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Individual fluctuations in toxin levels affect breeding site fidelity in a chemically defended amphibian

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Behaviours that influence habitat selection strongly determine species movement patterns. One component of animal behaviour that largely influences movement patterns and habitat choice is site fidelity. California newts (family Salamandridae) demonstrate remarkable site fidelity, typically homing to the same pool of a stream each breeding season. Individuals often occupy a specific pool throughout the breeding season, but some males shift among breeding pools, altering their set of potential mates, competitors, and predators. In this study, we measured dermal concentrations of the chemical defence compound tetrodotoxin (TTX) in recaptured male California newts (*Taricha torosa*) over five breeding seasons to evaluate whether relative TTX concentrations are associated with breeding site fidelity in the field. Our five years of field sampling indicates that TTX concentrations of individuals and group means fluctuate tremendously, implying that TTX is not a stable phenotypic trait. Despite such fluctuations, we found that an individual's relative TTX concentration explains fidelity to a breeding pool and suggests that newts may be able to assess both their own concentrations of TTX and that of conspecifics to make decisions about remaining in or abandoning a breeding pool. These results provide us a novel dimension to chemical defence phenotypes in nature and their ecological consequences, potentially requiring a re-evaluation of the coevolutionary dynamics of predation pressure on toxin-laden organisms.

1. Introduction

The constraints and mechanisms by which species select their breeding habitat is fundamental to their ecology, evolution, and conservation. The behaviours that influence habitat selection, for example, strongly determine species movement patterns, thereby affecting interactions with other organisms and the environment [1], fitness and local density [2], the spatial scale at which populations are regulated [3], metapopulation dynamics [4], and the evolutionary units within a population [5]. One key behaviour that largely influences movement patterns and habitat choice is site fidelity.

Site fidelity, whereby individuals tend to return to a previously occupied site, is a life-history strategy that occurs in many species of three phyla; chordata, arthropoda, and mollusca [6]. The behaviour may increase survival by allowing animals to efficiently relocate and use previously successful sites for breeding, feeding, and overwintering [7], as well as reducing relative energetic costs [8], vulnerability to predators [9], and risk of mortality [10] associated with moving to new areas. Although site fidelity is a behaviour that links individual- and population-level processes with demonstrated ecological and evolutionary consequences [11], the mechanisms by which it occurs remain poorly understood in most systems.

Some amphibians show remarkable site fidelity [12,13], and a considerable research effort spanning the last half-century has characterized its ecological consequences and underlying mechanisms. Some amphibian species are known to return year after year to the same pool or physical structures at a breeding site, with individuals often remaining in that microhabitat during the entire breeding period [14,15]. Other individuals may inexplicably abandon a breeding site, complicating explanations for the evolution and maintenance of site fidelity more generally.

Olfaction, as an orientation mechanism, is critical in guiding site fidelity [12,16]. Olfactory cues influence many amphibian behaviours [17] and often play an important role in the recognition of individuals, kin, and mates [18,19]. Salamanders use chemical cues to recognize and select habitat [20,21], mark territories [22], and detect oviposition site quality [23]. Scent marking, which is typically associated with territorial behaviour [22], may also offer intruders the means to assess the competitive quality of other males [24]. Although olfaction can guide amphibian site fidelity, the proximate underpinnings of movement behaviour once individuals reach a breeding site remain poorly understood and difficult to study in the field. Determining the processes that facilitate individuals returning to and maintaining a breeding locality, and why they may abandon these sites, will help to inform questions surrounding the phenomenon of site fidelity.

Newts of the genus *Taricha* are iteroparous, returning each spring to the same pond or reach of a stream to breed [12,25,26]. Newts are terrestrial most of the year and stream-breeding *Taricha* navigate 250–500 m or more between terrestrial habitats and breeding sites [27] with pinpoint precision, often entering their stream within 15 m of previous years [12,26]. Throughout the breeding season, individuals generally remain within a few metres of their breeding pool, and often do not migrate after entering the stream [25]. Olfactory cues aid terrestrial migration to breeding sites [28], and once there, chemical cues remain critical components for sexual and feeding behaviour [29].

All four currently recognized species of *Taricha* possess a neurotoxin, tetrodotoxin (TTX), that is considered to have evolved primarily as a chemical defence. Population variation of *Taricha* TTX concentrations has been observed across broad geographical scales [30], a pattern that is presumed to be the result of a coevolutionary arms race between newts and predatory *Thamnophis* garter snakes [31]. The prevailing hypothesis, whereby *Taricha* TTX levels escalate in response to *Thamnophis* resistance to TTX, rests, in part, on the assumption that the TTX phenotype is genetically based and evolutionarily capable of responding to natural selection. However, the means by which *Taricha* produce and maintain TTX is unknown. Because TTX is an alkaloid, it is presumably not the direct product of a specific gene or gene family. The TTX concentrations of the sister taxon of *Taricha*, *Notophthalmus* newts from eastern North America, also vary across broad geographical ranges [32], but with no apparent relationship to a predator [33]. Furthermore, it appears that other tetrodotoxic taxa produce TTX via endosymbiotic bacteria rather than via an endogenous biosynthetic or genetically controlled pathway [34].

