Reef fishes can recognize bleached habitat during settlement: sea anemone bleaching alters anemonefish host selection

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Understanding how bleaching impacts the settlement of symbiotic habitat specialists and whether there is flexibility in settlement choices with regard to habitat quality is essential given our changing climate. We used five anemonefishes (Amphiprion clarkii, Amphiprion latezonatus, Amphiprion ocellaris, Amphiprion percula and Premnas biaculeatus) and three host sea anemones (Entacmaea quadricolor, Heteractis crispa and Heteractis magnifica) in paired-choice flume experiments to determine whether habitat naive juveniles have the olfactory capabilities to distinguish between unbleached and bleached hosts, and how this may affect settlement decisions. All anemonefishes were able to distinguish between bleached and unbleached hosts, and responded only to chemical cues from species-specific host anemones irrespective of health status, indicating a lack of flexibility in host use. While bleached hosts were selected as habitat, this occurred only when unbleached options were unavailable, with the exception of A. latezonatus, which showed strong preferences for H. crispa regardless of health. This study highlights the potential deleterious indirect impacts of declining habitat quality during larval settlement in habitat specialists, which could be important in the field, given that bleaching events are becoming increasingly common.

1. Introduction

Coral reefs are becoming increasingly degraded, with local stressors such as declining water quality, overfishing, and outbreaks of invasive species and predators such as crown-of-thorns starfish being exacerbated by the global stressors of climate change and ocean acidification [1–4]. Ocean warming threatens the structure and function of coral reefs through episodes of bleaching, which are increasing in frequency and magnitude due to anthropogenic activity [5]. Bleaching is a general stress response that can be triggered by a variety of causes, and affects a range of organisms, including corals, sea anemones and clams [6,7]. It is characterized by the breakdown of the symbiosis between the animal host and its unicellular dinoflagellate algae (Symbiodinium spp.). Algae are expelled from the host and/or the photosynthetic pigments from within the symbionts are reduced, which leads to the animal becoming pale or white in appearance [6–8]. The breakdown in this relationship has implications for host health as these algae provide photosynthetic pigments that is used to supply a range of metabolic needs [9]. When stressors are extreme or last for an extended duration, the host may bleach and die; however, when conditions are less severe or persist for a shorter period, the host may bleach, remain alive and reacquire a healthy algal population [6].

While bleaching has obvious direct effects on the impacted organisms, the indirect effects on the ecosystem and other associated species, such as fishes, often vary [10–13]. For species that form obligate symbioses with microhabitats...
that are susceptible to bleaching, the impacts are amplified, as habitat quality and health are directly linked to survival. The symbiotic relationship among anemonefishes and their host sea anemones provides an ideal model for further exploring the consequences of habitat degradation, as the former are generally unable to survive in the wild without their hosts [14]. This association occurs on suitable reefs throughout the Indo-Pacific, in both shallow and mesophotic waters [15,16], with the greatest species richness occurring in Papua New Guinea [17]. Host specificity varies among the 28 species of anemonefishes, with some being extreme specialists that use only one host species and others being able to use a range or all 10 hosts [14,15].

All host anemones are susceptible to bleaching, and reductions in the abundance of both the anemones and their resident fish have been recorded following high-temperature anomalies [18–20]. Thus, flexibility in host use by anemonefishes may be crucial for survival during severe habitat degradation and subsequent community shifts [21]. Such plasticity is regularly observed in captivity, where anemonefishes can reside in unnatural sea anemone hosts, and even use other invertebrates such as soft corals as habitat [15,21]. In artificial situations, predation pressure is removed and therefore the protection provided by the anemones to the fish may no longer be vital. However, there has been one long-term field observation of Amphiprion clarkii using the soft coral Lobophytum sp. after bleaching impacted anemone populations in Japan [21]. As adult anemonefishes are highly site-attached [14,22], this may have occurred due to the need to find habitat within close proximity to the original settlement site.

The replenishment and persistence of coral reef fishes, such as anemonefishes, are reliant upon dispersing individuals finding and becoming established in a suitable habitat. The majority of reef fishes begin life as larvae that disperse among isolated adult populations [23]. The ability to find suitable habitat after this pelagic larval period represents a significant challenge. As predation pressure is estimated to be greatest during settlement [24], and inappropriate habitat selection has critical repercussions to fitness, survival and reproductive success, this process should be subject to strong selection pressure. Coral reef fish larvae have well-developed sensory and swimming capabilities [25], and evidence suggests that both sound [26,27] and olfactory cues such as those derived from resident conspecifics, coral tissues or symbiotic partners [28–35] are used to locate suitable habitat. Field observations and laboratory experiments have shown that larval recruitment of fishes, especially habitat specialists, is impacted by episodes of habitat degradation and bleaching [11–13,36].

