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# Fecundity pattern and spawning dynamics of the common cuttlefish *Sepia officinalis*

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## ABSTRACT

The fecundity pattern of common cuttlefish, *Sepia officinalis*, was assessed in Thermaikos Gulf, one of the species' main fishing grounds in the eastern Mediterranean. Female samples, collected with coastal fishing gears during the population spawning season (January–July), were analysed using a combination of histology and whole mount analysis of ovarian tissue. The potential fecundity was shown to vary seasonally, showing the highest values at the beginning of spawning, gradually dropping thereafter. Previtellogenic oocytes (<0.5 mm) were the most prevalent group occurring throughout the spawning period in all maturity stages. Low or null levels of oocyte recruitment during the spawning period in combination with seasonal drop in fecundity and clutch-specific egg production suggest that the fecundity pattern of cuttlefish displays similarities with the so-called 'determinate fecundity' pattern of fishes. Spent females end up having small reserves of secondary growth oocytes (pre- and vitellogenic) that will never be spawned. Potential annual fecundity was thus estimated as the difference in total fecundity between pre-spawners and spent females and equalled 2569 oocytes/eggs. For a spawning period of four months, the ratio between potential fecundity and clutch size (100–200 eggs) indicated 13–25 different spawning events with a mean interval of 5–9 days.

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## Introduction

The common cuttlefish, *Sepia officinalis* (Linnaeus, 1758), is a short-lived cephalopod spawning over a single period throughout its lifespan (monocyclic spawning; Rocha et al. 2001) followed by mass mortality of the adults (Bloor et al. 2013). Its life cycle is closely related to environmental factors and begins when mature females move inshore to spawn in shallow coastal waters, usually during winter and spring (Bloor et al. 2013). Hatchlings undergo a period of rapid growth, migrating to offshore grounds until the juvenile stage. Then, depending on the geographic location, this cohort will migrate back inshore to coastal grounds either to reproduce, such as in the Mediterranean and the Gulf of Tunis (Guerra and Castro 1988; Mangold 1963), or to feed, e.g. in the English Channel (Bloor et al. 2013). In the latter case, the maturing cohort will repeat its migration cycle once more and get back to inshore grounds to reproduce. The length of spawning season also varies over the species' distributional range, being longer in warmer waters such as those around the Iberian Peninsula, the Mediterranean and the Gulf of Tunis (Mangold

1963; Ezzedine-Najai 1985; Bloor et al. 2013). A stimulus for the onset or end of reproduction is the change in photoperiod, as it activates neuro-hormonal mechanisms that affect the physiology and behaviour of cuttlefish.

Egg production in common cuttlefish ranges from intermittent spawning with periods of inactivity as long as one month, to virtually continuous chronic spawning over many weeks (Boletzky 1987). The factors responsible for the length of pauses occurring in egg maturation and spawning are not yet known. In each spawning event females lay eggs one by one on various hard substrates including plants, sessile and mobile animals (Guerra et al. 2016) and artificial structures such as fishing pots (Gantias et al. 2021). A female can lay dozens of eggs at once, probably 150–200 (Boletzky 1987; Domingues et al. 2002), which roughly corresponds to the storage capacity of the genital coelom (Zatylny-Gaudin and Henry 2018). The stock of mature oocytes is replenished through asynchronous gametogenesis (Dursun et al. 2013; Salman et al. 2017) and females can lay several clutches of eggs in successive spawning events

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(Laptikhovsky et al. 2003, 2019). This breakdown of the reproductive effort in prolonged and successive egg maturations overcomes limitations in the holding capacity of the gonad, thus increasing annual egg production and spreading the mortality risk for the offspring (MacArthur and Wilson 1963).

Measurements of fecundity are of particular importance in assessments of population spawning energetics and reproductive dynamics, i.e. how the annual reproductive output is linked to recruitment (Ganias 2013; Ganias et al. 2015). The estimation of potential fecundity in cuttlefish, i.e. the total number of eggs that are laid before the programmed death of the animal (Laptikhovsky et al. 2003), presents difficulties. As shown above, mature oocytes only account for a fraction of annual reproductive output representing an underestimate of potential fecundity (Laptikhovsky et al. 2003). Moreover, previous studies both in wild populations (Laptikhovsky et al. 2019, 2003; Lin et al. 2019) and in captivity (Boletzky 1987) showed that females do not spawn the entire stock of oocytes, but spent individuals keep a reserve of healthy oocytes of various developmental stages in their ovaries (see also Salman et al. 2017). Thus, oocyte counts from mature females captured from the sea do not reveal how many eggs the individual has already laid – as they do not reveal how many of the early oocytes would have reached full maturity if the animal had continued to live and spawn (Boletzky 1987).

The safest calculation of potential fecundity derives from aggregate egg counts in tank-kept groups or generations of mature females. Forsythe et al. (1994) monitored a group of female cuttlefish for total egg production and estimated that about 12330 eggs

were laid over a 107-day period, averaging 3082 eggs per female. Of these, only 720 produced viable hatchlings. Hanley et al. (1998) report that spawning female cuttlefish laid about 2000 eggs over several weeks in largely open or flow-through culture systems, even if the number varied greatly. Laptikhovsky et al. (2003) estimated fecundity in wild captured cuttlefish through comparing the standing stock of oocytes between pre-spawning and spawning females and found that an ‘average’ spawning female released about 1000–3000 eggs. This value is comparable to fecundity estimates from captivity experiments, suggesting that intermittent spawning also occurs in natural cuttlefish populations, as suggested by Rocha et al. (2001).

