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RESEARCH ARTICLE

Abiotic factors that prompt major ecological transitions: Are fish on land to escape an intolerable aquatic environment?

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Abstract

1. Colonisation of novel habitats are important events in evolution, but the factors that initially prompt such ecological transitions are often unknown. The invasion of land by fish is an extreme habitat transition that offers an opportunity to empirically investigate the causes of major ecological transitions.
2. The intertidal ecotone—and rock pools in particular—have been an important staging ground for transitions onto land. Classic hypotheses focus on the adverse abiotic conditions of rock pools at low tide as the instigator of fish voluntarily stranding themselves out of water, which can then lead to the evolution of an amphibious lifestyle. To test these hypotheses, we studied the abiotic conditions of 54 rock pools on the island of Guam where there are various species of aquatic, amphibious and terrestrial blenny fishes.
3. We found little support for the expected deterioration of abiotic conditions in standing pools at low tide (salinity, pH and oxygen), and fish were not seen to be excluded from those pools that were found to exhibit poor abiotic conditions (temperature, salinity and pH). Hypoxia was the only factor that might account for the absence of blennies from certain rock pools.
4. Next, we experimentally measured oxygen depletion by an aquatic, mildly amphibious and highly amphibious species of blenny found on Guam in a simulated rock-pool to infer the proportion of rock pools at low tide outside the tolerable range of blennies. Rock pools were found to have oxygen levels within the requirements of most blennies and those of other marine fishes reported in the literature.
5. We conclude that the abiotic environment of rock pools alone was unlikely to have instigated the evolution of amphibious behaviour in blennies, at least on Guam. Instead, the broad range of abiotic conditions experienced in rock pools suggests these conditions could have primed amphibious blennies to better endure the novel conditions on land. Any ecotone typified by fluctuations or gradients in abiotic conditions is likely a key transitional environment for the invasion of novel habitats and, as such, are an important location for adaptive evolution and species diversification.

KEYWORDS
adaptive speciation, biological invasion, intertidal, physiology, respiration, thermoregulation, tidepool

1 | INTRODUCTION

Transitions in habitat are a critical ecological cog in the evolutionary engine of species diversification. Populations that establish in novel environments are invariably exposed to unique selection regimes that promote adaptive differentiation (e.g. Brewer et al., 2015; Lescak et al., 2015; McGee & Wainwright, 2013; Rainey & Travisano, 1998; Rosenblum, 2006). It is widely accepted that ecological adaptation is the source of much of the diversity in the natural world (reviewed by Schluter, 2009). This is supported by a large body of research documenting how phenotypic differences among closely-related species are predicted by the types of habitats occupied by those species (e.g. Collar et al., 2010; Goodman et al., 2008; Knope & Scales, 2013; Moen & Wiens, 2017; Nakazato et al., 2008; Seddon, 2005). Adaptive change within species has also been observed following the establishment of populations in areas with contrasting environmental conditions (e.g. Aguirre & Bell, 2012; Antonovics, 2006; Losos et al., 1997; Nosil et al., 2002; Szücs et al., 2017). What prompts habitat transitions to occur in the first place has been less studied. The putative frequency of ecological speciation (Funk et al., 2006; Nosil, 2012; Schluter, 2009), which often follows transitions in habitat, infers the colonisation of novel environments has been historically common in nature. However, this conflicts with ecological niche and coexistence theory (Yamamichi et al., 2022) that emphasise the exclusion of species from new environments because of restrictive abiotic (e.g. temperature, humidity, salinity, nutrients) and biotic conditions (e.g. predation, competition; e.g. Gotelli & McCabe, 2002; Redding et al., 2019; Stachowicz et al., 1999; reviewed by Chase & Leibold, 2003). A clearer understanding of the conditions that allow organisms to colonise new, sometimes drastically different, environments would help reconcile these contrasting evolutionary and ecological standpoints.

Much of the discussion around the colonisation of novel habitats centres on ecological release and ecological opportunity (Herrmann et al., 2021; Losos & Mahler, 2010; Pontarp & Petchey, 2018; Schluter, 2016; Stroud & Losos, 2016; Yoder et al., 2010). Here, organisms move into a new environment to escape competition or predation in the original habitat (Herrmann et al., 2021), or to exploit new or more accessible resources in the new habitat (Schluter, 2000). Both explain how populations might become established in a new area, but additional factors could still have facilitated the initial colonisation process. Chance might account for some individuals dispersing into a new area (Moore et al., 2008; Ward & Thornton, 2000), but successful establishment is contingent on propagule pressure (Simberloff, 2009) and specifically the frequent transition of large numbers of individuals (Britton & Gozlan, 2013; Hufbauer et al., 2013). This is likely to be especially true for the colonisation of a novel habitat where individuals are ecologically less suited to conditions and subsequently more prone to mortality. Colonisation is therefore more likely in the context of factors that maintain a consistent push of individuals from one habitat into another.

Adverse changes to the abiotic conditions of environments are a growing concern with the climate crisis (Gunderson et al., 2016; Harvey et al., 2022; Mi et al., 2023; Parmesan, 2006) and the extent to which species have the capacity to track shifting niche envelopes (Block et al., 2022; Engler et al., 2009) or adapt to new conditions (Arietta & Skelly, 2021; Hoffmann & Sgrò, 2011; Sinervo et al., 2010; Wooliver et al., 2020). More generally, deteriorating abiotic conditions could prompt organisms to move into adjacent environments. For example, seasonal fluctuations in temperature and rainfall are a common instigator of migration (Carbeck et al., 2022; Chowdhury et al., 2021; Monteith et al., 2011) and dispersal (Elias et al., 2004). Abiotic stressors can similarly push species to shift ranges (Somero, 2012; Wernberg et al., 2016) or temporarily shelter in nearby habitats that would otherwise be undesirable (Ord, 2023; Sullivan et al., 2015). In addition, individuals that occupy habitat edges or ecotones presumably have more opportunity to explore adjacent environments.

The intertidal ecotone encompasses one of the most dynamic environments on earth, with daily tide cycles exposing organisms to dramatic changes in both the amount of available habitat and the abiotic conditions experienced in that habitat. For example, marine fishes and other ectotherms that retreat to rock pools at low tide can experience large fluctuations in water temperature, pH, and other aspects of water chemistry (reviewed by Gunderson et al., 2016; Martin, 2015; Martin & Bridges, 1999; Richards, 2011; Sayer & Davenport, 1991; Wolfe et al., 2020). It is perhaps not surprising then that the intertidal zone is home to a significant overrepresentation of fish genera that are amphibious (meta-analysis by Ord & Cooke, 2016). Many of these fishes shelter out of the water under rocks or in crevices and seaweed until the return of high tide, rather than remaining in isolated rock pools (see Martin, 1993; Shorty & Gannon, 2013). Fish that cross the water-land boundary, however temporary, are examples of organisms making an extreme transition in habitat and offer a tractable model to study how and why individuals colonise novel environments (Ord & Cooke, 2016).

Classic hypotheses for the evolutionary origin of fish with amphibious behaviour generally centre on fish initially stranding themselves on land to avoid adverse water conditions (Martin, 1995; Sayer & Davenport, 1991; see also Turko et al., 2021 for other explanations). Of particular relevance for intertidal fishes in hot climates is the increase in water temperature in rock pools as the tide recedes (Gunderson et al., 2016; Martin & Bridges, 1999; McArley et al., 2018; Sayer & Davenport, 1991). Such temperature increases likely impact the aerobic activity of fishes (Kemp, 2009; McArley et al., 2018, 2019; Pörtner et al., 2017; Schulte, 2015), induce physiological stress (Davenport & Woolmington, 1981; Gunderson et al., 2016; Pulgar et al., 2005) and reduce oxygen solubility in pools (Martin, 2015; Martin & Bridges, 1999; Sayer & Davenport, 1991) leading to hypoxia (Davenport & Woolmington, 1981; Gobler & Baumann, 2016; McArley, Hickey, & Herbert, 2020). Other factors might include increases in salinity and pH (Gunderson et al., 2016; Martin, 2015; Martin & Bridges, 1999; Sayer & Davenport, 1991). Laboratory tests have confirmed elevated water temperature (Gibson et al., 2015) and aquatic hypoxia (Davenport & Woolmington, 1981; Tigert et al., 2022) can induce emergence onto land in some amphibious fish, and there are numerous studies documenting the abiotic conditions of rock pools (e.g. Johnson, 2001; Langenheder & Ragnarsson, 2007; Legrand et al., 2018; Martins et al., 2007; Morris & Taylor, 1983; Richards, 2011; Romanuk & Kolasa, 2002; Rosenfeld et al., 2019; Smit & Glassom, 2017; Truchot & Duhamel-Jouve, 1980; Wolfe et al., 2020). Hypoxia and elevated hydrogen sulphide from the decomposition of organic matter in mangrove swamps have also been linked to emersion behaviour in several fish species (Rossi et al., 2019; Turko et al., 2018). In the current study, we field test several key hypotheses of how the conditions of intertidal rock pools are expected to deteriorate as the tide recedes, and the capacity of both aquatic and amphibious fishes to tolerate those conditions. Our study is explicitly grounded within the context of the ongoing colonisation of land by intertidal blenny fishes (Ord & Cooke, 2016; Ord & Hsieh, 2011) and directly embedded in an ecological setting linked to the repeated, independent evolution of water-to-land transitions (specifically, the tropical intertidal zone; Ord & Cooke, 2016).

