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Climate-driven changes in life-history traits of the bastard grunt *Pomadasys incisus* (Teleostei: Haemulidae) in the north-western Mediterranean

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Abstract

This study evaluates the influence of sea water temperature on the life-history traits of the thermophilic bastard grunt *Pomadasys* incisus along the Catalan coast. We compared two key traits of this species, condition and reproduction, from populations in two separate areas with different thermal regimes: the Gulf of Roses (cold area) and the Ebre Delta (warm area). Specimens were collected monthly from each area between 2010 and 2012. The results were also compared with those given in the literature from sites where the species is more common, i.e. the southern Mediterranean and eastern Atlantic. Our findings indicate that, compared to the populations in warmer environments, the population inhabiting the colder waters appears to be in significantly poorer condition and there is some deviance in their life-history traits: egg size and quality being traded-off for higher egg numbers; lower lengths at maturity and alterations to spawning phenology. A certain degree of plasticity in life-history traits may favour the process of expansion and establishment of this thermophilic species into newly available but colder habitats of the north-western Mediterranean under a scenario of climate change.

Keywords: *Pomadasys incisus*, bastard grunt, condition, reproduction, sea warming.

Introduction

Sea warming is allowing northward expansion of thermophilic species in the Mediterranean (Francour *et al.*, 1994; Molinero *et al.*, 2005; Perry *et al.*, 2005; Sabatés *et al.*, 2006, 2012; Lloret *et al.*, 2014). It has been observed, in this context, that invading species may display deviant life-history traits showing, for example, great plasticity in growth and fecundity - as compared to well-established conspecific populations in other areas - which in periods of rapid population growth may favour the processes of expansion and establishment of these invading species in new habitats (Rosecchi *et al.*, 2001; Bøhn *et al.*, 2004; Ribeiro & Collares-Pereira, 2010). The extent of this plasticity in their life-history traits, which is a response to either fish density or environmental conditions, is dependent on adaptations to environmental variation which, in turn, are limited by the inherent physiological and behavioural characteristics of each fish species (Alcaraz & García-Berthou, 2007; Ribeiro & Collares-Pereira, 2010). In this sense, fish condition and reproduction are two key life-history traits that can help to characterize certain components of the environment in which fish exist and thus reveal changes - such as sea warming - in environmental conditions (Lloret *et al.*, 2002, 2012, 2014; Lloret & Planes, 2003; Pankhurst & King, 2010; Pankhurst & Munday, 2011).

The bastard grunt, *Pomadasys incisus* (Bowdich 1825), is a coastal demersal species inhabiting marine and brackish waters (Pajuelo *et al.*, 2003a), usually near sandy or muddy substrate, at depths between 10 and 100 m, although it is more commonly found not far from 50 m (Kapiris *et al.*, 2008). Its main distribution encompasses the eastern part of the Atlantic Ocean, from Angola to Gibraltar (including the Canary Islands, Madeira and the Cape Verde Islands). However, this subtropical and thermophilic species is currently spreading and establishing itself within the whole Mediterranean Sea (Bodilis *et al.*, 2013). This species naturally entered the Mediterranean Sea through the Strait of Gibraltar but the prevailing currents, sea warming and the availability of suitable soft substrate in relatively shallow waters has recently allowed *P. incisus* to establish itself in the north-western Mediterranean basin (Francour *et al.*, 1994; Pastor *et al.*, 2008; Bodilis *et al.*, 2013). *P. incisus* is an example of the latitudinal extensions and/or demographic increase of thermophilic fishes, which are taking advantage of the current climate change (Psomadakis *et al.*, 2012). In the Gulf of Roses and adjacent waters in particular (southern Gulf of Lyon), the bastard grunt has apparently become more frequent since the 1950s (Lloret *et al.*, 2011). However, landings statistics for this area are inexistent.

Information on the biology of *P. incisus* has so far been restricted to the Canarian Archipelago (Pajuelo *et al.*, 2003a, 2003b) and the Gulf of Tunis (Chakroun-Marzouk

& Ktari, 2006; Fehri-bedoui & Gharbi, 2008) where its natural subtropical habitats and warmer environmental conditions prevail. Therefore, since the shifts in the bastard grunt distribution appear to be the result of current climate change, this warm water species appears to be a good candidate for investigating the effects of sea warming (Francour *et al.*, 1994; Bodilis *et al.*, 2013). The overall aim of this study was to compare two key life-history traits - condition and reproduction - of the bastard grunt from two separate areas of the north-western Mediterranean with different thermal regimes in order to assess whether or not the particular life history traits of this thermophilic species may contribute to its expansion in the area.

Materials and Methods

Sampling locations

Samples were collected from two different areas with non-identical thermal regimes along the Catalan coast: the northern-most Gulf of Roses and the southern-most Ebre Delta (Fig. 1). The Gulf of Roses and surrounding waters have lower mean water temperatures than the Ebre Delta (Salat *et al.*, 2002). Therefore, the data on sea surface temperature (SST, in °C) from both sampling areas (the Gulf of Roses and the Ebre Delta) was retrieved from the International Comprehensive Ocean-Atmosphere Data Set (ICOADS) for the period 2010-2012 (ds540.0-Release 2.5) (Woodruff *et al.*, 2011). The data comprised individual daily mean SST observations for 1° latitude x 1° longitude units. In this study, we used the time series from two 1° squares in the north-western Mediterranean: the Gulf of Roses (42.0 to 43.0°N and 3.0 to 4.0° E) and the Ebre Delta (40.0 to 41.0°N and 0.0 to 1.0° E). The mean monthly values of SST were calculated by averaging daily temperatures. Monthly SSTs were indeed significantly higher in Ebre Delta than in the Gulf of Roses (ANOVAs, $p < 0.001$). In both areas, monthly SSTs began to increase from April to May, peaked in August, and then decreased during the coldest months from January to March (Fig. 2).

