Variable individual- and species-level responses to ocean acidification

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Abstract

Population responses to marine climate change are determined by the strength of the selection pressure imposed by changing climate, the genetic variability within the population (i.e. among individuals), and phenotypic plasticity within individuals. Marine climate change research has focused primarily on population-level responses, yet it is at the level of the individual that natural selection operates. We focused on individual-level responses of two bivalve species to ocean acidification (OA) at the earliest stage of the life-cycle. We measured sperm activity (swimming speed and percent motility) in the Boreal/Arctic *Macoma calcarea* and the temperate *Mytilus galloprovincialis* in response to two pCO_2 levels (380 and 1000 ppm). We also assessed sperm longevity under control conditions. Treatment effects on fertilization success were estimated using fertilization models. At the population level, simulated OA reduced *M. galloprovincialis* sperm swimming speed by 26%, percent motility by 42% and fertilization success by 46%, whereas *M. calcarea* was not significantly affected. Both species showed substantial variability among individuals in response to increased pCO_2. This variability was greatest in *M. galloprovincialis* ranging from non-significant effect to >77% reduction in fertilization success in response to OA, whereas *M. calcarea* responses varied from >8% increase in percent sperm motility to >26% reduction in swimming speed. Further, modeled fertilization success was negatively affected by simulated OA in 10 of 13 studied *M. galloprovincialis* males and in three of 10 *M. calcarea* males. We observed sperm longevities (82 h for *M. calcarea* and 25 h for *M. galloprovincialis* on average) far longer than the expected time-frame for efficient fertilization accounting for dilution of gametes. Assuming sperm activity is a heritable trait, our results suggest that the studied populations might be able to adapt to near-future OA through natural selection, although this would come at the cost of reduced genetic variability.

Introduction

The rate of anthropogenic climate change may be too rapid for sensitive marine taxa to adapt through new genetic mutation. Hence the response of populations to environmental perturbations will be largely determined by pre-existing genetic variability, the magnitude of the selection pressure caused by changing conditions, and plasticity within individuals [1–3]. Consequently, long-term population persistence will not be determined by the average response, but by the relative responses of genotypes within individuals, and the consequences of these responses for the broader gene pool [4, 5]. Genetic variability in traits affected by environmental factors is time-consuming to quantify. Among-individual variability in a measurable trait, however, can give clues as to the initial responses and longer-term adaptability of a population. Average responses to environmental perturbations, on the other hand, may indicate the general sensitivity of a population as well as the magnitude of selection pressure caused by these perturbations.

Most calcifying marine taxa pass through multiple life-stages, each of which may respond differently to selection pressures [6, 7]. Free-spawning marine broadcast-spawners shed their gametes directly into the water column, where fertilization takes place [8], and consequently selection can operate prior to fertilization. Fertilization in broadcast spawners is subject to strong individual selection pressure [9], and depends on complex interactions between gamete traits [10–12] and the environment [13, 14]. These interactions determine which gametes – and hence genotypes – meet and fertilize. Hydrodynamic processes lead to a rapid dilution of gametes causing conditions where gamete concentration may become too low for an efficient fertilization [9, 15–18]. The location of an individual within a population of sessile broadcast spawners, spawning synchrony, and the advection of gametes cause high variability in life-time reproductive success among individuals [9, 16–26]. Nonetheless, gamete traits also affect fertilization success [15,27,28]. In particular, percent motility and swimming speed of sperm affect gamete encounter-rate at small scales, favoring males with more active sperm [13, 28–30]. Higher swimming speed comes at the cost of reduced longevity, as germ cells have limited energy reserves and few or no homeostatic control mechanisms [30,31]. These processes are influenced by pH and temperature [32,33], and are presumed to be under genetic control. Consequently, these environmental factors are expected to influence fertilization success and thereby have substantial influence over the contribution of individuals to the next generation [5, 34–38].

