Alternative contexts of sex change with social control in the bucktooth parrotfish, *Sparisoma radians*

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Synopsis

Social control of sex change occurs in a variety of hermaphroditic fishes; upon removal of the dominant individual, the largest individual of the opposite sex typically changes sex and acquires mating priority with the remaining members of the social group. Social control may allow a phenotypically plastic response to social situations that convey cues about the relative advantages of functioning as one sex or the other, and should be advantageous in highly heterogeneous habitats such as coral reefs. Parrotfishes (family Scaridae) are dominant members of herbivorous coral reef fish assemblages, and numerous histological examinations of gonads have demonstrated the hermaphroditic life history of many species in the family. However, social control of sex change has never been conclusively demonstrated in the parrotfishes. To test a new version of the size-advantage model for sex change, we conducted removal experiments of dominant male bucktooth parrotfish, *Sparisoma radians*, in St. Croix, U.S. Virgin Islands. A total of seven females from five different reefs changed sex following removals, clearly demonstrating social control of sex change. In addition, all but one of those individuals changing sex were smaller than the largest females remaining in the harems, and this contrasts with nearly all previous studies of sex change in fishes. Sex change proceeds via a novel sequence of events when compared with previous studies. Rather than behavioral sex change preceding morphological sex change, the appearance of male coloration is followed by the development of male behavior that is fully expressed approximately 20 days after removal. We show how differing arrival rates of bachelor males at our study sites may facilitate alternative contexts of sex change, with sex change occurring within social groups in some locations and with bachelor males filling harem vacancies in other locations. Alternative contexts of sex change further illustrate the astonishing phenotypic plasticity in the social and mating behavior of parrotfishes.

Introduction

Protogynous hermaphroditism (sex change from female to male) is common in marine fishes (Warner 1984, Shapiro 1987, Francis 1992, Kuwamura & Nakashima 1998) and occurs in at least 14 fish families, 11 of which are found on coral reefs. The size-advantage model (Ghiselin 1969, Warner 1975, Warner et al. 1975) has been highly successful in explaining the adaptive significance of sequential hermaphroditism (functioning as one sex during one life phase and as the other sex during another) in animals. If reproductive success increases with size or age more rapidly for one sex, an individual that changes sex in the appropriate direction will have a higher lifetime reproductive success than one that does not change sex. If males gain in reproductive success with size or age faster than females, protogynous sex change is favored; the opposite situation favors protandry (sex change from male to female). The model predicts

protogyny where large males monopolize matings (Warner 1978, 1988). For example, many protogynous fishes have haremic social systems where large males control access to females (Robertson & Warner 1978, Warner & Robertson 1978) and where sex change is under social control (Fishelson 1970, Robertson 1972, Ross et al. 1983, Warner & Swearer 1991); upon removal of the dominant male, the largest remaining female changes sex and takes over the harem (Warner 1988, Ross 1990).

Social control of sex change may allow a phenotypically plastic response by individuals to social situations that convey cues about the relative advantages of functioning as one sex or the other (reviewed in Charnov 1982, Warner 1988). Such a response should be advantageous in highly heterogeneous habitats, such as coral reefs, that vary enormously in physical factors such as reef size, shape, exposure to currents and waves, and geological history, and in biological factors such as food availability and distribution, recruitment and mortality rates, population density, and population age and size structure (Shapiro 1991, Warner 1991). For example, as reef size increases, populations of the protogynous bluehead wrasse, *Thalassoma bifasciatum*, increase, while the mating success of large territorial males decreases, and the location of their mating territories shifts from preferred terminal ends of reefs to peripheral upcurrent areas (Warner & Hoffman 1980). The proportion of smaller males also increases with population size, apparently because higher densities of small males are better able to take over preferred spawning sites (Warner & Hoffman 1980). For *T. bifasciatum*, social control of sex change may allow smaller individuals to respond to their improved mating prospects in larger populations.

Social control of sex change occurs in a variety of protandrous (Fricke & Fricke 1977, Hattori 1991) and protogynous (Fishelson 1970, Robertson 1972, Moyer & Nakazono 1978, Shapiro 1980, Ross et al. 1983, Warner & Swearer 1991, Lutnesky 1994, Cole & Shapiro 1995, Sakai 1997) reef fishes, including anemonefishes (Pomacentridae), wrasses (Labridae), angelfishes (Pomacanthidae), sea basses (Serranidae), and gobies (Gobiidae). Though these studies are generally consistent with the pattern outlined above (initiation of sex change following removal or disappearance of a dominant individual), this previous work has also uncovered great variation in the timing and sequence of physical and behavioral changes experienced by sex-changing individuals. For example, anemonefish males may initiate sex change

shortly after removal of a dominant female but then require from 26 to 180 days to complete sex change and produce their first clutch of eggs (Fricke & Fricke 1977, Hattori 1991). In contrast, female *T. bifasciatum* initiate male-typical aggressive and courtship behaviors within minutes of the removal of large males, and will spawn in the male role on that same day (producing functional sperm 8 days later) (Warner & Swearer 1991).

Fishes of the family Scaridae (parrotfishes) are dominant and conspicuous members of herbivorous coral reef fish assemblages (Choat 1991), and numerous histological examinations of gonads have demonstrated the protogynous life history of nearly all members of the family (Robertson & Choat 1974, Choat & Robertson 1975, Robertson & Warner 1978, but also see Robertson et al. 1982). Despite having been the focus of numerous demographic, ecological, endocrinological, and behavioral studies (e.g. Reeson 1983, Cardwell & Liley 1991a, Colin & Bell 1991, McAfee & Morgan 1996), social control of sex change has never been demonstrated in the family.

