Behavioural responses of fish groups exposed to a predatory threat under elevated CO_2

Cattano C[1,2], Fine M[3,4], Quattrocchi F[5], Holzman R[4,6], Milazzo M[1,2]

[1] Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, Via Archirafi 20, I-90123 Palermo, Italy. C. Cattano: carlo.cattano@unipa.it

[2] CoNISMa, Piazzale Flaminio 9, 00197 Roma. Italy

[3] The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

[4] The Interuniversity Institute for Marine Sciences, Eilat 88103, Israel

[5] Institute for marine biological resources and biotechnologies. Via Vaccara, 61 - 91026 Mazara del Vallo (TP), Italy

[6] Department of Zoology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

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Abstract

Most of the studies dealing with the effects of ocean acidification (OA) on fish behaviour tested individuals in isolation, even when examining species that school. Here we evaluated the effects of elevated CO_2 concentrations (i.e. ~900 µatm) on the shelter use and group cohesion of the gregarious damselfish *Chromis viridis* using groups of sub-adults exposed to a predatory threat. Results showed that, under predatory threat, fish reared at elevated CO_2 concentrations displayed a risky behaviour (i.e. decreased shelter use), whereas their group cohesion was unaffected. Our findings add on increasing

evidence to account for social dynamics in OA experiments, as living in groups may compensate for CO_2-induced risky behaviour. boldness.

Keywords: Coral reef fish; group fish; Ocean Acidification; predation; shelter use; global change; risk assessment

1. Introduction

Oceans mitigate the effects of anthropogenic activities on the global climate by absorbing about a third of the atmospheric CO_2. However, this occurs at costs of changing physical and chemical seawater conditions (Doney et al., 2009). Increasing dissolved CO_2 concentration in seawater leads to carbonate chemistry changes and pH drop, a process known as Ocean Acidification (OA). Projections suggest that ocean partial pressure of CO_2 (pCO_2) could approximate to average 1000 µatm and pH will further drop by 0.3 units by the end of the century (Meinshausen et al., 2011).

During the last decade several experiments showed that fish reared at elevated pCO_2 levels display behavioural disruptions, including altered responses to visual, olfactory and auditory stimuli, reduced learning ability, increased boldness (i.e. the propensity to be explorative and to take risks) and decreased lateralization (reviewed in Nagelkerken & Munday, 2016; Cattano et al., 2018). Such observed disruptions may negatively affect ecological processes, like settlement and habitat selection (e.g. Devine & Munday, 2013; Rossi et al., 2015), foraging (e.g. Ferrari et al., 2012), predation avoidance (e.g. Ferrari et al., 2011a) and reproduction (Milazzo et al., 2016). The underlying mechanisms altered under OA are linked to impairments of the olfactory system and to the disruption of the GABAa receptor function, as a result of changes in ion gradients with acid base regulation under elevated CO_2 conditions (Nilsson et al., 2012; Heuer et al., 2016; Porteus et al., 2018; Williams et al., 2018). This evidence provides important insights on how OA might influence future responses of fish at the population level (Munday et al.,

2013a; Sunday et al., 2014; Lefevre, 2016). Despite this, other studies documented no effects of rising CO_2 on fish behaviour (e.g., Cattano et al., 2017; Jutfelt & Hedgärde, 2015; Sundin et al., 2017), suggesting that fish sensitivity to OA may be species-specific (Ferrari et al., 2011a), as well as being dependent upon the ecological context in which the experiments are conducted (e.g. Goldenberg et al., 2018).