Blennies are a diverse family of fishes that have experienced exceptional lineage diversification (e.g. just shy of the diversification rate documented for the extensive adaptive radiation of cichlids—Hundt et al., 2014; Near et al., 2013; Near & Thacker, 2024). There have been up to seven independent origins of amphibious behaviour in intertidal blennies (Ord & Cooke, 2016). The group consequently offers a unique opportunity to study the evolutionary and ecological origins of major transitions in habitat, with separate species representing various stages of colonisation (Ord & Cooke, 2016; Ord & Hundt, 2020; Platt et al., 2016). Most blennies are fully aquatic (the ancestral phenotype), but there are various species that are mildly to highly amphibious (transitional phenotypes), and those that are exclusively terrestrial and never voluntarily return to water (the derived phenotype; Ord et al., 2017; Ord & Cooke, 2016). Out of the water, amphibious and terrestrial blenny fish are restricted to exposed rocks within the splash zone (Ord & Cooke, 2016; Ord & Hsieh, 2011). Respiration still occurs through the gills and, to an extent, the skin (Brown et al., 1992; Martin & Lighton, 1989). This means that fish must remain moist to avoid asphyxiation. Beyond this restriction, blennies are highly agile on land (Ord & Hsieh, 2011) and easily locomote over the rocks by shuffling or hopping forward using their tail (see Hsieh, 2010). At low tide, this splash zone disappears and amphibious and terrestrial blennies retreat into moist rock holes and crevices, and in the case of amphibious blennies, return to rock pools.

The objective of our study was to determine the extent to which intertidal rock pools used by aquatic and amphibious blennies at low tide on the island of Guam exhibit the deteriorating abiotic conditions that are the basis of classic hypotheses in which fish emerged onto land to escape intolerable aquatic conditions (Sayer & Davenport, 1991). Specifically, we tested four non-mutually exclusive hypotheses formally defined by the Directed Acyclic Graphs in Figure 1a (DAGs; see Attia et al., 2022) and qualitatively described below:

H_1 *Temperature stress*: Solar radiation at low tide causes the temperature of the water to rise in isolated rock pools (Daniel & Boyden, 1975; Martin & Bridges, 1999; McArley et al., 2018; Smit & Glassom, 2017), mediated by the size of the pool (Buasakaew et al., 2021), and this leads to a heightened risk of thermal stress in fish caught within the pool (Davenport & Woolmington, 1981; McArley, Hickey, & Herbert, 2020; Sandrelli & Gamperl, 2023). This predicts a negative interaction between ambient air temperature at low tide ($T_{\text{air, low}}$) and the volume of isolated rock pools (RP_{vol}) to produce elevated water temperatures in pools at low tide ($T_{\text{water, low}}$) compared to high tide ($T_{\text{water, high}}$).

H_2 *Salinity stress*: As the temperature of isolated rock pools rises at low tide, there is an associated increase in water loss through evaporation (Brown et al., 1991; Martin & Bridges, 1999; Sayer & Davenport, 1991) that causes the salinity of pools to increase (Buasakaew et al., 2021; Daniel & Boyden, 1975), leading to physiological stress in fish caught within the pool (e.g. Przeslawski et al., 2015). This predicts greater salinity in rock pools at low tide (S_{low}) compared to high tide (S_{high}) and as a direct function of water temperature at low tide ($T_{\text{water, low}}$).

H_3 *pH stress*: As the temperature of isolated rock pools rises at low tide there is an associated increase in pH of pools (Buasakaew et al., 2021; Morris & Taylor, 1983) that is further exacerbated by photosynthesis from primary producers (Morris & Taylor, 1983; Wolfe et al., 2020), leading to physiological stress in fish caught within the pool (Evans et al., 1999; see also Przeslawski et al., 2015). This predicts greater pH in rock pools at low tide (pH_{low}) compared to high tide (pH_{high}), and as direct and interactive functions of both water temperature ($T_{\text{water, low}}$) and the abundance of primary producers (Pr_{abund}).

H_4 *Hypoxia*: As water temperature rises in isolated rock pools (e.g. McArley et al., 2018; Smit & Glassom, 2017), there is a progressive reduction in oxygen solubility in the water (Martin, 2015; Martin & Bridges, 1999; McArley, Sandblum, & Herbert, 2020) that heightens the risk of hypoxia in fish caught within the pool (Davenport & Woolmington, 1981; Sandrelli & Gamperl, 2023). This risk is offset by the presence of primary producers replacing oxygen within the pool (Daniel & Boyden, 1975; Graham, 1997; Morris & Taylor, 1983). This predicts an interaction between water temperature at low tide ($T_{\text{water, low}}$) and the absence of primary producers (Pr_{abund}) to cause a reduction in dissolved oxygen in pools at low tide ($O_{2\text{ low}}$) compared to high tide ($O_{2\text{ high}}$).

Each hypothesis similarly predicts an absence of blenny fishes within the pool as a function of the severity of the conditions recorded within the pool.

Our work was focussed on the island of Guam, which has the greatest diversity and abundance of aquatic and amphibious blennies of the locations so far studied in the Pacific and Indian Oceans (Ord & Cooke, 2016; Ord & Hundt, 2020; Platt et al., 2016). Guam is also home to a fully terrestrial blenny species (*Alticus arnoldorum*; Ord & Hsieh, 2011) and is the setting of several of the repeated,

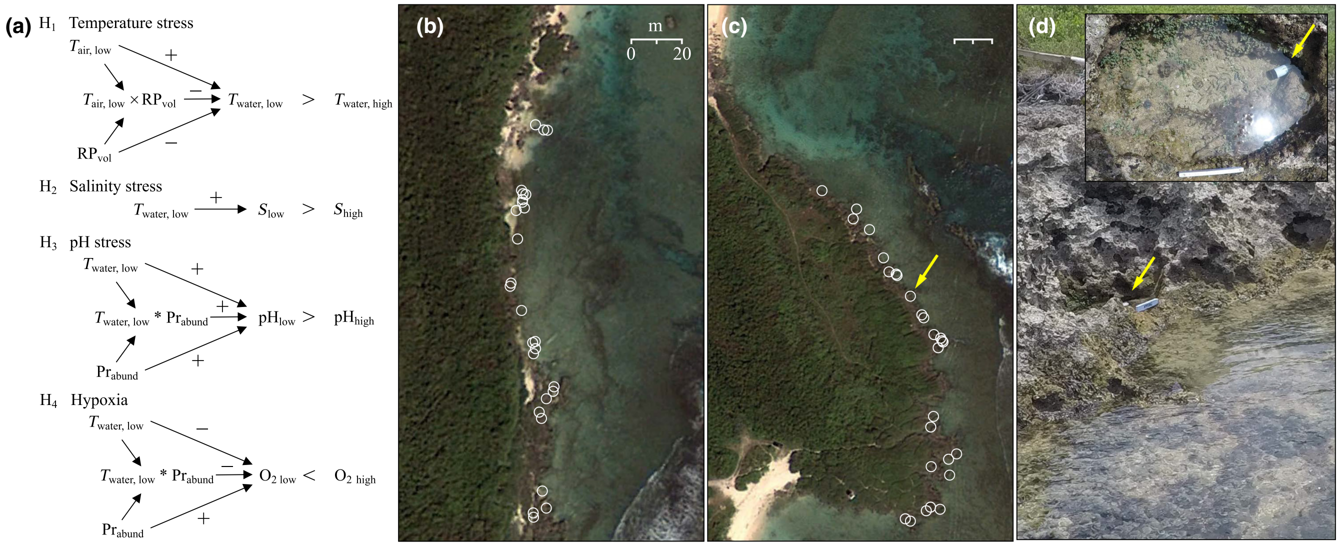


FIGURE 1 We tested (a) four classical hypotheses of how the daytime abiotic conditions of rock pools should deteriorate at low tide relative to high tide by sampling the environments of 54 rock pools near (b) Taga'chang beach and (c) Talofofo Bay on the island of Guam. Surveyed rock pools were those that were (d) fully enclosed and became isolated at low tide. H_1–H_4 refer to the four tested hypotheses involving abiotic conditions (see main text). Positive (+) and negative (−) interactions are shown between air and water temperature ($T_{\text{air/water}}$), water volume of rock pools (RP_{vol}), salinity (S), water pH (pH), or oxygen saturation (O_2) at either low or high ties, as well as the abundance of primary producers (Pr_{abund}). The yellow arrow in panel (c) highlights the position of the rockpool shown in panel (d), with the inset arrow pointed at a miniDOT logger. Also shown in the inset photo of (d) is a white folding ruler for scale (observed length in photo: 24 cm).