In order to compare the growth, condition and reproduction parameters of *P. incisus* between the two studied areas, samples were collected monthly from fishermen (coastal purse seiners) at Roses (Gulf of Roses) from July 2010 to September 2012 and at the port of Sant Carles de la Ràpita (Ebre Delta) from May to September 2012, shortly after the small-scale and trawl fishing vessels had landed their catches. It should be noted that fishermen were asked about the approximate location and depth where the specimens were caught in order to ensure that the origin of the samples is within the coastal waters of each study area. At the laboratory, we recorded total length (TL) to the nearest 0.5 cm and total weight (TW) to the nearest 1 mg. All samples were dissected and eviscerated, and the somatic or eviscerated body weight (SW), liver weight (LW) and gonad weight (GW) were obtained to the nearest 1 mg. The gonads were fixed in 4% buffered formalin for further histological processing and fecundity estimation.

Fish Condition and Reproductive Biology

The hepatosomatic index (HSI) was calculated for each individual as a function of somatic wet weight (SW) in order to avoid possible variations arising from the contents of the digestive tract. Thus, HSI was calculated as HSI = 100*(LW/SW), where LW and SW represent liver and somatic wet weights, respectively.

The gonadosomatic index (GSI) was also estimated for each individual using the formula GSI = 100*(GW/SW), where GW and SW represent gonad and somatic wet weights, respectively. Although the sex and reproductive status of specimens were macroscopically determined

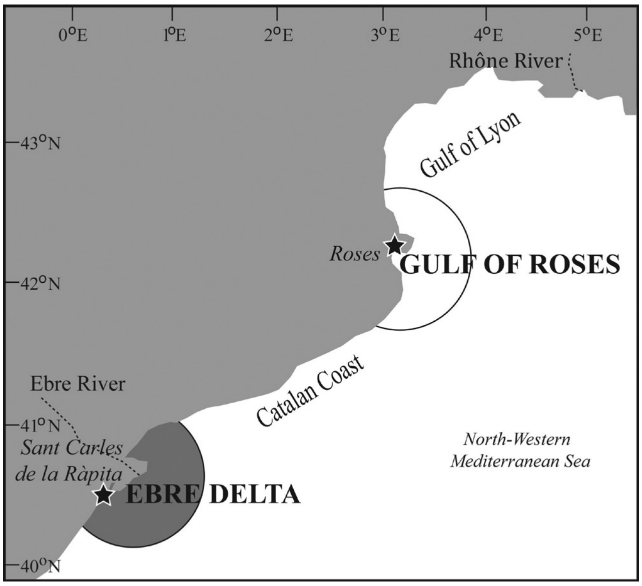

Fig. 1: Map of the Catalan coast showing the two study areas (the Gulf of Roses and the Ebre Delta), and the location of the fishing ports (Roses and Sant Carles de la Ràpita) where specimens of *Pomadasys incisus* were sampled.

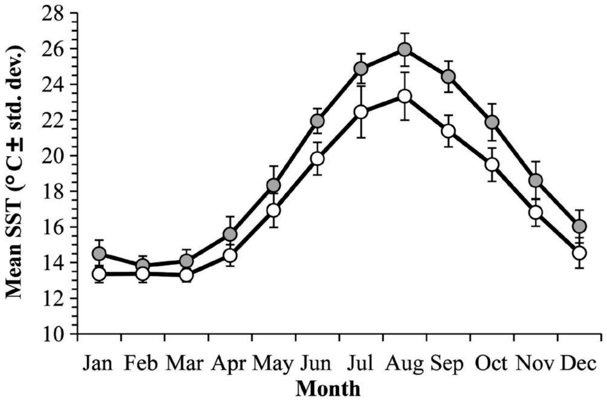

Fig. 2: Mean (± std. dev.) monthly variation of the sea surface temperatures (SSTs) in the Gulf of Roses (white circles) and the Ebre Delta (grey circles) during the study period (2010-2012).

initially, a histological analysis was also performed in order to provide a more accurate analysis of the reproductive characteristics and the annual reproductive cycle of *P. incisus*. Thus, a histological study of the gonads of each individual was carried out in order to determine the stages of development of their germ cells. Central portions (transverse sections) of the fixed gonads were dehydrated and embedded in paraffin, sectioned at between 3-8 μm, depending on their state of maturity, and stained with haematoxylin-eosin and Mallory's trichrome. The latter staining method highlights the zona radiata and its continuity, and facilitates the detection of atretic oocytes (Muñoz *et al.*, 2010). The maturation stages of the gonads were classified in line with Brown-Peterson *et al.* (2011) in order of developmental appearance as regenerating (RGN), early developing (EDEV), developing (DEV), spawning capable (SC), actively spawning (AS), and regressing (RGS).

In order to define sexual maturation as a function of body length, the L_{50} (body length at which 50% of the individuals were mature) was estimated separately for samples from both sampling areas. To predict the probability that an individual was mature based on its length, binary maturity observations (0 = immature, 1 = mature) and length (TL) were fitted to binary logistic models in order to construct maturity ogives (maturity-at-length probability plots) based on logistic equations using 1 cm length classes. Size at maturity was estimated initially for females and males.

