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Patterns of color phase indicate spawn timing at a Nassau grouper *Epinephelus striatus* spawning aggregation

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Abstract Nassau grouper *Epinephelus striatus* are a large bodied, top level predator that is ecologically important throughout the Caribbean. Although typically solitary, Nassau grouper form large annual spawning aggregations at predictable times in specific locations. In 2003, The Cayman Islands Marine Conservation Board established protection for a newly rediscovered Nassau grouper spawning aggregation on Little Cayman, British West Indies. The large size of this aggregation provides a unique opportunity to study the behavior of Nassau grouper on a relatively intact spawning aggregation. During non-spawning periods Nassau grouper display a reddish-brown-and-white barred coloration. However, while aggregating they exhibit three additional color phases: "bicolor", "dark", and "white belly". We video sampled the population on multiple days leading up to spawning across five spawning years. Divers focused a laser caliper equipped video camera on individual fish at the aggregation. We later analyzed the video to determine the length of the fish and record the color phase. Our observations show that the relative proportion of fish in the bicolor color phase increases significantly on the day leading up to the primary night of spawning. The increase in the proportion of the bicolor color phase from 0.05 early in the aggregation to 0.40 on the day of spawning suggests that this color phase conveys that a fish is behaviorally and physiologically prepared to spawn. Additionally, 82.7% of fish exhibiting dark or white belly coloration early in the aggregation period suggests that these color phases are not only shown by female fish as was previously posited [*Current Zoology* 58 (1): 73–83, 2012].

Keywords Nassau grouper, *Epinephelus striatus*, Spawning aggregation, Nuptial coloration, Spawning behavior

Fish communicate via a wide variety of pathways including auditory, olfactory, and visual exchanges. Visual communication in fish is often accomplished via changes in color patterns. Color change in fish has been well studied and has often proved useful in the study of a variety of conspecific interactions (Erisman and Allen, 2005; Kodric-Brown, 1998; Korzan et al., 2008; O'Connor et al., 1999; Price et al., 2008). Specifically, nuptial color changes (color changes associated with breeding) have captured the attention of behavioral ecologists, physiologists and evolutionary biologists as they are common among fish and often play a role in mate selection and successful spawn timing (Bakker and Mundwiler, 1994; Fujii and Noavles, 1969; Kodric-Brown, 1998; Seehausen et al., 2008). While many species representing many different mating systems exhibit color changes associated with breeding, color changes among species which aggregate to spawn may prove particularly interesting because of the aggregate nature of the behavioral cues and responses.

Many marine fish form spawning aggregations (Domeier and Colin, 1997; Sadovy de Mitcheson et al., 2008). By definition a spawning aggregation contains fish in much higher densities than they are typically found on home reefs. Logically, this also means that fish on a spawning aggregation will interact with conspecifics more frequently and in different ways than typically encountered during non-breeding periods. Fish may rely on color changes to communicate not only spawn timing, but also the motivation of behavior in conspecific interactions. Here, we investigate the role of color changes associated with breeding in the Nassau grouper (*Epinephelus striatus*, Bloch 1792) which form spawning aggregations but are solitary and territorial

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during the non-breeding season.

Nassau grouper are an iconic species found on reefs throughout the Caribbean. This large bodied top level predator is not only one of the most easily recognizable species on Caribbean reefs, but is also critically important in the maintenance of healthy reef communities (Mumby et al., 2006; Stallings, 2008). In addition to their ecological importance, Nassau grouper are also economically important to the region both as a fishery (Buchan, 2000; Ehrhardt and Deleveaux, 2007) and as an attraction for dive-related tourism (Rudd and Tupper, 2002).

Throughout much of the year Nassau grouper are solitary and territorial. However, during the winter months, on weeks surrounding the full moon, Nassau grouper form large spawning aggregations. While the timing of spawning varies latitudinally, within each individual aggregation the location and timing are specific and predictable. Today, Nassau grouper are listed by the International Union for the Conservation of Nature (IUCN) as threatened and aggregation fishing has largely been blamed for the species' decline (Albins et al., 2009; IUCN, 2010; Sadovy, 1999). Today, it is estimated that over 60% of Nassau grouper aggregations have been fished to the point where they no longer form and most of the remaining aggregations are severely depleted (Sadovy de Mitcheson et al., 2008).

Nassau grouper's large economic and ecological importance coupled with their marked decline has led to the implementation of several monitoring programs centered on Nassau grouper spawning aggregations. While the monitoring and associated research programs have contributed greatly to our understanding of many aspects of spawning aggregations, our understanding of the behavior of Nassau grouper while on the aggregation site has progressed little (although see Whaylen et al., 2004) since the first published descriptions by Smith (1972) and Colin (1992). An understanding of the behavior of Nassau grouper while on the aggregation site could help inform and direct the efforts of monitoring programs and possibly infer the occurrence of spawning.