The present paper assesses the fecundity pattern of *S. officinalis* in Thermaikos Gulf (North Aegean Sea), one of the main fishing grounds of cuttlefish in the eastern Mediterranean. Ovarian histology is combined with particle analysis of ovarian whole mounts to determine oocyte dynamics and the formation of succeeding egg clutches throughout the population spawning cycle. Estimation of clutch size was also performed in the field through monitoring egg deposition rates on the surface of fishing traps with spawning female cuttlefish placed inside. Present results are collated and compared with an array of recent studies in the E. Mediterranean (Laptikhovsky et al. 2003; Önsoy and Salman 2005; Dursun et al. 2013) to draw a comprehensive picture of the annual fecundity pattern of common cuttlefish and the factors affecting its reproductive potential.

## Materials and methods

A total of 391 female cuttlefish collected off Nea Michaniona fishing port (E. Thermaikos) during the period January–July 2019 were analysed (Table I). All individuals were captured by a chartered coastal fishing boat (8 m, 2.3 GT, 43 hp) deploying two types of trammel nets, with inner/outer panel mesh sizes of 72/360 mm and 64/320 mm, respectively, and 20 netting traps.

Captured cuttlefish were transferred to the laboratory and measured for dorsal mantle length (ML, mm) and total weight (TW, g). The specimens were dissected and assigned to a maturity stage according to the relative size and colour of gonads, following the five-stage macroscopic maturity scale of ICES (2010): I immature, IIa developing, IIb maturing, IIIa mature/spawning, IIIb spent. The ovaries were weighed to the nearest 0.1 g and placed in jars with 10% buffered formalin. The gonadosomatic index (GSI)

**Table I.** Monthly number of female cuttlefish sampled for biometric measurements and for fecundity analysis, i.e. ovarian whole mounts and histological procedures. Mean mantle length (ML, mm) is also provided with standard error in parentheses.

Month	Biometry	Fecundity	Mean ML
January	62	3	132.3 (±107.7)
February	66	7	144.5 (±90.3)
March	80	10	138.2 (±66.0)
April	50	9	130.4 (±50.5)
May	56	22	121.7 (±22.7)
June	66	8	118.6 (±24.5)
July	11	5	106.9 (±19.6)

was estimated as the ratio of gonad weight (GW, g) to TW expressed as percentage ( $GSI = WG/TW * 100$ ).

Reproductive dynamics was studied using a combination of ovarian histology and whole mount analysis in a random subset of 64 females from all sampling months. For histology, a small part of ovarian tissue was dehydrated in a graded ethanol series, cleared in xylol, and embedded in paraffin. Histological sections were cut at 5–7  $\mu$ m and stained with haematoxylin and eosin. The entire histological sections were digitized into high-resolution (2.3 MP) pictures using a 'Basler acA1920-40uc' microscopy camera, mounted on a Zeiss Axio Lab. A1 microscope, and Microvisioneer's *manualWSI* software. The oocytes of each specimen were assigned to stages of oogenesis, following the scale of Laptikhovsky and Arkhipkin (2001) for the squid *Loligo gahi*: stage-1 = secondary oogonia; stage-2 = primary growth; stage-3 = follicle cell multiplication; stage-4 = early yolkless; stage-5 = late yolkless; stage-6 = early vitellogenesis; stage-7 = late vitellogenesis; stage-8 = ripe egg. Oocyte atresia was also examined in the photomicrographs, following criteria suggested by Salman et al. (2017).

The same subset of specimens ( $n = 64$ ) was subject to ovarian whole mounts analysis. To estimate the fecundity of each specimen, a sub-sample of the ovaries was taken, weighed to the nearest 1 mg, and placed in a Petri dish with isotonic solution. The connective tissue and non-oocyte material were removed, and the oocytes were separated manually or with the use of a magnetic stirrer. Ovarian subsamples were digitally imaged using a Jenoptik Progress C3 camera mounted on a Euromex NZ 80 stereo microscope. Prior to digitalization, whole-mount subsamples were stained with haematoxylin to increase oocyte visibility (see also Mouchlianitis et al. 2020). The resulting images were analysed via particle analysis (Thorsen and Kjesbu 2001) in order to construct oocyte size frequency distributions (OSFD) using methods described in Ganiyas et al. (2010). Image processing and particle analysis were performed using ImageJ (imagej.nih.gov).

Total fecundity ( $F_T$ ) equalled the sum of secondary growth oocytes, i.e. from stage-3 onwards. In addition, the number of very large oocytes which were clearly distinguishable from the variable group of smaller oocytes (see Results) corresponded to advanced mode fecundity ( $F_{AM}$ ). Both  $F_T$  and  $F_{AM}$  were estimated using the gravimetric method as the ratio of the total number of oocytes, and oocytes of the advanced mode, respectively, of each sub-sample to sub-sample weight multiplied by total gonad weight. Relative total fecundity ( $FR_T$ ) and relative advanced mode

fecundity ( $FR_{AM}$ ) were estimated by dividing  $F_T$  and  $F_{AM}$  by total body weight, respectively. An additional sample of stage-IIIa females ( $n = 12$ ), captured at the spawning peak, were taken to the lab, measured for ML and TW and dissected to count the number of ripe eggs from the oviduct,  $F_{RE}$ .

