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## Variability in reproductive traits in the sex-changing fish, *Coris julis*, in the Mediterranean

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### Abstract

The quantity and quality of propagules, i.e. reproductive output, play a key role in the dynamics of exploited populations, with particular implications for sex-changing fish. Here, we studied for the first time the effects of maternal traits on the reproductive output of the Mediterranean rainbow wrasse *Coris julis*, a sequential hermaphroditic fish widely distributed in temperate marine coastal regions. The quantity of the reproductive output (batch fecundity) was significantly related to maternal size, which is of special interest in a species submitted to size-selective harvesting (recreational angling). However, egg quality (egg size) was not significantly related to any maternal factor and the spawning fraction was related to a seasonal pattern only. These results should contribute to improving estimations of the annual reproductive output and the stock assessment of this species. Certain implications for management are discussed.

**Keywords:** Batch fecundity, egg quality, maternal effects, Sex-change, Mediterranean, *Coris julis*.

### Introduction

The quantity and quality of reproductive output play a key role in understanding the population dynamics and life-history characteristics of exploited fish stocks (Hilborn & Walters, 1992; Marshall *et al.*, 2003). Reproductive output is usually estimated by measuring fecundity (Lambert, 2008). However, estimating the number of eggs released by a female during a year is challenging or even impossible, especially in indeterminate-fecundity fish species (Hunter *et al.*, 1989, 1992; Murua & Saborido-Rey, 2003). In such cases, estimating the spawning frequency and the number of spawning events within a spawning period (Lowerre-Barbieri *et al.*, 2011) is crucial for a proper estimation of annual egg production (Murua *et al.*, 2003). Besides, the quality of the reproductive output of a population refers to the egg and larval potential viability (Trippel, 1999). Therefore, both the ability of a female to produce viable eggs (Nissling *et al.*, 1998) and the egg's potential to produce viable fry (Kjorsvik *et al.*, 1990) play a key role in determining the fate of harvested populations.

The quantity and quality of the reproductive output are generally affected by the characteristics of the mother (Solemdal, 1997). Therefore, discriminating the role of the quantitative and qualitative maternal effects should

be considered as a correct way to estimate reproductive potential. Fish size is the most commonly reported maternal feature affecting reproduction, and both the quantity and quality of the reproductive output seem to improve in larger individuals (Birkeland & Dayton, 2005). However, the individual variability in reproductive timing is typically not considered when estimating reproductive output (Murua *et al.*, 2003; Murua & Saborido-Rey, 2003). Spawning frequency, however, has been shown to increase with both size and age (Ganias *et al.*, 2003; Claramunt *et al.*, 2007; Fitzhugh *et al.*, 2012) and is affected by other factors, such as the amount of energy available to the spawning females (Hunter & Leong, 1981; McBride *et al.*, 2013). Therefore, maternal factors exert a key influence in determining the reproductive output at individual level and, thus, at population level (Trippel & Neil, 2004), although they can be modulated by other environmental factors, such as seasonal trends (Trippel & Neil, 2004; Murua *et al.*, 2006; Treasurer & Ford, 2010).

Unfortunately, detailed information on reproductive output and the factors affecting output is limited in species with low interest for commercial fisheries, such as those that are only targeted by recreational fisheries (Alós *et al.*, 2013). This is the case for the Mediterranean rainbow wrasse, *Coris julis* (Linnaeus, 1758), a labrid species widely distributed in temperate coastal areas

(Froese & Pauly, 2013). *C. julis*, a low-value commercial fishery species, is one of the most frequently captured species in recreational fishing (Morales-Nin *et al.*, 2005; Cardona *et al.*, 2007; Lloret *et al.*, 2008). *C. julis* is a diandric protogynous hermaphrodite with two types of coloration (Bacci & Razzauti, 1958; Bruslé, 1987): *i*) an initial phase displayed by all females and a variable proportion of males (Bentivegna & Rasotto, 1983); and *ii*) a terminal phase displayed only by males. Little is known about the reproductive biology of *C. julis*, and available information is limited to a number of studies focusing on the process of sex change or sexual patterning (Bacci & Razzauti, 1958; Bentivegna *et al.*, 1985; Bruslé, 1987; Lejeune, 1987; Alonso-Fernández *et al.*, 2011).

