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ORIGINAL PAPER

Sex change as a survival strategy

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Abstract

Sequential hermaphroditism (sex change) is understood to be a strategy that maximizes lifetime reproduction in systems where one sex confers highest fitness early in life, and the other later in life. This strategy is evolutionarily stable despite costs to growth, survival, or current reproduction. Few studies have examined advantages of sex change outside of reproduction. The mangrove rivulus fish, *Kryptolebias marmoratus,* presents a unique system in which to study non-reproductive consequences of sex change because reproductive opportunity decreases significantly with sex change. In natural conditions, individuals develop as self-fertilizing simultaneous hermaphrodites. Some individuals change sex to male at various points after sexual maturity, even in isolation, essentially foregoing future reproductive assurance. In a large-scale experiment that examined fitness differences among individuals exposed to ecologically relevant environmental challenges, we found that individuals that change sex from hermaphrodite to male had overwhelmingly greater chances of survival compared to those that remained hermaphrodite. Furthermore, hermaphrodites derived from lineages with higher propensities to change sex experienced greater survival advantages by changing sex. Our results indicate that sex change may be a survival strategy, one with genotype-dependent consequences.

Keywords Sex change · Self-fertilization · Hermaphrodite · Sex allocation · Mangrove rivulus · *Kryptolebias marmoratus*

Introduction

The phenomena of hermaphroditism and sex change have been central to advancing our understanding of the fitness payoffs associated with different life history and sex allocation strategies. Sex allocation theory has generated numerous predictions regarding how

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simultaneous hermaphrodites should invest in male versus female function and the factors that might drive a switch in allocation strategies or, in sequential hermaphrodites, complete sex change (Charnov 1982). The core of sex allocation theory, and most models and experiments developed afterward, focus on the costs and benefits of simultaneous and sequential hermaphroditism with regards to reproductive success exclusively. Theories explaining the evolution and maintenance of sequential hermaphroditism (sex change) specifically seek to describe how and when individuals might experience higher reproductive success as the new sex (increasing their reproductive value, RV), thus justifying allocation of immediate resources away from growth, survival and/or current reproduction (Fisher 1930; Ghiselin 1969; Warner et al. 1975; Leigh et al. 1976; Charnov 1982; Warner 1988a).

Reproductive value models seek to understand how and when sex change maximizes lifetime reproductive fitness (Warner 1988a; Iwasa 1991; Allsop and West 2003). These models specifically include the reproductive success of an individual's past and present, and their future reproductive potential within the context of different life history traits as the new sex. An individual's reproductive value has often been investigated using size advantage models of sex change as the foundation; in these models, sex change exploits sex differences in size and growth rate to maximize reproductive opportunity (Warner 1988b; Munday et al. 2006a). However, variation in reproductive value can be independent of size and may rely on recruitment success (Breitburg 1986; Munday et al. 2006b) or sex differences in mortality rate (Charnov 1986; Iwasa 1991; Brooks and Iwasa 2010). For example, both models (Breitburg 1986) and experimental data (Munday et al. 2006b) indicate that recruitment and population size can affect the reproductive value of each sex in sequential hermaphrodites. In bluehead wrasse, *Thalassoma bifasciatum*, the reproductive value of primary males (those that do not develop as female first) is higher in large populations than in small populations (Munday et al. 2006b). In the sequential hermaphroditic Caribbean parrotfish, *Sparisoma radians,* territorial males suffer greater mortality than females, a difference that is exacerbated with increased body size. For a large female, this reduces the reproductive value of changing sex to male even when there is a large harem available for reproducing as the male (Clifton and Rogers 2008).

Regardless of an individual's initial sex and the potential gains associated with sex change, there are myriad costs: energetic costs of restructuring gonads (Charnov 1982), potential loss of reproductive output during and immediately after sex change (Hoffman et al. 1985; Iwasa 1991; Kazancıoğlu and Alonzo 2009; Yamaguchi 2016), misreading external cues about when it is beneficial to change sex (Hoffman et al. 1985), and mortality differences and/or growth differences that are sex-specific and offset immediate increases in reproductive fitness (Charnov 1986). These costs are not ubiquitous and may vary among individuals and/or depend on the environment but are essential to understanding the evolution and maintenance of sex change (Munday and Molony 2002). These costs are compared to the benefits of maximizing *lifetime* reproductive value, typically in species with traditional mating systems where outcrossing occurs between two separate individuals (Warner et al. 1975; Freeman et al. 1980; Aldenhoven 1986; Charnov 1987; Charnov and Anderson 1989; Munday et al. 2006a). Rarely, however, have the economics of sex change been considered in animals where the switch occurs between self-fertilizing hermaphrodites and individuals that invest only in one sex or the other, which may present a unique set of costs (Bauer 1987). For instance, sex change from self-fertilizing hermaphrodites to females, and the reverse, has been observed in populations of the pearl mussel, *Margaritifera margariteifera*, which also contain males (Bauer 1987). In this species, density-dependent sex change might maximize fitness by assuring reproduction (via selfing) at low densities when males are unlikely to be encountered frequently but eliminating the costs of maintaining male function at high densities. Currently, there are no experiments or models that attempt to understand how changing sex to *male* provides a fitness advantage superior to that of being a self-fertilizing hermaphrodite.

Another important issue is that the reproductive advantage of sex change might vary among individuals, based on characteristics they possess or environments they experience (Munday et al. 2006a). While the focus has been on identifying particular life history stages (or sizes) at which sex change would maximize lifetime reproductive success (Allsop and West 2003), there is accumulating evidence for other factors that govern these switches, e.g., social cues (Rodgers et al. 2007; Todd et al. 2019). In populations of the protandrous gastropod, *Crepidula fornicata,* size at sex change varies and is dependent on individual size, social conditions, and sex ratio (Hoch and Cahill 2012). Despite the importance of among-individual variation in the realized fitness benefit of sex change, and thus evolutionary responses to selection, there are still few empirical studies that consider this variation.

