

Repeated evolution of amphibious behavior in fish and its implications for the colonization of novel environments

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We know little about on how frequently transitions into new habitats occur, especially the colonization of novel environments that are the most likely to instigate adaptive evolution. One of the most extreme ecological transitions has been the shift in habitat associated with the move from water to land by amphibious fish. We provide the first phylogenetic investigation of these transitions for living fish. Thirty-three families have species reported to be amphibious and these are likely independent evolutionary origins of fish emerging onto land. Phylogenetic reconstructions of closely related taxa within one of these families, the Blenniidae, inferred as many as seven convergences on a highly amphibious lifestyle. Taken together, there appear to be few constraints on fish emerging onto land given amphibious behavior has evolved repeatedly many times across ecologically diverse families. The colonization of novel habitats by other taxa resulting in less dramatic changes in environment should be equally, if not, more frequent in nature, providing an important prerequisite for subsequent adaptive differentiation.

KEY WORDS: Amphibious fish, blenny, convergent evolution, intertidal zone, mudskipper, water–land invasion.

Despite the broad evolutionary implications of colonizing new environments for adaptive evolution and ecological speciation (Schluter 2000; Coyne and Orr 2004; Price 2008; Nosil 2012), there has been little study of how frequent transitions into new habitats might be in nature and what factors might prompt taxa to make those shifts in habitat in the first place (e.g., see Crisp et al. 2009 for a rare example on plants). Most work on colonization comes from the biological invasion literature that focuses heavily on pest species for obvious ecological and economic reasons (Kolar and Lodge 2001; Jeschke and Strayer 2006; Hayes and Barry 2008; Simberloff 2013). This work has been critical in highlighting several general factors that are likely broadly relevant for most colonization events in nature. For example, the degree to which environmental conditions differ among habitats has obvious implications for the likelihood of colonization for most taxa, as does the ability of would-be colonizers to physically access new areas (e.g., if geographic barriers can be circumvented in

some way). The number of ecologically similar taxa in an area can also have an impact on the ability of colonizers to successfully establish (Allen 2006; Colautti et al. 2006), which extends to the more general phenomena of how competition can influence community assembly (Emerson and Gillespie 2008) and the likelihood of character displacement occurring among interacting taxa (Pfennig and Pfennig 2012).

However, this literature has also highlighted that most populations fail to establish in new areas (e.g., Williamson and Fitter 1996). This raises the general question of how frequently transitions into new habitats occur in nature, especially the colonization of novel environments that are the most likely to instigate adaptive evolution. Such ecological transitions have clearly occurred repeatedly in the history of life given that virtually every environment on earth has some living organism occupying it. There is also increasing evidence that ecological speciation—divergent natural selection between environments that generates the evolution



of new species—is probably common in nature (Schluter 2009; Shafer and Wolf 2013). However, whether this ecological diversity reflects frequent colonization or the culmination of rare events over long periods of evolutionary time is unclear.

One of the most extreme examples of an organism transitioning into a novel environment, and one that has been a critical step in the history of life more generally (Laurin 2010), has been the colonization of land by fish. The change in medium—water to air—presents a number of critical challenges for respiration, metabolism, and locomotion. Intuitively, these challenges imply that leaving water should be difficult for fish and consequently quite rare. Well-known examples of amphibious behavior in fish such as the iconic mudskippers should therefore be viewed as special cases. However, several reviews have catalogued amphibious behavior in fish from a number of different families (e.g., Sayer and Davenport 1991; Graham 1997; Martin 2014; Pace and Gibb 2014), which implies amphibious behavior might actually be quite common. This has general implications for understanding how easily aquatic to terrestrial transitions might be for fish, both historically and for extant taxa, and offers a model system for studying the colonization of new environments in nonpest species more broadly. The notion of fish colonizing land may not be as unlikely as it might first appear, and successful ecological transitions in nature might be more likely than suggested by the failure of human-assisted introductions.

Unfortunately the incidence of amphibious behavior in fishes has rarely been considered within an explicit phylogenetic context (e.g., Morgans and Ord 2013) and no attempt has been made to formally quantify common ecological variables shared among amphibious fish (although there are qualitative reviews, such as Martin 1995 and Sayer 2005). It is therefore unknown whether reported examples of amphibious behavior tend to be clustered among a subset of closely related phylogenetic groups—which would suggest independent origins of amphibious behavior are more rare than might be inferred from the absolute numbers of taxa reported to exhibit such behavior—or whether amphibious fish share key ecological characteristics that might predisposed them to leave water. There has also been little attempt to formally quantify variation in amphibious behavior beyond coarse classifications of “passive” versus “active” emergence (Martin 2014). Fine-scale variation in amphibious behavior does exist among closely related species within several families (Morgans and Ord 2013; You et al. 2014), and this invites comparative study for assessing the evolutionary history of amphibious behavior.

Although almost all previous studies of amphibious behavior in fish have focused on the specific challenges associated with moving from a fluid to aerial environment (see Results), questions relating to how common ecological transitions by animals might be in nature and what factors might prompt those shifts

in habitat are equally applicable to this system. Furthermore, the dramatic nature of the transition should provide a powerful model for answering these questions that might otherwise be difficult to resolve through the study of less substantial ecological transitions. It is within this general context that we provide the first phylogenetic review of reports of amphibious behavior in fish, a meta-analysis of ecological variables common to these fish, and the only large-scale empirical and phylogenetic study conducted on amphibious behavior among closely related taxa within any fish group.

Our study was conducted in four parts. First, we compiled reports of amphibious behavior in fish from a major survey of published literature. These examples were then positioned onto a phylogeny to obtain a broad view of the phylogenetic diversity of amphibious behavior across all fishes and how frequently amphibious behavior might have evolved in fish. Second, the ecology of amphibious fish was then compared to all other species within the same families. This analysis focused on several ecological variables associated with factors previously implicated in prompting fish to emerge from water or that were likely to facilitate transitions between habitat types more generally:

- (1) Taxa tending to occupy the fringes of habitats or environments in which the abiotic conditions fluctuate widely should be taxa that tend to explore adjacent environments more readily, either because of opportunity (proximity) or necessity (adverse changes in abiotic conditions). In this sense, fish living in ecotones such as the intertidal zone or in freshwater systems close to terrestrial habitats (or that tend to contract or disappear entirely through evaporation) should be more likely to exhibit amphibious behavior compared to open water taxa.
- (2) Fish have been shown to voluntarily strand themselves out of water to avoid hypoxia in laboratory experiments (Martin 1996), which is believed to be a frequent cause of emergence in nature (Wells et al. 1984; Martin 1995; Turko et al. 2012; Urbina et al. 2014). The amount of dissolved oxygen in water is inversely correlated to temperature; as the temperature of water increases, the likelihood of hypoxia for fish also increases. Furthermore, aquatic fauna can be impacted by increases in temperature directly (Gibson et al. 2015) or through the reduction of closed water bodies from evaporation (e.g., rock pools and ponds; Ojha et al. 1979; Martin et al. 1993; Laberge and Walsh 2011). These issues are presumably most relevant for fish living in tropical regions. For example, a recent study revealed multiple invasions of land by true crabs, but only in tropical areas and because of the greater incidence of hypoxia in warmer climates prompt crabs to transition onto land (Giomi et al. 2014). This predicts that the incidence of

amphibious behavior in fish should be concentrated in the tropics compared to cooler climatic regions.