Nassau grouper can spend up to ten days on the aggregation site before finally spawning and returning to their home reef. While at the aggregation site Nassau grouper display three color phases in addition to the coloration typically displayed on their home reef: bicolor, white belly and dark (Colin, 1992; Smith, 1972; Whaylen et al., 2006; Whaylen et al., 2004). Because these color phases are typically only seen at the spawning aggregation when Nassau grouper are found in exceptionally high densities, it seems likely the fish are using these color phases to mediate aggressive interactions, indicate sex, and/or communicate reproductive readiness. Here we present five years of color phase observations taken from video of a Nassau grouper spawning aggregation in the Cayman Islands, British West Indies and investigate the relationship between fish length (as a proxy for age), color phase displays and the timing of peak spawning at the aggregation. Our intent is to gain insight regarding behavioral cues to individual readiness and the ultimate timing of group spawning.

1 Materials and Methods

1.1 Study area

This study was conducted off of the west end of Little Cayman Island, Cayman Islands, British West Indies. The Cayman Islands are located in the western Caribbean Sea, south of Cuba and northwest of Jamaica and are made up of three main islands; Grand Cayman, Cayman Brac, and Little Cayman. In 2001, an aggregation of Nassau grouper located off of the west end of Little Cayman Island was discovered by fishermen. In 2003, the Cayman Government imposed an 8-year fishing ban on all known Nassau grouper spawning aggregation sites, both current and historic, in order to allow for both population recovery and to identify appropriate management actions (Bush et al., 2004). Intense monitoring of the west end Little Cayman aggregation, including collection of diver-operated video images, began in 2004 as a partnership between the Reef Environmental Education Foundation (REEF) and the Cayman Islands Department of the Environment.

1.2 Video Collection and Analysis

A diver-operated video camera was mounted onto a laser bracket (caliper) to allow for the measurement of fish length. The lasers were calibrated to be 250 mm apart and be accurate at all distances up to 15.25m from the caliper. Diver observations indicated, and subsequent review of the video confirmed, that fish did not alter their behavior or coloration as the laser points were placed onto their sides. If a diver came within close proximity (< 2 m) of a fish and the fish changed color as a result of disturbance by a diver, the fish also tended to swim away from the diver, making it impossible to aim the laser calipers onto the fish. Close approach was generally not necessary due to the accuracy of the calipers even at 15 m distance. Fish were randomly selected for imaging as the diver swam a random transect through the area encompassed by the spawning aggregation and attempted to focus the laser caliper on each fish encountered**.** In all years other than 2010 (where two divers collected video) a single diver was responsible for collecting video on each dive. While it is possible that a single fish was measured more than once in a day, the large number of fish on the aggregation and the random transect swam by the diver collecting the video images made repeated measures of the same individuals during any given day unlikely. This was confirmed by the absence of daily repeated images of individually-marked fish.

All dives for the purpose of video analysis were conducted between 7 am and 12:30 pm and were well distributed within this time frame every year. Two dives were conducted per day with the first dive of the day dedicated to collection of video for analysis and the second dive to monitor for spawning activity. There were two days (February 13, 2009 and February 6, 2010) where two dives were conducted for the purposes of video analysis (Table 1). Dives were approximately 30 minutes in length with 20–25 minutes of bottom time per dive. Any spawning activity was recorded during evening dives conducted for the purposes of monitoring spawning.

Still video frames featuring a fish with the lasers showing clearly on the body were isolated from the video using Adobe Acrobat Pro® and the fish's length was determined using ImageJ software by measuring the known distance between the lasers to set the scale for the image and subsequently using the calibrated scale to measure the fish's length (total length, TL, see Archer et al. *In Press* for a complete description of length-determination methods; Rasband, 2009). In addition to measuring the length of the fish in each frame grab, the color phase of the measured fish was recorded along with the day and year that the image was captured on the spawning aggregation. The distribution of fish lengths collected via video-laser calipers was not significantly different than fish caught using hook and line off of the aggregation in the course of another study when compared using the Kolmogorov-Smirnov two sample test (D = 0.1356, $P = 0.0758$).

The color phases exhibited by Nassau grouper while on the spawning aggregation are: barred, bicolor, dark, and white belly (Fig. 1; Colin, 1992; Smith, 1972). The barred coloration is a reddish-brown and-white barred coloration and is the coloration Nassau grouper display on home territories during non-spawning periods. Fish in the bicolor coloration, first described by Smith (1972), are dark in color on the top of the fish and the belly has turned completely white, the bars typically seen on the fish have all but disappeared with a white stripe running through the eye. Fish in the dark coloration have darkened over their entire body to the point where bars are only slightly visible, if at all. Fish in the white belly color phase have a completely white belly similar to the bicolor phase, however the top of the fish has lightened and the typical barred pattern is visible but very faint.

