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Author Contributions
TLI, DLW, and EPE conceived of the study. DLW, TLI, and AD collected samples. TLI, AD, LS, PCW, EPE, and DLW collected data. TLI, DLW, and AD performed analyses. All authors contributed the writing and development of the manuscript.

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ABSTRACT

Understanding how organismal design evolves in response to environmental challenges is a central goal of evolutionary biology. In particular, assessing the extent to which environmental requirements drive general design features among distantly related groups is a major research question. The visual system is a critical sensory apparatus that evolves in response to changing light regimes. In vertebrates, the optic tectum is the primary visual processing center of the brain, and yet it is unclear how, or whether this structure evolves while lineages adapt to changes in photic environment. On one hand, dim-light adaptation is associated with larger eyes and enhanced light-gathering power that could require larger information processing capacity. On the other hand, dim-light vision may evolve to maximize light sensitivity at the cost of acuity and color sensitivity, which could require less processing power. Here, we use X-ray microtomography and phylogenetic comparative methods to examine the relationships between diel activity pattern, optic morphology, trophic guild, and investment in the optic tectum across the largest radiation of vertebrates—teleost fishes. We find that despite driving the evolution of larger eyes, enhancement of the capacity for dim-light vision generally is accompanied by a decrease in investment in the optic tectum. These findings underscore the importance of considering diel activity patterns in comparative studies and demonstrate how vision plays a role in brain evolution, illuminating common design principles of the vertebrate visual system.

Key words: comparative phylogenetics, physiological evolution, fish, brain evolution, diel activity patterns, vision

INTRODUCTION

The past several decades have provided unparalleled insights into the mechanisms that allow animals to adapt to the challenges posed by dim-light environments (Narendra et al., 2017, Warrant, 1999, Warrant, 2004, Land & Nilsson, 2012). Studies have yielded transformative insights into the anatomical (Palmer et al., 2017) and molecular basis (Viets et al., 2016) of vision, as well as the behavioural (Narendra et al., 2013, Nørgaard et al., 2008), ecological, and macroevolutionary dynamics of life in low light (Maor et al., 2017, Angielczyk & Schmitz, 2014, Gerkema et al., 2013, Hall et al., 2012, Schmitz & Motani, 2011, Tierney et al., 2017, Wu et al., 2016, Wu et al., 2017). However, few studies have investigated how evolutionary transitions to lifestyles characterized by low-light environments influence neural investment in the primary visual information processing center of the vertebrate brain: the optic tectum. Visual performance is an integrated result of the optical and physiological properties of the eyes, combined with neural processing of visual information in the retina itself and further downstream in the optic tectum. In all visually oriented vertebrates, the optic tectum receives substantial amounts of sensory input, and it is expected that the interplay of sensory information and data processing will significantly impact neural investment. While understanding optical and retinal adaptations to dim-light environments is fundamentally important, including the optic tectum in these analyses allows for a more complete understanding of both the evolution of the visual system and expectations of neural investment across the evolutionary history of the vertebrate brain.

In the continuum of light environments, bright, spatially, and chromatically complex habitats are a richer source of sensory information than dark, plain, and monochromatic

environments (Warrant & Johnsen, 2013). To effectively gather photic information across these environment types, vertebrates utilize two types of photoreceptors: 1) cones, which detect various energy wavelengths, some including UV, and 2) rods, which do not detect the same energy wavelengths as cones but are sensitive to movement and contrast in low light conditions (Fishelson et al., 2004). Photon abundance in bright (photopic) environments facilitates the use of cone photoreceptors with color discrimination and high visual acuity, allowing organisms to distinguish fine detail (Land & Nilsson, 2012). In contrast, the photon-limited environment of dim (scotopic) habitats allows vertebrates to make use of the rod photoreceptor system, which is characterized by much higher sensitivity modifications to improve image brightness (Land & Nilsson, 2012). This photopic-scotopic dichotomy of photon availability is a major axis of morphological and functional evolution of vertebrate eyes (Warrant, 2004), however the impact of diversification along this axis on the optic center of vertebrate brain remains unclear.

Both rods and cones converge onto retinal ganglion cells, the axons of which form the optic nerves and optic tracts that project to the optic tectum (Northmore, 2011). Light sensitivity is improved through increasing rod convergence to ganglion cells, and as a result, tens or even hundreds of rods may converge onto a single cell (Warrant, 1999, Hughes, 1977, Warrant, 2004, Joselevitch & Kamermans, 2009). In contrast, acuity is maximized by low convergence; therefore as little as one cone will interface with a single ganglion cell (Warrant, 1999, Hughes, 1977, Querubin et al., 2009, Kolb & Dekorver, 1991). This difference in visual information flow between the different cell types implies a relationship between visual information and patterns of retinal convergence: for a given eye size, scotopic vertebrates should have a much lower number of retinal ganglion cells than their photopic counterparts. If this is true, the optic tectum of scotopic vertebrates should also be relatively smaller.

Marine teleosts, which comprise 25% of the planet's vertebrate diversity, present an exemplary system for assessing general trends of neural investment following transitions to dim-light environments. Marine fishes such as bigeyes (Priacanthidae) or squirrelfishes (Holocentridae) represent some of the most iconic examples of a temporal niche in vertebrates and are just a few of the dozens of clades that have independently evolved true nocturnality (Dornburg et al., 2017a, Schmitz & Wainwright, 2011b). These nocturnal fish lineages generally have larger eyes than diurnal fishes relative to body size (Schmitz & Wainwright, 2011b, Goatley & Bellwood, 2009, Goatley et al., 2010), as well as a larger lens and pupil, which increases light gathering capacity and is evidence that vision is still an important modality in nocturnal species. However, whether transitions to nocturnality increase investment in the optic tectum remains unknown.

If nocturnal fishes predominantly use rod-vision with increased retinal convergence as predicted from physiological optics, we would expect that living life in the dark comes with a decreased cost to neural investment. There is evidence that this may be the case. Work on nocturnal cods (Gadidae) with larger eyes has repeatedly revealed a decrease in the size of the optic tecta (Kotrschal et al., 1998, Evans, 1940). In contrast, some lineages of diurnal fishes have vastly expanded their repertoire of cone cells to capture additional portions of the visible light spectrum (Losey et al., 2003), thereby increasing demands on visual processing. However, light availability may not be the only factor affecting teleost investment in the optic tectum; accounts in the literature suggest feeding behavior, in particular prey detection and predation avoidance, may affect tectal volume (Huber et al., 1997, Edmunds et al., 2016, Evans, 1940, Kotrschal et al., 2017, Huber & Rylander, 1991, Huber & Rylander, 1992). To date, no phylogenetic comparative analyses has attempted to examine the relationship between eye size, neural investment, and activity patterns across a broad representation of

marine teleosts, leaving us in the dark regarding how evolutionary changes in diel activity affect neural investment.

Here we use a time-calibrated phylogenetic comparative framework to assess the relationship between diel activity, visual morphology, and investment in the optic tectum. Using an information theoretic framework, we first assess the predictive power of activity and visual morphology on neural investment, expanding the candidate pool of predictors to also include the potential effect of trophic guild on neural investment. We next quantify overall patterns of neuro-visual phylomorphospace occupancy to assess the overlap and differences in phenotypic diversity between diurnal and nocturnal teleosts. Our results reveal how optic morphology drives investment and divestment in the optic tecta, providing a much-needed macroevolutionary perspective on how dim-light vision has impacted this region of the teleost brain.

METHODS

Specimens

Eye measurements and micro CT (X-ray microtomography) brain scans were collected for 111 individuals from 60 species (Data archived on Zenodo, DOI pending manuscript acceptance). Body mass was collected at the time of capture for all specimens scanned except *Orthopristis chrysoptera*. Eye measurements for 39 species were acquired from Schmitz and Wainwright (2011b) with measurements from an additional 21 species acquired from specimens collected in Hawaii. All fish were collected on scuba using dip nets or via rod and reel in Curaçao, Hawaii, or North Carolina in accordance with conditions stipulated in permits and in compliance with university standards of animal care and use (Macquarie University animal ethics permit 5201500020). Out of the 60 species used in this study, 44 are diurnal and 16 nocturnal (Dornburg et al., 2017a, Schmitz & Wainwright, 2011b). With

regard to feeding guilds, the diurnal group is composed of 66% benthivores (29), 11% piscivores (5), 14% planktivores (6), and 9% herbivores (4); the nocturnal group is composed of 63% benthivores (10), 31% piscivores (5), and 6% planktivores (1) (Supplemental Figure 1) (Froese & Pauly, 2014). Voucher photos or tissue samples of specimens were deposited in the Yale Peabody Museum of Natural History and the North Carolina Museum of Natural Sciences.

Eye measurements

Both left and right eyes for 1-4 individuals per species were measured following the methods described in Schmitz and Wainwright (2011b). Briefly, fish were deeply anesthetized in a solution of tricaine methanesulfonate (MS-222) in seawater, and each eye was photographed prior to removal to determine maximum and minimum pupil diameters. To measure eye diameter, eyes were individually removed and photographed next to a micrometer using a USB dissecting microscope attached to a laptop. Next, the lens was excised and photographed to determine lens diameter. Once both eyes had been removed and photographed, fish were rapidly decapitated and the head placed in a 10% formalin solution (mixed with seawater). All eye measurements were taken from photographs after decapitation to ensure that the fish would remain alive until brain preservation.

Fixation, staining and micro CT scanning

Heads or dissected brains were fixed in 10% formalin (in seawater) for at least three weeks before staining. Large heads (widest dimension greater than 40mm) were kept in 5% iodine potassium iodide (IKI). The remaining smaller heads were kept in 3.75% IKI, and dissected brains were kept in 1.5% IKI. Heads remained in stain for approximately four weeks; dissected brains one week. Just prior to micro CT scanning, tissues were removed from stain

and blot dried, then wrapped in low-density polyethylene plastic wrap to prevent desiccation and eliminate the interference of in-liquid leaching of IKI. Wrapped specimens were placed snugly inside polypropylene tubes, which were secured to the micro CT scanner base. Specimens were scanned using a Zeiss Xradia Versa XRM 510 micro CT scanner housed at the Okinawa Institute for Science and Technology Graduate University (OIST). All scans were set for 1-second exposure and 1001 projections with brains scanned at 50-60 kV, 4-5 W, and heads at 80-160kV, 7-10 W. Scans were approximately 50 minutes in duration. After scanning, specimens were returned to 10% formalin.