Given the extensive variation observed between individuals and populations [35], the role of TTX may well be more complex than that predicted purely by predator–prey interactions [36–38]. TTX appears to have selected for increased resistance in *Thamnophis* snakes [39], but it also appears to facilitate a diverse set of ecological processes. Various taxa rely on TTX as a feeding stimulant [40], sexual attractant [41], or antipredator chemical cue [29,42,43]. Specific to our study system, TTX from potentially cannibalistic adult California newts (*Taricha torosa*) causes conspecific larvae to flee and seek refuge [29,42]. TTX also affects the foraging behaviour of stream macroinvertebrate community members, and reduces movement and strike velocities of at least one macroinvertebrate predator [43].

In this study, we quantified TTX levels among individuals of a wild California newt population (*T. torosa*), and explored the relationship between TTX and site fidelity. We conducted longitudinal sampling of individual newts and used a multi-year mark–recapture study to track each individual's TTX concentrations and movement patterns within a stream across multiple years. We place these data into the context of an ongoing, multi-decadal study of newt population biology in southern California streams during a severe drought and a time of precipitous amphibian declines. Overall, our results demonstrate that site fidelity at the stream level is exceptionally strong, with no marked individual recaptured in adjacent streams over 20 years of intensive study and hundreds of marked and recaptured newts. However, consistent with other studies, we have observed that some individuals will move up- or downstream among breeding pools or occupy different pools across years, and we analyse these temporal movement patterns as a consequence of newt morphology and an individual's relative TTX concentrations. Given that TTX is both a defence compound and conspecific chemical cue, we designed our study to determine whether individual relative TTX concentrations influence site fidelity patterns in breeding male California newts. Our results indicate that (i) TTX levels of wild individuals fluctuate widely over time, (ii) mean TTX levels of our single breeding population span nearly the full range of *T. torosa* chemical defence levels previously measured across the entire species range, and (iii) adult relative TTX levels are linked to patterns of fidelity at breeding sites.

2. Methods

(a) Temporal patterns of individual breeding site fidelity

The breeding site history and fidelity of newts was reconstructed for two datasets of previously marked newts that were recaptured and sampled for TTX from 2011 to 2015 (electronic supplementary material, text). The first dataset included marked individuals sampled multiple times for TTX from 2011 to 2015 ($n = 32$). The second dataset consisted of marked newts that were sampled for TTX only once during 2011–2015 ($n = 68$). To determine the previous location of these newts prior to the first time they were sampled for TTX, we relied on a long-term mark–recapture survey dataset of 1 650 newts in Cold Creek that detailed where individuals were marked and recaptured from 1991 to 2015. Thus, every individual included in our analyses was previously caught, marked, and georeferenced at some point during 1991–2015. Using these data, we determined if the first TTX sampling location occurred in the same pool where the male had previously been captured. For newts that were sampled more than once, we referred to the previous sampling location to determine if the subsequent sample occurred in the same pool. We then used a binary variable to indicate whether the newt was found in the same pool (0) or had moved (1) at the time of sampling. These binary data were used as the categorical response variable in our separate analyses of these two datasets. For example, a previously marked newt sampled in 2011 from pool A, then 2012 from pool B, and 2014 from pool A would first be searched for in our survey dataset to determine where it was last found before 2011. If that male were last found in pool A in 2010, then the response variable would be coded as a ‘0’ in 2011, a ‘1’ in 2012, and a ‘1’ in 2014. Additionally, morphological and temporal data were included for each individual for each sampling event. Details of the morphological and temporal data are outlined under our Statistical methods section.

For the 68 individuals that were sampled only once, we realize that this set of individuals does not provide the same picture of temporal variation of chemical defence data and patterns of site fidelity as do individuals sampled two or more times. However, they nonetheless allow us to use a much larger sample size to evaluate the relationship between movement, morphological predictors, and TTX concentrations.

We also used the long-term mark–recapture survey data to understand breeding site fidelity at the population level. We compared recapture locations of these 1 650 newts and estimated the percentage of individuals recaptured in the same pool compared with those that were recaptured in different pools. To ensure that these two datasets are independent, we included only individuals recaptured, but never sampled for TTX between 1991 and 2015. Ultimately, these data provide us a much larger temporal context for our five years of TTX and movement data, thereby allowing us to determine whether the observed movement patterns of sampled individuals among pools from 2011 to 2015 was extraordinary relative to movement over the previous two decades.

(b) Statistical methods

We performed a series of analyses with classification models, first on our dataset composed of 32 individuals sampled for TTX multiple times, and then repeated the analysis with the data for the 68 individuals that were sampled for TTX only once. In general, the modelling approach evaluated relationships between *T. torosa* breeding pool fidelity and individual body condition, temporal variables, and TTX deviation (*TTX*), which is a measure of how much an individual’s TTX concentration on a sampling date deviated from that day’s mean TTX value of all newts sampled on that day (electronic supplementary material, S1 text). Thus, each individual sampled more than once had multiple *TTX* values, either positive or negative, for each date that they were sampled. Our decision to use an individual’s deviation from the mean on the day of sampling instead of absolute TTX concentrations was based on preliminary data that indicated individual and population concentrations fluctuated, which suggested there may be variation in the capacity of a chemical defence response at the individual level that could affect ecological or behavioural processes.