independent evolutionary origins of amphibious behaviour within the family (Ord & Cooke, 2016). More generally, the intertidal zone on Guam is typical of other island locations where other amphibious and terrestrial blennies have been documented (reviewed by Ord & Cooke, 2016). Our investigation was conducted in two stages. First, we measured the abiotic conditions and general abundance of blenny fishes in 54 isolated rock pools at low tide (benchmarked against their conditions at high tide) across two sites on Guam known to have abundant populations of aquatic and amphibious blennies (e.g., Platt et al., 2016; Figure 1b,c). Analyses showed the presence of blennies within pools at low tide was dependent on the amount of dissolved oxygen within pools, which prompted the next stage of our study. Second, we measured the oxygen depletion of seawater by the respiration of freshly caught blennies with fully aquatic (*Blenniella paula*), mildly amphibious (*Istiblennius lineatus*) and highly amphibious (*Entomacrodus striatus*) lifestyles in simulated rock pools. These data were then used to extrapolate the capacity of blennies to tolerate the progressive depletion of dissolved oxygen recorded in rock pools during the day at low tide in the first stage of the study. This last stage addressed the broader question of whether the deteriorating abiotic conditions of rock pools might have instigated the evolution of amphibious behaviour and the colonisation of land by blennies on Guam.

2 | MATERIALS AND METHODS

The work associated with this study was approved by the UNSW Animal Care and Ethics Committees through project 23/33A on 16 March 2023, and the University of Guam's Animal Care and Use Committee through project UOG23-01 on 4 May 2023. The collection of fish was approved by the Guam Department of Agriculture under licence SCR-23-006.

2.1 | Abiotic conditions of rock pools

Rock pools were selected based on the criteria of being fully enclosed pools that were isolated from seawater flushing at low tide. We concentrated our survey on pools at or immediately adjacent to the boundary of the intertidal and supralittoral zones, which is where transitions from water to land by ancestral aquatic blennies would have presumably occurred. Today, these pools are frequent sites of activity for both present-day aquatic and amphibious blennies, and are immediately adjacent to the exposed rocks, crevices and nesting holes frequented by the fully terrestrial species *Alticus arnoldorum* (Ord & Hsieh, 2011). Pools were located either at approximately sea level or elevated on the exposed reef to a height up to approximately 1 m above the intertidal substrate (all rock pools surveyed fit the definition of moderate to high tidepools of Richards, 2011). At high tide, all pools experienced full seawater inundation or extensive flushing. Rock pools were surveyed at northern coves from Tagachang Beach and near Talofofo Bay (from the northern shoreline of First Beach). A subset of rock pools meeting the criteria above was surveyed at Tagachang ($N=27$; Figure 1b), while an exhaustive survey was conducted at Talofofo ($N=27$; Figure 1c).

Rock pools were sampled during the day at or as close to low tide as possible and again at high tide within 3–6 days of the low tide sampling period. Sampling consisted of fully immersing a miniDOT Oxygen logger into the pool for at least 30 min at low tide (range: 32–123 min; median: 107 min) and 20 min at high tide (range: 19–46 min; median: 30 min), with the logger set to record water temperature and oxygen saturation every minute. A second measure of water temperature was taken to accompany estimates of salinity and pH using a calibrated handheld Cuteefun PH/TDS/EC/Salinity/Temp Digital Meter. Salinity was measured a second time using a Gain Express Refractometer ATC and was found to provide congruent estimates to the digital meter and were not considered further (Pearson's product–moment correlation: $df = 53$, $r = 0.97$ with a 95% confidence range of 0.95–0.98). Individual rock pool estimates of salinity from the digital meter were used to convert measures of oxygen recorded by the miniDOT loggers (mg/L) to percent air saturation (with elevation uniformly set to sea level and water temperature measured by the logger at the same time point as dissolved oxygen). This conversion was made using the miniDOTConcatenate software supplied with the logger. Ambient air temperature was measured using a WeatherMate hand-held thermo-windmeter (WM20). A qualitative estimate within pools of the abundance of aquatic and amphibious blennies and primary producers (predominately macro-algae) was taken at low tide (blennies absent, low, moderate and high in abundance coded as 0, 1, 2 and 3, respectively; primary producers absent, some and abundant coded as 0, 1 and 2, respectively). Rockpool volume was inferred from the measured length, width and depth of pools (in cm) using the formula for an ellipsoid (divided by two). The approximation of this formula to measure rockpool volumes was validated by manually emptying 33 of the 54 pools using a combination of plastic measuring jugs (1 and 3 L) and a 200 mL plastic syringe to extract all water from the pool. A regression analysis confirmed that the inferred volume provided a reasonable estimate of the true volume of the pool (Figure S1).

All statistical analyses were applied using R version 4.0.5 (R Development Core Team, R Foundation for Statistical Computing, Vienna). Analyses were linear regressions based on the DAGs defined in Figure 1a. All hypotheses explicitly assumed either an increase or decrease in estimates obtained at low tide relative to those taken at high tide (Figure 1a). As such, water temperature, air temperature, salinity, pH and percentage oxygen saturation were converted to delta scores (Δ) by subtracting values taken at low tide for a given pool from values taken at high tide for the same pool. Tests of salinity and pH were based on 40 rock pools (rather than 54) to exclude pools observed near seepage from freshwater springs that recorded unusually low salinity (<29.5% salinity; cut-off determined by the lower quantile of salinity values across the 54 rock pools). Two of the 54 rock pools were excluded from the test of minimum dissolved oxygen because of battery failure of the miniDOT logger (one logger used at low tide and another used at high tide).

In some cases, rock pools were seen to have an instance of flushing from fresh seawater at low tide (rare) or miniDOT loggers were later found to have reached their upper temperature limit (~36.5°C). Analyses were subsequently repeated with these pools excluded to evaluate the sensitivity of results obtained from the first set of analyses and are reported in the Supporting Information.

Finally, the qualitative abundance of blennies observed in rock pools at low tide as a function of water temperature, salinity, pH and minimum oxygen saturation was examined using a generalised linear model and a Poisson error distribution. The absence of blennies from certain rock pools would imply blennies either actively avoided these pools before the tide receded (which would presumably require prior experience of conditions in those pools at low tide and associated spatial memory: e.g. see Gibson, 1999) or left these pools once isolated at low tide and conditions had begun to deteriorate (e.g. Azevedo e Silva et al., 2022). The majority of blennies observed in rock pools at low tide were species that have previously been identified to exhibit some level of amphibious behaviour (*Praealticus labrovittas*, *P. poptae*, *Istiblennius lineatus*, *Entomacrodus striatus*, *E. sealei* and *Rhabdoblennius snowi*; Ord & Cooke, 2016), but several aquatic species were occasionally seen in rock pools as well (*Blenniella paula*, *B. caudolineata* and *Istiblennius edentulus*).

2.2 | Oxygen depletion by aquatic, mildly amphibious and highly amphibious blennies

To infer the proportion of rock pools that might become intolerable because of reduced oxygen, we estimated water oxygen depleted by aquatic and amphibious blennies in a simulated rockpool. This experiment was prompted by evidence that the presence of blennies in rock pools seemed to be influenced by the amount of minimum oxygen saturation recorded in pools at low tide (see Sections 2.1 and 3). We therefore selected three blennies encompassing the full range of aquatic and amphibious behaviour that had been observed within the rock pools at low tide: A fully aquatic (*Blenniella paula*: $N = 6$; 4 females and 2 males), mildly amphibious (*Istiblennius lineatus*: $N = 12$; 5 females and 7 males) and highly amphibious species (*Entomacrodus striatus*: $N = 6$; sex can only be determined by dissection; see Ord & Cooke, 2016 for quantitative estimates of amphibious behaviour expressed by these species). While there was an abundance of terrestrial blennies at the locations studied (*Alticus arnoldorum*), this species never returns to water and its inclusion in the above experiment was therefore not biologically meaningful.