To date, most of the assessments of CO_2-mediated effects on fish behaviour were carried out on isolated individuals, whilst whether and how OA conditions may affect behavioural responses of fish in group has only recently received attention (Lopes et al., 2016; Nadler et al., 2016a; Kwan et al., 2017; Maulvault et al., 2018). Group living occurs in the majority of animal taxa (Krause & Ruxton, 2002) and confers a number of benefits for individuals such as improved foraging opportunities, energy use, predator detection and predator avoidance (Pitcher & Parrish, 1993). More cohesive groups reduce the probability of predation through an enhanced "confusion effect" (Neill & Cullen 1974) and an improved ability for preys to detect a threat (Magurran et al., 1985). Some disrupted behaviours under elevated CO_2, such as lateralization, boldness, escape response, cue recognition and risk assessment, are known to play a key role in fish group cohesion and social interaction dynamics (Bisazza & Dadda, 2005; Ward & Currie, 2013; Seebacher & Kraise, 2017). Therefore, we can expect that under OA conditions, social behaviour and group formation could be affected potentially leading to negative consequences for the benefits of living in group. On the other hand, laboratory experiments suggest that living in a group may improve the physiological performance (Nadler et al., 2016b), the escape response (Domenici, 2010) and may reduce the cost of locomotion (Marras et al., 2015) of the individuals. Since the performance of an individual in isolation does not necessarily reflect the response of the same individual within a group (Domenici & Batty, 1997; Domenici, 2010; Semeniuk & Dill, 2005) it is essential to understand whether the behavioural alterations observed on single-individual experiments under OA conditions may occur

also when multi-individual fish groups face with elevated CO_2 conditions (Kwan et al., 2017; Nadler et al., 2016a).

In this study, we used fish groups to evaluate the effects of elevated pCO_2 levels predicted for the end of this century (i.e. ~ 900 µatm) on anti-predator responses of a gregarious coral-reef species. In particular, we aimed to test the hypothesis that elevated CO_2 concentrations affect the shelter use and the response to a food stimulus (both as proxies of boldness), along with the cohesion of fish groups exposed or not to a predatory threat. To this end, we used the blue-green damselfish *Chromis viridis*, a widespread Indo-Pacific planktivore fish forming groups above branching corals where they find shelter (Lecchini et al., 2005).

2. Material and Methods

2.1 Fish maintenance and CO_2 treatment

The experiment was carried out in the Red Sea Simulator (RSS) at the Interuniversity Institute for Marine Sciences (IUI, Eilat, Israel). The RSS is an open, flow-through aquaria system capable of manipulating environmental parameters, such as seawater temperature and pH (Bellworthy & Fine, 2018). A pH probe (SeaFET, Satlantic, Halifax, Canada) reports pH of nearby reef water to the system and the set value of the pH in the aquarium is a delta from the reef pH value. A two-armed robot, equipped with temperature (PT100), dissolved oxygen (VisiFerm DO ARC 120, Hamilton, Switzerland) and pH (POLILYTE PLUS ARC 120, Hamilton, Switzerland) probes on each arm, monitor each aquarium. In addition, each aquarium is equipped with permanent probes used to feedback the pH and temperature control. Total alkalinity (TA) in the aquaria was daily measured with titration until the day before the experiment (n = 4; Compact Titrosampler, Metrohm AG, Herisau, Switzerland). Dissolved inorganic carbon (DIC), Ω_{arag}, HCO_3^-, CO_3^{2-}, $CO_{2(aq)}$ and pCO_2 values were calculated from the pH_{NBS} and TA measurements using the

program CO_2SYS.xls (Pierrot & Wallace, 2006). The selected K_1 and K_2 carbonic acid dissociation constants were according to Mehrbach et al. (1973) as refit by Dickson & Millero (1987) and KSO_4 as determined by Dickson (1990). Seawater carbonate chemistry variables in Control and High CO_2 treatments are reported in Table 1.

Table 1. Summary of seawater carbonate chemistry variables recorded in the rearing tanks for Control and High CO_2 conditions. Salinity did not vary and was set at the average value of 40.7 ‰ for subsequent calculations. Temperature and pH data were averaged daily (n = 4). Total Alkalinity (TA) was assessed on discrete water samples (n = 4). Data are reported as mean (± S.E.).