In this study, we used sperm swimming speed and percent motility as model traits to compare individual- and species-level responses to CO_2-induced experimental ocean acidification (OA). We used two bivalve species in the comparison: the chalky macoma [*Macoma calcarea* (Gmelin, 1791)] is an Arctic to North-Atlantic species [39,40], whereas the Mediterranean mussel [*Mytilus galloprovincialis* (Lamarck, 1819)] has a temperate distribution [41, 42]. Both of these species are gonochoristic free-spawning broadcasters. *Mytilus galloprovincialis* is capable of inhabiting the inter-tidal zone where its gametes might experience a variable pH regime with values that may often reach as low as the predicted open ocean mean values for 2100 [pH drop of ~ 0.3 units, 43–45, but see 46]. *Macoma calcarea*, on the other hand, is solely a sub-tidal species, and the pH experienced by its gametes is presumed to be less variable in the short-term. However, *M. calcarea* is found in the Arctic waters, where OA is expected to be most pronounced during the coming centuries [47].

Sperm activity of *M. galloprovincialis* has been reported to respond negatively to OA levels

projected for 2100 [46], but to our knowledge, there are no published results on sperm activity or fertilization success for *M. calcarea*. Van Colen et al. [37] found a significant reduction in average fertilization success with a 0.6 pH unit drop in pH for the related species *Macoma balthica*, but a non-significant effect at pH levels comparable with this study. Both of those studies, however, focused on population-level responses and did not consider individual variability. The objectives of this study were to 1) identify whether increased pCO_2 affect sperm activity at the population-level; 2) quantify variation in these effects among individuals and species, and; 3) model the effects on fertilization success by using an established fertilization kinetics model (Model S [28], a revised version of the popular model by Vogel et al. [15]).

Materials and Methods

Collection sites and animal handling

Two species of bivalves were used for the experiments: *Mytilus galloprovincialis* obtained from a commercial mussel farm, Mahón, Menorca, Spain (2011-04-11 and 2011-04-14), and *Macoma calcarea* [depth 10-25 m, 69°29′50″N 18°53′35″E, 2011-03-29 and 2011-04-05, species identification using MT-CO1 (Laakkonen, unpublished data)] collected from Balsfjorden, Northern Norway. Studied species were not protected [48]. Sampling in Balsfjorden was conducted outside of protected areas using a Norwegian research vessel (R/V Hyas), and therefore no specific permissions were needed. *Macoma calcarea* were packed in coolers and transported to Mallorca, Spain, by an airplane the day after collection. Immediately after arrival, bivalves were transferred to storage tanks in temperature-controlled rooms set to 16 °C for *M. galloprovincialis* and to 4 °C for *M. calcarea*. Measurement of sperm activity was conducted within a week of collection using males that appeared to be in good condition.

Experimental setup

Experiments were conducted in temperature-controlled climate rooms. Temperature was controlled using separate incubators set to the collection site temperature (16°C for *M. galloprovincialis* and 2 °C for *M. calcarea*). The pH of artificial seawater (Instant Ocean Sea Salt) was manipulated by aerating water with pCO_2 concentrations of 380 ppm and 1000 ppm. These values correspond to the annual average atmospheric pCO_2 level of 2005 [49] and to the high-end open ocean projected levels for 2100 [43], respectively. The pCO_2 treatment was run for all males in the experiment (Table 1). Water for the experiments was collected from corresponding 10 L storage tanks (see Figure A1 in Vihtakari et al. [46]) in 2 L acid-washed and rinsed glass bottles every morning prior to spawning. Measurement of pH was conducted from these bottles using NIST buffer (4, 7, and 10) calibrated electrodes (Metrohm, 6.0262.100). In addition, pH was measured at 25 °C using a spectrophotometer (Jasgo 7800) following the standard operating procedure (SOP) 6b [50], as described in Vihtakari et al. [46]. Seawater samples for measuring total alkalinity (A_T) and dissolved inorganic carbon (DIC) were taken from larval cylinders that were run parallel to this experiment (see [46]). Temperature-adjusted carbonate chemistry parameters were calculated using CO2SYS [51] (CO_2 constant setting from Mehrbach et al. [52], refit by Dickson and Millero [53], $KHSO_4$ formulation from Dickson et al. [54]; Table 1 and S1).

