

Ocean acidification alters fish populations indirectly through habitat modification

Ivan Nagelkerken^{*}, Bayden D. Russell[†], Bronwyn M. Gillanders and Sean D. Connell

Ocean ecosystems are predicted to lose biodiversity and productivity from increasing ocean acidification¹. Although laboratory experiments reveal negative effects of acidification on the behaviour and performance of species^{2,3}, more comprehensive predictions have been hampered by a lack of *in situ* studies that incorporate the complexity of interactions between species and their environment. We studied CO₂ vents from both Northern and Southern hemispheres, using such natural laboratories⁴ to investigate the effect of ocean acidification on plant–animal associations embedded within all their natural complexity. Although we substantiate simple direct effects of reduced predator-avoidance behaviour by fishes, as observed in laboratory experiments, we here show that this negative effect is naturally dampened when fish reside in shelter-rich habitats. Importantly, elevated CO₂ drove strong increases in the abundance of some fish species through major habitat shifts, associated increases in resources such as habitat and prey availability, and reduced predator abundances. The indirect effects of acidification via resource and predator alterations may have far-reaching consequences for population abundances, and its study provides a framework for a more comprehensive understanding of increasing CO₂ emissions as a driver of ecological change.

Ecological communities are shaped by both direct and indirect effects whose combination is mediated by a changing environment^{5,6}. Although global change is anticipated to alter almost all known species interactions in the near future, the outcomes remain difficult to predict⁷. Not only may species be directly affected in their physiology and behaviour owing to increasing environmental stress^{8,9}, which could lead to altered species interactions, they may also be affected indirectly through changes to the resources on which they rely. Resource change drives fundamental shifts in key species which affect interactions with other species¹⁰; for example, global change can increase¹¹ or decrease⁴ habitat quality, and thus modify associated predator-refuge and prey availability, which together drive patterns of abundance¹². Here, we demonstrate the first example of where predicted changes to ocean environmental conditions increase the resources available to vertebrates, countering direct negative behavioural effects, and increasing survival and population size. The ways in which such direct and indirect effects of global change have the potential to modify population and community dynamics through altered species interactions is barely understood, and sometimes counter-intuitive, limiting our ability to predict species responses to anthropogenic environmental change and the potential cascading effects on ecosystem functioning^{13,14}.

The impacts of ocean warming on species performance has received much emphasis¹⁵, but ocean acidification due to increased

human CO₂ emissions has a similar potential to affect species performance², as well as interactions among species and the patterns of diversity and productivity they create^{11,16}. Elevated CO₂ has detrimental effects for invertebrate species that have skeletons made of calcium carbonate¹⁷, but acts as a resource for primary producers to drive major shifts in habitat¹¹. As well as modifying dominance of key habitats on which many species rely, elevated CO₂ has a diversity of effects, from altering physiology through to modifying behaviour^{2,3}. Surprisingly, vertebrate behavioural preferences can be reversed by elevated CO₂—for example, driving prey species to be attracted instead of deterred by their predators, or animals failing to identify and respond to ecologically important visual and olfactory cues of habitats, conspecifics and prey species³. Although behaviour underlies much of the demography, function and persistence of species, the ultimate effect on animal populations cannot be determined without considering the ubiquitous effect of the environment, particularly the powerful effects of habitat on their demography and abundance¹⁸. These environmental effects are not independent of changing climate and may well represent a set of indirect drivers of change that have largely been overlooked in previous predictions of climate on vertebrates. Although there is relatively well-developed theory and empirical evidence for the roles of direct and indirect effects on population dynamics of species in general^{6,10,19}, such effects are only recently being incorporated in the relatively new field of ocean acidification.

Here, we studied *in situ* changes in fish anti-predator behaviour (video recording fish escape response to a potential threat), fish population abundance and size-structure (visual surveys), fish–habitat associations (visual surveys of fishes within different habitats), resource availability (prey biomass and habitat availability), predator abundances (video transects), and habitat composition (visual estimations of habitat cover) at two locations: natural CO₂ vents in the Northern Hemisphere (Vulcano Island, Italy, Mediterranean Sea, seagrass ecosystem, mean pH \pm s.e.m. at control versus vent: 8.04 ± 0.02 versus 7.76 ± 0.12) as well as the Southern Hemisphere (White Island, New Zealand, South Pacific Ocean, rocky reef ecosystem, pH: 8.06 ± 0.02 versus 7.86 ± 0.02). We focused on territorial fish (Italy: *Gobius bucchichi*; New Zealand: *Forsterygion lapillum*) whose limited range of movement would restrict their benthic life to either acidified or control conditions.

