ECOLOGY

Naturally acidified habitat selects for ocean acidification–tolerant mussels

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Ocean acidification severely affects bivalves, especially their larval stages. Consequently, the fate of this ecologically and economically important group depends on the capacity and rate of evolutionary adaptation to altered ocean carbonate chemistry. We document successful settlement of wild mussel larvae (*Mytilus edulis*) in a periodically CO_2-enriched habitat. The larval fitness of the population originating from the CO_2-enriched habitat was compared to the response of a population from a nonenriched habitat in a common garden experiment. The high CO_2–adapted population showed higher fitness under elevated P_{CO_2} (partial pressure of CO_2) than the non-adapted cohort, demonstrating, for the first time, an evolutionary response of a natural mussel population to ocean acidification. To assess the rate of adaptation, we performed a selection experiment over three generations. CO_2 tolerance differed substantially between the families within the F_1 generation, and survival was drastically decreased in the highest, yet realistic, P_{CO_2} treatment. Selection of CO_2-tolerant F_1 animals resulted in higher calcification performance of F_2 larvae during early shell formation but did not improve overall survival. Our results thus reveal significant short-term selective responses of traits directly affected by ocean acidification and long-term adaptation potential in a key bivalve species. Because immediate response to selection did not directly translate into increased fitness, multigenerational studies need to take into consideration the multivariate nature of selection acting in natural habitats. Combinations of short-term selection with long-term adaptation in populations from CO_2-enriched versus nonenriched natural habitats represent promising approaches for estimating adaptive potential of organisms facing global change.

INTRODUCTION

Ocean acidification, caused by rising atmospheric CO_2 concentrations due to excess fossil fuel burning, severely affects many marine organisms (*1*). Calcifying organisms are especially affected by the change of ocean chemistry because their ability to form calcified structures is reduced. Bivalves are among the most vulnerable taxonomic groups because their $CaCO_3$-containing shells protect the animal from predation. In particular, their larval stages suffer from substantial reductions in growth and survival under elevated P_{CO_2} (partial pressure of CO_2) (*2*–*5*). This is likely a consequence of the high calcification rates during the formation of the first larval shell (*6*, *7*). Although the benthic life stage is able to compensate for the negative impact even of highly elevated P_{CO_2} (~3000 µatm) when food supply is abundant, early larval development is completely fueled by the limited energy provided by the egg and thus represents an important ecological bottleneck (*6*, *7*). Bivalves of the genus *Mytilus* have important ecological roles in boreal, benthic ecosystems and can contribute by up to 90% to epibenthic biomass in coastal habitats (*8*). In addition, their high economic value for aquaculture has stimulated a number of recent studies to estimate their adaptation potential to future ocean conditions (*4*, *9*–*11*). However, the relatively long generation time of bivalve species complicates multigenerational (MG) studies. Consequently, most studies until now have estimated evolutionary potential by quantifying variation of fitness-relevant traits such as growth during early development within and between locally adapted populations (*10*, *12*, *13*) or assessed transgenerational phenotypic plasticity (*10*). These studies did not provide a uniform picture on the potential of bivalves to adapt to ocean acidification. Modeling the rate of adaptation in a single population based on larval shell size variations within the first 60 hours of development under elevated P_{CO_2} (1000 µatm) suggested a low potential for adaptation when extrapolated over 50 generations (*8*). To capture longer time periods, comparison of populations naturally experiencing differing carbonate system conditions offers useful proxies for estimating adaptation [space-for-time substitution (*14*)]. In one study, the growth response of field-collected juvenile mytilid mussels originating from two populations differed under elevated P_{CO_2}, indicating local adaptation (*12*). In another, larval shell development of two *Mytilus* species was similar during exposure to varying carbonate system treatments, although one species originated from a habitat that encounters upwelling events associated with elevated P_{CO_2} (*13*). Although these studies suggested that bivalves can potentially adapt to rising P_{CO_2}, they lack a formal estimation of genetic versus nongenetic sources of variation. For example, transgenerational acclimation to elevated P_{CO_2} can substantially modulate fitness of offspring as observed in fish (*15*). In the oyster *Saccostrea glomerata*, a 5-week exposure of parental animals to elevated P_{CO_2} during gametogenesis enhanced the development and growth rates of F_1 and even F_2 offspring under acidified conditions (*9*, *16*). Selective breeding for aquaculture purposes substantially increased the productivity of *S. glomerata* and resulted in 25% improved growth within two to four generations (*17*). The higher developmental rates of this breeding line were also maintained under elevated P_{CO_2} compared to wild-type oysters (*10*). Therefore, rapid evolutionary responses in bivalves may enable adaptation to ocean acidification, but these MG selection studies using continuously elevated P_{CO_2} as selective agent are lacking so far.

To fill this gap, we performed a 3-year MG experiment to test whether the blue mussel *Mytilus edulis* can successfully adapt to ocean acidification and to estimate which mechanisms contribute to rapid evolutionary responses. The tested population inhabits the seasonally acidified Kiel Fjord, western Baltic Sea, which is characterized by low pH and elevated P_{CO_2} levels during the reproductive period of the species

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(*18*, *19*). This experiment was supported by field monitoring of carbonate chemistry variation in relation to mussel settlement patterns. In addition, we compared the Baltic population in a common garden experiment to the response of mussels from the North Sea, which is characterized by less variable pH conditions and higher seawater alkalinity due to higher salinity (*20*).

To investigate the time scale of adaptation to ocean acidification, we conducted two experiments to compare long-term adaptation between populations and processes of short-term adaptation within a population. We hypothesized that Baltic mussels have already adapted to high-CO_2 seawater and would better tolerate simulated ocean acidification than North Sea mussels. Furthermore, we hypothesized that selection for ocean acidification–tolerant specimens would increase the fitness of their offspring when exposed to acidified conditions.