Brain segmentation

Micro CT scans were visualized and virtually segmented using Amira 6.0 software. Segmentation allows regions of interest in the image layers to be labeled and volumetrically quantified. Total brain volume included from the olfactory bulbs (anterior) to the medulla (posterior) at the point where medullary structures fuse dorsally, which tends to coincide where cranial nerve X exits the brainstem or slightly posterior to this location. The left and right optic tecta were segmented independently. Volume was calculated using Amira 6.0 using metadata embedded within the micro CT file. Total brain volume included both right and left optic tecta. The relative optic tectum volume was the ratio of the sum of right and left optic tecta to total brain volume.

Comparative Analyses

Eyes of nocturnal fish are not only larger in diameter than those of diurnal fish; they also have greater depth, larger lenses, and changes in pupil shape (Schmitz & Wainwright, 2011b). By combining these axes of visual morphology, Schmitz and Wainwright (2011b) developed a metric (termed OPT3) that approximates where along the spectrum species lie

with respect to photopic (bright light) and scotopic (dim light) vision. OPT3 is the product of the ratio of lens diameter to eye diameter and the ratio of minimum to maximum pupil diameter

$$Opt3 = \left(ld * \min\left(pd\right)\right) / \left(ed * \max\left(pd\right)\right) \quad (1)$$

where ld is the lens diameter, pd the pupil diameter, and ed the eye diameter. We use this metric to quantify eye morphology for our comparative analyses.

Given that OPT3 was developed to distinguish between nocturnal and diurnal species, OPT3 and diel activity pattern are expected to be highly correlated variables (Supplemental Figures 2 and 3). Because OPT3 integrates eye morphology related to light gathering efficiency, we expect this continuous variable to be more informative than a dichotomous activity assignment (e.g., nocturnal, diurnal). We therefore use OPT3 and not diel activity in our models. Activity data was compiled from Schmitz and Wainwright (2011b) and Dornburg et al. (2017a).

For all comparative analyses, we used the time-calibrated phylogeny estimated by Rabosky et al. (Rabosky et al., 2013) as an evolutionary framework. This phylogeny is based on an analysis of 13 genes that capture the evolutionary divergences of 7822 fish species, including all but three lineages of our study. These species comprise *Equetus punctatus*, *Mulloidichthys flavolineatus,* and *Bothus mancus*, and represent recently diverged tipward lineages within the taxon sampling strategy of Rabosky et al (2013). To incorporate these missing lineages into our time-calibrated framework, we assembled cytochrome c oxidase subunit one (COI) sequences from genbank that included the missing species and a subset of their close relatives including at least two lineages that represent a divergence within the tree estimated by Rabosky et al. (2013; Supplemental Table 1). Divergences were time calibrated using secondary calibrations based on posterior distributions taken from the literature (e.g., (Near et al., 2012, Near et al., 2013); See Supplemental materials for more details) and

divergence times were estimated using BEAST v. 2.4.5. (Bouckaert et al., 2014) (see supplemental materials for details). Although mitochondrial markers such as COI have been shown to impact evolutionary divergences at deep time-scales in fishes, quantifications of phylogenetic information content for mitochondrial genomes (Dornburg et al., 2014) suggest that the tipward sampling of this strategy poses minimal risk of saturation based branch length errors while providing enough variable sites to achieve topological resolution and power for parameter estimation (Dornburg et al., 2014, Dornburg et al., 2017b). Resulting trees were grafted onto the phylogeny of Rabosky et al. (2013) and pruned to only include lineages sampled in our study.

We simultaneously visualized shared evolutionary history and patterns of convergence in the size of the optic tectum relative to OPT3 using a two-dimensional phylomorphospace (Sidlauskas, 2008). The internal nodes of the phylogeny were placed into the resulting morphospace using maximum likelihood-based ancestral states estimates for OPT3 and optic tectum. To assess how the resulting morphospace is partitioned between nocturnal and diurnal lineages, we used stochastic character mapping (Bollback, 2006) to reconstruct changes in diel activity across the phylogeny, mapping the resulting map onto the branch lengths of the phylomorphospace, selecting the best-fit model of the evolutionary transition rate matrix from the candidate pool of equal or asymmetric rates using corrected Akaike Information Criterion (AICc). For both nocturnal and diurnal lineages, a convex hull of morphospace occupancy was calculated and used to determine overall differences in trait diversity, coupled with a comparison of kernel density estimates (KDE) of the probability density of each trait for nocturnal and diurnal lineages. All analyses were conducted in phytools (Revell, 2012) using code from Federman et al. (Federman et al., 2016) with the exception of KDE analyses conducted using the sm package in R (Bowman & Azzalini, 2014).

We conducted comparative analyses using phylogenetic generalized least squares (PGLS) as implemented in the ape and geiger packages for R (Pennell et al., 2014, R Core Team, 2017, Paradis et al., 2004, Pinheiro et al., 2016). Volume and mass measurements were log transformed prior to analysis. We built models using both Brownian (BM) and Ornstein-Uhlenbeck (OU) error structures, with parameters for those models fit using the corBrownian and fitContinuous methods in ape and geiger, respectively. For each of these error structures we constructed five models with optic tectum volume as the dependent variable: one intercept-only model to represent the null hypothesis, one model with brain volume as the only predictor, one model with OPT3 and brain volume, one with feeding guild and brain volume, and finally one model containing brain volume, OPT3, and feeding guild. For these models, brain volume was calculated excluding optic tectum. We compared model fit using size corrected Akaike Information Criterion scores, AICc, a metric that assesses model fit while penalizing the addition of excess parameters. Parameter estimates and standard errors were calculated using model averaging (Mazerolle, 2014).

We also conducted a second set of PGLS analyses using total brain volume (including optic tectum) as the dependent variable to assess differences in total brain volume based on OPT3 (a continuous proxy for diel activity). We included all species except *Orthopristis chrysoptera* in this analysis because body mass data was missing for these specimens. Models for this analysis included an intercept-only model, a body mass only model, and a model with both body mass and OPT3. Models were built using both BM and OU error structures. In order to exclude the possibility that our results might be affected by the methods used to add species to the phylogeny (above), we repeated all analyses with the three additional species excluded. These results did not differ in any material way from the analysis of the full data set, and so only the results using the full data set will be discussed.

RESULTS

Visualizing changes in optic tecta volume across the phylogeny of sampled lineages depicts numerous independent decreases and increases in tissue mass investment (Figure 1A). In general, shifts in nocturnal activity correspond to decreases in the volume of the optic tectum (Figure 1A). In particular, nocturnal lineages such as moray eels (Muraenidae) (Figure 1B) have some of the smallest optic tectum volumes (Figure 1). In contrast, most diurnal lineages have larger optic tecta, with flatfishes demonstrating the largest of any sampled species (Figures 1A and 1C). Although changes in diel activity are generally linked with decreases in tecta volume, there are several notable exceptions. Diurnal triggerfishes (Balistidae) have some of the smallest optic tecta of any diurnal or nocturnal fishes (Figures 1A & 1D), while nocturnal silversides (Atherinidae) and squirrelfishes & soldierfishes (Holocentridae) possess optic tectum volumes that are on par with the larger volumes found in diurnal lineages (Figures 1A & 1E).

Visualization of the neural-visual phylomorphospace indicates a substantial reduction in the overall morphospace occupancy of nocturnal relative to diurnal lineages, with only a minor degree of overlap between the two (Figure 2). Quantification of the area of the convex hull area for diurnal versus nocturnal correspond to a 4.49-fold increase in combinations of OPT3 and optic tectum areas represented, with non-equal morphospace occupancy supported under a range of resampling strategies (Supplemental Figure 4). The majority of nocturnal lineages have converged in relative optic tecta volumes below 0.2 and OPT3 values as large or larger than those found in diurnal lineages (Figure 2). Moray eels represent some of the lowest optic tecta volumes and most divergent OPT3 values of the nocturnal lineages. In contrast, squirrelfishes & soldierfishes and the hardyhead silverside (*Atherinomorus stipes*; Atherinidae) have optic tecta values that are on par with those found in diurnal lineages (Figure 2). Of the diurnal lineages, most lineages appear closely clustered. Notable

exceptions include the scythe triggerfish (*Sufflamen bursa*; Balistidae), with both a low OPT3 and low optic tecta volume and flounders, which possess the most divergent OPT3 to optic tectum ratios and represent a major component of overall morphospace occupancy (Figure 2).

In general, models fit using the BM error structure outperformed their Ornstein-Uhlenbeck counterparts (Supplemental Table 2). Given the better fit of the BM based models and the consistency of results between BM and OU based models, we only discuss models constructed using the BM error structure. The intercept-only model representing the null hypothesis had a delta AICc of greater than two (147.04 ΔAICc) when compared to the best supported model (Table 1), favoring rejection of the null hypothesis (Burnham & Anderson, 2013). Model comparison of all models in the candidate pool support OPT3 as a strong predictor of investment in optic tectum with an AICc model weight of 0.91 for the model including only OPT3 and total brain volume as predictors. This model also substantially outperforms the model with brain volume as the sole predictor, with a delta AIC of 6.2. There was no demonstrable effect of feeding guild (model weight < 0.01).

There was support for an effect of visual morphology on optic tectum volume with a model-averaged coefficient of -1.72 +/- 0.59 SE for OPT3 (95% CI -2.87, -0.57). Higher values of OPT3 correlated positively with scotopic vision (dim-light vision) providing evidence that fishes more reliant on scotopic vision invest relatively less in optic tectum. However, we did not find that overall brain size in marine fishes correlates with OPT3. The model containing OPT3 had an AICc model weight of 0.50 and the intercept-only model weight was also 0.50. The difference in AICc was less than 2 (0.02 ΔAICc), providing no convincing support for increased fit with the inclusion of OPT3. Feeding guild did not have an overall effect on the volume of the optic tectum across all fishes, and examination of diurnal and nocturnal species separately also showed no robust pattern.

