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# Coloration is related to habitat in *Gymnothorax mordax*, a kelp forest predator

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ABSTRACT: Animal coloration serves a variety of functions, including communication and camouflage. We quantified hue, luminance, countershading, and pigmentation pattern of the California moray eel *Gymnothorax mordax* and determined whether coloration was correlated with the environmental variables of the kelp forest ecosystem. The California moray is an elongate, predatory, crevice-dwelling fish that does not rapidly change color. We photographed morays trapped at a variety of depths in 4 environmentally diverse sites around the Two Harbors isthmus on Catalina Island, California, USA. Depth, substrate type, cover type, horizontal visibility, and reef rugosity were recorded for the environment surrounding each trapped eel. We found that eels were lighter, redder, and yellower in shallow habitats with high percentages of sand, bare substrate, and seagrass. In habitats with greater substrate diversity, clearer water, and a higher percentage of boulder, morays were darker, greener, and bluer. Despite their benthic, crevice-dwelling behavior, we found that all individuals exhibited countershading, which was most extreme at the head and tail. Pigment spots became larger and more uniform in size as standard length increased, but few other size- or age-related color changes were found. We found little evidence that coloration is correlated with foraging success and instead speculate that coloration is established post-settlement in smaller size classes not examined in this study. This work shows that California morays exhibit a range of colorations and that hue and luminance are correlated with environmental variables in the Two Harbors region of Catalina Island.

KEY WORDS: Fish · Hue · Pigmentation pattern · Countershading · Muraenidae



## 1. INTRODUCTION

Coloration is used across the animal kingdom for a variety of purposes, including inter- and intra-specific communication, thermoregulation, and defense (Caro 2005, Cuthill et al. 2017). The term 'coloration' includes hue (the wavelength of reflected light), luminance (degree of lightness), countershading (ratio of dorsal to ventral luminance), and pigmentation pattern. Previous studies have examined coloration for both terrestrial and aquatic organisms (Cuthill 2019), primarily examining how coloration can be used as a predator avoidance mechanism (e.g. Eterovick et al. 1997, Johnsen & Sosik 2003, Cournoyer & Cohen 2011, Orton et al. 2018). Within fishes, coloration has been examined primarily for tropical species that are fusiform, rapidly change color, or those that have distinct color polymorphisms (Barry & Hawryshyn 1999, Marshall 2000, Gilby et al. 2015, Tyrie et al. 2015, Phillips et al. 2017). Bright colorations with many hues are more common in tropical ecosystems, where higher, more consistent light levels and increased water clarity may have allowed for the development of more dramatic colorations (Marshall 2000, Stevens & Merilaita 2009). A smaller subset of work has examined coloration in temperate fishes. Previous studies have shown correlations between coloration of fishes and abiotic environmental characteristics without explicitly examining background matching, such as for arc-eye hawkfish *Para-*

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*cirrhites arcatus* with multiple color polymorphisms (Whitney et al. 2018), Eurasian perch *Perca fluviatilis* in pelagic/littoral lake habitats (Kekäläinen et al. 2010), and coastrange sculpin *Cottus aleuticus* on different stream substrates (Whiteley et al. 2011). A study on King George whiting *Sillaginodes punctata* found that lower light conditions were correlated with brighter hues and more extreme countershading (Meakin & Qin 2012), while studies on threespine stickleback *Gasterosteus aculeatus* (Reimchen 1989) and brown trout *Salmo trutta* found a positive trend or no trend between coloration and water clarity/canopy cover (Westley et al. 2012).

Studies of coloration of aquatic predators are rare, though some work exists for penguins, seals, and cetaceans (Wilson et al. 1987, Caro et al. 2011, 2012). Few studies have examined coloration in predatory fishes, though Nassau groupers *Epinephelus striatus* apparently use rapid patterning changes to avoid detection by prey (Watson et al. 2014). Countershading, an aspect of coloration in which the ventral portion of an animal's body is lighter than the dorsum, may be an additional camouflaging tactic towards catching prey. When an animal is viewed from above or below, countershading may conceal an organism's shadow or break up the outline of an animal's body shape (Ruxton et al. 2004).

In this study, we examined coloration and its relation to habitat characteristics in a benthic, piscivorous, temperate reef fish with an extreme body plan. The California moray eel *Gymnothorax mordax* Ayers, 1859 is the only coastal moray species found off the coast of California, ranging from Point Conception, CA, USA, to southern Baja, Mexico (Fitch & Lavenberg 1971). They occupy most of Catalina Island's diverse coastal habitats, from kelp-dominated systems to sandy bays. As long-lived kelp forest predators capable of eating large prey whole, morays are thought to experience very little predation as adults (Higgins & Mehta 2018).

Similar to other reef-dwelling eels, California morays are primarily crevice-dwelling and nocturnal, characteristics that help organisms avoid detection by diurnal predators and prey without relying on coloration (Gilbert et al. 2005). Unlike the fusiform or deep-bodied fishes typically focused on in coloration studies, highly elongate fishes with reduced fins such as eels tend to roll/twist their bodies along the anterior–posterior axis exposing their ventrum as they swim through the water column (Donatelli et al. 2017). Natural history observations of adult California morays suggest a wide variety of colorations (Fig. 1) but no rapid color changes. In captivity, however, individuals of varying lengths (320–760 cm) have exhibited shifts to lighter colorations over the course of several months to years (R. Mehta unpubl. data), a morphological color change possibly driven by ontogenetic changes, diet, or UV light (Chen et al. 1994, Leclercq et al. 2010, Sköld et al. 2016).