The presence of hydrated oocytes and post-ovulatory follicles (POFs) was histologically determined in order to select suitable specimens for the analysis of fecundity. The oocyte size-frequency distribution for each spawning female was analysed in order to detect the batches of oocytes, since this may demonstrate different maturation patterns and associated fecundities (Kjesbu *et al.*, 1998). Thus, fecundity was estimated for each specimen found to be in either spawning capable (SC) or actively spawning (AS) phase - and without post-ovulatory follicles (POFs) - using the gravimetric method combined with image analysis as explained by Murua *et al.* (2003). With this aim, subsamples of about 150 mg were taken from the ovary; oocytes were separated from connective tissue using a washing process (Lowerre-Barbieri & Barbieri, 1993) and sorted by size through several sieves (from 1000 to 100 μm), which facilitated the subsequent work of counting and measuring oocytes using a computer-aided image analysis system (Image-Pro Plus 5.1). Subsamples were taken from sections of the middle part of the ovary since there were no significant differences in the number of most advanced oocytes per gram among the anterior, middle and posterior parts of the ovary of 10 specimens that were in the latest developmental stages (ANOVA, $F_{2,29} = 2.24$, $p = 0.125$). Thus, estimates of fecundity were based on batch fecundity (BF) - defined as the number of eggs spawned per batch - which was estimated according to Hunter *et al.* (1985) as $BF = GW*(Y/Sw)$, where GW is the gonad weight after fixation, Y is the number of hydrated oocytes in a weighted subsample of ovarian tissue and Sw is the subsample weight. Relative batch fecundity (RBF) was also calculated, as batch fecundity per gram of somatic weight of the fish. Only 98 females (41 from the Gulf of Roses and 57 from the Ebre Delta) met the histological criteria (actively spawning with hydrated oocytes and without POFs) for fecundity analysis.

In order to explore the size range for each oocyte developmental stage, the mean diameter of 200 oocytes from each stage was measured from the histological sections as the average of major and minor axes. Due to their irregular shape, the mean diameter of the hydrated oocytes was estimated separately after adding glycerine, which facilitates their differentiation.

The dry weight and diameter of hydrated oocytes were used to estimate the quality of the eggs, hence an approximation of the potential reproductive success (Brooks *et al.*, 1997). Thus, mean dry weights in mg per egg, were estimated by drying (for 24 h at 110°C) two replicates per sample of the eggs from a total of 20 actively spawning females per sampling area.

The prevalence of atresia, Pa (percentage of sexually mature females that have α-atretic vitellogenic oocytes) and the relative intensity of atresia, RIa (percentage of α-atretic vitellogenic oocytes in relation to the total number of vitellogenic oocytes) were estimated from observations at three different focal planes of different histological slides for each specimen (Kurita *et al.*, 2003).

Statistical Analyses

Firstly, generalized linear models (GLMs) (McCullagh & Nelder, 1989) were used to investigate the variation of fish condition with sex, stage of maturity and size. Therefore, GLMs were fitted to HSI and GSI as response variables; sex (females and males) and maturity (immature, developing, spawning capable, regressing, regenerating) were used as categorical predictor variables, while size (total length) was used as the continuous predictor variable. All predictors and their first order interactions were initially included in the GLM. Analysis of deviance to evaluate the significance (F-test) of the factors in the model was performed by a stepwise procedure, and the most appropriate error models were chosen on the basis of residual plots. GLMs incorporating sex and maturity as predictor variables accounted significantly for 42.3% of the deviance of HSI (ANOVA, $F_{9,595} = 49.48$, $p < 0.0.001$) and 65.4% of the variability in GSI (ANOVA, $F_{9,595} = 125.97$, $p < 0.0.001$). Because size did not significantly account for the deviance of either HSI or GSI ($p > 0.05$), we used the GLM approach to standardize HSI and GSI data only for the effects of sex and maturity by estimating the adjusted means of HSI and GSI for the variation of the covariables (sex and maturity).

Taking into account that the temporal scale of the samplings did not match entirely between study areas, i.e. in the Gulf of Roses from 2010 to 2012, while in the Ebre Delta only during 2012, and in order to carry out the comparative analyses between sampling areas, firstly a sub-dataset of the Gulf of Roses samples was created, which matched the temporal scale of the Ebre Delta sampling period (from May to September 2012). This analysis indicated that, even at this reduced temporal scale (2012), there were significant differences between sampling areas in the condition and reproduction variables such as, for example, GSI (ANOVA, $F_{1,407} = 4.56$, $p = 0.033$) or HSI (ANOVA, $F_{1,407} = 4.84$, $p = 0.028$). Secondly, there were insignificant interannual differences (2010, 2011 and 2012) among samples from the Gulf of Roses, e.g. GSI (ANOVA, $F_{2,266} = 2.13$, $p = 0.1209$) or HSI (ANOVA, $F_{2,266} = 2.51$, $p = 0.0832$). Overall, the significant differences between sampling areas, at the reduced temporal scale (2012), as well as the insignificant interannual differences (2010-2012) within the Gulf of Roses, supported the decision to group of three reproductive annual cycles from the Gulf of Roses in order to compare each variable with those of the Ebre Delta.

Subsequently, for all aforementioned variables (HSI, GSI, batch fecundity, oocytes diameter), the Shapiro-Wilk test was used to test the assumptions of normality and Levene's test was used to test the homogeneity of variances (Zar, 1996). If assumptions were met, ANOVA models were used to compare all indices between sexes (females vs. males) and sampling areas (Gulf of Roses vs. Ebre Delta). A p-value of $\alpha = 0.05$ or less was considered to be statistically significant. Moreover, if the ANOVA indicated significant differences, Bonferroni's multiple tests were applied for post hoc comparisons of significant effects (Sokal & Rohlf, 1995).

