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Moray eels are more common on coral reefs subject to higher human pressure in the greater Caribbean

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HIGHLIGHTS

Baited remote underwater videos and environmental DNA were used to assess morays

Market gravity had a strong positive effect on moray abundance

Morays and sharks were negatively associated

Lack of competitors and predators may explain increased morays on reefs near humans

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Moray eels are more common on coral reefs subject to higher human pressure in the greater Caribbean

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SUMMARY

Proximity and size of the nearest market ('market gravity') have been shown to have strong negative effects on coral reef fish communities that can be mitigated by the establishment of closed areas. However, moray eels are functionally unique predators that are generally not subject to targeted fishing and should therefore not directly be affected by these factors. We used baited remote underwater video systems to investigate associations between morays and anthropogenic, habitat, and ecological factors in the Caribbean region. Market gravity had a positive effect on morays, while the opposite pattern was observed in a predator group subject to exploitation (sharks). Environmental DNA analyses corroborated the positive effect of market gravity on morays. We hypothesize that the observed pattern could be the indirect result of the depletion of moray competitors and predators near humans.

INTRODUCTION

The size and proximity of the nearest market has a strong negative effect on predatory coral reef fishes, with some relief provided by closing reef areas to fishing (Cinner et al., 2018; Graham et al., 2017; MacNeil et al., 2020; Valdivia et al., 2017). This pattern has been observed on coral reefs worldwide, affecting commercially exploited predator taxa ranging from groupers to sharks (Cinner et al., 2018; Graham et al., 2017; MacNeil et al., 2020; Valdivia et al., 2017). Moray eels (Family Muraenidae; hereafter referred to as 'morays') are not readily detected in conventional reef surveys, and it is unknown how they respond to human impacts on coral reef systems (Chan, 2017; Gilbert et al., 2005; Mehta and Wainwright, 2007; Mouillot et al., 2013). Morays comprise nearly 200 species of functionally unique reef predators, with their elongate bodies and raptorial pharyngeal jaws enabling them to ambush relatively large fish, crustaceans, and octopuses in habitats with complex structure (Chan, 2017; Gilbert et al., 2005; Mehta and Wainwright, 2007; Mouillot et al., 2013). Morays have little to no commercial value because they are generally unpalatable and can contain toxic levels of ciguatera (Chan, 2017). We hypothesize that size and proximity of markets and closing reefs to fishing would therefore have no effect on morays.

The aim of the present study was to explore relationships between morays and two human-related factors that typically affect predatory reef fish: whether a reef is open or closed to fishing ('protection status') and the size and proximity of the nearest market ('market gravity'), as well as the interaction between these two factors. Market gravity is a measurement of human impact on a location that is a function of both accessibility in terms of travel times to markets and the size of these markets (i.e., human population size [Cinner et al., 2018]). We included reef structural complexity as a factor in the model, which generally has a positive effect on reef fish (MacNeil et al., 2020; Valdivia et al., 2017). We also explored relationships between sharks and these factors to enable comparison between morays and a commercially exploited predator taxon.

RESULTS AND DISCUSSION

Video sampling throughout the greater Caribbean was conducted as part of the Global FinPrint Project (https://globalfinprint.org/) and involved the deployment of 60-minute baited remote underwater video

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Table 1. BRUVS locations and corresponding anthropogenic and habitat factors

Location	Reef	Protection status	Market gravity	Reef complexity	No. BRUVS	Moray MaxN	Shark MaxN
Antigua and Barbuda	Barbuda – North	Open	8	1.53	42	0.05	0.43
	Barbuda – Palasar	Closed	9	2.11	45	0.13	0.71
Bahamas	Abaco – Bight of Old Robinson Bay	Open	19.02	1.54	61	0	0.1
	Abaco – Bight Reef	Open	18.41	1.8	68	0.03	1.04
	Abaco – Elbow Cay Reef	Open	22.2	1.71	57	0.04	0.54
	Abaco – Green Turtle Cay Reef	Open	19.52	1.28	61	0.05	0.79
	Abaco – Guana Cay Reef	Open	18.03	1.41	58	0	1.1
	Abaco – Water Cay Bay	Open	19.67	1.67	60	0	0.1
	Andros – North	Open	24.57	1.13	57	0.11	0.84
	Andros – South	Open	19.95	0.84	56	0	1.09
	Berry Islands – Chub Cay	Open	20.91	0.74	39	0.05	1.03
	Bimini – North	Open	79	1.44	53	0	0.7
	Bimini – South	Open	79	1.43	52	0	0.6
	Conception Island	Closed	4.4	0.23	42	0	0.83
	*Eleuthera – South	Open	9	N/A	0	N/A	N/A
	*Exumas – Middle	Closed	8.67	0.68	67	0.01	1.3
	Exumas – North	Open	11.47	1.02	67	0	1.21
	Exumas – South	Open	8	0.49	42	0	1.12
	New Providence – North	Open	1135.84	0.47	32	0.25	0.5
	New Providence – South	Open	134.33	0.29	38	0.08	1.03
	*San Salvador	Open	4.81	0.41	78	0.04	0.65
Barbados	Northeast	Open	124.75	0.16	29	0	0.24
	Northwest	Open	173.81	1.36	31	0.52	0.06
	Southeast	Open	295.89	2.05	35	1	0.57
	Southwest	Open	18665.35	0.91	35	0.51	0
Belize	Belize City	Open	106.64	1.46	39	0.26	0
	Glover's Reef East	Closed	80.45	2.06	123	0.23	0.92
	Glover's Reef West	Open	85.1	1.52	41	0.34	0.12
	Lighthouse Reef – Halfmoon Caye	Closed	52.19	1.34	43	0.02	1.09
	Lighthouse Reef – Sandbore Caye	Open	45.65	1.77	31	0.13	0.61
	South Water Caye	Open	119.56	1.36	106	0.09	0.23
British West Indies	Montserrat – West	Open	20.04	1.5	48	0.15	0.17
Colombia	Coastal – Isla Mangle	Open	133.2	1.62	50	0.16	0
	Coastal – Isla Tintipan	Open	126.6	1.74	50	0.14	0
	Coastal – Isla Grande	Closed	287.7	2.65	49	0.51	0.04
	Coastal – Isla Tesoro	Closed	267	2.88	50	0.58	0.16
	*Seaflower – Albuquerque	Open	12	1.14	50	0.02	1.28
	Seaflower – Serranilla	Open	1	1.14	63	0.03	1.37
	Seaflower – Providencia	Open	264.78	1.06	43	0.14	0.79
Cuba	Guanahacabibes – Maria la Gorda	Closed	70.56	3.13	40	0.15	0.2
	Guanahacabibes – Reef	Closed	66.9	3	40	0.08	1.8
	Punta Frances North	Closed	39	2.79	40	0.3	0
	Punta Frances South	Closed	100	3.38	40	0.25	0
	Jardines de la Reina	Closed	33.47	2.7	62	0.34	0.71