Residence in a diverse range of habitats, high site fidelity, an elongate body plan, and lack of rapid color-changing ability make California morays an interesting model for quantitatively examining coloration in a predatory fish. Here, we first aimed to use robust quantitative methods to characterize the variation in coloration for California moray eels in Two Harbors, CA, hypothesizing that eels would exhibit a gradient of colorations rather than distinct polymorphisms. We did not expect morays to exhibit countershading, as background matching in the water column or shape concealment does not seem to be advantageous for these nocturnal, benthic crevice-dwelling predators with a flexible body. Secondly, we examined whether hue, luminance, countershading, or pigmentation pattern were related to habitat. We hypothesized that morays would be greener and yellower in environments with more seagrass and sand, respectively, that overall body luminance would be darker in deeper and more rugose environments, and that pattern diversity would be positively related to substrate and cover diversity. Lastly, we hypothesized that coloration

Fig. 1. Moray eels around Catalina Island show a subtle, but diverse, range of hue, luminance, and pattern. These images have been corrected for white balance and exposure, but glare and injuries have not yet been masked out

would be correlated with feeding success, body condition, or mortality of adult eels.

## 2. MATERIALS AND METHODS

### 2.1. Sampling sites and trapping procedure

Eel trapping and environmental sampling took place on the western end of Santa Catalina Island, California, from June 26 to August 11, 2019. We sampled 4 sites: West Big Fisherman's Cove (West BFC), Intakes (located between Big Fisherman's Cove and Blue Cavern Point), Cat Harbor, and 4th of July Cove (Fig. S1 in the Supplement; www.int-res.com/articles/suppl/m677p067_supp.pdf). Three of the sites are within State Marine Conservation Areas (SMCA), with West BFC and Intakes falling within the Blue Cavern Onshore SMCA and Cat Harbor falling within the Cat Harbor SMCA. A previous study suggested that Intakes and 4th of July Cove have significantly different substrate types from one another (Higgins & Mehta 2018), while our qualitative observations from previous fieldwork suggested that Cat Harbor was shallower, sandier, and more turbid than the reef-dominated sites.

Eels were captured using custom-made double-chambered wire mesh traps with dimensions 36′ × 11′ × 9′ (~91 × 28 × 23 cm), which select for eels approximately 400–1200 mm in length. Traps were baited using a perforated water bottle containing 2 to 3 frozen anchovies. Each night, we set 4–6 traps at one of the 4 sites at depths between 2 and 10 m, the range of depths at which California morays are most common (Higgins & Mehta 2018). Each evening, we rotated the site at which traps were deployed. After 12–14 h of soak time, we checked each trap while on SCUBA for the presence of eels. Underwater environmental surveys were conducted around any trap in which eels were caught.

### 2.2. Environmental surveys

Underwater environmental surveys recorded trap depth, cover/substrate type, rugosity (i.e. substrate complexity), and horizontal visibility around each trap. When possible, each procedure was conducted 4 times per trap along each of the cardinal headings, then averaged.

To examine cover and substrate type, we conducted a uniform point of contact (UPC) survey following California Reef Check protocols (Freiwald et al. 2019). For each trap, the substrate size (sand, cobble, boulder, or reef) and cover type (articulated coralline algae, crustose coralline algae, red algae, encrusting red algae, brown algae, other brown algae, green algae, seagrass, or none) were recorded every 1 m along a 10 m transect (Freiwald et al. 2019). We then computed the proportion of each of the 4 substrate types and 9 cover types per trap. We additionally calculated 2 diversity indices for both substrate and cover using a Shannon-Wiener index (Shannon 1948).

Rugosity was measured using the linear distance method (Risk 1972, Luckhurst & Luckhurst 1978). A chain of known length was rolled out along each 10 m transect following the contour of the reef as closely as possible. We then recorded the surface distance along the transect that the chain reached. To calculate rugosity ($R$) index , we used the following equation:

$$R = \frac{C}{D} - 1 \quad (1)$$

Where $C$ is the length of the chain fully extended and $D$ is the average surface distance covered along the reef. Higher $R$ values indicate a more rugose reef.

Horizontal visibility was measured using the black disc method (Montes-Hugo et al. 2003). This method is advantageous when measurements are taken at varying light levels, as its reflectiveness depends less on above-surface conditions (Montes-Hugo et al. 2003). One diver swam away from the trap while holding a 30 cm diameter black disc attached to the end of the transect line that was clipped to a trap. The second diver remained at the trap and recorded the distance at which the black disc was no longer discernible from the background.

Due to the strongly colinear nature of the environmental data, we ran a principal component analysis (PCA) to reduce the dimensionality of the habitat data. First, we checked every environmental variable for normality by examining quantile-quantile plots. Then we applied a log transformation to depth, horizontal visibility, and rugosity and an arcsine transformation to the proportions for the 4 substrate types and 9 cover types. We then centered and scaled all values using the 'scale' function in R and imputed 3 missing depth values using the 'imputePCA' function in R package 'missMDA' (Josse & Husson 2016). We then performed a PCA using the 'PCA' function in package 'FactoMineR' (Lê et al. 2008). We extracted PCA components that explained >80% of variation, corresponding to the first 3 axes. PCA coordinates were used as numerical representations of the overall habitat of each trap.