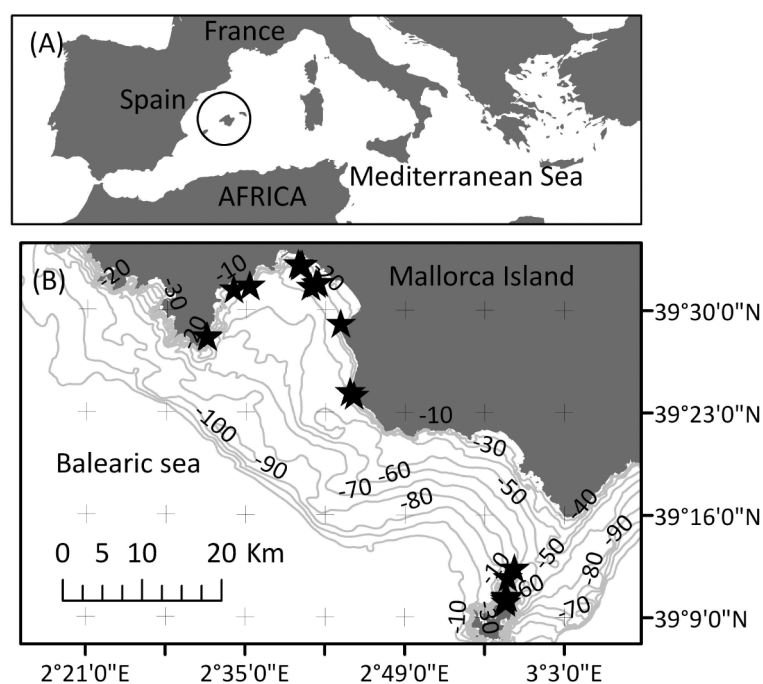
The outcome of the maternal effects on reproductive output are unknown in *C. julis*, even though they could play a major role in the case of protogynous species (Hawkins & Roberts, 2004; Fenberg & Roy, 2012). In sex-changing fish, size-dependent fishing activity preferentially targets large individuals. This bias alters the sex ratio and age structure of the population, which in turn appears to change the sex-change moment to earlier ages and render smaller females (Collins & McBride, 2011; Fenberg & Roy, 2012). Therefore, if the reproductive output of the females is related to the maternal size (Birkeland & Dayton, 2005), such biased harvesting pressure could decrease the population's reproductive output (Armstrong, 2001; Alonzo & Mangel, 2004; McBride *et al.*, 2008), which suggests special care in stock management (Brooks *et al.*, 2008). Therefore, the main objective of this study was to determine the annual reproductive output of *C. julis* females and identify its major

causes of variability. In addition to the likely existence of maternal effects, we also tested the possible existence of temporal variation in three specific reproductive parameters: *i*) batch fecundity; *ii*) egg quality and *iii*) spawning activity. This new information on reproductive characteristics may be useful for improving the estimation of biologically sound reference points.

## Material and Methods

### Biological sampling

*C. julis* individuals were collected during experimental fishing sessions along the south-western coast of Mallorca Island (north-western Mediterranean Sea; Fig. 1). The selected sites had optimal habitat characteristics for the studied species (10-20 m depth with a bottom habitat dominated by *Posidonia oceanica* seagrass). Individuals were captured using conventional recreational gear from February to October 2007, which fully covered the spawning season (Alonso-Fernández *et al.*, 2011). In total, 1,038 *C. julis* individuals were sampled (Table 1) and measured (Total length, TL; nearest mm). The gonads were removed and weighted (nearest 0.01 g) from all specimens and fixed immediately in a 10% solution of formalin buffered with  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (molar concentration = 0.046 M) and  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  (0.029 M). Central portions of the fixed gonads were extracted, dehydrated, embedded in paraffin, sectioned at 3  $\mu\text{m}$ , and stained with hematoxylin and eosin for microscopic analysis with a Leica Series RE digital microscope (Leica Microsystems, Wetzlar, Germany). Firstly, histological slides were examined to check sex. Then, for each ovary, the oocytes



**Fig. 1:** Map showing A) the spatial location of Mallorca Island and B) the sampling stations within the study area in the waters of Mallorca Island (NW Mediterranean).