Finally, the relationship between batch fecundity (BF) and total length (TL) was estimated by fitting power functions. Then, ANOVAs (for relative batch fecundity), ANCOVAs (for batch fecundity using TL as covariate) and paired Student's tests (for oocyte quality and relative intensity of atresia) were also used to find out whether, on average, differences between sampling areas, for the aforementioned variables, were statistically different (Zar, 1996).

Results

Of the 596 fish examined, a total of 267 specimens (158 females and 109 males) and 329 specimens (174 females and 155 males) of *P. incisus* were sampled in the Gulf of Roses and the Ebre Delta, respectively. The total length-frequency distributions ranged similarly in the two study areas from 13.0 to 29.0 cm (Fig. 3).

The standardized HSI were significantly higher on average in the Ebre Delta than in the Gulf of Roses (ANOVA, $F_{1,595} = 5.95$, $p = 0.015$), indicating better fish condition in the southern population (Table 1). Ebre Delta specimens also showed significantly higher average GSI compared to those from the Gulf of Roses (ANOVA, $F_{1,595} = 4.90$, $p = 0.027$), indicating that the reproductive investment was much higher in the southern population (Table 1).

With regard to the time of the year, it was found that the GSI of females and males of *P. incisus* peaked in July-September in similar fashion in both sampling areas, indicating that this species, in both our study areas, spawns only once per year (Fig. 4). This was later confirmed by the similar trends in maturity stages (expressed in frequency of occurrence) throughout the year; no differences between sexes or between sampling areas were observed in the maturation pattern of the gonads of this species (Fig. 4). The developing stages were observed mainly from May to June; then, the spawning activity began from July to September; subsequently the spawning activity ceased as the regressing stage became more evident; and finally from late September to April, the proportion of females at the regeneration stage increased. As stated before, similarities in the spawning phenology were observed between sampling areas; however, it was noticeable that spawning specimens began to appear simultaneously in July in both areas even though SSTs were considerably different, i.e. in the Ebre Delta spawning specimens appeared in catches at SSTs ranging from 24.0°C to 26.5°C, whereas in the Gulf of Roses spawning specimens began appearing at SSTs ranging from 19.3°C to 25.0°C.

The total lengths (TLs) of immature fish (n = 34) ranged from 13.0 to 18.0 cm, and the mean length at which 50% of females and males were mature (L_{50}) was lower in the Gulf of Roses than in the Ebre Delta (Table 1). An overall L_{50} (sexes and areas pooled) was estimated at 14.9 cm TL for the north-western Mediterranean (Gulf of Roses and Ebre Delta together), and no immature individuals were found with TLs greater than 19 cm.

For the two areas combined, the range of oocyte diameter at the different stages of development were as follows: cortical alveolar (CA, 100 – 200 μm), early

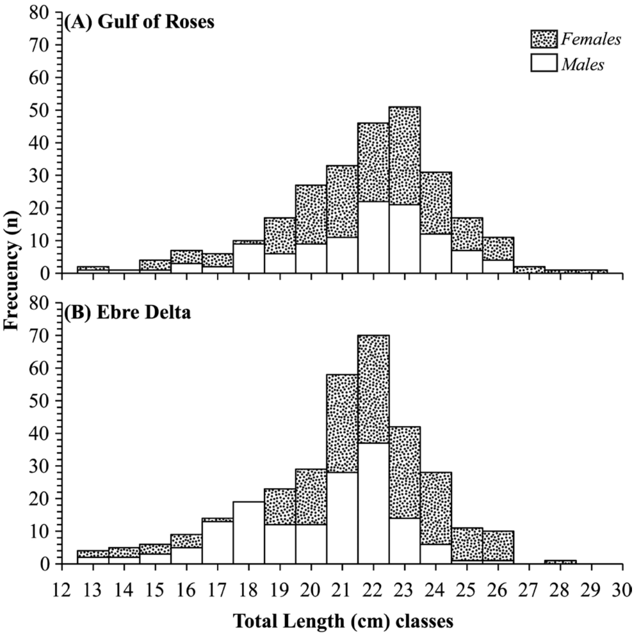

Fig. 3: Size-frequency distributions (1 cm TL interval classes) for specimens of *Pomadasys incisus* sampled for this study per sex at each sampling area: (A) Gulf of Roses and (B) Ebre Delta.

Table 1. Summary of the mean values (± std. dev.) for specimens of *Pomadasys incisus* and comparison tests evaluating the effect of the sampling area, Ebre Delta (ED) vs. Gulf of Roses (GR), on the following parameters: hepatosomatic index (HSI), gonadosomatic index (GSI), length at maturity (L_{50}, sexes pooled, cm TL), oocyte diameter (µm, for each developmental stage), egg quality (mg per egg), spawning season (months and temperature range), mean intensity of atresia (RIa, % vitellogenic oocytes in α-atretic state), batch fecundity (BF, eggs per spawning batch), relative batch fecundity (RBF, eggs per gram of body mass). P-values indicating significance are also shown where ** indicates $p < 0.05$; *** indicates $p < 0.001$, and NS indicates no significant difference.