Previous experiments designed to initiate social control of sex change in parrotfishes have been unsuccessful, perhaps because of the presence of nonterritorial bachelor males or neighboring territorial males ready to take over harems following dominant male removals (Dubin 1981, Clavijo 1982). Studies with *Centropyge* angelfishes suggest that alternative contexts of sex change may be associated with habitats that differ in the availability of males able to take over vacant territories (Aldenhoven 1986, Sakai 1997). When a dominant male disappears from a territory, sex change in remaining females may be delayed or completely absent in harems found in habitats that support a high number of bachelor males, or a high density of harems. In contrast, when harem vacancies arise where neighboring or bachelor males are rare, or where harem density is relatively low, sex change may readily occur from within the harem shortly after the vacancy develops. For example, females from isolated harems (>10 m apart from neighbors) of the rusty angelfish, *Centropyge ferrugatus*, changed sex when dominant males disappeared. In those harems that were located adjacent to one another, however, neighboring males took over vacant harems and remaining females did not begin to change sex until 12–78 days following takeover by neighboring males (Sakai 1997). The previous studies of alternative contexts of sex change help to identify factors that may critically affect the sex change process, while unsuccessful attempts at stimulating social control of sex change in parrotfishes

illustrate the complexity of sex change mechanisms in fishes and suggest the existence of additional factors that remain to be identified.

Additional evidence that illustrates this complexity of sex change mechanisms comes from studies of protogynous species in which females smaller than the largest females are those observed to change sex when provided an opportunity (Cole 1983, Muñoz & Warner 2003a,b). Working with one of these species (the bucktooth parrotfish, *Sparisoma radians*), Muñoz & Warner (2003a) proposed a new model for protogynous sex change (the expected reproductive success threshold [ERST] model) that has identified two additional factors that may sometimes be key to understanding sex change in fishes: sperm competition and size-fecundity skew. Size-fecundity skew occurs if the fecundities of the largest females are markedly higher than the aggregate of the other females in their social group, and together with sperm competition, can lead to situations where large females should defer sex change to smaller females.

In a test of the ERST model, Muñoz and Warner (2003b) conducted dominant male removal experiments of *S. radians*, in St. Croix, U.S. Virgin Islands. In this paper we present the behavioral and morphological details of sex change generated by these removal experiments and demonstrate conclusively for a member of the family Scaridae that dominant male removals can stimulate social control of sex change. In sex-changing individuals, male coloration appears well in advance (up to 12 days) of fully expressed male behavior (successful defense of territories and females, and attainment of spawns with high fertilization). We highlight another element of social control in that responses to male disappearance may occur under alternative contexts, depending on the opportunity for bachelor males to take over harem vacancies. These varied responses to opportunities for sex change in *S. radians* illustrate another facet of the extraordinary phenotypic plasticity characterizing the mating and social systems of parrotfishes (Thresher 1984, Shapiro 1991, Warner 1991, Muñoz & Motta 2000).

Methods

The bucktooth parrotfish

This study took place on patch reefs in Tague Bay (64°35′42″W, 17°45′45″N) on the northeast coast of St. Croix, U.S. Virgin Islands from April 1998 to August 2000. Details of the study site can be found in Gladfelter & Gladfelter (1978) and Muñoz & Warner (2003b). *S. radians* is a protogynous parrotfish in which all males have changed sex from a prior female phase (i.e. monandry). It is a common herbivorous fish in Caribbean seagrass beds and is one of the most abundant species in this habitat (Randall 1968, Weinstein & Heck 1979, Lobel & Ogden 1981). Populations of *S. radians* form isolated aggregations in seagrass habitats (Ogden & Zieman 1977, Robertson & Warner 1978), within which dominant, brightly colored males in terminal phase (TP) coloration defend contiguous territories containing harems of drab colored females in initial phase (IP) coloration. In the seagrass, non-territorial males roam across territories as 'bachelors' and can be in either the TP or IP coloration (the latter having changed sex but not color, Robertson & Warner 1978, Farm 1993). In St. Croix, *S. radians* also occurs on patch reefs interspersed throughout the seagrass community (see Gladfelter & Gladfelter 1978, Muñoz & Warner 2003b), where both large and small males maintain territories and harems. Spawning takes place in both of these habitats daily each afternoon. Territorial males normally spawn singly with a female (pair spawning), but will occasionally engage in sperm competition by rushing in and contributing sperm to an ongoing mating between a neighboring male and female ('streaking,' Warner & Robertson 1978). In contrast, bachelor males nearly always face sperm competition since most of their matings involve streaking a larger male and female pair, or group spawning in which several males mate simultaneously with a female (Robertson & Warner 1978, Farm 1993, Marconato & Shapiro 1996).