Morphological predictors were obtained from individual measurements performed at each sampling event. The first was body condition (*body*), the ratio of mass to snout–vent length (SVL) and the second was measured tail height (*tail*), which we included because it is a secondary sexual characteristic and an indicator of fitness [44]. The temporal variables included in our models consisted of the year of sampling (*year*), month of sampling (*month*), time between sampling events (*between*) to account for potential lag effects, and a categorical variable (*measure*), which indicates the cumulative number of samples collected from an individual during the five years. The predictor *measure* was incorporated to detect a potential effect of repeated sampling on TTX levels of the 32 newts because they were sampled more than once.

The first model implemented tree regression (*TREE* v. 1.0–29) [45] in *R* to identify predictors associated with breeding pool fidelity at Cold Creek. Tree regression uses a binary recursive partitioning method to construct a classification tree, hierarchically ranking predictor variables according to how much and at what level they explain variance in the response variable. This modelling approach is attractive because it is easily interpretable and indicates directionality and strength of a response. For our focal dataset of 32 newts, the tree regression model included the categorical temporal predictors *year*, *month*, and *between*; the morphological continuous variables *body* and *tail*; the predictor *measure*; the values for relative TTX concentrations, *TTX*; and the binary fidelity data, which were coded as the response variables. When analysing the dataset of 68 newts that were sampled only once, the predictors *between* and *measure* were deleted from our models because these males were only sampled once for TTX.

To better understand which variables predict male fidelity to a breeding pool, we constructed a second model that applied tree regressions using the RANDOMFOREST framework (v. 4.6-6) [46] in *R*. This algorithm iteratively and randomly removes variables to evaluate relationships between predictor and response variables. For both datasets, we constructed the models to perform 10 000 iterations and used the same predictors as in our tree regression models. We used a random forest model rather than more traditional linear regression methods because it is a non-parametric procedure that does not assume an underlying distribution of the data. It has also been shown to outperform traditional regression tests [47], to adequately assess clustered datasets with repeated measures [48], and to describe the role of continuous variables across a landscape [49]. Another benefit of random forest models is their ability to handle complex interactions between variables [50].

To assess the robustness of our models, we used the *predict* function in *R* and data from our focal set of 32 newts to generate predicted values of the number of instances individuals would move or show fidelity. These predicted and the observed values from the dataset of 32 newts were statistically compared using a chi-square test in a 2 × 2 contingency table. In addition, *p*-values for each predictor were estimated in *R* with the package *rfPermute* (v. 1.6.2) by producing a null distribution of 1 000 permutation replicates of the random forest model that was used for the analysis of the 32 newts sampled multiple times.

Finally, we computed matrices of Spearman’s rank correlations coefficients using the Hmisc (v. 3.9-3) framework [51] and *rcorr* function in *R* to test for correlations between TTX concentrations and measured individual morphological traits, which included mass, SVL, and tail height. We performed this analysis using our dataset of 32 individuals to determine whether greater individual TTX concentrations were associated with males having greater mass, size, or tail height.

3. Results

(a) Sampling effort

The 2011–2015 surveying and sampling effort was heavily male-biased, consisting of 465 encounters with males and 78 encounters with females. Of the total number of TTX samples collected during the sampling period, 73 of them were from 32 marked males repeatedly captured and sampled multiple times. An additional 68 samples came from marked males captured and sampled only once. The remaining number of TTX samples came from male newts that were not marked.

(b) Individual and group mean TTX concentrations

During the 2011–2015 sampling period, a total of 231 dorsolateral tissue samples were collected from breeding males to subsequently quantitate TTX concentrations within Cold Creek. Mean TTX concentrations through time are shown in figure 1. Individual male TTX concentrations ranged from