### 2.3. Moray measurements and photography

Once traps were brought to the surface, morphological data and photographs were taken for all eels. Morays were anesthetized in a solution of approximately 90 mg of tricaine methanesulfonate (MS-222) per liter of seawater. Although MS-222 has previously been shown to cause uniform darkening in skin luminance in small fishes (Wojan et al. 2019), anaesthetization was required to conduct measurements on eels, and MS-222 is preferable to clove oil in that it works rapidly (Wojan et al. 2019, R. Mehta unpubl. data). No qualitative changes in coloration were observed due to anesthesia. Eels were submerged and handled for only a few minutes, and this handling was consistent across all individuals. To ensure that each eel was only included once throughout our trapping efforts, we inserted a passive integrated transponder (PIT) tag (BIOMARK© MK165) into the tail muscle using 16-gauge injector needles (BIOMARK© #N165). For any successive catches in a site, we first scanned each individual with a PIT tag reader (BIOMARK© #601) to identify new individuals for our data set. The 15-digit serial number associated with each tag was used to link images, environmental data, and morphological data. We measured standard length (SL), body length (BL), and head length (HL) in mm. We calculated age using a previously developed von Bertalanffy head length–age curve (Mehta et al. 2020). We tested whether SL and age varied across sites by first log-transforming SL to meet assumptions of normality, then using an analysis of variance (ANOVA) test.

Each moray was photographed in a standard setting over a whiteboard with a scale bar using an Olympus Tough TG-5 camera in RAW setting (Fig. 2A). All photos were taken with fixed ISO 100 and aperture f/8.0 under natural lighting as suggested in Gray et al. (2011). In 79% of photos, we included a calibration grey card with 18% standard reflectance, which allowed coloration to be compared across all individuals. If the image did not contain a grey card, it was corrected using an image of a grey card taken in equivalent lighting conditions. We conducted white balance and exposure corrections using the MICA toolbox (Troscianko & Stevens 2015), a plug-in available for ImageJ (Rueden et al. 2017). A multi-spectral image was first generated for each RAW file, which corrected exposure and white balance using the 18% grey standard (Fig. 2B). We used GIMP 2.10 (The GIMP Development Team 2019) to mask out glare, reflection from the whiteboard, and shadows with a uniform white background (Fig. 2C). Only 10–15% of the body surface was masked out for any individual and typically occurred on the most extreme dorsal and ventral edges of the head and mid-body. We saved this new image as a JPEG.

### 2.4. Quantifying coloration

We quantified hue and luminance and conducted all statistical analyses in R 3.6.1 (R Core Team 2016). Hue and luminance were calculated through the function 'kMeansList' in R package 'colordistance'

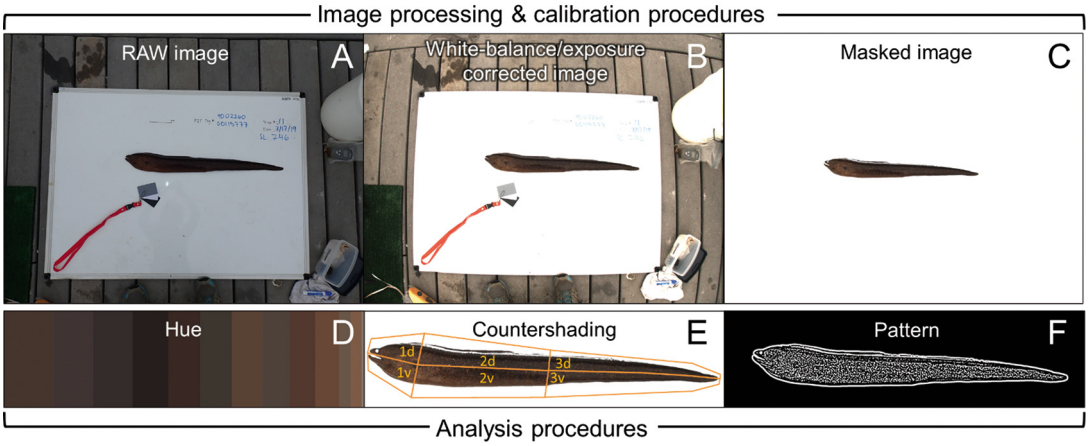


Fig. 2. Workflow for (A–C) image processing and (D–F) calibration and image analysis. (A) Raw, uncorrected original photograph including the 18% standard gray card used for white balance correction. (B) Photo after white-balance and exposure correction. (C) Glare, injuries, and background masked out with uniform white. (D) Each of the 13 clusters produced for each individual was described by a luminance ($L^*$), green–red ($a^*$) and blue–yellow ($b^*$) value. (E) Countershading was calculated by measuring the luminance value for the ventral (v) and dorsal (d) side of the head (snout to parabranchial opening, 1), the mid-body (from parabranchial opening to vent, 2) and the tail (vent to tip of tail, 3). (F) We examined dominant spot size and pattern diversity using ImageJ

(Weller & Westneat 2019). We converted from sRGB values to CIE Lab colorspace using a 'D65' standard daylight reference white. CIE Lab colorspace is typically recommended for coloration analyses because it is perceptually uniform (Weller & Westneat 2019). We chose to use K-means clustering over alternative methods because we were interested in extracting dominant color palette information for each individual to compare against environmental variables rather than comparing between individuals. Each of the clusters' centers were defined by a luminance value $L^\star$, a green–red value $a^\star$, and a blue–yellow value $b^\star$ along with the proportion of pixels in each cluster (Fig. 2D). Clusters were calculated by iteratively sampling 20 000 pixels from the image until convergence. Pure white values were ignored, but the $L^\star$, $a^\star$, and $b^\star$ channels were otherwise unbounded. We found that 13 clusters minimized total within-cluster sum-of-squares values while maintaining low runtimes. We then ran a PCA on each CIE Lab channel using the 'PCA' function in package 'FactoMineR' (Lê et al. 2008). We used values from the first PC axis as an overall $L^\star$, $a^\star$, and $b^\star$ value for each eel. We tested for coloration polymorphisms in hue and luminance using a Hartigan's dip test for multimodality (Hartigan & Hartigan 1985), using R package 'diptest.' We also examined whether coloration variables were correlated with one another through a series of linear regressions.