- (3) Fish that tend to be in close association with the substrate would presumably be more likely to possess features better suited for ground-based emergence onto land compared to pelagic fish. For example, the cylindrical body shape common to benthic fish seems ideal for reducing desiccation on emergence and facilitating terrestrial locomotion (Blob et al. 2008; Hsieh 2010).
- (4) Nevertheless, given the general difficulties of terrestrial locomotion for most fish (Hsieh 2010; Pace and Gibb 2011; Pronko et al. 2013), those that do not rely on the capture of mobile prey might more readily transition to a terrestrial lifestyle than carnivores.

Third, we focused on one family—Blenniidae—reported to have a large proportion of genera (14%; see Results) exhibiting amphibious behavior. Here, we conducted a systematic field survey quantifying differences in behavior among several closely related lineages from a variety of locations around the Pacific and Indian Oceans. These data were analyzed in conjunction with a newly created phylogeny to reconstruct the history of emergence onto land in this group. Within the Salariaiini division of blennies, there are known to be species exhibiting a range of amphibious behavior, from the marine ancestral phenotype of never emerging from water to a highly terrestrial phenotype in which adult fish rarely (if ever) voluntarily return to water (Brown et al. 1991; Bhikajee and Green 2002; Shimizu et al. 2006; Depczynski and Gagliano 2007; Ord and Hsieh 2011). Each of these species in effect represents snapshots of the progressive transition from water to land and offers an excellent opportunity to reconstruct the evolutionary history of the colonization of a novel habitat.

Finally, it was evident from the field survey of Blenniidae that there was considerable variation in amphibious behavior among individual blennies within taxa. To explore this further, we selected two of the most variable and best sampled taxa to examine whether the propensity to emerge from water was related to tide level or air temperature. We focused on these variables because past work on a highly terrestrial species of blenny showed activity was dependent on both tide and air temperature (Ord and Hsieh 2011). At low tide and during high temperatures, desiccation is likely an acute problem and blennies retreat into moist rock holes and crevices above the waterline (“terrestrial” blennies are more generally limited to the splash zone on land for the same reasons; Ord and Hsieh 2011). Similar behavior has been observed in amphibious fish from other families as well (amphibious mudskippers: Colombini et al. 1995). Furthermore, for blennies active on land, violent wave action at high tide tends to impede the ability of fish to move freely about on the rocks and this further

limits their activity. This suggests species that might otherwise be largely aquatic could in fact be capable of a high degree of terrestrial activity during those brief periods on land when conditions are potentially suitable for emergence (i.e., at mid-tide and moderate air temperatures), and this would have general implications for the overall diversity of amphibious behavior estimated across the group.

Materials and Methods

The Supporting Information accompanying this article provides additional details on methodology not covered in the sections below.

LITERATURE SURVEY

Two strategies were used to compile reports of amphibious behavior from the literature. First, a systematic search using ISI Web of Science and the topic terms “amphibious fish*,” “land fish*,” “terrestrial fish*,” “supra littoral fish*,” or “land dwelling fish*” across all years of publication and for all research areas in the “science technology” domain. The titles and abstracts of all publications identified by this search were assessed manually, and if potentially relevant, were downloaded through the UNSW library gateway for detailed review. Second, we used all previous review articles and several books on amphibious behavior in fish known to us to include additional species not initially uncovered by our own systematic literature search.

Once amphibious behavior was identified, we assigned a broad classification of the level of terrestrial activity exhibited by species: those reported to voluntarily strand themselves on land as water bodies recede (e.g., ebbing tides or ponds evaporating) were classified as “mildly amphibious, passively emerge,” those that actively leave water for short periods (minutes; e.g., to lunge at terrestrial prey or circumvent terrestrial obstacles) were classified as “mildly amphibious, actively emerge,” those that leave water and were active on land for extended periods (hours) were classified as “amphibious,” while those that spent the vast majority (or all) of their time out of the water and were highly terrestrially active were classified as “highly amphibious.” These classifications are provided for each species in Table S1. Species synonyms and current species names were based on those given by FishBase.org.

We then used the time-calibrated phylogeny developed by Betancur-R et al. (2015) to illustrate the distribution of any kind of amphibious behavior across all extant fishes at the family level. A formal ancestor state reconstruction was not possible for this aspect of our study; although we had data on the presence of amphibious behavior in species from the literature, we did not have companion data on the absence of amphibious behavior in other fishes. That is, it was impossible to know whether other

species lacked amphibious behavior (were exclusively aquatic) or whether they simply had not been studied to confirm (or refute) amphibious behavior.

META-ANALYSIS OF ECOLOGICAL FACTORS

Primary sources reporting amphibious behavior were also used to obtain information on general ecological attributes of species. That is, whether species were the following: aquatic, intertidal, estuarine, or freshwater (environment); tropical, subtropical, temperate, or polar (climate); demersal or pelagic (lifestyle); or carnivores, omnivores, herbivores, or detritivores (diet). In cases in which this information could not be obtained from primary sources, it was obtained from species descriptions in Fishbase.org.

With this information we evaluated whether certain ecological attributes tended to be associated with fish that exhibited some level of terrestrial activity. To this end, we compared the proportion of amphibious genera for a given ecological category to a null distribution of the proportion of genera that would be expected for that category given the general ecological diversity of fishes in those families (see Supporting Information). Amphibious genera were defined as those that had at least one species reported to exhibit any level of amphibious behavior. Some genera had amphibious species represented in more than one ecological category, which meant that proportions across categories summed to values greater than 1. Given this, comparisons were only made between the proportions of amphibious genera and the null expectation within, rather than across, ecological categories. Statistically distinguishable effects of the ecology of amphibious genera relative to the null were determined based on whether the 95% confidence intervals of proportions overlapped. These confidence intervals were computed using formula presented in Zar (1999).

PHYLOGENY OF AMPHIBIOUS BEHAVIOR IN BLENNY FISH

We conducted a large field study of the behavior of fishes within the Salariini division of the Blenniidae family to resolve the evolutionary history of terrestrial activity in this group. This study provided a fine scale view of how frequently amphibious behavior might have arisen in fish than was possible from the literature survey described in the previous section.

We focused on sampling blennies at seven geographic locations: three localities in the North Pacific (Guam, Okinawa, and Taiwan), two in the South Pacific (Rarotonga and Moorea/Tahiti), and two in the Indian Ocean (Mauritius and Seychelles). Surveys of behavior and specimen collection were made using snorkel for aquatic species, and by walking through the intertidal and supralittoral zone for amphibious and terrestrial species. Behavior was scored by the authors and several trained assistants using a

standardized key (Table S2; see also Supporting Information for other details).

DNA for both the mitochondrial and nuclear genomes was extracted from specimens collected by us on field trips and supplemented with marine specimens caught by colleagues on SCUBA (Table S4). Sequence data were collected from the following regions: 16S rRNA (16S), ATPase synthase subunits 8 and 6 (ATPase 6, 8), Cytochrome Oxidase subunit I (COI), recombination-activating gene 1 (RAG 1), zic family member 1 (zic1), hypothetical protein LOC564097 (Ptr), T-box brain 1 (tbr1), and super-conserved receptor expressed in brain 2 (sreb2). Details on DNA extraction, PCR, and sequencing methods are described in the Supporting Information.

The construction of the phylogeny was then made using three different methods: Maximum Likelihood, Bayesian Inference, and Coalescence-based Species-Tree estimation (Supporting Information Methods). Each method generally produced consistent topologies (Supporting Information Results). However, to illustrate ancestor state reconstructions of behavior, we selected the consensus tree from the Bayesian inference analyses of the partitioned concatenated dataset because this method resulted in better-supported phylogenies. This phylogeny was arbitrarily ultrametricized using Mesquite version 3.04 (Maddison and Maddison 2015). We also repeated ancestor state reconstructions using phylogenies based on maximum likelihood and coalescence-based species-tree methods, in addition to evaluating the potential impact of taxon sampling on our ancestor state reconstructions. Findings from these additional analyses are provided in full in the Supporting Information.