**Table 1.** *Coris julis*. Estimates of the total length (TL) range of the Mediterranean rainbow wrasse based on samples collected from the waters of Mallorca Island (NW Mediterranean) during the course of the reproduction assessment study (2007).

Month	Total individuals (n)	Length range (mm)	Females (n)	Length range (mm)
February	1	131-131	1	131-131
March	121	84-160	70	84-154
April	160	78-208	71	78-178
May	164	91-194	89	94-166
June	160	90-198	96	90-157
July	189	79-166	93	82-154
August	176	89-177	78	89-177
September	25	105-154	16	105-153
October	42	88-192	25	88-154
Total	1,038	78-208	539	78-178

of each slide were classified into stages of development using conventional histological criteria (West, 1990; Tyler & Sumpter, 1996).

### Batch fecundity

Batch fecundity (the number of eggs spawned per batch) was estimated for all females with hydrated oocytes and no newly collapsed post ovulatory follicles (which indicate recent spawning) according to previous recommendations (Hunter *et al.*, 1985). We used the gravimetric method, in which fecundity is determined as the product of gonad weight and oocyte density (number of hydrated oocytes per gram of ovarian tissue) (Murua *et al.*, 2003). An ovarian subsample of approximately 150 mg (which comprises approximately 15% of the gonad weight) was extracted, and the hydrated oocytes were separated manually and counted applying a semiautomatic image analysis protocol (Alonso-Fernández *et al.*, 2008).

### Egg quality

The dry weight (mg/oocyte) of the hydrated oocytes was used as a proxy of egg quality (Kjorsvik *et al.*, 1990; Kamler, 1992; Brooks *et al.*, 1997). The same hydrated oocytes obtained after batch fecundity estimation were used to determine their dry weight. Dry weight was determined after drying the sample for 24 h at 110°C. The mean dry weight of a single hydrated oocyte was estimated by dividing the dry weight of the sample by the number of hydrated oocytes per sample.

### Spawning activity

Histological indicators of spawning differ in their duration. These differences determine how they should be interpreted. Ovulation oocyte stages are extremely short-lived histological indicators and were used as the most conservative method to assess spawning time (Lowerre-Barbieri *et al.*, 2011). For example, in spotted sea trout, ovulation can last from 6 to 14 h (Brown-Peterson, 2003). Therefore, oocyte maturation stages (germinal vesicle migration to hydration) are interpreted as markers of

imminent spawning to assess the spawning fraction, i.e. the proportion of mature females spawning daily (Hunter & Macewicz, 1985; Brown-Peterson, 2003). Spawning fraction was then assessed using the proportion of actively spawning females within the total number of spawning capable females (Brown-Peterson *et al.*, 2011). After estimating the fraction of the population spawning on a single day, the time between spawning events is simply calculated as the reciprocal of the spawning fraction (Murua & Motos, 2006). Finally, spawning frequency at population scale results from dividing the number of days in the spawning season by the spawning interval (Murua *et al.*, 2003; Murua *et al.*, 2006).

### Data analysis

We used a generalised linear model (GLM) (McCullagh & Nelder, 1989; Zuur *et al.*, 2007) to evaluate the significance of the effects of maternal factors (i.e. fish total length) and seasonal trend (i.e. sampling month) on individual reproductive output (batch fecundity, oocyte dry weight and spawning fraction). Different distributions were assumed regarding the response variables: *i*) Negative binomial for batch fecundity (to address over-dispersed count data); *ii*) Gaussian for oocyte dry weight and *iii*) Binomial for spawning probability. Model assumptions were evaluated based on the study of residuals patterns (Zuur *et al.*, 2010). Step-wise backward selection was used to select the optimal model according to the Akaike information criterion (AIC) and likelihood ratio tests (Zuur *et al.*, 2007). All statistical analyses were conducted using R software, version 3.0 (R Development Core Team, 2013).