Fish were caught at mid-tide at Pago Bay next to the University of Guam's Marine Laboratory using aquarium hand nets and small weighted drop nets in water (*B. paula*) or in the splash zone on the exposed rocks above the waterline (*I. lineatus* and *E. striatus*). The experiment was conducted either in a shaded area set up on the beach under a tarpaulin strung between trees (*B. paula* and *E. striatus*) or in the outdoor facilities of the Marine Laboratory (*I. lineatus*). Within minutes of capture, each fish was placed individually into a plastic aquarium filled with 1 L of fresh seawater (taken directly from the bay or from the seawater tap at the Marine Laboratory) and housing a miniDOT Oxygen logger that recorded oxygen saturation every minute. Salinity, pH and water temperature were recorded using the digital meter before and after the experiment. There was no statistical difference in water temperature or salinity before and after the conclusion of the experiment (ANOVA: $F_{1,46}=2.13$, $p=0.15$ and $F_{1,46}=0.05$, $p=0.82$, respectively), although there was a decrease in pH from an average of 7.88 to 7.84 ($F_{1,46}=6.92$, $p=0.01$). Cardboard was positioned around the aquarium to visually isolate the fish and the aquarium was secured with a plastic lid that permitted air movement. Fish were monitored continuously for the first 10 min and then left for at least an additional 50 min, with welfare checks made visually every 20 min. Following this, fish were removed from the aquarium, placed in a moist sealable plastic bag, positioned flat against a white card and ruler, and photographed. This photograph was later used to measure the standard length of the fish with ImageJ (http://imagej.nih.gov/ij). Fish were released back at their point of capture.

The objective of this experiment was to provide a standardised simulation of a small barren rockpool (with no producers or other consumers present) and record the amount of oxygen depleted by a blenny over a timeframe comparable to the period pools were generally isolated at low tide. For measurements made at the beach, the first 10 min of data were excluded from analysis as an acclimation period from the stress of initially being caught and transported to aquaria. Measurements at the Marine Laboratory started right away because these fish had already undergone an acclimation period prior to the start of the experiment. To estimate oxygen depletion, we applied three alternative random regression models using the 'lme4' package version 1.1-28 (Bates et al., 2015). All models included a random intercept and slope for time grouped by individual (i.e. minutes spent in the experiment), and a random intercept for day of the experiment (fish were entered into the experiment on 1 of 5 days). To resolve model convergence problems, a Nelder–Mead parameter optimisation was applied. One model included a three-way interaction between time, standard length of each individual and species ID, a second model included a two-way interaction between time and standard length (and excluded species ID), while the third model included only time as the predictor (excluding standard length and species ID). Akaike Information Criteria with a correction for sample size, AIC_c, was computed for each model using the 'MuMIn' package version 1.46.0 (Bartoń, 2022). The model with the lowest AIC_c value was considered the best supported model and subsequently used to compute the coefficient estimate of oxygen depletion per minute.

These data were used to infer the proportion of rock pools that might become intolerable because of the progressive depletion of oxygen for an individual fish at various timepoints from peak low tide. The 39 rock pools that happened to have miniDOT oxygen loggers at precisely low tide were extracted from the set of 54 pools. The global regression line computed from the most credible model (previous paragraph) was then used to estimate the depletion of oxygen per minute based on three body sizes: the evolutionary optimal standard length of 8.9 cm inferred for aquatic intertidal blennies by Ord and Hundt (2020), the largest aquatic blenny (*B. paula*) included in the experiment of 11.6 cm, and the largest intertidal aquatic blenny species on Guam (*E. decussatus*) of 19.0 cm (data from Ord & Hundt, 2020). These body sizes were then used to extrapolate the progressive depletion of oxygen from the subset of 39 rock pools at 0, 30, 60 and 90 min from low tide. Tide level data for Pago Bay for the period rock pools were surveyed (4–11 May 2023; data from the US National Oceanic and Atmospheric Administration: www.tideandcurrents.noaa.gov) indicated 90 min was the likely maximum duration from low tide that pools might remain isolated (this corresponds to a king tide). The proportion of rock pools that might become intolerable because of progressive oxygen depletion from blenny respiration was tallied at each time point (0, 30, 60 and 90 min from low tide). This assumed that oxygen depletion from blenny respiration was not offset by the diurnal production of aquatic oxygen from producers within pools. As an alternative assessment, we extracted the minimum oxygen saturation recorded for every rock pool with available data ($N=52$), irrespective of time point from low tide, and tallied the percentage of rock pools reaching a likely intolerable benchmark of depleted oxygen. This test was more conservative in the sense it did not rely on statistically extrapolating the rate of oxygen depletion for any given body size and also incorporated the influence of primary producers on oxygen levels within pools.

For both assessments, two benchmarks were examined that could be expected to reflect some level of hypoxia. The first was the 'worst case' scenario of complete depletion of oxygen (zero percentage saturation). This benchmark would be biologically relevant for all species of fish, regardless of their tolerance to hypoxia. The second was the expected critical partial pressure of oxygen, P_{crit}, for marine aquatic fishes. This benchmark was computed from data compiled by Rogers et al. (2016). Data were standardised in units of kilopascals (kPa) and measured for various fish species in laboratory respiratory experiments. P_{crit} is a widely used threshold defining hypoxia tolerance, with the notion being partial oxygen pressures below this value likely lead to hypoxia. We extracted P_{crit} estimates from studies that tested marine fishes in water temperatures from 30 to 33°C (no studies were reported to have tested fish above 33°C). This range overlapped with the lower maximum temperatures measured in our rock pools (see Section 3). We obtained 13 P_{crit} values for nine marine species and entered these data into a random regression with species ID as a random intercept. The water temperature in which fish were tested was included as a predictor variable. We used the resulting regression equation to extrapolate an 'on-average' critical threshold of oxygen as a function of water temperature. In order to identify the proportion of rock pools at or below P_{crit}, we used the package 'respR' version 2.3.2 (Harianto et al., 2019) to convert minimum dissolved oxygen measures (initially recorded as mg/L by miniDOT loggers) of our rock pools (and subsequent estimates of oxygen depletion; see previous paragraph) into values of partial pressure of oxygen (in kPa) at sea-level elevation and based on measured rockpool salinity and maximum water temperature.

3 | RESULTS

3.1 | Abiotic conditions of rock pools and the presence of aquatic and amphibious blennies

3.1.1 | H_1: Temperature stress

The vast majority of rock pools were hotter at low tide than at high tide (mean difference in temperature, 95% confidence range: +1.20, 0.71–1.69°C; Figure 2a). Specifically, the water temperature was positively correlated with ambient air temperature at low tide, and there was partial evidence that this effect decreased with increased rockpool size (the 95% confidence range of the interaction term marginally overlapped 0; Table 1a; Figure 3a). Results were broadly consistent with the exclusion of those rock pools observed to have flushing at some point during sampling or where loggers reached their maximum recordable temperature (Table S1a). Overall, the conditions of rock pools at low tide were consistent with the hypothesis of temperature stress.

3.1.2 | H_2: Salinity stress

There was no evidence that the salinity of rock pools was higher at low tide compared to high tide (Figure 2a), if anything appeared to be lower at low tide (mean difference in salinity, 95% confidence range; all rock pools: *N* = 54, −2.76 parts per thousand (ppt), −4.13 to −1.40 ppt; excluding rock pools near freshwater spring seepage: *N* = 40, −0.79 ppt, −1.30 to −0.28 ppt). Furthermore, as the temperature of rock pools increased, there was a decrease (not an increase) in salinity, regardless of whether pools with or without flushing were included in the analysis (Table 1b; Table S1b). Overall, the conditions of rock pools at low tide did not support the hypothesis of salinity stress.