1.3 Statistical analysis

We examined patterns in the proportion of fish exhibiting each color phase using a multinomial logistic regression with the barred color phase as the reference category. Using an information theoretic approach we compared 14 unique multinomial logistic models that included all combinations of the predictor variables: day, length, year, an interaction between year and day, and second and third order polynomials of day (Table 2). We assumed that fish behavior is quite variable from year to year, and therefore included year as a predictor variable in all models tested. We compared models using the corrected Akaike Information Criterion (AICc, Hurvich and Tsai, 1989) and model weights.

Table 1 Summary of images analyzed per year with the minimum, maximum, mean, and standard deviation of lengths of the fish in mm measured, number of fish measured in each color phase

	Min	Max	Mean	*SD*	*n*	Ba	Bi	D	Wb	Dives	Date range	Peak	Full Moon
2004	397.10	874.48	625.36	88.63	283	23	85	80	95	4	Feb.8-11	Feb.11	Feb.6
2005	429.14	863.14	636.97	80.43	553	47	115	195	196	5	Jan.28-Feb.1	Jan.31 & Feb.1	Jan.25
2008	454.34	854.13	622.71	75.00	256	29	67	44	116	5	Jan.26-30	Jan.29-30	Jan.22
2009	374.80	846.28	587.79	77.83	446	62	129	79	176	5	Feb.11-14	Feb.14	Feb.9
2010	352.65	863.49	609.34	81.29	475	60	85	240	90	6	Feb.2-6	Feb.5 & 6	Jan.30
All years	352.65	874.48	616.11	82.53	2013	221	481	638	673	25	-	-	-

Ba=barred, Bi=Bicolor, D=dark, WB=white Belly. The number of dives, date range of observations, date of peak spawning, and the date of the full moon immediately preceding the spawning aggregation.

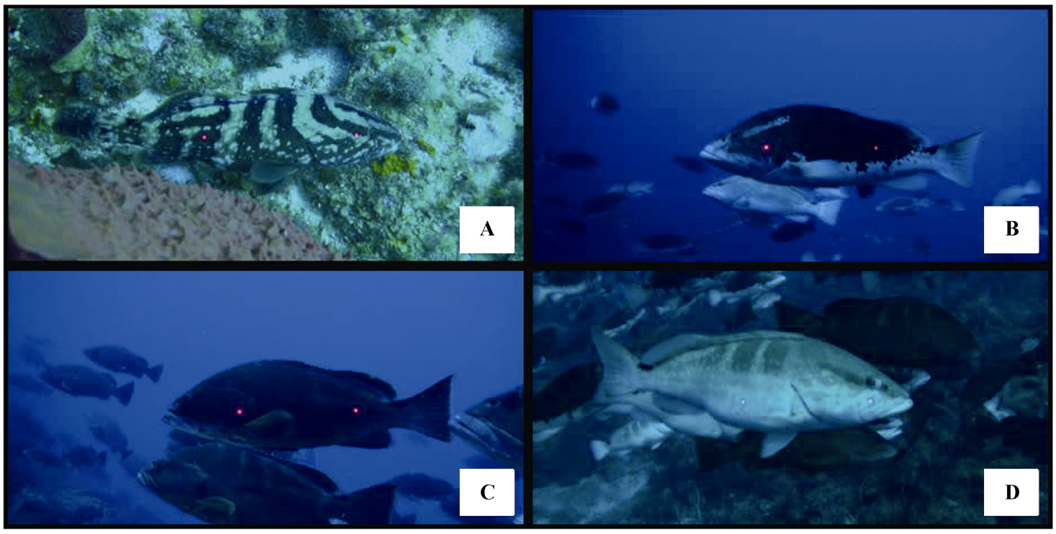

Fig. 1 The color phases exhibited by Nassau grouper while on the spawning aggregation
The colorations shown are A) barred, B) bicolor, C) dark, and D) white belly. The dots visible on the sides of the fish are the laser points used to determine the total length of the fish and are 250mm apart.

