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ELECTRONIC SUPPLEMENTARY MATERIAL AND DATA

Ocean acidification affects fish spawning but not paternity at CO_2 seeps

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Tables

Table S1. Seawater carbonate chemistry at High and Ambient pCO_2 sites off Vulcano Island and Cala Isola used to observe mating behavior, spawning and fertilization success of ocellated wrasse in 2012 (a) and 2013 (b). Multiple measurements of Salinity, Temperature and pH were made daily in each nesting site (between 10:00-16:00 h) both on different days before and on the same day of the behavioural observations. Data were analysed using linear mixed models (R package lme4 with REML) with site as a fixed factor and day as a random factor nested within sites. Results of significance tests (F-ratios and P values) with 3 nesting site levels for the 2012 survey (n=11 for Vulcano and n=14 for Cala Isola) and 2 levels for the 2013 survey in Vulcano (n=12) are reported for each variable. Means with different letters (a, b) differ significantly (P<0.05, pairwise contrasts).

		Vulcano Island		**Cala Isola**
		High CO_2	**Ambient CO_2**	**Ambient CO_2**
		38°25.184'N 14°57.696'E	38°25.248'N 14°57.853'E	38°12.341'N 13°15.490'E
(a) Survey 2012				
Salinity	*mean ±S.E.*	38.15(±0.02) [a]	38.12(±0.02) [a]	37.99(±0.04) [a]
$F_{2,33}$= 2.34, P=0.112	*range*	38.1-38.3	38-38.2	37.8-38.1
	median	38.1	38.1	38.09
Temperature (°C)	*mean ±S.E.*	19.38(±0.12) [a]	19.59(±0.13) [a]	20.37(±0.1) [b]
$F_{2,33}$= 12.62, P<0.001	*range*	18.6-20.1	18.9-20.1	19.89-20.68
	median	19.4	19.8	20.63
pH_{NBS}	*mean ±S.E.*	7.82 (±0.06) [a]	8.15(±0.01) [b]	8.18(±0.01) [b]
$F_{2,33}$= 15.79, P<0.001	*range*	7.36-8.06	8.08-8.22	8.13-8.23
	median	7.85	8.16	8.18
pCO_2 (µatm)	*mean ±S.E.*	1274(±244) [a]	480(±19) [b]	428(±10) [b]
$F_{2,33}$= 5.94, P<0.01	*range*	602-3516	390-578	368-488
	median	1034	463	424
Total Alkalinity (µmol kg^{-1})		2581	2607	2518
(b) Survey 2013				
Salinity	*mean ±S.E.*	38.15(±0.02) [a]	38.14(±0.02) [a]	
$F_{1,22}$= 0.04, P=0.84	*range*	38.1-38.25	38-38.3	
	median	38.1	38.1	
Temperature (°C)	*mean ±S.E.*	19.97(±0.09) [a]	19.54(±0.12) [b]	
$F_{1,22}$= 5.53, P<0.05	*range*	19.3-20.6	18.9-20.1	
	median	20	19.4	
pH_{NBS}	*mean ±S.E.*	7.83(±0.05) [a]	8.18(±0.01) [b]	
$F_{1,22}$= 48.63, P<0.001	*range*	7.57-8.06	8.09-8.23	
	median	7.89	8.2	
pCO_2 (µatm)	*mean ±S.E.*	1180(±153) [a]	421(±15) [b]	
$F_{1,22}$= 24.43, P<0.001	*range*	598-2112	368-550	
	median	922	406	
Total Alkalinity (µmol kg^{-1})		2545	2528	

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Table S2. Standard length (SL), wet weight (WW), and age estimations from otoliths of dominant males from nesting sites exposed to High CO_2 and Ambient CO_2 conditions off Vulcano Island and Cala Isola. Age of dominant males ranged between 2 and 3 years off the Vulcano CO_2 gradient, whilst ocellated wrasse nesting males from Cala Isola were all 3 years old, although slightly smaller. The asterisks indicate the genotypised individuals.