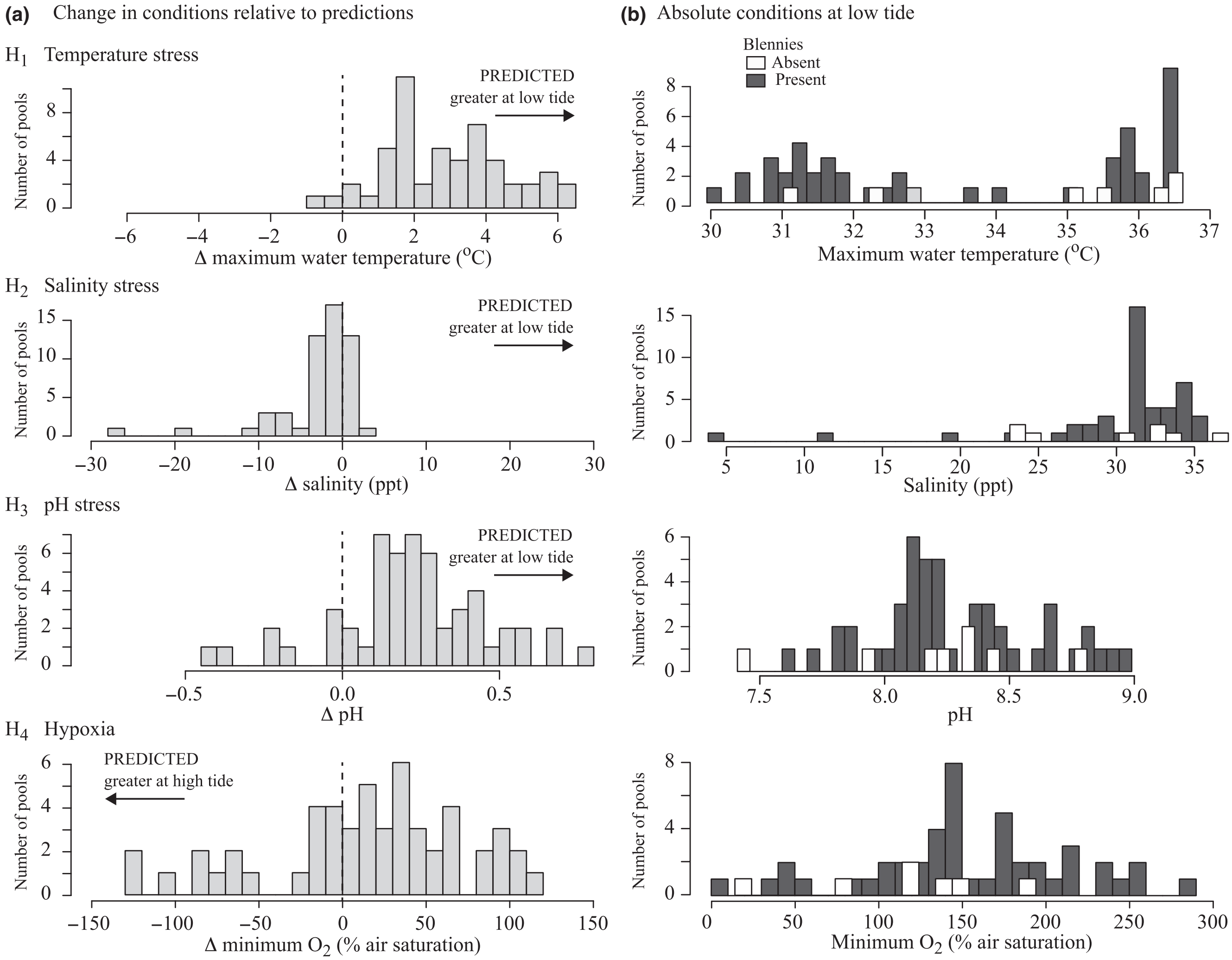


FIGURE 2 Daytime abiotic conditions within rock pools at low tide. (a) Changed conditions relative to high tide and expected direction consistent with deteriorating conditions predicted by Directed Acyclic Graphs for each hypothesis in Figure 1a. The dashed line corresponds to no change in conditions (i.e., identical values recorded at both low and high tide), with positive values corresponding to higher levels and negative values corresponding to lower levels recorded at low tide compared to high tide. (b) Distribution of aquatic and amphibious blennies across rock pools as a function of the absolute abiotic conditions recorded in pools at low tide.

TABLE 1 Factors associated with changed rockpool conditions at low tide versus high tide.

Variable	Estimate (low 95% CI, upper 95% CI)	*p*
(a) H_1 temperature stress ($N_{pools} = 54$)		
Intercept (Δ water temperature; °C)	−4.84 (−8.22, −1.45)	0.01
Δ Air temperature (°C)	0.98 (0.08, 1.88)	0.03
Log_{10}(pool volume; L)	0.60 (0.25, 0.95)	0.001
Δ Air temperature × $\log_{10}$(pool volume)	−0.08 (−0.17, 0.02)	0.10
(b) H_2 salinity stress ($N_{pools} = 40$)		
Intercept (Δ salinity; ppt)	−0.25 (−0.89, 0.39)	0.43
Δ Water temperature (°C)	−0.36 (−0.64, −0.07)	0.02
(c) H_3 pH stress ($N_{pools} = 40$)		
Intercept (Δ pH)	0.21 (0.05, 0.38)	0.01
Δ Water temperature (°C)	−0.003 (−0.07, 0.06)	0.94
Primary producer abundance (category 0, 1 or 2)	0.04 (−0.07, 0.15)	0.49
Δ Maximum water temperature × producer abundance	0.02 (−0.03, 0.07)	0.46
(d) H_4 hypoxia ($N_{pools} = 52$)		
Intercept (Δ minimum O_2; % saturation)	−77.8 (−135.8, −19.8)	0.01
Δ Maximum water temperature (°C)	32.8 (14.8, 50.8)	<0.001
Primary producer abundance (category 0, 1 or 2)	41.7 (1.00, 82.4)	0.04
Δ Maximum water temperature × producer abundance	−15.0 (−27.8, −2.28)	0.02

Note: Regression models are shown for the four tested hypotheses (H_1–H_4), with the relevant variables, 95% confidence intervals (CI) and *p*-values (*p*). In comparisons of salinity and pH (b, c), pools that had unusually low salinity and were observed near freshwater springs were excluded from the analysis. See also Table S1 for additional sensitivity analyses.

3.1.3 | H_3: pH stress

Most rock pools were higher in pH at low tide than high tide (mean difference in pH, 95% confidence range; all rock pools: $N = 54$, 0.23, 0.16–0.30, excluding rock pools near freshwater spring seepage: $N = 40$, 0.28, 0.21–0.34). However, there was no relationship between water temperature, primary producer abundance (or an interaction) and pH, regardless of whether pools with or without flushing were included in the analysis (Table 1c; Table S1c). Overall, the prediction associated with the underlying increase in rockpool pH at low tide (i.e., the product of elevated water temperature or photosynthesis by producers; Figure 1a) provides limited support for the hypothesis of pH stress.

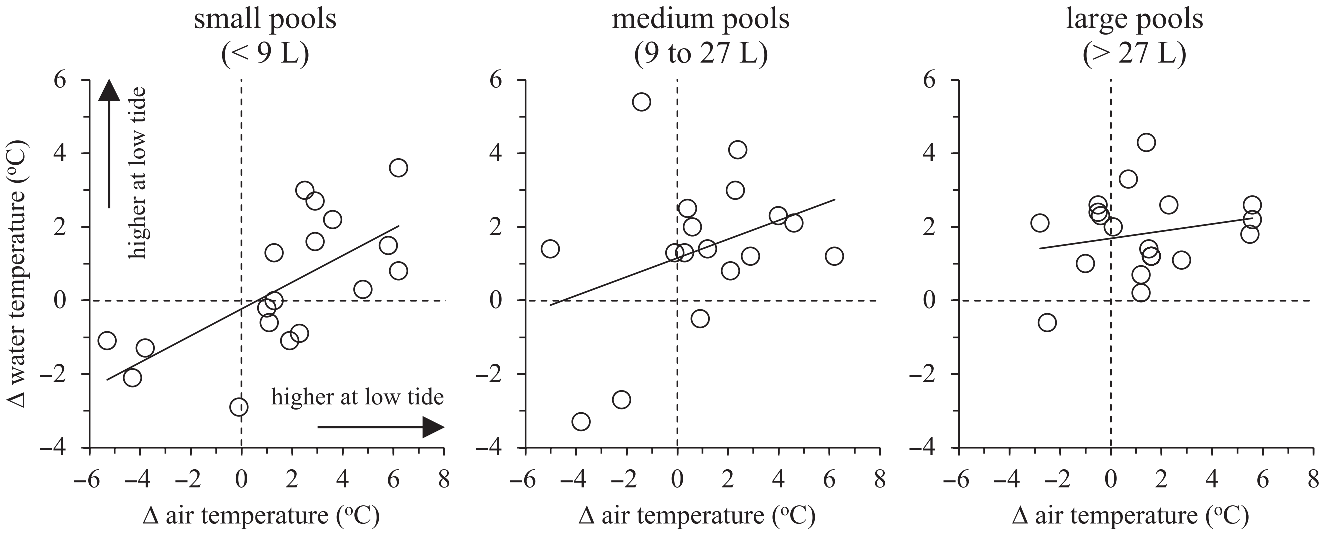
3.1.4 | H_4: Hypoxia

The minimum oxygen saturation of rock pools during the day seemed to be higher, not lower, at low tide than high tide, although this effect was not statistically distinguishable from zero in itself (mean difference in percentage oxygen saturation, 95% confidence range; all rock pools: $N = 52$, 15.6, −0.8 to 32.1; excluding rock pools near freshwater spring seepage: $N = 40$, 15.9, −4.0 to 35.9; excluding rock pools near freshwater spring seepage and miniDOT loggers hitting their maximum temperature limit: $N = 26$, 4.1, −20.0 to 28.2). There was a credible statistical difference in minimum oxygen saturation when the maximum water temperature measured by miniDOT loggers and the abundance of primary producers were considered together, and irrespective of whether pools with or without flushing or loggers reaching their maximum temperature limit were included or excluded in the analysis (Table 1d; Table S1d). Specifically, the minimum level of oxygen increased, not decreased, with maximum water temperature and this association with water temperature became progressively overridden by the abundance of primary producers observed in pools (Figure 3b). Overall, the elevated percentage oxygen saturation of rock pools at low tide during the day was not consistent with the hypothesis of hypoxia.

3.2 | Abundance of blennies within rock pools

The only credible predictor of blenny abundance in rock pools at low tide was the amount of oxygen, with rock pools higher in saturated oxygen possessing more blennies (Table 2). This provides some evidence that fish might avoid depleted rock pools or otherwise move out of those that become progressively depleted.