RESULTS

Field carbonate chemistry monitoring and larval settlement (Baltic Sea)

Monitoring of pH in Sylt and Kiel Fjord revealed higher and more stable pH in the North Sea habitat compared to the habitat of the Baltic population (fig. S1). Monthly mean pH in Sylt remained above 8, with maximum values recorded during spring bloom in April (Fig. 1A). In contrast, mean pH values declined to about 7.7 during the upwelling period in summer and autumn in Kiel Fjord. Our monitoring of mussel settlement on weekly deployed panels and continuously logged seawater P_{CO_2} revealed that bivalve larvae survived and settled in Kiel Fjord, which is characterized by elevated and fluctuating P_{CO_2}. The hourly averaged P_{CO_2} was 1087 ± 537 µatm and ranged between 266 and 2861 µatm over the whole monitoring period from mid-July to mid-September 2012 (Fig. 1B). Despite such high and fluctuating environmental P_{CO_2}, Baltic mussels settled successfully, with a peak of more than 1000 larvae settled per panel in early August (Fig. 1C) at elevated P_{CO_2} levels similar to those predicted for the average surface ocean of 2100. Because P_{CO_2} fluctuated rapidly due to upwelling events in Kiel Fjord, environmental conditions experienced by different larval cohorts differed significantly (fig. S2). Earlier settlers of the July cohort experienced only moderately elevated P_{CO_2} because larvae avoided the first pronounced upwelling peak at the beginning of August (Fig. 1C) (mean P_{CO_2}, 826 µatm; range, 266 to 1502 µatm). In contrast, larvae that settled at the end of August experienced P_{CO_2} levels between 443 and 2861 µatm (mean, 1191 µatm) during a calculated 27-day larval phase (Fig. 1C). Larvae settling in mid-September were again exposed to lower and more stable P_{CO_2} levels (larval phase, 25 days; mean, 859 µatm; range, 427 to 2225 µatm) (Fig. 1C). The number of days August and September cohorts were exposed to daily mean P_{CO_2} values above 1000 µatm differed, with 17 and 5 days corresponding to 63 and 20% of their estimated whole planktonic life phase, respectively.

Population comparison experiment (Baltic Sea versus North Sea)

The formation of the first larval shell [prodissoconch I (PD I)] (fig. S3) (*21*) was strongly delayed in both North Sea and Baltic Sea populations at high P_{CO_2}, which resulted in significantly reduced shell length compared to larvae from the control P_{CO_2} [two-way analysis of variance (ANOVA): population: $F = 1.6$, $P > 0.05$; P_{CO_2}: $F = 112.1$, $P < 0.01$]. However, Baltic mussel larvae were less affected and showed a smaller shell length reduction compared to North Sea larvae at elevated P_{CO_2} [−24% (Baltic Sea) versus −38% (North Sea) shell length compared to respective controls; population × P_{CO_2}: $F = 6.5$, $P < 0.05$] (Fig. 2A).

Growth patterns translated well into observed survival. Here, survival at 390-µatm P_{CO_2} did not differ between the two populations, but Baltic larval survival was higher at elevated P_{CO_2} (two-way ANOVA: population: $F = 0.8$, $P > 0.05$; P_{CO_2}: $F = 10.9$, $P < 0.01$; population × P_{CO_2}: $F = 6.9$, $P < 0.05$) (Fig. 2C and table S1). Subsequent shell growth rates were similar in Baltic larvae exposed to 390- and 2400-µatm P_{CO_2} (Fig. 2B; no data for North Sea larvae at 2400-µatm P_{CO_2} because of high mortality).

Three-year MG experiment (Baltic Sea)

F_0 and F_1 generation (2012).

The MG experiment utilized controlled genetic crosses of mussels collected from the more P_{CO_2}-tolerant Baltic Sea population to select for CO_2-tolerant and CO_2-sensitive families, which were used to elucidate the relative contribution of genetic and nongenetic environmental factors enabling adaptation to ocean acidification (Fig. 3B). PD I size was strongly reduced in F_1 larvae exposed to elevated P_{CO_2} and declined

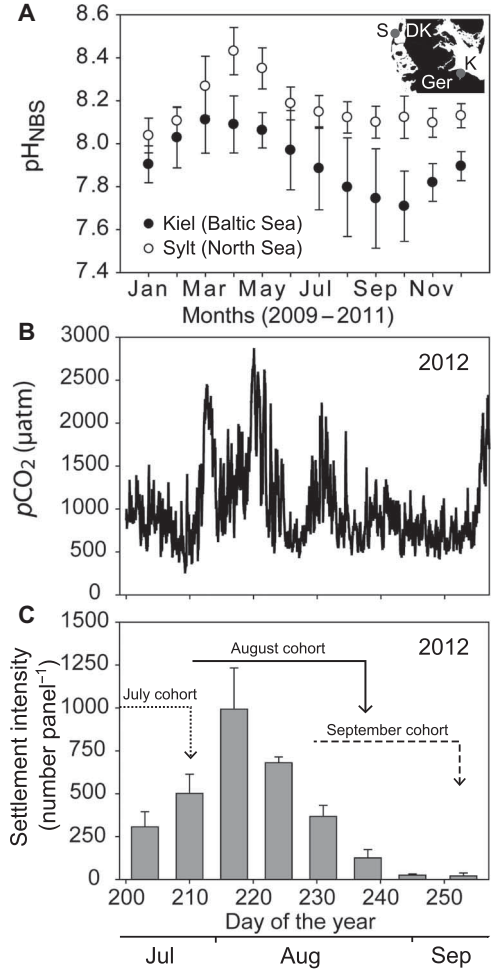

Fig. 1. Habitat carbonate system variability and juvenile settlement. (**A**) Averaged monthly pH values recorded from 2009 to 2011 in the habitats of the two tested populations. The inset depicts the location of the two habitats in North Sea and Baltic Sea (S, Sylt; K, Kiel; DK, Denmark; Ger, Germany). (**B**) Continuously measured seawater P_{CO_2} in summer 2012 in Kiel Fjord. (**C**) Mussel larval settlement intensity on panels between July and September. The arrows correspond to the estimated planktonic phases of larvae settled in July, August, and September. Values are means ± SD.