(Continued on next page)

Table 1. Continued

Location	Reef	Protection status	Market gravity	Reef complexity	No. BRUVS	Moray MaxN	Shark MaxN
Dominican Republic	Bayahibe	Open	249.23	0.49	42	0.07	0
	Boca Chica	Open	2661.95	0.56	44	0.11	0
	Buen Hombre	Open	312.93	1.89	40	0.13	0
	Santo Domingo – La Caleta	Closed	2962	2.34	40	0.38	0
French West Indies	Guadeloupe – Grande cul de Sac	Open	159.07	3.07	41	0.39	0.02
	Guadeloupe – Petit Terre	Closed	95.18	2.21	48	0.42	0.08
	Martinique – Reef 1	Open	226.9	2.96	42	0.76	0
	Martinique – Reef 2	Open	137.64	3.15	47	1.02	0
Jamaica	Coastal – Dragon Point	Open	330.54	1.54	25	0.44	0
	*Coastal – East Portland	Open	329.92	1.68	37	0.24	0
	Coastal – Negril	Closed	513.64	1.44	24	0.38	0
	Coastal – Ocho Rios	Open	377.82	1.24	34	0.15	0
	Pedro Bank	Open	15.58	3.12	54	0.28	0.67
Turks and Caicos	South Caicos – Back	Open	67	0.19	25	0	0.4
	South Caicos – Reserve	Closed	61.55	0.52	20	0	0.8
U.S.A.	Florida – Miami	Open	13,785.08	0.4	38	0.03	0.18
	Florida Keys – Lower Keys	Open	92.55	0.76	43	0.16	0.33
	Florida Keys – Tip	Open	125.67	1.5	40	0.23	0.45
	*Florida Keys – Middle Keys 1	Open	171.88	0.79	43	0.23	0.49
	Florida Keys – Middle Keys 2	Open	135.58	0.85	42	0.12	0.29
	Florida Keys – Middle Keys 3	Open	107.14	0.73	41	0.15	0.39
	Florida Keys – Upper Keys 1	Open	250.88	0.59	42	0.07	0.38
	Florida Keys – Upper Keys 2	Open	226.29	0.96	44	0.18	0.66
	Florida Keys – Upper Keys 3	Open	199.79	1.38	48	0.17	0.58

Reefs with an asterisk denote where eDNA samples were also collected. Protection status refers to open or closed to fishing. Market gravity score is derived from Cinner et al. (2018) 'Global Gravity of Coral Reefs Spatial Layer.' Reef complexity (0–5) is scored for each BRUVS (via BenthoBox online annotation tool [https://benthobox.com/]) and averaged per reef. Moray MaxN and Shark MaxN are the mean per reef.

systems (BRUVS) with ~1 kg of crushed oily fish in a bait cage within the field of view (see Transparent methods in Supplemental information). A total of 3,157 BRUVS were deployed between June 2015 and April 2019 across 67 reefs in 12 Caribbean nations (Table 1). BRUVS were set in randomized locations on each reef, which was defined in this study as a continuous patch of hard-bottom coral reef habitats of ~10 km^2 across a depth range of 2–40 m. Sightings of morays and sharks were converted to MaxN, which is the maximum number of individuals of each species observed in one frame per 60-minute BRUVS (Harvey et al., 2018). We fit a generalized linear model (GLM) with a Tweedie compound Poisson error structure, which explained 56% of the deviance in mean reef-level moray MaxN (all species combined) and included the factors: log-transformed market gravity (+), protection status (open [+] or closed [–] to fishing), reef complexity (+), and the interaction between log-transformed market gravity and protection status (+) (Table 2, Figure 1A). Reef complexity had the greatest effect ($F = 57.91$, $p < 0.0001$), followed by log-transformed market gravity ($F = 33.89$, $p < 0.0001$). Protection status ($F = 3.22$, $p = 0.08$) and the interaction between protection status and log-transformed market gravity ($F = 0.13$, $p = 0.72$) did not significantly affect reef-level moray MaxN. In a model exploring how the same factors affected shark MaxN log-transformed market gravity had the largest effect ([–]; $F = 27.50$, $p < 0.0001$), followed by reef complexity ([+]; $F = 8.10$, $p = 0.01$). Protection status (open [–] or closed [+] to fishing; $F = 0.03$, $p = 0.87$) and the interaction between protection status and log-transformed market gravity ([–]; $F = 0.64$, $p = 0.43$) had no significant effect on reef-level shark MaxN (Figures 1B, Table 3). There was no relationship between reef complexity and market gravity in our sampling (Figure 1C), but there was a negative correlation between morays and sharks at both the reef and national scale (Figures 1D and 2). A model where the factor log-transformed market gravity was replaced with reef-level shark MaxN explained 45% of the deviance in mean reef-level moray MaxN, where

Table 2. Tweedie GLM to predict moray abundance on BRUVS including market gravity

Factors	Degrees of freedom (DF)	Deviance	Residual DF	Residual deviance	F-value	p value	Deviance explained
Null			66	25.25			
log$_{10}$(Gravity)	1	5.01	65	20.24	33.89	<0.0001	0.20
Protection status	1	0.48	64	19.77	3.22	0.08	0.02
Reef complexity	1	8.56	63	11.20	57.91	<0.0001	0.34
log$_{10}$(Gravity) * Protection status	1	0.02	62	11.18	0.13	0.72	0.001
Sum of deviance explained							0.56

Analysis of deviance table for the Tweedie GLM to determine the effects of key anthropogenic (log-transformed market gravity score, protection status [open or closed to fishing]) and habitat (mean reef complexity score [0–5]) factors on the reef-level occurrence (mean MaxN) of morays on reefs throughout the greater Caribbean (*n* = 67 reefs). An asterisk denotes the interaction between factors.

mean shark MaxN had the greatest effect ([-]; *F* = 30.81, p < 0.0001), followed by reef complexity ([+]; *F* = 20.57, p < 0.0001) (Table 4, Figure 1D). Protection status did not significantly affect moray MaxN here either (*F* = 2.24, p = 0.14). Overall, the GLM with the factor log-transformed market gravity (AIC = −36.63) predicted moray MaxN better than the GLM with the factor shark MaxN (AIC = −22.84). A spatial co-occurrence probability model for shark and moray species was used to determine positive, negative, or random interactions at the reef-level using the R library 'co-occur' (Griffith et al., 2016). Spatial co-occurrence between sharks and morays was dominated by negative probabilities (Veech, 2013; Figure 3).