Variable	Source	Mean (± std. dev.) ED	Mean (± std. dev.) GR	Outcome	n	Test	Statistic	P
HSI	Pool	2.02 ± 0.57	1.58 ± 0.45	ED > GR	596	ANOVA	$F_{1,595} = 5.95$	**
GSI	Pool	3.28 ± 1.99	2.56 ± 1.91	ED > GR	596	ANOVA	$F_{1,595} = 4.90$	**
L_{50}	Males	15.3	14.1	ED > GR	264			
	Females	15.6	14.9	ED > GR	332			
Oocyte diameter	CA	154.9 ± 23.5	138.6 ± 19.6	ED > GR	400	ANOVA	$F_{1,399} = 56.20$	***
	Vtg-1	248.8 ± 24.7	228.9 ± 24.1	ED > GR	400	ANOVA	$F_{1,399} = 66.92$	***
	Vtg-2	346.0 ± 25.0	334.5 ± 27.6	ED > GR	400	ANOVA	$F_{1,399} = 18.88$	***
	Vtg-3	457.4 ± 26.7	434.3 ± 23.9	ED > GR	400	ANOVA	$F_{1,399} = 82.71$	***
	GVM	547.4 ± 24.1	529.2 ± 24.4	ED > GR	400	ANOVA	$F_{1,399} = 56.81$	***
	H	728.6 ± 57.1	700.4 ± 60.1	ED > GR	400	ANOVA	$F_{1,399} = 23.14$	***
Egg quality		0.124 ± 0.05	0.116 ± 0.08	ED > GR	40	Student's	t = 2.134	**
Spawning	SSTs	24.0-26.5	19.3-25.0	ED > GR	336			
	Period	Jul to Sep	Jul to Sep	=	336			
RIa		96.1 ± 8.1	95.5 ± 2.1	=	37	Student's	t = 1.788	NS
BF		7971 ± 4371	14033 ± 6912	GR > ED	98	ANCOVA	$F_{1,96} = 42.12$	***
RBF		51.1 ± 19.5	84.2 ± 27.6	GR > ED	98	ANOVA	$F_{1,97} = 48.81$	***

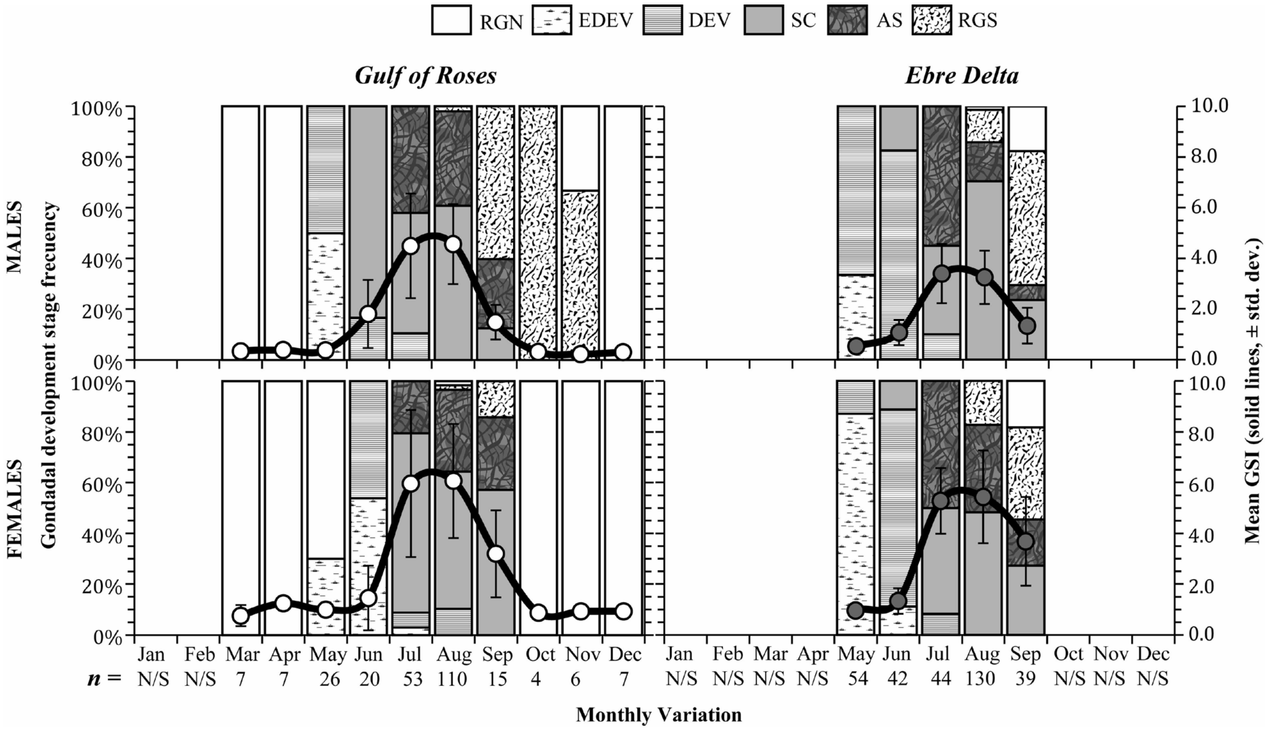

Fig. 4: Monthly frequency (percent abundance) of the ovarian and testis development stages, and mean (± std. dev.) variation in the gonadosomatic index (GSI) for specimens of *Pomadasys incisus* sampled in the Gulf of Roses and the Ebre Delta. Development stages: regenerating (RGN), early developing (EDEV), developing (DEV), spawning capable (SC), actively spawning (AS), and regressing (RGS). Sample number per month (n) is also given. N/S denotes periods in which no samples were caught.

vitellogenesis (Vtg-1, 200 – 300 μm), mid vitellogenesis (Vtg-2, 300 – 400 μm), advanced vitellogenesis (Vtg-3, 400 – 500 μm), germinal vesicle migration (GVM, 500 – 600 μm) and hydration (H, 600 – 900 μm). However, at each of these stages of development, the mean diameter of oocytes from the warmer Ebre Delta were significantly larger than those captured in the Gulf of Roses ($p < 0.001$) (Table 1).