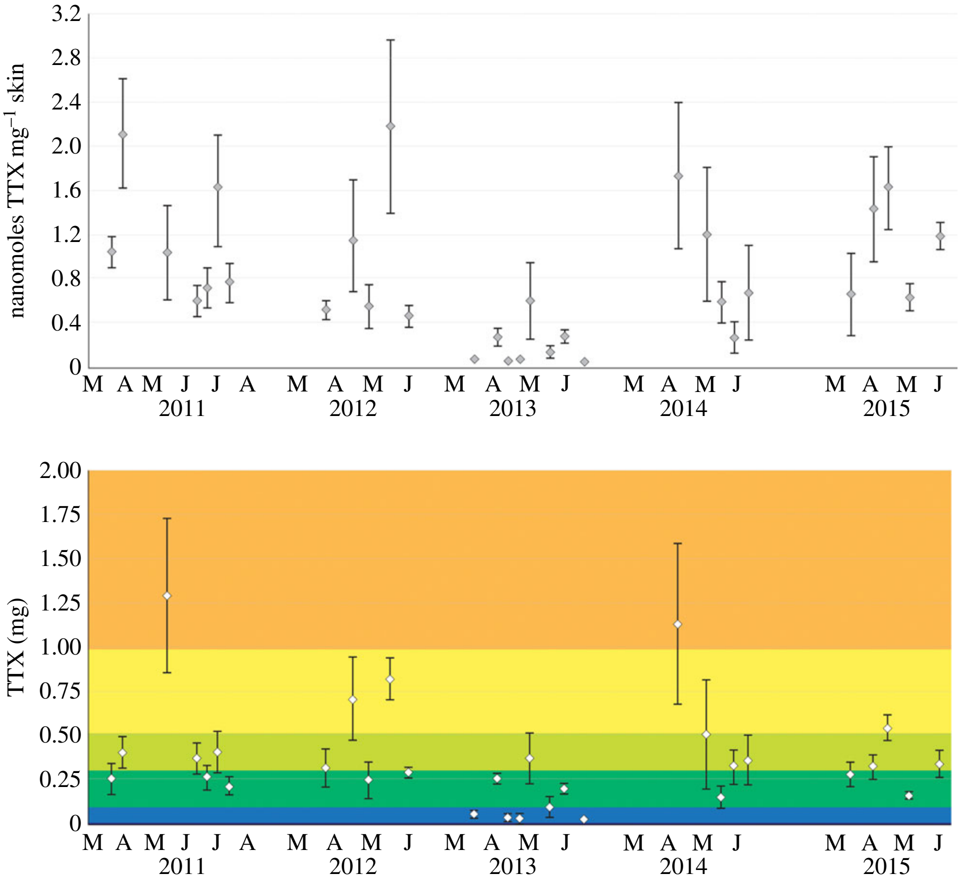

Figure 1. Fluctuations in mean TTX concentrations derived from adult male *T. torosa* in Cold Creek. Diamonds show mean male TTX concentrations (± s.e.) for each sampling date during 2011–2015. The measured TTX concentrations are presented in units of nanomoles TTX mg^{-1} skin [52] and estimated whole individual TTX concentrations in mg TTX [53]. Coloured segments in the bottom graph illustrate the scale of 'whole newt toxicity' from Hanifin *et al*. [53]. Regardless of units, mean TTX levels within this breeding population fluctuate nearly the full scale previously used to describe patterns of phenotypic variation across the entire species range.

0.00 to 5.72 nmol TTX mg^{-1} skin (electronic supplementary material, table S1). Daily mean TTX values varied within a season and between years, fluctuating from a high of 2.22 nmol TTX mg^{-1} on 15 May 2012, to a low of 0.03 nmol TTX mg^{-1} on 24 April 2013 (figure 1 and electronic supplementary material, table S2). Overall, the most extreme individual TTX deviation values were from newts sampled in March 2011 (+3.60) and May 2012 (−2.04).

(c) Temporal patterns of individual breeding site fidelity

All 32 individuals that were sampled multiple times for TTX were found in the 10 largest pools within Cold Creek, except one that was recaptured once in an intervening side pool (figure 2). Fidelity to a breeding pool was common year after year for the majority of individuals. Of the 32 individuals that were recaptured, approximately 68% were always recaptured in the same pool. Similarly, of the 68 individuals sampled for TTX only once, 42 individuals (62%) were recaptured in the same pool where they were originally captured. Finally, using the survey data collected from 1991–2015, we found that approximately 72% of newts were recaptured in the same pool across years.

(d) Movement among pools

Tree regression indicated that fidelity to a breeding pool is best explained by TTX deviation values and morphology (figure 3*a*,*c*). The most important correlate for both sets of data was an individual's relative TTX level (*TTX*), such that recaptures repeatedly occupying the same pool were associated with positive TTX deviation values. In our focal set of 32 individuals, males with deviation values greater than +0.38 nmol TTX mg^{-1} were recaptured in the same breeding pool. For individuals with TTX deviation values less than +0.38 nmol TTX mg^{-1}, fidelity was explained by a combination of both tail height and deviation values. Any male with a TTX deviation value less than +0.38 nmol TTX mg^{-1} and a tail that was less than 11.9 mm tended to move, whereas when tail height exceeded 11.9 mm, the fidelity of those males was explained further by *TTX* values. These males with taller tails tended to move if their *TTX* value was less than −0.28 nmol TTX mg^{-1} and show fidelity if *TTX* values exceeded −0.28 nmol TTX mg^{-1} (figure 3*a*). Similarly, a deviation value of +0.12 nmol TTX mg^{-1} best explained fidelity patterns in the 68 individuals sampled only once (figure 3*c*). Body condition was the next most important predictor of movement for this dataset of newts sampled only once. Overall, newts with below average TTX concentrations and shorter tails or low body condition ratios were often recaptured in different breeding pools over time.

The random forest models showed that individual *TTX* values and morphological predictors consistently explained fidelity to a breeding pool more than time related predictors (figure 3*b*,*d*). As with the tree regression models, *TTX* was the most important predictor, followed by tail height (*tail*) and body condition (*body*).

