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SOME ASPECTS OF THE SEX CHANGE AND REPRODUCTIVE BIOLOGY OF THE GROUPER, *EPINEPHELUS DIACANTHUS* (CUVIER ET VALENCIENSIS)[1]

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Chang-Po Chen, Hwey-Lian Hsieh, and Kun-Hsiung Chang (1980) Some aspects of the sex change and reproductive biology of the grouper, *Epinephelus diacanthus* (Cuvier et Valenciensis). *Bull. Inst. Zool., Academia Sinica* **19**(1): 11-17. Three hundred and eighty specimens of the grouper, *Epinephelus diacanthus* (Cuvier et Valenciensis), collected monthly from June 1975 to October 1977, were used in this study. This grouper is protogynous. Sex changes occurred during the non-reproductive period from age 2^+ to 6^+, but mainly between ages 2^+ and 3^+. Spawning occurred during April and May of each year. The minimum body size for reproductive activity is estimated as 125 mm (standard length). The fecundity ranged from 63×10^3 to 233×10^3, and is linearly related to the standard length.

The grouper, *Epinephelus diacanthus* is a dominant and economically important benthic fish in the coastal waters of northern Taiwan[14]. Its age and growth have been reported previously[4].

The groupers of the genus *Epinephelus* are well-known protogynous perciform teleosts of the family Serranidae[2,10,12]. The histological changes in the gonad during the process of sex change has been studied in detail for a number of groupers[2,10]. However, the relationship between the sex change and age composition is still not yet well understood due to the difficulties in determining age.

In this report, the sex change of the grouper, *E. diacanthus* and the relationship between the sex change and age composition are described. Other reproductive characteristics, such as the reproductive cycle, minimum body size for reproductive activity, and fecundity are also reported.

MATERIALS AND METHODS

A total of 380 specimens of grouper, *Epinephelus diacanthus* (Cuvier et Valenciensis) used in this study, were collected from waters off Northern Taiwan at monthly intervals from June 1975 to October 1977. Thorough collection data of this material has been described by Chen *et al.*[4].

Of the two forms of intersexuality within the genus *Epinephelus*, as recognized by Brusle and Brusle[2], only the ovarian intersexuality (with male tissue nests inside the female tissue) was clearly recognized as an intersex stage in this study. Testicular intersexuality (with occytes present in functional testes) was cited as male after Smith[10].

Fish age was cited from Chen, *et al.*[4]. In order to indicate the reproductive condition of the fish, the maturity factor (MF) was adopted. It is described as:

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$$MF = \frac{\text{Gonad weight}}{\text{Body weight without viscera}} \times 10^2.$$

A previous test on the distribution of ova revealed that ova are distributed homogeneously within the ovaries (Table 1) and that the development of ova follows a group synchronized type (Fig. 1)[16]. Those ova ready for release in the current spawning season are called maturing ova in this study. Thus, the fecundity of the fish was estimated by gravimetric method as described below: 0.1 g of ovary tissue was randomly selected from the mid-portion of either ovary. This was well mixed with 5 ml of tap water and a small subsample of about 2,000 ova was taken. The diameter of each ova, as small as 50 μ was measured under 30× with the Nikon dissecting microscope to determine the percentage of maturing ova ($p\%$). Then the total number of ova in this 0.1 g ovary tissue were counted (n). Therefore, the fecundity (F) was estimated as