Available settlement sites for recruiting anemonefishes will decrease as the incidence and extent of habitat degradation increases. While it is well documented that anemonefishes are able to distinguish among host anemones using chemical cues [34,35,37], it is not known if chemical cues are used by fishes to determine the bleaching status of the symbiotic hosts and if host health impacts settlement preferences. We therefore address the following questions. (i) Can juvenile anemonefishes distinguish between unbleached and bleached host anemones using chemical cues alone? (ii) Will a bleached anemone be selected as habitat? (iii) Are settlement preferences flexible when known host species are bleached and other unbleached non-host species are available?

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<thead>
<tr>
<th>E. quadricolor (13)</th>
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<td>A. clarkii (10)</td>
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<td>A. latezonatus (3)</td>
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<td>P. biaculeatus (1)</td>
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2. Material and methods

Five species of anemonefishes (Amphiprion clarkii, A. latezonatus, A. ocellaris, A. percula and Premnas biaculeatus) and three species of sea anemone (Entacmaea quadricolor, Heteractis crispa and H. magnifica) were tested to determine the role that chemical cues play in habitat recognition, and the potential settlement preferences of fishes towards unbleached versus bleached host and non-host species (table 1). Using Atema choice flume [28] experiments, we specifically tested responses by fishes to: (i) unbleached anemones versus seawater; (ii) bleached anemones versus seawater; (iii) unbleached versus bleached anemones of the same species; (iv) unbleached anemones versus bleached of different species; and (v) bleached versus bleached anemones of different species.

Experiments were done at Georgia Institute of Technology (GT), USA from the 12–24 October 2014. Each trial used 18–20 habitat naive captive-bred juveniles (all less than two months post-hatching), with a total of between 324 and 360 individuals per species. Fishes were reared at Sustainable Aquatics (Tennessee, USA) from three different breeding pairs of each species to account for genetic variability. Settlement stage juveniles were transported to GT and each species group was housed in individual 10 l aquaria within a 382 l recirculating seawater system with a 13 L : 11 D cycle. Before experimentation, fish were fed daily with 0.8 mm dry pellet hatchery diet (Sustainable Aquatics, TN, USA). Water quality measurements were taken daily and 50% water changes were done twice a week to ensure water quality was maintained. Seawater (26°C) used for maintaining the study species and in the experiments was artificial (30 psu, Instant Ocean, Blacksburg, Virginia). Once trials were completed, fishes were placed in different aquarium to ensure they were only used once.

Two unbleached and two bleached individuals (oral disc diameter more than 10 cm) of each species were soaked in 10 l of water for 2 h to generate the chemical cues [35]. The cues created were then used immediately in the trials. Anemones were sourced from a local saltwater aquarium supplier (The Fish Store, http://www.thefishstore.com; collection locations remain unknown), and housed in closed 90 l aquaria (124). The 124L aquarium with carbon filtration, a protein skimmer (Coralife Biocube, Central Aquatics, WI, USA) and actinic lights on a 13 L : 11 D cycle. Species were held separately without fish and fed twice weekly with prawn meat. Bleaching status was visually assessed using the colour health monitoring chart developed by Siebeck et al. [41]. Unbleached anemones had highly pigmented tentacles, whereas bleached anemones had tentacles that were white and lacked visible pigment. In some cases, anemones arrived bleached from the supplier, whereas in others bleaching was induced by gradually ramping the temperature (1–2°C per day) until full bleaching was achieved.
Using methods outlined by Gerlach et al. [29], a constant gravity-driven flow of 100 ml min\(^{-1}\) per channel was supplied from two 9.51 header tanks (which contained anemone cues or seawater) and maintained using flow meters (flume dimensions: 13 cm length × 4 cm width, 4 cm depth, working volume 308 ml, see [42] for further details). This flow rate ensured that the juveniles were not struggling to maintain their desired location within the flume, and therefore were able to respond to the chemical preferences being tested. Dye tests were done at each water change to ensure the two channels exhibited parallel water flow, and no areas of turbulence or eddies were present.

An individual fish was released in the downstream end of the flume where it was free to move to either side or swim towards the preferred water source. Each trial consisted of a 2 min habituation period, followed by a 2 min testing period, where the position of the fish on either the right or left side of the chamber was recorded at 5 s intervals (using a stopwatch and direct observation). Then a 1 min rest period was provided; during this time the water sources were switched from one side to the other, a measure to ensure a side preference was not being displayed by the fish. The 2 min habituation period and 2 min testing period were then repeated.

Kolmogorov–Simonov tests were used to compare the proportion of time that individuals spent in the stream of water containing the olfactory cue compared with the proportion of time that individuals spent in the stream of water containing the olfactory cues of the bleached anemone against untreated seawater. To do this Kolmogorov–Simonov tests compared the proportion of time individuals spent in the stream of water containing the olfactory cue of the bleached anemone against untreated seawater to the proportion of time that individuals spent in the stream of water containing the olfactory cues of the bleached anemone against untreated seawater.