Treatment	**T** (C°)	**pH_{NBS}**	**TA** (µmol $kgSW^{-1}$)	***p*CO_2** (µatm)	**HCO_3^-** (µmol $kgSW^{-1}$)	**CO_3^{2-}** (µmol $kgSW^{-1}$)	**$CO_{2(aq)}$** (µmol $kgSW^{-1}$)
Control	22.1	8.10	2503	506	1987	209	15
	(0.3)	(0.02)	(4.6)	(24)	(19)	(7)	(1)
High CO_2	22.1	7.88	2503	932	2162	138	28
	(0.4)	(0.05)	(3.7)	(113)	(29)	(12)	(3)

In April 2016, *C. viridis* sub-adults were collected by hand nets from the shallow reefs off the north-west side of the Gulf of Aqaba (29°30'16.9"N 34°55'07.4"E). Fish were transferred to the RSS where they were housed in 35 L aquaria at temperature conditions matching field conditions (daily average ~23°C). Successively, the two CO_2 conditions (Control and High *p*CO_2; Table 1) were randomly assigned to four different 35 L rearing tanks (the two High CO_2 tanks were independently dosed; dimensions of each tank: L: 40 x W: 25 x H: 35cm; fish density within each tank: 4.3 individuals l^{-1}; water flow 35 l per hour). Before running the experimental test, fish were reared at Control and High CO_2 conditions for five days,

which is a sufficient exposure time to observe OA-induced behavioural effects (Munday et al., 2010). Fish were daily fed with *Artemia* adults and nauplii, and food withheld for 12 hours before testing. Maintaining diel variability is the default setting in the RRS, hence seawater pH is not fixed at a certain level, but rather as a delta from incoming ambient reef water conditions (Bellworthy & Fine, 2018). During the exposure period, we recorded a daily pCO_2 variation of ~230 µatm (S.D.) in the High CO_2 condition (Table 1).

2.2 Experimental setup

Following the 5-days CO_2 exposure, the behaviour of C. *viridis* groups was assessed in three different 25 L tanks (see Fig. S1 for a schematic representation of the experimental arenas). Each tank was equipped with continuous water flow (1 l per hour) and subdivided in half by a vertical rigid mesh (1 mm mesh size) that allowed for water movement between the two sides of the tank. One side of each experimental tank hosted a group of *C. viridis* sub-adults and was equipped with an *Acropora* sp. (Scleractinia, Hexacoralia) colony, used as a shelter (surface: 44-48cm^2). The other tank side hosted the predator *Epinephelus fasciatus* or remained empty in the No-predator treatment. Each tank was surrounded on four sides by black plastic to avoid any other visual stimulus, which could potentially influence the damselfish behaviour.

2.3 Experiment and fish behaviour assessment

A group of three randomly chosen *C. viridis* individuals at a time from each CO_2 condition was transferred to one of the experimental tanks for each treatment (i.e. Predator and No-predator treatments). A total of 34 groups were used during the experiment (n: Control CO_2/Predator = 8, Control CO_2/No-predator = 10, High CO_2/Predator = 9, High CO_2/No-predator = 7). Each group of three damselfish sub-adults constituted an independent sample and was used only one time. For each trial, *C. viridis* individuals were habituated to the experimental arena for 15 minutes. This time period was preliminary assessed as

being sufficient for fish to leave the shelter and have a regular swimming behaviour. After this acclimation time, the behaviour of *C. viridis* group was recorded for 5 minutes using a digital video camera (GoPro Hero 3+) located above the tank. The videos were successively analyzed to obtain every 5 seconds the position of each fish and assess the fish group centroid. Then, the following variables were recorded: 1) sheltering time (percent time spent inside the shelter), calculated as the number of frames in which the fish group centroid was into the shelter out the total number of analyzed frames. 2) Distance from the Shelter, which is the mean distance (cm) of the fish group centroid from the shelter. 3) Inter-Individual Distance (IDD), which is a measure of the distance among the individuals calculated as the mean distance of each fish (center of mass) to all other group mates when the whole group was outside the coral (Kwan et al., 2017). At the end of the 5-minute observation, each fish group was approached with a plastic pipette to make fish take shelter into the coral. Then, a fixed amount of *Artemia* sp. adults was introduced in the experimental tank close to the dividing mesh using the same pipette (5 ml), and the time interval (s) each individual took to emerge from the shelter (TES) and reach the food was recorded during one additional minute. Total length (TL, cm) of each fish used in the experiment was obtained from video frames using ImageJ software (http://rsb.info.nih.gov/ij/). Fish individuals were measured when at the bottom of the tanks using a scale bar as a reference.