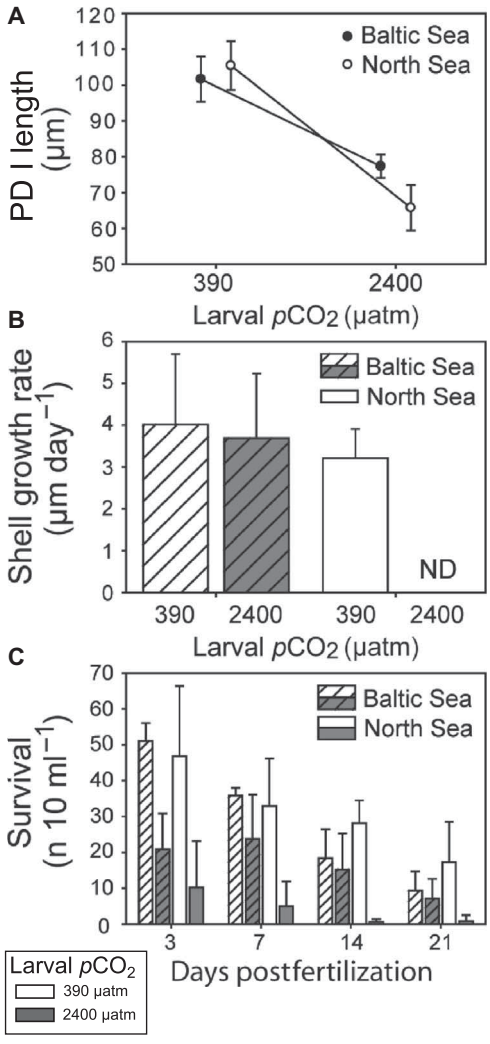

Fig. 2. Larval performance of Baltic Sea and North Sea populations exposed to elevated P_{CO_2}. (**A**) PD I length of both populations declined at high P_{CO_2}, but Baltic larval size was less affected (n = 22 to 73). (**B**) Daily shell growth was similar for both populations and P_{CO_2} treatments [no data (ND) for North Sea larvae at high P_{CO_2} due to low survival on days 14 and 21]. (**C**) Survival rapidly declined in North Sea larvae exposed to elevated P_{CO_2}, whereas Baltic Sea larvae were less affected by elevated P_{CO_2} from day 7 forward. Values are means ± SD; numbers in bracket state the number of measured individuals per P_{CO_2} treatment.

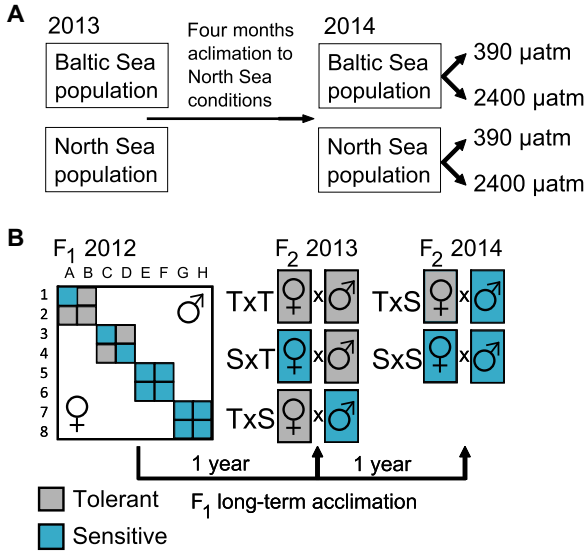

Fig. 3. Flow chart of the experimental approach. (**A**) Collection of mussels from North Sea and Baltic Sea population, acclimation to North Sea conditions from December 2013 to April 2014, and subsequent transfer, spawning, and exposure to two P_{CO_2} treatments. (**B**) Crossing schemes for F_1 and F_2 generation. In 2012, F_0 animals (eight dams and eight sires) were crossed pairwise to generate 16 full-sib F_1 families nested within four half-sib groups. Settled mussels were subsequently acclimated for 1 and 2 years. In 2013, F_2 was generated by performing single-specimen crosses using pure F_1 tolerant lines as well as tolerant and sensitive F_1 families selected in, and long-term acclimated to, 390-, 1120-, and 2400-µatm (tolerant only) P_{CO_2}. In 2014, F_2 was generated with three dams from four families of each line (tolerant from 390- and 2400-µatm P_{CO_2} acclimation and sensitive from 390-µatm P_{CO_2} acclimation only) crossed with one male from the tolerant family E6.