DISCUSSION

We find strong evidence that shifts to dim-light vision correspond with decreases in neural investment in the optic tectum. This finding is consistent with previous case studies focused on individual lineages of birds (Martin et al., 2007, Corfield et al., 2011, Gutierrez-Ibanez et al., 2013), primates (Barton et al., 1995, Barton & Harvey, 2000), and gadid fishes (Gadidae) (Evans, 1940). By examining the relationship between shifts in scotopic vision and neural investment across the largest clade of vertebrates, teleost fishes, our findings, taken with others (Wagner, 2001b, Wagner, 2001a), suggest this shift in investment to likely represent a general feature of vertebrate brain evolution.

Adjusting to the dark: lessons from the eyes of teleosts

Larger eyes can house more photoreceptors and may therefore be expected to collect larger amounts of sensory information. Under this scenario, the resulting increase in visual processing needs should drive a concomitant increase in the size of the optic tecta. Our results do not support this expectation. Instead we find that despite having larger eyes for given body size, scotopic vision reliant teleosts invest less in the optic tectum than photopic-reliant fishes (Figures 1 & 2). This lack of investment may be partially explained by the fact that visual information detectable in bright conditions such as color and ultra-violet are less detectable in dark conditions (Warrant & Johnsen, 2013). This precludes the need to invest greatly in neural tissue to process such information, suggesting that nocturnal fishes forego acuity and color spectrum sensitivity in order to maximize sensitivity to light. However, many nocturnal species are also somewhat active or must occasionally avoid predators during the day, requiring these lineages to also be able to navigate a bright environment (Ménard et al., 2008).

In contrast to nocturnal tetrapods, the majority of marine teleosts cannot cope with bright light by constricting their pupil. In essence the aperture of the teleost optical center is fixed, with only a few exceptions (Douglas et al., 1998). Further, a larger pupil is negatively correlated with depth of field (Keating, 2002) limiting the ability to focus on close objects. Our findings are consistent with previous work that supports nocturnal lineages disproportionately occupy areas of morphospace that include the largest pupils (Figure 2), suggesting a tradeoff between maximizing light sensitivity and visual acuity for nocturnal lineages (Schmitz & Wainwright, 2011b). How then do nocturnal lineages navigate a bright world? One solution may be the ability of some lineages to switch their sensory mechanism through retinomotor movements, a process analogous to a photographer switching light sensitivity settings (i.e., switching the International Standards Organization (ISO) scale settings). Although not widely studied, several independent studies have found that marine teleosts have the ability to change the position of their rod and cone photoreceptors. In bright conditions, the rods are withdrawn from incoming light and deactivated as they are surrounded by pigment. Cones, however, are fully exposed and functional. The opposite is true in dim conditions: rods are exposed while the cones are withdrawn and deactivated. Although intriguing, this physiological process is slow compared to pupillary light-mediation, requiring minutes to hours to accomplish (Hodel et al., 2006, Donatti & Fanta, 2007, Douglas et al., 1998) and does not explain the systematic decrease in optic tectum investment found in our study.

We propose two non-mutually exclusive hypotheses that could underlie the decrease in optic tectum investment found in our study yet still allow teleosts to navigate bright environments. First, the lower investment in optic tecta suggests that species more specialized in scotopic vision reduce the density of cones and favor rod-vision with higher retinal convergence, rather than adding more receptors. Such a neurophysiological change would

improve light sensitivity but reduce visual acuity not only as a result of increased convergence but also due to the inherent longer focal length of the larger eye. This strategy may explain the smaller optic tecta in scotopic-specialized species, as the information processing load of the optic tectum compared to more photopic-specialized species would be decreased. Second, it is possible that there are regional specializations of cell types across the retina. For example, eyes may feature an area with high densities of cones (photopic vision) surrounded by areas dominated by rods (scotopic vision); and/or different amounts of retinal convergence across the retina. This scenario is reminiscent of many-to-one mapping of form to function (Wainwright et al., 2005) and would enable numerous possible physiological solutions to dim-light vision while keeping visual processing costs low. Further studies of how teleosts physiologically and behaviorally cope with changes in light exposure are not only an interesting research frontier but are also of high importance for predicting how altered light regimes impact near-shore species in many of the world's rapidly developing coastal environments.

Ecology and the evolution of the optic tectum

Evolutionary transitions in trophic level have been repeatedly highlighted as driving changes in fish optic tecta, with tectum size increasing along a trophic gradient from planktivore to piscivore (Huber et al., 1997, Huber & Rylander, 1991, Huber & Rylander, 1992, Evans, 1940, Gonzalez-Voyer et al., 2009, Edmunds et al., 2016). For the lineages examined in this study, this result is not supported (Figure 1 & Table 1). Although it may be that this pattern is only true for lineages that share characteristics that are yet to be identified as relevant to this question. However, care should be taken to extrapolate the expectations of a trophic gradient as a general condition, as several environmental factors can offset or overturn this relationship.

Environmental factors such as turbidity, depth (a proxy for changes in light attenuation) and vegetation have all been suggested to erode the relationship between feeding ecology and optic tectum size (Evans, 1940, Davis & Miller, 1967, Edmunds et al., 2016, Kotrschal & Palzenberger, 1992, Gomahr et al., 1992). For example, recent work has reported a decrease in tectum size for several common diurnal North American freshwater piscivores (e.g., trout and bass) that hunt in low light conditions (Edmunds et al., 2016). These predators rely on olfaction to locate prey, which corresponds with an increase in the brain's olfactory bulb. A similar trend is evident in the nocturnal piscivores sampled in our study. Moray eels possess an extremely reduced optic tectum (Figures 1 & 2), a condition that has been used as a conceptual framework for expectations of the nocturnal fish brain (Yamamoto, 2017). Feeding on drifting or floating plankton is considered to require high spatial or temporal visual acuity (Hobson, 1991, Schmitz & Wainwright, 2011a). Diurnal zooplanktivores visually identify individual plankton before striking, requiring the ability to process the identity of difficult to resolve small and semi-transparent prey items. It is unclear whether nocturnal planktivores use a similar strategy and therefore may require higher acuity than nocturnal species of other feeding guilds.

The majority of fishes sampled in our study occur on coral reefs, an environment characterized by asymmetrical predation risks across temporal intervals. Diurnal species are under far less predation pressure than crepuscular and nocturnal lineages (Danilowicz & Sale, 1999). Nocturnal planktivores must forage in exposed environments, requiring visual acuity to detect incoming motion and early detection of ambush predators. Such early detection has been hypothesized to initiate rapid C-start and escape responses in fishes (Kotrschal et al., 2017). This "flee- early" strategy could theoretically drive an increase in tectum size as processing motion is primarily the domain of the optic tectum (Guthrie, 1990). For example, visual detection of predators has been demonstrated to promote site fidelity in refuge

selection in the nocturnal squirrelfish *Holocentrus*, favoring the selection of areas of the reef where incoming predators such as jacks, barracudas, or snappers can be detected more readily (Ménard et al., 2008). This raises a question: How generalizable is the hypothesis that predation impacts the evolutionary diversification of the optic tectum?

Recent investigations of how predation shapes the fish optic tectum has found evidence for an impact both at the species level (Kotrschal et al., 2017) as well as between closely related species (White & Brown, 2015). Given that visual processing is required for early detection as well as effective predator avoidance when schooling, nocturnal fishes may be in an evolutionary arms race with predators optimizing olfaction and other regions of the brains for effective hunting. Further, predation pressure has been found to drive overall patterns of brain size evolution in several vertebrate groups (Kondoh, 2010, Moller & Erritzoe, 2014), with recent work across the evolutionary history of frogs (Anura) demonstrating a strong effect of predation pressure on positive changes in optic tectum volume (Liao et al., 2015). As such, predation pressure may be a major force shaping the optic region of the vertebrate brain, and an under-appreciated axis of diversification driving general patterns of brain mosaicism.

Conclusion

It is increasingly clear that in vertebrates, spanning primates to fishes, common axes of diversification can promote repeated patterns of brain diversification within different neural regions (Barton & Harvey, 2000, Iwaniuk, 2004, Lefebvre & Sol, 2008, Hoops et al., 2017). Our study demonstrates several major patterns of neural investment associated with the teleost visual system. First, despite driving the evolution of larger eyes, transitions to

lifestyles characterized by dim-light vision generally drive a decrease in investment in the optic tectum. Second, there is a substantial shift and overall reduction of visual morphospace occupancy for nocturnal lineages, corresponding with convergence in large orbits and reductions in optic tectum size. These findings underscore the importance of considering diel activity patterns in comparative studies.

Across vertebrates, diel activity patterns are often deeply conserved over evolutionary timescales (Anderson & Wiens, 2017). As we continue to progress towards a synthetic understanding of the evolutionary pathways that have given rise to the compositional diversity of vertebrate brain, additional studies that consider transitions in temporal niche offer an exciting research frontier that promises new insights into patterns of neural investment and evolutionary-trade offs that have given rise to the diversity of the vertebrate brain. Such a perspective will not only illuminate general features of vertebrate evolution, but also be of potential high conservation importance for predicting the impact of environmental changes that alter the circadian rhythms of wildlife.

Competing Interests

The authors declare no competing financial interests.

Model	# Parameters	AICc	ΔAICc	AICcWt	Cum.Wt
Brain + OPT3	4	6.18	0.00	0.91	0.91
Brain + OPT3 + Guild	7	12.26	6.08	0.04	0.96
Brain only	3	12.38	6.20	0.04	1.00
Brain + Guild	6	17.60	11.42	0.00	1.00
Intercept only	2	153.23	147.05	0.00	1.00

Table 1. Model comparison results using the AICc information theoretic approach with the Brownian error structure. Brain: brain volume excluding optic tectum, OPT3: optic morphology, guild: piscivore, planktivore, herbivore, or benthivore, intercept-only: null model.