While Nassau grouper in the Little Cayman aggregation gather at the aggregation site on a predictable full moon (Whaylen et al., 2004) the precise day of peak spawning varies from year to year. This variation results in an inconsistent number of days prior to spawning during which times observations can be recorded. Restricting our analysis to three days prior to peak spawning up to the day of peak spawning maximized the number of years included in our analysis while at the same time ensuring continuous daily observational coverage within each year. To standardize among years we assigned a value of 0 to the day in each year when peak

Table 2 The models evaluated and their respective corrected Akaike Information Criteria (AICc) and Δ AICc values as well as model weight

Model	AICc	Δ AICc	Model weight
color~length+year+day+year*day+day*length+day^2+day^3	4740.113	0.000	0.8749
color~length+year+day+year*day+day^2+day^3	4744.055	3.942	0.1219
color~length+year+day+year*day+length*day+day^2	4751.680	11.567	0.0027
color~length+year+day+year*day+day^2	4755.176	15.063	0.0005
color~length+year+day+year*day+length*day	4760.518	20.405	<0.0001
color~year+day+year*day+day^2+day^3	4762.877	22.764	<0.0001
color~length+year+day+year*day	4765.207	25.094	<0.0001
color~year+day+year*day+day^2	4776.118	36.005	<0.0001
color~year+day+year*day	4787.531	47.418	<0.0001
color~length+year+day+day*length+day^2+day^3	4867.420	127.307	<0.0001
color~length+year+day+day^2+day^3	4870.272	130.159	<0.0001
color~length+year+day+day*length+day^2	4876.495	136.382	<0.0001
color~length+year+day+day*length	4878.224	138.111	<0.0001
color~length+year+day+day^2	4878.819	138.706	<0.0001
color~length+year+day	4881.507	141.394	<0.0001
color~year+day+day^2+day^3	4895.193	155.080	<0.0001
color~year+day+day^2	4905.732	165.619	<0.0001
color~year+day	4909.844	169.731	<0.0001
color~year+length	5117.599	377.486	<0.0001
color~year	5143.495	403.382	<0.0001

spawning was observed. Days leading up to spawning were assigned increasingly negative values. For example, the day before peak spawning would be assigned a day of -1. Nassau grouper may spawn over several nights while on the spawning aggregation, yet there are usually one or two nights when the fish exhibit much higher rates of spawning activity. For the purposes of this analysis we defined the peak night or nights of spawning as the night or nights when spawning bursts with gamete release were at least twice as frequent as other nights. Fish disperse quickly after the final night of peak spawning. Therefore, due to extremely small sample sizes we did not include observations from days after peak spawning in our analysis.

We evaluated the ability of the chosen model to accurately classify fish into a color phase using 10-fold cross validation. 10-fold cross validation is a technique which splits the data into 10 sets. A multinomial logistic regression using the selected formula is then run 10 times and each time one set of the original data is removed from the data set that builds the model. After each run the color phase was predicted for each fish in the set of data points that were not used to build the model. The overall error rate as well as color phase specific error rates for each run was recorded and then averaged. All analyses were conducted using R version 2.13.0 (R Development Core Team, 2011).

2 Results

We used a total of 2013 unique images in the analysis with the number of images per year ranging from 256 to 553 (2008 and 2005 respectively, Table 1). The length of fish used in the analysis ranged from 352.65 to 874.48 mm with a mean of 616.11mm and a standard deviation of 82.53mm. The distributions of lengths were similar across years (Fig. 2). The observed trends in proportion of fish exhibiting each color phase by day show that the dark and white belly color phases are more variable from year to year than either the barred or bicolor colorations (Fig. 3).

The data overwhelmingly supported a model with all predictor variables (color~year+day+length+year*day+length*day+day^2+day^3, model weight=0.87, Table 2). The results of the supported model show that, regardless of length, fish are less likely to exhibit three color phases; barred, dark, and white belly, as the day of spawning approaches while the likelihood of a fish exhibiting the bicolor color phase increases exponentially as spawning approaches (Fig. 4, Table 3). The exponential increase in the probability of a fish exhibiting the bicolor color phase is reflected by the relatively large coefficients estimated for the second and third order polynomials of day for bicolor fish (-2.6477 and -0.6776 respectively, Table 3).