Self-fertilization (i.e., selfing) is a common strategy in plants, not uncommon in invertebrates, and found in one genus of teleost fish (*Kryptolebias*). The mangrove rivulus fish (*Kryptolebias marmoratus*; hereafter ‘rivulus’) is a selfing hermaphrodite that can change sex to male via reabsorption of ovarian tissue, but cannot switch back to hermaphrodite (Harrington Jr 1961, 1971). A study designed to identify differences in fitness between outbred and inbred lineages was performed in this emerging model for integrative biology (Earley et al. 2012). Here, we report data that emerged from that study, which illustrates the possibility that sex change from a selfing hermaphrodite to male might provide a distinct survival advantage, even when future reproductive potential decreases significantly. Furthermore, we use longitudinal data on rivulus sex change under common laboratory condition to explore whether: (i) variation in the propensity to change sex exists among hundreds of genetically distinct lineages (Turner et al. 2006), and (ii) whether this variation in sex change propensity mediates the survival advantages of sex change.

Methods

Study organism

Rivulus inhabit mangrove forests in Florida, the Bahamas, and Central America (Davis 1990; Tatarenkov et al. 2017), with salinities varying from 0 to 60 parts per thousand (‰) (Taylor 2012). Populations consist of selfing hermaphrodites and males (androdioecy) (Harrington Jr and Rivas 1958). There is histological evidence that ovarian tissue matures first, leading some to suggest that pure females exist in rivulus populations (Cole and Noakes 1997) but there is no evidence that these individuals lay eggs before the testicular tissue develops. Thus, while the existence of pure females remains a possibility, it is more likely that young ‘females’ are hermaphrodites that have yet to develop spermatogenic tissue. Hermaphrodites and males are easily distinguishable; hermaphrodites’ color ranges from gray to dark brown with brown freckles, while males possess orange color on the body, often with orange freckles (Scarsella et al. 2018; Marson et al. 2019). Males result, predominantly, from adult hermaphrodites undergoing sex change, which can be induced by exposure to high temperatures (> 28 °C) coupled with changes in photoperiod (Harrington Jr 1971, 1975) but also occurs under common laboratory conditions (see Results). Sex change occurs at various ages after sexual maturity (personal observations). Primary males, those that develop directly as male with no sex change required, have also been induced in the lab by incubating eggs at low temperatures (Harrington Jr 1967; Ellison et al. 2015) but there is no evidence primary males occur in natural populations. Outcrossing between hermaphrodites seems unlikely; fertilization occurs internally (Sakakura et al. 2006) and attempts to mate pairs of hermaphrodites have been unsuccessful (Furness et al. 2015). Observed population-level heterozygosity must therefore be due to males fertilizing the few unfertilized eggs deposited by hermaphrodites (Harrington Jr 1963, 1971; Mackiewicz et al. 2006).

Rivulus likely disperse passively by rafting on debris (Taylor et al. 2008), which increases the probability that individuals might find themselves alone. Compared to other sex-changing vertebrates, none of which self, such dispersal adds additional costs to sex change in rivulus. Selfing hermaphrodites have 100% reproductive assurance, while sex change should reduce this value to significantly less than 50%, even in established populations. Twin Cays, Belize has exhibited the highest levels of observed heterozygosity by far (0.472–0.502 compared to 0–0.170 in most other populations), which is still significantly less than the expected heterozygosity of a randomly mating population (0.660–0.688, Tatarenkov et al. 2007). This indicates that males in established populations are not likely to experience significant reproductive opportunity. Attempts to mate hermaphrodites and males in the laboratory have had minimal success, primarily because hermaphrodites infrequently lay unfertilized eggs (Mackiewicz et al. 2006; Lomax et al. 2017). Given that sex change occurs when animals are isolated under controlled laboratory conditions and at varying sizes and ages, it does not appear to require social cues or occur at specific life history 'switch points'. For additional details about rivulus, refer to the Supplementary Material.

Genetic variation for sex change

Each month, from May 2012 to January 2019, the sex of every fish in the colony ($N = 29{,}282$ from 359 genetically distinct lineages), kept under common, controlled laboratory conditions (26.08 ± 0.003 °C ambient temperature, 25‰ artificial saltwater prepared with Instant Ocean® sea salt, and 12 h light:12 h dark photoperiod) was checked using external characteristics, primarily color (see above, and Scarsella et al. 2018). While alternative male phenotypes have been discovered in the field (Marson et al. 2019) we did not see evidence of this phenotype in our colony. Sex change propensity for each lineage was calculated as the proportion of hermaphrodites that changed sex to male.

Experimental background

An experiment was designed to test whether variation in fitness existed among individuals with different levels of heterozygosity (measured using 32 microsatellites, Mackiewicz et al. 2006); the overarching goal was to investigate inbreeding or outbreeding depression. From the 359 lineages described above, sixty-four lineages originating from the Florida Keys and Belizean Cays were chosen to maximize variation in heterozygosity in this experiment. We also attempted to include an equal number of lineages chosen from each region. While the results of the primary experiment will be reported elsewhere, we observed considerable differences in survival between males and hermaphrodites, which led to the emergent questions that we address in this study.