## Results

### Batch fecundity

Although the spawning season of *C. julis* lasts from April to July, we restricted the statistical analysis of batch fecundity variation to May and June due to the low number of samples available for the rest of the spawn-

ing period (Table 2). Mean batch fecundity (all values expressed as the mean $\pm$ sd) for the entire study period, May–July, was 2,362 $\pm$ 2,153 hydrated oocytes and the mean total length of the analyzed individuals was 119 $\pm$ 12 mm. Mean relative batch fecundity was 78 $\pm$ 53 hydrated oocytes/g of the total weight of the female, but batch fecundity significantly and exponentially increased with length (Table 3). In contrast, there was no significant difference between May and June (Table 3); thus, a single batch fecundity-size relationship was considered by pooling all fish regardless of the sampling date (Fig. 2, Table 3). Batch fecundity largely decline in July to 706 $\pm$ 193 hydrated oocytes.

### Egg quality

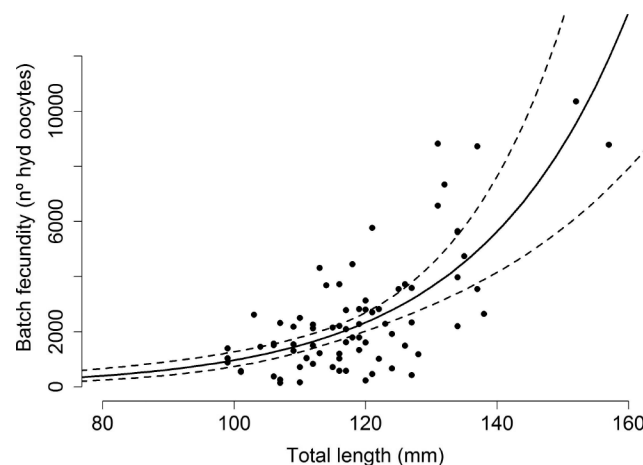
As for batch fecundity, we restricted egg quality variation analysis to May and June (Table 2). Mean oocyte dry weight was 0.0051 $\pm$ 0.0010 mg/oocyte in individuals with a mean total length of 119 $\pm$ 12 mm. The oocyte dry weight did not present any significant temporal trend (i.e. between-month differences). Similarly, regarding maternal factors, oocyte quality was not significantly related to fish size (Table 3).

### Spawning activity

We modelled the probability that a *C. julis* female would be at the spawning stage (assuming a ~24 h dura-

**Table 2.** *Coris julis*. Estimates of total length (mm), batch fecundity (n° hydrated oocytes) and egg dry weight (mg/egg) for the Mediterranean rainbow wrasse, based on sub-sampling selection for batch fecundity and egg quality estimations for the reproduction assessment study (2007).

Month	number (n)	Length range (mean $\pm$ sd)	Batch fecundity (mean $\pm$ sd)	Egg dry weight (mean $\pm$ sd)
May	32	118 $\pm$ 11	2,142 $\pm$ 1,964	0.0053 $\pm$ 0.0011
June	49	119 $\pm$ 12	2,782 $\pm$ 2,288	0.0050 $\pm$ 0.0009
July	5	122 $\pm$ 18	706 $\pm$ 193	0.0050 $\pm$ 0.0015
Total	86	119 $\pm$ 12	2,362 $\pm$ 2,153	0.0051 $\pm$ 0.0010



**Fig. 2:** *Coris julis*. Fish total length–batch fecundity relationship in the waters of Palma Bay and Cabrera Archipelago National Park (NW Mediterranean) during the study.

**Table 3.** *Coris julis*. Summary of parameters of the generalised linear models performed to test the relationship of maternal (total length in mm) and seasonal factors with batch-fecundity and spawning fraction. April was selected as the reference month.