To ensure identical CO_2 exposure duration, the entire experiment was carried out on a single day (from 8 a.m. to 6 p.m.). All fish were tested under control CO_2 water conditions, as recent evidence showed that experimental test water does not change high CO_2-induced behavioural effects (Munday et al., 2016). The experiment complied with IACUC approved guidelines for the use and care of animals in research at Bar-Ilan University, Israel.

2.4 Data analyses

Potential differences in experimental fish body size were tested by a two-way ANOVA (CO_2 treatment, 2 levels: Control and High; Predator, 2 levels: Presence, Absence). In order to analyze the shelter use (sheltering time and distance from the shelter), the group cohesion (IDD), and time to emerge from the shelter' (TES), three different analyses were used. Sheltering time was analyzed using a general linear model (GLM) with a binomial distribution, as the data were assessed as a proportion. The significance of treatments and their interaction were tested by the sequential likelihood ratio test (LR). To test the significance of CO_2 and Predator effects on the distance from the shelter and on the average distance among individuals (IID), the analysis of variance based on resampling (n=999) was used to overcome the unbalanced design issue (Warton et al., 2016), and was performed using the 'mvabund' package as above. The effects of CO_2 and Predator factors, along with their interaction, on TES were analyzed using a negative binomial GLM with log link function accounting for data overdispersion (Zuur et al., 2007). The LR test was used to test the significance of the treatments and their interaction under the null hypothesis of no association between these and the TES. Post-hoc analyses were performed for those models in which the interaction term was significant by the use of the 'lsmeans' (Lenth, 2016) and 'multcomp' packages (Hothorn et al., 2008). All the analyses were performed using the R software version 3.3.0 (R Core Team, 2016).

3. Results

Total length of fish used for the experiment (n=102) did not significantly differ between CO_2 treatments [mean TL: 2.89 (± 0.03 S.E.) cm and 2.95 (± 0.04 S.E.) cm in in Control and High CO_2 condition, respectively; ANOVA: $F_{1,98}$= 1.01, p>0.05)]. High pCO_2 levels did not affect the time spent inside the shelter ("sheltering time") by *C. viridis* sub-adults in the absence of the predator. However, when the predator was present, fish reared at high CO_2 concentrations spent 80% less time inside the shelter than fish exposed to Control CO_2 condition (Fig. 1a; CO_2 x Predator: LR=74.245, p<0.001; Table S1). On

average, in the absence of the predator, the fish groups from High CO_2 condition displayed a distance from the shelter slightly higher (+7%) than the control fish groups (Fig. 1b). This difference resulted more evident when fish were exposed to the predator threat, with fish groups from high CO_2 venturing ~30% farther from the shelter than control fish (Fig. 1b). Therefore, the distance from the shelter was significantly different for CO_2 and Predator factors separately (CO_2: $F=5.439$, $p<0.05$; Predator: $F=5.708$, $p<0.05$), but not for their interaction (Table S2).

Fish displayed similar group cohesion, in all CO_2 and predator treatments. However, the inter-individual distance (IID) significantly decreased (i.e. increased group cohesion) in presence of the predator in both CO_2 conditions (Figure 1c; CO_2: $F=0.519$, $p>0.05$; Predator: $F=6.305$, $p<0.05$; Table S4).

In the absence of the predator, when the food was introduced in the experimental arena, fish from both CO_2 conditions took a similar time to emerge from the shelter (i.e. TES; Fig. 1d), whilst under predatory threat fish from the High CO_2 condition emerged almost 5 times faster from the shelter than those from Control condition (Figure 1d; CO_2 x Predator: $LR=6.70$, $p<0.01$; Table S5).