(a) H_1 Temperature stress ($N_{\text{pools}} = 54$)



(b) H_4 Hypoxia ($N_{\text{pools}} = 52$)

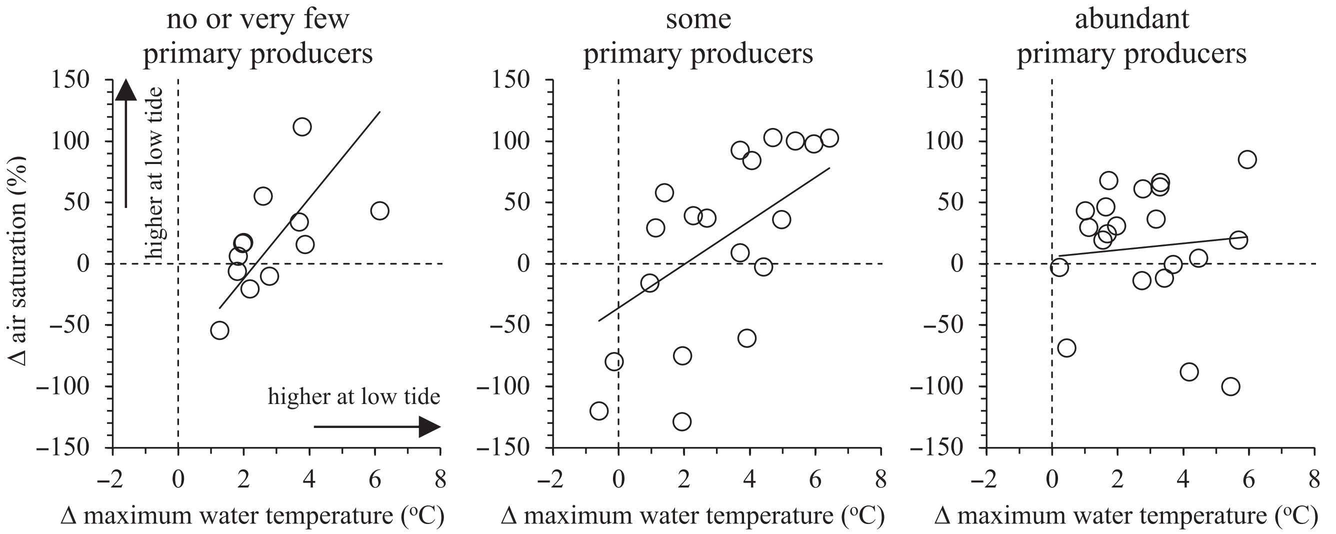


FIGURE 3 Details on how conditions in rock pools changed at low tide relative to high tide for (a) water temperature as a function of ambient air temperature and its interaction with pool size (H_1) and (b) the minimum amount of saturated oxygen as a function of water temperature and its interaction with producer abundance (H_4). Corresponding statistical analyses are presented in Table 1 and Table S1. Note that pool volume was grouped here based on percentile ranges into three general sizes for illustrative purposes only.

Yet the general presence of blennies across pools indicated a broad tolerance range (Figure 2b), with a moderate abundance of blennies even found in one rock pool with almost no oxygen (a minimum O_2 of 6.8%). Blennies seemed to be present in rock pools spanning the full range of water temperatures, pH and salinity as well (Figure 2b). In the case of salinity, while our hypothesis was specific to the impacts of elevated salinity at low tide (Figure 1a), stress could have been induced by reductions in salinity from the dilution of some pools by nearby freshwater springs (see Section 2). Yet even pools heavily diluted by freshwater (e.g. 4.4–11.3 ppt) were occupied by blennies (Figure 2b; see also Table 2) and visually did not appear adversely impacted by these conditions.

3.3 | Oxygen depletion by aquatic, mildly amphibious and highly amphibious blennies and the inferred number of intolerable rock pools at low tide

Model support and predictor effect sizes (*t*) indicated oxygen depletion was statistically comparable across the three blenny species tested (Table S2), with gross body size being the primary factor influencing the rate of oxygen depletion (Table S3). Specifically, larger fish depleted more oxygen per minute than smaller fish, regardless of species (Figure 4).

Extrapolating the oxygen depletion for three biologically and evolutionary-relevant body sizes (see Section 2) showed the vast majority of rock pools were well within the oxygen saturation

TABLE 2 Qualitative abundance of blennies in rock pools at low tide as a function of water temperature, pH, salinity and minimum oxygen saturation.

Variable	Estimate (low 95% CI, upper 95% CI)	*p*
Intercept (blenny abundance)	5.21 (−2.20, 12.95)	0.18
Water temperature (°C)	−0.05 (−0.23, 0.13)	0.61
pH	−0.55 (−1.70, 0.53)	0.34
Salinity (ppt)	0.01 (−0.03, 0.06)	0.62
Minimum O_2 (% saturation)	0.006 (0.001, 0.011)	0.02
$N_{\text{rock pools}} = 53$		

range that would likely be suitable for blennies or well above the P_{crit} threshold of most marine fishes (Table 3; Figure 5). It was only for the body size of the largest aquatic blenny on Guam (19.0 cm) that some rock pools might become entirely oxygen depleted after 60–90 min from low tide (Table 3a). Yet, this large aquatic species has never been observed in intertidal rock pools similar to those investigated by us. Nevertheless, even in the most liberal scenario, at least three quarters of rock pools remained within the tolerance of this largest species (e.g. after 90 min; Table 3b). An alternative assessment based on the minimum oxygen saturation recorded in rock pools, irrespective of time point from low tide and not considering oxygen depletion rates, showed over 90% of rock pools were above the critical threshold of marine fish (Table 3b). No rock pool was recorded to have become completely depleted in oxygen over the sampling period (although one rock pool observed with a moderate abundance of blennies reached 6.8%; see Section 3.2 and Figures 2b and 5).

4 | DISCUSSION

Determining why organisms transition from one environment into another is a critical starting point for understanding why species occur where they do and the frequency of adaptive diversification in nature. The colonisation of land by aquatic organisms has occurred repeatedly in the history of life (Damsgaard et al., 2020; Giomi et al., 2014; Graham, 1997; Kapoor et al., 2023; Minter et al., 2017; Vermeij & Motani, 2018) and represents a historic transition in habitat that ultimately heralded all terrestrial communities in existence today (Graham & Lee, 2004; Laurin, 2010; Vermeij & Dudley, 2000). Blennies are a contemporary system in which this transition across the aquatic-land boundary is likely ongoing (Ord & Cooke, 2016). Because of this, the group provides an opportunity to empirically test hypotheses of the potential factors that led aquatic organisms to leave water for a life on land, but also factors that prompt organisms to colonise novel environments more generally. Previous empirical studies on blennies have implicated ecological release in the repeated evolution of amphibious behaviour (Platt et al., 2016; and specifically from aquatic predation: Ord et al., 2017). Nevertheless, classical hypotheses primarily focus on the initial stranding of fish on land—a necessary first step in the evolution of amphibious behaviour—as a voluntary measure of 'last resort' to escape adverse abiotic conditions in standing pools of water (e.g. Mandic et al., 2009; Sayer & Davenport, 1991).

Rock pools are likely a key staging ground for the colonisation of land by intertidal aquatic organisms (Giomi et al., 2014; Graham, 1997; Martin & Bridges, 1999), including blennies and other fish (Ord & Cooke, 2016; Richards, 2011). We tested four hypotheses of how the abiotic conditions of rock pools should deteriorate with the receding tide (Figure 1a) and how these conditions should, in turn, prompt fish to avoid these pools. We found mixed support for the hypothesised mechanistic basis of how conditions should change in rock pools, and only one factor that seemed to account for the absence of blennies from certain pools on Guam.