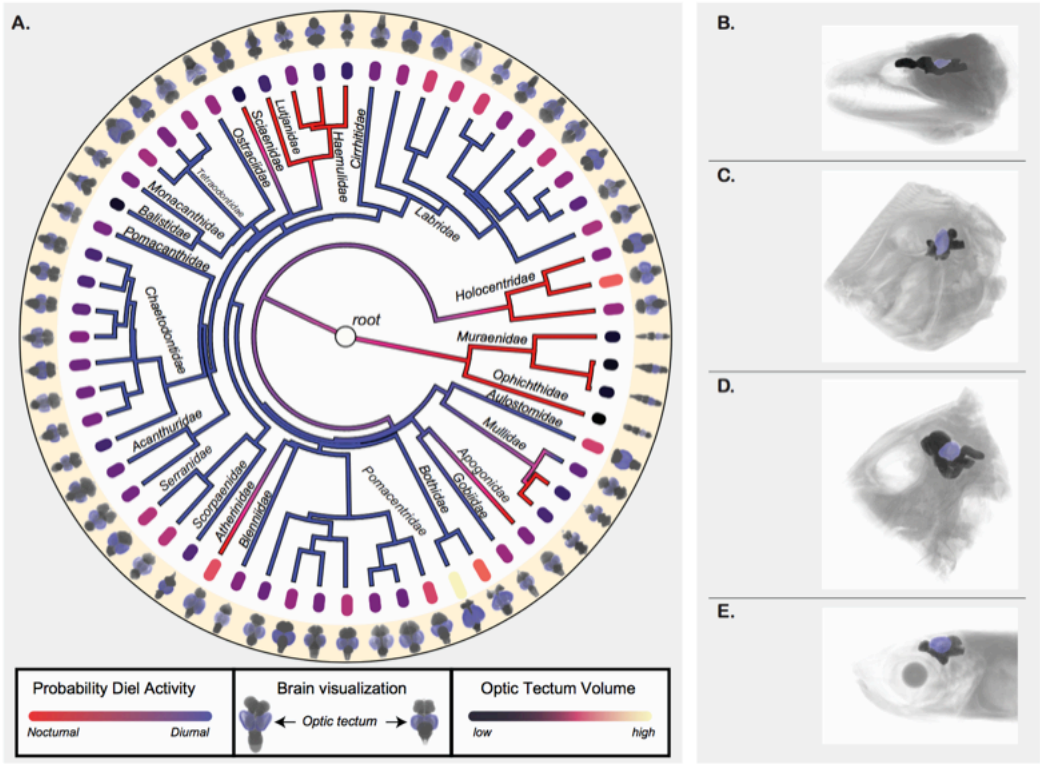

Figure 1. A) Phylogeny of species included in this study denoting diel activity patterns, relative investment in the optic tectum, and a 2D dorsal view of 3D-rendered brains with optic tecta in translucent purple and the rest of the brain in translucent gray. B-E) Translucent 3D-rendered heads with brain indicated in dark grey and optic tectum in purple for several key species discussed in the text: B) *Gymnothorax javanicus*; giant moray eel. C) *Bothus*

mancus; peacock flounder. D) *Sufflamen bursa*; scythe triggerfish. E) *Atherinomorus stipes*; hardhead silverside.

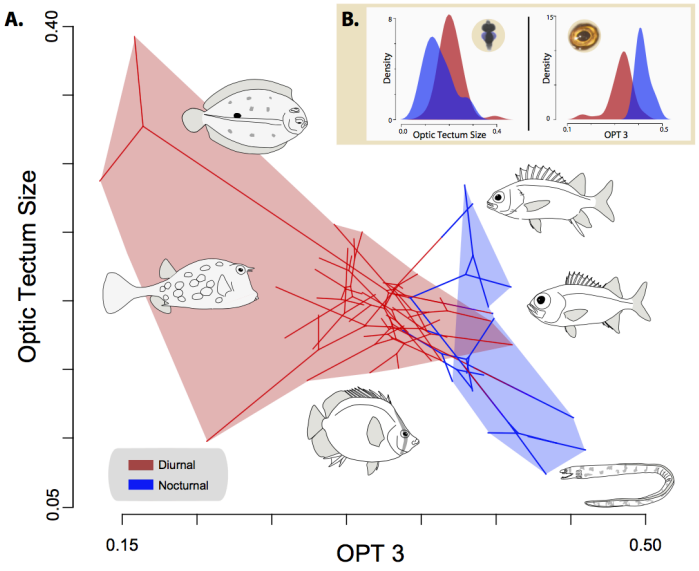

Figure 2. A) Neural-visual (Optic tectum-OPT3) phylomorphospace for nocturnal and diurnal lineages in our study. B) A comparison of kernel density estimates (KDE) of the probability density of each trait for nocturnal and diurnal lineages.

Anderson, S. R. & Wiens, J. J. 2017. Out of the dark: 350 million years of conservatism and evolution in diel activity patterns in vertebrates. *Evolution* **71**: 1944-1959.

Angielczyk, K. D. & Schmitz, L. 2014. Nocturnality in synapsids predates the origin of mammals by over 100 million years. *Proceedings of the Royal Society B: Biological Sciences* **281**.

Barton, R. A. & Harvey, P. H. 2000. Mosaic evolution of brain structure in mammals. *Nature* **405**: 1055-1058.

Barton, R. A., Purvis, A. & Harvey, P. H. 1995. Evolutionary radiation of visual and olfactory brain systems in primates, bats and insectivores. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **348**: 381-392.

Bollback, J. P. 2006. SIMMAP: Stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7**: 88.
Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A. & Drummond, A. J. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLOS Computational Biology* **10**: e1003537.
Bowman, A. W. & Azzalini, A. (2014) R package 'sm': nonparametric smoothing methods (version 2.2-5.4). pp., University of Glasgow, UK and Uni- versit`a di Padova, Italia.
Burnham, K. P. & Anderson, D. R. 2013. *Model Selection and Inference: A Practical Information-Theoretic Approach*. Springer New York.
Corfield, J. R., Gsell, A. C., Brunton, D., Heesy, C. P., Hall, M. I., Acosta, M. L. & Iwaniuk, A. N. 2011. Anatomical Specializations for Nocturnality in a Critically Endangered Parrot, the Kakapo (Strigops habroptilus). *Plos One* **6**.
Danilowicz, B. S. & Sale, P. F. 1999. Relative intensity of predation on the French grunt, Haemulon flavolineatum, during diurnal, dusk, and nocturnal periods on a coral reef. *Marine Biology* **133**: 337-343.
Davis, B. J. & Miller, R. J. 1967. Brain patterns in minnows of genus hybopsis in relation to feeding habits and habitat. *Copeia*: 1-&.
Donatti, L. & Fanta, E. 2007. Retinomotor movements in the Antarctic fish Trematomus newnesi Boulenger submitted to different environmental light conditions. *Revista Brasileira de Zoologia* **24**: 457-462.
Dornburg, A., Forrestel, E. J., Moore, J. A., Iglesias, T. L., Jones, A., Rao, L. & Warren, D. L. 2017a. An assessment of sampling biases across studies of diel activity patterns in marine ray-finned fishes (Actinopterygii). *Bulletin of Marine Science* **93**: 611-639.
Dornburg, A., Townsend, J. P., Friedman, M. & Near, T. J. 2014. Phylogenetic informativeness reconciles ray-finned fish molecular divergence times. *BMC Evolutionary Biology* **14**: 169.
Dornburg, A., Townsend, J. P. & Wang, Z. (2017b) Chapter One - Maximizing Power in Phylogenetics and Phylogenomics: A Perspective Illuminated by Fungal Big Data. In: *Advances in Genetics*, Vol. 100 (Townsend, J. P. & Wang, Z., eds.). pp. 1-47. Academic Press.
Douglas, R. H., Harper, R. D. & Case, J. F. 1998. The pupil response of a teleost fish, Porichthys notatus: description and comparison to other species. *Vision Research* **38**: 2697-2710.
Edmunds, N. B., McCann, K. S. & Laberge, F. 2016. Food Web Structure Shapes the Morphology of Teleost Fish Brains. *Brain Behav Evol* **87**: 128-38.
Evans, H. M. 1940. *Brain and body of fish; a study of brain pattern in relation to hunting and feeding in fish, by H. Muir Evans*. The Blakiston company, Philadelphia.
Federman, S., Dornburg, A., Daly, D. C., Downie, A., Perry, G. H., Yoder, A. D., Sargis, E. J., Richard, A. F., Donoghue, M. J. & Baden, A. L. 2016. Implications of lemuriform extinctions for the Malagasy flora. *Proceedings of the National Academy of Sciences* **113**: 5041-5046.
Fishelson, L., Ayalon, G., Zverdling, A. & Holzman, R. 2004. Comparative morphology of the eye (with particular attention to the retina) in various species of cardinal fish (Apogonidae, Teleostei). *Anat Rec A Discov Mol Cell Evol Biol* **277**: 249-61.
Froese, R. & Pauly, D., Editors (Eds.). 2014. FishBase. World Wide Web electronic publication. http://www.fishbase.org, version (04/2014),
Gerkema, M. P., Davies, W. I. L., Foster, R. G., Menaker, M. & Hut, R. A. 2013. The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proceedings of the Royal Society B: Biological Sciences* **280**.