ID number	Location	CO_2 level	SL(mm)	WW (g)	Age (years)
1*	Vulcano	High CO_2	70	8.44	2
2*	Vulcano	High CO_2	74	11.08	3
3*	Vulcano	High CO_2	81	12.44	3
4*	Vulcano	High CO_2	82	12.71	3
5*	Vulcano	High CO_2	78	10.43	2
6*	Vulcano	Ambient CO_2	82	11.78	2
7*	Vulcano	Ambient CO_2	81	11.23	3
8*	Vulcano	Ambient CO_2	83	13.29	3
9*	Vulcano	Ambient CO_2	80	12.29	3
10*	Vulcano	Ambient CO_2	79	8.68	2
11	Cala Isola	Ambient CO_2	73	8.75	3
12	Cala Isola	Ambient CO_2	71	7.50	3
13	Cala Isola	Ambient CO_2	67	6.42	3

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Table S3. Results of significance testing of main effects and interaction from the generalised least squares fit by maximum likelihood of the number of spawns by females (n. of spawns 10 min^{-1}) at nests exposed to two CO_2 conditions (**Nesting site**) in the presence and absence of satellite males (**Satellite**) off Vulcano island, followed by results of contrasts between high and ambient sites within the significant **Nesting site** x **Satellite** interaction. See methods for further details. There were no overall differences in the number of spawns by females at High vs Ambient CO_2. Thus there were no differences in nest attractiveness under different CO_2 levels, despite the influence of satellite males on female spawning.

Source	**df**	**F**	**P**
Nesting site (SI)	1	4.104	0.052
Satellite (SA)	1	1.076	0.520
SIxSA	1	12.041	**0.002**
Res	28		

PAIR-WISE CONTRASTS		
Term 'SixSA' for pairs of levels of factor 'Nesting Site'		
Within level 'Absent' of factor 'Satellite'	**t**	**P**
High, Ambient	4.107	**<0.001**
Within level 'Present' of factor 'Satellite'	**t**	**P**
High, Ambient	1.259	0.218

Term 'SixSA' for pairs of levels of factor 'Satellite'		
Within level 'High CO_2' of factor 'Nesting site'	**t**	**P**
Absent, Present	2.032	**0.052**
Within level 'Ambient CO_2' of factor 'Nesting site'	**t**	**P**
Absent, Present	3.150	**0.004**

Table S4. Statistical summary of nest composition, mating behaviour and spawning of ocellated wrasse at Ambient CO_2 Vulcano vs Ambient CO_2 Cala Isola (see methods for details).

Nest composition			df	t	p
	MaxN	Sneakers	1,22	0.726	0.472
		Females	1,22	1.429	0.161
	TotN	Sneakers	1,22	0.865	0.392
		Females	1,22	0.733	0.468

Nest success		df	F	p
	N. of spawns by females	1,22	0.759	0.393

Behavioural interactions of the dominant male				
	Courtship (%time)	1,22	0.0004	0.984
	N. of chasing events against other males	1,14	2.709	0.122

Inter-male competition		df	Chisq	p
	Dominant male spawn disruption	1	0.717	0.397
Inter-male competition		df	F	p
	Number of spawns	1,44	0.911	0.345

Table S5. Results of significance testing of main effects and interactions from the generalised least squares fit by maximum likelihood of the number of spawns 10 min^{-1}made by dominant males (pair spawns) and those involving accessory males (sneak spawns) (**Spawn type**) at nests exposed to two CO_2 conditions (**Nesting site**) in the presence and absence of satellite males (**Satellite**) off Vulcano island, followed by results of contrasts between high and ambient sites within the significant **Nesting site** x **Spawn type** interaction. Pair spawns were significantly lower at High CO_2 than Ambient CO_2 nests, whilst sneak spawns did not differ. In bold the interaction term further analysed by pairwise t-tests. * denotes a marginal p value with consistent inference under bootstrapping of confidence intervals; see methods for further details.