Batch fecundity (BF) ranged from 2738 to 35685 eggs per spawning batch in fish ranging from 18.5 to 29.0 cm TL (Fig. 5). The relationship between BF and TL was fitted to the following exponential regression models: in the Gulf of Roses ($BF = 0.0036 \cdot TL^{4.642}$, $r^2 = 0.623$, n = 41) and in the Ebre Delta ($BF = 0.0041 \cdot TL^{4.749}$, $r^2 = 0.635$, n = 57) (Fig. 5). Mean BF for each sampling area was estimated at 14033(± 6912) and 7971(± 4371) eggs per spawning batch for the Gulf of Roses and the Ebre Delta, respectively. BF was significantly higher in the Gulf of Roses than in the Ebre Delta (ANCOVA with TL, $F_{1,95} = 42.12$, $p < 0.0001$). Similarly, the mean relative batch fecundities (RBF) were estimated at 84.2 (± 27.6) and 51.1 (± 19.5) eggs per gram of body mass for the Gulf of Roses and the Ebre Delta (Fig. 6 A), respectively. The RBF was significantly higher in the Gulf of Roses than in the Ebre Delta (ANOVA, $F_{1,96} = 48.81$, $p < 0.0001$). *P. incisus* in the Gulf of Roses produced on average 40% more eggs per gram of fish (RBF) than fish in the southern Ebre Delta population.

As a measure of egg quality, the mean dry weight of hydrated oocytes in mg per egg, was estimated for this species at 0.116 (± 0.08) in the Gulf of Roses and 0.124 (± 0.05) in the Ebre Delta (Fig. 6 B), that is to say, the hydrated oocytes of *P. incisus* are significantly heavier for specimens from the Ebre Delta than those from the Gulf of Roses ($t = 2.134$, $p = 0.039$, n = 40).

Prevalence of atresia (Pa) was estimated at 15.2% of all mature females from the Gulf of Roses (n = 136) and 12.8% of all mature females from the Ebre Delta (n = 125). Signs of α-atresia were observed only during the regressing stage, with 95.5 ± 2.1% (Gulf of Roses) and 96.1 ± 8.1% (Ebre Delta) of their vitellogenic oocytes in α-atretic state; that is to say, there was no significant difference in mean intensity of atresia RIa ($t = 1.788$, $p = 0.082$, n = 37).

The development of P. incisus oocytes was considered to be asynchronous, since oocytes at different stages of development were simultaneously present in the ovary. Moreover, the variation in the stage-specific oocyte size-frequency distribution of *P. incisus* during the annual reproductive cycle indicated a lack of hiatus separating the yolked oocyte stock from the reservoir of unyolked oocytes (Fig. 7 A-C). These oocyte size-frequency distributions showed a continuous size-frequency development of oocytes, except for ovaries in the actively spawning stage, which, along with all the secondary growth stages, had a separate mode for the most advanced oocytes (> 500 μm) (Fig. 7 D-E). Only when hydration occurred just before ovulation, did most advanced oocytes outgrow the standing stock of vitellogenic oocytes and a separate mode of mature hydrated oocytes developed.

Discussion

The continuous oocyte size frequency distribution, the asynchronous development of oocytes, the lack of hiatus separating the yolked oocyte stock from the reservoir of unyolked oocytes and the fact that massive atresia was observed in post-spawning individuals are evidence of indeterminate fecundity and batch spawning of the bastard grunt (Murua & Saborido-Rey, 2003). However, the condition and repro-

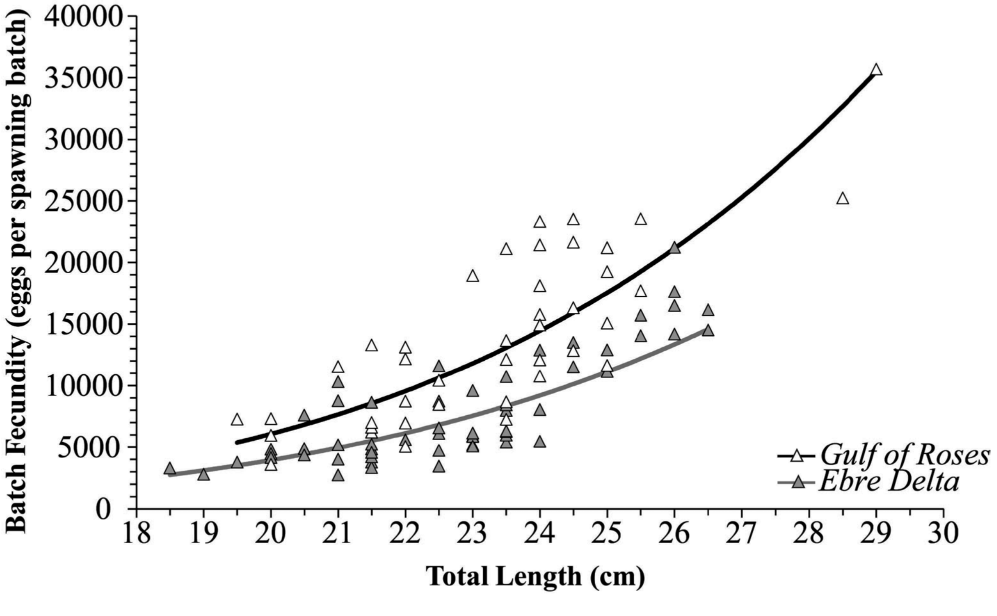

Fig. 5: Fitted regression model based on the relationship of batch fecundity with fish length for specimens of *Pomadasys incisus* sampled in the Gulf of Roses ($BF = 0.0036 \cdot TL^{4.642}$, $r^2 = 0.623$, n = 41) and the Ebre Delta ($BF = 0.0041 \cdot TL^{4.749}$, $r^2 = 0.635$, n = 57).