We did find that—as predicted (Figure 1a)—the standing water of rock pools at low tide increased in temperature as a function of ambient air temperature and rockpool size (Table 1a; Figure 3a). In fact, the temperature became so extreme in some pools that our data loggers reached their upper limit (~37°C; see Figure 2b). Yet temperature had little impact on the presence of blennies in rock pools (Table 2), with the vast majority of pools observed to have at least some aquatic and amphibious blennies, including the hottest pools (Figures 2b and 5; see also Brown et al., 1991). Furthermore, water temperature did not translate into the expected effects of increased salinity (via evaporation) or reduced oxygen saturation in pools (via temperature-dependent reductions in oxygen solubility; Figure 1a). Salinity instead decreased and saturated oxygen increased with water temperature (Table 1b,d). Rainfall impacts the salinity of most isolated rock pools at low tide (e.g. Johnson, 2001; Morris & Taylor, 1983), but the reduced salinity in our study was mainly the result of seepage from freshwater springs. It was not unusual to observe freshwater seepage flowing across the exposed rocks at low tide and flowing directly into isolated rock pools. While we have focussed on the classic hypothesis of stress associated with elevated salinity (Figure 1a), our observations of blennies within rock pools showed these fishes appeared tolerant to large drops in salinity as well (e.g. Figure 2b). Conversely, the amount of oxygen in rock pools was higher (not lower) when isolated at low tide compared to being completely submerged at high tide (Figure 2a; e.g. also Buasakaew et al., 2021). This was presumably because oxygen generated by algae and other primary producers in rock pools accumulated in pools when isolated at low tide and/or resulting from increased photosynthetic rates with increased water temperature (Figure 3b; Daniel & Boyden, 1975; Morris & Taylor, 1983; Richards, 2011). The pH of water was elevated in rock pools at low tide, but this was unrelated to water temperature (as hypothesised; Figure 1a) and represented only

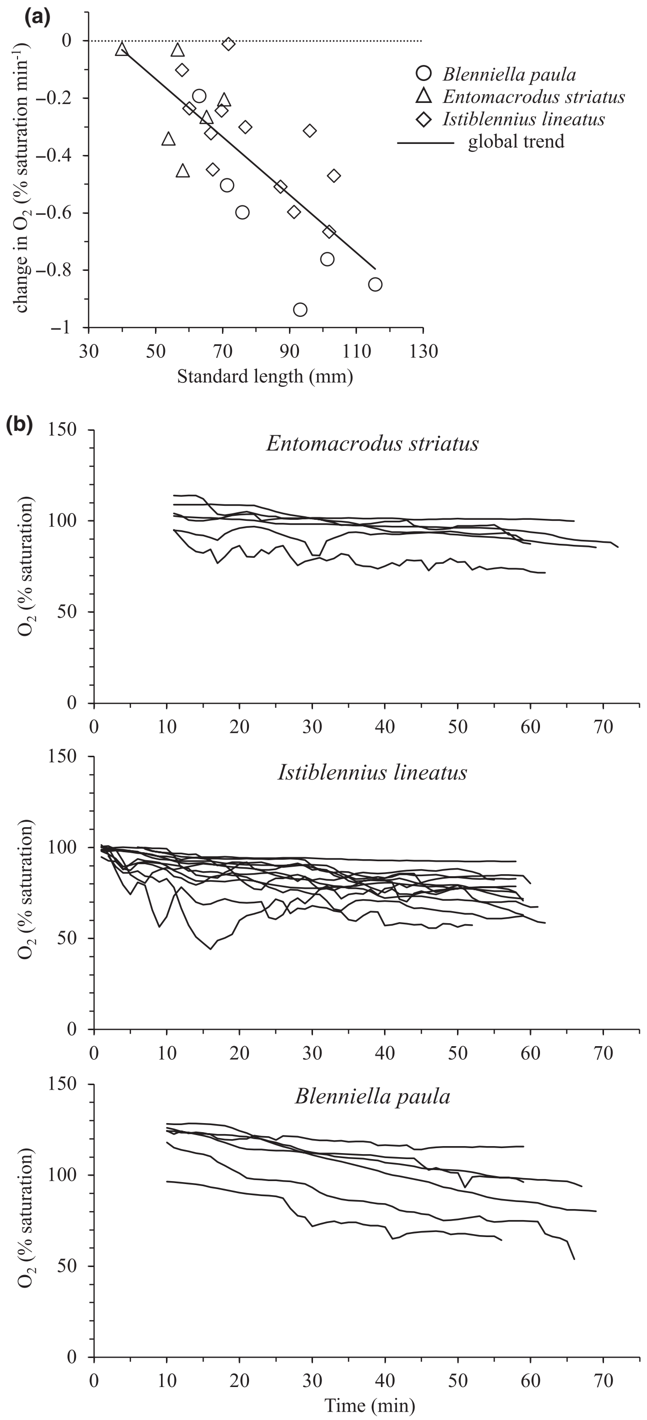


FIGURE 4 Oxygen consumption by blenny fish as a function of body size. The upper panel (a) shows individual regression coefficients of oxygen consumption (% saturation min^{-1}) for each fish tested in the simulated rockpool (housing 1 L of fresh seawater). Estimates and trend lines were computed from the model reported in Table S3. The lower panels (b) show individual traces of oxygen depletion for each fish tested. *Entomacrodus striatus* is a highly amphibious species, *Istiblennius lineatus* is a mildly amphibious species, and *Blenniella paula* is an exclusively aquatic species (see Ord & Cooke, 2016 for behavioural scorings).

a marginal increase in pH from that of high tide (a difference of just 0.2–0.3 units; e.g. Table 1c). Slight pH increases are consistent with higher photosynthetic rates, which reduce carbon dioxide dissolved in water. Neither differences in salinity nor pH were credible predictors of the abundance of blennies in rock pools (Table 2).

Taken together, either the underlying cause of how abiotic conditions should deteriorate in standing pools was not supported (salinity, pH and oxygen), or aquatic and amphibious blennies were still observed in reasonable abundances in rock pools despite poor abiotic conditions (temperature, salinity and pH). Hypoxia appeared to be the only situation that might account for the absence of blennies from certain rock pools (Table 2). But even here, our experimental tests inferred the vast majority of rock pools were almost certainly within the tolerable range for even the largest aquatic blenny species on the island. Oxygen depletion resulting from the respiration of an aquatic and two amphibious blenny species showed no credible differences among species. Instead, large fish depleted oxygen more quickly than small fish (Figure 4). We used the computed oxygen depletion from these blennies (Table S3) to calculate the likely amount of oxygen required to maintain a fish in a rockpool for varying amounts of time from low tide. Even for the longest period of 90 min (only possible during a king tide), most rock pools during the daytime were probably above the critical oxygen threshold for blennies (Table 3a,b). It remains unclear to what extent respiration by primary producers would deplete water oxygen in pools over night when photosynthesis is not possible. However, it would likely take many hours of isolation before hypoxic conditions would occur in rock pools (e.g. Truchot & Duhamel-Jouve, 1980), which rarely occur in intertidal habitats where amphibious and terrestrial blennies have evolved (Ord & Cooke, 2016). These experiments also showed a statistically distinguishable reduction in pH after an hour of respiration by blennies (during the day; see Section 2), and we can use these data to infer the potential change in pH for rock pools isolated overnight in the absence of producer photosynthesis. This drop in pH was an average of just 0.04 and unlikely to lead to any physiological stress for fish isolated in rock pools for prolonged periods at night. pH from respiration of primary producers can be expected to further decrease pH overnight, but again this would require many hours of isolation and still unlikely to result in pH levels outside the range recorded across rock pools in our study (see Truchot & Duhamel-Jouve, 1980).

Moreover, a projection of the critical partial pressures of oxygen (P_{crit}) required for respiration (compiled from Rogers et al., 2016) again showed most pools were well within the limits of most tropical marine fishes (Figure 5; Table 3b). Data on P_{crit} and subsequent emergence thresholds for amphibious intertidal sculpins (Mandic et al., 2009) and tropical mangrove killifish (Tigert et al., 2022) again support the general conclusion that most rock pools would almost certainly be within the tolerance range for these amphibious fishes as well. Unfortunately, direct comparison between blennies and these other amphibious groups is difficult because P_{crit} in sculpins and killifish were tested at water temperatures lower than those recorded in our study—12°C and 25°C, respectively—and P_{crit} tends to drop with decreasing water temperature (Figure 5; Table S4). P_{crit} also declines with decreasing salinity (Rogers et al., 2016) and

TABLE 3 Percentage of surveyed rock pools that would fall outside the likely tolerable range of saturated oxygen at low tide for three biologically and evolutionary relevant body sizes.

	Rock pools below threshold				
Standard length	**0 min at low tide (%)**	**30 min post-low (%)**	**60 min post-low (%)**	**90 min post-low (%)**	**Any time point (%)**
(a) Complete depletion					
8.9 cm	0.0	0.0	0.0	0.0	
11.6 cm	0.0	0.0	0.0	2.6	
19.0 cm	0.0	0.0	2.6	10.3	
Minimum O_2 recorded in pools					0.0
(b) Below or at critical partial pressure of oxygen (P_{crit})					
8.9 cm	0.0	0	2.6	2.6	
11.6 cm	0.0	2.6	2.6	2.6	
19.0 cm	0.0	2.6	7.7	25.6	
Minimum O_2 recorded in pools					7.5

Note: A standard length of 8.9 cm is the inferred evolutionary optimal size for an aquatic blenny (see Ord & Hundt, 2020); 11.6 cm is the largest blenny tested in our experiments—see Figure 4; while 19.0 cm is the average size of the largest aquatic blenny species, we have observed on Guam (data from Ord & Hundt, 2020). The percentage of pools at any time point relates to the minimum amount of saturated oxygen recorded directly in rock pools over the period of sampling. P_{crit} was inferred from data compiled by Rogers et al. (2016) on laboratory respirometry experiments using marine fish tested in water temperatures at or above 30°C. This threshold identifies rock pools with partial oxygen pressures likely to be hypoxic for most aquatic marine fishes (see Figure 5).