We analyzed countershading for the head (snout to parabranchial opening), the mid-body (from parabranchial opening to vent), and the tail (vent to tip of tail) (Fig. 2E). Each of these sections was further divided into a dorsal and ventral portion along the geometric midline of the fish. The luminance of the dorsal and ventral regions of each section was then quantified using the 'Pattern Color & Luminance Measurements' tool in the MICA Toolbox. We calculated a countershading ratio value for each of the 3 sections and the overall body by dividing the dorsal luminance by the ventral luminance. Countershading ratio values closer to 0 indicate more extreme countershading, values around 1 indicate no countershading, and values above 1 indicate reverse countershading. Countershading values for each body section were determined to be normally distributed through examinations of quantile-quantile plots. We tested whether countershading ratio values were significantly less than 1 (i.e. countershading present) using a 1-sample 1-tailed *t*-test. A repeated measure ANOVA test was used to determine if countershading varied among the 3 body regions within each individual, followed by a Tukey honest significant difference (HSD) test to examine which regions were driving differences.

Size-corrected dominant spot size and spot size diversity were quantified using the pattern and luminance measurements tool in the MICA Toolbox. Briefly, this tool uses a bandpass filtration method to determine the relative proportion of different pattern sizes across the entire body, termed 'pattern energy' (Fig. 2F). We ran this tool on the masked images with a filtration size range of 2–688 px, progressing by a factor of $\sqrt{2}$. We extracted the pixel size at which pattern energy was highest, equivalent to the dominant spot size. This spot size was converted from px to cm using the scale bar in each photograph. To size-correct dominant spot size, we ran a linear regression between SL and dominant spot size, then extracted the residuals of this relationship, thereby removing the effect of BL. Additionally, we calculated a spot size diversity index by dividing the sum of pattern energies by the maximum pattern energy. Low spot size diversity values indicated that a single spot size dominated the overall pattern.

To test if sites represented distinct suites of colorations, we ran 2 multivariate ANOVA (MANOVA) tests with the 3 hue values, 4 countershading values, and 2 pattern indices as predictor variables and site as the dependent variable. As Cat Harbor represented the most extreme habitat, we ran an additional MANOVA testing for differences just between Cat Harbor and the other 3 sites.

### 2.5. Relationship between coloration and habitat

To determine whether habitat is correlated with coloration, we ran a series of linear regressions between each of 6 response variables (PCA Axis 1 values for $L^\star$, $a^\star$, $b^\star$; countershading ratio across the entire body; size-corrected dominant spot size; and spot size diversity) and 5 predictors (environmental PCA Axes 1, 2, and 3, age, and log-transformed SL). Age and length were tested separately because growth rates of California morays have been shown to decline with age, such that age and SL are uncoupled in individuals >15 yr old, with head length being the more accurate predictor of age (Mehta et al. 2020). We did not run a regression analysis between dominant spot size and log-transformed SL since dominant spot size was already size-corrected. We categorized trapping site as a random effect. Regressions were estimated using restricted maximum likelihood (ReML) using the function 'lme' in R package 'nlme' package (Pinheiro et al. 2019). Conditional $R^2$ values were cal-

culated by the function 'rsquared' in R package 'piecewiseSEM' (Lefcheck 2016).

### 2.6. Correlation between coloration and foraging success

We used body condition as a first metric of foraging success. We calculated mathematical relationship between mass and SL for eels from the present study as well as 2163 eels captured around Catalina Island from 2015–2018 (Mehta et al. 2020). An expected mass was then calculated for each eel. The difference between the expected mass and observed mass formed the condition index for each individual, where positive values indicated that an individual was heavier for its length than expected. We then tested for significant linear relationships between all coloration variables and body condition.

As a second metric of foraging success, we examined whether coloration was correlated with the presence of gut contents or the number of prey items consumed. To determine recent feeding success, we manually palpated prey items from the stomach into the oral cavity, then recorded total number of prey items (Hyslop 1980). This method reflects prey recently consumed as complete digestion of fish prey representing 12% of moray mass is roughly 11–12 d (R. Mehta unpubl. data). We ran a series of logistic regressions with all coloration variables, age, and SL as predictor variables, and the presence of gut contents as the response variable. We ran a similar set of linear regressions with the number of prey items consumed as the response variable.

## 3. RESULTS

### 3.1. Moray sampling and environmental surveys

A total of 120 eels were photographed from 4 sites around Two Harbors, Catalina Island (Table 1). Eels captured ranged in length from 410–1118 mm (mean 674 mm). Age (calculated via HL) ranged from 7.8–23.3 yr (mean 11.3 yr). Log-transformed SL and age was not significantly different between sites (ANOVA, $F_{3,116}$ = 1.76 and $F_{3,115}$ = 1.43, respectively; both $p > 0.05$; Table S1).