Response	Explanatory	Estimate	Std. Error	t/Z value	p-value
<i>Batch fecundity</i>					
n=79	Intercept	2.4667	0.7615	3.24	0.0012
Deviance explained=39.46%	Length	0.0441	0.0064	6.89	<0.0001
<i>Spawning fraction</i>					
n=260	Intercept	2.1401	0.7475	2.86	0.0042
Deviance explained=8.71%	May	-0.1942	0.8141	-0.24	0.8115
	June	0.2464	0.8339	0.30	0.7676
	July	-1.5737	0.7959	-1.98	0.0480

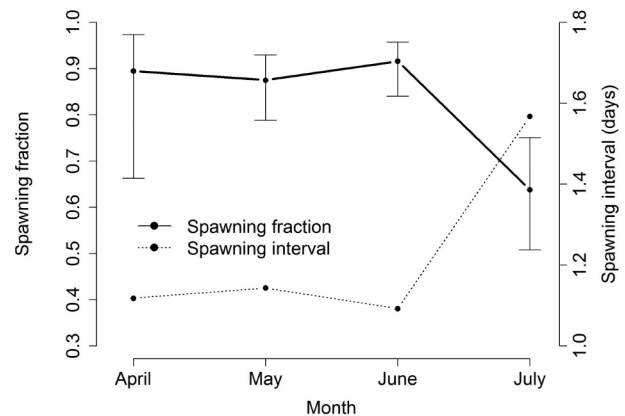


tion of the histological markers used) as a function of total fish length and seasonality (i.e. between-month differences). Female length was not significantly related to spawning probability, whereas between-month differences were significant (Table 3). The temporal pattern indicated a more or less stable spawning fraction along the spawning season but showed a significant decrease in July, the last month of the spawning season (Fig. 3). The spawning interval for each month was estimated to be 1.1 days from April to June and 1.6 days in July. For April and July it was not possible to construct a reliable batch fecundity-size relationship due to the low number of samples available. Therefore, we used a single batch fecundity-total length relationship for the complete spawning period based on statistical results (Table 3). Combining the point estimations for batch fecundity and spawning fraction, we estimated egg production per individual and month. A similar egg production rate per individual was estimated for all months, except for July, when females experienced a significant reduction of nearly 30% (Fig. 4). All this variation is due to monthly variation of the spawning fraction since batch fecundity was assumed to be constant throughout the period.

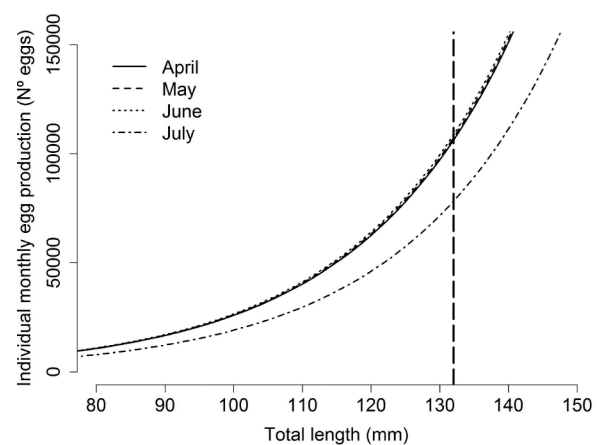
## Discussion

In this study, we investigated a fundamental but frequently neglected aspect of the population dynamics of *C. julis* in the NW Mediterranean Basin. As in many other species with no or limited interest for commercial fisheries (Alonso-Fernández *et al.*, 2011), information on the quantity and quality of the reproductive output is limited. However, *C. julis* is frequent in the creel of recreational anglers, and due to the growing interest for such fisheries in the Mediterranean, data on reproductive characteristics will play a key role in designing proper management and ensuring sustainable yield (Morgan, 2008). Here we describe, for the first time, the variability of the reproductive output of *C. julis* and the factors affecting output. We provide evidence of the existence of maternal effects (a positive exponential relationship between fish size and batch fecundity). This exponential relationship between fish size and number of eggs produced per batch should have a relevant influence on harvested populations of *C. julis* due to the strong size-selective mortality associated with this type of recreational (i.e. hook-and-line) fishing (Cerdà *et al.*, 2010) and the expected reduction in size and age of sex-change (Collins & McBride, 2011; Fenberg & Roy, 2012). Therefore, the results obtained here can be useful for ecologists and fisheries scientists and help them to gain a better understanding of the population dynamics of this widely distributed species in the Mediterranean (Marshall *et al.*, 2003).

Morgan (2008) demonstrated that fecundity data can be incorporated into indices of reproductive poten-



**Fig. 3:** *Coris julis*. Estimated monthly variation of the spawning fraction and spawning interval for each sampling period. Estimated parameters of the relationship are presented in Table 3. Vertical bars represent 95% confidence interval.