Source	**df**	**F**	**P**
Spawn type (SP)	1	4.900	0.031*
Nesting Site (SI)	1	4.744	0.034*
Satellite (SA)	1	0.284	0.596
SPxSI	1	8.929	**0.004**
SPxSA	1	9.545	0.003
SIxSA	1	13.370	<0.001
SPxSIxSA	1	2.119	0.151
Res	56		

PAIR-WISE CONTRASTS		
Term 'SPxSI' for pairs of levels of factor 'Nesting Site'		
Within level 'Pair' of factor 'spawning type'	**t**	**P**
High, Ambient	3.282	**0.002**
Within level 'Sneak' of factor 'spawning type'	**t**	**P**
High, Ambient	0.515	0.609
Term 'SPxSI' for pairs of levels of factor 'Spawn type'		
Within level 'High CO_2' of factor 'Nesting site'	**t**	**P**
Pair, Sneak	0.284	0.777
Within level 'Ambient CO_2' of factor 'Nesting site'	**t**	**P**
Pair, Sneak	3.329	**0.002**

Figures

Figure S1. Nest composition at different CO_2 levels off Vulcano Island. **(a)** Average maximum number (MaxN) of accessory males (sneakers and satellites) and females that were recorded within 15-sec frames of 10 min videos. **(b)** Average total number (TotN) of sneakers and females that were recorded within 10 min videos. Each 10-min video was subdivided on 15-sec frames. Within each frame we recorded the total number of females and accessory males (i.e. sneakers and satellites) participating to reproduction or visiting the nest within 1m diameter. TotN is the total number of individuals observed in a 10-min video. In both cases there are no differences between nesting sites (High CO_2 vs Ambient). These findings suggest that nests were similarly attractive in the two CO_2 conditions. Error bars ± 1 SE.