Like most parrotfishes and other diurnal herbivorous reef fishes, female *S. radians* are energy maximizers (Schoener 1971, Hoffman 1983, Sano 1993) and spend approximately 80–95% of their time feeding (Horn 1989, Bellwood 1995, van Rooij et al. 1996, Muñoz & Motta 2000) in small groups of 3–7 individuals. While remaining within a male's territory, these groups move freely throughout the seagrass beds and patch reefs. In contrast, male *S. radians* are time minimizers (Schoener 1971, Hoffman 1983, Sano 1993) that spend most of their time actively patrolling the borders of their territories and maintaining social dominance over females, and much less time foraging than females (Clavijo 1982, Hoffman 1983, Sano 1993, Muñoz & Motta 2000). Female-distinguishing coloration in *S. radians* includes a broadly blue–green base and axil of the pectoral fins, together with a blue edge

of the opercle. IP males do not possess these markings although they superficially resemble females in their overall drab coloration. In contrast, the distinctive coloration of TP males consists of a diagonal bicolored band of blue and orange running from the corner of the mouth, rimming the lower edge of the eye, and extending a short distance beyond the eye (Randall 1968).

Experimental procedure

Prior to experimental removals, we surveyed populations to obtain quantitative estimates of parameters identified by the ERST model (Muñoz & Warner 2003a) that may critically affect the sex change process. We determined local population densities, harem sizes, male and female sizes, and incidence of sperm competition. We also collected females shortly before the spawning period and expressed their eggs to determine female size-fecundity relationships (Muñoz & Warner 2003b). We removed dominant males from a total of 22 harems on eight patch reefs in an attempt to initiate sex change in the remaining harem members (Muñoz & Warner 2003b). We confined our removals to large fish on patch reefs because the larger size of reef fish, the larger size-skew possible in reef harems, and the lower densities of harems on reefs (Muñoz & Warner, unpubl. data) allowed us to observe the effects of removals more easily on patch reefs than in seagrass. Following removals, we conducted daily searches throughout the day that monitored experimental patch reefs for sex-changing individuals and quantified the number of roving bachelors attempting to take over the experimental harems. In both removal experiments (see below), we aimed to increase the probability that harem takeover would come from within the reef harem. Therefore, bachelors were removed and transplanted to distant reefs when seen. In one experiment (large and small male removal, 18 harems on seven reefs) we removed all males larger than 7 cm total length (TL) (the average size of seagrass males, n = 43, a group of fish substantially smaller than females on reefs, Muñoz & Warner, unpubl. data) that were resident or appeared in the harem area, so that only the smallest males were available for mating with remaining females.

We identified individuals changing sex by the emergence of male coloration and/or behaviors. Once these transitional individuals were identified, they were observed daily during the spawning period to determine the onset of their participation in mating, and spawns between transitionals and females were monitored for streaker male participation. We validated our observations of external morphology and male behavior by tracking the fertilization rate and sperm concentration of unstreaked pair spawns between transitionals and females over several consecutive days. The onset of substantial fertilization rates (i.e. >80%, approximately the median fertilization rate of unstreaked pair spawns in non-experimental fish, see the section on 'Results') was taken as an indicator of completed sex change. We followed procedures outlined in Shapiro et al. (1994) and collected gametes that were spawned to determine fertilization rates and sperm concentrations per spawn. These collections were taken as near as was possible to the onset of male reproductive activity by transitionals. Briefly, we caught spawns seconds after gamete release in the water column with large reinforced plastic bags. Gametes were subsampled from the bag to estimate fertilization rate and sperm concentration per spawn. For fertilization rate, eggs were left in seawater to develop for approximately 5 h, at which point fertilized eggs are easily distinguished by a clearly visible fertilization envelope. Eggs were then counted and the number of fertilized *versus* unfertilized eggs was recorded. For estimates of sperm concentration, we stained the sperm with rose bengal, preserved them with formaldehyde, concentrated them onto membrane filters with a Millepore filtration unit, and counted them under a compound microscope. Fertilization rates and sperm concentrations were estimated only from samples containing at least 20 eggs. We also caught spawns of reef-based fish from non-experimental patch reefs to characterize normal fertilization dynamics for comparison with transitionals.

In one additional experiment on a separate patch reef (large male only removal), we removed only the largest dominant males from four harems on the reef, leaving several males that were larger than 7 cm TL (the average size of seagrass males). In this way we were able to determine the effect on the dynamics of sex change of the continued presence of medium-sized reef males (those territorial males smaller than most of the large females remaining on the patch reef but larger than males in the seagrass). In this experiment, we addressed the possibility that any incidence of sex change in our previous experiments may have been an artificial result of a lack of all but the smallest males.

To confirm that any sex change observed was the result of our removals (i.e. to control spontaneous sex change), we monitored 14 harems on four additional unmanipulated patch reefs. These observations

began 54 days following the start of our experiments. We determined the identities of resident fish through unique natural color markings, individual sizes, and home range locations. We searched for transitional individuals and censused all fish every 3 days for 1 month.

Analyses

Prior to parametric analyses, data were tested for normality with the Kolmogorov–Smirnov (Lilliefors' correction) test and for homoscedasticity with the Levene Median test. Consistently non-normal or heteroscedastic data were analyzed with non-parametric procedures (Siegel & Castellan 1988).

Results

Behavioral details of sex change

We chose eight different patch reefs for experimental removals and characteristics of their residents are detailed in Muñoz & Warner (2003b). Harems on control patch reefs showed characteristics within the range of experimental reefs (Muñoz & Warner 2003b). Sex change occurred in 7 of 22 harems from five different patch reefs following removal of dominant males. Strikingly, all but one of the sex changers were smaller than the largest females remaining in the harems (Figure 1; also see Muñoz & Warner 2003b), demonstrating that overall, the largest female *S. radians* do not change sex. The process of sex change was virtually identical in 6 of the 7 instances, and proceeded in the following sequence for both experiments.