Fishes exposed long-term to elevated CO₂ and reduced pH (Δ pH -0.20 and -0.28 units at White Island and Vulcano Island, respectively) at CO₂ vents showed a slower escape speed from a perceived threat at both locations (Fig. 1a and Supplementary Tables 1 and 2; $p = 0.037$ and $p = 0.001$, respectively), irrespective of habitat. No CO₂ effect was present for distance fled (Fig. 1b). Performing our experiments *in situ*, we show for the first time that increased risk-taking behaviour appears habitat-dependent at CO₂

Southern Seas Ecology Laboratories, School of Biological Sciences and The Environment Institute, DX 650 418, The University of Adelaide, Adelaide, South Australia 5005, Australia. [†]Present address: The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong SAR. *e-mail: ivan.nagelkerken@adelaide.edu.au

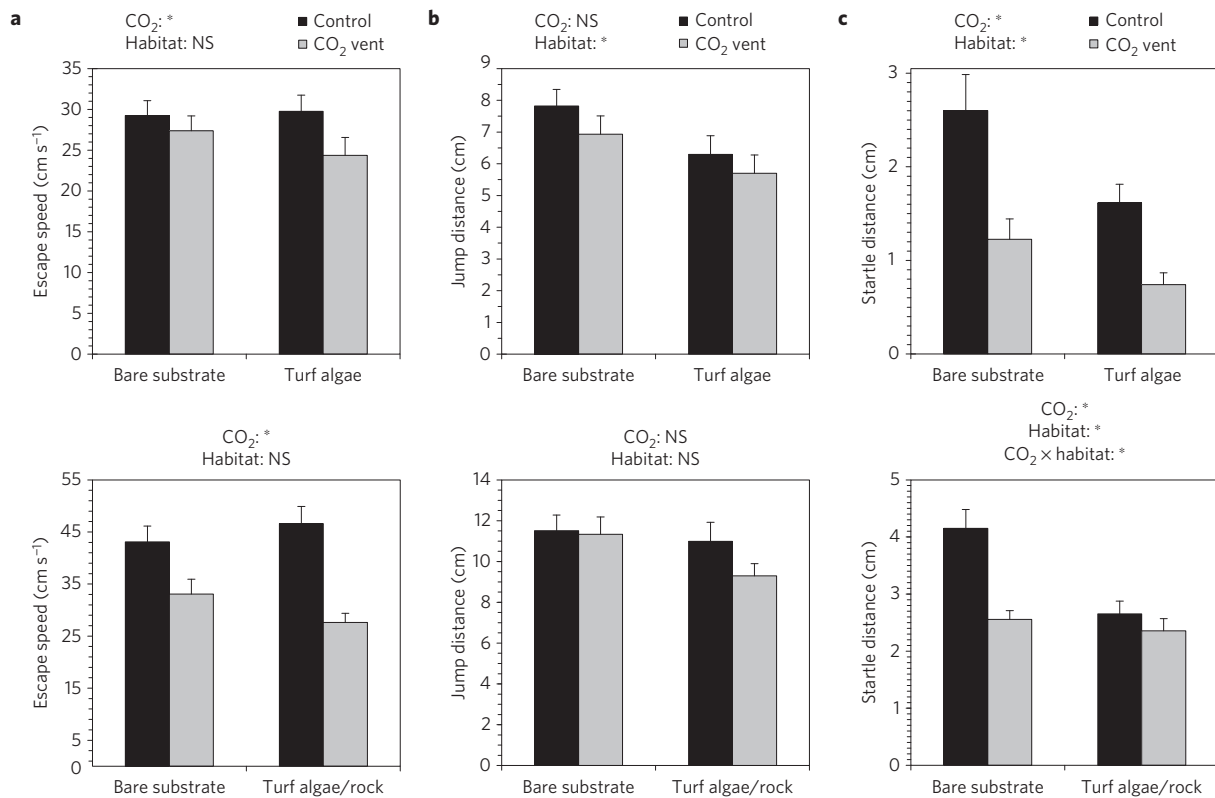


Figure 1 | *In situ* escape responses (mean \pm s.e.m.) by fish from an approaching threat at White Island (top panels; $N = 146$ fish per response) and Vulcano Island (bottom panels; $N = 209$ fish) CO_2 vent and control sites. **a**, Escape performance: escape speed and **b**, jump distance (distance fled). **c**, Escape behaviour: distance at which fish initiated a flight response. x axes indicate the habitats in which the various responses were tested. *, significant ($p < 0.05$); NS, not significant.

Table 1 | Hypotheses that could explain the observed increase in fish populations at CO_2 vents.

Hypothesis	Supported?	Supporting observation
Altered anti-predator behaviour due to CO_2	No	Anti-predator response lower at vents, leading to potentially higher mortality rates.
Altered habitat preference	No	Same relative fish densities in the different habitats at control versus vent sites.
Habitat shifts	Yes	Increase in cover of main utilized habitats at vents.
Changes in habitat complexity	Yes	Increase in biomass of main utilized habitat at vents.
Changes in the landscape of fear	No	Decrease in startle distance at vent versus control sites at Vulcano Island (with more predators at control sites) is smaller than at White Island (with similar predator densities at control and vent sites); risk avoidance is lower at vents but fish densities higher.
Altered predation pressure	Possibly Yes	White Island: nonsignificant trend of elevated predator density at control versus vent sites. Vulcano Island: fewer predators at vents, but predicted effects of habitat shifts are larger than that of other CO_2 effects such as predation.
Competition	No	No change in density of competing fish species at vents.
Food abundance	Yes	Increase in abundance of prey items at vents.
Fishing pressure	No	Selected species are too small to be targeted by fishing.

vents. Elevated CO_2 and habitat vegetation independently delayed the flight response of fish on an approaching threat (Fig. 1c and Supplementary Tables 1 and 2; CO_2 effect: $p = 0.001$ for White Island and Vulcano Island; habitat effect: $p = 0.003$ and $p = 0.002$, respectively). Importantly, at Vulcano Island the difference in risk-taking behaviour at vent versus control sites in risky, open habitats disappeared in the presence of shelter ($\text{CO}_2 \times$ habitat interaction: $p = 0.013$).