from 112 ± 6 µm at 390 µatm to 94 ± 7 µm and 78 ± 8 µm at 1120 and 2400 µatm, respectively (ANOVA: P_{CO_2}: F = 165.1, P < 0.001) (Fig. 4A). Family-specific PD I shell length varied substantially, and the calculated heritability for this trait was 0.56 [confidence interval (CI), 0.27 to 0.81], 0.47 (CI, 0.24 to 0.81), and 0.53 (CI, 0.23 to 0.83) at 390-, 1120-, and 2400-µatm P_{CO_2}, respectively. Subsequently, larvae from all P_{CO_2} treatments grew at comparable rates and thus reached similar sizes at the end of the planktonic phase (Fig. 4C). Because of the large variance in final larval survival between families, our study showed no significant difference in larval survival between 390- and 1120-µatm P_{CO_2}, but did reveal a drastic reduction at 2400 µatm (Fig. 4E and table S2). Similarly, larvae from all families successfully settled at the two lower P_{CO_2} levels, but only the offspring of five families (classified as "tolerant" families A2, B1, B2, C4, and D3) metamorphosed into juveniles at 2400 µatm (Fig. 3B). Successful metamorphosis and thus tolerance correlated positively with PD I size because shell length was slightly larger in tolerant compared to sensitive families at 2400 µatm (82 ± 5 µm versus 76 ± 8 µm) (two-way ANOVA: sensitive versus tolerant: F = 1.5, P > 0.05; P_{CO_2}: F = 365.7, P < 0.001; sensitive versus tolerant × P_{CO_2}: F = 5.5, P < 0.05) (Fig. 4A).

Following settlement, juveniles of all families were transferred into a flow-through experimental system and raised for 1 year (2012–2013) at the respective P_{CO_2} until the next spawning season (Table 1). During that time, no mortality was observed and F_1 juveniles from all families grew to shell sizes of about 25 mm within 1 year irrespective of P_{CO_2} treatment or family type [390 µatm, 24.7 ± 3.7 mm; 1120 µatm, 26.0 ± 2.7 mm; and 2400 µatm, 24.8 ± 3.0 mm (tolerant families only)] (ANOVA: F = 0.216, P > 0.05).

First F_2 generation (2013).

Crosses of F_1 specimens were carried out to test (i) whether developmental acclimation of F_1 families conferred environment-specific benefits in relation to offspring P_{CO_2} (that is, transgenerational plasticity) and (ii) whether tolerance has a genetic component that could be crossed into the genetic background of sensitive families (Fig. 3B). Maternal investment, measured as egg production of F_1 dams and egg diameter, did not change under elevated P_{CO_2} (fig. S4). Fertilization success was not significantly affected by P_{CO_2} levels, irrespective of whether it was assayed in tolerant or sensitive families. In crosses between tolerant F_1 parents (T×T), PD I size of F_2 larvae was similar at control P_{CO_2}, irrespective of parental rearing history. However, at high P_{CO_2}, PD I sizes were larger for offspring from tolerant F_1 families raised at elevated P_{CO_2} compared to larvae from control P_{CO_2}–treated parents (see parental P_{CO_2} × offspring P_{CO_2} interaction effects in table S2). Shell size of offspring from F_1 dams selected

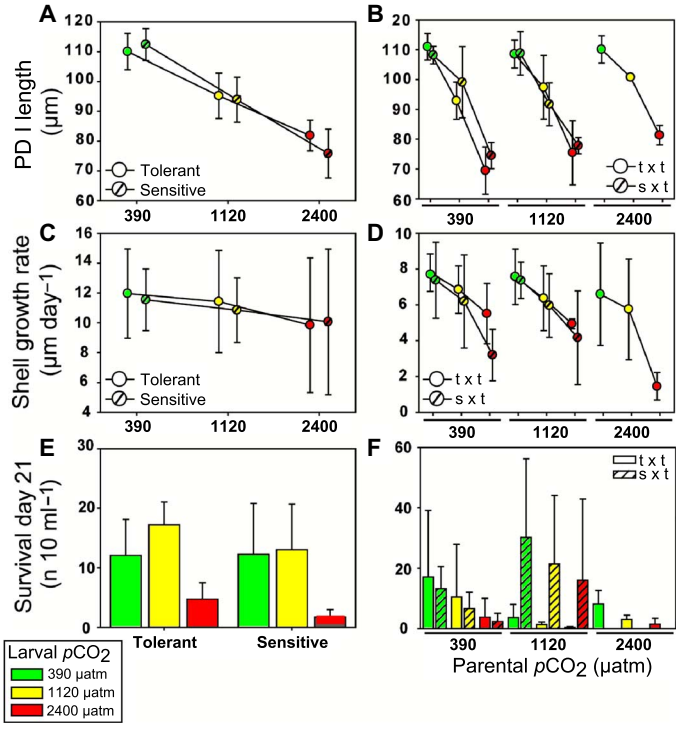

Fig. 4. Larval performance of F_1 and F_2 animals exposed to elevated P_{CO_2}. (**A**) PD I length declined with increasing P_{CO_2} but remained larger in tolerant F_1 families ($n = 314$ to 620) and (**B**) partly recovered in tolerant (T×T) but not sensitive (S×T) F_2 offspring from high P_{CO_2}–treated parents (n = 902 to 1314). (**C**) Larval daily shell growth at elevated P_{CO_2} was not affected in F_1 offspring (**D**) but decreased in F_2 larvae and further deteriorated with high-CO_2 exposure of parental animals. (**E**) Larval survival decreased at high P_{CO_2} but was higher in tolerant families compared to sensitive F_1 families. (**F**) Parental selection and long-term acclimation at high P_{CO_2} did not improve survival in the F_2 generation. Values are means ± SD; numbers in brackets state the number of measured individuals per P_{CO_2} treatment.