Recording animals on BRUVS is partially dependent on a positive response to bait, which can be inhibited in predator-rich habitats by predator avoidance behavior (Sherman et al., 2020) and interspecific competition (Sabando et al., 2020). It is therefore important to compare detections from multiple survey methods, including techniques that don't require a behavioral response from the subject (Sherman et al., 2020). Six reefs subject to a range of market gravity (4.81–329.92) were surveyed for moray extra-organismal environmental DNA (eDNA) by vacuum filtering 14–21 replicate 2L water samples collected at random locations per reef across hydrophilic polyethersulfone filters and using universal 12S teleost fish primers (Miya et al., 2015) to generate sequence reads. We reasoned that eDNA surveys would be less sensitive than BRUVS to any difference in moray behavior between reefs because eDNA detection does not depend on individual morays approaching a bait source. A total of 19,168 moray sequence reads, belonging to at least six species, were obtained (Table S1). Each water sample was scored from 0 to 3 representing the minimum number of individual morays detected. We conservatively assumed that all detections of the same species on one filter were from one individual, thus our metric is equivalent to moray species richness per sample. A GLM with a negative binomial error distribution explored the relationship between this metric and 'high gravity' (log-transformed market gravity >2; East Portland, Jamaica and Middle Florida Keys, U.S.A.) and 'low gravity' categories (log-transformed ∼ 1; Middle Exumas, San Salvador, Cape Eleuthera [Bahamas] and Albuquerque Island in the Seaflower Biosphere Reserve [Colombia]). Market gravity explained 16.9% of the deviance in the minimum number of moray individuals detected per sample (p = 0.01; Table 5). Significantly more individuals were detected in samples collected on reefs subject to high market gravity (at least six moray species were detected overall, with 1–3 individuals in 17.6% of water samples) than reefs subject to lower market gravity (at least two moray species were detected overall, with 1–2 individuals in 3.5% of samples; Table S1).

This study is the largest effort to date to assess moray occurrence on coral reefs. The positive effect of increasing reef complexity was expected and likely reflects moray preference for reef structures that provide both refuge and prey ambush opportunities (Gilbert et al., 2005). While a preference for complex structure is typical of Caribbean coral reef predators (Valdivia et al., 2017), the positive effect of market gravity on moray sightings is unusual. This factor or its components (local human population density, proximity to humans) nearly always have a strong negative effect on reef predators (Cinner et al., 2018; Graham et al., 2017; MacNeil et al., 2020; Valdivia et al., 2017). We hypothesize that the positive effect of market gravity on morays could be an indirect result of a local reduction in moray predators, competitors, or both on reefs subject to higher human pressure. The negative correlation between shark and moray MaxN we observed at the reef and national level is consistent with this 'release' hypothesis, with uniformly low moray MaxN observed in jurisdictions where sharks are protected at the national level and common on all sampled reefs ('Shark Sanctuaries', *n* = 21 reefs in The Bahamas and Sea Flower Biosphere Reserve,
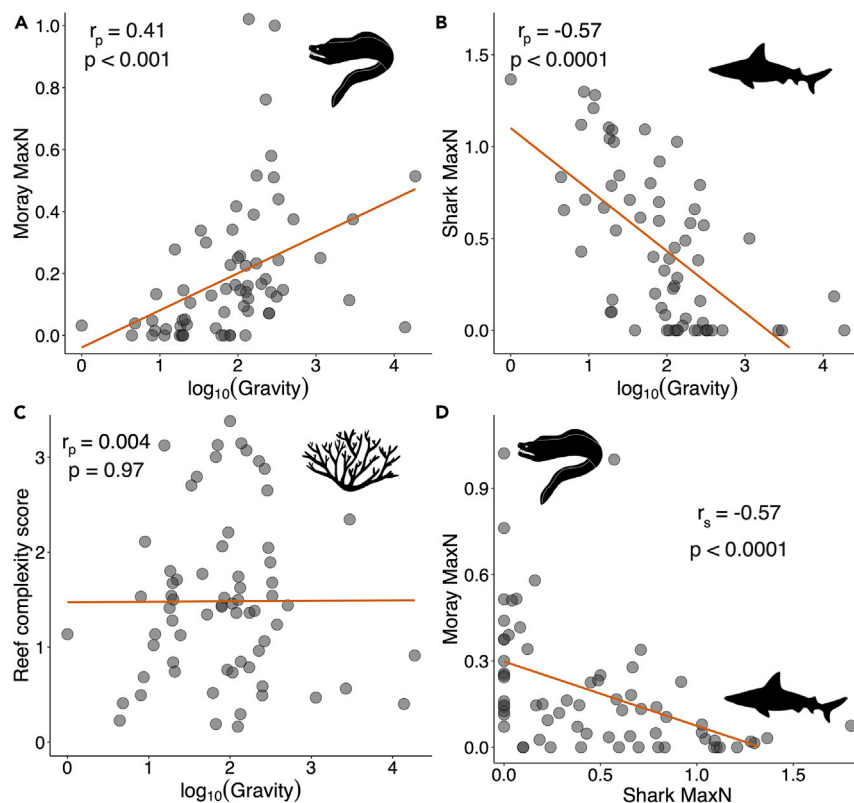
Figure 1. Relationships between market gravity, morays, sharks, and reef complexity in the Caribbean
(A) Relationship between market gravity and moray occurrence (n = 67 reefs in 12 nations).
(B) Relationship between market gravity and shark occurrence on the same reefs.
(C) Relationship between market gravity and mean reef complexity.
(D) Relationship between moray and shark MaxN. Red lines depict the linear trendlines.
Correlation coefficients and p values calculated via Pearson (A, B, C) and Spearman's rank (D; non-linear relationship).