Habitat surveys were completed on 57 traps (Table 1). The 4 sites exhibited variation in environmental features (Fig. 3). Cat Harbor was the most distinct of the 4 sites, characterized by sandy, shallow, highly turbid habitat with low rugosity. 4$^{th}$ of July was the least rugose and had the lowest substrate diversity. Cover was primarily comprised of brown algae and cobble. Conversely, Intakes had high rugosity, high substrate diversity, and high coverage diversity, with a mixture of reef and boulder. Habitat

Table 1. Sample sizes for both eels and traps per site, along with the average number of eels captured per trap

| Site | N eels | N traps | Age range (yr) | Size range (mm) | N eels with gut contents |
|---|---|---|---|---|---|
| 4$^{th}$ of July | 30 | 14 | 7.8–19.4 | 442–1018 | 7 |
| Cat Harbor | 32 | 15 | 7.8–23.3 | 414–1118 | 8 |
| Intakes | 26 | 11 | 9.1–19.4 | 556–922 | 6 |
| West Big Fisherman's Cove | 32 | 17 | 7.9–19.0 | 415–1004 | 5 |

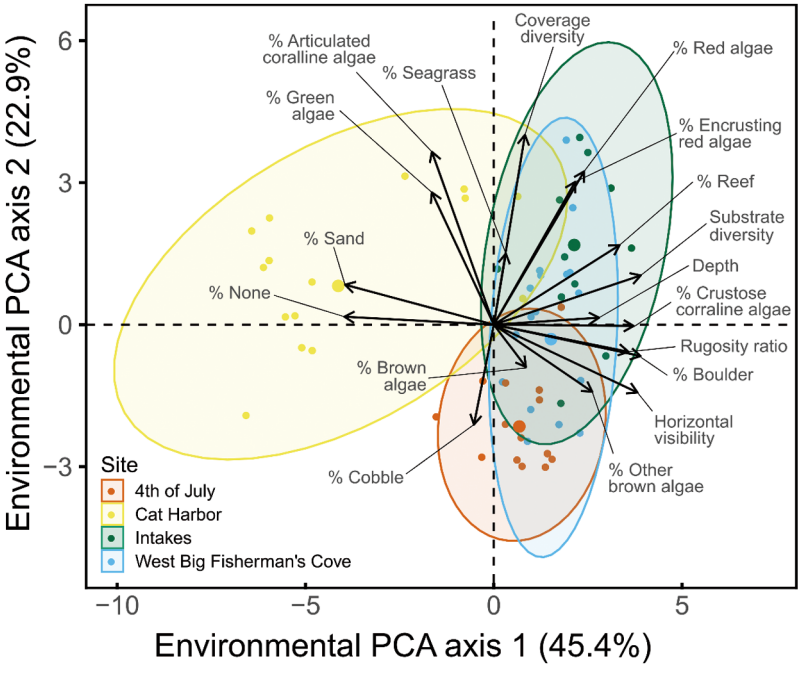


Fig. 3. Environmental principal components analysis (PCA) showing Axes 1 and 2, which together explain 66.9% of environmental variation among traps. Axis 3 explained an additional 15.9%. The 4 sites chosen successfully capture the diverse array of habitats occupied by California morays, with Cat Harbor representing the most distinct site. Ellipses are calculated based on a multivariate normal distribution

in West BFC resembled both 4th of July and Intakes. Axes 1, 2, and 3 of the environmental PCA together described 84.0% of variation among trap environments (Fig. 3, Table S2).

### 3.2. Quantifying coloration

We quantified 4 aspects of coloration: hue, luminance, countershading, and pattern. Hue and luminance were represented by the first principal components analysis for $L^*$ (dark to light), $a^*$ (green to red), and $b^*$ (blue to yellow) value, which have a maximum range of −100 to 100. Eels varied from 1.5 to 80.0 in the $L^*$ channel, −6.5 to 50.5 in the $a^*$ channel, and −7.5 to 38.8 in the $b^*$ channel. All 3 channels were strongly correlated with one another ($p < 0.0001$; Table S3). Neither hue nor luminance was correlated with age or SL ($p > 0.05$). We found no evidence of distinct polymorphisms in hue or luminance (Hartigan's dip test, $p > 0.05$); instead, eels fell along a gradient for each channel (Fig. 4). We tested whether sites represented different suites of colorations through 2 MANOVAs, first between all sites, and second between Cat Harbor and all other sites.

Eels showed an average countershading ratio (CR) of 0.95 across all body regions, and countershading was significantly less than one in all body regions (1-tailed $t$-test, df = 370, $p < 0.0001$). Countershading values were normally distributed. CR varied significantly between the head (snout to branchial opening), mid-body (gill opening to vent), and tail (vent to tip of tail) (Fig. 5). This trend occurred both within individual eels and when averaged across all eels (repeated measures ANOVA, $F_{2,245} = 60.17$, $p < 0.0001$; Table S1), with all regions being significantly different from one another (Tukey HSD test, SE = 0.02, $p < 0.0001$). The head showed the most extreme degree of countershading (mean CR = 0.85), followed by the tail region (mean CR = 0.93). Countershading of the tail was positively related to SL so that longer eels had more extreme countershading in their tails ($p < 0.01$, slope = −0.2; Table S3). However, age was not significantly related to tail countershading ($p > 0.05$). On average, the mid-body region showed reverse countershading, in which the dorsal side was lighter than the ventral side (mean CR = 1.07). However, the mid-body region also exhibited the greatest variation in CR values, with a SD of 0.22, nearly twice that of the head (SD = 0.12) and tail (SD = 0.15).