A subset of individuals from all lineages experienced each of four treatment conditions (see Supplementary Material, Figure S1). At least four individuals from each lineage experienced each treatment. Three treatments had constant water availability with 1) 25‰

salinity (control); 2) low salinity (10‰); 3) high salinity (40‰); the final, tidal treatment had 4) 25‰ salinity with water reduced from 350 to 10 mL for 5.5 h twice daily. 25‰ water was the control because this is the average salinity in which most wild fish are caught (average from 274 field sites: 26 ± 0.44‰, unpublished data, also see Taylor 2012) and in which all fish are maintained in the colony. All experimental individuals were collected as eggs from progenitors that were one or two generations removed from the wild. Details on egg collection, hatchling rearing conditions, and all pre-experimental procedures can be found in the Supplementary Material. At 90 days post-hatch (dph), the median time to sexual maturity among our lineages, fish were transferred to treatment tanks with a large marble-sized ball of Poly-fil provided as egg-laying substrate, which was checked for eggs once every 7 days. Details about tank construction can be found in the Supplementary Material.

Each individual was checked for male external characteristics every 30 dph, including and after 90 dph. Also, the date of death, if prior to 180 dph, was recorded. Individuals, whether hermaphrodite or male, that survived until the end of the treatment period (180 dph) were euthanized by immersion in 4 °C water, measured for mass and length, and dissected ($N = 675$). The gonad was identified as either an ovotestis (hermaphrodite) or testis (male) following Scarsella et al. (2018). Using the criteria also described in Scarcella et al. (2018), external morphological characters accurately predicted the gonadal sex of the individuals. The University of Alabama Institutional Animal Care and Use Committee approved all procedures (IACUC Protocol # 15-04-0102) in accordance with the Animal Care Guidelines from the American Society of Herpetologists and Ichthyologists (https://asih.org/sites/default/files/2018-05/guidelines_herps_research_2004.pdf) and the Guide for the Care and Use of Laboratory Animals (2010).

Statistical analysis

The original experiment resulted in 866 animals (from 64 lineages) surviving until entering treatment at 90 dph. To determine if the incidence of sex change (Y/N) was associated with treatment, lineage, hatch season, and/or hatch month (nested within season), all fixed effects, a generalized linear model (GzLM) with a binomial distribution, logit link function, and Firth adjusted maximum likelihood estimates (i.e., accounting for biases associated with sex change being rare in some lineages and common in others) was conducted. Statistical significance was assessed with a likelihood estimated Chi square and associated p-values. Months were binned into four seasons as follows–winter: December-February; spring: March–May; summer: June–August; autumn: September–November.

To determine if variation in survival (Y/N) was associated with treatment, sex, and the propensity of each individual's lineage to change sex (% male in colony), a GzLM with a binomial distribution and logit link function was conducted. Differences among levels of the treatment × sex interaction were assessed using a priori contrasts (with χ^2 reported), with adjusted (α_{adj}) using sequential Dunn-Šidák adjustments to account for compounding Type I error; α_{adj} is reported when adjustments modified statistical significance. 'Tank' was initially included as a random effect in the above analyses but there were no differences in survival or sex change among tanks within a treatment ($\chi^2 \leq 0.92$, $P = 0.34$, using *lme4*, *car*, and *emmeans* packages in R Studio Fox and Weisberg 2011; Bates et al. 2015; RStudioTeam 2016; R Core Team 2018; Lenth 2019). All remaining models were constructed in JMP® (JMP®, Version 14 Pro 2019).

Table 1 Summary of lineages derived from four major geographical regions across the range of *Kryptolebias marmoratus*

	No. lineages	No. animals/ lineage-range	No. animals/ lineage–mean (median)	% Male-range	% Male-mean (median)
Belize	87	8–1467	88 (43)	0–87.5	30.5 (30.8%)
Florida Keys	133	6–399	68 (50)	0–66.7	17.4 (15.4%)
Central/South Florida	113	6–1436	83 (45)	0–83.3	13.5 (10.4%)
Bahamas	26	7–1488	123 (35)	0–57.1	11.4 (7.1%)

The number of genetically distinct lineages per region is shown in addition to the minimum and maximum (range), average, and median number of animals per lineage used to calculate sex change propensity (percent [%] male). The minimum and maximum (range), average, and median % males produced, under common garden conditions, from adult hermaphrodite sex change is shown and summarizes the data visualized in Fig. 1

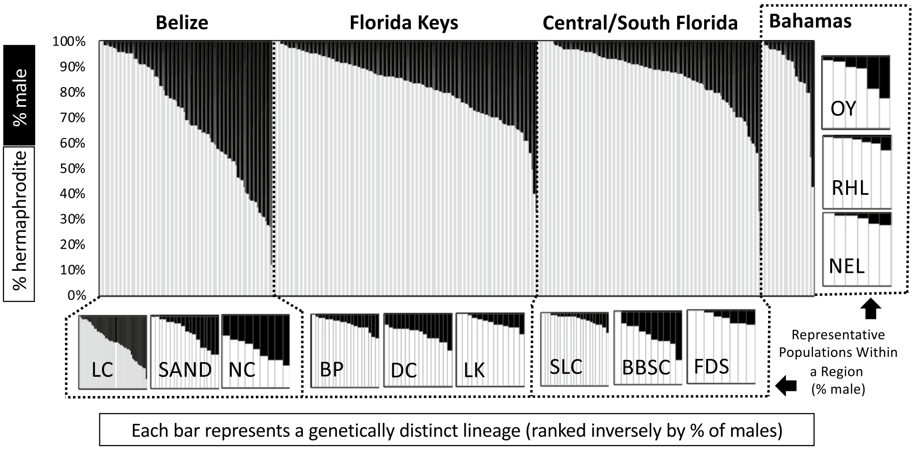

Fig. 1 Extensive variation in the propensity to sex change (percent [%] secondary males produced via sex change under common laboratory conditions) among 359 genetically distinct *K. marmoratus* lineages (29,282 individuals) derived from the four major geographical regions across the species' range (Belize, Florida Keys, Central/South peninsular Florida, and Bahamas). Each of the 359 columns is split into a white portion (% hermaphrodites) and a black portion (% males) that sum to 100%. The smaller insets show representative populations within each region; *LC* Long Caye, *SAND* Sandbore Caye, *NC* Northern Caye, *BP* Big Pine Key, *DC* Dove Creek, Tavernier, *LK* Long Key, *SLC* St. Lucie County, *BBSC* Bunche Beach, San Carlos Bay Preserve, *FDS* Fort de Soto Preserve, *NEL* North Eleuthera Island, *RHL* Reckley Hill Lake, San Salvador, *OY* Oyster Pond, San Salvador. See Table 1 for summary statistics