Colombia; Figure 2). Local shark MaxN had a significant negative effect in the moray GLM, which was further supported by the inverse relationship between sharks and morays as demonstrated by their negative co-occurrence probabilities at the reef-level (Figure 3). Morays were less likely to occur on reefs where several shark species (Caribbean reef, nurse, blacknose, tiger, blacktip, unknown) were present. While more research on the ecological relationships between morays, sharks, and other predators is needed to further test this hypothesis, it is mechanistically viable because a wide variety of shark species include morays in their diet (Delorenzo et al., 2015; Estupiñán-Montaño et al., 2017; Lowe et al., 1996; McElroy

Table 3. Tweedie GLM to predict shark abundance on BRUVS

Factors	Degrees of freedom (DF)	Deviance	Residual DF	Residual deviance	F-value	p value	Deviance explained
Null			66	47.61			
$\log_{10}$(Gravity)	1	12.47	65	35.14	4.49	<0.0001	0.26
Protection status	1	0.01	64	35.12	2.88	0.87	0.0003
Reef complexity	1	3.68	63	31.45	28.26	0.01	0.08
Protection status * $\log_{10}$(Gravity)	1	0.29	62	31.16	0.64	0.43	0.01
Sum of deviance explained							0.35

Analysis of deviance table of Tweedie GLM to determine the effects of key anthropogenic (protection status [open or closed to fishing], log-transformed market gravity score) and habitat (mean reef complexity score [0–5]) factors on the reef-level occurrence (mean MaxN) of sharks on reefs throughout the greater Caribbean (n = 67 reefs). An asterisk denotes the interaction between factors.
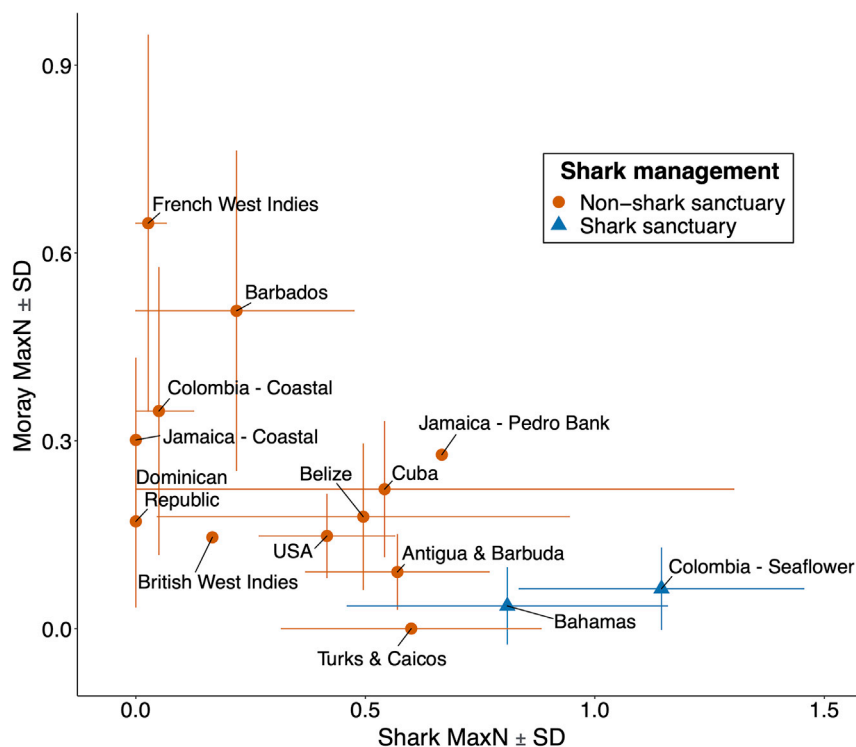
Figure 2. Relationship between mean country-level occurrence of sharks and morays on BRUVS
Jurisdictions where all shark fishing and trade is prohibited at the time of sampling are labeled as 'Shark sanctuaries'. Red circles denote non-shark sanctuaries, blue triangles denote shark sanctuaries, and vertical and horizontal error bars denote standard deviation.

et al., 2006; Sears and Sikkel, 2016; Torres-Rojas et al., 2010; Wetherbee et al, 1996, 1997) (e.g., Families: Carcharhinidae, Sphyrnidae, Ginglymostomatidae) and probably also compete with them for prey. Anecdotal observations at St. Paul's Rocks (Brazil) linked the decline of sharks around the island with a subsequent increase in sightings of the whitespot moray (*Muraena pavonina*) (Luiz and Edwards, 2011). Nonetheless, the better fit of the market gravity model than the substituted shark MaxN model suggests that there may be other factors that are contributing to the positive effect of market gravity on morays. It is possible that removals of moray predators and competitors other than sharks (e.g., large teleosts) may also be important. Other viable hypotheses outside of release that could potentially explain this pattern include that prey availability is greater near humans or that human activities benefit morays (e.g., if fishing provides opportunities for morays to scavenge discarded catch).

It is possible that morays are less inclined to approach BRUVS on reefs where other predator taxa are common due to elevated predation risk or to avoid aggressive interactions with competitors (Sherman et al.,

Table 4. Tweedie GLM to predict moray abundance on BRUVS including shark abundance

Factors	Degrees of freedom (DF)	Deviance	Residual DF	Residual deviance	F-value	p-value	Deviance explained
Null			66	28.00			
Shark MaxN	1	7.24	65	20.76	30.81	<0.0001	0.26
Protection status	1	0.53	64	20.23	2.24	0.14	0.02
Reef complexity	1	4.83	63	15.40	20.57	<0.0001	0.17
Sum of deviance explained							0.45