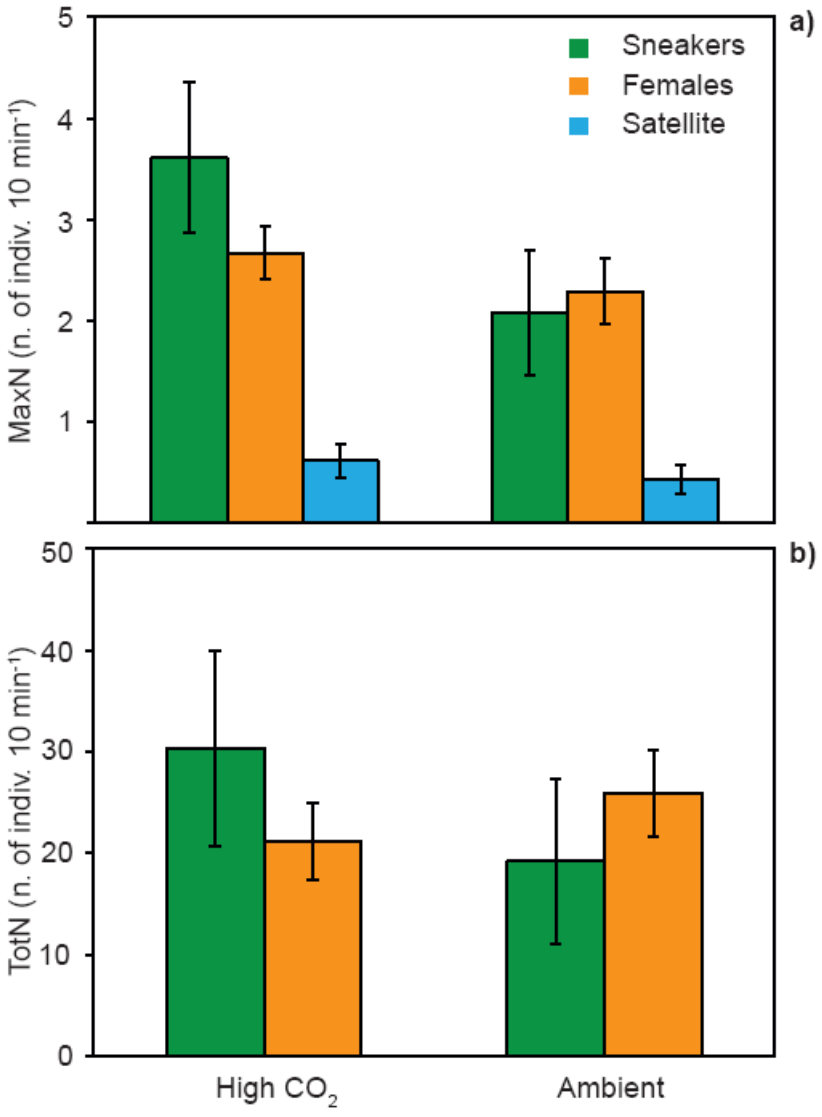


Figure S2. Number of spawns by females at two CO_2 levels off Vulcano Island. Nest success seemed to be unaffected by CO_2 levels as there were no differences in the number of spawns by females between nesting sites (High CO_2 vs Ambient). However, the presence/absence of the satellite males at the nest had opposite effects on the number of spawns by females at High vs. Ambient CO_2 nests respectively. Means with different letters (a, b, c) are significantly different in pair-wise t-tests (see Table S2 for full analyses). Error bars ± 1 SE.

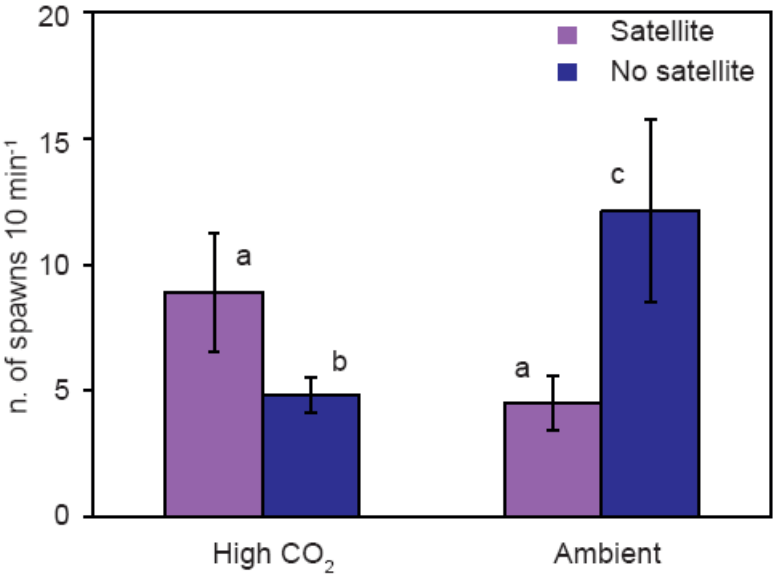


Figure S3. Eggs size in nests exposed to different CO_2 levels. Measurements were carried out on eggs randomly collected from five nests at each nesting site off Vulcano island (n=200 eggs). Error bars ± 1 SE.