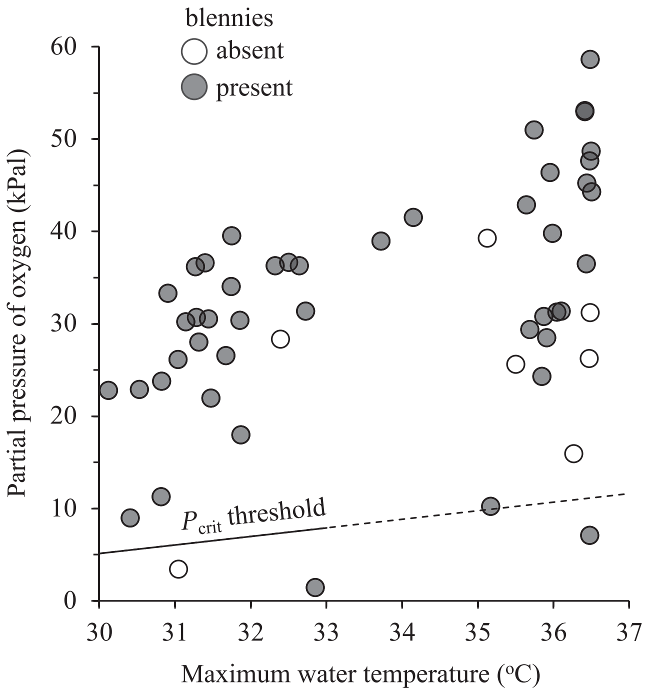


FIGURE 5 The number of rock pools below the computed P_{crit} threshold of partial oxygen pressure inferred for tropical marine fish over temperatures 30–33°C (solid trend line; Table S4; data from Rogers et al., 2016) and extrapolated to 37°C (dashed trend line). Partial oxygen pressures (kPa) are a conversion of rockpool minimum dissolved oxygen (mg/L), salinity and water temperature. Aquatic and amphibious blennies were observed in most rock pools, including those below the inferred and at the hottest rockpool temperatures (see also Figure 2b).

killifish occupy brackish habitats (Tigert et al., 2022). Nevertheless, regardless of the oxygen threshold assumed—based on the complete depletion of oxygen from pools (Table 3a) or P_{crit} from laboratory respiratory studies (Table 3b)—oxygen does not appear to be a limiting factor. Rather, the vast majority (83%) of rock pools were hyperoxic (i.e. >100% air saturation) with many (19%) acutely so (>200% air saturation; see Figure S2). Hyperoxia can expand the aerobic capacity of fishes to mitigate anaerobic stress from (for example) heat stress (reviewed by McArley, Sandblum, & Herbert, 2020). This suggests blennies might have greater capacity to tolerate high water temperatures at low tide, which would account for the prevalence of blennies in even the hottest rock pools we surveyed (Figure 2b).

The results of our study combined with past work on blennies imply that biotic pressures were probably the primary instigator of blennies emerging from water and ultimately facilitating the evolution of amphibious and terrestrial behaviour. The activity of amphibious blennies in and out of the water on other islands has been closely linked to the presence of aquatic predators: as the abundance of predatory fish increases in water, particularly with the incoming tide, amphibious blennies progressively move out of the water onto land (Ord et al., 2017). A comparison of life history traits across aquatic, amphibious and terrestrial blennies on Guam (the location of the current study) has also revealed predictable changes in growth rate and reproduction consistent with improved survival as species become more terrestrial (Platt et al., 2016; see also Platt & Ord, 2015). Predation on land is still a concern for blennies (Morgans & Ord, 2013), but considerably less so than those experienced in water (Ord et al., 2017). These data, combined with findings from the current study, suggest an ecological release from aquatic predation has likely been the main driver of both historic (Platt et al., 2016) and ongoing (Ord et al., 2017) transitions onto land by blennies.

In addition to sculpins and killifish, another obvious comparison to blennies is mudskippers in the family Gobiidae. Mudskippers are mildly amphibious using the scale applied to blennies (as are sculpins and killifish; Ord & Cooke, 2016) and are thought to have originated from two independent aquatic ancestors (Steppan et al., 2022). This is in stark contrast to the multiple independent transitions to land in blennies (Ord & Cooke, 2016). Blennies do not appear to be acutely sensitive to adverse abiotic conditions in rock pools (Figure 2b). While anecdotal observation of blennies experiencing the worst conditions suggested increased respiratory rate and reduced activity in the hottest and lowest oxygenated pools, we did not observe aquatic surface respiration or emergence from rock pools at low tide (e.g. see Mandic et al., 2009). An argument could be made that these blennies would eventually succumb to stress if conditions became protracted. Our computed maximum duration of isolation was 90 min, and only during a king tide. However, even during such a prolonged isolation most rock pools would remain tolerable for most blennies (Table 3a,b). Other intertidal environments routinely experience longer periods of isolation (e.g. several hours to days) and greater fluctuations in rockpool abiotic conditions (Truchot & Duhamel-Jouve, 1980; reviewed by Wolfe et al., 2020), but we focussed on the context specifically relevant for the origins of amphibious behaviour in Blenniidae. To this end, the intertidal zone on Guam is typical of the climate and intertidal topography for all of locations where we have studied amphibious and terrestrial blennies (Okinawa, Taiwan, Rarotonga, French Polynesia, Mauritius and Seychelles; reviewed by Ord & Cooke, 2016), as well as those on other islands where amphibious blennies have been reported to occur (La Réunion and Christmas Island; Fricke et al., 2024 and Allen, 2000, respectively). Given this, we consider our study to have been conducted in the same biological context where amphibious behaviour most likely evolved in this fish family. Our data suggests voluntary stranding to avoid a deteriorating aquatic environment with the receding tide probably cannot account for the repeated origins of amphibious behaviour in this family. Yet the intertidal zone has been an important transitional environment for the invasion of land by fishes more generally (Ord & Cooke, 2016). Living in such a dynamic ecotone that oscillates constantly between the extremes of high and low tide has presumably primed the broad abiotic tolerances of blennies, and potentially other intertidal fishes as well (Azevedo e Silva et al., 2022; Mandic et al., 2009; Rummer et al., 2009). This, coupled with blennies' flexibility in other characteristics (e.g. diet; Ord & Hundt, 2020), has likely allowed blennies to endure the terrestrial conditions to ultimately escape aquatic predation.

Ecotones that fluctuate in abiotic conditions (mangroves, estuaries) or are characterised by a steep abiotic gradient (grasslands, marshlands) are potentially important staging grounds for major transitions in habitat. While not necessarily pushing organisms into adjacent habitats to escape poor abiotic conditions (consider Sullivan et al., 2015), these transitional environments instead select for higher ecological tolerances that ultimately enable the colonisation of novel habitats. As such, ecotones are potentially important environments in generating adaptive diversification (e.g. Cooke et al., 2014; Mullen & Hoekstra, 2008; Rosenblum, 2006). Troublingly, ecotones are also under threat from the current climate crisis (Alahuhta et al., 2011; Gonzalez et al., 2010; Helmuth et al., 2002; Mayle et al., 2007; Oliveras & Malhi, 2016; Smale et al., 2019; Tittensor et al., 2010) and other human impacts (Friess et al., 2019). Their decline is of concern for the loss of both present-day and future biodiversity.

AUTHOR CONTRIBUTIONS

Terry J. Ord and Elizabeth A. Surovic surveyed rock pools; Terry J. Ord, Iker Irisarri, Elizabeth A. Surovic and Diego F. B. Vaz collected fish for the simulated rockpool experiment; Terry J. Ord, Iker Irisarri and Elizabeth A. Surovic performed the experiment; Terry J. Ord, Iker Irisarri and Diego F. B. Vaz obtained research approvals and permits; Iker Irisarri and Terry J. Ord funded the work, and Diego F. B. Vaz provided logistical support for all aspects of the work; Terry J. Ord performed analyses and wrote the initial draft of the paper, with all authors contributing to final revisions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.ht76hdrrd (Ord et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Factors associated with changed rockpool conditions at low tide versus high tide, excluding pools seen to be periodically flushed with seawater at low tide (a, d) as well as near freshwater springs (b, c).

Table S2. Support of models accounting for combinations of body size and species identity and their relation to oxygen consumption per minute in three species of blenny.

Table S3. Factors associated with the depletion of oxygen (% air saturation) by blenny fishes.

Table S4. The computed P_{crit} threshold as a function of water temperature estimated from marine fishes tested at 30–33°C.

Figure S1. There was a strong, positive association between the measured volume of rockpools (calculated from manually emptying pools with measuring jugs) and the volume inferred from the formula of half an ellipsoid using the length, width and depth of pools.

Figure S2. Minimum percentage air saturation of rockpool water as a function of minimum dissolved oxygen at low tide shows most rockpools were hyperoxic (>100% saturation).

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