Subsequent to removal of large dominant males, remaining females from all experimental harems began aggregating *en masse* and roving about the patch reef, beyond the borders of their home ranges that had been identified in previous surveys. This aggregation was most evident during the daily spawning period. In the interval between male removal and the sex change of a female, remaining females spawned either with smaller males remaining on the patch reef or resident in the seagrass. The largest females frequently spawned with numerous (from 2 to >15) males in group spawns, and the mean number of males in these multi-male spawns was greater than the mean number of males in streaked multi-male spawns prior to removals (mean ± SE number of males: post removal, 4.18 ± 0.66 males, n = 28; prior to removals, 2.19 ± 0.19 males, n = 88;
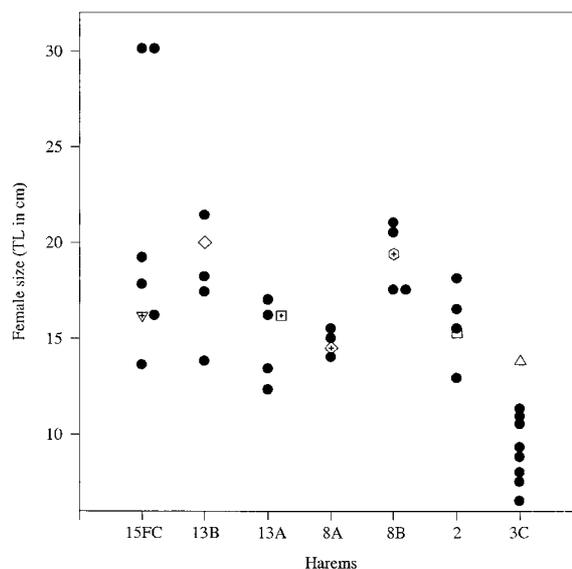

Figure 1. Harem size and size rank of *S. radians* females that changed sex for seven separate harems. Different open symbols correspond to different individuals that changed sex, and these symbols correspond to the same individuals in Figures 2 and 3. The female from harem 3C (open triangle) initiated sex change during the 'large male only' removals (experiment 2). Horizontally adjacent symbols are similarly sized females. Data from Muñoz & Warner (2003b).

Mann–Whitney Rank Sum test, T = 2237.0, $n_1 = 28$, $n_2 = 88$, p < 0.0001). Duration of courtship for these large females, though not explicitly measured, appeared longer and more intense than courtship and spawning prior to removals. After a female had completed sex change, most, but not all remaining females eventually began spawning with the new male. Those females that did not spawn with the new male continued to spawn with smaller reef or seagrass males.

On those patch reefs where sex change did occur, we initially identified transitional females by observing atypical female behavior (i.e. repeated sighting of a female feeding solitarily in a restricted area of the former territory). These atypical females were frequently the recipients of aggression by small males. Within 3 days, close-range (0.5 m) observations revealed the male-typical bicolored band rimming the eye in conjunction with the female-typical blue pectoral fin base, a combination only observed in experimentally induced transitional fish (although we presume it to occur during natural sex change). Following its emergence at the eye, the color band gradually became more distinct, and the blue-green at the pectoral base slowly faded, until the transitional individual was in full male coloration

(see Randall 1968 for more coloration details). Starting from the moment of dominant male removal, the average time to the first appearance of transitional coloration (presence of male eye band with female blue pectoral fin base) was 9.3 ± 0.76 days (n = 6, Figure 2). Despite the onset of male coloration and behavior, typical male behavior (described above) was not observed until nearly 20 days after removals. For example, for 1–2 weeks following the first appearance of male coloration, transitionals did not patrol territories, were chased by smaller males and larger females, and continued to maintain a restricted home range (see below). Additionally, although sex-changing fish
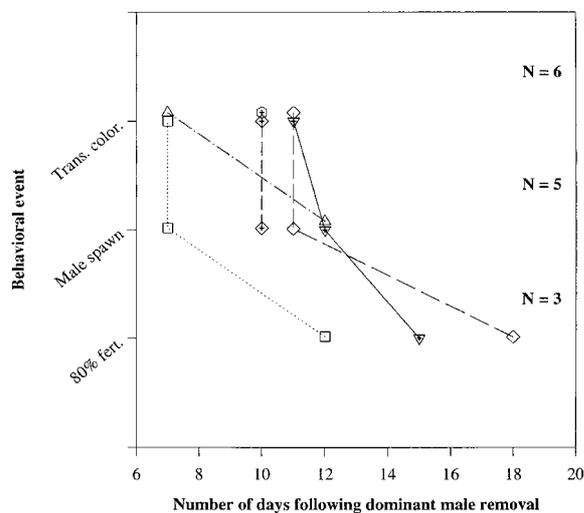

Figure 2. Timing of behavioral events by transitionals following dominant male removals. 'Trans. color.' is the first appearance of transitional coloration. One female (8B) was observed directing male courtship behavior (lateral head shakes, also described as 'quivering' [Robertson & Hoffman 1977] or 'body-flicking' [Clavijo 1982]) toward another female while still completely in female coloration. The date of this observation was used in place of the date of first appearance of transitional coloration. 'Male spawn' is the first appearance of a transitional spawning in the male role. '80% fert.' is the first occurrence of an unstreaked pair spawn involving a transitional in which captured gametes revealed a fertilization rate of at least 80%. Sample sizes decrease because not all behavioral events were observed for all transitionals, and sample sizes start at six because one female (13A) who changed sex was excluded from the comparisons. This female was the second female to change sex on a single patch reef (13), and her timing of sex change was probably influenced by the previous sex change of female 13B (see Muñoz & Warner 2003b). Timing of behavioral events following removals for female 13A was markedly delayed compared to other transitionals (e.g. transitional coloration: 27 days; male spawn: 29 days).

were observed to spawn as a male for the first time an average of 10.4±0.93 days (n = 5, Figure 2) following removals, the spawning rushes were not well coordinated (i.e. females would frequently begin the rush without the transitional), and fertilization rates were low (<80%) until 12–18 days post removal (Figure 2).