$$F = n \times 10 \times \text{ovary weight } (g) \times p\%$$

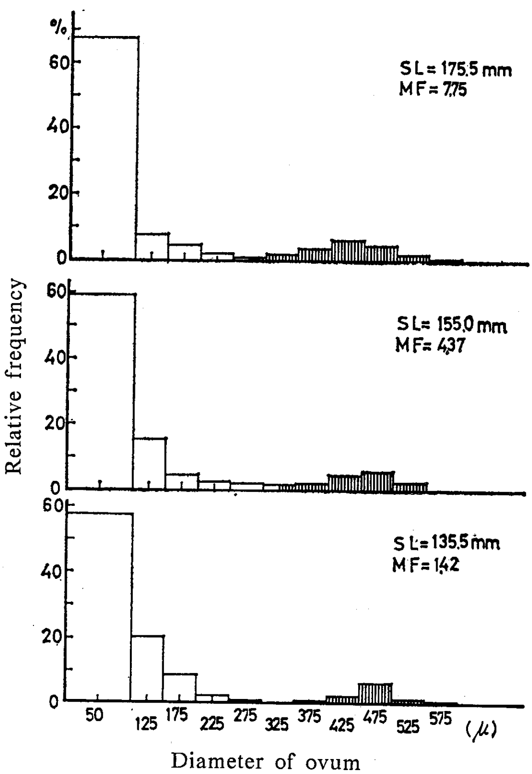

Fig. 1. The distribution of diameter of ovum. Vertical lines indicate maturing ova. SL: Standard length, MF: Maturity factor.

TABLE 1

Mean size values (μ) of ova in different parts of the ovaries

Specimens/MF		Left ovary			Right ovary		
		Pro-	Mid-	Post-	Pro-	Mid-	Post-
1.	775	467	473	458	472	470	468
2.	437	455	442	436	451	439	430
3.	142	476	474	478	474	470	473

Note: A two-way, fixed model Anova shows no significant difference among parts of ovary (F, $1.28 < F_{0.05,(5,10)}$, 3.33); but significant difference among specimens (F, $44.04 > F_{0.01,(2,10)}$, 7.56)

RESULTS

1. Sex change

Among 380 individuals examined, there were 236 females, 91 males and 41 intersexes. 12 others were sex-unidentified due to their abnormal gonads or errors in sampling. 11 individuals of those sex-identified fish were age-undetermined. The standard lengths of these fish were plotted in each age class (Fig. 2). Males were clearly larger than females in all age classes. 357 fish were classified according to their sex and age classes. (Table 2). All 1-year-old fish were females. Females still dominated over males in the 2-year-old and 3-year-old classes, then, declined sharply in older age classes. However they were still evident even in the oldest age class, the 7-year-old class in this study. The intersexual fish appeared first in the 2-year-old class and continued in abundance to the next age class, then decreased gradually but did not occur beyond the 6-year-old age class. The first male emerged in the 2-year-old class. Males increased quickly and took over females' dominant position after 4-year-old.

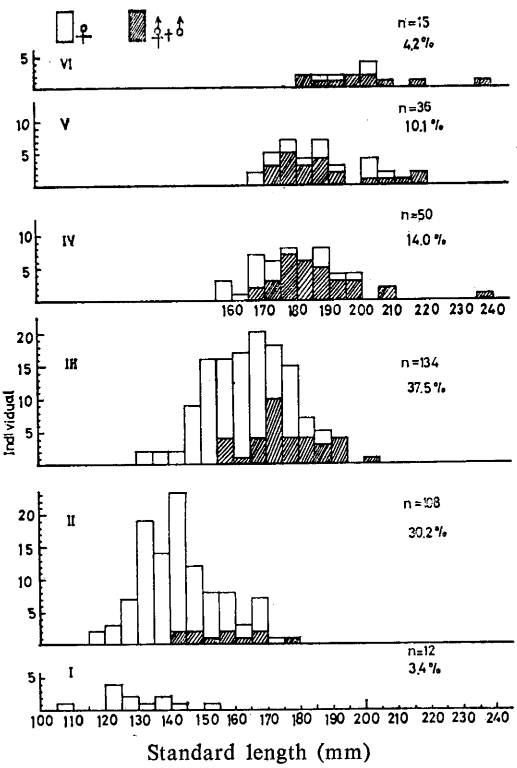

Fig. 2. The standard length distribution of the grouper.

TABLE 2
Sex composition in different age classes of *E. diacanthus*

age	female (%)	Intersex (%)	Male (%)	Total	Uniden-tified
1	12 (100)	0	0	12	0
2	94(88.7)	11(10.4)	1 (0.9)	106	2
3	93(71.0)	17(13.0)	21(16.0)	131	3
4	14(29.8)	7(14.9)	26(55.3)	47	3
5	12(34.3)	4(11.4)	19(54.3)	35	1
6	3(21.4)	1 (7.1)	10(71.4)	14	1
7	1(50.0)	0	1(50.0)	2	0
Total	229(66.0)	40(11.5)	78(22.5)	347	10

As shown in Fig. 3, intersexual fish existed mainly in the fall season and completely vanished in April and May, which is the spawning season of *E. diacanthus* (see later).