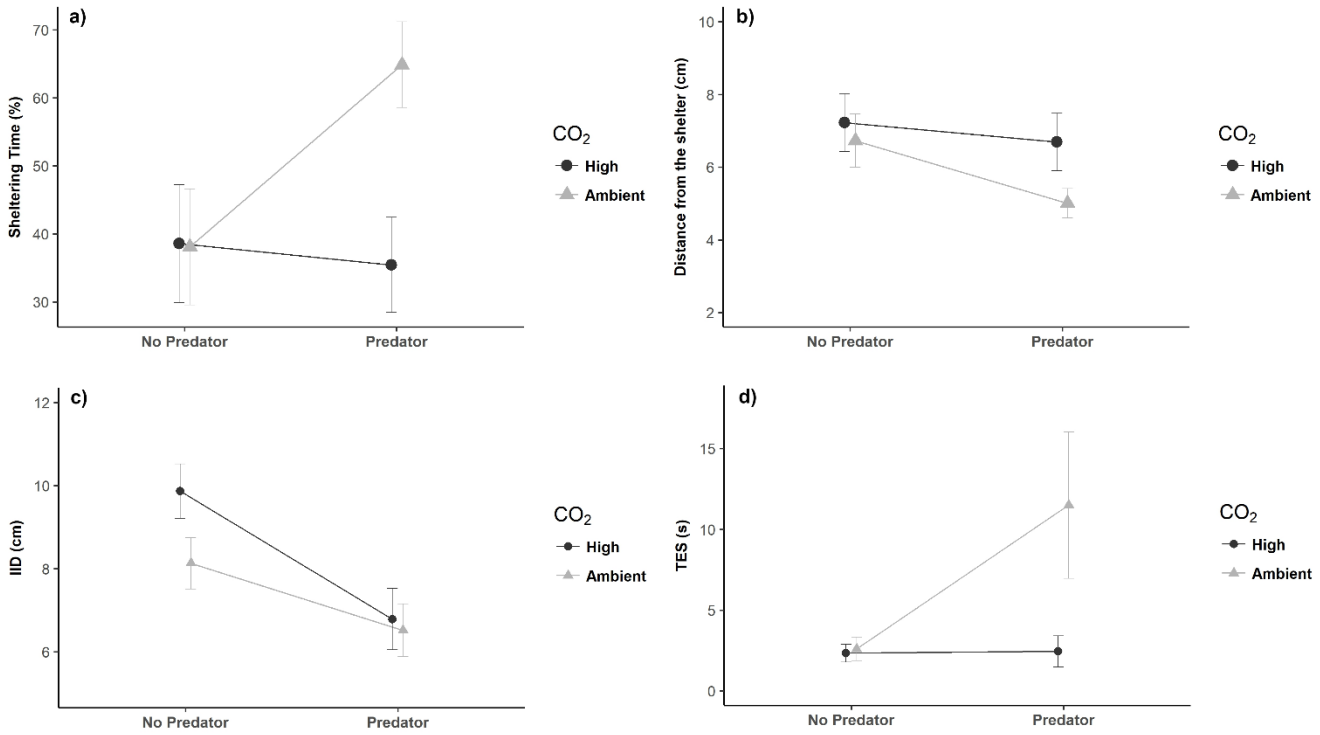

Figure 1 – CO_2 effects on the a) mean (±S.E.) sheltering time (time spent inside the shelter), b) distance from the shelter, c) Inter-Individual Distance (IID) among fish and d) time to emerge from the shelter (TES) displayed by groups of *C. viridis* sub-adults in presence/absence of their predator. See table S1-S4 for full statistical results and pair wise contrasts. Sample size for the independent individual groups tested was: n=10 for Control/No-predator treatment; n=7 for High CO_2/No-predator; n=8 for Control/Predator treatment and n=9 for High CO_2/Predator treatment.