No sex change was observed in 15 of the 22 experimentally manipulated harems during 53 days of post-removal observations. Reproductive behavior in these harems was similar to behavior observed in those harems with sex change, in the interval between male removal and the sex change of a female: remaining females simply continued spawning with small seagrass or reef TP males, and in most cases were incorporated into the harems of adjacent smaller males. In a few cases we witnessed large females making short migrations out of their home ranges to spawn with males in the seagrass, and then returning to the reef (also see Muñoz & Warner 2003b).

Changes in spawning coordination and harem control

Spawns captured on 3–4 consecutive days for two transitionals in the large and small male removals demonstrated a gradual increase in fertilization rate and sperm output (and presumably testicular function), which confirmed the sex change process suggested by behavioral and external morphological changes (Figure 3). Taken together, changes in fertilization rate and sperm output support the general observation of an increase in spawning coordination with time. For example, note the trend toward increases in fertilization rate with time when examining data for both transitionals (Figure 3a). However, although transitional female 13B (diamond) showed increases in both fertilization rate and sperm output, instances of high and low fertilization rates for transitional female 15FC (triangle) do not correspond perfectly with high and low sperm output for this individual. These data suggest that both increasing coordination and sperm output contribute to increasing fertilization rates. Information on the fertilization dynamics of non-experimental mature fish (Figure 4) also suggests an effect of increasing coordination over time by transitionals. Pair spawns for mature fish that were not parasitized by intruding males revealed fertilization rates ranging from <1% to 99% (median = 82.6%, n = 45). Although mature fish released an average of 18.80 ± 1.26 million sperm per spawn with fertilization rate ≥80% (n = 29), the
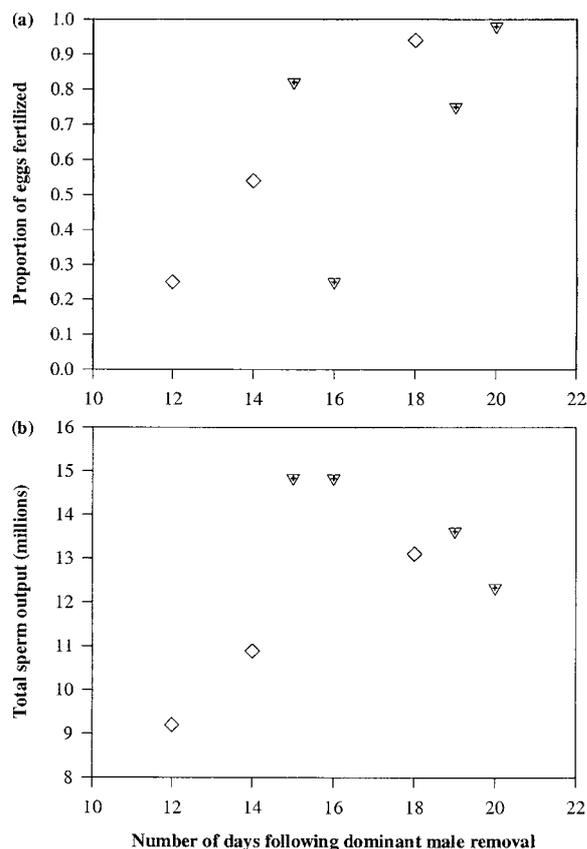
Figure 3. Changes in transitional female *S. radians* fertilization rate and sperm output following dominant male removals. Diamonds and triangles represent transitional individuals 13B and 15FC, respectively, and each data point represents a single spawn. For approximately two weeks post removal, there is an increasing trend in the proportion of spawns streaked (see Figure 5) and a significant increase in the number of males associated with multi-male spawns (see the section on 'Behavioral details of sex change'). Additionally, females typically spawn only once per day. Together, these factors make it difficult to capture spawns between transitionals and females that are not streaked. (a) Proportion of eggs fertilized *versus* days following dominant male removal. (b) Number of sperm released per spawn *versus* days following dominant male removal.

proportion of eggs fertilized from mature fish tended to surpass 80% after approximately 8 million sperm (Figure 4). By contrast, fertilization rates of transitionals were comparatively lower despite the production of more than 8 million sperm per spawn (Figure 3).