Goatley, C. H. R. & Bellwood, D. R. 2009. Morphological structure in a reef fish assemblage. *Coral Reefs* **28**: 449-457.
Goatley, C. H. R., Bellwood, D. R. & Bellwood, O. 2010. Fishes on coral reefs: changing roles over the past 240 million years. *Paleobiology* **36**: 415-427.
Gomahr, A., Palzenberger, M. & Kotraschal, K. 1992. Density and distribution of external taste-buds in cyprinids. *Environmental Biology of Fishes* **33**: 125-134.
Gonzalez-Voyer, A., Winberg, S. & Kolm, N. 2009. Social fishes and single mothers: brain evolution in African cichlids. *Proceedings of the Royal Society of London B: Biological Sciences* **276**: 161-167.
Guthrie, S. D. M. (1990) The physiology of the teleostean optic tectum. In: *The Visual System of Fish*, (Douglas, R. & Djamgoz, M., eds.). pp. 279-343. Springer Netherlands, Dordrecht.
Gutierrez-Ibanez, C., Iwaniuk, A. N., Lisney, T. J. & Wylie, D. R. 2013. Comparative study of visual pathways in owls (Aves: Strigiformes). *Brain Behav Evol* **81**: 27-39.
Hall, M. I., Kamilar, J. M. & Kirk, E. C. 2012. Eye shape and the nocturnal bottleneck of mammals. *Proc Biol Sci* **279**: 4962-8.
Hobson, E. S. (1991) Trophic Relationships of Fishes Specialized to Feed on Zooplankters above Coral Reefs. In: *The Ecology of Fishes on Coral Reefs*, (Sale, P. F., ed.). pp. 69-95. Academic Press, San Diego.
Hodel, C., Neuhauss, S. C. F. & Biehlmaier, O. 2006. Time course and development of light adaptation processes in the outer zebrafish retina. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* **288A**: 653-662.
Hoops, D., Vidal-García, M., Ullmann, J. F. P., Janke, A. L., Stait-Gardner, T., Duchêne, D. A., Price, W. S., Whiting, M. J. & Keogh, J. S. 2017. Evidence for Concerted and Mosaic Brain Evolution in Dragon Lizards. *Brain, Behavior and Evolution* **90**: 211-223.
Huber, R. & Rylander, M. K. 1991. Quantitative histological studies of the optic tectum in six species of Notropis and Cyprinella (Cyprinidae, Teleostei). *J Hirnforsch* **32**: 309-16.
Huber, R. & Rylander, M. K. 1992. Quantitative histological study of the optic-nerve in species of minnows (cyprinidae, teleostei) inhabiting clear and turbid water. *Brain Behavior and Evolution* **40**: 250-255.
Huber, R., vanStaaden, M. J., Kaufman, L. S. & Liem, K. F. 1997. Microhabitat use, trophic patterns, and the evolution of brain structure in African cichlids. *Brain Behavior and Evolution* **50**: 167-182.
Hughes, A. (1977) The Topography of Vision in Mammals of Contrasting Life Style: Comparative Optics and Retinal Organisation. In: *The Visual System in Vertebrates*, (Crescitelli, F., ed.). pp. 613-756. Springer Berlin Heidelberg, Berlin, Heidelberg.
Iwaniuk, A. N. 2004. Brood parasitism and brain size in cuckoos: a cautionary tale on the use of modern comparative methods. *Int. J. Comp. Psychol.* **17**: 17.
Joselevitch, C. & Kamermans, M. 2009. Retinal parallel pathways: Seeing with our inner fish. *Vision Research* **49**: 943-959.
Keating, M. P. 2002. *Geometric, physical, and visual optics,* 2nd ed ed. Butterworth-Heinemann, Boston.
Kolb, H. & Dekorver, L. 1991. Midget ganglion cells of the parafovea of the human retina: A Study by electron microscopy and serial section reconstructions. *Journal of Comparative Neurology* **303**: 617-636.
Kondoh, M. 2010. Linking learning adaptation to trophic interactions: a brain size-based approach. *Functional Ecology* **24**: 35-43.
Kotrschal, A., Deacon, A. E., Magurran, A. E. & Kolm, N. 2017. Predation pressure shapes brain anatomy in the wild. *Evolutionary Ecology* **31**: 619-633.

Kotrschal, K. & Palzenberger, M. 1992. Neuroecology of cyprinids - comparative, quantitative histology reveals diverse brain patterns. *Environmental Biology of Fishes* **33**: 135-152.
Kotrschal, K., Van Staaden, M. J. & Huber, R. 1998. Fish brains: evolution and environmental relationships. *Reviews in Fish Biology and Fisheries* **8**: 373-408.
Land, M. F. & Nilsson, D. E. 2012. *Animal Eyes*. OUP Oxford.
Lefebvre, L. & Sol, D. 2008. Brains, Lifestyles and Cognition: Are There General Trends? *Brain Behavior and Evolution* **72**: 135-144.
Liao, W. B., Lou, S. L., Zeng, Y. & Merila, J. 2015. Evolution of anuran brains: disentangling ecological and phylogenetic sources of variation. *J Evol Biol* **28**: 1986-96.
Losey, G. S., McFarland, W. N., Loew, E. R., Zamzow, J. P., Nelson, P. A., Marshall, N. J. & Montgomery, W. L. 2003. Visual Biology of Hawaiian Coral Reef Fishes. I. Ocular Transmission and Visual Pigments. *Copeia* **2003**: 433-454.
Maor, R., Dayan, T., Ferguson-Gow, H. & Jones, K. E. 2017. Temporal niche expansion in mammals from a nocturnal ancestor after dinosaur extinction. *Nature Ecology & Evolution* **1**: 1889-1895.
Martin, G. R., Wilson, K. J., Wild, J. M., Parsons, S., Kubke, M. F. & Corfield, J. 2007. Kiwi Forego Vision in the Guidance of Their Nocturnal Activities. *Plos One* **2**.
Mazerolle, M. J. 2014. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.00. http://CRAN.R-project.org/package=AICcmodavg.
Ménard, A., Turgeon, K. & Kramer, D. L. 2008. Selection of diurnal refuges by the nocturnal squirrelfish, Holocentrus rufus. *Environmental Biology of Fishes* **82**: 59-70.
Moller, A. P. & Erritzoe, J. 2014. Predator-prey interactions, flight initiation distance and brain size. *J Evol Biol* **27**: 34-42.
Narendra, A., Kamhi, J. F. & Ogawa, Y. 2017. Moving in Dim Light: Behavioral and Visual Adaptations in Nocturnal Ants. *Integr Comp Biol* **57**: 1104-1116.
Narendra, A., Reid, S. F. & Raderschall, C. A. 2013. Navigational Efficiency of Nocturnal Myrmecia Ants Suffers at Low Light Levels. *PLOS ONE* **8**: e58801.
Near, T. J., Dornburg, A., Eytan, R. I., Keck, B. P., Smith, W. L., Kuhn, K. L., Moore, J. A., Price, S. A., Burbrink, F. T. & Friedman, M. 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proceedings of the National Academy of Sciences* **110**: 12738-12743.
Near, T. J., Eytan, R. I., Dornburg, A., Kuhn, K. L., Moore, J. A., Davis, M. P., Wainwright, P. C., Friedman, M. & Smith, W. L. 2012. Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National Academy of Sciences* **109**: 13698-13703.
Nørgaard, T., Nilsson, D.-E., Henschel, J. R., Garm, A. & Wehner, R. 2008. Vision in the nocturnal wandering spider Leucorchestris arenicola (Araneae: Sparassidae). *Journal of Experimental Biology* **211**: 816.
Northmore, D. P. M. (2011) Optic tectum. In: *Encyclopedia of Fish Physiology: from Genome to Environment*, (Farrell, A., ed.). pp. Elsevier.
Palmer, B. A., Taylor, G. J., Brumfeld, V., Gur, D., Shemesh, M., Elad, N., Osherov, A., Oron, D., Weiner, S. & Addadi, L. 2017. The image-forming mirror in the eye of the scallop. *Science* **358**: 1172.
Paradis, E., Claude, J. & Strimmer, K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**: 289-290.
Pennell, M. W., Eastman, J. M., Slater, G. J., Brown, J. W., Uyeda, J. C., FitzJohn, R. G., Alfaro, M. E. & Harmon, L. J. 2014. geiger v2.0: an expanded suite of methods for

fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* **30**: 2216-2218.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & Team, R. D. C. 2016. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-128, http://CRAN.R-project.org/package=nlme. *R Foundation for Statistical Computing, Vienna*.
Querubin, A., Lee, H. R., Provis, J. M. & O'Brien, K. M. B. 2009. Photoreceptor and ganglion cell topographies correlate with information convergence and high acuity regions in the adult pigeon (Columba livia) retina. *Journal of Comparative Neurology* **517**: 711-722.
R Core Team (2017) R: A Language and Environment for Statistical Computing. pp. R Foundation for Statistical Computing, Vienna, Austria.
Rabosky, D. L., Santini, F., Eastman, J., Smith, S. A., Sidlauskas, B., Chang, J. & Alfaro, M. E. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications* **4**.
Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217-223.
Schmitz, L. & Motani, R. 2011. Nocturnality in Dinosaurs Inferred from Scleral Ring and Orbit Morphology. *Science* **332**: 705.
Schmitz, L. & Wainwright, P. C. 2011a. Ecomorphology of the eyes and skull in zooplanktivorous labrid fishes. *Coral Reefs* **30**: 415-428.
Schmitz, L. & Wainwright, P. C. 2011b. Nocturnality constrains morphological and functional diversity in the eyes of reef fishes. *Bmc Evolutionary Biology* **11**.
Sidlauskas, B. 2008. Continuous and arrested morphological diversification in sister clades of characiform fishes: A phylomorphospace approach. *Evolution* **62**: 3135-3156.
Tierney, S. M., Friedrich, M., Humphreys, W. F., Jones, T. M., Warrant, E. J. & Wcislo, W. T. 2017. Consequences of evolutionary transitions in changing photic environments. *Austral Entomology* **56**: 23-46.
Viets, K., Eldred, K. & Johnston, R. J., Jr. 2016. Mechanisms of Photoreceptor Patterning in Vertebrates and Invertebrates. *Trends Genet* **32**: 638-659.
Wagner, H. J. 2001a. Brain areas in abyssal demersal fishes. *Brain Behav Evol* **57**: 301-16.
Wagner, H. J. 2001b. Sensory brain areas in mesopelagic fishes. *Brain Behav Evol* **57**: 117-33.
Wainwright, P. C., Alfaro, M. E., Bolnick, D. I. & Hulsey, C. D. 2005. Many-to-One Mapping of Form to Function: A General Principle in Organismal Design? *Integr Comp Biol* **45**: 256-62.
Warrant, E. 2004. Vision in the dimmest habitats on earth. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **190**: 765-89.
Warrant, E. J. 1999. Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Res* **39**: 1611-30.
Warrant, E. J. & Johnsen, S. 2013. Vision and the light environment. *Current Biology* **23**: R990-R994.
White, G. E. & Brown, C. 2015. Microhabitat use affects brain size and structure in intertidal gobies. *Brain Behavior and Evolution* **85**: 107-116.
Wu, Y., Hadly, E. A., Teng, W., Hao, Y., Liang, W., Liu, Y. & Wang, H. 2016. Retinal transcriptome sequencing sheds light on the adaptation to nocturnal and diurnal lifestyles in raptors. *Scientific Reports* **6**: 33578.
Wu, Y., Wang, H. & Hadly, E. A. 2017. Invasion of Ancestral Mammals into Dim-light Environments Inferred from Adaptive Evolution of the Phototransduction Genes. *Scientific Reports* **7**: 46542.

Yamamoto, N. (2017) Adaptive Radiation and Vertebrate Brain Diversity: Cases of Teleosts. In: *Brain Evolution by Design. Diversity and Commonality in Animals.*, (Shigeno, S., Murakami, Y. & Nomura, T., eds.). pp. 253-271. Springer, Tokyo.