4. Discussion

Here we show that short-term exposure to elevated CO_2 concentrations did not affect the shelter use, the time to reach the food and the group cohesion in *C. viridis* sub-adults, but instead led to increased boldness and risky behaviour in presence of a predatory threat. Indeed, fish groups from high CO_2 condition exposed to a predator almost doubled the time spent outside the shelter, ventured on average 30% farther from the branching coral and responded to the food stimulus almost 5 times faster than fish groups reared at control condition. When fish face with a threatening situation, they typically exhibit

some behavioural responses to minimize the risk of predation, such as increased sheltering, decreased feeding and activity levels (Lima & Dill, 1990; Ferrari et al., 2010). Elevated CO_2 concentrations affect such anti-predator responses altering threat perception through sensorial and neurological impairments. As a result, threat-exposed fish display riskier behaviours under OA conditions than under ambient CO_2 concentrations. In this regard, our findings are consistent with previous studies showing that acute exposure to elevated pCO_2 levels affects the innate ability of fish to recognize a predatory threat, leading to increased boldness (Lönnsted et al., 2013; Munday et al., 2010, 2012, 2013b).

We documented damselfishes under high CO_2 condition did not change their response to the food stimulus either in the presence or in the absence of the predator. By contrast, fish reared at control CO_2 water remained close to the shelter and reached the food five-fold slower than in the absence of the predator, hence displaying a typical behavioural change in response to the predatory threat. Similarly, a recent experiment showed that juveniles of *Amphiprion percula* reared at elevated CO_2 conditions did not change their feeding activity in presence of a predator cue, displaying a riskier behaviour under predatory threat than fish reared at ambient CO_2 conditions, regardless of food resources availability (McMahon et al., 2018). Our findings support the evidence that threat perception is impaired under OA conditions (Ferrari et al., 2012; Chivers et al., 2014). Such behavioural disruption makes prey more vulnerable to predation, potentially affecting population dynamics of many species (Munday et al., 2010; Ferrari et al., 2011a, b). However, experimental evidence showed that an increased prey vulnerability to predation may disappear when both predators and prey are concomitantly exposed to high CO_2 conditions (Allan et al., 2013), suggesting that the extent to which predator-prey dynamics will be affected under OA conditions will depend upon differences in tolerance of the interacting species and upon their adaptation ability. In addition to this, recent evidence suggested that diel variation of pCO_2 levels might reduce the severity of behavioral alterations caused by elevated CO_2. For instance, the negative effects on lateralization and predator cue recognition observed in two coral reef species exposed to elevated

stable CO_2 conditions (~1000 µatm) were mitigated when using fluctuating pCO_2 levels during acclimation (Jarrold et al. 2017). In the present study fish were exposed to diel CO_2 fluctuation, therefore we expect the detrimental behavioural effects we observed on *C. viridis* under OA conditions could be more severe under stable elevated CO_2 conditions.

If in one hand becoming bolder might increase the risk for fish of being preyed, on the other a bolder behaviour could increase the time spent in foraging activity leading to enhanced growth rate and increased body size. This in turn may decrease mortality due to predation, as capture success would decline with increasing prey length (e.g. Miller et al., 1988). Therefore, we suggest OA may affect the species trade-off between predation avoidance and food obtaining, which greatly relies on risk perception (McMahon et al., 2018; Nagelkerken & Munday, 2016).

Our results also documented no differences in the group cohesion (measured as the average distance among individuals of each group) displayed by damselfish sub-adults from different CO_2 conditions. Such finding is consistent with a recent study showing that neither constant nor oscillating CO_2 affected the inter-individual distance in shoals of *Chromis punctipinnis* juveniles (Kwan et al., 2017). By contrast, other recent evidence reported negative CO_2-induced effects on shoaling behaviour of *Argyrosomus regius* juveniles (Maulvault et al., 2018). Lopes et al., (2016) showed that *Atherina presbyter* larvae exposed for 7 days to elevated CO_2 displayed decreased shoal cohesion, but this effect was reversed after a 21-day exposure. These studies suggest that effects on group cohesion may be temporary and context-dependent, changing with species, habitat or ontogenetic stage.