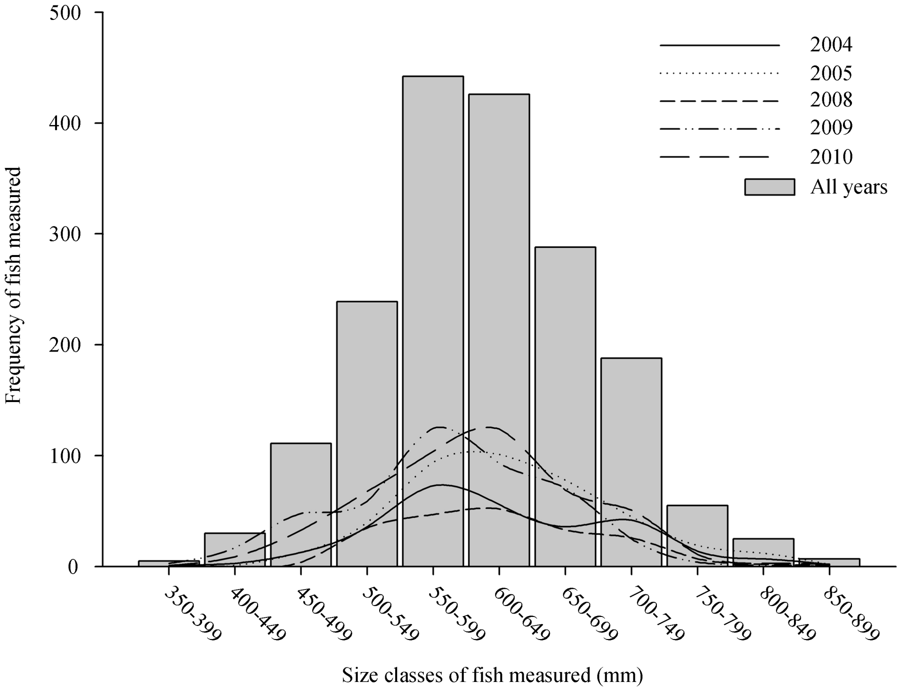

Fig. 2 Length distribution of fish used in the analysis for all years combined as well as all years individually

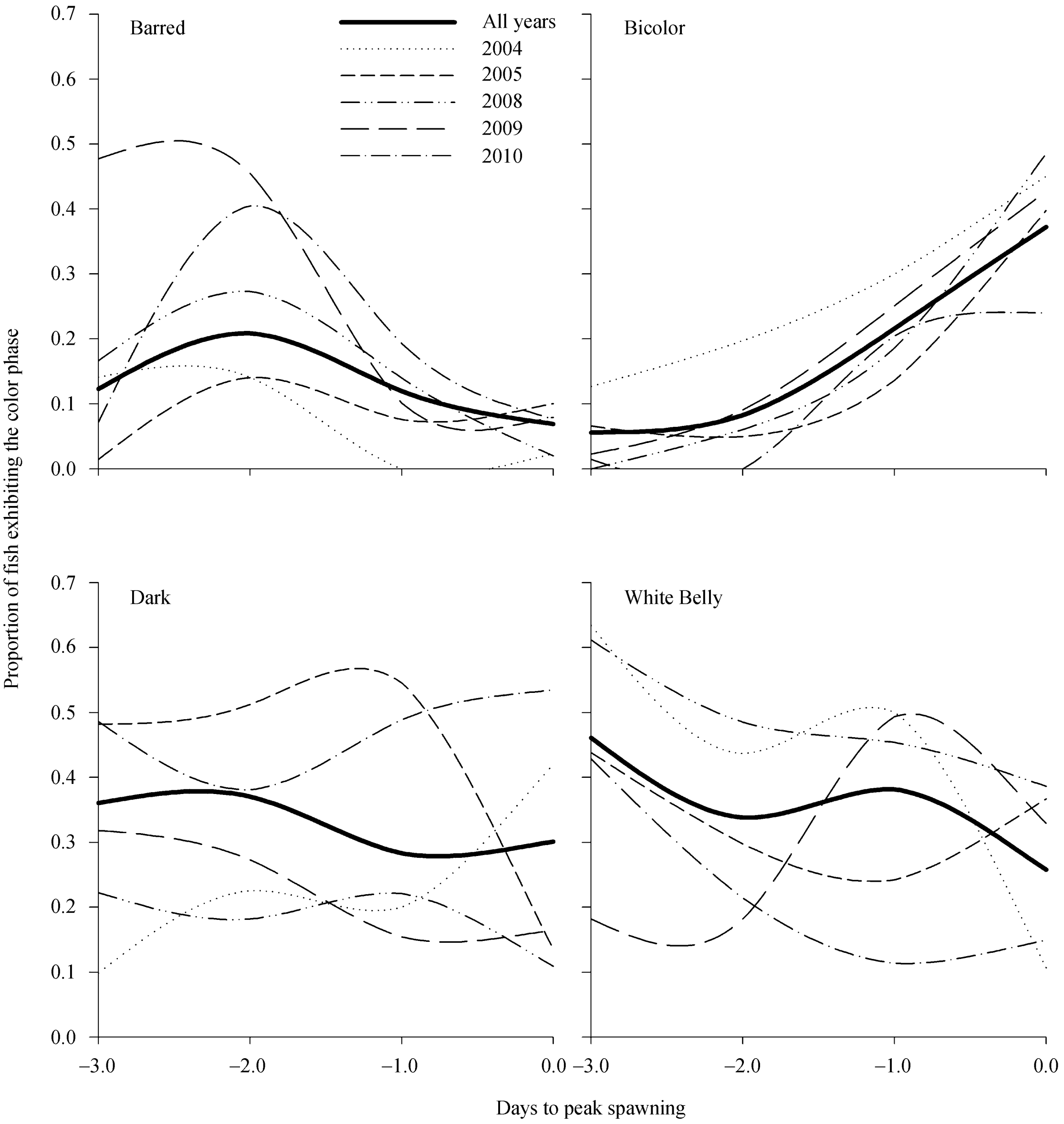

Fig. 3 The observed proportion of fish in each color phase by day and year

As we assumed inter-annual variation in fish behavior did contribute to the structure of the model. The coefficients for all combinations of years and color phases, with the exception of 2008 and white belly, are significantly different from zero (see Table 3 for estimates and confidence intervals). For example, the dark color phase resulted in two years (2004 and 2010) actually showing the reverse of the trend exhibited in other years (Fig. 4).