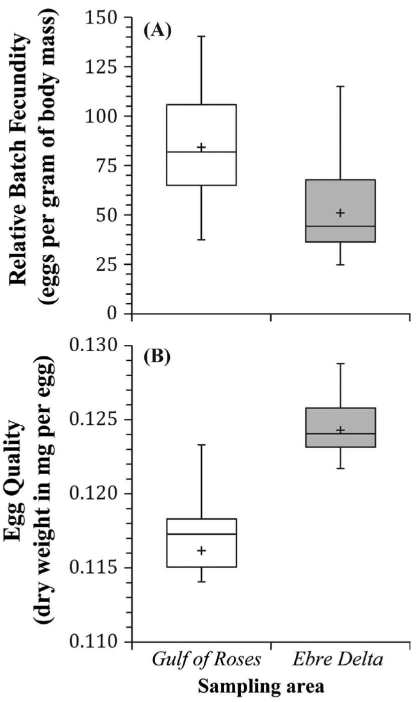

Fig. 6: Mean relative batch fecundities (A) and mean dry weights of hydrated oocytes (B) for *Pomadasys incisus* per sampling area are shown as interquartile box range (25-75%), with mean (+) and median (⊟)whiskers (represented as Q1 and Q3 deviations from the smallest and largest observation, respectively).

ductive traits of the two NW Mediterranean populations of *P. incisus* in this study show that this thermophilic species displays great spatial plasticity in its life-history traits.

Our observations point to different reproductive strategies among populations inhabiting different thermal regimes: in the relatively warmer waters of the Ebre Delta, *P. incisus* develops considerably fewer eggs per batch compared to the population inhabiting the relatively colder waters of the Gulf of Roses. However, in the Ebre Delta, oocytes at all stages of development were significantly larger and also heavier (at hydration) than those of the Gulf of Roses. Thus, the strategy of *P. incisus* in the warmer waters of the Ebre Delta is to spawn better quality eggs at the expense of quantity, while in the colder waters of the Gulf of Roses the strategy is inverted, with a greater number of eggs spawned at the expense of quality. Similar differences in egg quantity and quality among populations inhabiting different temperature regimes have been also reported for the anchoveta *Engraulis ringens* off the Chilean coast. In this case, larger eggs were spawned in the southern population at the cost of a reduction in fecundity, which was attributed to the different temperature conditions in the spawning habitats (Castro *et al.*, 2009; Leal *et al.*, 2009). Moreover, the significantly higher HSI and GSI levels found in the Ebre Delta compared to the Gulf of Roses indicate that, in warmer waters, *P. incisus* is in better condition and can invest relatively more energy reserves in reproduction than is the case in colder waters. In general terms, better conditioned fish also have a higher reproductive potential, e.g. see Lambert & Dutil (2000) and Lloret *et al.* (2007). This supports the idea that the plasticity of the life history traits of *P. incisus* helps to optimize the survival of its offspring under the environmental conditions that occur in areas inhabited by this species.This evidence of life-history traits differing between two populations of *P. incisus* inhabiting different water temperature regimes is supported by comparison with the life-history traits observed in populations inhabiting the more southern and warmer waters of the central Mediterranean coast and the eastern Atlantic. For example, the mean length-at-maturity (14.9 cm TL) in our study in the north-western