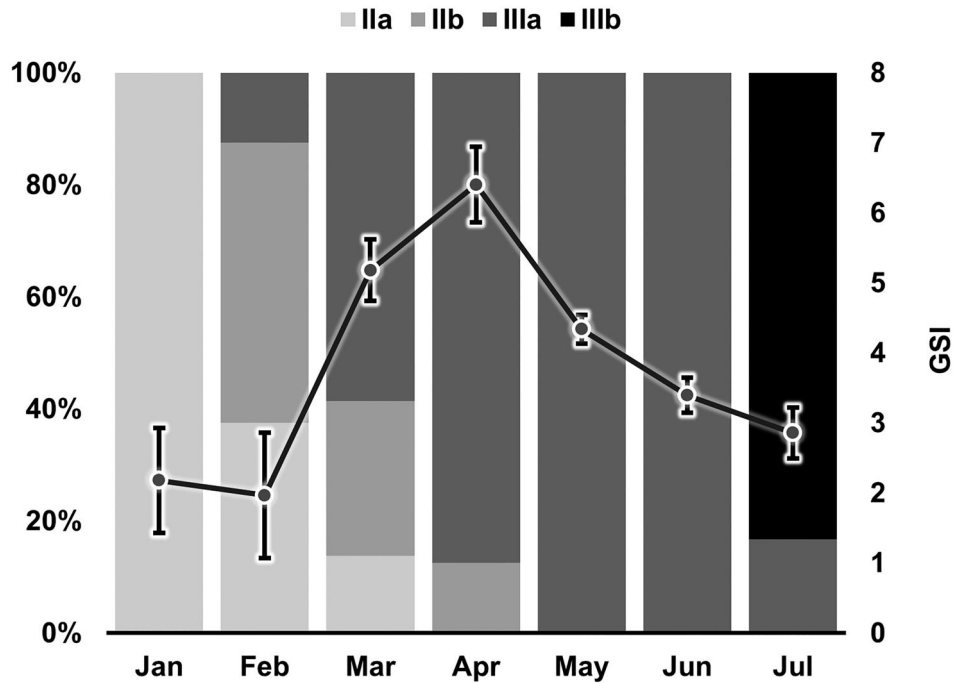
### Field survey

Female cuttlefish captured alive from trammel net hauls (hereafter called 'baits') were placed singly in one fishing trap of a string which in total consisted of 10 traps (for details see Ganiyas et al. 2021). A total of four such trap-string operations were performed during peak spawning months (May and June). One of the trap-strings operated for 23 h and the other three for  $\sim 48$  h. Before each operation the traps were examined to be free of cuttlefish eggs. At retrieval, cuttlefish egg clusters were identified on various parts of the traps (mesh and metal frame) and their total number was counted. A representative number of eggs was detached with the utmost care from the traps and maximum diameter was measured using a calliper and their development stage was assessed macroscopically according to the 3-point scale of Zatylny-Gaudin and Henry (2018). These females were measured for ML and TW and  $F_{RE}$ .

### Results

Mantle length (ML) varied from 71–214 mm (average 125.9, s.d.  $\pm 2$  mm). No immature individuals (stage I) were found while developing (stage IIa) and maturing individuals (stage IIb) were identified from January to March and from February to April, respectively (Figure 1). Spawning individuals (stage IIIa) were observed throughout the sampling period except from January. In June, only spawning individuals were recorded while in July most individuals were categorized as spent (stage IIIb). The gonadosomatic index (GSI) varied from 0.3–8.6% (average  $3.86 \pm 0.18\%$ ) while its monthly values followed a similar trend to the seasonal breakdown of maturity stages (Figure 1). Specifically, an initial period of steep increase between January and April was followed by a period of gradual decrease until the end of sampling. In that respect, the prevalence of spawning individuals coincided with a gradual decrease in ovarian mass.

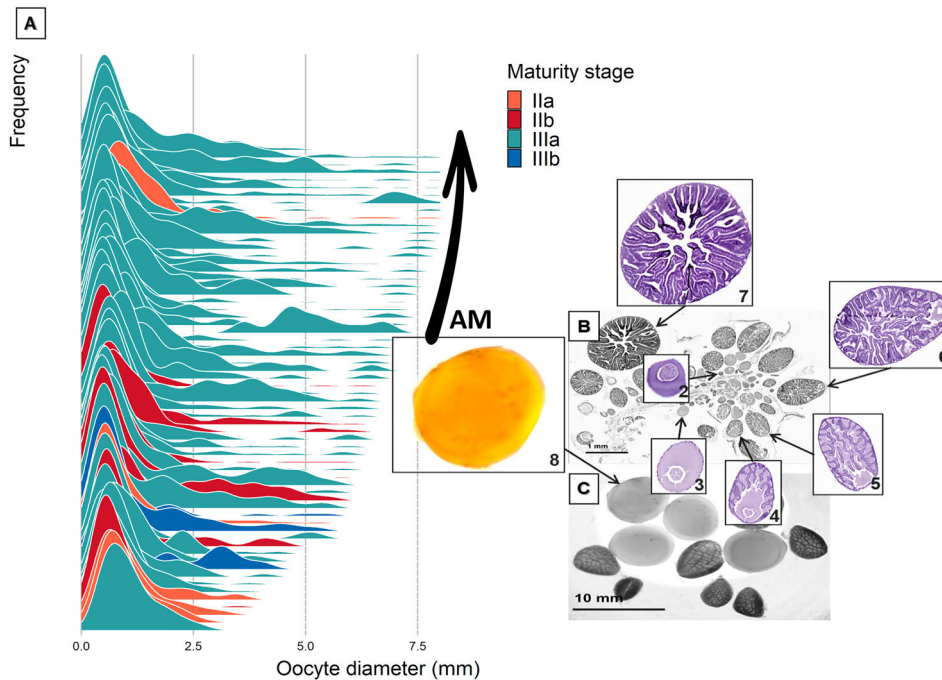
Oocyte size varied from 0.01 to 8.9 mm, while small oocytes of about 0.5 mm were the most abundant group at all maturity stages (Figure 2). Only six of the eight stages of oogenesis were found in the histological sections since neither secondary oogonia (stage-1)



