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Research

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Pelagic larval duration predicts extinction risk in a freshwater fish clade

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Pelagic larval duration (PLD) can influence evolutionary processes ranging from dispersal to extinction in aquatic organisms. Using estimates of PLD obtained from species of North American darters (Percidae: Etheostomatinae), we demonstrate that this freshwater fish clade exhibits surprising variation in PLD. Comparative analyses provide some evidence that higher stream gradients favour the evolution of shorter PLD. Additionally, similar to patterns in the marine fossil record in which lower PLD is associated with greater extinction probability, we found a reduced PLD in darter lineages was evolutionarily associated with extinction risk. Understanding the causes and consequences of PLD length could lead to better management and conservation of organisms in our increasingly imperiled aquatic environments.

1. Introduction

Larval characteristics can greatly influence the dynamics of organismal diversification [1–3]. For instance, pelagic larval duration (PLD), or the time an aquatic larva spends in the water column, might have influenced the likelihood of extinction in many fossil marine organisms [4–6]. However, evolutionary divergence in PLD and its potential relationship with extinction risk have rarely been examined in extant, freshwater organisms partly because freshwater organisms often lack a pelagic stage [1]. Furthermore, extant organisms are by definition the clades that have not gone extinct. Therefore, assessing the relationship between PLD and the likelihood of extinction in living groups has been difficult. However, human activity is precipitating a global wave of extinction, and we used the freshwater fish group known as darters (Percidae: Etheostomatinae) to take advantage of this anthropogenic ‘experiment’ to comparatively examine the causes and consequences of evolutionary divergence in PLD.

In marine systems, a longer PLD is thought to be advantageous for colonizing new habitats, and marine larvae often spend 30–60 days in the water column [1,4–6]. However, the unidirectional flow characterizing many freshwater systems could readily cause pelagic fish larvae to be swept downstream from suitable habitats [7]. This could be why freshwater fish often have a short or completely lack a pelagic larval stage [1,8]. Because elevation gradients structure flow regimes in freshwater systems, these gradients could shape the evolution of PLD in freshwater organisms. If a freshwater group showed substantial variation in PLD length and had evolved to exploit a range of stream gradients, phylogenetic comparative methods could be used to test whether increased gradients are linked to the evolution of shorter PLD.

Darters are a clade containing approximately 200–250 species of North American stream-dwelling fishes that exploit habitat gradients ranging from

steep headwater mountain streams to slow-moving coastal bayous [9,10]. Many darter species are also imperiled [10], and a combination of anthropogenic impacts and inherent biological attributes could be responsible for the high extinction risk of many darter species. For instance, human water use and habitat degradation have impaired the small ranges of many darter species [9,11]. Additionally, despite any advantage a shorter PLD might confer to species inhabiting higher stream gradients, the evolution of a shorter PLD might also be associated with a greater risk of extinction. To test these hypotheses, we examined the phylogenetic associations among stream gradients, extinction risk and darter PLD.

2. Material and methods

(a) PLD measurements

Twenty darter species were reared at Conservation Fisheries, Inc. in Knoxville, Tennessee to obtain estimates of species PLD. Species were examined either because of their conservation status or local availability. PLDs for three additional species were obtained from the literature [8,11–14]. To determine PLD, tanks with breeding darters were routinely checked for spawning. Following spawning, eggs were examined under a microscope for development, transferred to a shallow tray if fertilized, and monitored for hatching. Once the larvae hatched and began swimming, they were transferred to a pelagic rearing tub marking pelagic stage day 0. Black 94 l tubs were used to raise the pelagic larvae as these tubs reduced phototactic behaviour as well as maintained sufficient aeration and current at this sensitive stage. Larvae were monitored daily for water column position. When 50% of larvae settled to the bottom of the tub, the PLD was determined to have ended [11].

(b) Phylogenetic reconstruction

To estimate the phylogenetic relationships among the 23 darter species and two outgroups (*Perca flavescens* and *Sander vitreus*), we compiled sequence data available on GenBank (see electronic supplementary material, table S1) for three loci. The loci included the mitochondrial cytochrome *b* (*Cytb*), nuclear S7 ribosomal protein intron 1 (S7) and nuclear recombination activating protein 1 (*RAG1*) exon 3. We first ran a partition homogeneity test implemented in PAUP 4.0b10 to test for topological incongruence among the loci [15]. Using each locus as a single partition across 100 replicates that each had 100 replicates of random taxon addition, we did not identify any significant incongruence among the loci ($p = 0.20$). Therefore, we performed a concatenated analysis of all three loci with sequence alignments and models of molecular evolution taken from Near *et al.* [10]. Using BEAST v. 1.7.5 [15], we ran a Bayesian analysis on the dataset partitioned by gene for 10 million generations with sampling every 5000 generations. We used TRACER v. 1.5 [16] to estimate the effective sample size (ESS) of parameters in the MCMC. ESS values more than 200 were considered adequate.

