.

among size classes were rather similar to those of ODIM (Fig. 5b).

### GSI and size frequency distribution of oocytes of ripening ovaries

Gonadosomatic index analysis included the whole size range studied, which showed a similar seasonal bimodal tendency as those found in *IF*, *BF*, ODIM, and ODAM; and also fluctuated closely with $F_{ib}$ rate. Mean GSI ranged from 4.49 in April to 8.11 in February. There were significant differences only among the maximum GSI values of the winter and summer period with those of weakened months ($P < 0.05$; Fig. 6). The shape of the size frequency distribution of ripe oocytes also varied considerably during the study period. Figure 7 shows the predominant oocyte size frequency distribution for each month studied. Modal sizes were similar during the winter and summer months, whereas a clear asymmetry in the modal size was observed during the weakened months.

### Egg size relationship

Data of all females bigger than 26 cm total length were pooled to study the ODIM and ODAM relationships, because previous results showed that oocyte diameter was not influenced by female size. Regressions analyses showed strong linear relationships, with $r^2$ values being quite high and significant over the entire annual cycle. There were no significant differences (ANCOVA, $P > 0.05$) between the slopes for all months studied, and multiple comparison tests detected significant differences (ANCOVA, $P < 0.05$) in the intercepts only between both April and November with the remaining months (Table 2).

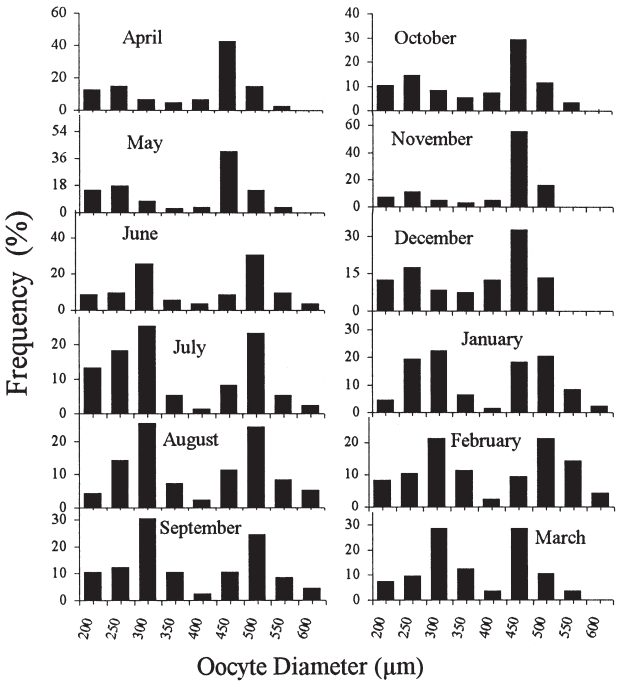


**Fig. 7** Oocyte size frequency distribution of 12 ripening ovaries of *Sardinops sagax* with the same maturity stage, which had the major monthly occurrences during 1992–1993 reproductive period in northern Chile.

## DISCUSSION

### Fecundity and egg size

Great quantities of oocytes with smaller diameters occurred during the winter months in all size classes, which was in contrast to the summer months when the opposite was observed, with the exception of the smaller size range which showed higher fecundity in winter than in summer. Pizarro has shown that newly recruited fishes spawn only during the winter months.[12] The presence of reproductive younger fish in the summer months might indicate that those smaller fishes able of spawning seem to do so with a lower reproductive potential. The clear decrease in oocyte number in whole length range in August is difficult to explain. Beyond a possible methodological error in the estimations, the presence of environmental anomalies being responsible of this failure cannot be discarded. Although it is not the purpose of the present study to understand these effects, it is important to underline certain points described by Claramunt *et al.*[13] and Herrera *et al.*[20] Their studies during the same reproductive periods have attributed thermal anomalies provoked by the El Niño event in 1992–1993 as the main factor explaining the fall in the reproductive parameters in the