We used R version 3.2.4 (R Development Core Team, R Foundation for Statistical Computing, Vienna) and the package “phytools” version 0.5-20 (Revell 2012) and the option “fastAnc” to reconstruct the evolutionary history of terrestrial behavior using maximum likelihood. These estimates were visualized onto the phylogeny using “contMap.” Two sets of reconstructions were performed: one based on the mean behavioral score computed for each taxon, and a second based on the highest behavioral score recorded for each taxa. The first provided a general view of how average behavior might have changed over evolutionary time, whereas the second analysis had the potential to provide a clearer picture of the true level of emergence behavior exhibited by taxa (see Results and Discussion). Both sets of reconstructions were performed on the consensus tree and 1000 evenly spaced samples of the posterior distribution to accommodate phylogenetic uncertainty (after discarding the first 25% as burn-in). A custom-written R function (provided in the Supporting Information) was then used to count the number of independent evolutionary transitions to a highly amphibious/terrestrial lifestyle (a behavioral rating of ≥ 3.0) or to a mildly amphibious lifestyle (≥ 1.0) across phylogenies.

PLASTICITY IN AMPHIBIOUS BEHAVIOR:

Entomacrodus striatus AND *Praealticus labrovittas*

We focused on two mildly amphibious species—*E. striatus* and *P. labrovittas*—that had been intensively surveyed ($N = 64$ and 59 , respectively) at the same sites on the island of Guam. We then made a qualitative comparison of the propensity for terrestrial activity in these two species to a highly terrestrial species—*Alticus arnoldorum*—at the same sites as a function of tide and air temperature (data from Ord and Hsieh 2011; *A. arnoldorum* is considered to be a “terrestrial” fish in the sense that it likely spends its entire adult life out of the water on land).

We used a model selection approach to evaluate the level of support for alternative models that assessed the independent and combined effect of tide level and air temperature relative to an intercept-only null model. All models were applied within the R environment using the “lm” base function with a Poisson error distribution to accommodate the structure of the count data. Model support was assessed using the second-order Akaike’s information criterion, AIC_c , calculated using formula presented in Burnham and Anderson (2002). We also computed AIC_w to provide a general measure of the relative support for a given model to all others considered. Effect sizes in the form of t -values are reported to indicate the magnitude and direction of effect for those models with ΔAIC_c values ≤ 2.0 .

Results

DISTRIBUTION OF AMPHIBIOUS BEHAVIOR ACROSS FAMILIES OF ALL FISHES

Reports of amphibious behavior were widespread, occurring in 130 species from 73 genera (Table S1). Although the precise number of independent evolutionary origins of emergence behavior cannot be determined (see Methods), there are clearly many separate instances across the phylogeny of fishes (Fig. 1). For example, there were 33 families with at least one species that exhibited some level of amphibious behavior and many of these are likely examples of the repeated evolution of terrestrial activity. The majority of these reports were of species observed out of water for a matter of seconds or minutes. However, four families had species spending extended periods (hours or days) out of the water: Gobiidae (specifically mudskippers), Blenniidae (rockskippers), Synbranchidae (eels), and Labrisomidae (four-eyed blenny). The Supporting Information provides additionally summary of the main research foci and taxonomic coverage of the literature on amphibious behavior.

ECOLOGICAL ASSOCIATIONS WITH AMPHIBIOUS BEHAVIOR AMONG GENERA

A considerable proportion of amphibious genera were those making excursions onto land from the intertidal zone, and this

proportion was significantly higher than would be expected based on the general proportion of intertidal fish genera in these families (Fig. 1). Conversely, there were substantially fewer amphibious genera from aquatic or estuarine environments given the proportion of fish genera occupying these environments. Most amphibious genera were tropical, but there were still many representatives from other climate regions as well (Fig. 1), including reports of amphibious behavior in polar species (the capelin, *Mallotus villosus*, and rock gunnel, *Pholis gunnellus*; Table S1). Furthermore, these climatic distributions were virtually identical to the general distribution of fish genera in these families (i.e., climate was not a specific predictor of amphibious behavior). The vast majority of amphibious genera were demersal, but again this was a general property of fish genera in these families (Fig. 1). Finally, there were significantly fewer carnivorous amphibious genera than expected given the proportion of carnivorous fish in these families (Fig. 1).

EVOLUTIONARY HISTORY OF AMPHIBIOUS BEHAVIOR WITHIN BLENNY FISH

Results of the construction of the phylogeny are provided in the Supporting Information. Alternative ancestor state reconstructions accounting for missing genera or using phylogenies constructed with alternative methods are also reported in the Supporting Information and recovered similar numbers of convergences in behavior as described below (Figs. S6–S8).

By the most conservative estimate, ancestor reconstructions of mean behavior inferred at least three independent convergences on a highly amphibious or terrestrial lifestyle in the Salarinian blennies, specifically in *E. caudofasicatus*, *E. striatus* (Okinawa), and the monophyletic group of *Andamia* and *Alticus* (Fig. 2). There was also some evidence for additional convergences on a mildly amphibious phenotype—for example, most notably in select populations of *Istiblennius lineatus* and *Praealticus margaritarius*—and potentially a reversal back to an aquatic lifestyle in the broader monophyletic group encompassing *Istiblennius* and *Blenniella*, and possibly the remaining *Praealticus* lineages (Fig. 2).

However, most taxa sampled exhibited a wide range of behaviors, and with only few exceptions (e.g., *Andamia* and *Alticus* were almost always exclusively classified as highly terrestrial, whereas *Blenniella* were always classified as aquatic). Reconstructions based on the highest behavioral score for taxa, which possibly represents the true propensity to emerge from water, inferred as many as seven convergences on a highly amphibious/terrestrial lifestyle (Fig. 3). All these taxa seemed to share a common ancestor that was likely to have been quite amphibious at the outset, and again several lineages appear to have returned to a fully aquatic lifestyle (Fig. 3).