(c) Comparative analyses

To test the hypothesis that stream gradient is associated with PLD, we determined the stream gradient ($\Delta\text{m}/\text{km}$) of 257 geo-referenced collection sites (see electronic supplementary material, table S2) spanning the distribution of the 23 species. We used the software TOPO NORTH AMERICA v. 9.0 (Delorme, 2010) to determine elevation change between 500 m upstream and 500 m downstream of each site's GPS location. Because

these stream gradient measurements generally exhibited a long tail of high values for the distribution of sites where a species occurred, we used the species median from all sites for comparative analyses. Extinction risk for the darter species was determined based on their presence or absence on the International Union for Conservation of Nature (IUCN) Red List which highlights species at a relatively high risk of extinction [17]. The species *Etheostoma sitikuense* and *Etheostoma marmoripinnum* were considered to be Red Listed because they recently were elevated from the Red Listed *Etheostoma percnum*. Prior to comparative analyses, PLD values were square root transformed.

We ran comparative analyses on 100 topologies inferred to have the best likelihood scores from the posterior distribution of trees generated. This distribution of 100 trees was used to account for phylogenetic uncertainty in branch lengths and relationships among the species while ensuring topologies sampled during the MCMC with low likelihood were not examined. Within this framework, we examined the correlation of phylogenetic independent contrasts of median stream gradient and PLD in the CRAN package *ape* [18] implemented in R [19]. We then examined the phylogenetically corrected association of Red List status, coded as a categorical state, with transformed PLD, a continuous variable, using the 'brunch' function from the *caper* CRAN package [20]. The brunch algorithm was used to examine evolutionary associations between combinations of continuous and categorical data while accounting for the lack of phylogenetic independence of phenotypes. Brunch identified and calculated contrasts for all variables at nodes at which the two descendent lineages were assigned to alternative Red List categories. A linear model was then formulated with the degrees of freedom equal to the number of inferred transitions between categories of extinction risk. If lower values of PLD were associated with extinction risk, we would expect a significant association between PLD and IUCN status.

3. Results

The PLDs ranged from 0 to 60 days in the darter species examined (figure 1). The phylogenies we reconstructed mirrored relationships recovered in Near *et al.* [8], and this phylogenetic structure suggests PLD values have repeatedly increased and decreased during darter diversification. Over the 100 phylogenetic topologies, stream gradient ranged from marginally to significantly correlated with darter PLD ($r = 0.38 \pm 0.04$; $p = 0.087 \pm 0.042$). Thirteen of the species examined were on the IUCN Red List and 10 were not. The species values of PLD for the imperiled ($\mu = 12 \pm 3$) and non-imperiled ($\mu = 25 \pm 4$) darters were generally divergent. The evolution of a lower darter PLD was significantly associated with extinction risk (d.f. = 7; $p = 0.038 \pm 0.013$).

4. Discussion

The evolutionary variation in darter PLD is substantial (figure 1). Components of the darter radiation have lost a pelagic stage while others have surprisingly lengthy PLDs. Similar to the condition found in many large freshwater groups [21], darter species like *Etheostoma boschungii* and *E. percnum* lack a PLD, becoming benthic immediately after hatching and effectively spending no time in the water column. Conversely, other species like *Etheostoma variatum* and *Percina aurantiaca* have larval durations similar to many marine fish that exhibit PLDs lasting over a month [4].