Predicted versus observed values did not significantly differ from one another (χ^2, d.f. = 1, $p = 0.84$). All estimated

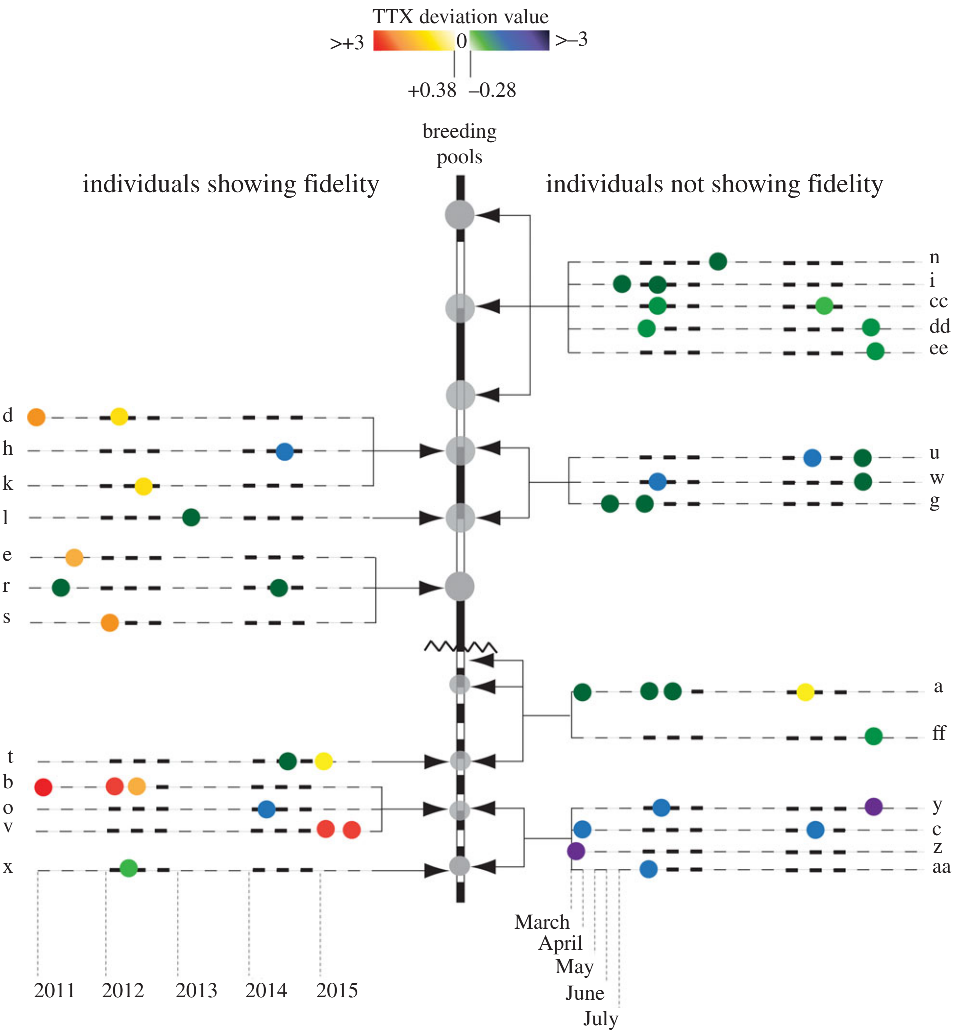

Figure 2. Individuals with above average TTX levels tend to show fidelity to a breeding pool. Coloured circles show individual TTX deviation values, a calculation of how much an individual's TTX concentration on a sampling date deviated from the mean TTX concentration of all newts sampled on that same day. Individuals recaptured in the same pool (left side) over our five-year study tend to have greater positive *TTX* values compared with individuals recaptured in different pools (right side). The vertical segmented bar represents the length of the study site in the Cold Creek Preserve, with each alternating segment scaled to a 20 m section of the stream (note the break in scale). Each horizontal dashed line represents an individual newt (identified by letter, see electronic supplementary material, table S1) and the five-sampling period. Arrows point to the breeding pool(s) (grey circles) where an individual was recaptured. Using the values from tree regression results, only measurements and individuals with deviation values exceeding $+0.38$ and less than -0.28 are plotted.

p-values generated in *rfPermute* for every predictor exceeded the 0.05 α level except the predictor *TTX* ($p = 0.001$), which was our measure of relative TTX concentrations (electronic supplementary material, figure S1). Test statistics from Spearman's rank analyses indicated that no morphological trait was significantly correlated with TTX concentrations (*mass*: $p = 0.29$; SVL: $p = 0.49$; *tail height*: $p = 0.99$).

4. Discussion

Although breeding site fidelity is a well-documented phenomenon in a number of amphibian species, a more proximate understanding of why some individuals repeatedly use the same site and others do not remains obscure. In a few cases where it has been studied, individual attributes have been identified as a potential causal explanation. For example, Kopecký *et al.* [54] observed that male Alpine newts (*Mesotrition alpestris*) with high body condition tended to move among adjacent, human-created breeding sites, perhaps, because such movement enhances the most fit male's ability to gain increased access to mates. However, most studies simply document the fraction of individuals that move among breeding sites [55]. If we are to understand why some individuals have strong site fidelity and others do not, and use that information to better understand metapopulation dynamics and conserve declining species as land fragmentation and loss of aquatic habitat occur, a more mechanistic grasp of site fidelity is necessary.