Spawning of *M. galloprovincialis* was induced by a combination of temperature shock and fluoxetine (see [46] for more details). Sperm activity measurements for this species were made within 4.5 h of spawning (Table S3). Sperm of *M. calcarea* was collected by strip-spawning [55], and 10-35 µL of sperm was mixed with 1.5 mL of corresponding experimental water leading to an average suspension of $1.58 \times 10^7 \pm 4.1 \times 10^5$ (SE, n = 23) sperm mL^{-1} across all studied males in the experiment. Sperm activity of *M. calcarea* was measured within 1 h of activation (Table S3). Sperm suspensions were held in Eppendorf tubes in incubators set to the relevant treatment temperature (Table 1). After initial measurements, sperm longevity was accessed only in *control* conditions, because we were not able to control pH in Eppendorf tubes beyond initial measurements. Sperm activity was measured once or twice per day until no motile sperm cells were observed.

Measuring sperm activity and fertilization assays

Sperm swimming speed was determined from replicated digital video clips of sperm suspension (see Videos S1-S2) using 4-chamber slides (Leja, Netherlands). Measurements were randomized across treatments and, conducted within 5 min. Sperm swimming speed [velocity straight line (VSL)] was measured for each path in 1 s long video clips using CellTrak 1.3 (Motion Analysis Corp., CA, USA). A motility threshold (definition of when a sperm cell was classified as motile) was determined from sperm speed histograms of single video clips from *control* conditions and set to 10 $\mu m\,s^{-1}$ for *M. galloprovincialis* and to 9 $\mu m\,s^{-1}$ for *M. calcarea*. Sperm swimming speed was determined for each video clip by averaging the measured sperm path speeds after excluding non-motile sperm. Percent sperm motility was calculated as a percentage of motile sperm in each video clip. Video clips with fewer than 15 and more than 300 sperm paths were excluded from the dataset as these yielded unreliable measurements. The resulting number of replicates varied between five and 11 for start measurements (Table S3). See Vihtakari et al. [46] for further details about sperm activity measurements. Sperm longevity was estimated as the last observation of continuously swimming sperm for each male under *control* pCO_2 treatment (see Videos S4-S5).

Fertilization experiments were conducted to examine whether the studied sperm was capable of fertilizing eggs. Sperm from every *M. galloprovincialis* fertilized eggs successfully and the eggs developed to early trochophore stage, at which point the experiment was terminated. In contrast, *M. calcarea* did not produce viable embryos. The eggs of this species, which were also stripped, were clearly irregular and immature. Nonetheless, both of the two tested *M. calcarea* males successfully fertilized eggs, although the eggs did not develop beyond the 8-cell stage.

Numerical methods

Log-transformed response ratios (LnR) were used to access effects of increased pCO_2 [56, 57]. Mean LnR and 95% confidence intervals (CIs) were calculated using equations 1 and 2 from Hedges et al. [56]. To account for low sample size, CIs were calculated using a t-distribution instead of a z-distribution. LnR values values (and their upper and lower 95% CIs) were subsequently back-transformed to yield response ratios (R, also called 'effect size') and reported as percentages. Mean species responses were analyzed using mean values for each male as replicates.

Variability among and within males was examined by response ratios, which were calculated for each male separately using video clips as replicates.

Fertilization success (F) was calculated using Model S [28, 58, 59], a revised version of the model by Vogel et al. [15]. For all comparisons, model parameters were set to be constant with the exception of sperm swimming speed and egg diameter (61 µm for *M. galloprovincialis* [60], and 95 µm for *M. calcarea* [61]). The model was run using swimming speed and percent motility from each replicate video clip after multiplying the initial sperm concentration (S0) by the proportion of motile sperm. Afterwards, a mean fertilization model was calculated for each male and treatment. The sperm concentration that yielded 50% of maximum F in *control* treatments ($F_{50Control}$ in [5]) was chosen to compare fertilization success among treatments. Response ratios were calculated as described above.