Elevated CO_2 modified habitat composition at both vent locations and drove habitat shifts towards simplified systems. At White Island, the control reefs consisted of a habitat mosaic of kelp, bare hard substratum (urchin barrens) and turf algae (Fig. 2a). At the vent sites, the habitat mosaic was eliminated in

favour of turf algae and loss of kelp (Supplementary Table 3a; CO_2 effect: $p = 0.001$). Also, biomass of turf algae was over 100% greater at vent sites (mean \pm s.e.m. at vent versus control sites: 0.7 ± 0.3 versus $0.3 \pm 0.1 \text{ g cm}^{-2}$; analysis of variance (ANOVA): $F_{1,33} = 20.57$, $p = 0.001$). The increase in biomass was also reflected as an increase in turf height, increasing overall structural complexity of this habitat. At Vulcano Island, a habitat shift emerged from seagrass domination at control sites towards macroalgal/sand domination at vent sites (Fig. 2b and Supplementary Table 3b; CO_2 effect: $p = 0.001$). Habitats shifted towards those habitats with which the fishes were positively associated (Fig. 2c,d and Supplementary Table 3; habitat effect White Island: $p = 0.001$; Student–Newman–Keuls (SNK) *post hoc* test ($p < 0.05$): fish

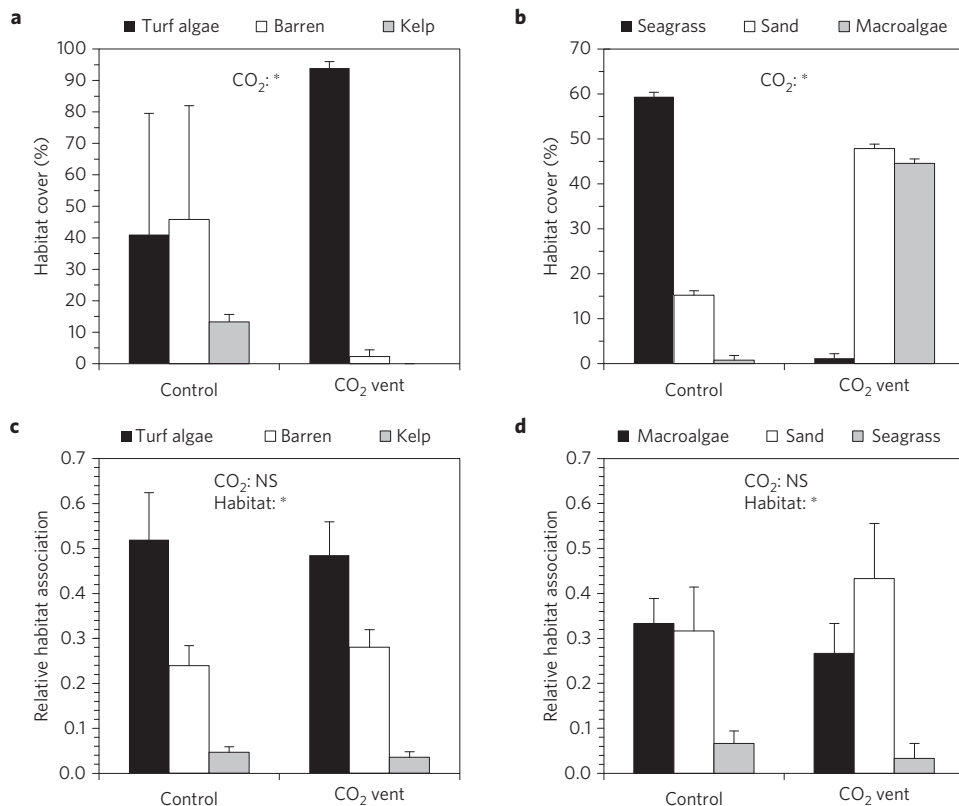


Figure 2 | Habitat composition and fish–habitat association at CO₂ vent and control sites. a,b, Cover of different habitats (mean \pm s.e.m.) at White Island ($N=4$ transects across sites and treatments) (a) and Vulcano Island ($N=60$ quadrats) (b). **c,d,** Relative fish density (mean \pm s.e.m. density, as a proportion of the maximum density recorded within treatments) in each of three habitats at White Island ($N=60$ quadrats across sites and treatments) (c) and Vulcano Island ($N=60$ quadrats) (d). *, significant ($p < 0.05$); NS, not significant.

density in turf algae > barrens > kelp; habitat effect Vulcano Island: $p=0.020$; *post hoc*: fish density in macroalgae = sand > seagrass).