Results

Over 6 + years of quantifying sex change in laboratory animals, we documented extensive variation in the propensity to change sex among genetically distinct lineages (assessed via 32 microsatellite loci; Mackiewicz et al. 2006) representing the entirety of the species' geographical range (Table 1, Fig. 1). Furthermore, in the current study, there was a significant overall effect of experimental treatment on the probability of sex change (Table 2). Sex

Table 2 Summary of the generalized linear model for sex change from hermaphrodite to male

Model Effects	χ^2	df	P value
Treatment	12.3	3	**0.0063**
Lineage	159.6	63	**< 0.0001**
Season	23.2	3	**< 0.0001**
Month [season]	6.87	8	0.5507

Region refers to the geographic origin of the lineage (i.e., Belize, Florida Keys, Central/South Florida, Bahamas). Season and month refer to the hatch date. Statistically significant effects ($P < 0.05$) are shown in bold. Whole model test data and the parameter estimates can be found in the appendix, Table S1

change occurred in 25.9%, 23.3%, 21.3%, and 13.9% of animals in low salinity, control, tidal, and high salinity treatments, respectively. Animals in high salinity had a significantly lower probability of changing sex than low salinity animals ($\chi^2 = 12.0$, df = 1, $P = 0.0005$), and a marginally lower probability of changing sex than controls ($\chi^2 = 5.4$, df = 1, $P = 0.020$, $\alpha_{adj} = 0.01$); no other treatment comparisons were significant ($\chi^2 < 3.0$, df = 1, $P > 0.09$). Lineages also varied significantly in their propensity to change sex ($\chi^2 = 159.6$, df = 63, $P < 0.0001$), which supports our findings in the larger colony (Fig. 1).

Sex was a highly significant determinant of survival across most treatments. Animals that transitioned to male were significantly more likely to survive than animals that remained hermaphrodite in high salinity ($\chi^2 = 15.7$, df = 1, $P < 0.0001$) and tidal ($\chi^2 = 9.7$, df = 1, $P = 0.0019$) treatments (Table 3, Fig. 2a). The same trend was observed in low salinity but was not significant ($\chi^2 = 2.9$, df = 1, $P = 0.09$), while there were no sex differences in survival in controls ($\chi^2 = 1.3$, df = 1, $P = 0.26$). Animals that remained hermaphrodite varied in their survival by treatment (Fig. 2a), which explains the important overall effect of treatment on probability of survival (Table 3). Hermaphrodites in high salinity were significantly less likely to survive than hermaphrodites in control ($\chi^2 = 62.6$, df = 1, $P < 0.0001$), low salinity ($\chi^2 = 57.2$, df = 1, $P < 0.0001$), and tidal ($\chi^2 = 50.3$, df = 1, $P < 0.0001$) treatments. Hermaphrodites were also less likely to survive in the tidal treatment compared to control ($\chi^2 = 4.3$, df = 1, $P = 0.039$; $\alpha_{adj} = 0.017$), an effect rendered not significant after Dunn-Šidák adjustments. There was not a

Table 3 Summary of the generalized linear model for survival to 180 dph, with the addition of lineage sex change propensity (and its interactions with the main effects of sex and treatment) as a fixed effect

Model effects	χ^2	df	P-value
Treatment	6.79	3	0.0787
Sex	36.2	1	**< 0.0001**
Treatment × sex	3.75	3	0.2892
Sex change propensity (% male in colony)	1.88	1	0.1707
Sex change propensity (% male in colony) × treatment	2.62	3	0.4542
Sex change propensity (% male in colony) × sex	8.36	1	**0.0038**
Sex change propensity (% male in colony) × treatment x sex	5.03	2	0.1695

Statistically significant effects ($P < 0.05$) are shown in bold. Whole model test data and the parameter estimates can be found in the appendix, Table S1