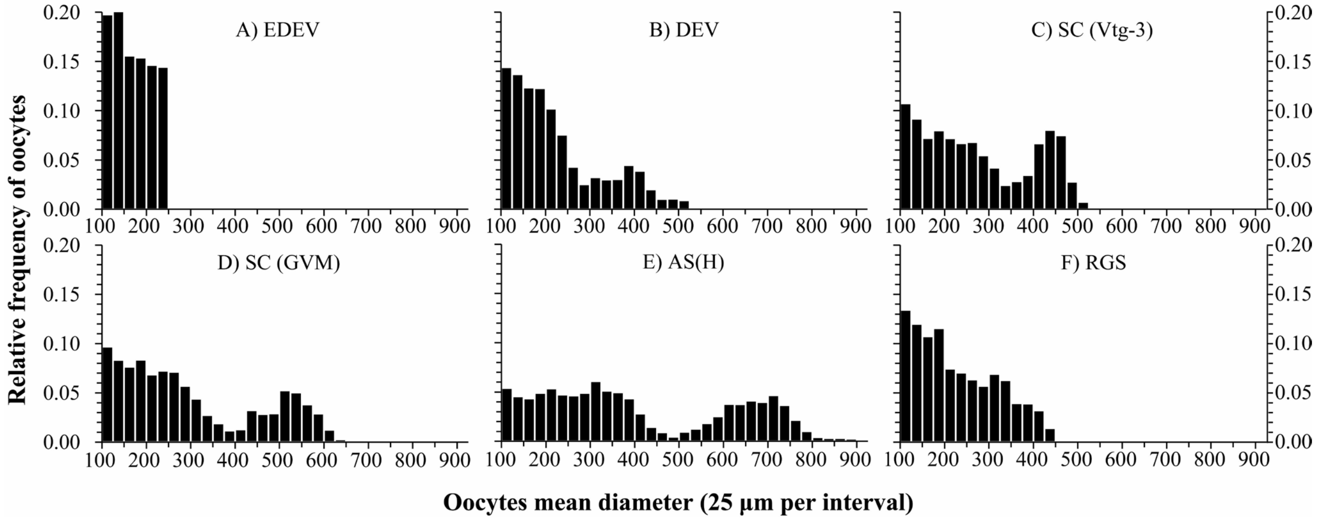

Fig. 7: Oocyte size-frequency distributions (per cent abundance per 25 µm diameter class) of a 'standard' 25 cm TL female of *Pomadasys incisus* through subsequent gonadal development stages: (A) early developing (EDEV), (B) developing (DEV), (C) spawning capable (SC-advanced vitellogenic), (D) spawning capable (SC-germinal vesicle migration), (E) actively spawning (AS-hydration), and (F) regressing (RGS). Each distribution corresponds to an individual fish.

Mediterranean was much lower than those reported for warmer water populations inhabiting the Canarian Archipelago (18.3 cm TL) (Pajuelo *et al.*, 2003b) and the Gulf of Tunisia (15.33-16.13 cm TL) (Chakroun-Marzouk & Ktari, 2006; Fehri-bedoui & Gharbi, 2008). The size-at-maturity of fish is known to vary spatially and temporally and is usually closely related to total population abundance over the life of a cohort, with cohorts maturing at a smaller size when population size is low (Rijnsdorp, 1993; Morgan & Bowering, 1997; Morgan & Colbourne, 1999). Furthermore, water temperature has been assumed to affect only body growth and so the effects of temperature on maturation and reproductive effort appear to be indirect via the optimization of life-history traits in fish populations (Charnov & Gillooly, 2004).

The increased fecundity of *P. incisus* in the colder and the northernmost waters of the Mediterranean Sea, along with reduced size-at-maturity compared to warmer populations of the North African coast and the Canarian Archipelago, might be an adaptation aimed at maximizing the reproductive output at an earlier age during the ongoing northward spread of this species in the Mediterranean. This strategy has also been observed and documented during the invasion of the vendace *Coregonus albula* in the subarctic Pasvik watercourse, and has been seen as 'a successful achievement of an effective pioneer strategy' (Bøhn *et al.*, 2004). Therefore, the plasticity of its life-history traits probably allows *P. incisus* to respond to the environmental pressures exerted by the new habitats and might play an important role in its successful establishment in northern areas of the Mediterranean Sea.

Our results indicate that spawning of *P. incisus* in the north-western Mediterranean is probably not triggered by a specific temperature since spawning began in both sampling areas simultaneously at the beginning of the warmest season (July) despite a difference in water temperature of approximately 4°C. Nevertheless, in the warmer waters of the eastern Atlantic and the southern central Mediterranean, spawning of *P. incisus* takes places during a more prolonged season from June to November (Chakroun-Marzouk & Ktari, 2006; Fehri-bedoui & Gharbi, 2008) than in the north-western Catalan Sea (July to September according to our study; Table 1) or even all year round in the Canarian Archipelago (Pajuelo *et al.*, 2003b). In a scenario of sea warming in the Mediterranean, *P. incisus,* as well as other invading warm water fish species, might take advantage of any increase in sea temperature to change its seasonal phasing of reproduction and hence improve its colonization capabilities.

It has been shown that invading species may display deviant life-history traits as compared to well-established conspecific populations in optimal environmental conditions since adapting to new ecosystems requires bioenergetic trade-offs resulting in variations in fish growth and reproduction (Rosecchi *et al.*, 2001; Bøhn *et al.*, 2004). Therefore, the plasticity of the life-history traits of *P. incisus* may allow this thermophilic species to response to environmental pressures from the new habitats and might play an important role in the northward spread and successful establishment within the Mediterranean Sea. It has been suggested that the present warming of the Gulf of Lyon and the availability of suitable soft substrate in these shallow waters allowed *P. incisus* to establish itself in the NW Mediterranean Sea recently (Francour *et al.*, 1994; Pastor *et al.*, 2008; Bodilis *et al.*, 2013). Despite the fact that nowadays *P. incisus* can be caught in the Gulf of Roses and the adjacent waters of the southern Gulf of Lyon throughout the year (but considerably in larger abundances during the warmest season), its actual regional proliferation and deviant life-history traits are enhancing its establishment, along with the warmer environmental conditions, within the study area.

Although the distance (about 300 km) between sampling sites is relatively short, the larval transport from the Ebre Delta to the north is unlikely since the dominant current, the Northern Current, flows in opposite direction, from the colder northern waters south-westwards along the continental slope (Millot, 1990; Sabatés *et al.*, 2004, 2007). It is also doubtful that continuous interchanges of mature individuals occur between the northern and southern areas due to the inshore and demersal nature of *P. incisus* as juvenile /adult and the considerable distance between these two studied areas. However, future molecular studies could clarify the population's connectivity and the dispersal pathway, which has been followed by *P. incisus* during its northward spread in the Mediterranean Sea.

In summary, our results show that the plasticity of two key life-history traits (condition and reproduction) of *P. incisus* in relation to different sea water temperature regimes, may contribute to the successful establishment of this thermophilic species into new, colder habitats in a scenario of climate change, thus facilitating its northward expansion in the Mediterranean Sea. This could also well be the case for other warm water species that are expanding into the increasingly warmer waters of the Mediterranean Sea. In addition, our results also support the idea that fish condition indices and their reproductive parameters can help to provide indicators of the impacts of environmental factors and habitat characteristics (including the impact of climate change) on the abundance and productivity of marine species (Lloret *et al.*, 2014).

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