Results

The population-level effects of increased pCO_2 on sperm swimming speed, percent motility, and modeled fertilization success of *M. galloprovincialis* were significantly negative, whereas similar significant effects were not observed for *M. calcarea* (Figure 1). There was substantial variability among males in response to increased pCO_2 in both species (Figure 1). This variability was largest in *M. galloprovincialis* response ratios varying from a non-significant 100.5% effect (1.005 ×) to a 10 × reduction ($\bar{R}$ = 10.1%, CIs 5.4-18.7%) in percent sperm motility, and from a non-significant 98.6% to a 2 × reduction ($\bar{R}$ = 48.7%, CIs 36.2-65.4%) in sperm swimming speed (Figure 1, Videos S1-S2). The effect of increased pCO_2 on percent sperm motility and swimming speed was significantly negative in 10 of 13 and 7 of 13 males *M. galloprovincialis* males, respectively (Figure 1). Increased pCO_2 led to a significant positive effect on percent sperm motility in one of 10 *M. calcarea* males and to significant negative effects in two males. Mean effect sizes ranged from 134.4% to 70.5% for *M. calcarea* males, which was less than that for *M. galloprovincialis*. Sperm swimming speed responses were significantly negative in two *M. calcarea* males, with a minimum response ratio of 63.1% (53.6-74.3%). Increased pCO_2 had a significant negative effect on modeled fertilization success in 10 of 13 *M. galloprovincialis* males (Figure 1). The response ratios varied from 15.3% (CIs 10.3-22.6%) to 83.8% (CIs 67.0-104.7%). Significantly negative pCO_2 effects on modeled fertilization success were observed in three of 10 *M. calcarea* males. One *M. calcarea* male showed a positive effect that was marginally non-significant ($\bar{R}$ = 122.4% CIs 99.5-150-6%).

Sperm longevity was estimated only in control pCO_2 conditions and at different temperatures for each species (Table 1, Figure 2, Videos S3, and S5). Average sperm longevity for *M. calcarea* [81.7 ± 11.3 h (se, n = 9)] was longer than that for *M. galloprovincialis* [24.7 ± 3.5 h (se, n = 13)]. Males demonstrated variability in sperm longevity: four *M. galloprovincialis* males had a markedly shorter sperm longevity (∼ 10 h) than the rest (25.2-46.8 h, Figure 2, Table S2). In *M. calcarea*, sperm longevity among males ranged between 22.5 and 142.6 h (Table S2).

Discussion

We observed substantial variability among individuals in response to simulated ocean acidification in both studied species (Figure 1). The variability in response to increased pCO_2 was greater in *M. galloprovincialis* ranging from statistically non-significant to strongly negative than in *M. calcarea* for which responses varied from significantly positive to significantly negative. Fertilization modeling predicted a significant negative effect of acidification on fertilization success for the majority of *M. galloprovincialis* (10 of 13), but only for three of ten *M. calcarea* males (Figure 1). Among-individual variability in response to OA comparable to that found in *M. calcarea* has been reported for a sea-urchin [5], and an oyster [62], whereas similar strong variability as for *M. galloprovincialis* was previously observed in a polychaete [63]. These results suggest that among-individual variability in response to global change is likely a norm, rather than an exception. Sperm from males less affected by acidification are likely better adapted to fertilize eggs in a future ocean, and therefore – assuming these beneficial sperm traits are heritable – the offspring of these males will become more abundant in the gene pool [63].