**Figure 1.** Monthly breakdown of maturity stages (IIa: developing; IIb: pre-spawning; IIIa: spawning; IIIb: spent) and seasonal evolution of gonadosomatic index (GSI) of common cuttlefish, *Sepia officinalis*, in Thermaikos Gulf. Vertical bars: standard error.

nor ripe eggs (stage-8) were identified in histological specimens (Figure 2). The latter could only be observed in whole mounts, due to their very large size (Figure 2). The intensity of primary growth oocytes (stage-2) was

particularly low in all individuals (mean 3%) irrespective of size, sampling time and maturity stage. Previtellogenic oocytes (i.e. oocytes up to stage-5 with size <0.5 mm) were the most prevalent group occurring



**Figure 2.** (A) Oocyte size frequency distributions in ascending maximum oocyte size for the 64 common cuttlefish, *Sepia officinalis*, specimens per maturity stage; arrow indicates the clearly distinguished advanced mode of oocytes (AM). (B) Histological section of a cuttlefish ovary with magnified stages of oogenesis 2, 3, 4, 5, 6 and 7, scale bar 1 mm; (C) Oocytes from a formalin-preserved ovarian whole mount with magnified stage of oogenesis 8; scale bar 10 mm.

throughout the spawning period in all maturity stages (i.e. pre-spawning, spawning and post-spawning individuals) at high intensities, above 70% (Figure 3). The fraction of stage-4 oocytes decreased moderately but significantly ( $P < 0.01$ ) from January to July from 37% to 23% (Figure 3). On the other hand, the intensity of atresia increased during the sampling period from 5% in January to 17% in July.

Oocytes larger than 5 mm formed a distinct advanced mode (AM) which was clearly distinguishable from the versatile population of smaller oocytes, in mature/spawning (stage IIIa) females (Figure 2). The advanced mode consisted of stage-8 oocytes and its fecundity,  $F_{AM}$ , displayed similar values to the number of ripe eggs from the oviduct,  $F_{RE}$  (Figure 4A). Specifically,  $F_{AM}$  values ranged from 2 to 156 oocytes (average  $34.2 \pm 3.5$  oocytes) while  $F_{RE}$  values ranged from 4 to 193 (average  $39.2 \pm 12$  eggs) while the two fecundity measures did not differ significantly (Kruskal–Wallis test:  $P > 0.1$ ). Furthermore, both  $F_{AM}$  and  $F_{RE}$  were not found to be correlated with ML (Figure 4B).

Total fecundity ( $F_T$ ) and relative total fecundity values ( $FR_T$ ) ranged from 193 to 12233 oocytes (average  $2305 \pm 291$  oocytes) and from 0.5 to 51 oocytes per gram (average  $11.5 \pm 1.2$  oocytes per gram), respectively. Total fecundity had its highest value in January–February (4865 oocytes); the two months were pooled due to low number of fecundity specimens in January. Total fecundity considerably dropped until April to less than 2000 oocytes and stabilized thereafter till the end of sampling, in July (Figure 5A). When specimens were split into two size cohorts (smaller and larger than 125 mm) two distinct fecundity patterns emerged. In larger females, fecundity peaked at the onset of sampling while fecundity in smaller females lagged by 1–2 months, peaking in March (Figure 5A). Moreover, total fecundity varied significantly according to maturity stage (Mann–Whitney test:  $P < 0.01$ ), being higher for stage-IIb and lower in stage-IIIb females (Figure 5B).

The egg deposition experiment revealed that cuttlefish produce their eggs in a clutch specific manner (Table II). In all four trap-string operations, only the traps that were baited with a female cuttlefish had eggs on their surface. This finding indicated that all these eggs had been deposited by the baits. The bait which remained in the trap for 23 h had deposited 150 eggs and had a reserve of 34 ripe eggs in its oviduct. This individual displayed the largest ML and the smallest egg size amongst the tested baits. The remaining three baits which remained in the trap for almost twice as long, had deposited 70, 100 and 200