**3. Results**

(a) Responses of anemonefishes to unbleached and bleached sea anemones versus seawater

All anemonefishes preferentially selected unbleached sea anemones that they associate with in the field over seawater, spending more than 82% of their time in the anemone cue \((p > 0.001\) in all cases; figure 1; electronic supplementary material, table S1). When presented with cues from non-host species, all fishes chose seawater \((p < 0.001\) in all cases), spending less than 30% of their time in the anemone cue. These same patterns were observed regardless of host health with all fish choosing bleached host chemical cues over seawater, and seawater over non-host chemical cues.

While all fishes preferred the chemical cues derived from the host compared with seawater irrespective of the anemone’s bleaching status, the strength of the fish’s response towards the chemical cues of unbleached host tissue compared with the response towards bleached host tissue often differed (figure 1). *Amphiprion clarkii* was the only species to show no difference in the time spent in cues from known unbleached or bleached hosts versus seawater \((E.\quad quadricolor\quad p = 0.23,\quad H.\quad crispa\quad p = 0.135,\quad H.\quad magnifica\quad p = 0.771)\). By contrast, *A. latzeonatus* selected unbleached *H. crispa* 96 ± 0.6% of the time over seawater, whereas the bleached equivalent was only selected 84 ± 1.3% of the time \((p < 0.01)\); *A. percula* selected unbleached *H. crispa* 95 ± 1.0% of the time, while spending 71 ± 1.2% of the time in the bleached *H. crispa* cue \((p < 0.001)\); and *P. biaculeatus* spent 100 ± 0% of their time in cues from unbleached *E. quadricolor* and 97 ± 0.3% in bleached cues \((p < 0.001)\). Interestingly, *A. ocellaris* and *A. percula* spent significantly more time in bleached *H. magnifica* cues versus...
seawater than in unbleached cues versus seawater ($p < 0.001$ in both cases), and so did *A. latezonatus* in the *E. quadricolor* versus seawater trials ($p = 0.01$, figure 1).

(b) Responses of anemonefishes to unbleached versus bleached sea anemones of the same species

All anemonefishes displayed very strong preferences for unbleached known host anemones when tested simultaneously against bleached anemones of the same species, spending between 94 and 100% of their time in the cues generated from the former ($p < 0.001$ in all cases; figure 2; electronic supplementary material, table S1). When unbleached and bleached non-hosts of the same species were trialled against each other, fishes displayed no preference for either cue ($p > 0.05$ in all cases; figure 2; electronic supplementary material, table S1).

(c) Responses of anemonefishes to unbleached versus bleached sea anemones of differing species

*Amphiprion clarkii* and *A. percula* showed strong preferences for unbleached over bleached hosts ($p < 0.001$ in all cases; figure 3; electronic supplementary material, table S1). By contrast, *A. latezonatus* selected *H. crispa* over *E. quadricolor* regardless of health ($p < 0.001$ in both cases). Fishes, with the exception of *A. clarkii*, which uses all sea anemones tested, selected unbleached or bleached hosts rather than non-host species ($p < 0.001$ in all cases). When two non-hosts were tested simultaneously, fishes typically showed no preference towards either cue (figure 3; electronic supplementary material, table S1).

(d) Responses of anemonefishes to bleached versus bleached sea anemones of differing species

Anemonefishes showed differing responses when they were presented with cues from two different known hosts that were bleached (figure 4; electronic supplementary material, table S1). Both *A. clarkii* and *A. percula* showed no preference and spent equal time in the cues produced by bleached hosts ($p > 0.05$ in all cases). By contrast, *A. latezonatus* showed strong preferences for bleached *H. crispa* over bleached *E. quadricolor*, spending $71 \pm 1.1\%$ of their time in these cues ($p < 0.001$ in all cases). When a bleached host was tested against a non-host anemone, all fishes selected the bleached host ($p < 0.001$ in all cases); and when two non-hosts were tested against each other no preference was shown for either cue ($p > 0.05$ in all cases; figure 4; electronic supplementary material, table S1).

4. Discussion

Bleaching events are increasing in frequency and severity, and are one of the major contributors to the decline in the diversity of coral reef communities [5,7]. This study used paired-choice flume experiments to examine the consequences of sea anemone bleaching during settlement of an iconic group of reef fishes. We found that juvenile anemonefishes (*Amphiprion clarkii, A. latezonatus, A. ocellaris, A. percula* and *Premnas biaculeatus*) have olfactory capabilities that allow them to distinguish between unbleached and bleached host sea anemones, and that although bleached anemones will be selected as habitat, this generally only occurs when other host options are unavailable. Bleached hosts were always preferentially selected over unbleached non-host sea anemones, indicating that the need for species-specific hosts during settlement is more powerful than the health of the habitat. In some cases, the potential adverse impacts of settling on a bleached host in the field may only be short-term, as depending on the causative stressor, anemones can recover [19].