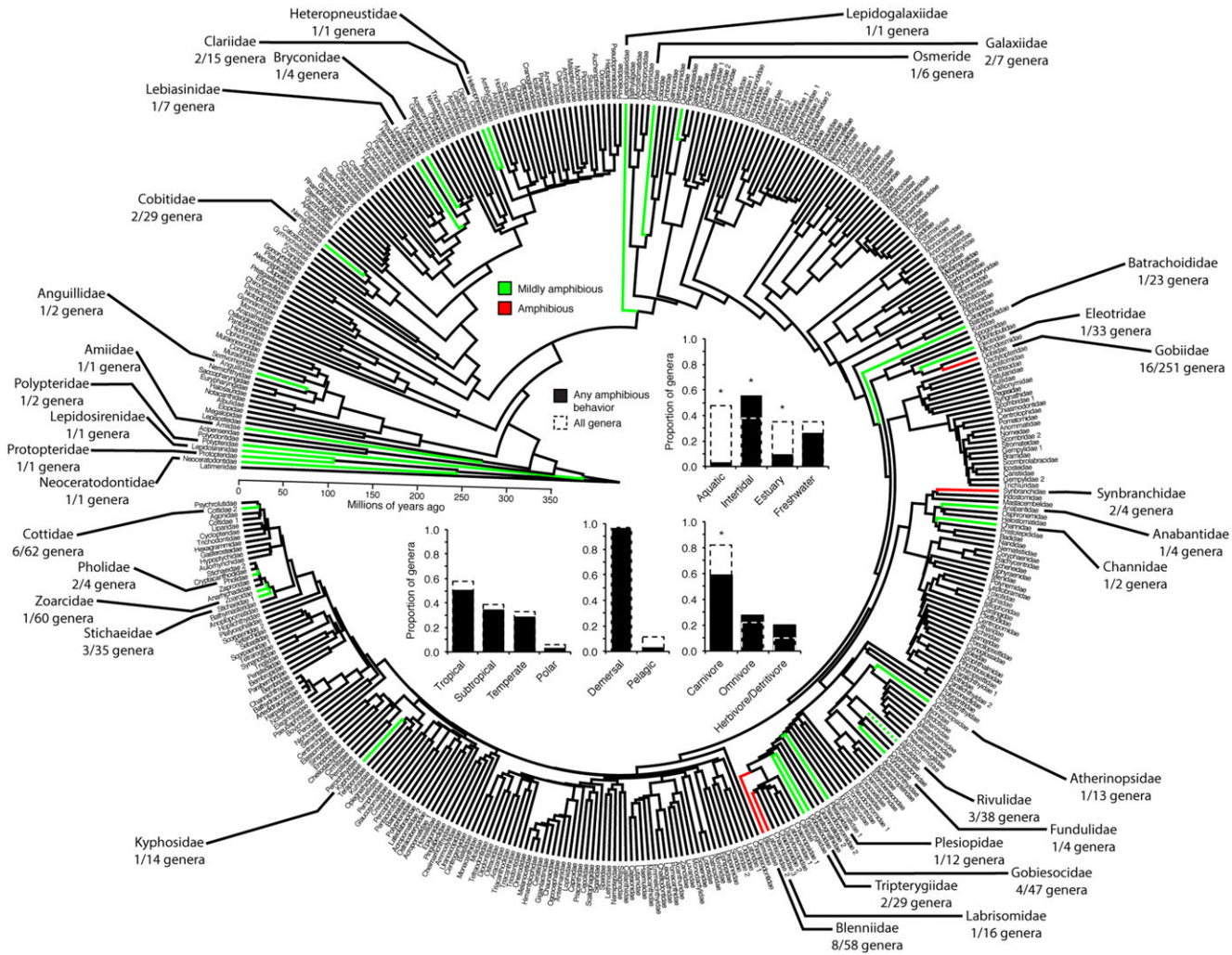


Figure 1. Phylogeny and general ecology of amphibious behavior in all fishes. Highlighted in color are the 33 families in which at least one species has been reported to exhibit amphibious behavior (see Table S1). Color codes provide a general indication of the maximum level of amphibious behavior reported for species within those families. This ranges from “mildly amphibious” in green—species known to voluntarily strand themselves on land as water bodies recede, or actively leave water for brief terrestrial excursions (lasting seconds to minutes)—to “amphibious” in red—species that are highly active on land for extended periods (hours; see Table S1 for details). Also shown is the number of genera represented by amphibious species relative to the total number of described genera within each family. For example, in Gobiidae there were 16 genera of the total 251 genera described within this family with species reported to be amphibious. In histograms, the black bars show the proportion of genera with at least one amphibious species categorized by habitat, climate, habit, or diet (see Table S1). Some genera had amphibious species represented in more than one category, so proportions across categories sum to a value greater than 1. Dashed bars in histograms indicate the proportion of all described genera—amphibious and nonamphibious—within these same families. Asterisks highlight instances in which the number of amphibious genera within a given category differs significantly from the total number of genera in those families that occur in this category more generally. The phylogeny was provided by Ricardo Betancur-R and is described in Betancur-R et al. (2015).

TIDE AND TEMPERATURE EFFECTS ON TERRESTRIAL ACTIVITY IN *E. striatus* AND *P. labrovittas*

The influence of tide and air temperature was equivocal for *E. striatus* with the null, tide, and temperature models all receiving comparable support ($\Delta AIC_c \leq 2.0$; Table 1A). The effect sizes were largest for the influence of tide, which suggested peaks in activity at mid-tide (Fig. 4B) similar to that of the highly

terrestrial species *A. arnoldorum* (Fig. 4A). However, the magnitude of the effect of tide was not large ($t < 2$; Table 1) and the null model was the best-supported model overall. Results for *Praeaalticus labrovittas* were clearer with overwhelming support for the influence of temperature on emergence behavior (Table 1B): activity out of water increased with temperature up to approximately 28°C and then decreased at higher temperatures