Simulated ocean acidification (increased pCO_2) had substantial and negative population-level effects on *M. galloprovincialis* sperm activity (previously published in Vihtakari et al. [46]) and modeled fertilization success (Figure 1E). Similar, significant population-level reductions in sperm activity and fertilization success were not observed in *M. calcarea* (Figure 1B, D, and F). Interestingly, the response was more negative in inter-tidal *M. galloprovincialis*, whose gametes may already occasionally experience as low pH values as projected for 2100 [43, 45, 64], than in *M. calcarea* (Figure 1). This could indicate that variable conditions might select for a higher level of phenotypic variation as several environmental stressors might act simultaneously [65], although data on more species are needed to draw such conclusions. In many marine invertebrates sperm are stored immotile in the acidic environment of the testis, which inhabits sperm respiration and metabolism [5,33,66]. The increase in pH upon release is partly responsible of activation of mitochondria in sea-urchin sperm [67]. Although sperm activation in marine bivalves appears to be more complex, pH is likely to contribute to sperm activation [66]. The weaker pH gradient than in the controls could therefore partly explain the lower percentage of motile sperm in increased pCO_2 treatment. Adult acclimation in experimental conditions might affect the responses of offspring to ocean acidification through transgenerational plasticity [68–70] as gametes inherit nutrition and other bioactive materials from their parents [71, 72]. Since we did not acclimate studied males, we cannot assess the possible consequences of such effects.

Our results for *M. calcarea* are similar to those of Van Colen et al. [37], who found a non-significant decrease in fertilization success of the related species *Macoma balthica*, at a similar pH reduction (ΔpH $\sim$0.3 units). Nonetheless, Van Colen et al. [37] found a significant 11% reduction in fertilization success at ΔpH of 0.6 units. It is possible that the population-level fertilization success in *M. calcarea* could also be negatively affected by this high pH differences as our modeling results indicate that some individuals were negatively affected by the studied 0.3 unit drop in pH (Figure 1F). Other reported acidification effects on fertilization success of bivalves include a significant reduction for *Saccostrea glomerata* starting from a pCO_2 level projected for the end of this century (600 ppm) [35], and a reduction for *Crassostrea gigas* at a far-future ΔpH of 0.7 units [36]. Havenhand and Schlegel [62], on the other hand, found no

significant population-level effects on sperm activity or fertilization success of *C. gigas* for a pH reduction similar to that used in this study (≈ 0.35 units) – a result that is consistent with Barros et al. [36]. Negative population-level effects of OA on fertilization success have been reported for echinoderms [5, 34, 73, 74], corals [38, 73], and a polychaete [63] among free-spawning marine invertebrates, whereas Caldwell et al. [33] reported a positive OA effect on sperm activity of a sea urchin.

A recent review concludes that fertilization in benthic marine invertebrates could be relatively robust to OA [75]. Considering that we found signs of a negative effect of acidification on sperm activity for one species, and that negative effects have been reported on a wide range of benthic taxa, this might not be a good generalization, at least not on a population-level. Although it is clear that population-level effects of OA on fertilization kinetics of marine invertebrates vary among species, populations, and gamete concentrations, our results together with the literature suggest that OA is likely to decrease the sperm performance in a range of benthic marine invertebrates resulting to a lower fertilization success in sperm limited low density populations [18, 30, 38, 58]. The consequences of these reductions are unpredictable considering the complex fertilization kinetics in marine broadcast spawners [9, 12, 14, 17, 23].