In our study, an individual's relative TTX concentrations best explained site fidelity patterns, such that males with low TTX levels relative to other males concurrently occupying the stream tended to be recaptured in different pools. Roughly, two-thirds of newts were repeatedly recaptured in the same pool during our 2011–2015 study, while the remaining third moved among pools separated by more than 20 m. From our analyses, these movement patterns are strongly correlated with relative TTX concentrations. Tree regression and random forest models on both sets of recapture data indicate that temporal patterns do not explain these differences. Rather, relative TTX concentrations and morphological features best explain fidelity to a breeding pool. The similarity in model results for the two different datasets, which cover different temporal periods, provides strong evidence of the importance of TTX in site

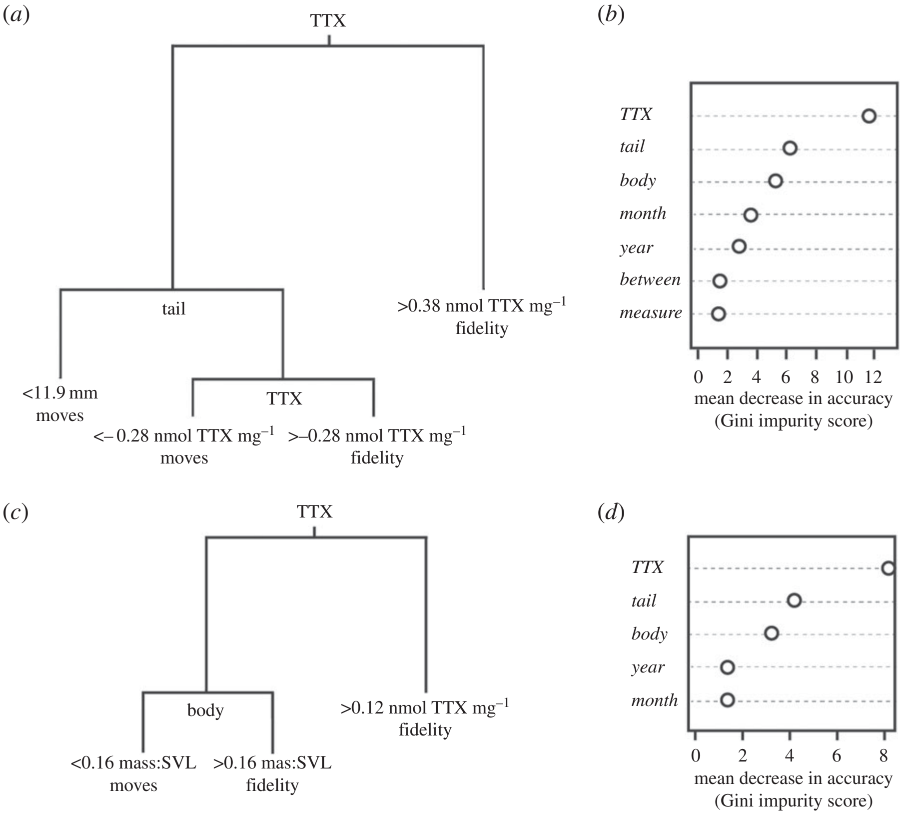

Figure 3. Tree and random forest model results indicate relative TTX levels are most important in explaining fidelity. (*a*,*c*) Tree regressions depict the relationship between breeding pool fidelity and individual's relative TTX concentrations and morphology for the focal set of 32 newts sampled multiple times (*a*) and the 68 individuals sampled once (*c*). Overall, the results indicate that males with greater relative TTX concentrations tend to remain in the same pools. Variables in the random forest models are shown for the 32 newts sampled multiple times (*b*) and only once (*d*), ordered top-to-bottom from most to least important in explaining fidelity. Both random forest models indicate that relative TTX is the most important predictor in explaining breeding site fidelity, followed by morphological variables.

