cubation at 37°C, with continuous stirring, the reaction was started by adding enzyme solution to a final concentration of approximately 1 nmole/ml; the oxidation rate in the absence of the substrate was recorded for 30 to 40 seconds. The substrate (25 or 50 μM) was then added in 5 μl of acetone, and the oxidation of NADPH was recorded for 30 to 60 seconds.

9. These values are comparable to those determined for dimethylaniline (15 μM), the substrate commonly used for determining the amine oxidase activity of this enzyme and methimazole (6.7 μM) (*6, 7*), the substrate used for assaying its sulfur oxidase activity. Since, at saturation, all substrates for this monooxygenase appear to be oxygenated at the same velocity (*6*), the rates listed in Table 1 presumably reflect differences in K_m rather than V_{max}. In some cases, velocity is probably limited by solubility.
10. Product identification was carried out by first oxidizing 50 μM disulfoton, phorate, or phorate fortified with 1.7×10^5 dis/min of methylene-[^{14}C]phorate (specific activity, 9.7 mCi/mmole) under optimum conditions. In the presence of NADPH, the reaction was essentially complete in 2 to 3 minutes, and the reaction mixture was immediately extracted twice with chloroform. The product was identified by one- and two-dimensional thin-layer chromatography on silica gel, with development in five different solvent systems.
11. Compounds 1, 5 through 9, 23, 25, 26, 29, 30, and 32 through 34 were purchased from Chem Service; compounds 1, 5, and 7, from City Chemical Co.; compounds 20 and 21, from Fairfield Chemical Co.; and compound 31, from Matheson, Coleman and Bell. Compounds 2 through 4, 27, and 28 were donated by Mobay Chemical Corp.; compounds 12 and 13, by the Environmental Protection Agency. Compounds 10, 11, 14 through 19, 22, and 24 were synthesized according to established procedures.
12. This is a metabolite of fonofos in vivo [P. W. Lee, R. Allahyari, T. R. Fukuto, *Pestic. Biochem. Physiol.* **9**, 23 (1978)].
13. J. E. Casida, R. A. Gray, H. Tilles, *Science* **184**, 573 (1974); J. P. Hubbell and J. E. Casida, *J. Agric. Food Chem.* **25**, 404 (1977); J. E. Casida, E. C. Kimmel, H. Ohkawa, R. Ohkawa, *Pestic. Biochem. Physiol.* **5**, 1 (1975); Y. S. Chen and J. E. Casida, *J. Agric. Food Chem.* **26**, 263 (1978).
14. R. A. Neal, *Rev. Biochem. Toxicol.* **2**, 131 (1980).
15. Supported by grants ES-07046 and ES-00044 from the National Institute of Environmental Health Sciences. We thank W. C. Dauterman and A. A. Nomeir for their advice and for providing compounds 17 through 19, 22, and 24. We also thank D. M. Ziegler, who provided all the purified enzyme used in these studies. This is paper No. 6341 of the Journal Series of the North Carolina Agricultural Research Service.

12 March 1980; revised 21 April 1980

Serial Female Sex Changes After Simultaneous Removal of Males from Social Groups of a Coral Reef Fish

Abstract. *The simultaneous removal of three to nine males from large social groups of* Anthias squamipinnis *led to close to a one-to-one replacement of the removed males by sex-reversing females. The females changed sex serially within each group with a mean interval between successive onset times of 1.9 days. The timing of sex change is thus not independent for each fish but is influenced by the events surrounding other sex reversals within the group.*

Female-to-male sex reversal can be initiated in several species of marine fish by the removal of a male either from spatially well-defined, bisexual social groups (*1–3*) or from less tightly structured aggregations (*4*), depending on the species. This phenomenon has been examined carefully in small, single-male groups where the removal of one male has led to the sex reversal of one female (*5, 6*). The sexually dichromatic, serranid fish *Anthias squamipinnis* (Peters) is a protogynous hermaphrodite that lives in sedentary, bisexual social groups (*2, 7, 8*). When females of this species reverse sex, their coloration, behavior, and gonadal histology change in well-defined sequences (*5, 9*). This report shows (i) that the simultaneous removal of N males from large *A. squamipinnis* social groups leads to the sex reversal of N females, that is, there is close to a one-to-one replacement of sex-reversing females for removed males and (ii) that the multiple sex reversals initiated within a social group by the simultaneous removal of multiple males do not begin at the same time but are evenly spaced in onset, approximately 2 days apart, that is, the timing of sex change is not independent for each fish but is influenced by other sex reversals or by the events initiating other sex reversals within the group.