2. Reproductive cycle

The maturity factor of all different sexual classes was averaged monthly, and then plotted (Fig. 4). The sharp peak occurring during April or May in each year indicates that the

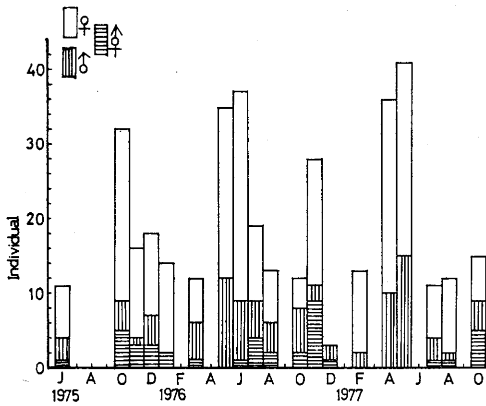

Fig. 3. The monthly change in the sex composition in the grouper.

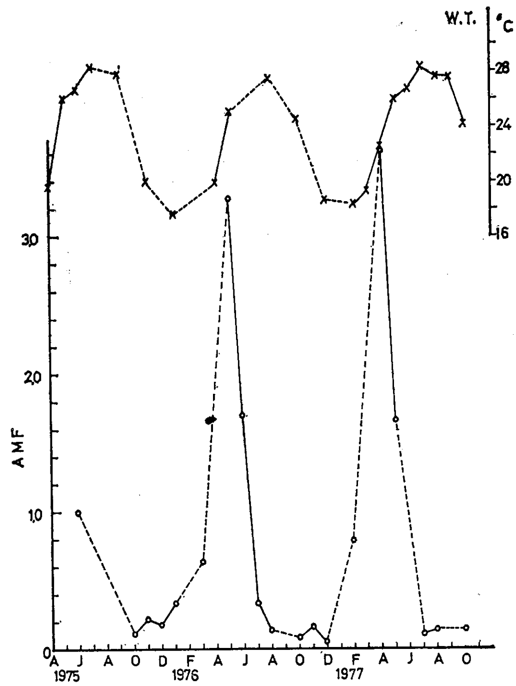

Fig. 4. The monthly change in the averaged maturity factor (AMF) and water temperature (WT).

spawning season of *E. diacanthus* is from late spring to early summer.

3. Minimum biological size

The maturity factors (*MF*) of females collected in April and May were plotted against their standard lengths. As shown in Fig. 5, most of the mature fish had a *MF* greater than 4.0. Among the smallest matured fish, one fish in 123.5 mm *SL* (2-year-old) had a *MF* of 4.3 and one 126 mm *SL* had a *MF* of 7.2 (1-year-old). Therefore, the minimum size of maturity of this grouper is estimated to be approximately 125 mm in standard length (*SL*).

4. Fecundity

Fourteen fish with *MF* values about 5.0 were used to estimate fecundity. Table 3 shows the results of the fecundity estimated. All 14 fish had the same distribution pattern in their diameter of ova and their percentages of maturing ova ranged from 6% to 19%. Fig. 6 reveals that only fish in the 2 and 3-year-old age classes had a higher percentage of maturing ova, however in these same classes, some individuals had a correspondingly low percentages of maturing ova present.

The fecundity of the grouper ranges from 63×10^3 to 233×10^3 and a linear relationship between the fecundity and standard length of the grouper is indicated in Fig. 7. The equa-

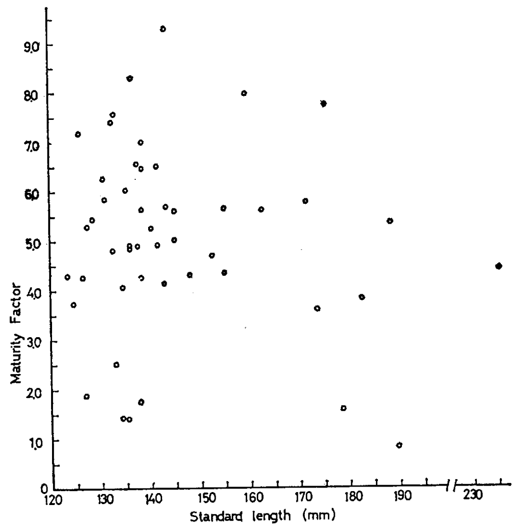

Fig. 5. The grouper's minimum biological size estimated by the standard length (SL) and maturity factor (MF) in spawning season.