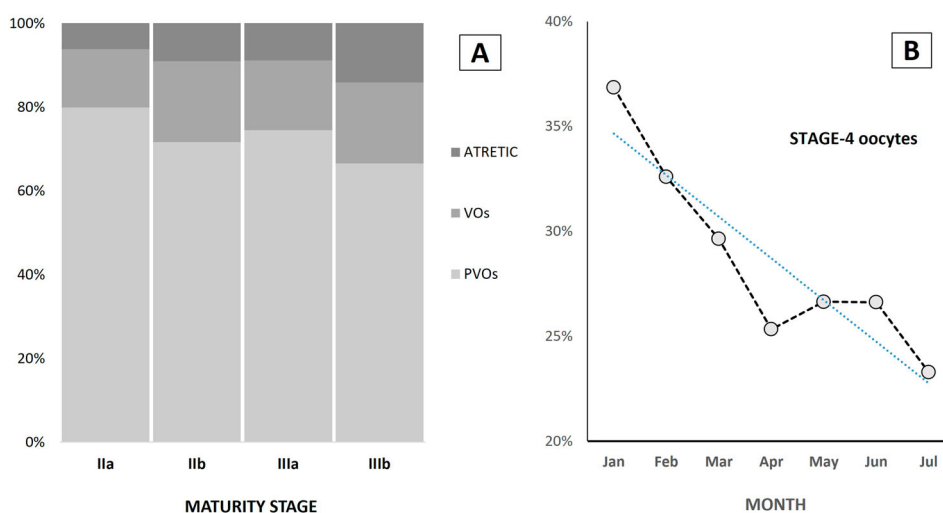
eggs and had 41, 5 and 1 ripe eggs, respectively, in their oviduct. Thus, the latter two individuals had deposited almost their total number of ripe eggs. This finding suggests that cuttlefish produce their eggs gradually in a clutch-specific manner, i.e. the oviduct is evacuated from the ripe eggs of the current clutch before being refilled by the eggs of the next clutch, within a period of at least two days. Judging from the sum of ripe and deposited eggs per female, clutch size roughly equals 100–200 eggs.

## Discussion

The present study covered the spawning period of common cuttlefish in the eastern Mediterranean, which similarly to other areas (Izmir Bay: Akyol et al. 2011; off Mauritania: Lin et al. 2019; English Channel: Laptikhovsky et al. 2019) extends from late winter to mid-summer. No immature individuals were captured while spawning individuals (stage IIIa) were the most prevailing maturity stage during all sampling months except from January. Samples originated from coastal fishing gears (trammel nets and fishing traps) which target the mature fraction of the population during its migration to coastal spawning grounds (Pierce et al. 2010; Bloor et al. 2013). Ganias et al. (2021) showed that the cuttlefish population in Thermaikos Gulf consists of two size/age cohorts (smaller and larger than 125 mm) while a demographic shift occurs during the spawning season with larger individuals being steadily replaced by smaller individuals. The present study shows that this shift, which has also been described for other common cuttlefish populations (e.g. Izmir-Bay: Önsoy and Salman 2005), may affect the reproductive potential of the population since the two cohorts display differences in their seasonal fecundity pattern.

The fecundity pattern was assessed using a combination of histology and image analysis techniques of ovarian whole mounts. In particular, total fecundity ( $F_T$ ) was shown to vary seasonally in a different manner between the two size cohorts. In  $>125$  mm individuals,  $F_T$  showed its highest values at the beginning of the sampling period in January–February and then it dropped towards the end of spawning, in late spring and summer. In smaller individuals, the  $F_T$  peaked in March, and again decreased thereafter towards the end of spawning.

Despite differences in the timing of fecundity variation, oocyte dynamics were similar for the two size/age cohorts. None of the analysed specimens displayed secondary oögonia (stage-1) while the intensity of primary growth oocytes (stage-2) was particularly

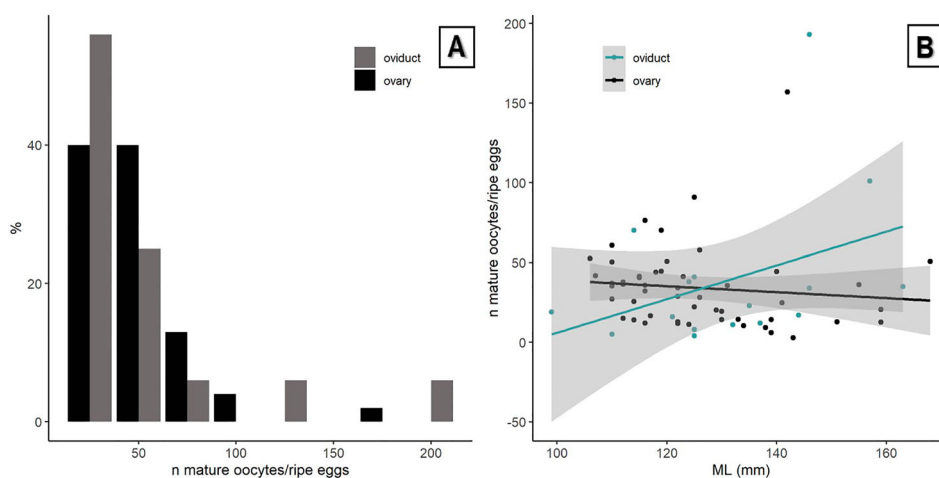


**Figure 3.** Ovarian histology survey of common cuttlefish, *Sepia officinalis*. (A) Per cent fraction of atretic, vitellogenic (VOs) and pre-vitellogenic (PVOs) oocytes per maturity stage. Monthly variation of the per cent fraction of stage-4 oocytes, i.e. early yolkless oocytes.