**Fig. 4:** *Coris julis*. Individual monthly egg production estimations. The vertical line represents species size at 50% sex change in the Balearic Islands, NW Mediterranean (Alonso-Fernández *et al.*, 2011) to illustrate a likely threshold size for egg production rate at population level.

tial, which can have a great impact on estimates of reference points and recognition of stock status. Furthermore, Lambert (2008) has emphasized the importance of adding measures of fecundity to other parameters for exploited marine fish stocks, in order to improve assessment of reproductive potential. In this respect, this is the first study carried out to demonstrate the existence of a relationship between batch fecundity and fish size for *C. julis* total egg production. Our results on the number of eggs released per batch are close to those reported for other members of the Labridae family, such as *Notolabrus fucicola*, for which  $75 \pm 35$  hydrated eggs per gram of female have been described (Harwood & Lokman, 2006), while considerably different from others, such as *Semicossyphus pulcher*, with  $38 \pm 28.5$  hydrated eggs per gram of female (Loke-Smith *et al.*, 2012). Generally, fecundity and female length are well related (Birkeland &

Dayton, 2005). However, in species with indeterminate fecundity (i.e. batch spawners), this relationship is usually estimated from batch fecundity rather than potential fecundity, which introduces an additional level of variation (between-batch variability) and tends to reduce the explanatory power of fish length in predicting fecundity (Wootton, 1990; Fitzhugh *et al.*, 2012). This seems to be the case for *C. julis*, for which there is clear evidence of a positive exponential relationship between fecundity and fish size, but the predictive power of this relationship is relatively poor. Seasonal patterns in egg production have been previously reported in fish species with determinate (Trippel and Neil, 2004) an indeterminate (Macchi *et al.*, 2004; Murua *et al.*, 2006) fecundity strategies. These changes are generally associated with fish condition and nutritional status of females (Trippel & Neil, 2004; Murua *et al.*, 2006). During peak spawning activity in *Coris julis*, May and June (Alonso-Fernández *et al.*, 2011), no temporal changes were detected. However, batch fecundity at the end of the spawning season suffered a large decline, in agreement with other indeterminate fecundity species (Macchi *et al.*, 2004). Despite this decreasing trend at the end of the spawning season, it is not possible to draw robust conclusions due the low number of samples available. Increased sampling effort is required throughout the entire spawning season in order to obtain a sample size that is adequate for statistical modelling of egg production.

We refer to “egg quality” as “egg size” (expressed as oocyte dry weight) under the assumption that “bigger is better” (Birkeland & Dayton, 2005). Maternal effects on egg quality have been widely recognized in temperate fish species (Marteinsdottir & Steinarsson, 1998; Vallin & Nissling, 2000); however, several counter-examples have been documented (see references in Kamler, 1992). In *C. julis*, the maternal effects (female size) were not significant within the sampled size range. In this respect, it has been suggested that there are likely confounding factors associated with different environmental cues (temperature and/or salinity) that they could obscure the relationship between egg and fish size (Chambers, 1997). Moreover, considering the short life-span and size range of *C. julis* females (i.e. sex change occurs at approximately 4 years old and around 13 cm in the area studied; Alonso-Fernández *et al.*, 2011; Linde *et al.*, 2011), the maternal effects on egg quality are difficult to detect in comparison to species with long life-spans, such as Sebastes spp (Berkeley *et al.*, 2004).

In addition to the estimates of batch fecundity per individual, an estimation of the annual realized fecundity in indeterminate species is needed to estimate the number of batches released within the spawning season, i.e. spawning frequency (Murua *et al.*, 2003; Lowerre-Barbieri *et al.*, 2011), which is estimated at population level from the fraction of females at the spawning stage at a given time (Armstrong & Witthames, 2012). The spawning