TABLE 3
The fecundity of the female grouper, *E. diacanthus*

Specimens (mm)	SL	Age	Body weight (without viscera) (g)	MF	1 gonad weight (g)	2 No. of ova in 0.1 g. gonad	3 Ratio of maturing ova (%)	4 Total no. of ova 4=1×2×10	5 Fecundity ×10³ 5=3×4
1.	126.0	1	56.74	7.2	4.08	25,826	6.04	1,053,700	64
2.	132.5	2	57.91	7.4	4.30	11,259	18.62	484,137	90
3.	135.5	2	60.98	6.0	3.68	22.492	13.68	827,705	113
4.	138.5	2	71.66	6.5	4.64	17,886	14.13	829,910	117
5.	140.5	2	69.44	5.3	3.65	29,621	9.77	1,081,166	106
6.	141.5	2	76.74	4.9	3.78	16,289	15.79	615,724	97
7.	143.5	2	73.34	5.7	4.19	24,264	9.92	1,016,661	106
8.	143.5	3	69.23	9.3	6.43	20,468	9.48	1,316,092	125
9.	155.0	3	106.90	5.7	6.05	18,500	11,59	1,119,250	130
10.	158.0	4	102.02	5.2	5.33	28,175	12.04	1,501,727	180
11.	159.5	4	119.19	8.0	9.05	15,893	8.92	1,438,316	128
12.	171.5	5	126.12	5.8	7.31	20,096	12.23	1,469,017	180
13.	175.5	3	135.86	7.8	10.53	13,095	16.50	1,378,903	227
14.	188.5	6	191.53	5.4	10.25	21,222	10.71	2,175,255	233

Note: SL, standard length; MF, maturity factor.

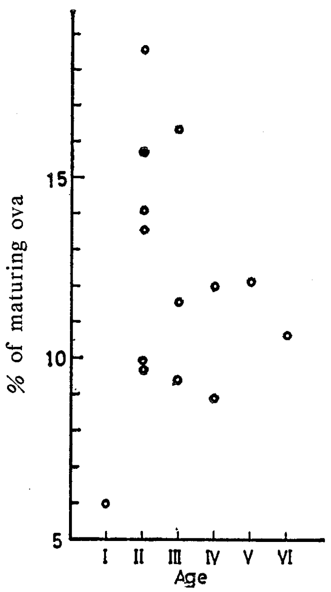

Fig. 6. The relationship between the percentage of maturing ova and the age of the grouper.

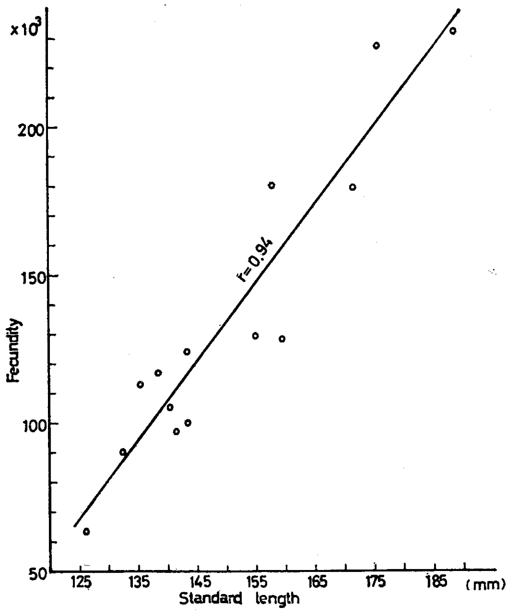

Fig. 7. The linear relationship between the fecundity and standard length of the grouper.