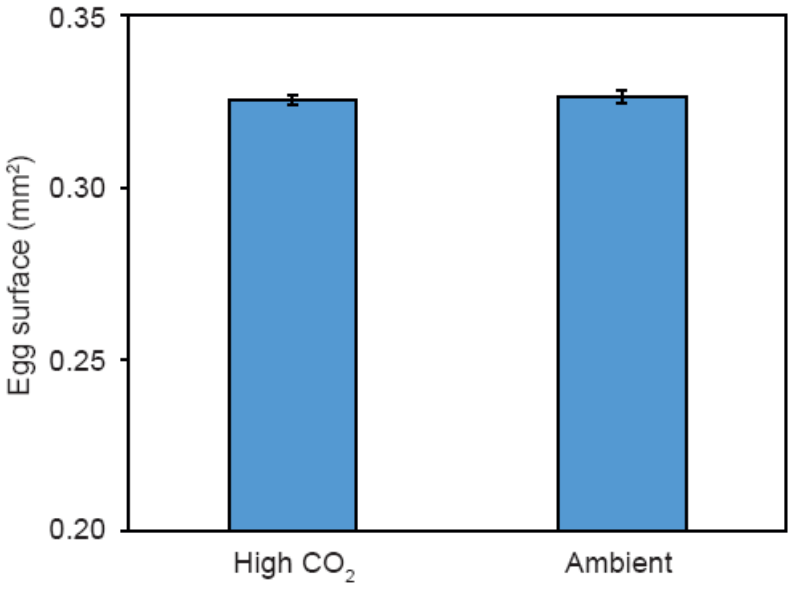


Figure S4. Number of eggs/nest at different CO_2 levels. Nests exposed to different CO_2 levels (n=5 for each condition) had a similar number of eggs, suggesting that the reproductive output was similar under present-day and end-of-century CO_2 concentrations. Error bars $\pm$ 1 SE.

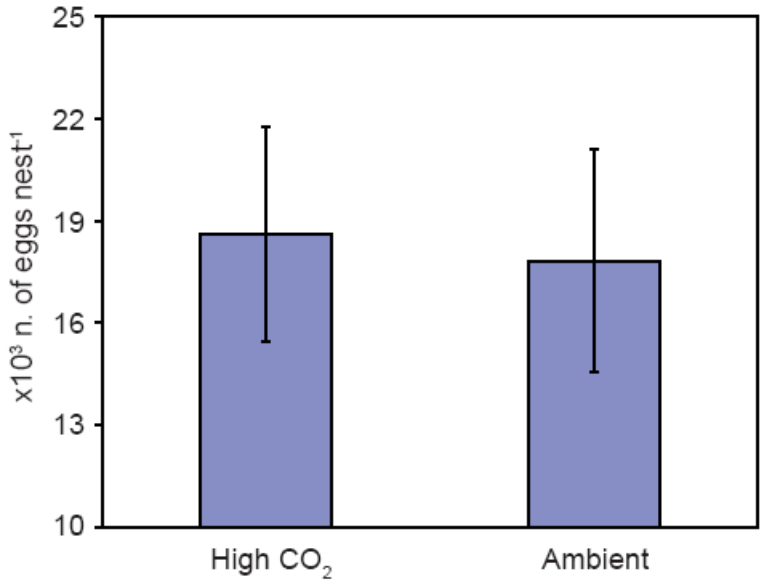


Figure S5. Number of dominant male spawn disruptions at different CO_2 levels. The number of spawn disruptions made by the dominant male did not differ between nests exposed to different CO_2 levels (High CO_2 n=18; Ambient CO_2 n=14). Error bars ± 1 SE.

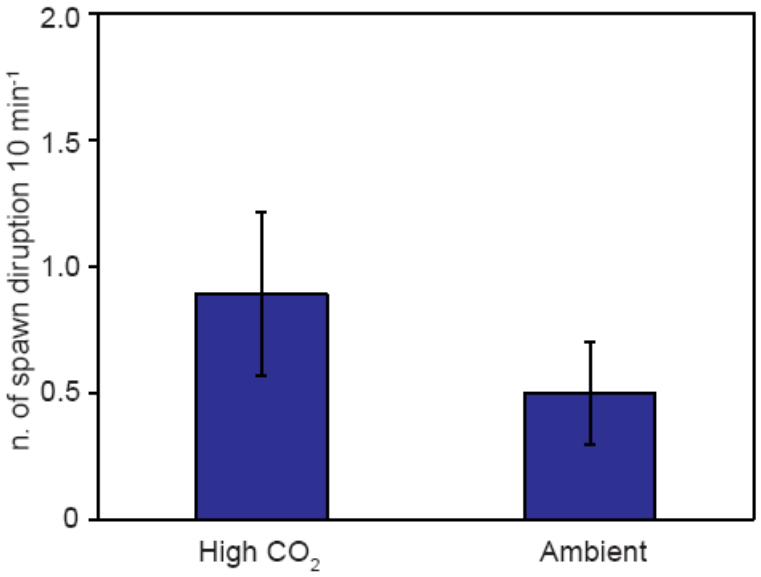
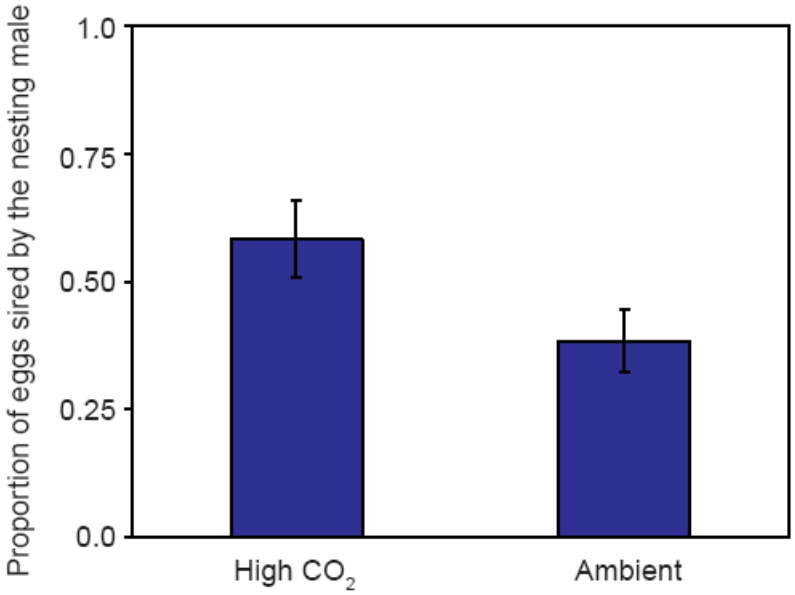