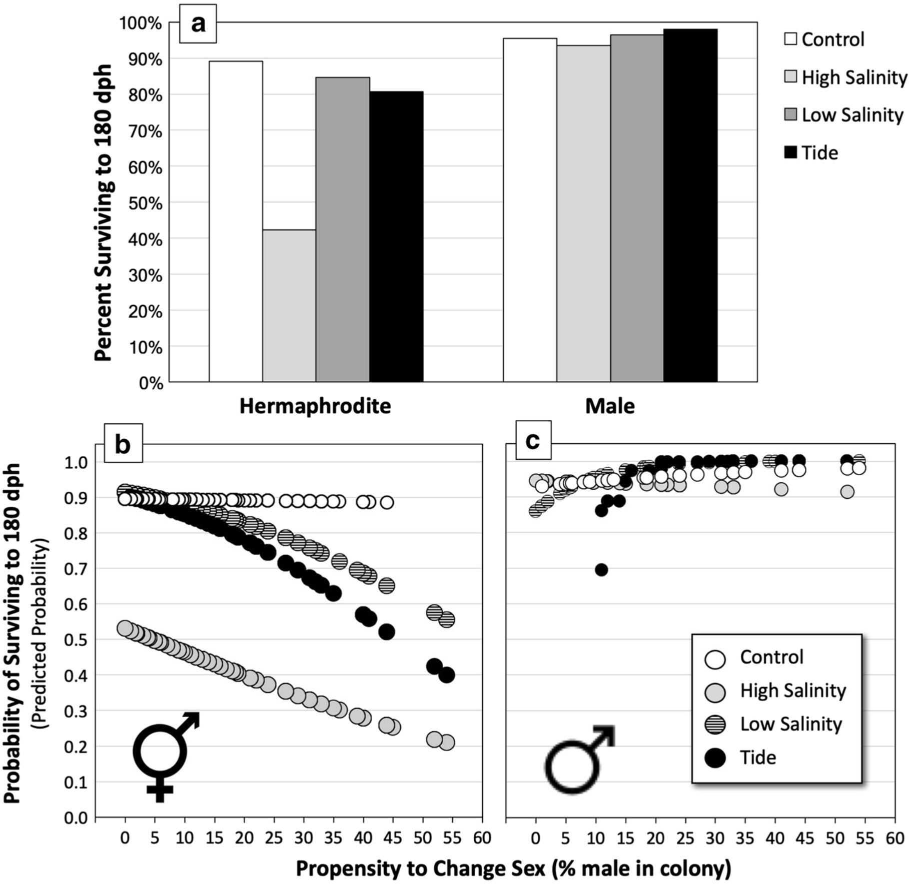

Fig. 2 **a** Sex differences and treatment differences in the probability of surviving to the end of the experiment (180 dph); and probability of survival as a function of the lineage's sex change propensity (% males in the colony) for both **b** hermaphrodites and **c** males. Probabilities calculated from model in Table 3

difference in hermaphrodite survival between low salinity treatment and either control ($\chi^2 = 1.3$, df = 1, $P = 0.26$) or tidal ($\chi^2 = 0.9$, df = 1, $P = 0.35$) conditions. However, in the control treatment, all individuals from lineages that produce males at a frequency greater than 45% changed sex (indicated by missing data points for hermaphrodites derived from those lineages in the control; Fig. 2b). Male survival was not treatment-dependent ($\chi^2 < 0.98$, df = 1, $P > 0.32$; Fig. 2a).

Differences in survival between the sexes depended on the lineage's propensity to change sex (lineage propensity x sex; Table 3, Figs. 2b, c). Hermaphrodites from high-male-producing lineages that did not change sex experienced significantly lower survival than hermaphrodites from low-male-producing lineages that did not change sex; this pattern was particularly pronounced in tidal and salinity treatments (Fig. 2b). Animals that changed sex to male were essentially assured survival, regardless of their lineage's propensity to change (Fig. 2c).

For details on how hatching season affected probabilities of sex change, refer to Table 2 and the Supplementary Materials.

Discussion

Historically, theoretical models and experiments focused on sex change have sought to identify how and when sex change might be beneficial with respect to maximizing current and near-future reproductive success (Ghiselin 1969; Warner 1975; Leigh et al. 1976; Charnov 1982; Allsop and West 2003). More recent models and empirical studies, have included sex differences in mortality as well as the effects of the environment and population changes to calculate a more comprehensive reproductive value (Breitburg 1986; Charnov 1986; Charnov and Anderson 1989; Iwasa 1991; Munday et al. 2006a; Brooks and Iwasa 2010). However, sex change in self-fertilizing hermaphrodites has garnered little attention. Here, we demonstrate that sex change can maximize survival, despite being associated with greatly diminished lifetime reproductive opportunity. According to current theories on sex allocation (Maynard Smith 1978; Charnov 1982, 1987), changing sex from a self-fertilizing hermaphrodite to an obligate outcrossing male should be a very unsuccessful strategy without important concessions, such as an equivalent number of unfertilized eggs available for outcrossing as there are self-fertilized eggs, or a significant survival advantage. This is exactly what appears to happen in rivulus, one of only two vertebrate species that utilizes self-fertilization as its primary reproductive strategy. Since its rediscovery and description in Harrington Jr and Rivas (1958), very little progress has been made towards understanding why sex change occurs in this predominantly selfing species, and whether it might represent an evolutionarily stable strategy. As there are no obvious signs of inbreeding or outbreeding depression (Gresham et al. unpublished data), sex change for facultative outcrossing appears to be unlikely, but experiments have tested and continue to test this hypothesis (Ellison et al. 2011, 2012, 2013; Molloy et al. 2011). The other hypothesis is that changing sex comes with such a significant survival advantage that it trumps the loss of reproductive assurance. In this study, animals that changed sex to male were significantly more likely to survive the treatment period of 180 days across all conditions, but especially in the most challenging of conditions, the high salinity treatment. Male survival did not vary among treatments; almost every single male survived (96%). Hermaphrodite survival did, however, vary with treatment. Individuals that remained hermaphrodite showed significantly lower survival in the high salinity treatment than all other treatments, and those in the tidal treatment showed significantly lower survival compared to controls. It is important to note that we also found significantly fewer males in the high salinity treatment, suggesting that 40‰ might be so stressful that animals die before they have the chance to change sex.