Our observations of spawns by transitionals over time further illustrate the increasing spawning and social coordination of transitionals. A greater number of spawns tended to be streaked following the onset
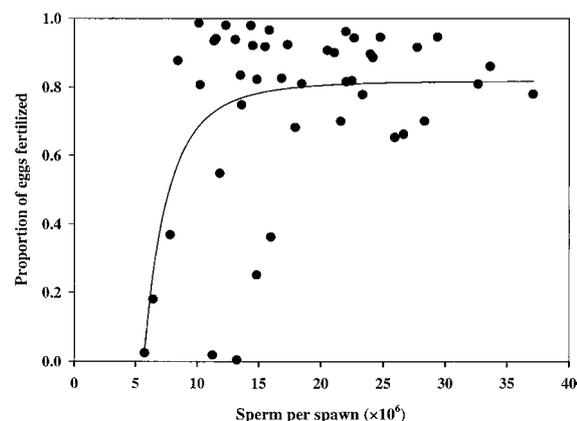

Figure 4. Proportion of eggs fertilized on non-experimental patch reefs from 45 pair spawns as a function of the amount of sperm contributed by a male. The curve is a modified hyperbolic relation fitted by the Marquardt–Levenberg algorithm (Press 1992), where proportion of eggs fertilized $= 0.82 - (241.57/(1 + 0.60\text{sperm})^{1/0.26})$.
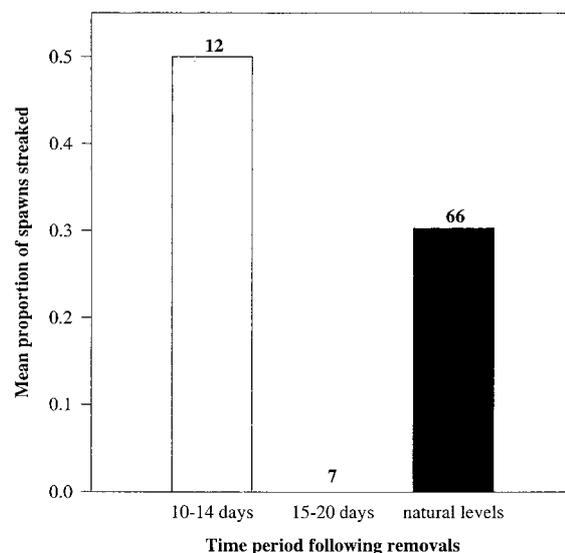

Figure 5. Changes over time in streaking intensity experienced by transitionals over patch reef habitat. Bars correspond to spawns observed within the indicated number of days following removal of dominant males. 'Natural levels' of streaking intensity were determined on non-experimental and pre-manipulation patch reefs (see Muñoz & Warner, unpubl. data). Numbers above bars are sample sizes.

of participation by transitionals in mating as males (Figure 5). However, after approximately 2 weeks had passed following removals, transitionals gained control of defense of females, and the proportion

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of spawns streaked declined (Figure 5). A similar response occurred in the adjacent seagrass habitat (mean proportion of spawns streaked in the seagrass: 10–14 days: 0.6, n = 10; 15–20 days: 0.47, n = 38; natural levels: 0.46, n = 175).

To summarize the large and small male removal experiment, sex change in *S. radians* begins with easily observable behavioral changes in which transitionals behave like neither females nor males. Behavioral changes are followed by morphological changes in color from female to male, concurrent with the development of male-typical behavior, and culminating with successful spawning (>80% fertilization rate) and harem/territory defense. Despite these general patterns, 10 days after removal one behaviorally transitional female in full female coloration (8B) was observed displaying male courtship behavior to another female; the pair later spawned, accompanied by two streakers. The following day the same transitional (still in female coloration) was again seen to spawn in the male role, and on that same day later observed to engage in 15 separate spawning rushes in the female role. Unfortunately, we were not able to collect unstreaked spawnings from this individual, so we could not determine the actual gamete production.

When only the largest males were removed, a single female changed sex, and the pattern of sex change was similar to the previous experiment. Male coloration was first noted 7 days post removal and the first spawn as a male occurred 5 days later, well within the range seen on the other experimental patch reefs (Figure 2). This similarity suggests that the other instances of sex change did not simply result from a lack of all but the smallest males. One difference from the large and small male removal, however, was that this was the only instance where the sex-changer was the largest remaining female in the harem. In the interim following male removal before sex change was completed, remaining males expanded their territories to include adjacent harems from which a male had disappeared. Later, the female that changed sex acquired a subgroup of these aggregate harems (see Shapiro 1979, Sakai 1997).

Despite consistency in the sequence of behaviors surrounding sex change, the spatial location of experimental patch reefs within Tague Bay may have had an effect on the timing of sex change. There was a trend for transitional coloration to appear sooner following removals with increasing distance from the barrier reef enclosing Tague Bay (Figure 6a). This timing of sex change may have been influenced by
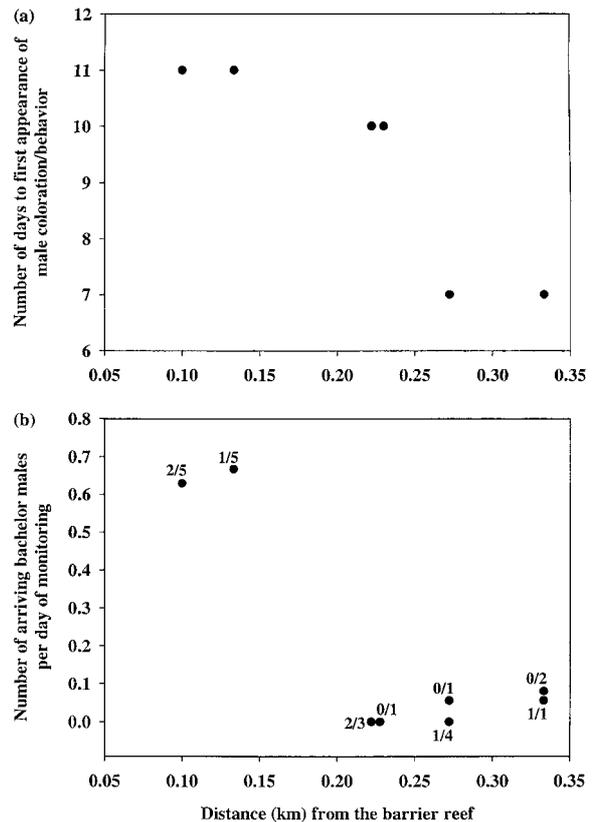