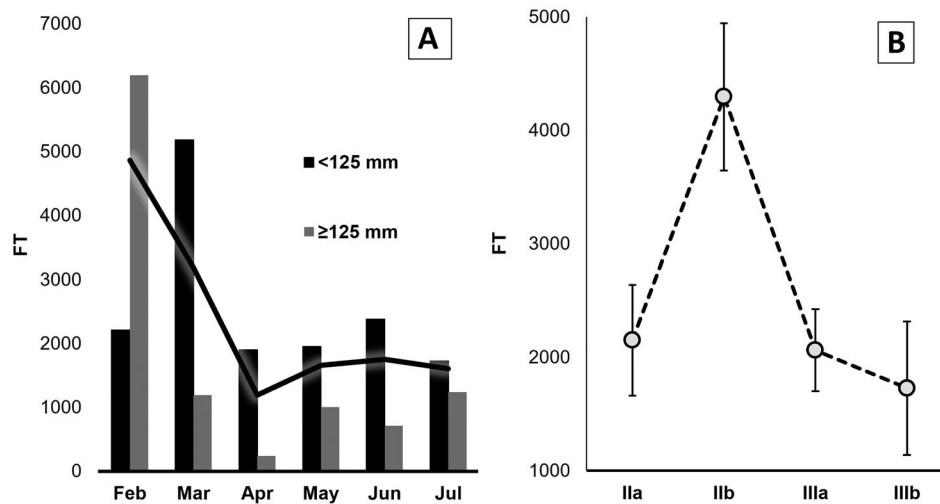
low in all individuals irrespective of size, sampling time and maturity stage. Previtellogenic oocytes (i.e. stage-3 to -5 with size <0.5 mm) were the most prevalent group (60–82%), occurring throughout the spawning period in all maturity stages. Even if their overall fraction remained unchanged, stage-4 oocytes, i.e. early yolkless oocytes with a follicular epithelium that begins to penetrate into the oocyte, steadily decreased during the sampling season. Lin et al. (2019) describe a similar drop in the fraction of small oocytes from developing to mature/spawning cuttlefish off Mauritania. Laptikhovsky et al. (2003) observed similar OSFD for cuttlefish collected from three different locations off Turkish coasts, with small, <0.5 mm oocytes again being the most prevalent group. According to the

authors, their number decreased at maturation, attaining some 40–50% of the total oocyte stock in spawning females. The complete absence of stage-1 and the low fraction of stage-2 oocytes in combination with the seasonal drop in the fecundity of secondary growth oocytes – which is indicative that they are not replenished – suggests that oocyte recruitment during the spawning period is very poor or even null.

At the other endpoint of the OSFD, an advanced group of oocytes above 5 mm was clearly distinguishable in the composite plot from all analysed specimens. These were the mature oocytes (Boletzky 1987; Laptikhovsky et al. 2019), i.e. oocytes at the final stage of development (stage-8, based on the scale of Laptikhovsky and Arkhipkin 2001) that are still held within



**Figure 4.** (A) Frequency distribution of the number of mature oocytes and ripe eggs from the ovary and the oviduct, respectively, of cuttlefish specimens. (B) Relationship between mantle length (ML) and the number of mature oocytes and ripe. Least squares regression lines are shown.



**Figure 5.** (A) Monthly variation of total fecundity ( $F_T$ ) values in cuttlefish specimens: solid line: total; bar graph: per size class; (B) variation of  $F_T$  per maturity stage; vertical bars: standard error.

follicular sheaths (Laptikhovskiy et al. 2019) and which were only discernible in whole mounts and not in histological specimens due to their very large size. According to Laptikhovskiy et al. (2019) mature oocytes need to be distinguished from ripe ovulated eggs that are not connected to the gonad anymore and which are accumulated in the oviduct and ready to be spawned. Mature oocytes displayed the same size and had the same characteristic ovoid shape and bright yellow colour with ripe eggs. The numbers of mature oocytes and ripe eggs were complementary, suggesting that the two groups were in a dynamic equilibrium state. Thus, ripe eggs are produced in a clutch-specific manner, i.e. the oviduct is refilled by the eggs of the next spawning episode after the eggs of the current spawning episode have been fully deposited.

Clutch-specific egg production was further supported from the field experiment where spawning females gradually deposited, within at least 48 h, their stock of ripe eggs until the oviduct was almost completely evacuated; two of the females that were kept for two days inside the pot had deposited 100 and 200 eggs on the pot having 5 and 1 ripe eggs left in their oviduct, respectively. Spawners from our regular samples had on average 39 (+/-) ripe eggs in their oviduct which was 4–5 orders of magnitude

lower than the number of eggs deposited on the traps. The maximum number of ripe eggs found in the oviduct of a female spawner ( $n = 193$ ) was very close to the maximum number of newly deposited eggs on a trap ( $n = 200$ ), suggesting that the oviduct can host the entire clutch before it is deposited.

In that respect, clutch size in cuttlefish should optimally be estimated by counting the number of eggs deposited in single spawning episodes; this could be done by monitoring individual spawners either in the lab (Boletzky 1987) or in the field, e.g. *in situ* fishing pots (Bouchaud 1991; present study). In his detailed tank experiment, Boletzky (1987) showed that a small female cuttlefish (80 mm ML) deposited clutches of 40–60 eggs in spawning episodes that lasted 1–2 days at irregular intervals which ranged from few days to many weeks. The smaller clutch size of this female could either be attributed to smaller body size compared with our samples ( $125.9 \pm 2$  mm) and/or to the effect of captivity (e.g. stress). Similarly to our study, Bouchaud (1991) estimated clutch size for the cuttlefish population of the Bay of Biscay using the number of eggs deposited on pots by individual females. Clutch size in this previous study was 50–150 eggs which is close to present estimates. On the other hand, calculations based on dissections and counts of mature oocytes and ripe eggs require that the two groups belong to the same clutch, which still needs to be confirmed.

Low or null levels of oocyte recruitment during the spawning period in combination with seasonal drop in fecundity and clutch-specific egg production suggest that the fecundity pattern of cuttlefish displays similarities but, also, important differences with the so-called 'determinate fecundity' pattern in fishes

**Table II.** Results of the field experiment showing the mantle length (ML) and number of ripe eggs ( $F_{RE}$ ) of the baits and the number and size of eggs deposited on the traps.