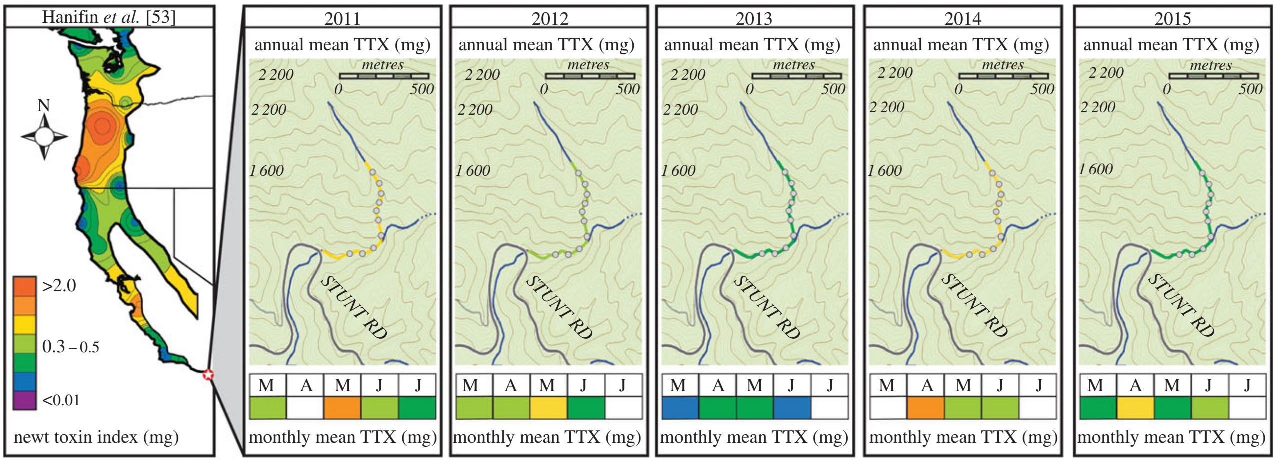

Figure 4. The measured mean TTX concentrations of one breeding population in southern California show fluctuations annually and monthly, spanning nearly the entire scale observed across the geographical range of Hanifin *et al*. [53]. Each annual mean is presented as a colour-coded stream according to newt toxin index values from Hanifin *et al*. [53]. A breakdown of the annual mean by month for each year shows broad variation of TTX concentrations over time that would otherwise be overlooked using only annual means. Mean TTX concentrations from each sampling date further highlight the fluctuations occurring at smaller timescales (figure 1).

selection and breeding pool fidelity for *T. torosa*. Furthermore, the percentage of newts (approx. 72%) recaptured in the same pool during our mark–recapture surveys from 1991 to 2015 is similar to the percentages of newts that exhibit site fidelity in both analysed datasets, further supporting the interpretation that our focal set of 32 individuals sampled during 2011–2015 is a reasonable representation of the population.

An additional striking result of this study is the amount of phenotypic variation in TTX concentrations observed within individuals (electronic supplementary material, figure S2 and table S1) and a breeding population (figures 1 and 4). In general, broad geographical variation in *Taricha* TTX concentrations has been explained as a result of a coevolved relationship with a TTX-resistant predator [36] and to a lesser extent, as a response to selection pressure from other predators [37,38]. However, mismatch between predator resistance and newt chemical defences has been observed in over one-third of sampled populations (88). While such geographical patterns may be representative, they are all based on a sample from a single point in time or uncontrolled sampling that groups data across broad temporal ranges. Population means may be important, but based on our results, it appears that an individual's TTX concentrations, as well as chemical defence levels of an entire breeding population, fluctuate over space and time under natural conditions (figures 1 and 4). Moreover, our data indicate that sample timing and the individuals sampled are major components of population 'toxicity' (figures 1 and 4). Both these aspects have largely been overlooked when describing the geographical patterns of phenotypic variation in chemical defences of *Taricha*. The fluctuations in TTX concentrations in newts at our study site span almost the entire range of previously measured values in populations across much of California (figures 1 and 4; electronic supplementary material, figure S2). Thus, it seems reasonable that fluctuating TTX levels may be an important aspect of observed mismatch between newt TTX concentrations and predator resistance. Ultimately, variation of TTX concentrations in other populations should be assessed across time to better understand what variables drive fluctuations.

The ecology and evolution of *Taricha* and TTX have primarily been viewed through the lens of a predator–prey relationship. Researchers have invested years of work into understanding what processes may have shaped predator resistance and the underlying proximate responses [39,53]). However, the processes that drove variation of TTX concentrations within populations and the observed mismatch between predators and *Taricha* have remained relatively vague. Placed within the general context of a predator–prey model, the complexity of *Taricha* and TTX as an independent study system may have been obscured in part by assuming that newt chemical defences are (i) stable through time, (ii) genetically capable of responding to selection pressure from predators, and (iii) endogenously biosynthesized. There is limited evidence for a pathway by which *Taricha*, or any other animal biosynthesizes or regulates the production of TTX, although one has been proposed [56]. Alternatively, newt TTX may be the product of endosymbionts, as it is in many other taxa [34]. If TTX is obtained from or influenced by local environmental factors, it may still have evolutionary significance. For example, poison dart frogs (*Dendrobates (Oophaga) pumilio*) source defensive compounds from the environment, yet show considerable variation in toxin levels across the Bocas Del Toro Islands in Panama. However, aposematic coloration, which is likely heritable [57,58] appears to have evolved to match population levels of defensive compounds. Whether environment plays any role in shaping *Taricha* chemical defences remains relatively unexplored. Enzymes responsible for TTX biosynthesis in newts have not been discovered, and only one study has attempted to amplify potential bacterial sources [59]. Over the last several decades, researchers have tried to determine the proximate mechanisms that allow *Taricha* to produce or maintain TTX, but there are no definitive conclusions. Recent efforts evaluating the maintenance of TTX in newts under captive conditions have reached contradictory results. Studies of *Taricha* demonstrate that individual TTX concentrations are sustained and vary in captivity regardless of diet [60], which the authors invoke as evidence of a de novo source for TTX production in *Taricha*. However, TTX is not present in other tetrodotoxic newts (genus *Cynops*) reared in captivity from egg [61]. Thus, it remains possible that individual fluctuations and population variation of TTX concentrations is entirely or largely environmentally induced, or that TTX is a metabolic by-product.