Analysis of deviance table for the Tweedie GLM to determine the effects of key anthropogenic (protection status [open or closed to fishing]), habitat (mean reef complexity score [0–5]), and ecological (mean shark MaxN) factors on the reef-level occurrence (mean MaxN) of morays on reefs throughout the greater Caribbean (*n* = 67 reefs).
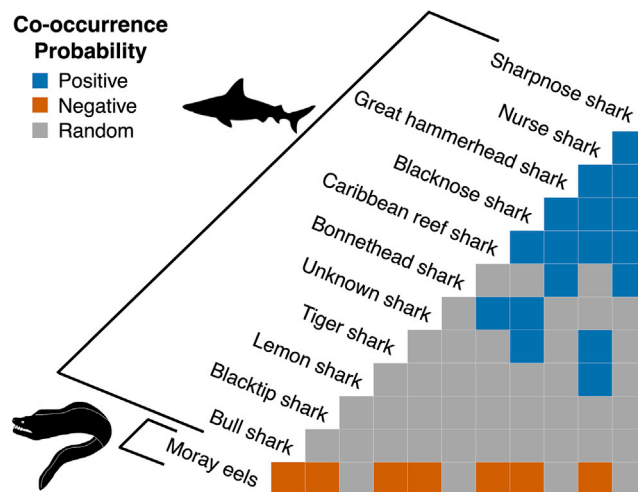
Figure 3. Co-occurrence probability matrix between morays and different shark species on BRUVS
Each cell represents the spatial co-occurrence probability of a pair of species. Significant probabilities are denoted by color (red: negative, blue: positive). Non-significant co-occurrence is denoted by the color gray.

2020). Indeed, we found that mean moray MaxN was significantly lower on BRUVS sets where at least one shark was also recorded than on sets where sharks were absent (negative binomial GLM, p < 0.001; Figure 4 and Table S2). However, shark presence on BRUVS sets did not always deter moray detection: in 98 analyzed BRUVS when sharks and morays co-occurred, they did not overlap in frame on 76 occasions (76.6%). Moreover, on 23 BRUVS where morays and sharks occurred in frame together, the moray left the field of view and did not return after a shark arrived in only five instances (22%; Video S1). Morays generally ignored sharks when they were able to remain partially hidden in reef structure, when the shark(s) passed in the background, or when the individual shark was small, perhaps because the shark was too small to be a direct threat (Video S2). Detection of moray eDNA also exhibited the same pattern observed in the BRUVS; morays were detected less often in water samples from reefs subject to low market gravity. This indicates that instead of just hiding more often (in which case their eDNA would still be detected), and consequently not being visually detected on BRUVS, morays were also less common at these sites. The combined eDNA and BRUVS results argue for reduced moray populations and changes in their behavior on reefs with lower market gravity and more predators.

Morays are functionally unique in that they are able to move undetected within complex reef structure and ambush relatively large prey (Gilbert et al., 2005; Mehta and Wainwright, 2007; Mouillot et al., 2013). Changes in moray abundance and the risk of moray ambush from reef structure could affect prey populations and corresponding risk-sensitive behavior. It may also reduce cooperative hunting with other fishes (e.g., grouper [Epinephelinae]) that capture prey flushed out of structure by morays (Bshary et al., 2006). Yet the ecological roles and importance of morays are not as extensively studied as most other reef predator taxa (Gilbert et al., 2005; Mehta and Wainwright, 2007; Mouillot et al., 2013). We show that eDNA and BRUVS are potentially informative survey approaches for these cryptic predators, which are frequently undercounted using underwater visual censuses (Gilbert et al., 2005). Our study indicates that morays are more common on Caribbean coral reefs subject to high market gravity, making them unusual among the major predator taxa (Cinner et al., 2018; Graham et al., 2017; MacNeil et al., 2020; Valdivia et al., 2017). A better understanding of moray ecology is needed to assess the causes and consequences of these profound changes in Caribbean coral reef predator assemblages, while also acknowledging that emerging export markets for morays (Mañez and Paragay, 2013) and human impacts that reduce reef complexity and prey biomass may ultimately deplete morays close to people.

Limitations of the study

This study used a large sample of BRUVS set randomly on reefs distributed throughout the greater Caribbean and provided corroboration of the BRUVS results with more limited eDNA sampling. Our eDNA sampling sites (n = 6) were opportunistically selected and half of the sites were within The Bahamas.

Table 5. Negative binomial GLM to predict moray eDNA detection

Factors	Degrees of freedom (DF)	Deviance	Residual DF	Residual deviance	p-value	Deviance explained
Null			116	38.72		
$\log_{10}$(Gravity)	1	6.54	115	32.19	0.01	0.17

Analysis of deviance table for the negative binomial GLM to determine the effect of log-transformed market gravity (low [~1] or high [> 2]) on the minimum number of moray individuals detected in water samples (n = 118) collected across 6 reefs in the greater Caribbean (U.S.A., Jamaica, Bahamas, Colombia [Seaflower Biosphere Reserve]).

Expanding the number and geographic coverage of reefs sampled for moray eDNA could overcome this limitation in the future. Although BRUVS were deployed during daylight hours in this study, it is suggested that some species of reef-associated morays and sharks actively forage during twilight and night hours (Bardach et al., 1959; Chapman et al., 2007; Gruber et al., 1988). Further studies could therefore incorporate nighttime surveys to explore the relationship between these species under low-light conditions. This study focused on testing for associations between morays and three factors that generally have strong effects on reef fish: market gravity, protection status, and reef complexity. While these factors explain much of the deviance in moray sightings on BRUVS there are many other important factors that could be investigated. For example, our model does not include any metric for prey availability. Morays can have broad diets that include a diversity of reef fish, crustaceans, and octopus, some of which are not well sampled using BRUVS and may require targeted sampling efforts using other methods. There remain several potential mechanisms that could be driving the positive association between moray occurrence and market gravity. We discussed a 'release' hypothesis in relation to the loss of sharks, but this could also apply to the loss of other potential predators and competitors. Our study only compared morays and sharks due to limitations on the time to annotate BRUVS for other species, and it would be insightful to also model the co-occurrence of morays, sharks, and other large predators on BRUVS in order to resolve the strongest interactions. It is also a viable hypothesis that human activities increase the prey available to morays. Although increased market gravity typically reduces overall reef fish biomass (Cinner et al., 2018), this does not necessarily translate to fewer prey for morays. It is possible that low biomass reefs are composed of more suitable prey species for morays than high-biomass, predator-rich reefs. It is also possible that human activities directly enhance moray presence (e.g., if morays frequently scavenge fishery discards or if SCUBA tour operators feed morays). Overall, future studies should model the relationships of a wider variety of potential moray MaxN covariates to better resolve the key drivers behind the association detected in our analyses. Additional information on moray ecology and their location-specific interactions with humans are necessary to select and quantify the most appropriate potential covariates.

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Demian Chapman (dchapman@fiu.edu).

Materials availability

No materials were newly generated for this paper.