The confidence intervals for the coefficient for length for all color phases did not overlap zero which means that larger fish were less likely to be observed in the barred color phase (Table 3). Larger (and thus older) fish are also more likely to exhibit the dark or white belly color phase than the bicolor coloration (Table 3). The interaction between length and proximity to spawning did not affect the probability that a fish will exhibit the bicolor color phase. However, larger fish are more likely to exhibit the white belly or dark color phases as spawning approaches (Table 3).

When examined graphically the trends in observed proportion of fish exhibiting each color phase by day (Fig. 3) match well with the modeled probability of a fish exhibiting each color phase by day (Fig. 4). The chosen model was able to accurately classify an average of 47.19% of new observations. The model performed the worst when classifying fish in the barred coloration with an average of only 13.57% of observations classified correctly. The model performed much better for bicolor

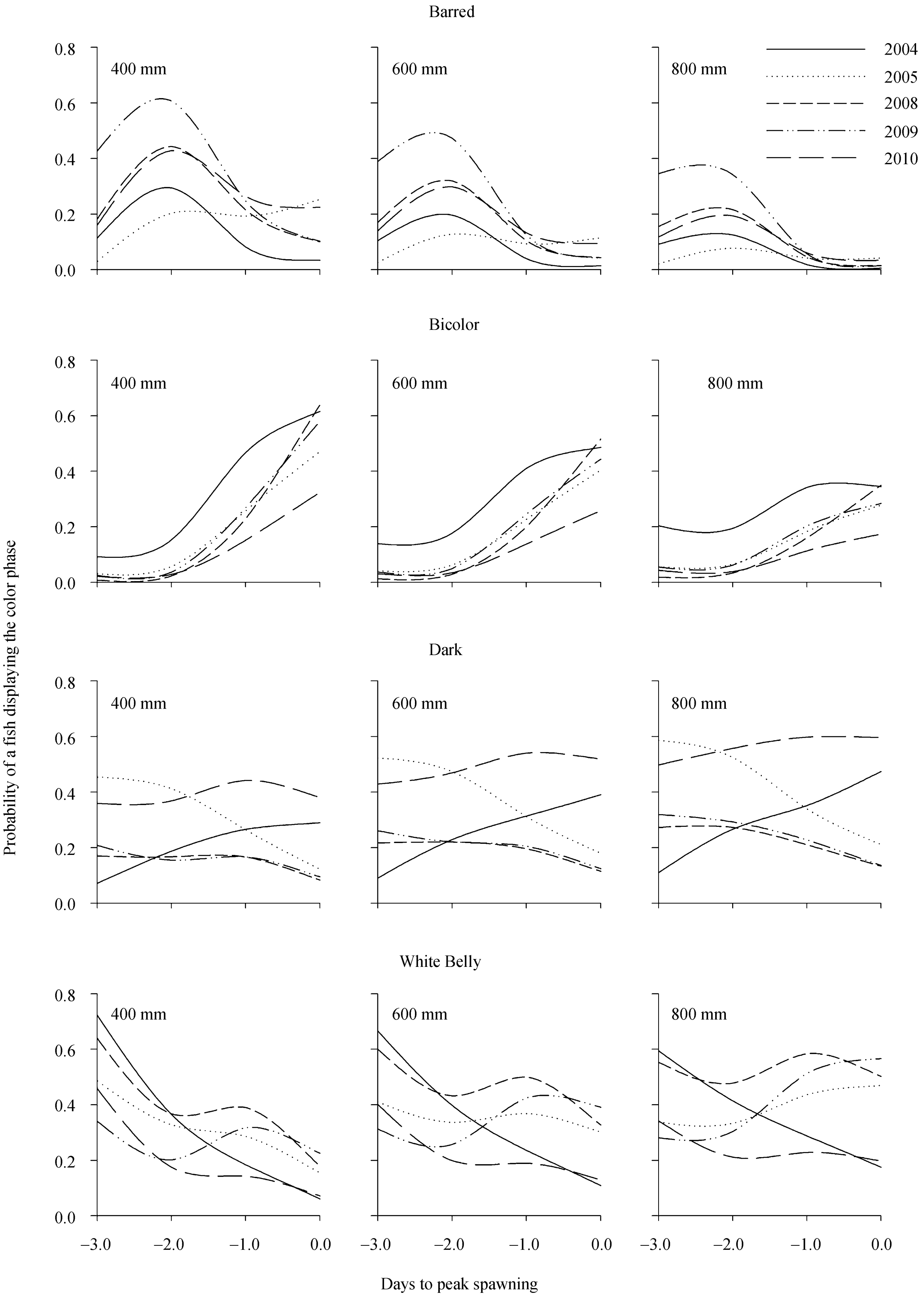

Fig. 4 Daily changes in the probability of a fish exhibiting each of the color patterns as spawn time approaches, based on the results of the most parsimonious model (color~year+day+length+year*day+day^2+day^3)