Diet composition of fish was similar between control and vent sites at White Island (ANOVA for CO₂ effect: $F_{1,51} < 0.01$, $p=1.000$) and was dominated by amphipods, which were also by far the most abundant of the prey items collected. The biomass of invertebrate prey in turf habitat was slightly higher at vent (mean \pm s.e.m.: 4.7 ± 0.9 mg cm⁻²) than at control (3.7 ± 0.7 mg cm⁻²) sites at White Island (ANOVA, $F_{1,33} = 20.57$, $p=0.001$).

Abundances of both species of fish were more than twice as high at vent compared to non-vent sites (Fig. 3a and Supplementary Table 4; CO₂ effect: $p < 0.001$ for both White Island and Vulcano Island). This was not driven by any changes in fish–habitat associations due to CO₂ (Fig. 2c,d). Elevated fish densities were reflected throughout all post-settlement life stages with lack of a CO₂ effect on the size–frequency distribution of fishes (Fig. 3c,d; Kolmogorov–Smirnov two-sample test: $p=0.964$ and $p=0.808$ for White Island and Vulcano Island, respectively). Other site-attached fish species (triplefins) at White Island showed no such increase in density (mean density \pm s.e.m. at control versus vent sites: 7 ± 3 versus 6 ± 1 per 20 m², respectively; t -test, $t(10) = 0.314$, $p=0.760$). Growth rates of fish were similar at vent and control sites at White Island (Supplementary Fig. 1).

We separated habitat-shift effects from other CO₂ effects by scaling average fish densities within habitats by total cover of these habitats (that is, combining Fig. 2a,b with Fig. 2c,d; see Methods). On the basis of these calculations, the predicted habitat-shift versus other CO₂ effects on fish densities were of the same magnitude at White Island, but the habitat-shift effects were much stronger than other CO₂ effects at Vulcano Island. Predicted increases in fish densities at White Island were $+6.8$ m⁻² (increasing from

6.6 to 13.5 m⁻²) due to other CO₂ effects, and $+6.8$ m⁻² (adding to a further increase from 13.5 to 20.2 m⁻²) due to habitat-shift effects. At Vulcano Island, predicted increases in fish densities due to other CO₂ effects were $+0.2$ m⁻² (increasing from 0.3 to 0.5 m⁻²), and $+1.3$ m⁻² (adding to a further increase from 0.5 to 1.8 m⁻²) due to habitat-shift effects. Predator abundances (Fig. 3b), representing one of several potential other habitat-independent indirect effects, were significantly higher at control than vent sites at Vulcano Island (adjusted t -test, $t(3) = -5.451$, $p=0.012$), with a trend of higher abundances at control sites at White Island ($t(5) = 1.563$, $p=0.185$). Also the number of transects containing predators was higher at control than vent sites at Vulcano ($\chi^2(1) = 4.5$, $p=0.034$), but not at White Island ($\chi^2(1) = 0.6$, $p=0.439$).

Because most species show complex interactions with their environment and with other species, it has been difficult to develop realistic experiments that represent these important interactions, particularly for vertebrates²⁰. Our study at two disparate natural CO₂ vents shows that the effects of ocean acidification, which incorporate a multitude of ecological processes, can have opposing outcomes for population abundance relative to those predicted by laboratory experiments alone.

Elevated p CO₂ in the ocean is predicted to have a negative effect on the physiology of animals, resulting in energetic trade-offs⁸. Laboratory experiments have shown different and sometimes opposite effects of ocean acidification on animal physiology²¹, which may be related to species-specific capacity for pH regulation. In our study we found a reduction in escape behaviour at vent sites, either driven by reduced predator abundances and/or by neurological effects of elevated CO₂. Escape responses were slower at vents where predators were fewer. However, fishes at White Island (with fewer predators) showed a larger reduction in startle