Specimen	ML (mm)	$F_{RE}$	N eggs	Egg size (mm)	Time (h)
#1	132	34	150	0.478	23
#2	125	41	70	0.666	47
#3	110	5	100	0.568	46
#4	110	1	200	0.517	48



(Hunter et al. 1992; Kjesbu 2009; Ganias 2013). In particular, annual fecundity in determinate spawners is fixed prior to the onset of the spawning season while total fecundity – i.e. the standing stock of secondary growth oocytes to be spawned during the current season – steadily drops during the season as oocyte batches are subsequently released. As a consequence, late season spawners and post-spawners end up having few or no oocytes at all in their ovaries, respectively. Also, there is no massive atresia (i.e. mopping up) as happens with indeterminate spawners which continue recruiting new oocyte batches until the end of spawning. The seasonal fecundity pattern of determinate spawners consists of a preliminary ‘built-up phase’ followed by a ‘stabilization phase’ which lasts up to the onset of spawning and finally a ‘decrease phase’ which lasts up to the cessation of spawning (see Hunter et al. 1989). The seasonal variation of fecundity in younger (<125 mm) cuttlefish matched this seasonal pattern obviously because the present study also covered the pre-spawning phase of this cohort. On the other hand, this study missed the ‘fecundity built-up’ phase of the larger cohort.

Spent females (stage IIIb) end up having small reserves of secondary growth oocytes (pre- and vitellogenic) that will never be spawned and total fecundity is not zeroed but it stabilizes to rather low levels which is indicative of spawning cessation. Therefore, in contrast to determinate spawning fishes where potential annual fecundity ( $F_p$ ) equals the standing oocyte stock of pre-spawners,  $F_p$  in cuttlefish should be estimated as the difference in total fecundity between pre-spawners (stage IIb) and spent females (Stage IIIb). Using this approach, the estimated  $F_p$  was 2569 eggs which is close to the 3082 eggs/female estimated by Forsythe et al. (1994) who monitored total egg production in two tank-cultured female cuttlefish over a 107-day period. Laptikhovskiy et al. (2003) compared the  $F_p$  of pre-spawning and spawning females and found that an ‘average’ spawning female (mean body weight 844.5 g) released about 1000–3000 eggs before being caught. The ratio between  $F_p$  and clutch size suggests that intermittent spawning (see Rocha et al. 2001) in the North Aegean cuttlefish population consists of 13–25 different spawning events; this range of values includes the 17 spawning events estimated by Boletzky (1987) in his 4-month tank experiment.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Ethics approval

Samplings and animal processing were performed with the permission of the Fisheries Department, Directorate of Agricultural Economy and Fisheries, of the Region of Central Macedonia.

## Data/Code availability

Data will be made available on reasonable request.

## Authors’ contribution

The paper was conceived by K.G.; I.F.K. participated in the fieldwork, conducted the biometric measurements, prepared the histological sections and did whole-mount analyses. Statistical analysis and figure preparation were performed by K.G. and I.F.K. The manuscript was written by K.G. with additional input from I.F.K.

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## References

- Akyol O, Tellibayraktar B, Tevfik C. 2011. Preliminary results on the cuttlefish, *Sepia officinalis*, reproduction in Izmir Bay (Aegean Sea). *Journal of Fisheries Sciences*. 5:122–130. doi:10.3153/jfscom.2011015.
- Bloor ISM, Attrill MJ, Jackson EL. 2013. A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish (*sepia*