Data and code availability

The BRUVS sampling locations and corresponding ecological, anthropogenic, and habitat factors are listed in Table 1. The number of moray eel sequence reads obtained from water samples are listed in Table S1.

METHODS

All methods can be found in the accompanying Transparent methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102097.
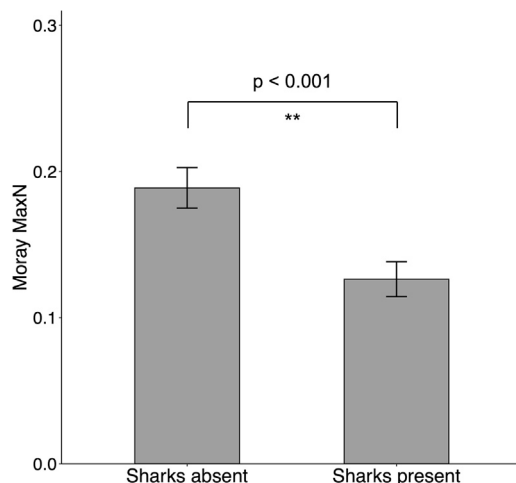
CellPress
OPEN ACCESS

Figure 4. Set-level abundance of morays in the presence or absence of sharks

Mean moray MaxN $\pm$SE on BRUVS sets (n = 2,052 BRUVS across 51 reefs in 11 nations) where sharks were absent or at least one shark was present, on reefs where at least one shark was detected. p value calculated via negative binomial GLM (Table S2).

AUTHOR CONTRIBUTIONS

D.D.C. and K.I.F. conceived the BRUVS moray study. D.D.C., M.R.H., E.S.H., M.H., C.A.S., M.A.M, and M.G.M. led Global FinPrint. D.D.C., B.D.P., and J.B. conceived of the eDNA study. All authors contributed to the design and execution of Global FinPrint and/or eDNA sampling. J.B. and B.D.P. conducted eDNA laboratory work and eDNA bioinformatics. BRUVS modeling was conducted by G.M.C. with guidance from E.A.B. eDNA modeling was conducted by K.I.F. with guidance from E.A.B. The manuscript was initially written by G.M.C., J.B., and D.D.C. All authors read and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental information

Moray eels are more common on coral

reefs subject to higher human

pressure in the greater Caribbean

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Supplementary Information

Transparent Methods

All research was conducted in accordance with protocols of Florida International University's Animal Care and Use Program. Data were collected using baited remote underwater video systems (BRUVS) because of their ability to capture of variety of marine predators and benthic habitat, replicability on a large spatial scale, and non-extractive nature (Harvey et al., 2018). BRUVS were deployed in shallow waters (2–40 m) throughout 2015-2019. The sampling area at each location was a continuous or near continuous hard bottom patch of ~ 10 km^2 (hereafter referred to as a 'reef') and was selected based on the operational range of the vessel used for sampling and access through a local collaborator. BRUVS were comprised of a single video camera (e.g. Sony Handycam DCR-HC52, GoPro HERO2/3/4) fixed on a stainless steel, rebar, or PVC frame. Baits consisted of ~ 1 kg of crushed oily fish (e.g., species from the Families Scombridae or Clupeidae). The deployment coordinates for the first BRUVS in each sampling session (determined by the number of units available, with ~ 3 sampling sessions of four units per day being typical) was determined using a random coordinate generator from a map of the reef constructed using ArcMap software (ArcGIS 10) or Google Earth and the University of New Hampshire Cooperative Extension KML Tools Project (https://extension.unh.edu/kmlTools/index.cfm). The remaining units were then separated by a minimum of 500 m from BRUVS deployed during the same sampling session randomly or haphazardly. BRUVS were deployed during daylight hours and in-water personnel guided the BRUVS on deployment to ensure an unobstructed FOV and to avoid damage to live coral. BRUVS were retrieved after at least ~ 70 minutes to ensure a continuous 60 minutes of video from the time of settlement. All sites were sampled with ~ 50 BRUVS deployments made over

the course of ~ 10 successive days with the exception of Turks and Caicos where the BRUVS were deployed over four months. Videos analysts time-logged all moray eel (Family Muraenidae; hereafter referred to as 'morays') and shark sightings during the 60-minute post-settlement period. Experienced observers made all species-level identifications. Although green (*Gymnothorax funebris*), spotted (*G.moringa*), purplemouth (*G.vicinus*), goldentail (*G.miliaris*), viper (*Enchelycore nigricans*) and chain (*Echidna catenata*) morays were all positively identified on BRUVS it was frequently difficult to visually resolve species with confidence, especially for species other than greens. We therefore collapsed all morays into one category for analysis. We also grouped sharpnose sharks that co-occur in the western Atlantic (*Rhizoprionodon terraenovae* and *R. porosus*) since vertebral counts or genetics are need to identify to species level (Mendonça et al., 2011; Todd et al., 2004). We determined MaxN for every species or species group, which is an index of relative abundance representing the maximum number of individuals of each species seen on any given frame of a BRUVS set (Ellis and DeMartini, 1995). This parameter is the standard reporting metric for BRUVS that avoids double counting the same individual should they leave the field of view and return (Ellis and DeMartini, 1995). While MaxN exhibits hyperstability (i.e. counts remain high as true abundance increases) this is primarily an issue at very high true abundances (> 20 individuals (MacNeil et al., 2020; Schobernd et al., 2014), which is unlikely to bias our comparisons given low MaxN of morays and sharks observed in this study (Mean MaxN < 1 [morays] or 2 [sharks] for all reefs in this study). MaxN was then summed for all moray species to give a generic 'moray MaxN' for each BRUVS. The same process was followed for sharks. We did not exclude any shark or moray species given the wide variety of shark species that could prey upon or compete with morays (see references in main text). 'Unknown shark' and 'unknown moray' were assigned when the

species identity could not be visually determined, usually because the sighting was in the background of the FOV. Unknowns were only counted when there were no other sightings of the taxon on the BRUVS. If there were sightings of an identified shark or moray on the BRUVS we conservatively assumed that the unknown was the same individual.