Supplemental Methods and Results

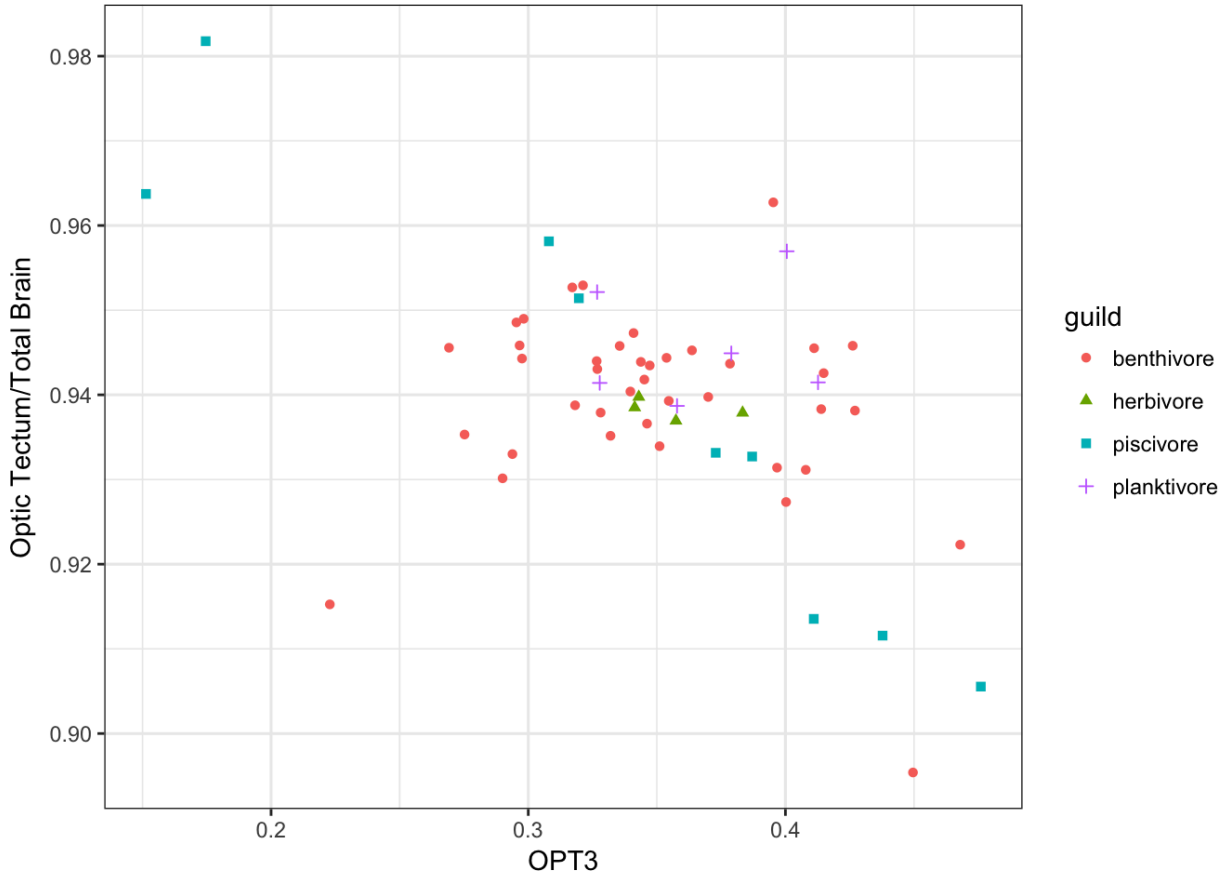

Figure S1. Trophic guild membership and species averages for eye morphology (OPT3) related to photopic (lower numbers) and scotopic (higher numbers) vision and how it relates to investment in the optic tectum relative to total brain.

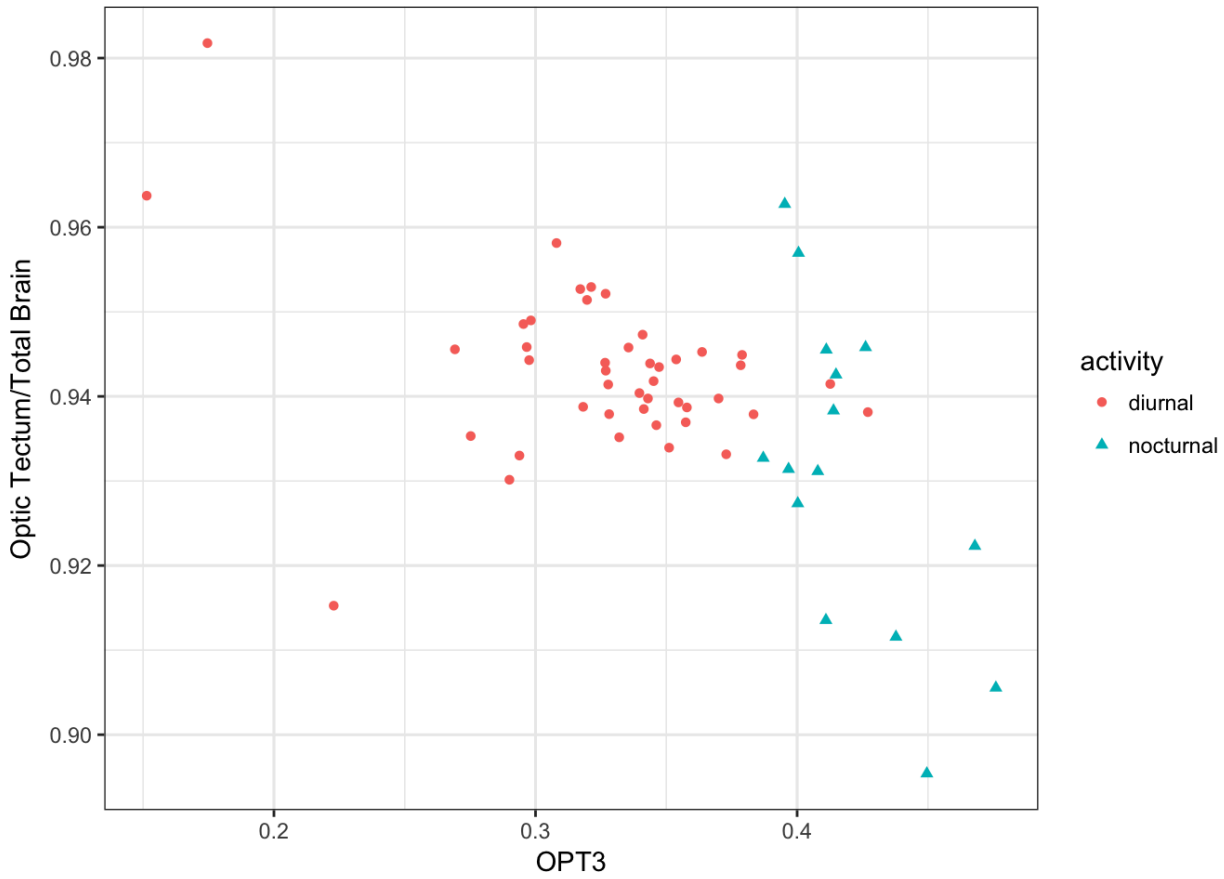

Figure S2. Diel activity pattern and species averages for eye morphology (OPT3) related to photopic (lower numbers) and scotopic (higher numbers) vision and how it relates to investment in the optic tectum relative to total brain.

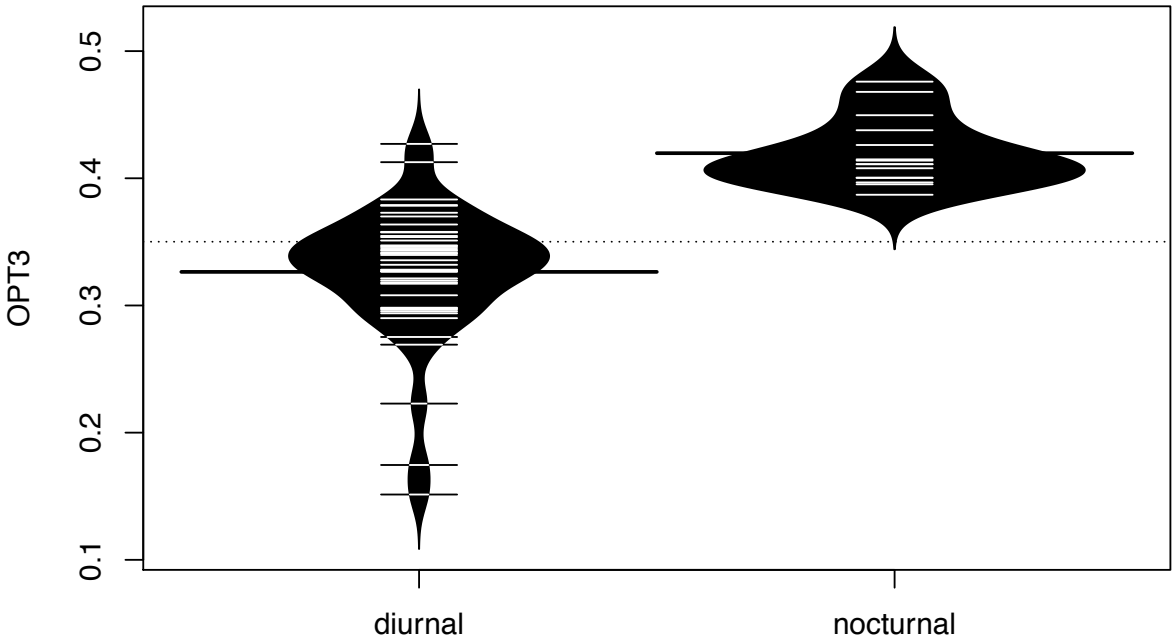

Figure S3. Distribution of measures of OPT3 for diurnal and nocturnal species of teleost fishes with white lines indicting the species average OPT3.