The results are presented by color phase as well as a theoretical fish at three different lengths, 400, 600, and 800 mm, chosen to represent the youngest, average, and oldest fish on the aggregation respectively. Model fits for all five years of available data are also presented.

Table 3 Maximum likelihood estimates and confidence intervals for the parameters of the multinomial logistic regression model with the formula color~length+year+day+year×day+day^2+day^3

	Bicolor			Dark			White Belly		
	Estimate	Confidence Interval		Estimate	Confidence Interval		Estimate	Confidence Interval	
Length	0.0032	0.0027	0.0037	0.0059	0.0053	0.0064	0.0073	0.0068	0.0078
2005	−2.2828	−2.4981	−2.0675	−2.8764	−3.1174	−2.6354	−1.0815	−1.2872	−0.8757
2008	−1.0572	−1.2828	−0.8316	−2.3423	−2.5852	−2.0995	−0.0188	−0.2452	0.2075
2009	−1.1675	−1.3797	−0.9553	−2.2250	−2.4873	−1.9627	0.2002	−0.0039	0.4042
2010	−2.5378	−2.7455	−2.3300	−1.6206	−1.8348	−1.4063	−1.7343	−1.9540	−1.5147
day	−0.8939	−1.0608	−0.7270	−0.6764	−0.8504	−0.5025	−2.5900	−2.7607	−2.4192
day^2	−2.6477	−2.8590	−2.4364	−1.4615	−1.6889	−1.2341	−1.9175	−2.1237	−1.7112
day^3	−0.6776	−0.7493	−0.6060	−0.3784	−0.4436	−0.3132	−0.5048	−0.5665	−0.4431
2005*day	−0.8340	−1.0998	−0.5681	−2.0177	−2.2159	−1.8194	−0.6726	−0.8641	−0.4810
2008*day	0.6230	0.2880	0.9580	−0.9094	−1.1460	−0.6727	0.1923	−0.0302	0.4148
2009*day	0.4902	0.1779	0.8025	−0.6554	−0.8888	−0.4221	0.7581	0.5487	0.9676
2010*day	−0.2427	−0.5747	0.0893	−0.9614	−1.1640	−0.7589	−0.3113	−0.5096	−0.1131
length*day	0.0002	−0.0006	0.0010	0.0014	0.0006	0.0022	0.0024	0.0016	0.0032

The reference category for the regression is the barred coloration and the reference level for the factor year is 2004.

and white belly fish with 40.75 and 51.71% classified correctly respectively. The model performed the best for the dark color phase with an average of 58.94% of observations classified correctly.

3 Discussion

Color changes and their role in visual communication and reproduction has interested scientists in a variety of fields and led to breakthroughs in our understanding of everything from behavior to speciation in fish (Kodric-Brown, 1998; Korzan et al., 2008; Price et al., 2008; Seehausen et al., 2008). While nuptial coloration has been studied in fish with a wide range of reproductive strategies, coloration of fish that aggregate has not received much attention. Nuptial color changes in fish that aggregate are particularly interesting because individuals presumably respond to the cumulative behaviors of all members in the aggregation. Additionally, many fish that aggregate are solitary during the non-reproductive periods of the year. Therefore, aggregating fish must not only convey information regarding spawn timing but must also negotiate social interactions that are uncommon in the non-reproductive period. Although many fish, including Nassau grouper, are capable of changing color instantaneously (Colin, 1992) trends in the exhibition of color phases can provide insight into the behavior and communication of fish on an aggregation. Our analysis of color phase data from a Nassau grouper spawning aggregation shows that the fish are likely using color changes to convey physiological readiness and willingness to spawn and perhaps to mediate social interactions as well.

The barred coloration is the Nassau grouper's typical coloration off of the spawning aggregation when the fish actively maintain and defend territories. If the barred coloration conveys territoriality, then fish faced with the necessity of relinquishing their territories in favor of close proximity to conspecifics for the purposes of spawning may choose to adopt another color pattern in order to avoid unnecessary conflict. It is thus not surprising that barred coloration displays decrease as the night of peak spawning approaches.