There is an alternative hypothesis to the sex-dependent survival differences that we observed. It is possible that only those individuals that are more viable can change sex; they are fit enough to live *and* change sex. Those individuals that are less viable either die or cannot expend the resources required to successfully change sex. To determine whether only more viable individuals can change sex, we ran a post hoc analysis that tested whether the incidence of sex change was influenced by body condition at 90 dph, treatment, and the interaction. Here, body condition at 90 dph is being used as a proxy for pre-treatment viability. In this model, there was only a treatment effect, as demonstrated in primary results (Table 2). Neither body condition ($\chi^2 = 0.6$, df = 1, $P = 0.44$) nor the interaction of body condition and treatment ($\chi^2 = 4.9$, df = 3, $P = 0.18$) were significant predictors of sex change.

Hermaphrodite survival was also dependent on the lineage's propensity to change sex. Previous reports indicating the potential for genotypic variation in the frequency of sex change (Harrington Jr 1971; Turner et al. 2006) led us to measure how many fish of each lineage changed sex under common laboratory conditions, and we have been recording these data for ~ 7 years. There is incredible variation among lineages in their propensity to change sex, from many lineages that have never produced males to lineages that produce up to 87.5% males (Fig. 1). Our data suggest that lineage-specific propensities to change sex are an important determinant of survival for hermaphrodites; individuals derived from lineages that change sex at higher rates and that remained hermaphrodite experienced decreased survival regardless of treatment. However, it is not the case that all individuals of a single lineage will change sex to male or change sex at the same time, suggesting potentially strong epigenetic influences on the 'decision' to change sex. Somewhat paradoxically, this within-lineage variation remains, even under circumstances where some individuals will likely perish if they remain hermaphrodite (e.g., in high salinity environments). We are also not sure which environmental cues trigger sex change. Harrington Jr (1971) reported increases in sex change in response to shortened days; responses were also lineage- and age-dependent. Although individuals change sex under a 12 h light: 12 h dark photoperiod in the laboratory colony, variation among lineages in the propensity to change sex hints at lineage- and age-specific sensitivities to whatever cues the fish are using. We hope that future work will focus on both among- and within-genotype variation in the frequency of sex change, which will have important implications for understanding the evolution of sexually labile reproductive strategies.

For transitions from selfing hermaphrodite to male to be evolutionarily stable, the survival advantage of sex change that we report must be coupled with opportunities for reproduction. Males must have opportunities to pass on their sex-changing alleles, which requires that at least two things occur in wild populations. First, hermaphrodites in the population must lay unfertilized eggs. Second, males must find these unfertilized eggs, and fertilize them externally (hermaphrodite selfing occurs internally). Sex-changing alleles can also be passed on to the next generation if individuals produce offspring through selfing prior to sex change. An emergent hypothesis could then be that individuals 'destined' to change sex would have significantly higher reproductive output prior to the switch. In the Supplementary Material, we detail initial evidence indicating that this is not the case for rivulus; eventual sex-changers have lower odds of laying eggs and, for those that laid eggs prior to the switch, similar rates of egg-laying compared to individuals that remained hermaphrodite. Thus, reproduction does not necessarily happen before sex change, and certainly not at a rate that is likely to balance the costs of losing reproductive assurance altogether upon changing sex to male. An alternative hypothesis is that most sex change occurs after reproductive senescence; egg production has ceased but there is still opportunity to outcross as male. Currently, there is conflicting laboratory evidence for this hypothesis. Harrington Jr (1971) observed that one lineage would change sex when older if exposed to low temperatures, while a different lineage would change sex when older if exposed to high temperatures. Mackiewicz et al. (2006) used "nearly senescent" hermaphrodites in their attempt at outcrossing, but 94% of the individuals still laid fertilized eggs. These studies reaffirm the vast variation among lineages with respect to both their propensities to change sex and their reproductive longevity. To estimate the real cost of sex change in this species, further empirical studies are needed to determine the adaptive value of sex change under different environmental conditions and whether lineages with a higher propensity to change sex are more or less fecund in early life.

Harrington Jr (1963) first described the frequency of fertilized and unfertilized egg production by some hermaphrodites. Unfertilized eggs provide opportunities for males to reproduce, assuming the males can find these eggs. We do not yet know if some hermaphrodites lay more unfertilized eggs than others. We also do not know how males find unfertilized eggs. We do know that hermaphrodites collected from Twin Cays preferred to associate in a simulated crab burrow with a male, rather than alone or with another hermaphrodite (Martin 2007). Still, hermaphrodites do not lay an abundance of unfertilized eggs in the presence of males, though there is evidence that males attempt to initiate courting behaviors in the presence of a hermaphrodite (Luke and Bechler 2010). These behaviors are similar to behaviors observed in other rivulids like the elaborate courtship behaviors of *Austrolebias reicherti* (García et al. 2008). Mackiewicz et al. (2006) attempted to mate males with hermaphrodites and produced only two outcrossed offspring out of thirty-two (i.e., 30 were produced via selfing). Nakamura et al. (2008) also attempted in vitro outcrossing with similarly low success; all but one egg surgically removed from hermaphrodites was already fertilized, suggesting that self-fertilization happens very soon after egg maturation. When and which hermaphrodites lay unfertilized eggs, and how males find unfertilized eggs are important avenues for future research to help us understand how sex change (and the male sexual phenotype) is maintained by natural selection.