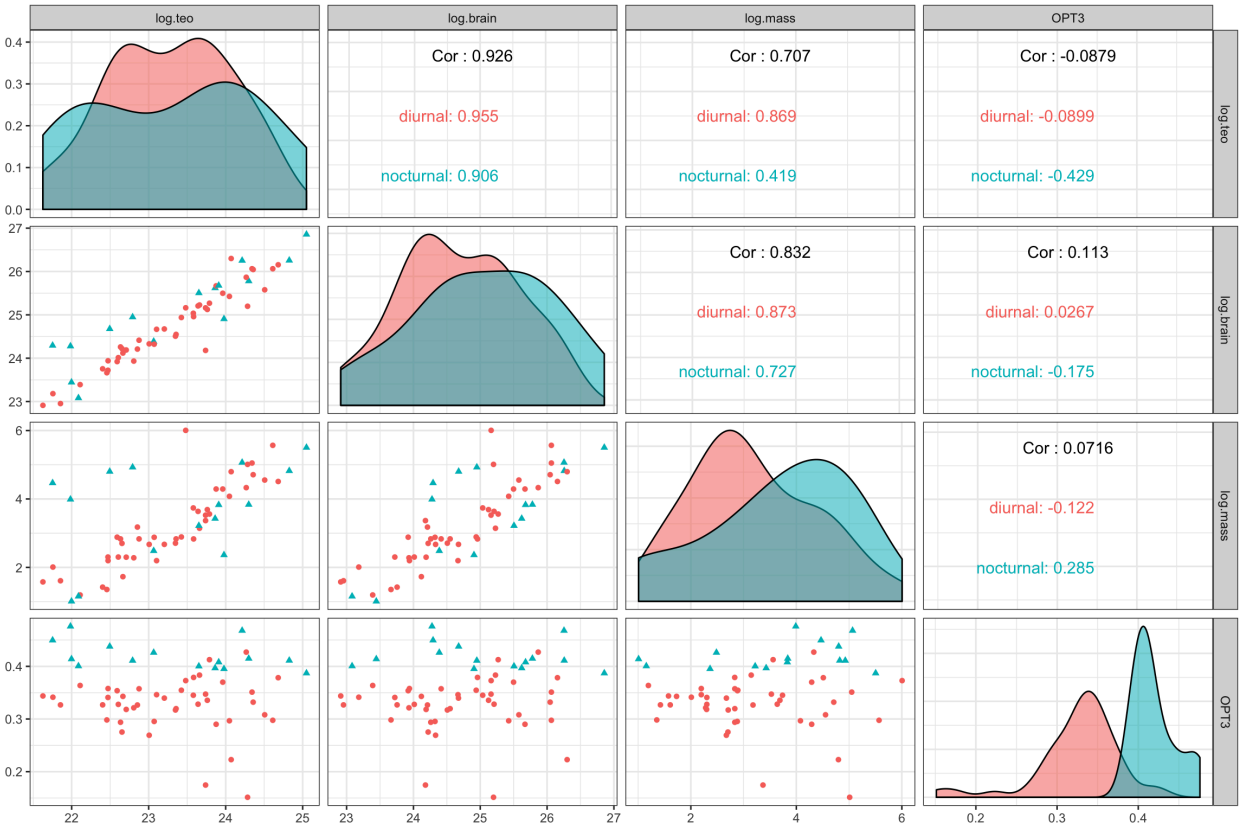

Figure S4. Variables used in PGLS analysis, coded by diel activity period.

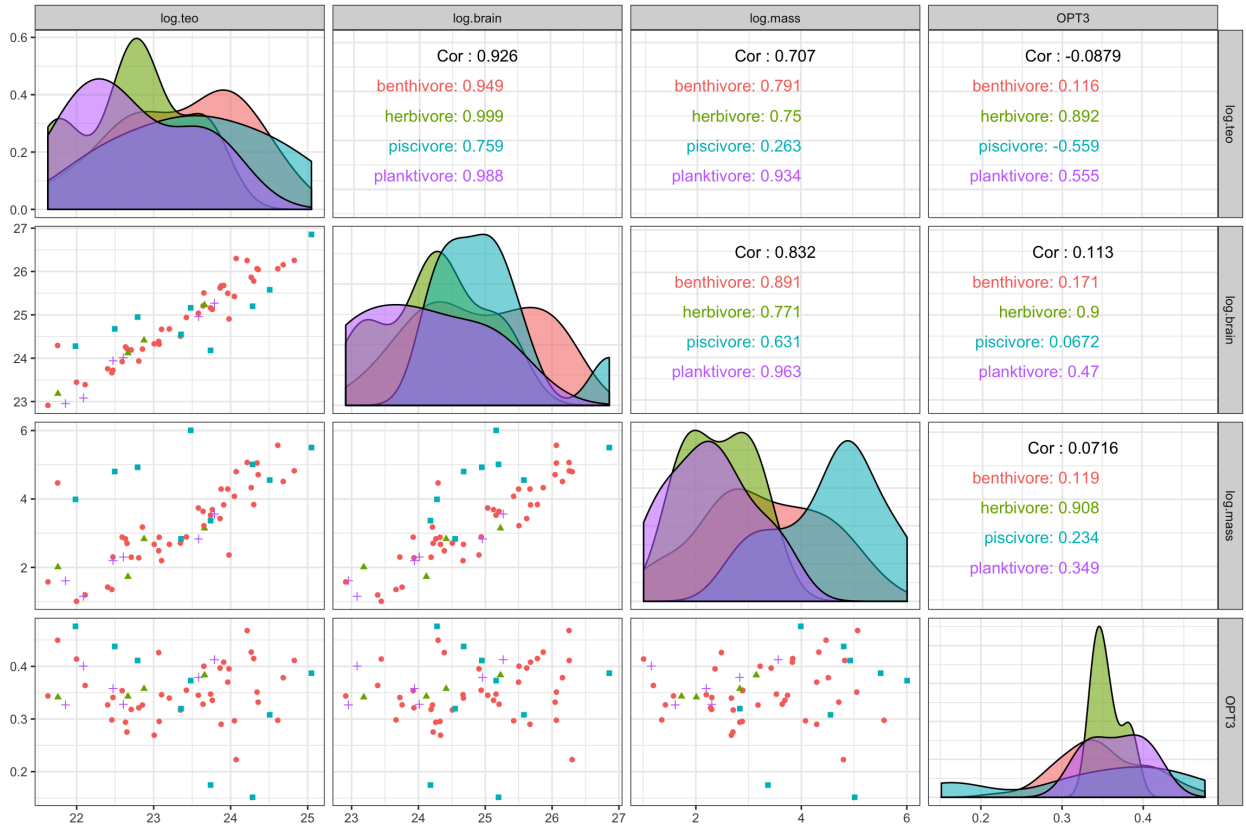

Figure S5. Variables used in PGLS analysis, coded by trophic guild.

Sampling additional taxa

Although the supertree of Rabosky et al. (2013) samples 7822 species of fish, six species we sampled here were not present in this tree. However, for three of these species (*Ostracion meleagris*, *Istiblennius zebra*, and *Ulaema lefroyi*) an information equivalent branch with a taxon unsampled for brain or visual data was available for interchanging taxon identity. This is process equivalent to interchanging human and lemur data when only a single primate lineage is present in a supertree of mammals. In both cases, the sampled branches reflect the same divergence (e.g., "Boxfishes").

For taxa that were highlighted in the primary text as having no information equivalent branches available (*Bothus mancus, Equetus punctatus*, *Mulloidichthys flavolineatus*), we assembled three datasets that allowed us to respectively capture recent divergences in flatfishes, drums, and goatfishes (Supplemental Table 1) as well as divergences that overlapped with the taxon sampling strategy of the tree used by Rabosky et al. (2013). For each dataset, divergence time estimates were generated using BEAST v.2.4.5 (Bouckaert *et al.*, 2014) using a model of uncorrelated rates that follow a lognormal distribution (UCLN) for all analyses with a birth-death prior on rates of cladogenesis. For each dataset, we conducted two independent Markov Chain Monte Carlo (MCMC) runs for 100 million generations that sampled every 10000 generations. Convergence and mixing were assessed by visual examination of the chain likelihoods and quantification of effective samples sizes (ESS) for each parameter in Tracer 1.6. For all parameters, ESS values that exceeded 200 were deemed as indicative of effective sampling of the posterior distribution.

Divergence times were calibrated using either primary or secondary calibrations from the literature. For the addition of *Bothus mancus*, the flatfish, the fossil †*Oligobothus pristinus*, was used to calibrate the stem of Bothidae (divergence between *Bothus* and *Pseudopleuronectes*). This fossil stems from the Rupelian aged Lower Dysodilic shales of Piatra Neamt, Romania, and is placed based on the presence of myorhabdoi (Baciu DS, 2002). This fossil was given an offset of 30 Ma with a 95% soft upper bound of 34.4 million years corresponding to its use a calibration 29 in Near et al. (2012) and 19 in Near et al. (2013). To place *Equetus punctatus,* the spotted drum, we calibrated the crown Scianidae using a secondary calibration based on the 95% HPD interval of divergence time estimates from Near et al. (2013) and Lo et al. (2015), using a normal distribution with an offset of 30 Ma and sigma of 0.5. Finally, to place *Mulloidichthys flavolineatus,* the goatfish, we used the estimated divergence times from Near et al. (2013) between *Parupeneus* and *Pseudupeneus* to place a normal prior distribution with an offset of 21.7 and sigma of 2.1.

The effect of taxon sampling on diurnal morphospace occupancy

To determine the influence of taxon sampling on the estimated differences between nocturnal and diurnal lineages we conducted a series of random subsampling analyses of our data that sampled diurnal lineages in proportion to the nocturnal lineages sampled in our study (n=17). For each set of analyses, we controlled for the minimum number of major clades (families) represented in each draw from 50% to 88% (9-15 families). Once the target number of major clades was reached, additional diurnal taxa were sampled at random until the target number of total species was reached. The convex hull of the morphospace for diurnal lineages was then quantified and divided by the convex hull of the nocturnal lineage morphospace. Values of 1 indicated equal morphospace occupancy (Null) while values below or above 1 indicated a reduced or expanded morphospace respectively. For each level of taxon sampling, the above procedure was repeated 5000 times to determine whether a hypothesis of equal or lesser morphospace occupancy for diurnal lineages could be rejected.