**Table 2** Linear regression coefficients for the relationship of the oocyte diameter of intermediate mode (ODIM) on oocyte diameter of most advanced mode (ODAM) of ripening ovaries close to hydration stage of *Sardinops sagax* in northern Chile, during 1992–1993 spawning season

| Month | *a* | *b* | $r^2$ | *Sb* | *F* | *n* |
|---|---|---|---|---|---|---|
| April | 302.12* | 0.79 | 0.82 | 0.03 | 420.5 | 57 |
| May | 317.12 | 0.78 | 0.96 | 0.02 | 1283.0 | 45 |
| June | 351.58 | 0.81 | 0.81 | 0.04 | 326.5 | 69 |
| July | 308.05 | 0.83 | 0.94 | 0.04 | 503.3 | 57 |
| August | 309.82 | 0.82 | 0.91 | 0.03 | 914.1 | 71 |
| September | 320.42 | 0.78 | 0.88 | 0.03 | 536.2 | 67 |
| October | 297.84* | 0.84 | 0.95 | 0.02 | 1385.7 | 57 |
| November | 292.15* | 0.75 | 0.94 | 0.04 | 526.3 | 28 |
| December | 326.02 | 0.76 | 0.82 | 0.06 | 650.5 | 82 |
| January | 335.29 | 0.77 | 0.88 | 0.08 | 285.6 | 49 |
| February | 320.95 | 0.8 | 0.92 | 0.02 | 771.4 | 73 |
| March | 317.26 | 0.79 | 0.95 | 0.03 | 1502.23 | 60 |

Females bigger than 26 cm of total length were included. *Sb*, estimate error of the slope.
* $P < 0.05$.

winter months for this species. They also suggested that this failure might have been a compensatory reply to the warm water intruding just at the beginning of the reproductive process.

Clear seasonal variations were found in oocyte size in both the smaller yolked oocytes and the yolked oocytes in advanced vitellogenesis. It is well known that the egg size produced by multiple spawner fishes (*Sardina*, *Sardinops*, *Engraulis*, etc.) vary seasonally – the largest eggs being spawned during the local winter and the smallest during the local summer.[3] However, the causes as to why there is a major fall in oocyte size during those months of less reproductive activity are still unknown. Blaxter and Hunter have proposed that the seasonal decline of egg size in multiple spawner fishes can be attributed to either a reduction in energy reserves over the spawning season, a change in the partitioning of energy between growth and reproduction, or a seasonal change in the age structure of the spawner.[3] Additionally, Douglas and Economu have suggested that temperature is the main regulator of oocyte size,[22] and Claramunt *et al.*[13] have shown that this hypothesis is valid interseasonally for *S. sagax* in northern Chile. Furthermore, Hislop observed in studies of *Merlangius merlangius* that larger female fishes mature and spawn before the smaller-sized ones.[23] He suggested that this might be the reason for the decrease in the mean oocyte size as the season progresses; however, he also found a reduction in oocyte size as the reproductive season progressed. This last fact was confirmed in the present study, while incorporating some additional elements such as variations in oocyte production being independent of female size.

Conversely, some investigators have suggested that the biological basis for oocyte regulation might be pre-ovulatory atresia,[24,25] which can actuate, at the end of the breeding season, as a 'mopping up' process, particularly in those fish with asynchronous ovaries or multiple clutches.[26] In spite of fact that the atresia ratio was not determined in the present study, the similarity in fluctuations between IM and MAM found here might indicate that the seasonal decline in egg size might be triggered very early during vitellogenesis. Furthermore, slope homogeneity in the ODAM–ODIM relationship indicates that despite fluctuations in oocyte production as the reproductive season progresses, modal displacement of the oocytes remains constant.