The ability to recognize bleached sea anemones during settlement is advantageous, as depending on severity, bleaching can lead to host mortality [20,43], and consequent fish mortality due to obligate nature of the symbiosis [14,15,18]. If the bleached host remains alive, post-settlement mortality may not be an issue, as juvenile, sub-adult and adult *A. polymnus* numbers were not affected after mild bleaching in Bootless Bay, Papua New Guinea [19]. However, settlement onto a bleach host may have implications for the future reproductive success of the fish, as habitat degradation can result in a reduced egg production [19].

Another potential deleterious effect of settling onto a bleached host would be the loss of visual crypsis. Fishes are more conspicuous against the white background of a
bleached anemone, which could lead to increased predation [19,44]; however, the protection afforded by sheltering in the anemone’s nematocyst-laden tentacles [14] may help negate this. While chemical crypsis has not been investigated in anemonefishes, many organisms have evolved mechanisms to blend in with the chemical components of their habitat [45]. Because our study has shown that anemonefishes have the olfactory capabilities to detect differences between bleached and unbleached hosts, bleaching may also alter chemical crypsis. Furthermore, behavioural changes have been documented in *A. akindynos* that occupy bleached hosts, and it has been suggested that these changes may increase predation susceptibility [44].

While all five anemonefish species were attracted to bleached hosts rather than seawater, the strength of this attraction often varied when compared with the responses found during trials that used unbleached hosts. Some fishes spent more time in unbleached host cues versus seawater than in bleached cues versus seawater, and vice versa. *Amphiprion clarkii* was the only species that spent equal time in cues from unbleached or bleached hosts versus seawater. Because host anemones are typically rare on reefs [20,46–49], anemonefishes

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**Figure 3.** Anemonefish responses to chemical cues from unbleached and bleached sea anemones of differing species. *Entacmaea quadricolor*: unbleached (dark blue), bleached (light blue); *Heteractis crispa*: unbleached (orange), bleached (yellow); *Heteractis magnifica*: unbleached (dark green), bleached (light green); tick marks above the bars indicate known associations; crosses indicate associations that do not naturally occur in the field. *n* = 18–20, data are means ± s.e.

**Figure 4.** Anemonefish responses to chemical cues from bleached versus bleached sea anemones of differing species. *Entacmaea quadricolor*, light blue; *Heteractis crispa*, light yellow; *Heteractis magnifica*, light green. Tick marks above the bars indicate known associations; crosses indicate associations that do not naturally occur in the field. *n* = 18–20, data are means ± s.e.
have relatively short pelagic larval durations of 7–22 days [50–52], and predation pressure is likely to be high during this time; settling on the first host encountered, even if bleached, may be beneficial as rejecting bleached habitat may mean that settlement does not occur. Our study provides laboratory-based comparative observations and insights into how anemonefishes may respond to bleaching, which are further supported by field observations, as Saenz-Agudelo et al. [19] found no evidence of A. polyactis larvae avoiding bleached anemones in the field. By contrast, field experiments have shown that some other pomacentrids prefer live coral over bleached habitat at the settlement stage [36], and that recruitment can be reduced during large-scale bleaching events [12].

Anemonefishes showed differing responses when presented with cues from two bleached hosts. Amphiprion clarkii, a generalist species that uses all 10 host anemones, and A. percula, which is more specialized in terms of host use [15], both showed no preference between bleached hosts that were tested against each other. By contrast, A. latezonatus, which uses E. quadriradix and H. crispa as habitat [38], showed a strong preference for the latter species, and was the only fish to preferentially select a bleached over an unbleached host. Heteractis crispa has been suggested to serve as important nursery habitat for A. latezonatus in the Solitary Islands Marine Park, Australia (A. Scott and H. Malcolm 2016, personal communication), and similarly for A. bicinctus in the Red Sea [53].

5. Conclusion

This laboratory-based study furthers our understanding of the information used during coral reef fish larvae settlement, specifically investigating the role of habitat quality in the selection of settlement sites by habitat specialists. All five anemonefishes tested were able to recognize bleached habitat using chemical cues alone, and all but A. latezonatus preferentially selected unbleached versus bleached host anemones when tested concurrently. Anemonefishes did not show any flexibility in terms of host use regardless of bleaching status, which could be deleterious to populations during severe habitat degradation events that lead to host mortality. The ability of anemonefishes to determine the health status of their species-specific host could be particularly important given that bleaching events are becoming more common, and recruitment occurs during the warmer months [54–56] when thermal bleaching is most likely to occur [5]. These findings highlight how specialists that associate with bleaching-susceptible habitat may potentially respond to the indirect impacts of climate change.

References