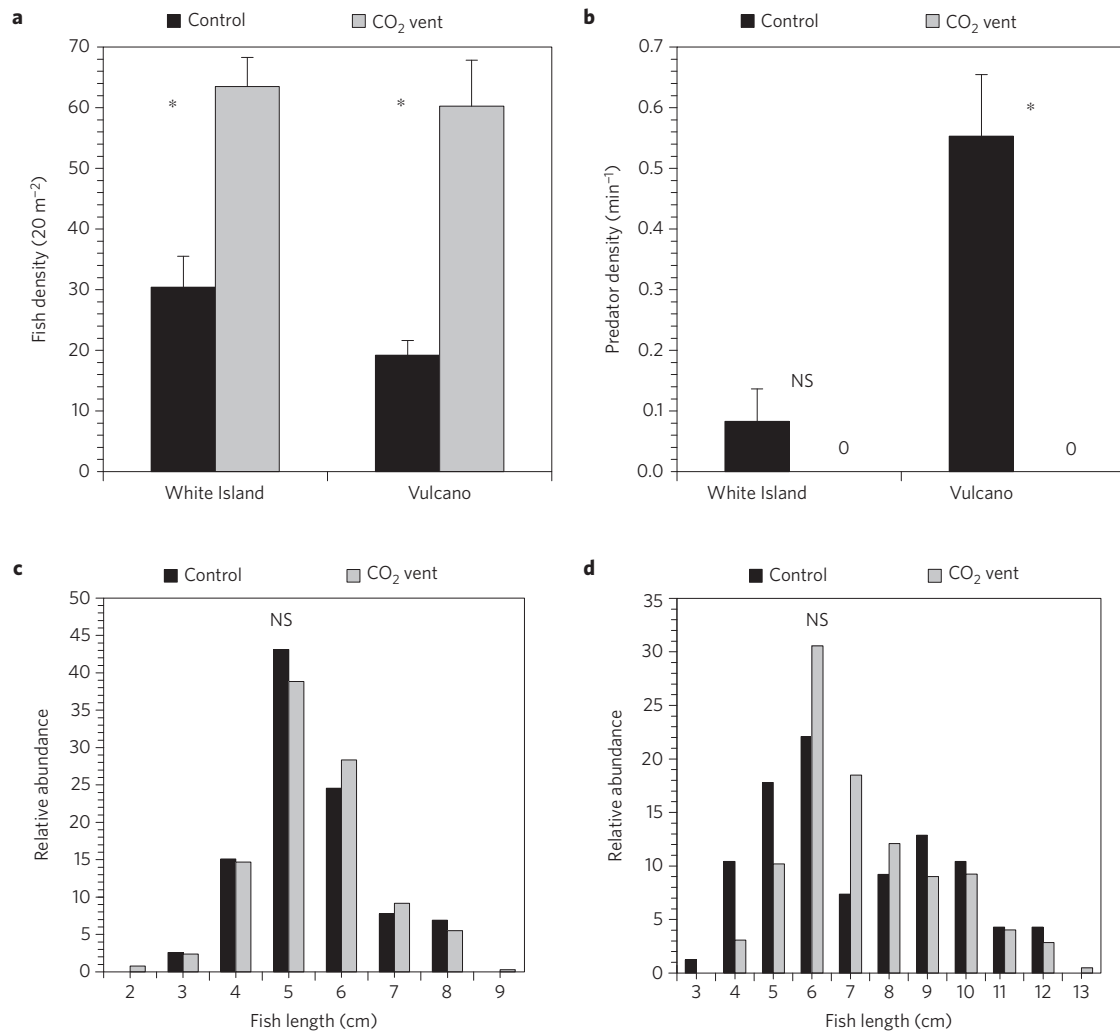


Figure 3 | Population structure of fish and predators at CO₂ vents and control sites. a,b, Density (mean \pm s.e.m.) of fish ($N=12$ and 12 transects at White Island and Vulcano Island, respectively, across sites and treatments) (**a**) and predators ($N=12$ and 8 transects, respectively) at both locations (**b**). **c,d**, Fish size–frequency distribution at White Island (**c**) and Vulcano Island (**d**). *, significant ($p < 0.05$); NS, not significant.

distance between control and vent than at Vulcano Island (with more predators), suggesting at least a partial direct effect of elevated CO₂. Neurological effects of elevated CO₂ on escape response have been observed for tropical fishes tested in the laboratory²² and for fishes collected from vents and tested in on-board aquaria²³, and a slower response to a rapid predator attack might increase mortality risk^{24,25}. However, performing our experiments *in situ*, we show for the first time that in the presence of shelter there was no difference in escape response at vents versus control sites, illustrating how consideration of species–habitat interactions is key to determining how animal populations respond to ocean acidification.

Growth–survival trade-offs are ubiquitous in nature and are strong drivers of animal behaviour, species interactions and population dynamics²⁶. Habitat shifts may affect species through both changes in growth rates (for example, through altered prey abundances) and survival rates (for example, through altered shelter availability or predator abundances). Regime shifts towards more simplified macroalgal-dominated systems are occurring globally in various ecosystems (for example, coral reefs, kelp forests, seagrass beds). They are often driven by human-induced disturbances such as overfishing, eutrophication and global warming, leading to less diverse, less productive and less resilient systems that provide fewer ecosystem services²⁷. CO₂ vents facilitated habitat shifts, leading to significant increases in key resources (macroalgae–sand habitats

at Vulcano Island, turf habitat and higher food abundances at White Island) and reductions of predators, which together drive population increases of some fishes.

Our study suggests at least three mechanisms that can mitigate the negative effects of ocean acidification (Table 1), although for other species with different resource requirements elevated CO₂ can create negative effects, depending on which habitat and resource types are being gained and lost⁴. Alternative explanations for population increases include reduced competitor abundances at vents, but these could not have been the only drivers of the pattern observed (Table 1). Transgenerational acclimation, evolutionary adaptation and epigenetic flexibility may further mitigate the negative effects of ocean acidification, but our current knowledge is very limited and the responses may differ between traits^{28,29}.