Results of our resampling procedure universally rejected a hypothesis of equal or lesser morphospace occupancy in every set of analyses ($0.00098 < p < 0.045$). Taxonomic diversity did have an impact on quantified differences. When 50-65% of the families were sampled, a twofold difference in morphospace occupancy between diurnal and nocturnal lineages represented the median expected difference, with secondary peaks in the range of 3-fold difference (Figure S6). By 75% family representation, median values suggested a 3.5-fold difference in convex hull areas (Figure S6). While these results do demonstrate that quantification of the exact difference in morphospace occupancy between nocturnal and diurnal lineages is sensitive to taxon sampling of major marine fish clades, these results strongly support that regardless of taxon sampling strategy, morphospace occupancy between these two groups is not expected to be equal.

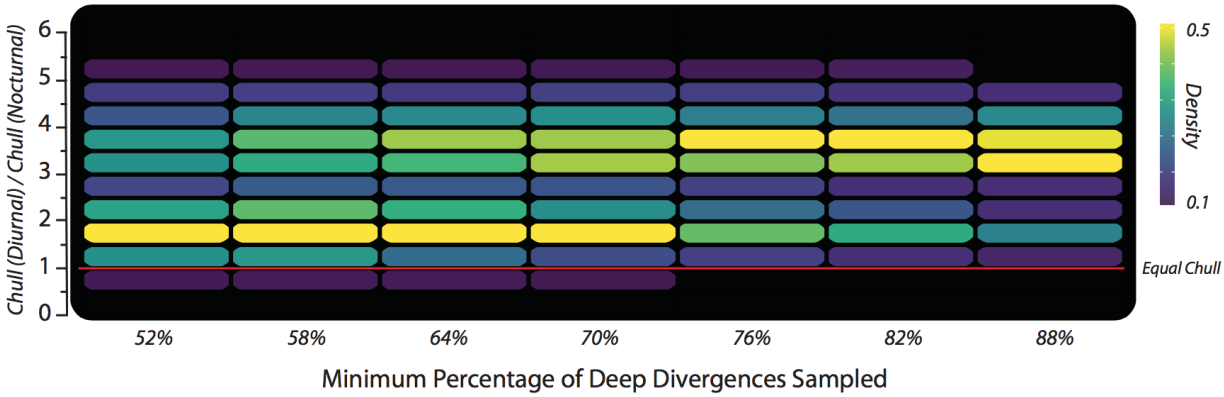

Figure S6: Results of subsampling procedure assessing the impact of decreased taxon sampling on quantification of the convex hulls of morphospace occupancy for diurnal versus nocturnal lineages. Percentages correspond to the minimum number of deep (family) level divergences captured by each subsampling. Colors correspond to the density of values based on 1000 randomizations. Chull = Convex hull.

Family	*Taxon*	*Genbank ID*	*Family*	*Taxon*	*Genbank ID*
Bothidae	*Bothus mancus*	*JQ431490.1*	*Mullidae*	*Upeneus guttatus*	*554792385*
Bothidae	*Bothus pantherinus*	*KP194285.1*	*Mullidae*	*Upeneichthys vlamingii*	*70723084*
Bothidae	*Bothus podas*	*KM538239*	*Mullidae*	*Upeneus japonicus*	*1002677338*
Bothidae	*Bothus_lunatus*	*KF929670.1*	*Mullidae*	*Upeneus luzonius*	*926820302*
Bothidae	*Bothus ocellatus*	*JQ839976.1*	*Mullidae*	*Upeneus moluccensis*	*224581657*
Bothidae	*Bothus maculiferus*	*JQ840775.1*	*Mullidae*	*Upeneus nigromarginatus*	*807059683*
Bothidae	*Bothus leopardinus*	*EU513618.1*	*Mullidae*	*Upeneus parvus*	*383388831*
Bothidae	*Bothus myriaster*	*NC_030365*	*Mullidae*	*Upeneus pori*	*563425672*
Mullidae	*Mulloidichthys auriflamma*	*155965044*	*Mullidae*	*Upeneus suahelicus*	*930269120*
Mullidae	*Mulloidichthys ayliffe*	*554792091*	*Mullidae*	*Upeneus sulphureus*	*1043337146*
Mullidae	*Mulloidichthys flavolineatus*	*375585980*	*Mullidae*	*Upeneus supravittatus*	*930269128*
Mullidae	*Mulloidichthy*	*297525546*	*Mullidae*	*Upeneus*	*151975789*

	s martinicus			*tragula*	
Mullidae	*Mulloidichthys vanicolensis*	*151975775*	*Mullidae*	*Upeneus vittatus*	*1041927192*
Mullidae	*Mullus argentinae*	*383388507*	*Paralichthyidae*	*Citharichthys sordidus*	*JQ354049.1*
Mullidae	*Mullus auratus*	*584296311*	*Paralichthyidae*	*Citharichthys darwini*	*JX516097.1*
Mullidae	*Mullus barbatus*	*307342080*	*Paralichthyidae*	*Paralichthys californicus*	*KT247728.1*
Mullidae	*Mullus surmuletus*	*930580548*	*Paralichthyidae*	*Paralichthys lethostigma*	*KF930227.1*
Mullidae	*Parupeneus barberinoides*	*223368582*	*Paralichthyidae*	*Paralichthys dentatus*	*KF930226.1*
Mullidae	*Parupeneus barberinus*	*161777780*	*Paralichthyidae*	*Paralichthys albigutta*	*JQ842633.1*
Mullidae	*Parupeneus bifasciatus*	*296746999*	*Pleuronectidae*	*Pseudopleuronectes americanus*	*KT073234.1*
Mullidae	*Parupeneus chrysonemus*	*112292605*	*Paralichthyidae*	*Etropus microstomus*	*JX516090.1*
Mullidae	*Parupeneus ciliatus*	*151975531*	*Paralichthyidae*	*Etropus crossotus*	*KF929880.1*
Mullidae	*Parupeneus cyclostomus*	*1041927198*	*Sciaenidae*	*Argyrosomus regius*	*JQ623911.1*
Mullidae	*Parupeneus forsskali*	*227935147*	*Sciaenidae*	*Cynoscion othonopterus*	*KC208685.1*
Mullidae	*Parupeneus fraserorum*	*116608019*	*Sciaenidae*	*Cynoscion reticulatus*	*KC208680.1*
Mullidae	*Parupeneus heptacanthus*	*296746967*	*Sciaenidae*	*Equetus punctatus*	*KF929859.1*

Mullidae	*Parupeneus indicus*	*116608021*	*Sciaenidae*	*Johnius dussumieri*	*FJ384685.1*
Mullidae	*Parupeneus insularis*	*381279622*	*Sciaenidae*	*Larimichthys crocea*	*FJ237998.1*
Mullidae	*Parupeneus macronemus*	*328486658*	*Sciaenidae*	*Larimus pacificus*	*KC208688.1*
Mullidae	*Parupeneus multifasciatus*	*227936615*	*Sciaenidae*	*Leiostomus xanthurus*	*KF930027.1*
Mullidae	*Parupeneus pleurostigma*	*227937084*	*Sciaenidae*	*Menticirrhus elongatus*	*KC208687.1*
Mullidae	*Parupeneus rubescens*	*328486678*	*Sciaenidae*	*Micropogonias megalops*	*KC208689.1*
Mullidae	*Parupeneus spilurus*	*70723092*	*Sciaenidae*	*Micropogonias megalops*	*KC208675.1*
Mullidae	*Parupeneus trifasciatus*	*359326535*	*Sciaenidae*	*Protonibea diacanthus*	*FJ238008.1*
Mullidae	*Pseudupeneus grandisquamis*	*294989292*	*Sciaenidae*	*Stellifer lanceolatus*	*KF930465.1*
Mullidae	*Pseudupeneus maculatus*	*386366747*	*Sciaenidae*	*Totoaba macdonaldi*	*KC208684.1*
Mullidae	*Pseudupeneus prayensis*	*959315921*	*Sciaenidae*	*Umbrina cirrosa*	*JQ624013.1*

Supplemental Table 1: Genbank identifiers for additional sequences used in this study.

Model	# Parameters	AICc	ΔAICc	AICcWt
bm: OPT3 + brain	4	6.18	0.00	0.84
ou: OPT3 + brain	4	10.87	4.69	0.08
bm: OPT3 + brain + trophic guild	7	12.26	6.08	0.04
bm: brain	3	12.38	6.20	0.04
ou: OPT3 + brain + trophic guild	7	17.31	11.13	0.00
bm: trophic guild	6	17.60	11.42	0.00
ou: brain	3	20.04	13.86	0.00
ou: trophic guild	6	26.13	19.95	0.00
ou: intercept only	2	145.52	139.34	0.00
bm: intercept only	2	153.23	147.05	0.00

Supplemental Table 2: Model comparison using the AICc information theoretic approach for small sample size to determine the best fit error structure for our analyses explaining optic tectum volume: brownian motion (bm) or Ornstein-Uhlenbeck (ou) error structure. OPT3: optic morphology, trophic guild: piscivore, planktivore, herbivore, or benthivore, brain: brain volume minus optic tectum, intercept-only: null model.

References

Baciu DS, C.B. 2002. The fossil flatfishes (Teleostei: Pleuronectiformes) from the Oligocene of Piatra Neamt (Romania). *Oryctos* **4**: 17–38.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., *et al.* 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**: e1003537.

Lo, P.-C., Liu, S.-H., Chao, N.L., Nunoo, F.K.E., Mok, H.-K. & Chen, W.-J. 2015. A multi-gene dataset reveals a tropical New World origin and Early Miocene diversification of croakers (Perciformes: Sciaenidae). *Mol. Phylogenet. Evol.* **88**: 132–143.

Near, T.J., Dornburg, A., Eytan, R.I., Keck, B.P., Smith, W.L., Kuhn, K.L., *et al.* 2013. Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proc. Natl. Acad. Sci. U. S. A.* **110**: 12738–12743.

Near, T.J., Eytan, R.I., Dornburg, A., Kuhn, K.L., Moore, J.A., Davis, M.P., *et al.* 2012. Resolution of ray-finned fish phylogeny and timing of diversification. *Proc. Natl. Acad. Sci. U. S. A.* **109**: 13698–13703.

Rabosky, D.L., Santini, F., Eastman, J., Smith, S.A., Sidlauskas, B., Chang, J., *et al.* 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat. Commun.* **4**: 1958.