Surprisingly, greater TTX concentrations do not appear to be a correlate of male body condition, and larger males do not appear to have greater concentrations of TTX. Prior empirical studies by Brodie *et al.* [36] found that TTX concentrations (mg TTX cm^{-2} tissue) were exponentially related to SVL in the congener *T. granulosa*, but that relationship was primarily driven by differences between adults and juveniles. A recent study by Kraemer *et al.* [32] evaluated TTX concentrations in the red-spotted newt (*Notophthalmus viridescens*) across a wide geographical range and found a significant positive relationship between TTX concentrations (mg TTX g^{-1} tissue) and mass. In our study, TTX concentration fluctuates independent of body condition or morphological features. Although individual body condition and tail height were important predictors in our models of breeding site fidelity, the correlation across 32 individuals between absolute TTX concentrations and morphology was low and non-significant. This suggests that morphological traits may be important not only for mate choice [44], but also together with TTX concentrations, may affect male intraspecific interactions.

Overall, our results indicate that individual TTX levels fluctuate and that fidelity is strongly associated with individual changes in relative TTX levels. One explanation we offer for these two observations is that males may be able to gauge and respond to competitive attributes (i.e. TTX levels) of resident conspecifics via tetrodotoxic cues. Newts as a group undergo complex and extremely competitive courtship processes [62] and empirical studies of the sister taxon to *Taricha*, *N. viridescens* found that males rely on chemical cues from other breeding males to facilitate social behaviour during breeding [63]. Thus, olfactory cues from conspecifics in breeding pools may provide critical information that male *T. torosa* use to make decisions about investing in chemical defences and movement behaviour. Male wall lizards (*Podarcis hispanica*) employ conspecific chemical cues to evaluate competitors [64], allowing competitively inferior males to avoid areas occupied by competitively superior males. Tetrodotoxic chemical cues from adult *T. torosa* are already known to provide critical information to conspecifics [29,42]. These cues cause larvae to flee and seek refuge, which appears to be an antipredatory response to potential cannibals. Given the observed roles of TTX in chemical communication, it seems reasonable that tetrodotoxic chemical cues may also affect adult behaviours. Adults of other chemically defended taxa use toxic compounds as both a defence and intraspecific chemical cue. For example, lepidopterans sequester pyrrolizidine alkaloids as defences, but sexual attractants derived from pyrrolizidine alkaloids are also important in mate selection [65], so much so that males lacking chemical defences were much less successful in mating. Relevant to our study, a male with increased TTX concentrations may have greater defences, but also greater appeal to mates. This seems likely, given that male TTX concentrations over the length of our study repeatedly increased during the peak of breeding in March through April (figure 1). Although intriguing, the general explanation we have offered requires understanding (i) the potential costs of modulating TTX levels relative to the costs associated with moving and (ii) the energetics of mating. Both remain necessary components to determining how individuals balance the energetic demands of mating and the presumed costs of modifying TTX levels. Once such costs are established, it would be worthwhile to explore whether individuals with low concentrations are benefitting from conspecifics without paying a presumed physiological price of increasing or maintaining higher relative TTX levels.

In general, we found that relative TTX levels are strongly associated with breeding site fidelity, such that individuals with below average relative TTX levels were recaptured in novel breeding sites much more than those with above average relative TTX levels. Thus, the social context of TTX appears to be an important contributor to the migration probability and breeding biology of newts, rather than TTX purely serving an antipredator function. Although currently protected as a Species of Special Concern in central and southern California [66], *T. torosa* continues to face challenges ranging from habitat loss to urbanization to non-native predators, stream siltification, wildfires, drought, and emerging diseases. Strong site fidelity is thought to have evolved as an effective life-history strategy, but if individuals home to degraded habitats, then populations may continue their trend towards local extinction [67–69]. Understanding the interaction between TTX variation, breeding site fidelity, and antipredator behaviour should assist evolutionary ecologists and conservation managers working to attenuate amphibian declines in California. Whatever the final answers, it does not appear that the *Taricha* TTX phenotype is stable, nor that selection from predators fully explains phenotypic variation of this trait and its ecological role in the evolution of newts.

Ethics. All research presented in the manuscript was conducted in accordance with all applicable laws and rules set forth by their governments and institutions and all necessary permits were in hand when the research was conducted.

Data accessibility. The datasets supporting this article have been uploaded as part of the supplementary material.

Authors' contributions. G.M.B. designed the study, conducted fieldwork, performed analytical laboratory work, carried out statistical analyses, and drafted the manuscript; D.B.G. helped design the study, assisted with analytical laboratory work, and helped draft the manuscript; H.B.S. helped design the study and helped draft the manuscript; L.B.K. helped design the study, conducted fieldwork, and helped draft the manuscript. All authors gave final approval for publication.

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