Forty-eight social groups (*7, 10*) of *A. squamipinnis* were identified with numbered floats and visually censused for the number of adult males and females, at a water depth of less than 15 m on the northeast reef of Apo Island, near Dumaguete City, Negros Oriental, Philippines, in July and August 1978. From this sample, 15 control groups and 11 experimental groups were selected and matched for approximate similarity in size and adult composition. Groups ranged from 2 adult males and 13 adult females to 50 males and 294 females, with a median group containing 10 males and 61 females. In general, from each control group one male was removed. From each experimental group three to nine males were removed (*11*) within an 8-hour period. After the males were removed, the females of each group were observed daily to within 2 m and scrutinized for the earliest changes in coloration indicative of sex reversal. Previous laboratory and fieldwork on 44 socially initiated sex reversals had specified the precise sequence of color changes to be expected in five body regions (*2, 9*). The first day of recognizable color change was recorded as the day of onset of sex change. The sex-reversing individuals were distinguished within each group primarily by the progress and degree of advancement of their respective coloration changes. Experimental groups were observed daily for 15 to 25 days and control groups for 12 to 24 days after male removals. A female was said to have changed sex if her color changed from characteristically female to typically male. This criterion was thought sufficient because 96.9 percent of individuals ($N = 130$) in an earlier study showed full correspondence between the gender of external color pattern and the histologically determined gonadal gender and because 100 percent of individuals ($N = 45$) that were observed to change from female to male coloration contained testes with histological evidence of a prior ovarian state (*3*).

After 58 males were removed from the experimental groups, 57 females changed sex. In each group, very close to the same number of females changed sex as the number of males removed (Fig. 1). Similarly, in most of the control groups a single female changed sex after the removal of one male, resulting in a mean of 1.17 sex reversals per male removed. The median onset day for sex reversals was day 3 after male removal, with a range of 1 to 7, in the control groups, and day 5, with a range of 1 to 16, in the experimental groups. The variance in day of onset for the 57 sex reversals in the experimental groups was 6.7 times as great as the variance in the control groups (variance ratio test, $P < .001$, two-tailed) and the onset day for experimental groups was significantly higher than the onset day for control groups (Mann-Whitney U test, $P < .001$, two-tailed). In the experimental groups, when the first, second, third, fourth, and so on successive sex reversals within each group were examined separately, the onset day varied directly with the order of sex reversal within the group (Fig. 2). The sex reversals from the control and experimental groups were unlikely to have come from a single, homogeneous population (Fig. 2) (Kruskal-Wallis one-way analysis of variance, $P < .001$; single factor analysis of variance, $P < .001$, two-tailed). The mean onset day for the one sex reversal in the control groups was significantly lower than the mean onset day for the third, fourth, fifth, sixth, and seventh plus sex reversals in the experimental groups

0036-8075/80/0905-1136$00.50/0

(Dunnett test, $P < .01$, one-tailed). When the 11 experimental groups were examined separately, the median number of days separating the onsets of successive sex reversals ranged from 1 to 6, with a median separation time for the 11 median values for these groups of 2 days. When the 46 intervals separating successive sex reversals in all 11 groups were considered together, the median separation time was 1.5 days, the mean was 1.9 days, and the standard deviation was 1.9 days.

Thus, generally, for each of multiple males removed from a group, one female changed sex. When multiple males were removed simultaneously from a group, multiple sex reversals succeeded one another in onset with a mean interval of 1.9 days. These results were not predicted by a detailed analysis of intragroup behavior before and after male removal in small, single-male groups (*2, 5*) and seemed unlikely to be predicted by existing models for the evolution of the proper time of sex change (*4, 12*). While some large, multimale groups are divided into two or three spatially separated subgroups (*7*), one-to-one replacement suggests an additional, high degree of intragroup social structure in large groups.

One simple hypothesis is that large groups consist of an aggregation of spatially defined clusters of individuals, each containing one male and multiple females, with sex reversal controlled independently within each cluster. The removal of the male from several clusters would separately induce one female to change sex within each cluster and one-to-one replacement would result. In such a case, the onset of sex reversal should be independent for each cluster. There should be no difference in onset times between each of the multiple sex reversals after removal of many males and no difference between those onset times and the onset times of single sex reversals after removal of single males. This prediction is belied by the present results. Furthermore, while identified males, in one study, showed overlapping but statistically distinct home ranges within a large group, identified females moved in no simple relation to the home ranges of specific males (*13*). The multiple cluster hypothesis, then, appears not to apply.

These results might be partially explained if the group were organized, not into spatially defined clusters, but into behavioral subsets of individuals, each involving one male and multiple females. If the rapidity of onset of sex reversal depended on the value of some measure of interaction between members of the subset, and if subsets were hierarchically arranged within the group, with interaction levels within each subset determined by the subset's relative rank in the group, then multiple male removals would produce multiple sex reversals whose onsets would be spaced in accordance with the difference in rank between the subsets whose males were removed. The difficulty with this hypothesis is that it predicts a different, definite onset time for each subset within the group. It is most unlikely that a haphazard removal of three to nine males from groups containing up to 50 males would invariably select males from high ranking subsets with very rapid onset times. Yet, in each group, the first sex reversal showed onset 1 to 7 days after male removal (Fig. 2).