Indirect effects represent powerful effects in ecology^{5,30} and their study is likely to reveal some surprising outcomes that cannot be predicted by the sum of direct effects of climate change. Moreover, direct and indirect effects may work in either the same or opposite directions, altering the readily detectable outcomes of direct effects alone. Incorporation of both direct and indirect effects, therefore, enables more comprehensive predictions of ecological change so they do not create the false impression that the more readily studied direct effects, however complex, produce large effects relative to other processes.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

I.N. and S.D.C. conceived and designed the experiments. I.N. and S.D.C. performed the experiments. I.N., B.D.R. and S.D.C. analysed the data. B.D.R. and B.M.G. contributed materials/analysis tools. All authors contributed to writing the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to I.N.

Competing financial interests

The authors declare no competing financial interests.

Methods

Study sites. The study encompassed two islands containing natural subtidal CO₂ vents and hereafter are referred to as 'locations' (Supplementary Fig. 2), where one was located in a seagrass-dominated embayment of an island in the Northern Hemisphere (a single vent at Vulcano Island, Mediterranean Sea) and one at a rock-dominated island in the Southern Hemisphere (a multiple-vent site at White Island, New Zealand).

At Vulcano Island, the vent is characterized by a single large CO₂ vent located in Levante Bay (Supplementary Fig. 2), with a slowly increasing gradient in pH with increasing distance from the vent. Two sites close to the vent and two control sites away from the vent were selected. The two vent sites represented approximate end-of-the-century projections in pH reduction of 0.28 units, on average, due to ocean acidification (Supplementary Table 5) based on the RCP8.5 scenario of greenhouse gas emissions³¹; water temperature did not differ between control and vent sites. Long-term variability of pH and other physico-chemical variables in the Bay are reported elsewhere³². Hydrogen sulphide released from the main bubbling area does not extend to the north-eastern part of the Bay and sulphate levels are typical of oceanic waters³². The substratum of the shallow parts of the Bay (1–5 m depth) at control sites was characterized by a mosaic of seagrass (mainly *Cymodocea nodosa*), turf-forming macroalgae rooted in the sediment (<10 cm in height; mainly *Caulerpa prolifera*), cobbles, small rocky reefs, and sandy substratum.

White Island is a volcanic island located in the Bay of Plenty of the North Island of New Zealand (Supplementary Fig. 2). Two independent vent and two control sites were identified along the north-eastern coast of the Island. The CO₂ plumes at vent sites were ~24 × 20 m in dimension and located at 6–8 m depth. The control sites were located adjacent to the vents (>~25 m away) where pH levels represented ambient oceanic conditions. pH levels at the two vent sites represented approximate end-of-the-century projections in pH reduction of 0.20 units, on average, and were not confounded by elevated temperatures (Supplementary Table 5). Measurements from different time periods showed similar pH values, suggesting the vents are relatively stable over time. Sulphate levels at the vent sites do not differ from control sites (mean ± s.e.m.: 1,157 ± 11 versus 1,154 ± 13 ppm, respectively³³) but are slightly higher than the regional and global oceanic averages (mean ± s.e.m.: 1,083 ± 4 versus ~904 ppm, respectively³³). The study area represents a rocky reef ecosystem, and the substratum at control sites was characterized by a mosaic of kelp (*Ecklonia radiata*), turf-forming macroalgae (<10 cm in height), and hard-substratum sea urchin barrens devoid of vegetation.

Vents are known to fluctuate in CO₂ release and this may potentially affect biological responses. Studies on fishes have shown that behavioural effects to elevated CO₂ manifest from one to four days after the onset of exposure, and recovery takes 8–48 h (ref. 34). Therefore, behavioural impairment to CO₂ operates at longer timescales than the typical short-term extremes that are observed at vents, including our study sites³². This is also true for the other processes we studied, as fish population dynamics, habitat shifts, and changes in prey abundances operate at even longer timescales. Hence, the similar responses observed in our study at two disparate vent systems are more likely to be the result of longer-term exposure to mean conditions rather than short-term extremes. Moreover, geochemical studies at our two vent locations both concluded that these vents are suitable for studies on ocean acidification^{32,33}. Nevertheless, it should be noted that, for some traits or ecological processes, sporadic exposure to extreme conditions can also have an impact.

Carbonate chemistry measurement and analysis. The CO₂ concentration in the water was calculated using the values of temperature, salinity, pH_{NBS} and total alkalinity (TA) measured in the field. The software CO2SYS was used to estimate seawater pCO₂ with constants K1 and K2 from Mehrbach³⁵ and refit by Dickson and Millero³⁶. Alkalinity was measured by dynamic endpoint titration using a Titrande (Metrohm) titrator. During the study, values for standards were successfully maintained within 1% accuracy from certified reference materials from A. Dickson (Scripps Institution of Oceanography). All samples were collected from 14 to 18 September 2013 at Vulcano Island and from 18 to 21 November 2013 at White Island; additional data was added for 2 May 2013 and 9–11 February 2015 at White Island. The pH_{NBS} was measured daily at Vulcano Island with a portable probe SG2-ELK SevenGo (Mettler Toledo) and at White Island with the multi-meter and logger Sonde 6600V2 (YSI), which was calibrated daily. TA samples were collected at each site on three different days, fixed with mercuric chloride, and preserved in Duran glass bottles (Schott) until analysis, according to standard operating procedures³⁷. Salinity was measured with a SR6 refractometer (Vital Sine).