Pattern results confirmed that moray pigmentation is best described as 'mottled' rather than 'uniform' or 'disruptive' (Barbosa et al. 2008), with pattern energies forming a curve rather than a staircase or flat line (Fig. S2). We determined that spots remained proportionally very small in relation to overall body

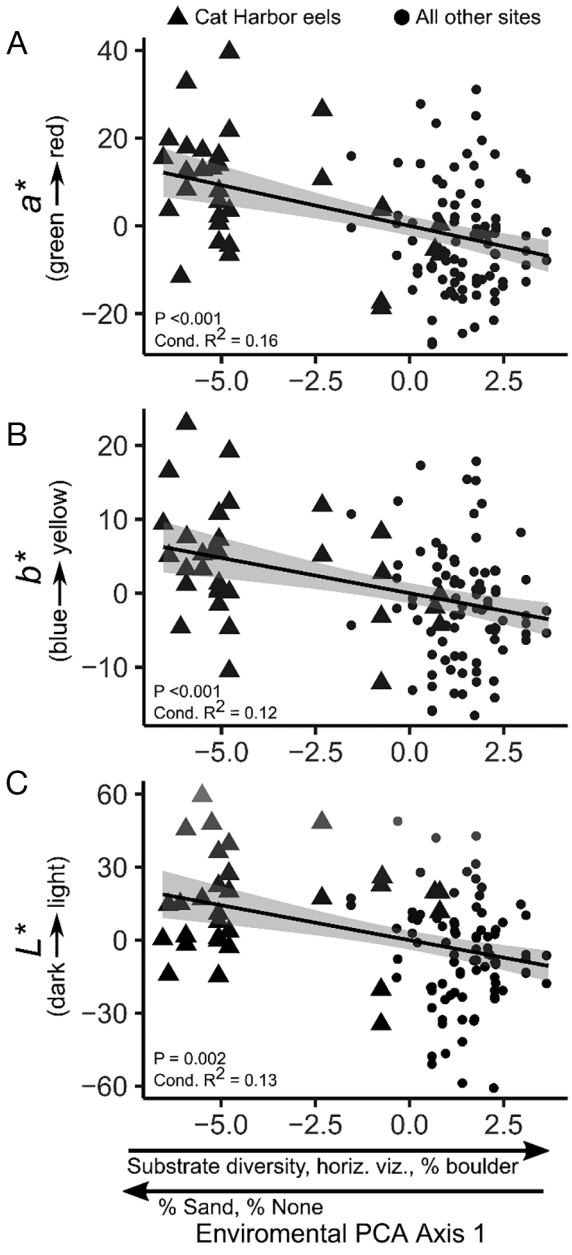


Fig. 4. Moray eel (A,B) hue and (C) luminance is predicted by environmental characteristics. Eels are redder, yellower, and lighter in sandy habitats with bare substrate. Alternatively, eels are greener, bluer, and darker in habitats with high substrate diversity, clearer water, high percentages of boulder, and high rugosity (see Table S2 for variable loadings). Triangles represent eels from Cat Harbor. Conditional $R^2$ values represent variance explained by both fixed effects and trapping site. Point colors represent actual CIE Lab scores, with green–red ($a^*$) and blue–yellow ($b^*$) values shown at luminance ($L^*$) of 25 (the mean across all individuals) to improve visualization

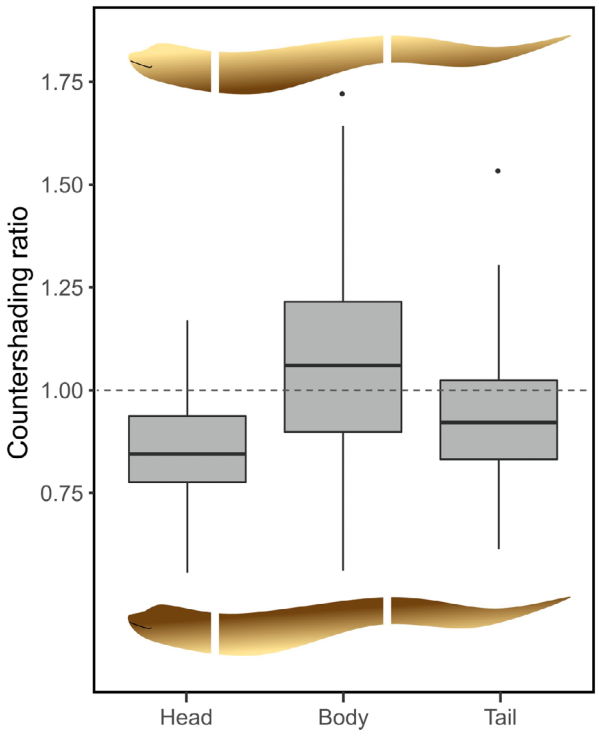


Fig. 5. Countershading varies significantly between the head, middle body, and tail regions (repeated-measures ANOVA, post-hoc Tukey HSD test, $p < 0.0001$). Both within individual eels and across all eels, the head and tail show the most extreme degree of countershading (countershading ratio <1). This may be related to moray behavior of keeping the head (and occasionally tail) outside of crevices. Bar: median; box: interquartile range (IQR) spanning the 25th–75th percentiles; whiskers: 1.5× above/below IQR; dots: outliers. Dashed line at 1.0 indicates no countershading

length, even for large individuals (linear regression, $p < 0.001$, slope = 0.005; Table S3). Spot size diversity was negatively correlated with SL, indicating that as eels grow, their spots become more uniform in size ($p < 0.001$, slope = –0.001; Table S3). Additionally, dominant spot size was negatively correlated with spot size diversity, in that individuals with larger spots had lower spot size diversity ($p < 0.001$). However, no other correlations were seen between coloration variables.

When considered separately, all 4 sites were indistinguishable from one another in terms of coloration except for countershading of the head (MANOVA, $F_1 = 5.42$, $p = 0.02$; Table S4). When Cat Harbor was compared separately from the other sites, $a^*$ and pattern spot size distinguished Cat Harbor from all other sites (MANOVA, $F_1 = 3.71$ vs. 1.02, $p < 0.001$ vs. $p = 0.004$; Table S4), with Cat Harbor eels being redder and having a slightly higher pattern of spot size diversity.