Sperm longevities estimated for *M. calcarea* (82 h at 2 °C on average, Figure 2) and *M. galloprovincialis* (25 h at 16 °C) in this study are remarkably long considering that effective fertilization is expected to occur within minutes from spawning due to rapid dilution of gametes [9, 16, 17]. Nevertheless, these results are not exceptional in the literature: Powell et al. [76] found that sperm of an Antarctic bivalve *Laternula elliptica* were capable of fertilizing eggs more than 90 h after spawning at $\sim$ 0 °C. The highest sperm longevity values in this study (4-6 days for *M. calcarea*) were of a similar order of magnitude as those found by Alavi et al. [66] for Pacific oyster *C. gigas* (4-6 days), Manila clam *Ruditapes philippinarum* ($\sim$ 7 days), and Japanese scallop *Patinopecten yessoensis* (2-4 days), all at room temperature. Previous longevities reported reported for near-shore bivalves are considerably shorter (*Mytilus edulis*: $>$ 5 h, [77]; *Cerastoderma edule*: 4-8 h, [78]). However, sperm longevity, i.e. the measure of how long sperm remain motile, does not measure the fertilization capability of sperm and consequently might lead overestimating the actual fertilizability of sperm. To account for this caveat, we report sperm longevity as the last time when we observed continuously swimming sperm cells similar to the initial measurements and the fertilization experiments conducted to check whether sperm was capable of fertilizing eggs (Figure 2, Videos S1, S3, S4, and S5). In this study, sperm cells reached immotile state on average in 9 days for *M. calcarea* and in 38 h for *M. galloprovincialis*. These extreme longevities could partly be explained by the high concentration of studied sperm [$\bar{x} = 1.58 \times 10^7$ sperm mL^{-1}], which could lead to sperm being restricted by space and therefore not swimming as actively as in the field [31]. Also reduced motility caused by sperm cells sedimenting at the bottom of Eppendorf tubes could decrease the amount of energy consumed compared to the natural environment. Due to filming of sperm cells, usage of any lower concentrations was not possible, and consequently imitating the natural environment was difficult. Nevertheless, our results together those from the literature [66, 76, 78] indicate that bivalve sperm might stay active far longer than the time-frame of efficient fertilization due to hydrodynamic processes leading to a rapid dilution of gametes [9, 17, 18]. This is an interesting observation and poses a question whether this phenomenon is an evolutionary artifact, and would not contribute to an increased fertilization success, or whether long-lived sperm could be of an

advantage in certain conditions. Powell et al. [76] suggested that long-lived sperm could increase the effectiveness of synchronized mass spawning events in *L. elliptica* by allowing time for sperm densities to reach the levels needed for high fertilization success. Although this hypothesis could also apply for *M. galloprovincialis* and *M. calcarea*, there is currently not enough field-data to identify the potential reasons for sperm remaining active longer than the dilution of gametes in bivalves.

In this study, we observed negative population-level effects of ocean acidification on sperm swimming speed, percent motility and modeled fertilization success in *M. galloprovincialis*. We also observed a substantial among-male variability in these responses varying from significantly positive in percent sperm motility to >26% reduction in swimming speed among *M. calcarea* males, and from statistically non-significant to a 10-fold reduction among *M. galloprovincialis* males. Population-level responses may be useful for detecting traits that are vulnerable to climate change, but they cannot indicate how individuals within that population will respond to natural selection, and therefore tell us little about the adaptive capacity of a population to future conditions. The key determinant for a species' success in future oceans is the extent of genetic variability in traits that are susceptible to climate change, and thereby inter-individual variability could mitigate the effects of climate change on future populations [4, 63]. Inter-individual variability in response to global change is likely a norm, rather than an exception, and the responses of populations in the future may therefore differ from the average response of a population today. Nonetheless, strong selection favoring individuals that are robust to climate change will reduce overall genetic variability, and thereby persistence, of a species [65, 79, 80]. Consequently, the long-term effects of strong selection for acidification on population (and species) viability are difficult to predict. Importantly, it should be remembered that there are as yet no data linking robustness of sperm performance under environmental change to robustness of the resulting individual during the remainder of its life-cycle. The possibility that gamete, larval, and adult robustness are co-evolved is an exciting one that deserves further attention.