Our data strongly suggest that, at least in some species, sex change may not evolve solely for reproductive fitness gain, but also as a survival strategy in the face of harsh environmental conditions, such as high salinity and low water availability. Our data also support the growing consensus that, in contrast to the size-advantage and dimensionless invariant models (Warner 1988b; Charnov and Anderson 1989; Charnov and Skúladóttir 2000; Allsop and West 2003; Buston et al. 2004), there is not an absolute value that determines when an individual should change sex or that determines associated fitness gains. There may be significant variation within the species/population with respect to when and whether sex change occurs, and this variation might be evolutionarily stable. Our colony data tentatively support the idea that considerable variation in the propensity to change sex is maintained among genotypes within each population (insets, Fig. 1). The questions of 'how' and 'why' remain open and deserve a focused empirical effort. Mechanisms underlying such variation likely involve genetic effects and epigenetic effects (i.e., individuals derived from the same homozygous lineage might have slightly different embryonic/early life experiences that predispose them more or less to sex change). Rivulus is an incredibly unique organism whose sexual habits remains mysterious, especially when it comes to the evolution and maintenance of sex change. This study forwards alternative explanations for sex change, those that are not directly related to increasing reproductive fitness.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Aldenhoven JM (1986) Different reproductive strategies in a sex-changing coral reef fish *Centropyge bicolor* (Pomacanthidae). Mar Freshw Res 37:353–360

Allsop DJ, West SA (2003) Life history: changing sex at the same relative body size. Nature 425:783–784

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Bauer G (1987) Reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*. J Anim Ecol 56:691–704

Breitburg DL (1986) Effect of variability in recruitment on selection for protogynous sex change. Am Nat 128:551–560

Brooks M, Iwasa Y (2010) Size-dependent sex change can be the ESS without any size advantage of reproduction when mortality is size-dependent. Theor Popul Biol 78:183–191

Buston PM, Munday PL, Warner RR (2004) Evolutionary biology—sex change and relative body size in animals. Nature 428:U1

Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton

Charnov EL (1986) Size advantage may not always favor sex change. J Theor Biol 119:283–285

Charnov EL (1987) On sex allocation and selfing in higher plants. Evol Ecol 1:30–36

Charnov EL, Anderson PJ (1989) Sex change and population fluctuations in pandalid shrimp. Am Nat 134:824–827

Charnov EL, Skúladóttir U (2000) Dimensionless invariants for the optimal size (age) of sex change. Evol Ecol Res 2:1067–1071

Clifton KE, Rogers L (2008) Sex-specific mortality explains non-sex-change by large female *Sparisoma radians*. Anim Behav 75:1–10

Cole KS, Noakes DLG (1997) Gonadal development and sexual allocation in mangrove killifish, *Rivulus marmoratus* (Pisces: Atherinomorpha). Copeia 1997:596–600

Davis WP (1990) Field observations of the ecology and habits of mangrove rivulus. Ichthyol Explor Freshw 1:123–134

Earley RL, Hanninen AF, Fuller A, Garcia MJ, Lee EA (2012) Phenotypic plasticity and integration in the mangrove rivulus (*Kryptolebias marmoratus*): a prospectus. Integr Comp Biol 52:814–827

Ellison A, Cable J, Consuegra S (2011) Best of both worlds? association between outcrossing and parasite loads in a selfing fish. Evolution 65:3021–3026

Ellison A, Wright P, Taylor DS, Cooper C, Regan K, Currie S, Consuegra S (2012) Environmental diel variation, parasite loads, and local population structuring of a mixed-mating mangrove fish. Ecol Evolut 2:1682–1695

Ellison A, De Leaniz CG, Consuegra S (2013) Inbred and furious: negative association between aggression and genetic diversity in highly inbred fish. Mol Ecol 22:2292–2300

Ellison A, Rodrı CM, Moran P, Breen J, Swain M, Megias M, Hegarty M et al (2015) Epigenetic regulation of sex ratios may explain natural variation in self-fertilization rates. Proc R Soc B 282:20151900

Fisher RF (1930) The genetical theory of natural selection. Oxford University Press, Oxford

Fox J, Weisberg S (2011) An R companion to applied regression. Sage, Thousand Oaks

Freeman DC, Harper KT, Charnov EL (1980) Sex change in plants: old and new observations and new hypotheses. Oecologia 47:222–232

Furness AI, Tatarenkov A, Avise JC (2015) A genetic test for whether pairs of hermaphrodites can cross-fertilize in a selfing killifish. J Hered 106:749–752

García D, Loureiro M, Tassino B (2008) Reproductive behavior in the annual fish *Austrolebias reicherti* Loureiro and Garcia 2004 (Cyprinodontiformes: Rivulidae. Neotropical Ichthyology 6:243–248

Ghiselin MT (1969) The evolution of hermaphroditism among animals. Q Rev Biol 44:189–208

Guide for the care and use of laboratory animals (2010) National Academies Press

Harrington RW Jr (1961) Oviparous hermaphroditic fish with internal self-fertilization. Science 134:1749–1750

Harrington RW Jr (1963) Twenty-four-hour rhythms of internal self-fertilization and of oviposition by hermaphrodites of *Rivulus marmoratus*. Physiol Zool 36:325–341

Harrington RW Jr (1967) Environmentally controlled induction of primary male gonochorists from eggs of the self- fertilizing hermaphroditic fish, *Rivulus marmoratus* Poey. Biol Bull 132:174–199

Harrington RW Jr (1971) How ecological and genetic factors interact to determine when self-fertlizing hermaphrodites of *Rivulus marmoratus* change into functional secondary males, with a reappraisal of the modes of intersexuality among fishes. Copeia 1971:389–432

Harrington RW Jr (1975) Sex setermination and differentiation among uniparental homozygotes of the hermaphroditic fish *Rivulus marmoratus* (Cyprinodontidae: Atheriniformes). In: Reinboth R (ed) Intersexuality in the animal Kingdom. Springer, Berlin, pp 249–262

Harrington RW Jr, Rivas L (1958) The discovery in Florida of the cyprinodont fish, *Rivulus marmoratus*, with a redescription and ecological notes. Copeia 1958:125–130

Hoch JM, Cahill AE (2012) Variation in size at sex-change among natural populations of the protandrous hermaphrodite, *Crepidula fornicata* (Gastropoda, Calyptraeidae). Mar Biol 159:897–905