and raised under 2400-µatm P_{CO_2} increased by 11.9 µm or 17% compared to that of offspring of 390-µatm acclimated F_1 dams (Fig. 4B and table S2). In contrast to the F_1 generation, F_2 larval growth rates in general were slower and particularly reduced at high P_{CO_2} and additionally declined when raised from F_1 exposed to elevated P_{CO_2} during long-term acclimation (Fig. 4D and table S2). Furthermore, larger PD I size of F_2 larvae at 2400-µatm P_{CO_2} had no positive effect on larval survival, in contrast to observations in the F_1 generation (Fig. 4F and table S2). Selection of tolerant phenotypes in the F_1 generation thus only had a positive transgenerational effect on PD I size but did not improve the mean population fitness of their F_2 offspring. Rather, offspring from mothers acclimated at control P_{CO_2} conditions (390 µatm) showed higher survival rates, indicating no positive effects of parental acclimation to high P_{CO_2} on offspring survival (table S2). In contrast to the results for crosses between tolerant families (T×T), crosses between tolerant mothers and sensitive fathers (S×T) resulted in similar PD I sizes of F_2 larvae, irrespective of parental P_{CO_2} treatment (Fig. 4B and table S2). Crosses between tolerant mothers and sensitive fathers showed an increased survival when compared to T×T crosses, especially at 1120-µatm P_{CO_2} (Fig. 4F and table S2).

Second F_2 generation (2014).

The observed responses for fecundity, PD I, larval growth rates, and survival were largely confirmed when generating the second F_2 generation from the same F_1 animals in the subsequent year (Fig. 3B and fig. S5). When kept for another year in the experimental system at their respective P_{CO_2} treatment (2013–2014), fecundity and egg sizes were affected neither by parental P_{CO_2} treatment nor by family type (sensitive/tolerant F_1 families; figs. S4, B and D, and S5A). F_2 offspring from high P_{CO_2}–selected parental F_1 animals showed a nonsignificant trend toward larger PD I size (fig. S5B and table S3). Shell growth rates were reduced in all high P_{CO_2}–treated larvae but were again affected neither by parental acclimation P_{CO_2} treatment nor by family type (fig. S5C). Similarly, survival of larvae was negatively affected by elevated P_{CO_2} and not improved by selection of tolerant F_1 parents (T×T) or the prolonged high-P_{CO_2} acclimation of F_1 animals (fig. S5D and table S3).

DISCUSSION

The high sensitivity of bivalve larvae to elevated P_{CO_2} (*3*, *4*) suggests that selective pressures should be strong and populations should rapidly adapt to the prevailing local P_{CO_2} levels. However, evidence for this hypothesis is circumstantial (*9*, *12*, *13*). We used two different approaches to study the adaptation potential of mussels to ocean acidification. First, we performed a population comparison (PC) experiment to test for existing differences in tolerance to ocean acidification. Second, we assessed how rapidly tolerance can be acquired by selection of tolerant phenotypes or transgenerational plasticity in an MG experiment.

Population comparison experiment (Baltic Sea versus North Sea)

In an experimental common garden approach, larval performance of North Sea mussels under low and elevated P_{CO_2} was compared to that of larvae from the Baltic Sea population. On the phenotypic level, adaptation to elevated P_{CO_2} in Baltic mussels was indicated by increased survival under elevated P_{CO_2} and higher capacity to maintain PD I formation rates compared to the more sensitive North Sea mussels. The experiment revealed that naturally and locally deviating ocean carbonate chemistry characteristics influence the responses of blue mussel populations to experimental ocean acidification, most likely reflecting local adaptation to prevailing environmental conditions on longer time scales (*22*–*24*). In general, larval calcification was strongly impaired by elevated P_{CO_2}; this impairment was even more pronounced than reported at comparable P_{CO_2} for fully marine populations (*5*, *25*). This reflects our choice of the highly selective environment in the brackish Baltic Sea, where conditions for calcification are less favorable (*7*). Calcification of bivalve larvae is not directly affected by P_{CO_2}, but is sensitive to lowered pH and availability of inorganic carbon (HCO_3^-; C_T) as a substrate for calcification, which correlates with seawater Ω [calcium carbonate saturation state (*5*, *7*, *26*)]. The low alkalinity and thus low C_T concentrations of the Baltic Sea result in lowered carbon availability and Ω and therefore synergistically enhance the negative effects of elevated P_{CO_2} on larval calcification (*7*). As a result of this intensified selection pressure, Baltic mytilid mussels have successfully adapted to adverse conditions for calcification.

Calcification of PD I coincides with the highest relative calcification rates of all bivalve life stages, which makes this ontogenetic stage most vulnerable to external carbonate system perturbations (*5*–*7*). The correlation of higher calcification rates and survival of tolerant Baltic mussels suggests that PD I calcification is mechanistically linked to survival and therefore directly to fitness. PD I sizes were similar in both populations under control P_{CO_2} when the external carbonate chemistry did not limit calcification; thus, growth and development were potentially limited by

Table 1. Carbonate chemistry during the larval experiments and the long-term acclimation. pH on total scale and C_T were measured (n = 163), and A_T, Pco_2, [CO_3^{2-}], and $\Omega_{Aragonite}$ were calculated using CO2SYS. NBS, National Bureau of Standards.