Figure 6. Spatial effect of patch reef location within Tague Bay on the dynamics of sex change in *S. radians*. (a) First appearance of transitional coloration as a function of patch reef location relative to the barrier reef. Each data point represents a female, and the sample size starts at six for reasons explained in Figure 2. (b) Bachelor male arrival rate as a function of patch reef location relative to the barrier reef. Each data point represents a patch reef, and fractions next to data points indicate the number of harems that experienced sex change (numerator) out of the total number of harems on the reef (denominator).

the arrival rate of bachelor males because more bachelor males appeared following removals on those patch reefs adjacent (within 0.1 km, see Figure 1 of Gladfelter & Gladfelter 1978) to the barrier reef than those patch reefs further away (approximately 0.33 km) from the barrier (mean ± SE number of arriving bachelor males per day of post-removal observation, adjacent patch reefs: 0.648 ± 0.019, n = 2; distant patch reefs: 0.032 ± 0.015, n = 6; t test: t = 21.8, p < 0.0001, df = 6, Figure 6b). We did not explicitly quantify the process of bachelor male harem takeover, *per se*, because attempts were made to remove all arriving bachelors (see section on 'Methods'). However, during the removal process we witnessed bachelors spawning

with remaining females. Additionally, on one reef we were unsuccessful at removing a bachelor despite repeated attempts at capturing this individual. During these attempts, this fish was consistently seen in the same location with a large aggregate harem of females. Given the two previous observations, we assume that bachelors that were allowed to remain on a territory would have acquired at least a portion of the harem of remaining females.

Finally, no transitionals were seen during censuses of 14 harems on four unmanipulated control patch reefs, suggesting that our removals were responsible for the incidences of sex change that occurred.

Discussion

Our removal experiments clearly demonstrate social control of sex change in *S. radians* and provide the first field evidence for this process in the family Scaridae. These results complement prior histological studies that documented widespread occurrence of protogynous hermaphroditism in the family (Choat & Robertson 1975, Robertson & Warner 1978, Robertson et al. 1982), but that lacked experimental manipulations to demonstrate the contexts under which sex change occurs. Perhaps the most striking result of our removals is the size of females that initiate sex change, contrasting with virtually all previous studies of sex change in fishes (e.g. reviews by Shapiro 1984, Warner 1984, Kuwamura & Nakashima 1998, but see Robertson & Warner 1978, Cole 1983, Cole & Shapiro 1995). In most cases, the largest females in a harem do not change sex when provided an opportunity. Instead, smaller females change sex and fill harem vacancies, and Muñoz & Warner (2003a,b) argue that sperm competition and female size-fecundity skew can create conditions where the largest females have higher fitness (i.e. produce more zygotes) by continuing to function as females.

In general, sex change in *S. radians* proceeds via a novel sequence of events when compared to previous studies of social control of sex change. Previous work with other protogynous hermaphrodites has demonstrated that the appearance of male aggressive and reproductive behavior in a female usually occurs quite rapidly subsequent to the disappearance of a dominant male (immediately – 1.5 h post removal), followed by conversion of female to male coloration and gonadal tissue (e.g. Robertson 1972, Moyer & Nakazono 1978, Warner & Swearer 1991). In contrast, male coloration

appears in transitional *S. radians* up to 12 days before transitionals are able to successfully defend their territories, dominate females, and attain spawns with high fertilization (i.e. fully express male behavior). This novel sequence of events could be related to the pattern of sex change in which females smaller than the largest females change sex.

When smaller females initiate sex change, they may not be immediately able to dominate all members of their harem by size alone, possibly because of insufficient levels of male gonadal hormones produced by their transitional gonads. Elevated levels of gonadal hormones (and the accompanying elevated levels of aggression) later in the course of sex change may be necessary for full control of harem members by smaller transitionals. Testosterone is known to regulate the expression of sexual and aggressive reproductive behaviors (review in Balthazart 1983), and the frequency or intensity of reproductive aggression as an effect of testosterone appears to be strongest in situations of social instability, such as the formation of dominance relationships (Wingfield et al. 1990). Stoplight parrotfish, *S. viride*, showed elevated levels of the teleost androgens testosterone and 11-ketotestoterone (11-KT) when faced with territorial intrusions, and 11-KT levels were also elevated in transitional individuals that were captured while changing sex (Cardwell & Liley 1991a,b). The lag between the appearance of male coloration and fully expressed male behavior may reflect the development of the testis as it matures sufficiently to respond to male aggression and sexual behavior of females by increasing testosterone (Wingfield et al. 1990) and 11-KT (Brantley et al. 1993). Increasing testosterone and 11-KT, in turn, will increase aggression in transitional *S. radians* and may aid their ability to dominate remaining harem members, despite the larger size of the latter. A validation of sexual state with external coloration through histological studies of transitional fishes (including *S. radians*) would improve our understanding of the effect of changes in gonad state on changes in body coloration and behavior.