We investigated the possibility that the presence of sharks directly inhibited moray detection on BRUVS. First, we compared the MaxN of morays on BRUVS where a shark was also detected with that on sets where a shark was not detected ($n = 2,052$ total), excluding reefs where no sharks were detected in our sampling (16/67 reefs, 23.4%; see main text Figure 4). We determined the significance of the factor shark presence or absence (1/0) on set-level moray abundance (MaxN) using a negative binomial generalized linear model (GLM) with the 'MASS' library in R (Table S2) (R Development Core Team, 2020; Ripley et al., 2019). We also directly investigated moray response to shark presence on 98 sets where they co-occurred (in Bahamas, Belize, Barbados, Cuba, Colombia [Coastal and Seaflower Biosphere Reserve], Jamaica [Pedro Bank], Antigua and Barbuda, French West Indies, and the U.S.A). In most sets sharks and morays did not co-occur in frame during the 60-minute deployment (76.6%), usually because sharks typically make infrequent, short duration passes and then depart FOV for the remainder of the set. Sharks and morays were observed in frame together on 23 BRUVS. These sets were viewed at 1X play speed from the first co-occurrence to the end of the 60-minute deployment to determine whether or not the moray departed and did not return after the shark arrived. In five instances the moray left frame and did not return, although it was not always clear that this was a direct response to shark arrival (ESM 1). In one additional case a moray departed frame after being approached by a nurse shark (*Ginglymostoma cirratum*) but it returned before the 60 minutes elapsed.

Six reefs were sampled for moray eDNA: Islamorada in the Middle Florida Keys [U.S.A., $n = 14$ 2-L water samples], East Portland [Jamaica, $n = 20$], Exuma Cayes Land and Sea Park, middle Exumas ($n = 20$), San Salvador ($n = 23$), and Cape Eleuthera ($n = 20$) [all in The Bahamas], and Albuquerque Island [Seaflower Biosphere Reserve, Colombia, $n = 21$], with the two former being adjacent to more dense human populations and the four latter adjacent to less populated areas (Center for International Earth Science Information Network - Columbia University, 2018). The four latter sites are also within shark sanctuaries that have among the highest shark MaxN recorded in the region (see main text Figure 2). Water sampling was not conducted on the same days as BRUVS sampling to avoid contamination of samples with bait residues. From 14-21 seawater samples (2 L each) were collected manually with a Kemmerer type water sampler at randomized locations, with sampling coordinates obtained in the same manner as described for the BRUVS on each of these reefs. All water samples were taken ~ 1 m off the sea floor. Vacuum filtration was carried out immediately on the vessel with a peristaltic pump (www.fondriest.com). The hydrophilic polyethersulfone (PES) filters (Pall Corporation; 47-mm diameter; 0.45-μm pore size) containing sample filtrates were stored in sterile 5.0-ml cryogenic screw-cap files containing ethanol or silica beads. Silica beads function as a desiccator, drying out the filters, reducing DNA degradation (Bakker et al., 2017). Subsequently the filters were stored at -20 °C until extraction using the DNeasy PowerSoil Kit (www.qiagen.com), following the manufacturers' protocol. Genomic DNA was eluted into 100μl and frozen at -20 °C until further processing. At all stages of sample collection and laboratory procedures single use disposable gloves were used. Surfaces, sampling devices, and filtration equipment were cleaned with 50% bleach, and all laboratory work was carried out inside a

laminar flow hood. Filter extraction and library preparation were performed at Jonah Ventures, Boulder, CO, USA (www.jonahventures.com) in dedicated controlled eDNA laboratories.

Concentrations of a subset of extracted genomic DNA were measured using a Qubit 4 Fluorometer (Thermo Fisher Scientific), prior to PCR amplification. The MiFish-U primer set (Miya et al., 2015), targeting a ~ 171 bp fragment of the mitochondrial 12S rRNA region was used for the amplification of teleost eDNA. Both forward (5'-GTCGGTAAAACTCGTGCCAGC-3') and reverse (5'-CATAGTGGGGTATCTAATCCCAGTTTG-3') primers contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 25 μl PCR reaction was prepared according to Promega (Madison, WI) PCR Master Mix specifications, which included 12.5 μl Master Mix, 0.5 μM of each primer, 1.0 μl of gDNA, and 10.5 μl DNase/RNase-free water. All PCR amplifications were done in triplicate reactions using the following PCR profile: initial denaturation at 95 °C for three minutes, followed by 45 cycles of 20 seconds at 98 °C, 30 seconds at 60 °C, and 30 seconds at 72 °C, and a final elongation at 72 °C for ten minutes. In order to determine amplicon size and PCR efficiency, each reaction was assessed by electrophoresis, running the products through a 2% agarose gel and visualized on a UV light platform. Amplicons were subsequently cleaned by incubation with Exo1/SAP for 30 minutes at 37 °C, followed by inactivation at 95 °C for five minutes. A second PCR was performed on the cleaned amplicons from the first stage PCR reaction in order to give each sample a unique 12 nucleotide index sequence, yielding a total length of 346 bp. The indexing PCR included Promega Master mix, 0.5 μM of each primer and 2 μl of template DNA. The PCR profile included an initial denaturation of 95 °C for three minutes followed by eight cycles of 95 °C for 30 sec, 55 °C for 30 seconds and 72 °C for 30 seconds. To test for successful barcoding, the

indexed PCR products were visualized on a 2% agarose gel. Sample library pools were sequenced on an Illumina NovaSeq platform at the Texas A&M University sequencing center using the 6000 SP Reagent Kit (500 cycles). Necessary quality control measures were performed at the sequencing center prior to sequencing. Sequences were processed using the JAMP pipeline (https://github.com/VascoElbrecht/JAMP) and raw reads were demultiplexed with 'iu-demultiplex' v2.3 (https://github.com/merenlab/illumina-utils). Forward and reverse reads from each sample were paired end merged with Usearch v11.0.667 (www.drive5.com/usearch/download.html). Sequence reads 130-210 bp were retained using Cutadapt v1.18 (https://cutadapt.readthedocs.io/en/v1.18/colorspace.html) after primer removal. Quality filtering was carried out using expected error filtering (max ee=0.5) as implemented in Usearch. Reads affected by sequencing and PCR errors were removed using the unoise3 algorithm (www.drive5.com/usearch/manual/cmd_unoise3.html) with an alpha value of five. Denoising was applied to each individual sample and Exact Sequence Variants (ESV) compiled in an ESV table including sequences and read counts for each sample. Taxonomy assignment was performed by mapping each ESV against the MitoFish database containing complete and partial mtDNA reference data, using Usearch_global with maxaccepts 0 and -maxrejects 0 to ensure mapping accuracy. All resulting sequences were subsequently blasted in GenBank to ensure that potential species were not missed, as many western Atlantic fish 12S sequences that are deposited in GenBank are not yet available in the MitoFish database. Consensus taxonomy was generated from the hit tables, by first considering 100% matches and then decreasing in 1% steps until hits were present for each ESV. In the respective 1% bracket, taxonomy present in at least 90% of the hits was reported. A 'NA' was reported if several taxa match the ESV. To reduce errors caused by misidentified taxa, the bracket was increased to 2% if matches of 97% or