### Previous spawning

It is important to note that the $F_{ib}$ rate followed the same seasonal trend as other reproductive parameters characterized by earlier studies, such as GSI and mature female ratio.[7,11,12,20] Likewise, in the present study the mean GSI of ripe ovaries also showed seasonal fluctuations following a similar tendency with $F_{ib}$ rate. Some questions arise from these facts. It is well known that during the reproductive period of *S. sagax* there are females with active and spent ovaries. This is the cause for the fluctuation of mean GSI of the population,[10,12,27] particularly for the lower values of GSI in those months of lower reproductive activity, due to the low occurrences of female fish with ripening ovaries. Then, why does the GSI of ripe ovaries show a similar decrease?

The findings of the present study showed that while seasonal spawning progresses there is a decrease in oocyte production to the most advanced mode (Fig. 8) despite the asynchronic trait of the ovaries. It is also necessary to discard the influence of fish size because the same tendency was observed in all size classes. Hence, we suggest that the effect of the previous spawning is an important factor responsible for the intra-annual decline of batch fecundity for this species. For example, Tascheri and Claramunt established that for *S. sagax* the same relative energy is used by ovaries in the spawning peaks of winter and summer, and that a lower amount of relative energy is destined for the reproductive organs in those months of lower reproductive activity.[27] If we take this point of view, it is necessary to ask the question: To what extent can *S. sagax* support two

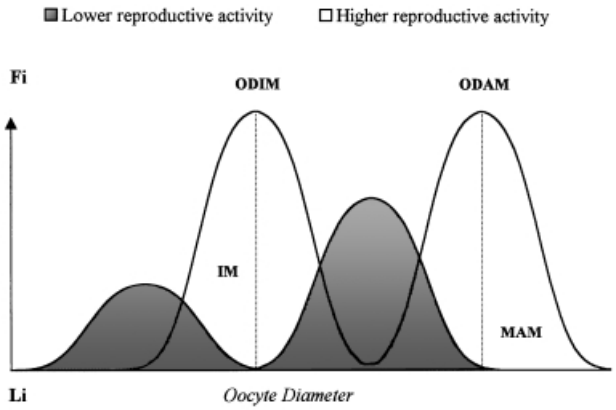


**Fig. 8** Theoretical model illustrating the variations in the modal components of ripening ovaries of *Sardinops sagax* during the periods of lower and higher reproductive activity. Fi, frequency distribution; Li, lower limit used in the frequency distribution; IM, intermediate mode; MAM, most advanced mode; ODIM, mean oocyte diameter of intermediate mode; ODAM mean oocyte diameter of most advanced mode.

intensive reproductive periods? Is it the same stock spawning twice during the extended spawning season or are there two separate stocks spawning only one at a time? Despite previous studies having reported the existence of only one spawning stock, based on the existence of two peaks in variables such as GSI and mean gonad weight, it seems that further studies should be carried out to find an answer. For example, Hunter and Leong described that approximately two-thirds of all annual spawning of northern anchovy *Engraulis mordax* was sustained from fat reserves stored the previous year during the annual spring bloom and that only the remaining one-third was consumed during the spawning season.[2] Blaxter and Hunter have suggested that similar patterns probably exist in other multiple spawners with a prolonged spawning season.[3] Hence, does *S. sagax* have a high capacity for feeding and reproducing simultaneously? These questions lead us to a fundamental problem of partitioning energy supplies between metabolism and reproduction;[3] however, attempts at clarification for the species *S. sagax* are still poorly developed.

Finally, we can point out that considerable aspects have arisen from the synchronism, which tends to a maximization, of both batch and intermediate fecundity of ripening ovaries of this species. These fluctuations are clearly synchronized with variations in oocyte size; hence, we might expect that any fluctuation in a reproductive parameter could result in changes in the output product (oocyte size). In these findings the size frequency distribution of intra-ovarian ripe oocytes became an important tool, which should be continually improved. The interrelations between modal components, the constant displacement process of partial yolked to advanced yolked oocytes, and the role of ODAM in their quantification could be used in future attempts to model this process.

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