### 3.3. Relationship between coloration and habitat

Hue ($a^*$ and $b^*$) and luminance ($L^*$) were significantly correlated by environmental PC axis 1 ($p < 0.05$), representing habitat (Table S3). Eels in habitats with greater substrate diversity, clearer water, greater percentages of boulder, and greater rugosity were greener, bluer, and darker (Fig. 4). Conversely, eels were redder, yellower, and lighter in shallow habitats with high percentages of sand and bare substrate (Fig. 4). Since we found that countershading varied between body regions, we tested for environmental correlations for each section individually. However, none of the countershading variables, size-corrected dominant spot size, or spot size diversity were correlated with habitat as represented by the 3 environmental PC axes ($p > 0.05$).

### 3.4. Correlation between coloration and foraging success

We tested for the effect of coloration on body condition and predation success. Only 27 eels (22.5%) from all 4 sites had stomach contents (Table 1). We found no relationship between any of the 6 coloration variables (hue, luminance, average countershading across the entire body, and pattern) and the 3 metrics of foraging success (body condition, the presence of gut contents, or the number of gut contents) ($p > 0.05$). Instead, age and SL were the strongest predictors of foraging success. Longer, older eels were in better condition, more frequently had prey in their stomachs, and had more prey items in their stomachs ($p < 0.05$; Table S3, Fig. S3).

## 4. DISCUSSION

### 4.1. Quantifying coloration

We found that California morays exhibit a gradient of colorations rather than distinct polymorphisms, and there is strong correspondence between hue and luminance and environmental variables. A few of our initial hypotheses were supported: eels were lighter and yellower in areas with high percentages of sand and bare substrate, while eels in rugose environments with clear water, high substrate diversity and high percentages of boulder were darker and bluer. Although the environmental surveys did not measure coloration of the actual habitat, we hypothesize that sand is yellower than other types of substrate

and thus has some correspondence with moray hue. However, we found no correlation between seagrass and hue, nor between pattern diversity and substrate/cover diversity. Similarly, while red hues appear darker underwater due to rapid wavelength attenuation through water (Jerlov 1976, Johnsen 2002), California morays were redder in shallower environments. One potential explanation may be the strong correlation between the $a^*$ and $b^*$ channels: eels that were yellower were also redder. Consequently, we hypothesize that California morays have several strategies to avoid detection by predators and prey depending on habitat: individuals in areas with an abundance of potential hiding places may depend more on behavior to avoid detection, but individuals in sand-dominated habitats with fewer crevices, like Cat Harbor, may rely more on coloration.

Although the MS-222 likely affected all eels similarly, Gray et al. (2011) demonstrated that this anesthetic can decrease overall coloration variation. Future studies examining fitness or communication as it relates to eel coloration should consider the effects of MS-222 on overall color variation. Future work examining fitness consequences or intraspecific communication should attempt to photograph eels prior to and after anesthesia to determine if there are differences in coloration.

We show that California morays have a mottled pigmentation pattern. Our initial hypothesis that mottling might be a form of concealment in habitats with a diverse array of substrate and cover types was unsupported. An alternative hypothesis could be that morays in temperate ecosystems are more frequently mottled. However, among subtropical and temperate species in the genus *Gymnothorax*, pigmentation pattern varies from uniform, with little patterning (e.g. *G. prasinus*, *G. unicolor*) to disruptive, with reticulations, spots, or bars (e.g. *G. minor*, *G. prionodon*, *G. ypsilon*) (Froese & Pauly 2019). Only a few species (*G. porphyreus*, *G. obesus*) qualitatively exhibit the same type of mottling as the California moray.

In California morays, it appears that controls on pigmentation cause smaller spots to coalesce into larger spots of more uniform size as BL increases but that spot size remains proportionally very small in comparison to overall BL. Similar changes in pigmentation patterns have been observed in *G. favagineus*, whose spot number increases with length while relative spot size remains constant (Chen et al. 1994). In zebrafish, reaction-diffusion or mechanical mechanisms could be responsible for skin patterns in fish across development, including spots that coalesce over time (Caballero et al. 2012, Watanabe & Kondo 2015). In addition to genetic controls (Kelsh 2004), growth rate, body shape, interactions between pigment cells, and epigenetics all affect the rate and type of pattern development (McClure & McCune 2003, Caballero et al. 2012) in fishes.

Countershading, one of the traits we investigated as part of the suite of coloration traits, is often associated with fish species having a fusiform shape and residing in the water column (Ruxton et al. 2004), where it is hypothesized to assist with camouflaging organisms when viewed from above or below in low turbidity environments. Other eels with different life histories exhibit countershading, such as European eels *Anguilla anguilla* in the migratory, pelagic silver eel phase (Pankhurst & Lythgoe 1982) and a heterenchelyid mud eel *Pythonichthys* cf. *macrurus* which exhibits an unusual infaunal, flatfish-like lifestyle (Martinez & Stiassny 2017). However, California morays are benthic, nocturnal, non-migratory, and do not maintain a constant body posture when in the water column, and we did not expect to find strong countershading. While we found that the countershading is not as striking as in European eels or *P. macrurus*, all individual morays examined exhibited countershading and that location on the body, rather than habitat, predicted countershading intensity. We also observed reverse countershading in the middle region of the body. Variations in countershading intensity across the 3 body regions may correspond with the amount of time morays expose each body section during the day, as morays frequently protrude their heads, and occasionally tails, out of crevices. Alternatively, countershading variations across the length of the body (including reverse countershading in the mid-body) could assist with shape concealment of this elongate fish in the water column. Fish that are primarily active at dawn/dusk have been shown to exhibit countershading, despite the low-light environment, with countershading potentially providing camouflage through shape concealment (Nemtzov et al. 1993). These results suggest that there may be functional advantages of countershading even for benthic, elongate, crevice-dwelling organisms.