	Temperature (°C)	Salinity (g kg^{-1})	Pco_2 treatment (µatm)	C_T (µmol kg^{-1})	pH (total scale)	Measured pH (NBS scale)	A_T (µmol kg^{-1})	Pco_2 (µatm)	[CO_3^{2-}] (µmol kg^{-1})	$\Omega_{Aragonite}$
F_1 larvae 2012	17.7 ± 0.1	15.5 ± 0.3	390	1802 ± 46	7.97 ± 0.02	8.17 ± 0.07	1884 ± 51	508 ± 14	76.6 ± 4.9	1.23 ± 0.08
			1120	1889 ± 78	7.65 ± 0.01	7.75 ± 0.06	1897 ± 76	1128 ± 82	38.6 ± 1.2	0.62 ± 0.02
			2400	1995 ± 50	7.39 ± 0.03	7.46 ± 0.05	1944 ± 51	2114 ± 108	22.6 ± 1.4	0.36 ± 0.02
F_2 larvae 2013	17.1 ± 0.2	16.0 ± 0.4	390	1916 ± 86	8.03 ± 0.20	8.16 ± 0.07	2026 ± 90	476 ± 181	99.8 ± 14.8	1.60 ± 0.24
F_2 larvae 2014			1120	2056 ± 54	7.64 ± 0.15	7.69 ± 0.01	2063 ± 87	1264 ± 167	44.1 ± 33.5	0.71 ± 0.53
			2400	2078 ± 27	7.43 ± 0.15	7.43 ± 0.02	2032 ± 28	2093 ± 379	28.7 ± 15.2	0.46 ± 0.24
	18.8 ± 0.1	16.0 ± 0.2	390	1875 ± 12	8.05 ± 0.03	8.19 ± 0.03	1989 ± 17	440 ± 24	99.6 ± 5.3	1.62 ± 0.09
Long-term acclimation (2012–2014)			2400	2039 ± 25	7.40 ± 0.07	7.55 ± 0.07	1991 ± 13	2160 ± 358	25.0 ± 4.3	0.41 ± 0.07
	11.4 ± 4.3	15.1 ± 2.1	390	2044 ± 125	7.84 ± 0.07	8.02 ± 0.06	2064 ± 122	734 ± 108	46.2 ± 12.1	0.71 ± 0.20
			1120	2108 ± 118	7.57 ± 0.05	7.71 ± 0.07	2068 ± 118	1381 ± 136	25.7 ± 6.7	0.40 ± 0.11
PC			2400	2258 ± 257	7.33 ± 0.06	7.44 ± 0.09	2146 ± 253	2515 ± 382	15.7 ± 4.6	0.24 ± 0.07
	15.5 ± 0.1	28.5 ± 0.2	390	2160 ± 14	8.02 ± 0.02	8.16 ± 0.01	2334 ± 14	462 ± 26	136.0 ± 6.0	2.14 ± 0.09
			2400	2411 ± 30	7.33 ± 0.01	7.46 ± 0.01	2357 ± 29	2588 ± 45	31.4 ± 0.6	0.49 ± 0.01

other physiological processes. Because PD I is formed before development of the larval feeding apparatus, a substantial fraction of the limited energy stored in the bivalve egg is needed for shell formation even under favorable carbonate system conditions (*6*, *27*). Because calcification generates protons, which need to be excreted by means of an active transport process, disproportional up-regulation of shell formation could challenge the larval energy budget. A more efficient energy allocation into PD I formation may thus explain larger and maintained PD I size under elevated Pco_2 in the adapted Baltic compared to North Sea mussels.

Three-year MG experiment (Baltic Sea)

Although common garden experiments offer a means to test for the existence of local adaptation, only experiments performed over multiple generations can give insights into the rate and mechanistic basis of the adaptation process. Earlier studies using oysters as model organisms revealed that parental preexposure to elevated Pco_2 resulted in faster growth and development of larvae under high Pco_2 when compared to larvae generated from parents that were acclimated to control conditions (*9*). Therefore, transgenerational phenotypic plasticity needs to be considered as an important factor that can modulate the response of bivalves to ocean acidification (*28*).

In our MG experiment performed with the Baltic Sea population, we observed a large variance in response to elevated Pco_2 among the CO_2-sensitive and CO_2-tolerant families. Our high-resolution environmental Pco_2 monitoring in the habitat of the population revealed rapidly fluctuating Pco_2. Thus, different cohorts of larvae can be exposed to either high or low Pco_2 during the sensitive planktonic larval phase, indicating that not all individuals from the Kiel Fjord population were selected in a high-Pco_2 environment. It is likely that this environmental heterogeneity selects for maintenance of variance of CO_2 tolerance and genetic diversity in this population. The role of temporal heterogeneity of selection pressures for maintaining genetic diversity has historically been underestimated, although the scaling of phenotypic change with time strongly suggests that fluctuating selection pressures are the rule rather than the exception (*29*). Especially when generations overlap and selection pressures vary across life stages, fixation of alleles by selective sweeps becomes unlikely (*30*). The high sensitivity of larvae to elevated Pco_2 compared to adult mussels along with several yearly cohorts observed in the fjord fits this condition for maintaining genetic diversity. The low predictability of selective environmental Pco_2 levels in the Baltic population makes tracking of these fluctuations by heritable trait changes unlikely and should rather select for bet hedging (*31*) or mechanisms of

plasticity, particularly for transgenerational plasticity with the early life stages that are affected here (*10*, *32*).