Contrary to our expectations, OA levels did not affect *C. viridis* group cohesion, which was higher in the presence than in the absence of the predator. Under a predatory threat, preys typically increase group cohesion (Sogard & Olla, 1997). Indeed, both group living and group cohesion may represent an important anti-predator strategy improving threat detection, reducing individual risk and enhancing

acquisition of information by preys (Pitcher & Parrish, 1993). High cohesive groups display increased ability of detecting a threat (Ward et al., 2011) and are less sensitive to predation through an enhanced "confusion effect" (Neill & Cullen, 1974). Moreover, groups confer a number of additional benefits to individuals, such as greater foraging success (Pitcher & Parrish, 1993) and reduced physiological costs associated to swimming (e.g. Marras et al., 2015). Very likely, our results suggests that exhibiting a high group cohesion under OA may compensate the risk associated with a bolder behaviour displayed in presence of the predator. In summary, here we show that sub-adults of a widespread tropical damselfish do alter their perception of predatory threat when exposed to short-term elevated CO_2, whereas the group cohesion seems to be unaffected. The bold behaviour displayed by CO_2-treated fish in the presence of a predator threat could lead to increased mortality, with potential consequences for population replenishment (Munday et al., 2010; Ferrari et al. 2011b). On the other hand, fish could benefit from a bold behaviour spending more time in feeding-related activities than shy fish under a predatory risk, hence potentially increasing their size and altering their vulnerability to predation (Miller et al., 1998). This study also emphasizes the importance of the social context when assessing the responses of gregarious fish species to environmental disturbances.

An additional intriguing result of our experiment is that when the predator was not present we did not detect any OA-induced effects on fish group boldness (sheltering time, shelter distance and TES between the CO_2 conditions). This finding suggests that the extent to which fish responses are affected under elevated CO_2 may depend on the ecological context in which the experiment is conducted (Goldenberg et al., 2018). Similarly, some recent wild-based evidence reported that scaling up responses of single species experiments to multi-species interactions (e.g. predator vs prey) may change the ecological outcome under OA conditions (Nagelkerken et al., 2017). We recognize some limitations of the present study, as we were not able to compare the anti-predator behaviour displayed by multi-individual groups versus single individuals. We also caution that our findings should be generalized under multiple

stressors condition (e.g. with concomitant changes in O_2 availability and temperature), as well as cannot exclude transgenerational acclimation to CO_2 effects (e.g. Munday 2014). In conclusion, our results advocate the need to increase the number of studies dealing with CO_2 effects on chronically exposed group-living fish along natural CO_2 gradients. Indeed, field investigations could better reflect the complexity of organism's interactions, their adaptation to natural variability in pH and pCO_2, and may further explain the role of acclimation and adaptation processes in a context of a changing ocean.

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Supplementary Data

List of contents:

Table S1 – Binomial GLM and pairwise comparisons on the sheltering time (proportion of time spent inside the shelter) displayed by groups of *Chromis viridis* sub-adults in the different CO_2 (Control and High) and Predator treatment combinations. ns: not significant. Significant results are in bold. LR= log-likelihood ratio statistic, DF=degree of freedom; p=p value; SE= Standard error.

Sheltering time

	LR	DF	p
Predator	74.730	1	**< 0.001**
CO_2	56.488	1	**< 0.001**
Predator x CO_2	74.245	1	**< 0.001**

Pair-wise contrasts

	Estimate	SE	z value	p
No Predator, High CO_2 – Predator, High CO_2	0.1342	0.10433	1.286	ns
No predator, High CO_2 – No predator, Control CO_2	0.01994	0.10134	0.197	ns
No predator, High CO_2 – Predator, Control CO_2	-1.07891	0.10731	-10.054	**<0.001**
Predator, High CO_2 – No Predator, Control CO_2	-0.11425	0.09537	-1.198	ns
Predator, High CO_2 – Predator, Control CO_2	-1.21311	0.10169	-11.929	**<0.001**
No Predator, Control CO_2 – Predator, Control CO_2	-1.09886	0.09862	-11.142	**<0.001**

Table S2 –Analysis of variance showing the effects of CO_2 and Predator treatments on the distance from the shelter displayed by groups of *Chromis viridis* sub-adults. Significant effects (p-value) were calculated by sequential F test comparing each model with a null model basing on resampling iterations (n=999). ns: not significant. Significant results are in bold. DF=degree of freedom; p=p value; Fstat= F statistic.