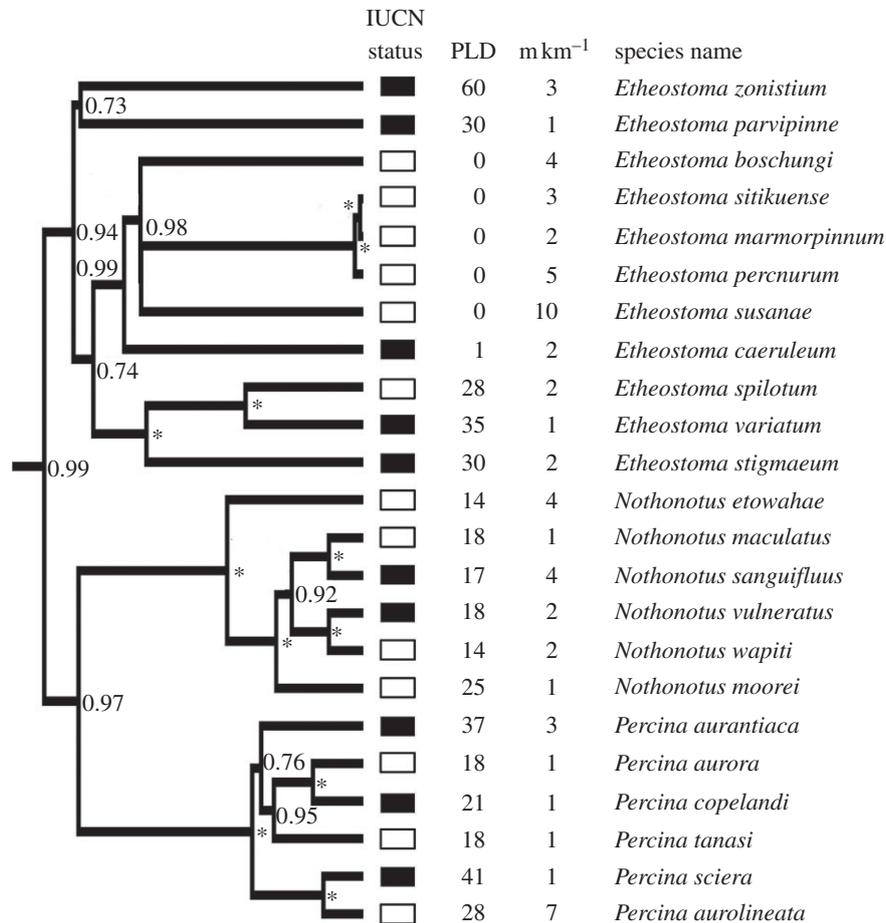


Figure 1. Phylogenetic association between IUCN Red List status and pelagic larval duration (PLD). The phylogeny is reconstructed using the genes *Cytb*, *S7* and *Rag1*. Asterisks to the right of nodes represent 1.00 posterior probability support and support for nodes over 0.50 is also given. Rectangles next to the tips indicate IUCN status (black, not listed, white, listed). The mean PLD and median stream gradient of each species are also given.

Although the significance (p -value) of the phylogenetic correlation between stream gradient and PLD length depended on the topology used, trade-offs associated with environmental variables like stream gradients could be shaping the evolution of darter PLD. Longer darter PLD might increase population connectivity via dispersal in large, slow rivers. Alternatively, a reduced time in the water column might be particularly advantageous in high gradient streams with rapid flows, reduced pelagic zones and little suitable downstream habitat. Despite the potential advantages, reduced dispersal associated with lower PLD probably leads to isolated populations with small ranges that are less stable and more vulnerable to environmental changes [22].

The association of shorter darter PLD with Red List inferred extinction risk (figure 1) is consistent with inferences from marine paleontological studies [1,3,6]. Some caution in accepting this result is probably warranted as the Red List might not perfectly reflect 'natural' extinction factors, but this measure of extinction risk did allow us to perform one of the first tests of this relationship in extant taxa. Furthermore, in many fossil taxa, it is only feasible to test whether the qualitative presence or absence of a larval stage influences extinction [1–3], and our continuous estimates of darter PLD allowed a more fine-scaled parsing of the putative association between PLD and extinction. Because both paleontological and neontological inferences provide incomplete views of evolution [23], increased examination of traits like PLD in extant taxa will provide a more robust

understanding of mechanisms influencing macroevolutionary patterns of diversification.

The relationship between PLD and imperilment provides a putative link between a larval characteristic and species persistence. PLD is probably related to range size in darters, and the range size could causally link PLD to extinction risk [1]. However, because of the patchiness of stream habitats and the variable density of streams within different geographical areas, the evolution of range size is difficult to quantify rigorously in many freshwater systems [9]. Additionally, since the range size of a species depends explicitly on how a lineage is diagnosed taxonomically, the vagaries of species delimitation also make the range size difficult to quantify [24]. Because of the relationship of PLD to range size in many marine and freshwater environments that are facing intensifying anthropogenic modifications, we predict that species with a reduced PLD will generally suffer greater extinction risk. Learning more about PLD and other crucial life-history traits of organisms inhabiting our increasingly imperiled aquatic environments should lead to better management and conservation.

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Data accessibility. DNA sequences: electronic supplementary material, table S1. Stream Gradient Measurements: electronic supplementary material, table S2.

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