In both experiments (the MG and PC experiments), selection for tolerance to high P_{CO_2} correlated with a higher capacity to reach larger PD I sizes in the F_1 generation. Calculated heritabilities for this trait (0.23 to 0.83) were within the range of values previously reported for mytilid larvae [0.09 to 0.9 (*8*, *33*)]. The relatively larger PD I size of tolerant compared to sensitive families was also passed on to the F_2 generation, thus showing a heritable component. This suggests that the ability to form the PD I shell even under adverse environmental conditions can be an important fitness trait. Although transgenerational plasticity could partly compensate the negative effects of elevated P_{CO_2} levels for PD I formation rates within one generation (Fig. 4B), the absence of an effect on F_2 survival implies that PD I size alone cannot be used as a trait for reliable modeling of the evolutionary response of population mean fitness (*9*). Similar results were obtained for oysters when selection of larvae under 856-µatm P_{CO_2} did not improve the survival of their F_2 offspring under the same P_{CO_2} treatment (*16*). Increased performance observed in marine organisms under moderately elevated P_{CO_2} can probably be attributed to transgenerational phenotypic plasticity (TGP). TGP has been suggested to function as a short-term buffering mechanism to alleviate the effects of adverse environments before genetic adaptation can fill the fitness deficit. TGP has been shown to even persist over several generations in a range of species (*10*, *15*, *34*, *35*). TGP can manifest itself in altered animal performance with beneficial effects on growth and fecundity (*34*–*37*) or, in the case of bivalves, via modification of shell formation processes (*38*). More specifically, TGP can modulate, for example, respiratory capacity (aerobic scope) by acting upon mitochondrial properties. TGP thereby enables animals to adjust crucial physiological processes to the changed environment (*35*–*37*, *39*). Maternal effects can play a central role in passing TGP from one generation to the next (*35*, *37*). The rapid recovery of the PD I size of offspring from high P_{CO_2}–treated dams under acidified conditions (Fig. 4A) could result from such maternally driven TGP as well. In contrast, the absence of a positive effect on F_2 survival suggests that F_1 larval fitness is dependent on specific combinations of genotypes and nonheritable components.

Although significant adaptive responses may not necessarily be detectable on the whole-organism level within the three generations investigated in this study, they likely have contributed to the higher fitness of the Kiel Fjord population compared to North Sea mussels over longer time scales. Although high-CO_2 fluctuations in this habitat have increased only within recent decades as a result of eutrophication, adverse conditions for calcification due to lower alkalinity compared to the North Sea have prevailed for thousands of years (*20*, *40*). The high mortality of bivalves during the sensitive larval phase and the very high effective population size of mussels in the Baltic Sea (*41*) should have efficiently selected for beneficial mutations that increased population fitness. In support of this view, changed allele frequencies in response to elevated P_{CO_2} have been observed in sea urchin larvae within only 7 days of exposure (*24*). In our study, selection of tolerant F_1 specimens did not improve F_2 survival, which corresponds to findings obtained with oysters (*16*). However, selective breeding of high-yield oysters for aquaculture purposes resulted in significantly improved ocean acidification tolerance as a side effect within just four generations (*10*, *17*). The absence of a beneficial effect of selection in our study could be due to the small number of individuals used for the genetic crosses, which reduced the standing genetic variation present in the F_1 generation. However, a large standing variation is needed as a prerequisite for selection (*24*). Consequently, future experiments would need to use a larger number of individuals or families to lower the risk of detrimental genetic drift to more closely resemble the genetic variability present in populations, enabling rapid adaptation (*42*). This is particularly important for coastal habitats such as Kiel Fjord, which are characterized by large abiotic variability that could lead to high genetic variation within a single population.

In conclusion, several lines of evidence suggest a potential of *Mytilus* populations to adapt to elevated CO_2. This conclusion is supported by (i) the different sensitivity of Baltic Sea and North Sea populations in response to a natural P_{CO_2} gradient and (ii) a heritable component of calcification performance in early larval development observed in the MG experiment. Mussel larvae from the Baltic were characterized by higher CO_2 tolerance that correlated with higher ability to form the PD I shell under CO_2 stress. In concurrence with these data, our MG experiment revealed that selection for settlement in high-P_{CO_2} environments correlated with retention of PD I formation capabilities in F_1 animals. However, selection of tolerant F_1 phenotypes and long-term acclimation of F_1 specimens in our MG study did not significantly improve F_2 offspring survival. Consequently, prediction of adaptation potential based on short-term experiments and single traits within a population and generation appears to be highly speculative. Future experiments need to be performed over multiple generations to obtain a detailed understanding of the rate of adaptation and the underlying mechanisms to predict whether adaptation will enable marine organisms to overcome the constraints of ocean acidification.

MATERIALS AND METHODS

Kiel Fjord seawater P_{CO_2} was continuously monitored using a HydroC CO_2 sensor [Kongsberg Maritime AS (*43*)] mounted on a floating platform in about 1-m water depths. Abundance of settled bivalve larvae was assessed weekly on 5 cm × 5 cm manually roughed, replicated polyvinyl chloride panels (n = 4) suspended in the fjord in about 50-cm water depth.

For the PC experiment, *M. edulis* from Kiel Fjord (Baltic Sea) were transferred to List/Sylt (North Sea) and suspended in net cages along with North Sea specimens to acclimate to North Sea conditions. Acclimation lasted from December 2013 to April 2014 when all specimens were transferred back to Kiel and used for spawning the next day.

For the MG experiment, adult *M. edulis* were collected in Kiel Fjord in 2012 and kept overnight in a flow-through seawater setup under control conditions. Spawning was induced by a moderate heat shock (5°C) using heaters. Parental (F_0) animals (eight dams, A to H; eight sires, 1 to 8) were crossed pairwise in a reduced North Carolina I cross under control conditions to generate 16 full-sib families within four half-sib groups. Embryos were transferred into three experimental P_{CO_2} levels (390, 1120, or 2400 µatm). All families with successful settlement at 2400-µatm P_{CO_2} were considered as tolerant (5 of 16), and the remaining families (11 of 16) were termed "sensitive." Juveniles were transferred to a flow-through setup under constant P_{CO_2} until the next spawning season. The setup consisted of a header tank, which steadily supplied the experimental aquaria with seawater from Kiel Fjord. A *Rhodomonas* suspension was pumped into the header using a peristaltic pump and provided food to the experimental aquaria. Each aquarium was separately aerated with pressurized air with a P_{CO_2} of either 390, 1120, or 2400 µatm. Animals grew to average sizes of about 25 mm and sexual maturity within 1 year.