Distance from shelter			
	Fstat	DF	p (Resampling)
NULL			
Predator	5.708	1	**0.03**
CO_2	5.439	1	**0.028**
Predator x CO_2	0.249	1	ns

Table S3 - Analysis of variance showing the effects of CO_2 and the Predator treatments on the average distance among fish (Inter-individual distance, IID). Significant effects (p-value) were calculated by sequential test F test comparing each model with a null model basing on resampling iterations (n=999). ns: not significant. Significant results are in bold. DF=degree of freedom; p=p value; Fstat= F statistic.

Inter-Individual Distance			
	Fstat	DF	p (Resampling)
NULL			
Predator	6.305	1	**0.016**
CO_2	0.519	1	ns
Predator x CO_2	0.486	1	ns

Table S4 - Negative binomial GLM and pair-wise comparisons on time to emerge from the shelter (TES) displayed by groups of *Chromis viridis* sub-adults during the feeding time in the different CO_2 (Control and High) and Predator treatments combinations. ns: not significant. Significant results are in bold. LR= log-likelihood ratio statistic, DF=degree of freedom; p=p value; SE= Standard error.

Time to emerge from shelter

	LR	DF	p
NULL			
Predator	8.48	1	**0.0035**
CO_2	8.58	1	**0.0033**
Predator x CO_2	6.70	1	**0.0096**

Pair-wise contrasts

	Estimate	S.E.	z value	p
High CO_2, No Pred – Control CO_2, No Pred	-0.10821	0.40871	-0.265	ns
High CO_2, No Pred – High CO_2, Pred	-0.04652	0.4171	-0.112	ns
High CO_2, No Pred – Control CO_2, Pred	-1.59505	0.42740	-3.732	**<0.001**
Control CO_2, No Pred – High CO_2, Pred	0.06169	0.36365	0.170	ns
Control CO_2, No Pred – Control CO_2, Pred	-1.48684	0.37537	-3.961	**<0.001**
High CO_2, Pred – Control CO_2, Pred	-1.54853	0.38453	-4.027	**<0.001**

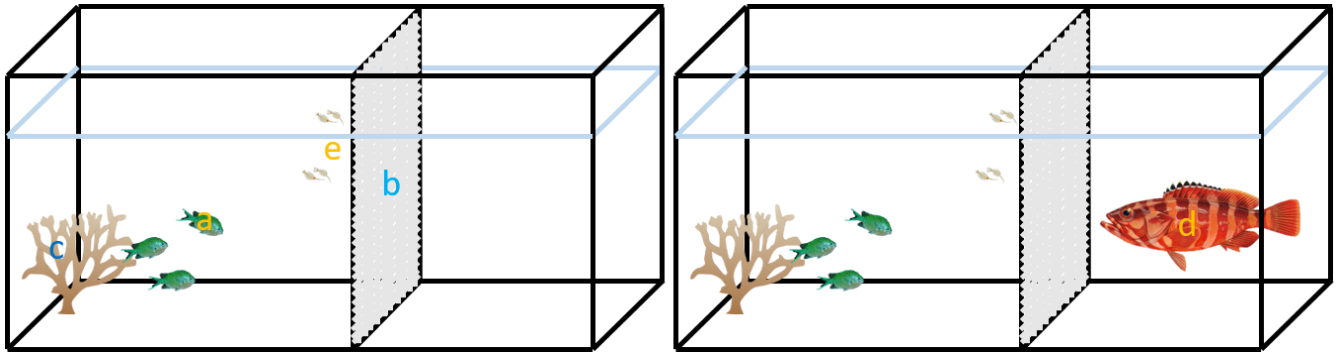

Fig. S1 - Schematic representation of the experimental arenas used for the assessments of *C.viridis* group behaviour without predatory threat (left) or in presence of the predator (right) after a 5-day exposure to Control or High CO_2 concentrations. a): *C. viridis* sub-adult group; b): rigid mesh (1 mm mesh size); c): coral shelter; d): predator; e) food stimulus (*Artemia* sp. adults).