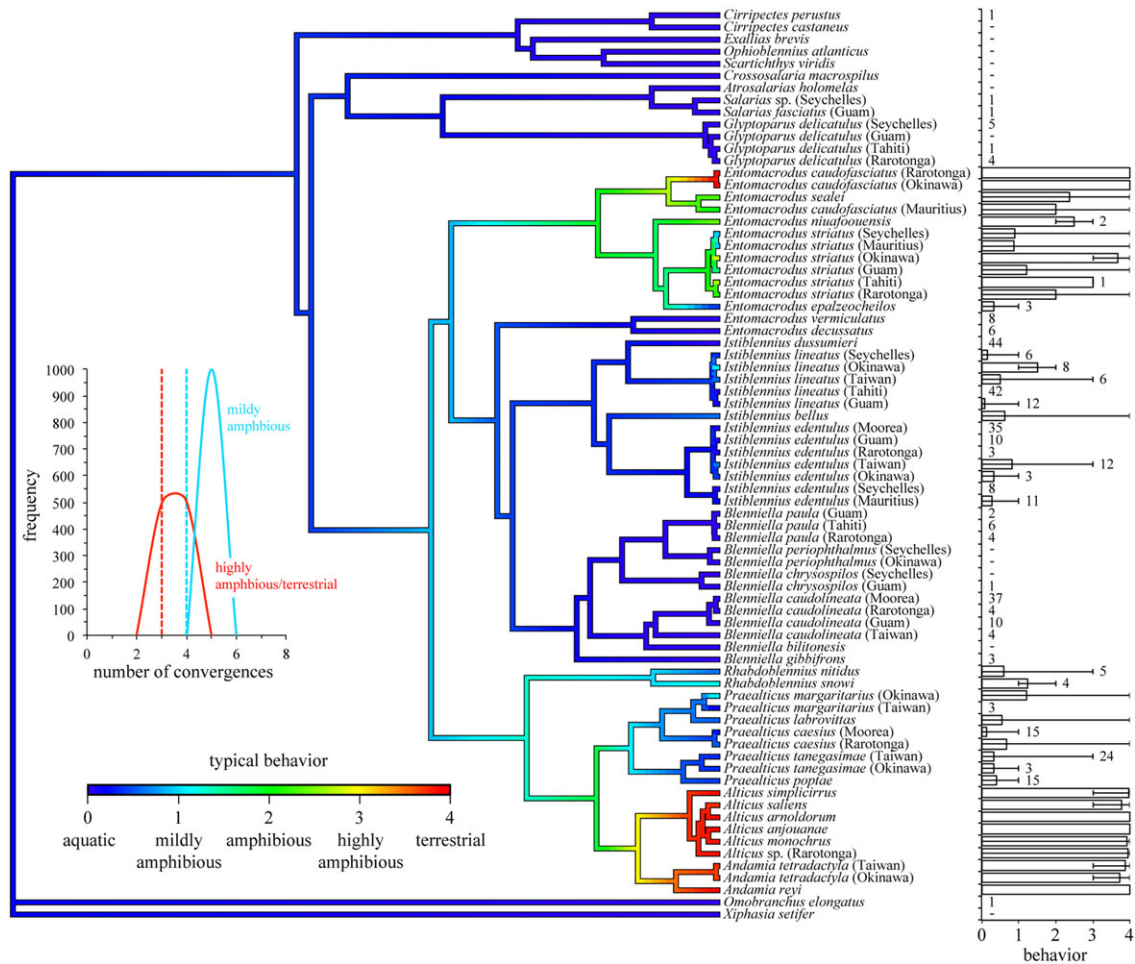


Figure 2. The evolution of amphibious behavior in Blenniidae. The phylogeny shown is the Bayesian inference of the partitioned concatenated dataset (see Supporting Information for reconstructions based on other methods). Reconstructed onto this phylogeny is the mean (typical) behavioral rating measured for each taxa based on the coding described in Table S2. The histogram at the tips of the phylogeny provides the mean, minimum, and maximum values of this rating. Numbers alongside histogram bars are the number of individuals sampled within taxa. Those taxa with “-” are known to be exclusively aquatic and were included in the phylogeny using tissue samples from donated specimens (all collected using SCUBA). The inset reports the number of convergences in a highly amphibious/terrestrial lifestyle or mildly amphibious lifestyle recovered across the consensus tree (dashed vertical lines) or from 1000 samples of the posterior distribution used to create the phylogeny (solid lines).

(Fig. 4C), a trend that was generally comparable to the abundance of the highly terrestrial species *A. arnoldorum* at the same sites (Fig. 4A).

Discussion

The repeated evolution of amphibious behavior has occurred many times across the phylogeny of fishes. There are at least 33 families in which one or more species has been reported to exhibit some level of terrestrial activity and many of these are likely to be independent origins of amphibious behavior (Fig. 1). Furthermore, within one of those families—Blenniidae—our phylogenetic reconstructions of behavior from field observations inferred at least three and as many as seven convergences on a

highly terrestrial lifestyle (Figs. 2 and 3). It is possible then that repeated origins of amphibious behavior have occurred within other families as well (e.g., Gobiidae). Our data also suggest that many otherwise aquatic fish might still exhibit a propensity for terrestrial activity under certain conditions. At least one primarily aquatic blenny was found to actively emerge onto land during conditions that have been previously shown to correspond to peak activity periods for more terrestrial fishes (mid-tide and moderate air temperatures; Fig. 4, Table 1; e.g., Colombini et al. 1995; Ord and Hsieh 2011). Classifications of behavior given to fish may subsequently depend on when those fish were observed, with the implication being that amphibious behavior could be even more widespread than currently reported in the literature.

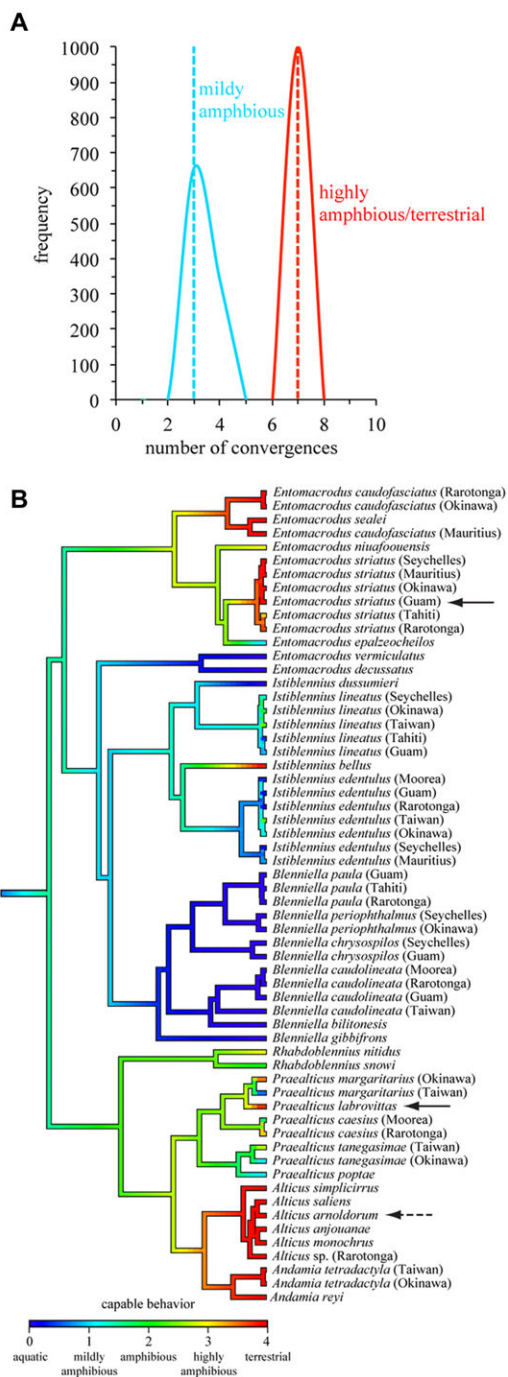


Figure 3. Evolution of amphibious behavior in Blenniidae based on the highest behavioral rating recorded for taxa. These ratings likely reflect the true potential for emergence behavior in a given taxa. See Table S2 for description of coding used. The top panel (A) reports the number of convergences in a highly amphibious/terrestrial lifestyle or mildly amphibious lifestyle recovered across the consensus tree (dashed vertical lines) or from 1000 samples of the posterior distribution used to create the phylogeny (solid lines). The bottom panel (B) provides a detail of the reconstruction based on the consensus tree (comparable to that of mean behavior shown in Fig. 2). Arrows highlight the position of taxa detailed in Fig. 4.

It is also worth noting the apparent cluster of amphibious behavior in some of the most ancient fish lineages; the lobefinned lungfish (Neoceratodontidae, Protopteridae, and Lepidosirenidae) and several ancient ray-finned fishes (Polypteridae and Amiidae) are some of the closest living relatives to the early tetrapods that made the first forays onto land towards the end of the Devonian, and that ultimately led to the evolution of all land dwelling vertebrates (Graham and Lee 2004; Laurin 2010). Such transitions from an aquatic to terrestrial environment have obviously been critical evolutionary steps in the history of life. However, our data show that crossing the water–land interface is not evolutionarily exceptional in itself, at least in the case of extant fish. Yet there are only a handful of instances in which organisms—plant, insect, or vertebrate—have gone on to proliferate into fully fledged terrestrial radiations (Vermeij and Dudley 2000; Laurin 2010). Our study suggests this is unlikely because of any major constraint on making the initial transition to land, given that terrestrial activity appears common in many living fishes. Indeed, some of the most obvious challenges that would be faced by an aquatic fish on land, such as those associated with respiration and other physiological requirements (Sayer 2005; Martin 2014), locomotion (Pace and Gibb 2014), and reproduction (Martin et al. 2004), seem to have been readily overcome.

An obvious explanation for the limited distribution of extant fishes on land to areas adjacent to water is the continued risk of desiccation (Sayer 2005). The vast majority of amphibious fishes belonged to families that were overwhelmingly demersal and, although not an exclusive characteristic of amphibious fish specifically (Fig. 1), many tended to exhibit the typical benthic cylindrical body shape that should facilitate terrestrial locomotion (Hsieh 2010; Gibb et al. 2013) and help reduce desiccation (Horn and Riegle 1981; Sayer 2005). Critically, however, respiration for most amphibious fish continues to involve gills and to some extent occurs through the skin (Sayer 2005; Uchiyama et al. 2012; Martin 2014), and the maintenance of moisture on these respiratory surfaces is needed to facilitate gaseous exchange and avoid asphyxiation. Terrestrial crustaceans have been particularly successful in evolving strategies for avoiding desiccation and maintaining effective respiration using gills (e.g., Farrelly and Greenaway 1987, 1992), but the possession of an exoskeleton has clearly been important in their establishment on land in this regard. The early tetrapod colonizers such as Tiktaalik were also at an advantage with the possession of a primitive lung as well as gills prior to the permanent transition to land. In this way, they were also less susceptible to respiratory problems associated with desiccation (Laurin 2010).