In 2013, individual crosses of F_1 specimens were carried out within tolerant families (dams: A2, B1, C4 × sires: D3) and between tolerant and sensitive families (tolerant dams: A2, B1, C4 × sensitive sires: E6; sensitive dams: F5, G7, H8 × tolerant sires: D3). The sex bias in mussels

(*44*) resulted in only 4 of the 16 families with male offspring and 12 exclusively female families, which reduced the number of potential crosses. P_{CO_2} during fertilization and the larval phase corresponded to tolerant sires' acclimation P_{CO_2} (exception tolerant dams × sensitive sires), and larvae were exposed to all P_{CO_2} levels in a fully crossed experimental design. In 2014, the response of 390- or 2400-µatm acclimated tolerant families and 390-µatm acclimated sensitive families was compared. For this purpose, eggs from three individuals from each of four tolerant dam families (A2, B1, B2, and C4) acclimated to 390- or 2400-µatm P_{CO_2} or females from the sensitive dam families (F5, G7, H7, and H8) acclimated to 390-µatm P_{CO_2} were pooled in equal numbers. Eggs were fertilized at 390- or 2400-µatm P_{CO_2}, corresponding to the treatment P_{CO_2} during the larval phase, with the sperm of one sire of family E6 acclimated to 1120 µatm.

Larval experiments were performed in a constant-temperature room at 19°C (population experiment, 16°C) at the Helmholtz Centre for Ocean Research Kiel. For fertilization, excess sperm was added, which does not allow testing for effects of P_{CO_2} on fertilization success. After assessing fertilization success in replicated measurements, embryos were transferred into the experimental units at an initial density of 10 larvae ml^{-1}. Experimental units were filled with 0.2-µm filtered seawater from Kiel Fjord. Weekly, 60% of the water volume was exchanged. In 2012 and 2013, larvae were fed daily with *Isochrysis* and *Rhodomonas* (days 7 to 21) or with *Rhodomonas* only (2014) cultured in F/2 or Provasoli enriched seawater (PES) medium, respectively. Larval survival and shell growth were assessed on days 2, 7, 14, and 21 after fertilization. Samples were immediately fixed, or living specimens were collected individually using a pipette, counted and subsequently fixed in 4% paraformaldehyde, and buffered with 4 mM $NaHCO_3$. Pictures of eggs and larvae were taken under a stereomicroscope with a MicroPublisher 3.3 RTV camera and analyzed for shell length using the Image-Pro Plus 5.0.1 software.

Monitoring of pH_{NBS}, salinity, and temperature was carried out twice or once a week in the larval experiments and the juvenile long-term acclimation, respectively. Weekly, water samples were analyzed for C_T using an AIRICA CT analyzer (Marianda) or for A_T using a Metrohm 862 Compact Titrosampler. Sample pH was determined on NBS or total scale using seawater buffers. Carbonate system speciation was calculated using the CO2SYS program using published $KHSO_4$, K_1, and K_2 dissociation constants (*45*–*47*).

Statistical tests are based on replicate means and were analyzed by ANOVA using R. Genetic variation of size was based on individual measurements and was analyzed by generalized linear mixed models (GLMMs) containing animal and replicate tank as random effects. See the Supplementary Materials for a detailed method description.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/4/e1602411/DC1

Supplementary Materials and Methods

fig. S1. Geographic origin of the two tested populations from Kiel Fjord in the Baltic Sea and the island of Sylt in the North Sea.

fig. S2. Analysis of the P_{CO_2} data from Fig. 1B on P_{CO_2} levels experienced by larvae settling in July, August, and September in Kiel Fjord (54°19.8′N; 10°9.0′E).

fig. S3. Picture of an *M. edulis* larva, with an approximate shell length of 120 µm, at the PD I stage 2 days after fertilization.

fig. S4. Egg diameter and fecundity of F_0 and F_1 dams.

fig. S5. F_1 egg diameter and F_2 larval performance in 2014.

table S1. Statistical analyses of population experiment.

table S2. Main effect contrasts from Bayesian GLMMs.

table S3. Statistical analyses of the transgenerational experiment in 2014.

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Acknowledgments: We would like to thank U. Panknin for algae culturing and monitoring of mussel cultures during the 3-year experimental period; A. Resteu and I. Podbielski for assisting during the larval experiment; and R. Asmus (AWI Sylt), V. Saderne, and C. Hiebenthal [Kiel Marine Organism Culture Centre (KIMOCC)] for providing data and supporting carbonate system monitoring. T. Reusch is acknowledged for his comments on an earlier version of the manuscript. **Funding:** This work was supported by the German Federal Ministry of Education and Research (BMBF)–funded project BIOACID II [subproject 3.7 (FKZ03F0655B) and subproject 3.4 (FKZ 03F0655A)] and is a contribution to the PACES (Polar regions and coasts in a changing earth system) research programme of the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research. **Author contributions:** J.T., K.M.W., and F.M. conceived the study, analyzed the data, and wrote the manuscript with the help of all coauthors. J.T., L.S.S., K.H., H.S., and M.D. conducted the experiments. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 30 September 2016
Accepted 28 February 2017
Published 26 April 2017
10.1126/sciadv.1602411

Citation: J. Thomsen, L. S. Stapp, K. Haynert, H. Schade, M. Danelli, G. Lannig, K. M. Wegner, F. Melzner, Naturally acidified habitat selects for ocean acidification–tolerant mussels. *Sci. Adv.* **3**, e1602411 (2017).

Science Advances

Naturally acidified habitat selects for ocean acidification–tolerant mussels

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Sci Adv **3** (4), e1602411.
DOI: 10.1126/sciadv.1602411