Study species. A major drawback of using natural CO₂ vents as an experimental area representing future acidified ecosystems is that many animals move in and out of the vent areas and are therefore not continuously exposed to high CO₂. To avoid this limitation we focused on site-attached species that occupy a territory directly after settlement, show little movement, and have small home ranges. For both vent

locations this was an acceptable approach, as the benthic fish communities were dominated by gobies (Gobiidae) and triplefins (Tripterygiidae). All of these species maintain territories of a few m² and are highly site-attached^{38,39}. We focused on the benthic species that showed highest abundances at each study location (*F. lapillum*, common triplefin and *G. buccichi*, Buccich's goby). The common triplefin is a habitat generalist found commonly in most habitats⁴⁰.

In addition, we determined densities of all other triplefin species at White Island, as potential competitors of the common triplefin.

We also quantified potential predators at both locations in roving transects using a video camera. At Vulcano, the only conspicuous predators were juveniles (~20 cm total length) of the relatively site-attached grouper *Serranus scriba*, which appeared to associate with rocks in seagrass habitat. No other free swimming predators were observed during any snorkel surveys. At White Island, the only conspicuous predators were relatively site-attached scorpionfish (*Scorpaena* spp.) and hiwi hiwi (*Chironemus marmoratus*); roving predators were not seen during these transects or other SCUBA surveys.

Fish escape behaviour and performance. We designed a device that mimicked the approach of a potential threat while recording the fish's escape behaviour (Supplementary Fig. 3). The device was a cubical frame made of white PVC pipes. A GoPro camera was attached to the top of the frame. A black iron rod was attached to the top of the frame and extended ~60 cm forward from the camera. A metal ruler of 30 cm was attached to the end of the rod, pointing downwards so that the bottom half of the ruler was in view of the camera's field. The recordings were taken at a speed of 30 s per frame.

To elicit an escape response by the fishes, the tip of the ruler was lowered vertically towards the head of a randomly selected individual until the ruler reached the substratum. The camera was recording continuously and captured the entire threat approach and escape process. Both species showed very similar behaviour towards the approaching threat. The response of a fish was to first direct its eyes towards the approaching ruler, followed by a fast jump with a few tail flips when the ruler approached too close, before the fish settled back onto the substratum several centimetres away. The test was performed under animal ethics approval # S-2013-150.

A total of 209 individuals were tested at Vulcano Island (control: 107 fish; vent: 102 fish; 14–15 September 2013) and 146 individuals at White Island (control: 73 fish; vent: 73 fish; 20 November 2013). For the video recording of each individual fish we used the program VLC media player 2.0.1 to quantify: distance from the approaching ruler at which the fish initiated its escape response, distance covered during the escape, and duration of the escape, which was transformed to escape speed by dividing escape distance by escape duration. In addition, the habitat in which the fish resided during the mimicked attack was recorded as bare, non-vegetated substrate, turf algae, or small rocks. The escape was defined as from the moment at which the fish started its jump until it landed back onto the substratum. Because the fish were always approached from their side, their forward escape response was generally in a direction parallel to the line of sight (that is, escaping either towards the left- or right-hand side of the camera's view, rather than towards or away from the camera). The distance at which the fish initiated their escape was measured as the distance between the top of the head and the tip of the ruler, using the gridded ruler as a reference for the magnification. Likewise, the distance moved during the escape was measured from the recording. All measurements were done by forwarding the recording frame by frame (1/30 s). The escape speed was calculated by dividing the escape distance by the number of frames to complete the escape.

Fish population structure. Abundance and total body length of the two species (common triplefin at White Island and Buccich's goby at Vulcano Island) and other triplefin species (only at White Island) were visually quantified in replicate 2 × 10 m belt transects on snorkel at Vulcano Island and SCUBA at White Island (*n* = 3 transects at each of the two control and two vent sites at each of the two locations, for a total of six control and six high-CO₂ transects at each location).

Predators were quantified at both locations in roving transects (~4–5 min each) using a video camera. At Vulcano Island, two replicate transects at each site were performed at control (four total) and vent (four total) sites. At White Island, six video transects were performed at control sites and six transects at vent sites.

To determine whether CO₂ had a direct effect on fish habitat association, we quantified abundances of the common triplefin at White Island and Buccich's goby at Vulcano Island in 1 m² quadrats in different microhabitats. At each control and vent site at each location, ten quadrats (five per site) were randomly deployed in each of three microhabitats (30 quadrats in total at each site at each location): rocky barrens, turf macroalgae and kelp at White Island, and fleshy macroalgae (mainly *C. prolifera*), seagrass and sand at Vulcano Island.