higher were present, and no family level taxonomy was returned. All filters were scored as 'positive' or 'negative' for moray sequences regardless of the number of reads or number of moray ESVs detected to be as conservative as possible. There were three moray positive samples in 14 samples from the Florida Keys, U.S.A., 3/20 from East Portland, Jamaica, 2/20 from Exumas, Bahamas, 1/23 from San Salvador, Bahamas, 0/20 from Cape Eleuthera, Bahamas, and 0/21 from Albuquerque Island, Colombia. We used the number of distinct moray species (ESVs) present in each positive sample (ranging from 1-3) as a minimum estimate of the number of individual morays detected since it was not possible to resolve how many individuals of each species were detected with these sequences.

Quantification and statistical analysis

The mean MaxN of morays on each reef was modeled using a generalized linear model (GLM) fitted with a Tweedie compound Poisson error structure using the R libraries 'tweedie', 'statmod', and 'cplm' [5, 9–11]. Tweedie is a type of compound Poisson distribution, which has been used in modeling continuous data with excess zeroes (Shono, 2008; Zhang, 2013). To assess the effect of several potentially important anthropogenic, habitat, and ecological parameters on mean MaxN of morays across all BRUVS on a reef, we included the following factors in the GLM measured at the reef-level: (1) market gravity, (2) protection status (open or closed to fishing), (3) reef complexity, and (4) the interaction between market gravity and protection status. We then replaced market gravity with reef-level shark MaxN to avoid modelling collinear factors. We selected these habitat and anthropogenic factors because they are the strongest predictors of overall predator biomass on Caribbean coral reefs identified by previous studies and we were primarily interested in how morays respond to them compared to other predator taxa (Stallings, 2009; Valdivia et al., 2017). Reef complexity was estimated from

a still frame for each BRUVS using the BenthoBox online annotation tool (https://benthobox.com) where 4x5 gridded squares were scored 0-5 for relief (Polunin and Roberts, 1993; Wilson et al., 2007), and the mean relief score per reef was calculated as a measure of complexity. Market gravity scores were determined for each reef using calculations found in the 'Global Gravity of Coral Reefs Spatial Layer' (Cinner et al., 2018), which defines market gravity as the human population within 500-km of each reef divided by the squared travel time to the reef, which estimates the accessibility of reefs to humans and associated fishing pressure. Market gravity was log-transformed for analysis. Protection status was a categorical variable where the reef was either classified as a fished (open) or no-take (closed) area (i.e., marine reserve status [http://www.mpatlas.org/]). Sharks were used as a proxy for potential predators and competitors because a wide variety of species are documented to include morays in their diet, including relatively small shark species (see references in main text), and they were the focal taxon of Global FinPrint (www.globalfinprint.org). We used the mean MaxN of sharks observed on the reef from the same BRUVS dataset as a measure of their relative abundance. A model with the same factors (market gravity, protection status, reef complexity, and interaction between market gravity and protection status) was also conducted for shark MaxN.

A spatial co-occurrence probability model for shark and moray species was used to determine positive, negative, or random interactions at the reef-level. Presence-absence data from the BRUVS was used to test whether pair-wise interactions between species were random or non-random using the R library 'co-occur' (Griffith et al., 2016). Significantly non-random interactions ($p < 0.05$) were determined via default model specifications. Species pairs that did not occur more than once were excluded (thresh = TRUE). This type of probabilistic model

approach has very low Type I and Type II error rates due to its lack of reliance on computer-algorithm-based randomization (Veech, 2013).

The effect of log-transformed reef-level market gravity scored as low (~ 1) or high (> 2) on the minimum number of individual morays detected on eDNA filters (conservatively assumed to be equal to the total number of species detected per filter) was modeled across six reefs using a GLM with a negative binomial error distribution (NBD) using the R library 'MASS' (R Development Core Team, 2020; Ripley et al., 2019). The NBD was selected due to the high proportion of zeroes in the data. By estimating a dispersion parameter, the NBD can appropriately account for abundance data that are overdispersed rather than distributed at random where a Poisson distribution would be suitable (Davis et al., 2011; White and Bennetts, 1996). Residual diagnostics were checked using the R library 'DHARMa' as standard residual plots often fail to detect NBD assumption violations (Hartig, 2020).

Supplemental references

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Table S1. Moray eel environmental DNA results, Related to Table 5. Number of moray sequence reads obtained across water samples obtained in 6 reefs in Jamaica (JAM), United States (USA), Bahamas (BAH), and Colombia (COL). Sample sizes (N) refer to number of 2L water samples collected and analyzed per reef.

Species	E Portland (JAM, $n = 20$)	FL Keys (USA, $n = 14$)	San Salvador (BAH, $n = 23$)	Exumas (BAH, $n = 20$)	Cape Eleuthera (BAH, $n = 20$)	Alberqueque (COL, $n = 21$)
Anarchias similis	2440	0	0	0	0	0
Enchelycore nigricans	0	2370	0	0	0	0
Gymnothorax spp.	753	73	0	714	0	0
Gymnothorax miliaris	349	5994	0	0	0	0
Gymnothorax vicinus	3297	1917	665	596	0	0
Total moray reads	6839	10354	665	1310	0	0

Table S2. Model results for predicted moray abundance, Related to Figure 4. Analysis of deviance table for the negative binomial GLM to determine the effect of shark presence (1) or absence (0) on the set-level relative abundance (mean MaxN) of morays on reefs throughout the greater Caribbean where at least one shark was detected ($n = 2,052$ BRUVS across 51 reefs in 11 nations).

Factors	Degrees of Freedom (DF)	Deviance	Residual DF	Residual Deviance	p-value	Deviance explained
Null			2051	1070.90		
Shark	1	10.98	2050	1059.90	<0.001	0.01

presence or absence						