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Figures

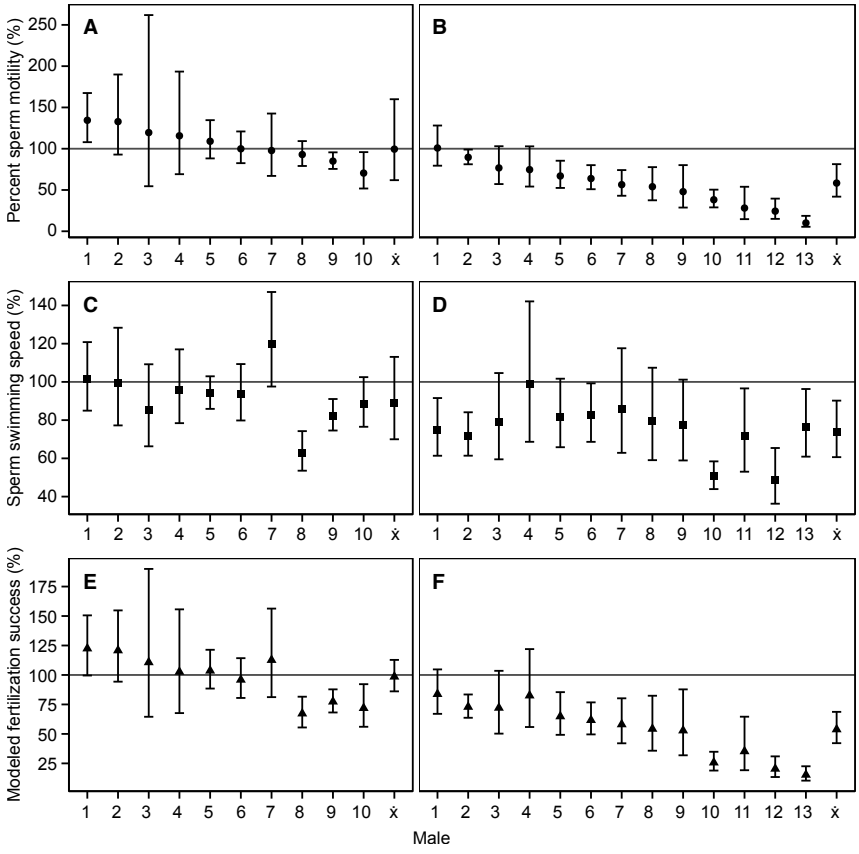

Figure 1. Effect sizes (R) of increased pCO_2 for percent sperm motility (A-B), swimming speed (C-D), and modeled fertilization success (E-F). Panels in the first column (A, C & E) represent *M. calcarea*, and panels in the second column (B, D & F) indicate *M. galloprovincialis*. Males along x-axis are ordered based on average effect size for percent sperm motility, and $\bar{x}$ presents the average effect over males for each species. Error bars represent 95% confidence intervals. The line at 100% indicates no significant treatment effect where error bars cross this line. Fertilization success values were calculated using sperm concentrations that give 50 % of maximum fertilization success in *control* treatment ($F_{50Control}$ in Schlegel et al. [5]).

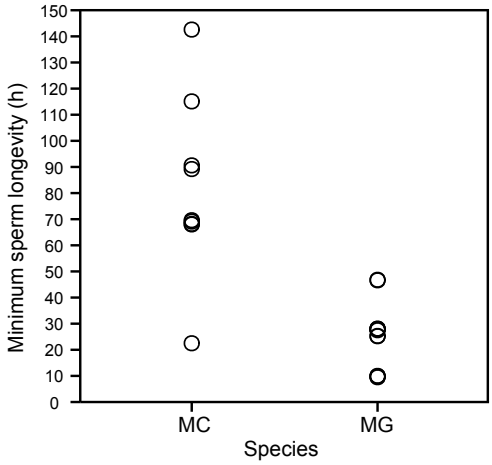

Figure 2. Sperm longevity of *M. calcarea* (MC) and *M. galloprovincialis* (MG). Each open circle represents a time (h) for the last observation of continuously swimming sperm for an individual male.