Hoffman SG, Schildhauer MP, Warner RR (1985) The costs of changing sex and the ontogeny of males under contest competition for mates. Evolution 39:915–927

Iwasa Y (1991) Sex change evolution and cost of reproduction. Behav Ecol 2:56–68

JMP®, Version 14 Pro. 2019. SAS Institute Inc., Cary

Kazancıoğlu E, Alonzo SH (2009) Costs of changing sex do not explain why sequential hermaphroditism is rare. Am Nat 173:327–336

Leigh EG, Charnov EL, Warner RR (1976) Sex ratio, sex change, and natural selection. Proc Natl Acad Sci 73:3656–3660

Lenth R (2019) emmeans: estimated marginal means, aka least-squares means. R package, version 1.3.2. https://CRAN.R-project.org/package=emmeans

Lomax JL, Carlson RE, Wells JW, Crawford PM, Earley RL (2017) Factors affecting egg production in the selfing mangrove rivulus (*Kryptolebias marmoratus*). Zoology 122:38–45

Luke KN, Bechler DL (2010) The role of dyadic interactions in the mixed-mating strategies of the mangrove rivulus *Kryptolebias marmoratus*. Curr Zool 56:6–17

Mackiewicz M, Tatarenkov A, Perry A, Martin JR, Elder JF, Bechler DL, Avise JC (2006) Microsatellite documentation of male-mediated outcrossing between inbred laboratory strains of the self-fertilizing mangrove killifish (*Kryptolebias marmoratus*). J Hered 97:508–513

Marson KM, Taylor DS, Earley RL (2019) Cryptic male phenotypes in the mangrove rivulus fish, *Kryptolebias marmoratus*. Biol Bull 236:13–28

Martin SB (2007) Association behaviour of the self-fertilizing *Kryptolebias marmoratus* (Poey): The influence of microhabitat use on the potential for a complex mating system. J Fish Biol 71:1383–1392

Maynard Smith J (1978) The evolution of sex. Cambridge Univ Press, Cambridge

Molloy PP, Nyboer EA, Côté IM (2011) Male-male competition in a mixed-mating fish. Ethology 117:586–596

Munday PL, Molony BW (2002) The energetic cost of protogynous versus protandrous sex change in the bi-directional sex-changing fish *Gobiodon histrio*. Mar Biol 141:1011–1017

Munday PL, Buston PM, Warner RR (2006a) Diversity and flexibility of sex-change strategies in animals. Trends Ecol Evol 21:89–95

Munday PL, Wilson White J, Warner RR (2006b) A social basis for the development of primary males in a sex-changing fish. Proc R Soc B Biol Sci 273:2845–2851

Nakamura Y, Suga K, Sakakura Y, Sakamoto T, Hagiwara A (2008) Genetic and growth differences in the outcrossings between two clonal strains of the self-fertilizing mangrove killifish. Can J Zool 86:976–982

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rodgers EW, Earley RL, Grober MS (2007) Social status determines sexual phenotype in the bi-directional sex changing bluebanded goby *Lythrypnus dalli*. J Fish Biol 70:1660–1668

RStudioTeam (2016) RStudio: integrated development for R. RStudio Inc, Boston

Sakakura Y, Soyano K, Noakes DLG, Hagiwara A (2006) Gonadal morphology in the self-fertilizing mangrove killifish, *Kryptolebias marmoratus*. Ichthyol Res 53:427–430

Scarsella GE, Gresham JD, Earley RL (2018) Relationships between external sexually dimorphic characteristics and internal gonadal morphology in a sex-changing fish. J Zool 305:133–140

Tatarenkov A, Gao H, Mackiewicz M, Taylor DS, Turner BJ, Avise JC (2007) Strong population structure despite evidence of recent migration in a selfing hermaphroditic vertebrate, the mangrove killifish (*Kryptolebias marmoratus*). Mol Ecol 16:2701–2711

Tatarenkov A, Lima SMQ, Earley RL, Berbel-Filho WM, Vermeulen FBM, Taylor DS, Marson K et al (2017) Deep and concordant subdivisions in the self-fertilizing mangrove killifishes (*Kryptolebias*) revealed by nuclear and mtDNA markers. Biol J Lin Soc 122:558–578

Taylor DS (2012) Twenty-four years in the mud: What have we learned about the natural history and ecology of the mangrove rivulus, *Kryptolebias marmoratus*? Integr Comp Biol 52:724–736

Taylor DS, Turner BJ, Davis WP, Chapman BB (2008) A novel terrestrial fish habitat inside emergent logs. Am Nat 171:263–266

Todd EV, Ortega-Recalde O, Liu H, Lamm MS, Rutherford KM, Cross H, Black MA et al (2019) Stress, novel sex genes and epigenetic reprogramming orchestrate socially-controlled sex change. Sci Adv 5:eaaw7006

Turner BJ, Fisher MT, Taylor DS, Davis WP, Jarrett BL (2006) Evolution of “maleness” and outcrossing in a population of the self-fertilizing killifish, *Kryptolebias marmoratus*. Evol Ecol Res 8:1475–1486

Warner RR (1975) The adaptive significance of sequential hermaphroditism in animals. Am Nat 109:61–82

Warner RR (1988a) Sex change in fishes: hypotheses, evidence, and objections. Environ Biol Fishes 22:81–90

Warner RR (1988b) Sex change and the size-advantage model. Trends Ecol Evol 3:133–136

Warner RR, Robertson DR, Leigh EG (1975) Sex change and sexual selection. Science 190:633–638

Yamaguchi S (2016) Time required for sex change in teleost fishes: hormonal dynamics shaped by selection. J Theor Biol 407:339–348