Figure S6. Paternity of dominant nesting males at different CO_2 levels. Proportion of eggs sired by nesting males based on strict exclusion analysis. Dominant males were the fathers of embryo fish ca 20% more often at High CO_2 but this was not statistically significant from the ambient conditions (unpaired t-test, t = 2.05, p = 0.074). Error bars ± 1 SE.



SUPPLEMENTARY DATA

Mean (± S.E.) of nest composition, mating behaviour, spawning, egg size, number of eggs and paternity of the ocellated wrasse at at High and Ambient CO_2 off Vulcano Island and at Ambient CO_2 at Cala Isola.

CO_2 condition	**High CO_2**	**Ambient CO_2**	**Ambient CO_2**
Location	**Vulcano Island**	**Vulcano Island**	**Cala Isola**
Sample size (n)	**18**	**14**	**10**
MaxN sneakers	3.61 (±0.74)	2.07 (±0.62)	2.9 (±0.81)
Ntot sneakers	30.33 (±9.65)	19.21 (±8.76)	33.7 (±15.4)
Nmax females	2.67 (±0.26)	2.29 (±0.32)	3 (±0.45)
Ntot females	21.11 (±3.79)	25.93 (±4.27)	31.2 (±6.7)
Nmax satellites	0.61 (±0.16)	0.43 (±0.14)	0.1 (±0.1)
Courtship (time%)	15.76 (±2.09)	21.12 (±3.29)	21.25 (±3.83)
N. of chasing events*	12.58 (±3.18)	16.57 (±3.12)	8.22 (±3.4)
N. of dominant male spawn disruptions	0.89 (±0.32)	0.5 (±0.20)	0.3 (±0.21)
N. of spawns by females	5.72 (±1.47)	10.93 (±2.55)	7.4 (±2.88)
N. of spawns by dominant males	2.61 (±0.67)	8.79 (±2.4)	5.8 (±2.16)
N. of spawns by accessory males	3.11 (±1.19)	2.14 (±0.54)	1.6 (±0.98)
Eggs size (mm2)**	0.325 (±0.002)	0.327 (±0.002)	-
N. of eggs $nest^{-1}$ ***	18621 (±3156)	17833 (±3275)	-
Dominant male paternity (%)***	58.2 (±7.52)	38.34 (±6.1)	-

* Vulcano High CO_2 n=12; Vulcano Ambient CO_2 n=7; Cala Isola Ambient CO_2 n=10. ** Vulcano High CO_2 n=200; Vulcano Ambient CO_2 n=200. *** Vulcano High CO_2 n=5; Vulcano Ambient CO_2 n=5.