Tables

Table 1. Number of males exposed to the treatments (n) and corresponding sea water parameters. Measured seawater parameters: approximate temperature (T, in °C), measured salinity (S), average pH measured directly from treatment water using sensors (pH_{NBS}), and average pH measured from storage tanks at 25 °C using spectrophotometer (pH_T) ± standard error. Sensor measurements are relative to NBS scale and calculated using daily values. Spectrophotometer measurements are relative to total scale and calculated using replicate samples. Given pH value was based on one replicate in cases where standard error is missing. Total alkalinity (A_T, in µmol kg^{-1}) was measured for MC and calculated for MG using DIC values (see Table S1). Calculated seawater parameters after correcting for treatment temperatures (T) using CO2SYS: pH relative to total scale (pH), pCO_2 of seawater (pCO_2, in µatm), calcite saturation state (Ω_{Ca}), and aragonite saturation state (Ω_{Ar}). Species abbreviations are given in brackets after each species.

		Measured					Calculated*			
Species and treatment	n	T	S	pH_{NBS}*	pH_T**	A_T	pH	pCO_2	Ω_{Ca}	Ω_{Ar}
M. calcarea (MC)										
Control	10	2	28	7.94 ± 0.01	7.92 ± 0.01	2575 ± 19	8.28	250	3.9	2.4
Acidified	10	2	28	7.53 ± 0.06	7.50 ± 0.01	2743 ± 14	7.82	854	1.6	1.0
M. galloprovincialis (MG)										
Control	13	16	35	8.09 ± 0.01	8.10 ± 0.01	3173	8.10	481	6.0	3.9
Acidified	13	16	35	7.64 ± 0.02	7.67	2969	7.73	1189	2.7	1.7

*Corrected for experimental temperature.

** Measured at 25 °C.

Supporting Information

Table S1. Water chemistry sample overview over studied males. The first two letters in Male column represent the species (see Table 1 for the species abbreviations) and the number the male number from Figure 1. S = measured salinity in treatment tanks, pH_{NBS} = sensor measurement directly from treatment water, pH_T = spectrophotometer measurement from storage tanks, A_T = total alkalinity measurement from larval cylinders, and DIC = dissolved inorganic carbon measurement from larval cylinders. A number in pH_{NBS}, pH_T, A_T, and DIC columns represent the number of replicate measurements. Missing values indicate that measurement was not conducted. Results of the measurements are presented in Table 1.

Table S2. Longevity data for Figure 2. Columns from the left: Species = corresponding species (see Table 1 for the species abbreviations); Male = the male number from Figure 1, and $\bar{x}$ indicates species average; Time = time of the last observation of continuously swimming sperm in hours.

Table S3. Experimental data averaged over males. The first two letters in Male column represent the species (see Table 1 for the species abbreviations) and the number the male number from Figure 1. pCO_2 = air pCO_2 level, T = temperature (°C), pH_{NBS} = pH in NBS scale, Time = average time from spawning when measurements were conducted (min), C = sperm concentration at the beginning of measurements (sperm μL^{-1}), and n = number of replicate film clips included in the analyses. Average and standard deviation for percentage sperm motility (Motility, %), sperm swimming speed (Speed, $\mu m\, s^{-1}$) and number of sperm paths (Paths) per replicate film clip are given in $\bar{x}$ and σ columns respectively.

Video S1. Video clip of *M. galloprovincialis* (MG10) sperm under control conditions during initial sperm activity measurements. The video clip shows continuously swimming sperm in good condition.

Video S2. Video clip of *M. galloprovincialis* (MG10) sperm under increased pCO_2 during initial sperm activity measurements. Sperm is clearly affected by the treatment (compare with Video S1)

Video S3. Video clip of *M. galloprovincialis* (MG3) sperm under control conditions 46 h after induced spawning. The video clip show several continuously swimming sperm cells.

Video S4. Video clip of *M. calcarea* (MC5) sperm under control conditions during the initial measurements. The video clip show several continuously swimming sperm cells in good condition.

Video S5. Video clip of *M. calcarea* (MC5) sperm under control conditions 115 h after extraction of gametes. One cell adjacent to the lower left corner is swimming continuously. Many cells are still swimming, although not as continuously as in Video S4, and therefore not recorded as "continuously swimming sperm".