The interpretation of the general decrease of dark and white belly colorations is less obvious. Both of these colorations are not seen off of the spawning aggregation and while they tend to decrease as spawning approaches they never reach proportions as low as the barred coloration. Additionally, the proportion of fish exhibiting the dark color phase actually increased as spawning approached in two of the five years analyzed. Anecdotal information suggests that many spawning rushes are led by a female fish in the dark color phase and some have suggested that both dark and white belly colorations are exhibited only by females (Colin, 1992; Sadovy and Eklund, 1999). However, due to the high proportion of fish exhibiting the dark color phase early in the aggregation period it is unlikely that the color phase simply indicates a willingness to spawn. Additionally, 82.7 %

of the fish observed three days before spawning displayed either the dark or white belly color phase. If only females exhibited these color phases our results would suggest a sex ratio heavily skewed towards females. Yet sex ratios at aggregations of Nassau grouper have typically been found to be close to 50:50 (Sadovy and Eklund, 1999) and there is evidence that the sex ratio at the West end Little Cayman aggregation, where this study was conducted, is slightly skewed towards males with a 1:1.6 female to male sex ratio (Whaylen et al., 2004). Therefore, while the dark and white belly color phase are associated with spawning in that they only occur while the fish are on the spawning aggregation, they likely convey information other than the fish's readiness or willingness to spawn. It is unlikely that these colorations indicate the sex of the fish. Therefore, since both color phases do not appear to convey a readiness to spawn and are likely not indicators of sex, it may be that they help to mediate social interactions the fish experience while on the aggregation but that are unlikely to occur in their territories during the remainder of the year. They may simply be different forms of non-aggressive coloration. Additional behavioral observation of the fish on the aggregation is necessary to interpret what information these colorations convey.

The exponential increase in the proportion of fish exhibiting the bicolor color phase as the day of spawning approaches suggests that this color phase is used to convey both physiological readiness and behavioral willingness to spawn. This result is also consistent with previous observations made by Smith (1972), Colin (1992), and Whaylen et al. (2006) who all report that during spawning nearly all fish exhibit the bicolor color phase. While Nassau grouper begin to aggregate on winter full moons throughout the Caribbean, on the west end Little Cayman aggregation the actual day (or days) of spawning can be anywhere from one to ten days after the full moon (Tucker et al., 1993; Whaylen et al., 2006). While it is unknown exactly why the fish choose a specific evening to spawn, it is clear that by the time that evening comes the majority, if not all, the fish are ready to spawn. The images analyzed for this paper were taken during the day, while Nassau grouper spawn at dusk and for a short time into the night. Therefore, the large increase in fish exhibiting the bicolor phase on the day of spawning may indicate that the fish use the bicolor color phase to convey readiness to spawn. The lower proportion of larger fish that exhibit the bicolor color phase in the days leading up to spawning would seem to support this interpretation (mean proportions of 0.05 ± 0.06 *SD* three days before spawning compared to 0.40 ± 0.04 *SD* on the day(s) of peak spawning, Fig. 3). Spawning is a physiologically costly exercise (Semmens et al., 2006) and lifetime fitness likely increases if females spawn only when conditions are favorable (Rideout et al., 2005). The high cost associated with spawning combined with the results of studies showing selection pressure for honesty in nuptial coloration (Price et al., 2008; Seehausen et al., 1999) suggest that fish displaying the bicolor color phase are willing to spawn that evening. Presumably, if the fish are waiting for environmental conditions to cue their spawning (Heppell et al., *In Press*; Heppell et al., 2008), and bicolor coloration conveys willingness to spawn, then older and more experienced fish would only change into the bicolor phase when conditions were right and spawning would occur.

The number of Nassau grouper aggregations remaining, let alone healthy aggregations, has decreased dramatically in recent decades (Sadovy de Mitcheson et al., 2008). Because of this, we are rapidly losing the chance to study the behavior of aggregating fish in the absence of fishing and large population declines. Nonetheless, a nuanced understanding of spawning behavior may prove extremely useful when developing monitoring and recovery plans as well as assessing recovery and in predicting the timing of spawning (Fitzsimons and Nishimoto, 1995; Mesa-Avila and Molina-Borja, 2007; Shumway, 1999). For example, the fact that the proportion of fish exhibiting the bicolor phase increases exponentially until the day of spawning may prove useful in focusing effort during future monitoring efforts. If funds or access to the aggregation site limit the number or timing of dives possible at the aggregation site, spawning may be inferred if a rapid increase in the fish in bicolor coloration is observed during the day. Alternatively, researchers may reduce the cost of monitoring programs by conducting evening dives only when the proportion of bicolor fish begins to increase. In situations where placing divers in the water is unsafe such as when aggregations occur beyond safe diving depths, or to supplement observations collected by divers, drop cameras and/or remotely operated vehicles may be used to asses color changes and if necessary infer spawning.

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