Others have suggested competition from resident taxa or predation has been important limiting factors to terrestrial establishment for aquatic organisms (Vermeij and Dudley 2000). Competition from ecologically similar species and increased predation

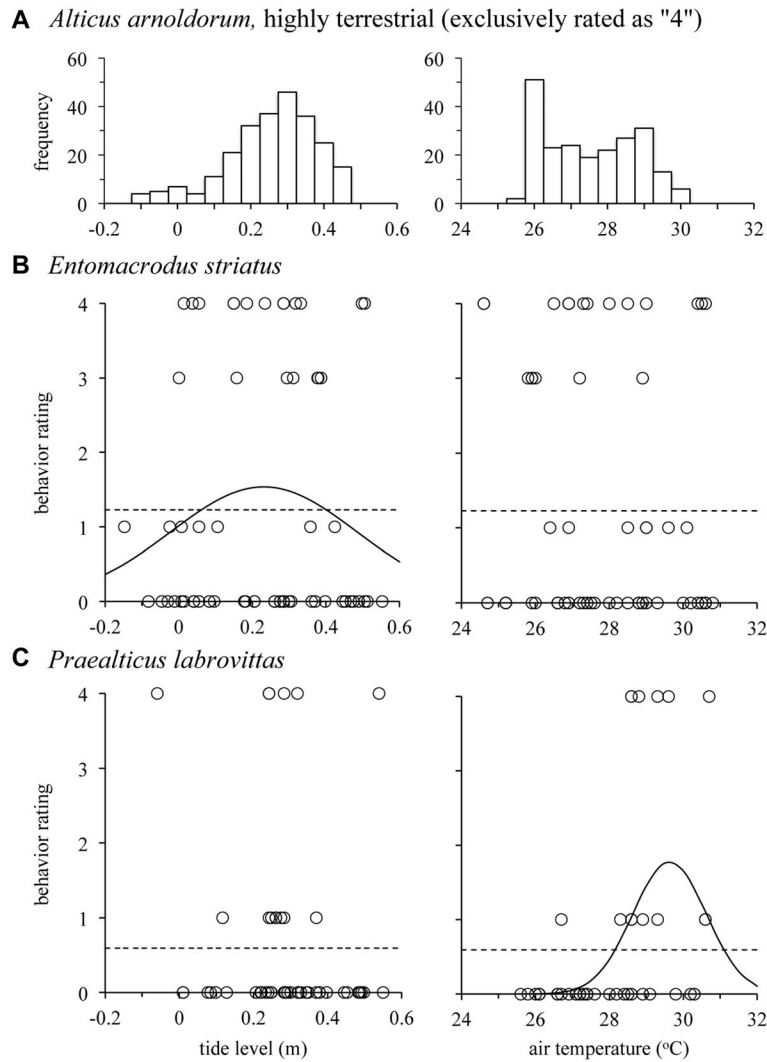


Figure 4. Environmental correlates of emergence behavior in two mildly amphibious taxa from Guam: (B) *Entomacrodus striatus* and (C) *Praealticus labrovittas*. The dashed horizontal lines indicate the mean behavioral rating for each species. Solid lines highlight trends identified by model selection in Table 1. Shown for comparison is the change in abundance for the island's single highly terrestrial species, (A) *Alticus arnoldorum*. Activity in this highly terrestrial species has been shown to be dependent on fluctuations in tide level and air temperature, with the greatest activity concentrated at mid-tide and moderate temperatures (see Ord and Hsieh 2011). All three species are sympatric with one another at the same locations on Guam.

pressure in new environments are common factors that reduce colonization success in introduced species (Allen 2006; Colautti et al. 2006; Yeh et al. 2007). Conversely, competition and predation are the basis of a key hypothesis of why taxa might move into novel environments as well; because doing so results in an "ecological release" from competition or predation in ancestral environments (Bolnick et al. 2010; Refsnider et al. 2015). Competition and predation are clearly important variables determining establishment success in contemporary settings (Schoener and Spiller 1995; Allen 2006; Colautti et al. 2006; Yeh et al. 2007) and the types of species that coexist in communities more generally (Robertson 1996; Emerson and Gillespie 2008; HilleRisLambers

et al. 2012), so it seems reasonable that these factors might have also played a role in transitions between aquatic to terrestrial environments.

Whether competition or predation have limited the distribution of extant fishes on land is difficult to test. In the case of the amphibious fishes of Blenniidae, the likelihood of encountering novel predators in a terrestrial environment appears to have been circumvented through the evolution of cryptic body coloration in aquatic ancestors (i.e., blennies were already well camouflaged in their aquatic environment such that they should still be cryptic to most visual-oriented predators on land as well; Morgans and Ord 2013). There is also some evidence of changes in life history that

Table 1. Support for alternative statistical models of terrestrial activity measured in the field for two mildly amphibious species of blenny.

	AIC _c	ΔAIC	AIC _w	<i>t</i> tide	<i>t</i> tide ²	<i>t</i> temperature	<i>t</i> temperature ²
<i>A Entomacrodus striatus</i> , <i>N</i> = 62							
Null	224.5	0.0	0.45				
Tide + tide ²	225.1	0.7	0.33	1.774	-1.824		
Temperature + temperature ²	226.4	1.9	0.17			-0.158	0.111
Tide + tide ² + temperature + temperature ²	229.0	4.5	0.05				
<i>B Praealticus labrovittas</i> , <i>N</i> = 54							
Null	137.7	35.3	0.00				
Tide + tide ²	139.1	36.7	0.00				
Temperature + temperature ²	102.4	0.0	0.88			2.879	-2.834
Tide + tide ² + temperature + temperature ²	106.5	4.1	0.12				

might be consistent with shifts in competition and predation, but these would reflect an ecological release from these factors following the transition to land, not vice versa (Platt and Ord 2015; Platt et al. 2016).

The oceanic islands we surveyed typically only had a single highly terrestrial species, although several amphibious species that were capable of exhibiting terrestrial activity were often present (Fig. 3). Okinawa and Taiwan were the only islands where two highly terrestrial species were found, but these species tended to only occur in high abundance at separate sites from one another. It is possible that the presence of another highly terrestrial fish in itself limits emergence of other fishes. However, we observed both *E. striatus* and *P. labrovittas* to frequently emerge onto land at the same sites on Guam, despite the presence of one another and the high densities of an existing highly terrestrial species (*A. arnoldorum*; Fig. 4). Evidently, any effect of competition or predation on the distribution of amphibious fish on land is likely complex.

It has also been argued that freshwater habitats have been important transitional environments for promoting shifts between aquatic and terrestrial habitats because they represent low predation and low competition environments (Vermeij and Dudley 2000). We found no evidence for this in our study. There were many examples of freshwater amphibious fish, but no more than would be expected given the number of fish genera in freshwater systems generally. Instead, the intertidal zone appears to be an important environment associated with amphibious behavior in extant fish. The intertidal zone fluctuates widely in abiotic conditions, with daily tide circles exposing organisms to both aquatic and terrestrial conditions. There were many reports of intertidal fish becoming “voluntary” stranded out of water in shallow pools or under moist rocks at low tide (Table S1). Moreover, water temperature tends to increase as the tide recedes, particularly in tide pools that become isolated at low tide. Elevations in water temperature can have adverse impacts on marine fauna in itself (Gibson

et al. 2015), but it also reduces the amount of dissolved oxygen in water and can lead to hypoxia (Giomi et al. 2014). Experimental manipulations of increasing water temperature or reducing water oxygen have both induced emergence behavior in fish in captivity (Martin 1996; Gibson et al. 2015). Many amphibious fish occur in tropical climates, and although this does not appear to have been a key aspect associated with terrestrial activity per se (Fig. 1), both voluntary stranding and active emergence to escape adverse abiotic conditions in warm rock pools at low tide would seem to be obvious evolutionary precursors to a more permanent transition to land.