- officialis). *Advances in Marine Biology*. 65:1–65. doi:10.1016/B978-0-12-410498-3.00001-X.
- Boletzky S. 1987. Fecundity variation in relation to intermittent or chronic spawning in the cuttlefish, *Sepia officinalis* L. (Mollusca, Cephalopoda). *Bulletin of Marine Science*. 402:382–387.
- Bouchaud O. 1991. Recherches physiologiques sur la reproduction de la seiche, *Sepia officinalis* (Mollusque, Cephalopode, Sepiidae), dans le secteur Mor Braz-Golfe du Morbihan (Sud Bretagne). Rennes: L'université de Rennes.
- Domingues PM, Sykes A, Andrade JP. 2002. The effects of temperature in the life cycle of two consecutive generations of the cuttlefish *Sepia officinalis* (Linnaeus, 1758), cultured in the Algarve (South Portugal). *Aquaculture International*. 10 (10):207–220. doi:10.1023/A:1022148802078
- Dursun D, Eronat EGT, Akalin M, Salman MA. 2013. Reproductive biology of pink cuttlefish *sepia orbignyana* in the aegean sea (eastern Mediterranean). *Turkish Journal of Zoology*. 37:576–581. doi:10.3906/zoo-1209-22
- Ezzedine-Najai S. 1985. Fecundity of cuttlefish, *Sepia officinalis* L. (Mollusca: Cephalopoda) from the gulf of Tunis.
- Forsythe JW, DeRusha RH, Hanlon RT. 1994. Growth, reproduction and life span of *Sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. *Journal of Zoology*. 233:175–192. doi:10.1111/j.1469-7998.1994.tb08582.x
- Ganias K. 2013. Determining the indeterminate: evolving concepts and methods on the assessment of the fecundity pattern of fishes. *Fisheries Research*. 138:23–30. doi:10.1016/j.fishres.2012.05.006
- Ganias K, Lowerre-barbieri SK, Cooper W. 2015. Understanding the determinate – indeterminate fecundity dichotomy in fish populations using a temperature dependent oocyte growth model. *Journal of Sea Research*. 96:1–10. doi:10.1016/j.seares.2014.10.018
- Ganias K, Perri R, Moditsis A. 2021. Cuttlefish and squid egg deposition patterns on artificial devices and trap-like gears: implications for offspring survival and population management. *ICES Journal of Marine Science*. 78:1614–1622. doi:10.1093/icesjms/fsab062
- Ganias K, Rakka M, Vavalidis T, Nunes C. 2010. Measuring batch fecundity using automated particle counting. *Fisheries Research*. 106:570–574. doi:10.1016/j.fishres.2010.09.016
- Guerra A, Castro B. 1988. On the life cycle of *Sepia officinalis* (Cephalopoda, Sepioidea) in the ria de Vigo (NW Spain). *Cahiers de Biologie Marine*. 29:395–405.
- Guerra Á, Hernández-Urcera J, Garci ME, Sestelo M, Regueira M, Gilcoto M, González ÁF. 2016. Spawning habitat selection by the common cuttlefish *Sepia officinalis* in the Cíes Islands (Northwest Spain). *Fisheries Research*. 183:44–54. doi:10.1016/j.fishres.2016.04.023
- Hanley JS, Shashar N, Smolowitz R, Bullis RA, Mebane WN, Gabr HR, Hanlon RT. 1998. Modified laboratory culture techniques for the European cuttlefish *Sepia officinalis*. *The Biological Bulletin*. 195:223–225. doi:10.2307/1542850
- Hunter JR, Macewicz BJ, Kimbrell A. 1989. Fecundity and other aspects of the reproduction of sablefish, *Anoplopoma fimbria*, in central California waters. *Calif Coop Oceanic Fish Invest Rep*. 30:61–72.
- Hunter JR, Macewicz BJ, Lo NCH, Kimbrell A, Chyan-Huei Lo N, Kimbrell CA. 1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fish Bull*. 90:101–128.
- ICES. 2010. Report of the Workshop on Sexual Maturity Staging of Cephalopods (WKMSCEPH), 8-11 November 2010, Livorno, Italy. 97 pp.
- Kjesbu OS. 2009. Applied fish reproductive biology: contribution of individual reproductive potential to recruitment and fisheries management. Pages 293-334 in T. Jakobsen, M. J. Fogarty, B. A. Megrey, and E. Moksness, editors. *Fish reproductive biology*. Wiley-Blackwell S. Fish Reprod Biol Implic Assess Manag 293–332.
- Laptikhovskiy VV, Arkhipkin AI. 2001. Oogenesis and gonad development in the cold water loliginid squid *Loligo gahi* (Cephalopoda: Myopsida) on the falkland shelf. *Journal of Molluscan Studies*. 67:475–482. doi:10.1093/mollus/67.4.475
- Laptikhovskiy V, Barrett C, Firmin C, Ouréns R. 2019. Adaptations of the common cuttlefish *Sepia officinalis* L. to cold water reproduction in the English channel. *Journal of Shellfish Research*. 38:629. doi:10.2983/035.038.0314
- Laptikhovskiy V, Salman A, Önsoy B, Katagan T. 2003. Fecundity of the common cuttlefish, *Sepia officinalis* L. (Cephalopoda, Sepiidae): A new look at an old problem. *Scientia Marina*. 67:279–284. doi:10.3989/scimar.2003.67n3279.
- Lin D, Xuan S, Chen Z, Chen X. 2019. The ovarian development, fecundity and hypothesis on spawning pattern of common cuttlefish *Sepia officinalis* off Mauritania. *Fisheries Research* 210:193–197. doi:10.1016/j.fishres.2018.08.003
- MacArthur RH, Wilson EO. 1963. An equilibrium theory of insular zoogeography. *Evolution* (N Y). 17:373. doi:10.2307/2407089.
- Mangold K. 1963. Biologie des cephalopodes benthiques et nectoniques de la Mer Catalane. *Vie et Milieu*. 13:295.
- Mouchlianitis F, Minos G, Ganias K. 2020. Timing of oocyte recruitment within the ovulatory cycle of Macedonian shad, *Alosa macedonica*, a batch spawning fish with indeterminate fecundity. *Theriogenology*. 146:31–38. doi:10.1016/j.theriogenology.2020.01.050.
- Önsoy B, Salman A. 2005. Reproductive biology of the common cuttlefish *Sepia officinalis* L. (Sepiida: Cephalopoda) in the Aegean Sea. *Turkish Journal of Veterinary and Animal Sciences*. 29:613–619.
- Pierce GJ, Allcock L, Bruno I, Jereb P, Lefkaditou E, Malham S, Moreno A, Pereira J, Piatkowski U, Rasero M, et al. 2010. *Cephalopod biology and fisheries in Europe*.
- Rocha F, Guerra A, Gonzalez AF. 2001. A review of reproductive strategies in cephalopods. *Biological Reviews of the Cambridge Philosophical Society*. 76:291–304. doi:10.1017/S1464793101005681
- Salman A, Akalin M, Sen H. 2017. Residual Eggs in Post-Mortem *Sepia officinalis* (Mollusca: Cephalopoda).
- Thorsen A, Kjesbu OS. 2001. A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. *Journal of Sea Research*. 46:295–308. doi:10.1016/S1385-1101(01)00090-9
- Zatylny-Gaudin C, Henry J. 2018. Egg-Laying in the cuttlefish *Sepia officinalis*. In: *Biological resources of water*.