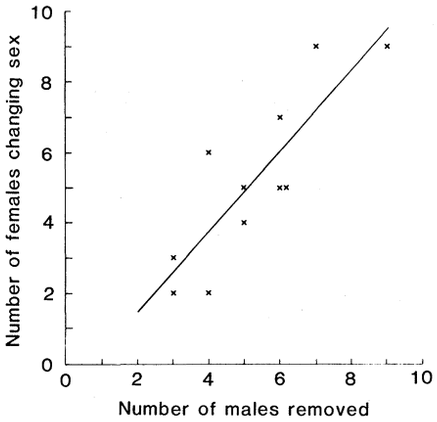

Fig. 1. The number of females that changed sex after the removal of three to nine males from each of 11 social groups of *A. squamipinnis*. Each data point represents one social group. The number of females changing sex was correlated with the number of males removed (Spearman rank order correlation, $r_s = .82$, $P < .01$, two-tailed). The straight line ($y = -0.93 + 1.16x$) is a linear regression fitted to the data points.

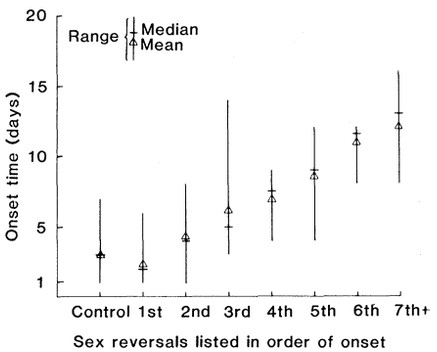

Fig. 2. The onset time, in days after male removal, for the coloration changes (i) of single sex reversals in large control groups after the removal of a single male and (ii) of seven or more successive sex reversals in large groups of *A. squamipinnis* after simultaneous removal of many males. Sample sizes were, for the controls, $N = 15$; first, $N = 11$; second, $N = 11$; third, $N = 9$; fourth, $N = 8$; fifth, $N = 7$; sixth, $N = 4$; and seventh plus, $N = 7$.

Alternatively, if females were hierarchically arranged in multimale groups, as they are in small, single-male groups (*2, 5*), the first female to begin changing sex may interact with a lower ranked female in such a way as to delay the onset of sex reversal of the lower ranked fish by approximately 2 days; or the second fish to change sex may depend on some interaction from the first sex-reversing fish before its sex reversal could begin, and the particular interaction at question may require several days for the first fish to perform. This sort of hypothesis could possibly explain the delay in onset time between successive reversals, but not the one-to-one replacement of sex-reversing females for lost males. Some combination of a behavioral subset structure and stimulation or suppression between hierarchically arranged females may provide the solution.

DOUGLAS Y. SHAPIRO

Department of Marine Sciences, University of Puerto Rico, Mayaguez 00708

References and Notes

1. L. Fishelson, *Nature (London)* **227**, 90 (1970); J. T. Moyer and A. Nakazono, *Jpn. J. Ichthyol.* **25**, 25 (1978); D. R. Robertson, *Science* **177**, 1007 (1972).
2. D. Y. Shapiro, thesis, University of Cambridge (1977).
3. _____, *J. Zool. (London)*, in press.
4. R. R. Warner, D. R. Robertson, E. G. Leigh, *Science* **190**, 633 (1975).
5. D. Y. Shapiro, *Adv. Study Behav.* **10**, 43 (1979).
6. D. R. Robertson, thesis, Queensland University (1974). In protandrous species the removal of one female leads to the sex change of one male [H. Fricke and S. Fricke, *Nature (London)* **266**, 830 (1977); J. T. Moyer and A. Nakazono, *Jpn. J. Ichthyol.* **25**, 101 (1978)].
7. D. Y. Shapiro, in *Proc. Int. Coral Reef Symp. Third* (Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Fla., 1977), pp. 571–577.
8. D. Popper and L. Fishelson, *J. Exp. Zool.* **184**, 409 (1973).
9. D. Y. Shapiro, *Bull. Mar. Sci.*, in press.
10. A social group was defined as an aggregation of fish occupying a definable water volume over a fixed site and surrounded by a much larger water volume containing few or no fish of the same species.
11. Males were individually speared, placed in plastic bags, and removed from the group. In some instances, males were hit with the spear but could not be captured. The number of males listed as removed, in all cases, represents the sum of the number speared and captured and the number speared and never seen again within the group. In three control groups, to ensure that one male was definitively removed from the group, a second male was speared when a first speared male could not be captured.
12. R. R. Warner, *Am. Nat.* **109**, 61 (1975); E. G. Leigh, Jr., E. L. Charnov, R. R. Warner, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3656 (1976); E. L. Charnov and J. Bull, *Nature (London)* **266**, 828 (1977).
13. D. Y. Shapiro, in preparation.

14. I thank P. L. Colin, M. E. Leighton-Shapiro, Y. Sadovy, R. E. Waldner, and E. H. Williams, Jr., for critical reviews of the manuscript, and A. Alcala of Silliman University for facilities in the Philippines. Supported by NIMH grant R03 MH 31962 and grant 8319 from the Penrose Fund of the American Philosophical Society.

12 October 1979; revised 2 May 1980