### 4.2. Correlation between coloration and foraging success

Our results indicate that coloration is not correlated with either body condition or the presence/number of prey items. Instead, we found that age and SL

were the strongest predictors of body condition, presence of gut contents, and number of prey items. Past work has shown that as California morays grow, they can consume proportionally larger prey and have larger bite forces (Higgins et al. 2018). Thus, body size appears to be a bigger influence on foraging success than coloration.

We viewed coloration through a human-based system and did not measure the visual systems of potential prey or predators of eels, and thus, biological conclusions regarding the benefit of coloration are not possible. Modeling the visual systems of predators and prey is ultimately required for assessing how morays appear to other organisms in the system (Endler 1990, Olsson et al. 2018). Current evidence suggests that while color vision may be more common in marine fishes than previously thought, the extent and type of color vision in fish is likely limited (Levine & MacNichol 1982, Marshall & Vorobyev 2003, Marshall et al. 2019). California morays feed primarily on kelp bass *Paralabrax clathratus* (Higgins et al. 2018). Kelp bass possess double cones, which indicates some ability to distinguish colors during the daytime (Schwassmann 1968), though they may also be used for detecting luminance differences (Lythgoe 1980, Pignatelli et al. 2010). Along with color vision, fish differ in their achromatic vision (i.e. ability to distinguish between luminance signals), predominantly used for detecting edges and patterns (Siebeck et al. 2014). It appears that for some fish, the visual processing pathways for hue and luminance are linked (Siebeck et al. 2014), but fish likely vary widely and may adapt to different environmental conditions (Carleton et al. 2020). While no studies quantify achromatic vision for morays, studies on serranids related to kelp bass show that this clade of fishes is relatively sensitive to luminance differences (Caves et al. 2017) but fall well below human abilities.

### 4.3. Selection on morays <400 mm

Coloration selection in eels in size classes smaller than those selected by our traps is the most likely explanation for the correlation between coloration and the environment. Unfortunately, it is also the selective force for which we have the least evidence. Traps used in this study target eels >400 mm, corresponding to morays approximately 7 yr and older. Thus, trap selectivity precluded us from examining how early coloration is established. It is unknown what mechanisms may influence coloration of smaller eels, though predation or post-settlement color change are the most likely. While adult California moray eels likely have very few natural predators around Catalina Island due to their position as apex predators, no data exists for the frequency with which small eels are consumed. Though seals and sea lions have been shown to feed on eels in other regions (Goodman-Lowe 1998, Berry et al. 2017), these marine mammals are not abundant around the Two Harbors area of Catalina Island, and they do not appear to feed on California morays (Shane 1994, LeBoeuf 2002). Sharks have also been observed feeding on eels in other areas (Béguer-Pon et al. 2012, Sears & Sikkel 2016). However, historical diet studies on 2 of the most likely shark predators, the leopard shark *Triakis semifasciata* and the blue shark *Prionace glauca* showed no indication that these species consume eels (Talent 1976, Tricas 1977, Smith 2001). While white sharks *Carcharodon carcharias* are known to visit Catalina Island (Jorgensen et al. 2012), only younger individuals are thought to predate upon bottom-dwelling fishes (Dewar et al. 2004), and no studies have reported eels in their diets. However, diet studies of sharks from the last 30 yr (the approximate lifespan of the California moray) are limited. While conspecific predation does occur, it makes up only a small percentage of moray diets (Higgins et al. 2018, Mehta et al. 2020). Increased trapping effort for smaller eels would provide more information on how coloration is established in earlier stages, particularly in post-settlement individuals.

### 4.4. Habitat self-selection

It is unlikely that California morays are self-selecting microhabitats within each site based on hue alone. First, California morays are probably colorblind, similar to other *Gymnothorax* species that have only one type of color-sensitive cone cell (Wang et al. 2011). The Kidako moray *G. kidako* and giant moray *G. javanicus* also have very small optic tectum volumes and unusual olfactory bulb structures, suggesting that these species hunt primarily by smell rather than sight (Yamamoto 2017, Iglesias et al. 2018). While California morays could theoretically still self-select habitat based on luminance, our results indicate that environmental characteristics associated with ambient light levels such as depth and rugosity were only weakly correlated with luminance.

Second, eels have very high site fidelity. Mehta et al. (2020) reported that of 1311 eels captured around

Santa Catalina from 2015–2018, only 17 individuals were captured in more than one cove. Thus, eels do not appear to be migrating between coves to find suitable habitat. This suggests that other mechanisms (such as mortality, predation, or post-settlement color changes) influence the establishment of coloration.

### 4.5. Conclusion

In summary, we found that there are differences in the coloration of individual California moray eels inhabiting the Two Harbors area of Catalina Island, California. Eels from Cat Harbor, a site characterized by sandy habitats with few hiding places, had the most distinct colorations. For a crevice-dwelling, nocturnal fish, we found a surprising correlation between hue and luminance and environmental characteristics of the habitat. Interestingly for a benthic fish whose body position in the water column is not static, morays exhibited countershading across their entire body surface, but most pronounced in the head and tail. Results indicated that coloration does not improve foraging success of adult eels, and we subsequently hypothesized that coloration has some advantage in early life stages.

Other benthic reef fishes in southern California waters do not exhibit the same suite of coloration characteristics as California morays. However, scorpionfishes *Scorpaena* spp. and cabezon *Scorpaenichthys marmoratus* qualitatively exhibit some degree of mottling and/or countershading. Other predatory fishes in California show distinctly different coloration patterns between the juvenile and adult stage, including the California sheephead *Semicossyphus pulcher* and giant sea bass *Stereolepis gigas*. Coloration may be a previously overlooked characteristic of importance for many nearshore fishes in California.

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