The phenomenon of a fish out of water might seem extraordinary given the dramatic differences between an aquatic and terrestrial environment, but it is in fact quite common. This implies ecological transitions that result in less extreme changes in environmental conditions should be equally or more common in nature. Although we found characteristics that did seem to occur disproportionately in fish that frequented terrestrial environments, the diversity of ecologies exhibited by amphibious fish was nonetheless striking: forays onto land have occurred from a range of different aquatic environments, in all climate regions, and by species with various types of diet. Perhaps the most noticeable similarity, however, is the dominance of transitions from the intertidal zone. Ecotones in nature are common and intuitively any area in which different environments meet could act as important ecological and evolutionary staging grounds for making transitions into new areas. The intertidal zone appears to have been one such ecotone for the evolution of amphibious behavior in fish. More generally, ecotones could prove to be important generators of biodiversity by priming taxa for a wider range of environmental conditions.

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DATA ARCHIVING

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LITERATURE CITED

- Allen, C. R. 2006. Predictors of introduction success in the South Florida avifauna. *Biol. Invasions* 8:491–500.
- Betancur-R, R., G. Orti, and R. A. Pyron. 2015. Fossil-based comparative analyses reveal ancient marine ancestry erased by extinction in ray-finned fishes. *Ecol. Lett.* 18:441–450.
- Bhikajee, M., and J. M. Green. 2002. Behaviour and habitat of the Indian Ocean amphibious blenny, *Alticus monochrus*. *Afr. Zool.* 37:221–230.
- Blob, R. W., W. C. Bridges, T. Maie, R. A. Cediell, M. M. Bertolas, M. B. Ptacek, M. L. Julius, and H. L. Schoenfuss. 2008. Morphological selection in an extreme flow environment: body shape and waterfall-climbing success in the Hawaiian stream fish *Sicyopterus stimpsoni*. *Integr. Comp. Biol.* 48:734–749.
- Bolnick, D. I., T. Ingram, W. E. Stutz, L. K. Snowberg, O. L. Lau, and J. S. Paull. 2010. Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proc. Biol. Sci.* 277:1789–1797.
- Brown, C. R., M. S. Gordon, and H. G. Chin. 1991. Field and laboratory observations on microhabitat selection in the amphibious red sea rockskipper fish, *Alticus kirki* (family Blenniidae). *Mar. Behav. Physiol.* 19:1–13.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodal inference: a practical information-theoretic approach. 2nd edn. Springer, New York.
- Colautti, R. I., I. A. Grigorovich, and H. J. MacIsaac. 2006. Propagule pressure: a null model for biological invasions. *Biol. Invasions* 8:1023–1037.
- Colombini, I., R. Berti, A. Ercolini, A. Nocita, and L. Chelazzi. 1995. Environmental factors influencing the zonation and activity patterns of a population of *Periophthalmus sobrinus* Eggert in a Kenyan mangrove. *J. Exp. Mar. Biol. Ecol.* 190:135–149.
- Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland.
- Crisp, M. D., M. T. K. Arroyo, L. G. Cook, M. A. Gandolfo, G. J. Jordan, M. S. McGlone, P. H. Weston, M. Westoby, P. Wilf, and H. P. Linder. 2009. Phylogenetic biome conservation on a global scale. *Nature* 458:754–756.
- Depczynski, M., and M. Gagliano. 2007. Andaman blennies bathe in the tropical sun rather than in the water. *Coral Reefs* 26:677.
- Emerson, B. C., and R. G. Gillespie. 2008. Phylogenetic analysis of community assembly and structure over space and time. *Trend. Ecol. Evol.* 23:619–630.
- Farrelly, C. A., and P. Greenaway. 1987. The morphology and vasculature of the lungs and gills of the soldier crab, *Mictyris longicarpus*. *J. Morph.* 193:285–304.
- . 1992. Morphology and ultrastructure of the gills of terrestrial crabs (Crustacea, Gecarcinidae and Grapsidae): adaptations for air-breathing. *Zoomorphology* 112:39–49.
- Gibb, A. C., M. A. Ashley-Ross, and S. T. Hsieh. 2013. Thrash, flip, or jump: the behavioral and functional continuum of terrestrial locomotion in teleost fishes. *Integr. Comp. Biol.* 53:295–306.
- Gibson, D. J., E. V. A. Sylvester, A. J. Turko, G. J. Tattersall, and P. A. Wright. 2015. Out of the frying pan into the air-emersion behaviour and evaporative heat loss in an amphibious mangrove fish (*Kryptolebias marmoratus*). *Biol. Lett.* 11:20150689.
- Giomi, F., M. Fusi, A. Barausse, B. Mostert, H.-O. Portner, and S. Cannicci. 2014. Improved heat tolerance in air drives the recurrent evolution of air-breathing. *Proc. Biol. Sci.* 281:20132927.
- Graham, J. B. 1997. Air-breathing fishes: evolution, diversity and adaptation. Academic Press, San Diego, CA.
- Graham, J. B., and H. J. Lee. 2004. Breathing air in air: in what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? *Physiol. Biochem. Zool.* 77:720–731.
- Hayes, K. R., and S. C. Barry. 2008. Are there any consistent predictors of invasion success? *Biol. Invasions* 10:483–506.
- HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of coexistence theory. *Annu. Rev. Ecol. Evol. Syst.* 43:227–248.
- Horn, M. H., and K. C. Riegler. 1981. Evaporative water loss and intertidal vertical distribution in relation to body size and morphology of stichaeoid fishes from California. *J. Exp. Mar. Biol. Ecol.* 50:273–288.
- Hsieh, S. T. 2010. A locomotor innovation enables water-land transition in a marine fish. *PLoS One* 5:e11197.
- Jeschke, J. M., and D. L. Strayer. 2006. Determinants of vertebrate invasion success in Europe and North America. *Global Ecol. Biogeogr.* 12:1608–1619.
- Kolar, C. S., and D. M. Lodge. 2001. Progress in invasion biology: predicting invaders. *Trend. Ecol. Evol.* 16:199–204.
- Laberge, T., and P. J. Walsh. 2011. Phylogenetic aspects of carbamoyl phosphate synthetase in lungfish: a transitional enzyme in transitional fishes. *Comp. Biochem. Physiol. Part D Genomics Proteomics* 6:187–194.
- Laurin, M. 2010. How vertebrates left the water. University of California Press, Berkeley, CA.
- Maddison, W. P., and D. R. Maddison. 2015. Mesquite: a modular system for evolutionary analysis. Available at <http://mesquiteproject.org>
- Martin, K. L. M. 1995. Time and tide wait for no fish: intertidal fishes out of water. *Env. Biol. Fishes* 44:165–181.
- . 1996. An ecological gradient in air-breathing ability among marine cottid fishes. *Physiol. Zool.* 69:1096–1113.
- . 2014. Theme and variations: amphibious air-breathing intertidal fishes. *J. Fish Biol.* 84:577–602.
- Martin, K. L. M., T. M. Berra, and G. R. Allen. 1993. Cutaneous aerial respiration during forced emergence in the Australian salamanderfish, *Lepidogalaxias salamandroides*. *Copeia* 1993:875–879.
- Martin, K. L. M., R. C. van Winkle, J. E. Draiss, and H. Lakisic. 2004. Beach-spawning fishes, terrestrial eggs, and air breathing. *Physiol. Biochem. Zool.* 77:750–759.