season of *C. julis* lasts approximately 4 months, when most females spawn daily or every 2 days. A proper estimate of the spawning fraction is highly dependent on correct definition of the spawning stage (Uriarte *et al.*, 2012). Different histological spawning markers have different life-spans (i.e. are observable for more or less time after ovulation or oocyte hydration Lowerre-Barbieri *et al.*, 2011; Armstrong & Witthames, 2012). For example, in warm waters, an oocyte of *Lutjanus campechanus* was found to take approximately 10 h to fully hydrate (Jackson *et al.*, 2006), and oocytes of *Cynoscion nebulosus* can last 6–14 h from ovulation to spawning (Brown-Peterson, 2003). In contrast, in deep sea species such as *Hippoglossus hippoglossus*, the complete hydration process can take 35–55 h (Finn *et al.*, 2002). Egg size can also have an effect on timing of hydration, for instance McBride *et al.* (2003) reported a 30–36-hour period for final oocyte maturation for *Hemiramphus brasiliensis* (2.0–3.5 mm egg diameter) in southern Florida. Therefore, considering the temperature range of the Mediterranean Sea, *C. julis* oocyte size and the histological markers used, the assumption that a specific *C. julis* female has ovulated within 24 h seems reasonable. The high rate of females found at the spawning stage suggests that spawning events in *C. julis* take place often, almost every day, and that the high rate remains relatively constant throughout the spawning season. With such a high frequency of batches, our results, i.e. that the probability of being in the spawning stage does not depend on fish size, are not surprising. These results are in contrast with the general patterns observed in indeterminate fecundity species, which shows that the spawning fraction is positively size/age related (Claramunt *et al.*, 2007; Ganas *et al.*, 2007; Uriarte *et al.*, 2012). However, in species that spawn every day there is a boundary effect that obscures our interpretation.

The clear effects of fish size on egg quantity (batch fecundity) should play an important role in the dynamics of an exploited population. *C. julis* is a sedentary species with high site fidelity and a small home range (Palmer *et al.*, 2011), which makes this species particularly vulnerable to harvesting-related depletion at small spatial scales (Palmer *et al.*, 2011). Therefore, spatially patchy harvesting pressure can directly reduce abundance and, thus, reproductive potential (i.e. the total egg production) of the stock. Secondly, due to the small mouth size of *C. julis*, recreational harvesting is highly size selective, with large fish being more vulnerable than smaller ones (Cerdà *et al.*, 2010). In protogynous species, this size-selective mortality is indirectly sex selective, introducing a greater bias in operational sex ratios, i.e. males (older and larger) are more vulnerable to local depletion than females (younger and smaller) (Hamilton *et al.*, 2007). According to the “size advantage model” (Ghiselin, 1969), the largest females and fastest growers (Linde *et al.*, 2011) change sex in response to a modification of the social

structure of the population. Therefore, a higher probability of removing larger males could lead to a reduction of not only the total female population but, more importantly, large females. In fact, this expected response to anthropogenic harvesting has been confirmed in *C. julis*, and a larger and higher proportion of terminal males has been observed inside marine protected areas in the Mediterranean, where fishing activity is prohibited (Harmelin *et al.*, 1995). Therefore, size-dependent harvesting activity can induce a “dwarfing” and “juvenescence” of females inhabiting harvested populations (Collins & McBride, 2011), which can induce a strong decrease in the reproductive output of exploited populations of protogynous hermaphrodites (Hawkins & Roberts, 2004; Hamilton *et al.*, 2007). Populations with less fecund females will be more vulnerable to over-exploitation (Birkeland & Dayton, 2005), and effective management should incorporate some replenishment mechanism to alleviate the depletion of the number of individuals harvested and maintain sustainable density. In this sense, marine protected areas can play a role due to the limited movement of *C. julis* (Palmer *et al.*, 2011).

Finally, this study deepens our understanding of the reproductive biology of *C. julis* and is key for developing future estimations of stock reproductive potential. Additionally, we have identified additional areas of research that will complement the findings reported here, such as *i*) confirming the assumption made for the duration of spawning markers and *ii*) completing more detailed demographic studies of other reproductive parameters in order to understand the spatio-temporal variation of reproductive output. This information should prove useful to managers, especially because the implementation of relatively small marine protected areas in the Mediterranean has become more popular recently (Francour *et al.*, 2001). These positive factors could steer the exploitation of *C. julis* stocks in a direction that favours the sustainable development of a recreational fishery.

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