By contrast in other species, when the largest female remaining in a harem initiates sex change, she should be immediately able to dominate other smaller members of her harem (since dominance is related to size, Archer 1987, Taborsky 1998) and thus can engage in aggressive dominant male behaviors without delay. This may explain why surgical gonad removal did not prevent or discernibly alter female-to-male behavioral sex change in the largest resident female *T. bifasciatum*,

a wrasse that changes sex according to the traditional sequence (Godwin et al. 1996). Dubin (1981) also noted that transitional haremic redband parrotfish, *Sparisoma aurofrenatum*, were solitary and secretive while changing sex. Sex change in relative seclusion may simply be related to the haremic social system, and the difficulty that transitional males face in claiming a harem and minimizing aggression from remaining males. Unfortunately, Dubin (1981) provided no data on the relative sizes of transitionals and females in the social groups she studied.

The pair spawns that we captured from fish on non-experimental patch reefs for comparison with spawns by transitionals highlight another difference between reef populations in St. Croix, and in this case, conspecific seagrass populations in Puerto Rico. Marconato & Shapiro (1996) studied sperm production and fertilization rates of seagrass *S. radians* and found that seagrass fish achieved higher fertilization rates with lower quantities of sperm per spawn (>80% fertilization with approximately 3 million sperm compared with 8 million sperm in St. Croix) and released lower amounts of sperm per pair spawn, on average, than reef populations in St. Croix (8.94 versus 18.8 million, respectively). The disparities between locations likely arise because *S. radians* males release increasing amounts of sperm with increasing female body size (perhaps to counteract the positive relationship between female size and the proportion of spawns streaked, Marconato & Shapiro 1996), and because dramatic size differences exist between seagrass and reef fish. Puerto Rican seagrass fish range from 23 to 88 mm TL (Marconato & Shapiro 1996) while reef populations in St. Croix range from 60 mm to greater than 300 mm TL, with some larger reef-based females 1.5–2 orders of magnitude more fecund than smaller females (Muñoz & Warner 2003b).

Although certain aspects of sex change in *S. radians* seem to differ from previous studies of social control of sex change, similarities with previous work also exist. The social dynamics that occurred following the second set of (large male only) removals mirror closely the process of harem-fission sex change known from other protogynous fishes (e.g. Shapiro 1979, Sakai 1997, and references therein). Before sex change by transitionals was completed, remaining males expanded their territories to include adjacent harems from which a male had disappeared. Later, the newly sex-changed male acquired a subgroup of this aggregate harem.

Another similarity that sex change in *S. radians* appears to share with previous studies is the emergence of alternative contexts of sex change mediated by the frequency of bachelor male arrival (Aldenhoven 1986, Sakai 1997). For example, previous work with Caribbean parrotfishes (*S. aurofrenatum*, striped parrotfish, *Scarus iserti*, and princess parrotfish, *Scarus taeniopterus*) has shown spatial variation in the frequencies of bachelors on offshore *versus* inshore reefs, and dominant male removals at a variety of locations failed to initiate sex change in females remaining in social groups (Dubin 1981, Clavijo 1982). Following removals, females either remained in their territories while neighboring or bachelor males took control (often within 24 h), or females abandoned their territories. These authors concluded that sex change in these species differs from other protogynous hermaphrodites (Robertson 1972, Shapiro 1980, Moyer & Nakazono 1978, Sakai 1997) in that it takes place outside the social unit (the harem territory). Females were believed to leave the harem and reef in response to unknown cues and change sex to become bachelors in the hopes of eventually acquiring a territory. Our removal experiments provide an alternative explanation.

We found a decreasing trend in the number of days following male removal before the first appearance of a transitional fish as distance from the barrier reef increased. Transitionals tend to appear faster on those patch reefs further away from the barrier reef, where the frequency of bachelor arrival is lower. On two experimental patch reefs (15 and 13) located approximately 0.1 km from the extensive barrier reef that bounds Tague Bay, it was necessary to repeatedly remove roving bachelor males that wandered in and attempted to take over the harems. The barrier reef may provide abundant habitat for an apparently steady supply of bachelor males. If we had not repeatedly removed bachelors arriving to reefs 13 and 15, sex change on these patch reefs probably would not have occurred. This suggests that environmental heterogeneity in the arrival rates of bachelors across Tague Bay may produce local conditions that facilitate alternative contexts of sex change and harem takeover, as are known from *Centropyge* angelfishes (Aldenhoven 1986, Sakai 1997). On some reefs in Tague Bay, females left without dominant males may defer sex change to avoid the risk of initiating sex change only to have bachelor males arrive and take control of the harem before sex change is completed. We do not know the actual context that stimulates the initiation of sex change to the bachelor status. However, under natural conditions roving bachelors may quickly fill harem vacancies as they arise near the barrier reef (as in the studies by Dubin [1981]

and Clavijo [1982]), whereas sex-changed males may recruit from within harems to fill vacancies that arise on more isolated reefs further away from the barrier reef.

We have presented the first demonstration of social control of sex change in the family Scaridae and have provided a detailed description of the physical and behavioral changes accompanying the process in *S. radians*. Sex change following the disappearance of a dominant male proceeds via a novel sequence of events. Rather than behavioral sex change preceding morphological sex change, in *S. radians* morphological change occurs well in advance of fully developed male behavior. We have also shown how differing arrival rates of bachelor males at our two study sites may facilitate alternative contexts of sex change, with sex change occurring within social groups in some locations and with bachelor males filling harem vacancies in other locations. Alternative contexts of sex change may be a common feature in the parrotfishes, a group well known for their phenotypically plastic social and mating systems (Thresher 1984, Shapiro 1991, Warner 1991, Muñoz & Motta 2000).

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