Fish ages of the common triplefin were determined from otolith analyses. Otoliths were embedded in resin, sectioned transversely and placed on microscope slides. Sectioned otoliths were viewed under a compound microscope (Leica DMLB) and age estimated by counts of growth increments.

Fish diet composition and food abundance. Gut content analysis was performed for the common triplefin collected at control (ten fish) and vent sites (nine fish) at White Island. Abundance of their prey was estimated from a total of 40 cores of turf at White Island ($n = 10$ at each replicate vent and control site). A small circular core (diameter 4.25 cm) was used to take samples of the turf algae growing on the rocky reefs. Cores were closed with a lid underwater to prevent escape of prey organisms. The algae collected in each core were thoroughly washed above a sieve and the total weight of all invertebrates per sample determined. The vast majority (>90%) in terms of numbers and weight comprised amphipods.

Habitat composition. Samples of turfs from White Island (that is, as described above) were dried with a paper towel and weighed to quantify fresh weight, which was used as an indication of amount of biotic structure per unit surface area.

Overall habitat composition (that is, percent habitat cover) at the vent and control sites of White Island were recorded using line intercept transects (25 m), recording the substratum beneath the transect line. One transect was quantified at each of the two control and vent sites. At Vulcano Island, photographs were taken of quadrats (25 × 25 cm) at control ($n = 15$ at each of two sites) and vent ($n = 15$ at each of two sites) sites. Percent habitat cover was estimated visually from each photograph.

Data and statistical analyses. For the analysis of startle distance, escape distance and escape speed (Fig. 1), 2-way ANOVAs treated CO₂ concentration (control versus vent) and habitat (bare substrate versus turf algae/rock) as orthogonal and fixed factors. Escape speeds at White Island were log-transformed. PERMANOVA was used to perform the tests.

For the analysis of habitat shifts (Fig. 2a,b), permutational MANOVAs tested for differences between CO₂ concentrations (control versus vent; fixed factor) using percentage cover of individual habitats as a dependent variable (turf algae, barren, kelp at White Island, and seagrass, sand, macroalgae at Vulcano Island). For Vulcano Island, site was treated as a random factor and nested within CO₂ treatment. Habitat cover was fourth-root transformed and a resemblance matrix was constructed using Bray–Curtis similarity. Permutation of the residuals was done under the full model.

To test whether the association between fish and habitat was the same between control and vent sites at White Island (Fig. 2c,d), 3-way ANOVAs tested for differences in fish density based on an arcsine transformation, with habitat as a fixed factor and site as a random factor nested within CO₂ concentration. Because for this analysis we specifically tested for differences in abundance among habitats for the two CO₂ concentrations rather than direct effects of elevated CO₂ on total fish abundance, we transformed fish densities relative to the maximum density found within each CO₂ treatment. GMAV was used to perform the statistical tests.

Fish densities (Fig. 3a) were log-transformed and differences between CO₂ concentrations tested with a 2-way nested ANOVA for each species separately, with site nested within CO₂ concentrations (random factor), using PERMANOVA. In addition, we developed a formula to predict changes in fish densities due to CO₂-driven habitat shifts as opposed to other factors, by scaling fish densities within habitats (using density data that formed the basis for Fig. 2c,d) by relative habitat cover (Fig. 2a,b). To calculate predicted control fish abundances, fish densities within habitats were multiplied by the respective habitat cover and summed across habitats. The same calculation was used for fish densities and habitat cover at vent sites, to reveal expected change in fish densities due to the sum of habitat-dependent and habitat-independent CO₂ effects. Finally, fish densities within habitats at control sites were multiplied by habitat cover at vent sites to

reveal predicted change in fish densities due to habitat-independent CO₂ effects. By comparing these three different densities, predicted changes in fish densities due to habitat shifts and changes due to habitat-independent CO₂ effects could be evaluated.

Differences in competitor densities (all other triplefin species) at White Island (results reported in the main text) between control and vent sites, were tested with an independent sample *t*-tests. Differences in predator densities between control and vent sites (Fig. 3b) were tested with an independent sample *t*-test adjusted for lack of homogeneity of variances (due to multiple zero counts at vent sites) by lowering the degrees of freedom (automatically performed by SPSS). In addition, we tested for the difference in number of transects with and without predators between the two treatments using a contingency table and the Yates's continuity correction. Differences in size–frequency distribution of fish (Fig. 3c,d) between control and vent sites were tested with a 2-sample Kolmogorov–Smirnov test for each species separately. IBM SPSS Statistics 20 was used to perform the above tests.

ANOVA was used to test for differences in biomass of turfs between control and vent sites at White Island, as reported in the main text. *Post hoc* pooling⁴¹ of site with the residual ($P > 0.25$) enabled a more powerful test of the main effect (vent versus control). The same approach was used to test for differences in biomass of invertebrate prey between control and vent sites at White Island. Differences in diet composition between control and vent sites was tested with a 2-way ANOVA using treatment (control versus vent) and prey composition (three taxa of encountered prey) as fixed factors. PERMANOVA was used to perform the tests.

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