- Morgans, C. L., and T. J. Ord. 2013. Natural selection in novel environments: predation selects for background matching in the body colour of a land fish. *Anim. Behav.* 86:1241–1249.
- Nosil, P. 2012. *Ecological speciation*. Oxford Univ. Press, Oxford, U.K.
- Ojha, J., N. Mishra, M. P. Saha, and J. S. D. Munshi. 1979. Bimodal oxygen uptake in juveniles and adults amphibious fish, *Channa* (= *Ophiocephalus marulius*). *Hydrobiologia* 63:153–159.
- Ord, T. J., and S. T. Hsieh. 2011. A highly social, land-dwelling fish defends territories in a constantly fluctuating environment. *Ethology* 117:918–927.
- Pace, C. M., and A. C. Gibb. 2011. Locomotor behavior across an environmental transition in the ropefish, *Erpetoichthys calabaricus*. *J. Exp. Biol.* 214:530–537.
- . 2014. Sustained periodic terrestrial locomotion in air-breathing fishes. *J. Fish Biol.* 84:1–22.
- Pfennig, D. W., and K. S. Pfennig. 2012. *Evolution's wedge: competition and the origins of diversity*. University of California Press, Berkeley, CA.
- Platt, E. R. M., and T. J. Ord. 2015. Population variation in the life history of a land fish, *Alticus arnoldorum*, and the effects of predation and density. *PLoS One* 10:e0137244.
- Platt, E. R. M., A. M. Fowler, and T. J. Ord. 2016. Land colonisation by fish is associated with predictable changes in life history. *Oecologia*. In press. DOI 10.1007/s00442-016-3593-6.
- Price, T. D. 2008. *Speciation in birds*. Roberts and Company, Greenwood Village, CO.
- Pronko, A. J., B. M. Perlman, and M. A. Ashley-Ross. 2013. Launches, squiggles and pounces, oh my! The water-land transition in mangrove rivulus (*Kryptolebias marmoratus*). *J. Exp. Biol.* 216:3988–3995.
- Refsnider, J. M., S. D. Roches, and E. B. Rosenblum. 2015. Evidence for ecological release over a fine spatial scale in a lizard from the White Sands formation. *Oikos* 124:1624–1631.
- Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3:217–223.
- Robertson, D. R. 1996. Interspecific competition controls abundance and habitat use of territorial Caribbean damselfishes. *Ecology* 77:885–899.
- Sayer, M. D. J. 2005. Adaptations of amphibious fish for surviving life out of water. *Fish Fisheries* 6:186–211.
- Sayer, M. D. J., and J. Davenport. 1991. Amphibious fish: why do they leave water? *Rev. Fish Biol. Fisheries* 1:159–181.
- Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford Univ. Press, New York.
- . Evidence for ecological speciation and its alternative. *Science* 323:737–741.
- Schoener, T. W., and D. A. Spiller. 1995. Effect of predators and area on invasion: an experiment with island spiders. *Science* 267:1811–1813.
- Shafer, A. B. A., and J. B. W. Wolf. 2013. Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology. *Ecol. Lett.* 16:940–950.
- Shimizu, N., Y. Sakai, H. Hashimoto, and K. Gushima. 2006. Terrestrial reproduction by the air-breathing fish *Andamia tetractyla* (Pisces: Blenniidae) on supralittoral reefs. *J. Zool.* 269:357–364.
- Simberloff, D. 2013. *Invasive species: what everyone needs to know*. Oxford Univ. Press, New York.
- Turko, A. J., C. A. Cooper, and P. A. Wright. 2012. Gill remodelling during terrestrial acclimation reduces aquatic respiratory function of the amphibious fish *Kryptolebias marmoratus*. *J. Exp. Biol.* 215:3973–3980.
- Uchiyama, M., M. Komiyama, H. Yoshizawa, N. Shimizu, N. Konno, and K. Matsuda. 2012. Structures and immunolocalization of Na⁺, K⁺ -ATPase, Na⁺/H⁺ exchanger 3 and vacuolar-type H⁺ -ATPase in the gills of blennies (Teleostei: Blenniidae) inhabiting rocky intertidal areas. *J. Fish Biol.* 80:2236–2252.
- Urbina, M. A., A. S. Meredith, C. N. Glover, and M. E. Forster. 2014. The importance of cutaneous gas exchange during aerial and aquatic respiration in galaxiids. *J. Fish Biol.* 84:759–773.
- Vermeij, G., and R. Dudley. 2000. Why are there so few evolutionary transitions between aquatic and terrestrial ecosystems? *Biol. J. Linn. Soc.* 70:541–554.
- Wells, R. M. G., M. E. Forster, and A. S. Meredith. 1984. Blood oxygen affinity in the amphibious fish *Neochanna burrowsius* (Galaxiidae: Salmoniformes). *Physiol. Zool.* 57:261–265.
- Williamson, M., and A. Fitter. 1996. The varying success of invaders. *Ecology* 77:1661–1666.
- Yeh, P. J., M. E. Hauber, and T. D. Price. 2007. Alternative nesting behaviours following colonisation of a novel environment by a passerine bird. *Oikos* 116:1473–1480.
- You, X., C. Bian, Q. Zan, X. Xu, and E. Al. 2014. Mudskipper genomes provide insights into the terrestrial adaptation of amphibious fishes. *Nat. Commun.* 5:5594.
- Zar, J. H. 1999. *Biostatistical analysis*. Prentice Hall, Upper Saddle River, NJ.

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Supporting Information

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Figure S1. Emphasis of past research on amphibious fishes (see Table S1) in relation to (a) topic area or (b) species focus of the seven most commonly studied groups.

Figure S2. Individual gene tree phylogenies generated using maximum likelihood for the (a) mitochondrial (mtDNA) genome and the following nuclear genes: (b) recombination-activating gene 1 (RAG 1), (c) zic family member 1 (zic1), (d) hypothetical protein LOC564097 (Ptr), (e) T-box brain 1 (tbr1), and (f) super-conserved receptor expressed in brain 2 (sreb2).

Figure S3. Consensus phylogeny constructed from the concatenated dataset using maximum likelihood.

Figure S4. Consensus phylogeny constructed from the Bayesian inference of the partitioned concatenated dataset using Mr Bayes.

Figure S5. Coalescent-based Bayesian species tree generated with all mitochondrial and nuclear loci using *BEAST.

Figure S6. The evolution of amphibious behavior in Blenniidae based on the Bayesian inference of the partitioned concatenated dataset that includes all original taxa used to create the phylogeny, in addition to 11 missing genera (highlighted by the dashed lineage branches).

Figure S7. The evolution of amphibious behavior in Blenniidae based on the Maximum Likelihood phylogeny.

Figure S8. The evolution of amphibious behavior in Blenniidae based on the Coalescent-based Bayesian species tree.

Table S1. Documented cases of amphibious behavior in 130 species from 33 families of fish.

Table S2. Scoring of varying degrees of amphibious behavior in Blenniidae.

Table S3. Primers 5'–3' (for nested PCRs, R1: PCR step 1, R2: PCR step 2), annealing temperatures (TD: Touch down), and sequence length for all amplified loci.

Table S4. Species used in this study: sample location, voucher and tissue reference number, and GenBank accession numbers.