tion of this regression is estimated below:

$$F = -265.2036 + 2.6570 \times SL \qquad (1)$$

DISCUSSION

Ghiselin (1969) proposed three models to explain what conditions would give rise to the types of hermaphrodism in animals(6). In these models, only the *size advantage model* applies to animals exhibiting sequential hermaphrodism in populations not subject to chronic or periodic low densities. Ghiselin suggested that when fecundity increases more rapidly with age in one sex than the other, then individuals should be born into the sex where fecundity increases less rapidly with age, then change later to the sex where age is more advantageous. In general, fecundity of female fishes increases geometrically with length, weight, or age. The relationship usually is of the form, $F=aL^b$, where F is fecundity, L is length and a & b are constant(1). In this study, however, the fecundity of the female *E. diacanthus* was found to increase only arithmatically. The fact that females are the first born and exhibit this arithmatic fecundity relationship should encourage later studies to be made of the male fecundity relationship to determine if a clear Ghiselin model exists.

However, after simulating various conditions for sequential hermaphrodism, Warner (1975) emphasized that "Factors which favor the evolution of protogyny are those which tend to depress male fecundity values at early ages, such as inexperience, territoriality, or female mate selection." and also noted that "Selection for protogyny can also exist when female fecundity decreases with age, although this situation seem rare in the field."(15). In *E. diacanthus*, no information can be cited concerning the depression of male fecundity values at early ages, but work with other fishes may suggest this. For example, a spawning aggregation of the Nassau grouper, *E. stratus*, dominated by older fish was observed by Smith (1972)(13), although no pairing, spawning or other social behavior were observed in his short diving period. Spawning aggregation undoubtedly has adaptive value by assuring that a large number of gametes are released in the same area at the same time, thus enhancing the chance of fertilization. But if the Nassau grouper mates randomly in the spawning aggregation, there is no advantage for change in sex. Additionally, when hermaphrodism exists, protoandry might be expected to be found in a randomly mating population[15]. In contrast, the Nassau grouper exhibits protogyny. Moreover, some coral fishes such as the bluehead wrasse, *Thalassoma bifasciatum*, the parrotfish, *Sparisoma rubripinne*[9] and the *Anthias squamipinnis*[5], have both group spawning and pair spawning within a spawning aggregation. The younger males participate in group spawning, and the more dominant males in pair spawning. Older males would tend to be both more dominant and more experienced. The fecundity of these pair spawning males would be higher than that of group spawning males. If this condition also exists in the spawning aggregation of groupers, then the adaptive advantages of the sex change could be well understood.

The sexual succession of *E. diacanthus* belongs to the incompletely metagonus category of Smith[11]. The sex change, instead of occurring completely at one particular age, is a continuous process occurring during age 2^+ to age 6^+, therefore, the presence of unusually large females in the samples is expected. Brusle and Brusle (1975) also noted that some larger *E. guaze* and *E. aeneus* specimens were still functional females[2].

The intersexes of *E. diacanthus* were found only during non-reproductive periods (Fig. 2 and 3). Studies with *E. aeneus*, *E. guaza*[2] and other groupers[10] also agreed. Apparently the transformation to the male phase occurs soon after the spawning season and is finished before the next spawning season, thus providing a system as efficient as gonochrism in preventing self-fertilization[3].

As in many groupers, the spawning season of *E. diacanthus* is correlated with the water temperature (Fig. 4).

The grouper *E. diacanthus* is one of the smaller species in the family of Serradiea, compared to *Mycteroperca microlepis* which mature as females during their fifth or sixth year and transform to males during their 10th or 11th year[8], therefore, this grouper will be an excellent choice for the study of the mechanism and the evolutionary adaption of sex change.

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擬青石斑之性轉換及其生殖生物學之研究

陳章波　謝蕙蓮　張崑雄

本研究之擬青石斑 (*Epinephelus diacanthus* (Cuvier et Valenciensis)) 取材自民國 64 年 6 月到民國 66年 10月，由臺灣北部海域採得的 380 尾標本。擬青石斑爲先雌後雄，性轉換發生在非生殖季節。主要性轉換發生在 2^+ 歲到 3^+ 歲之間。每年 4～5 月間是產卵期。最小成熟體長估計爲 125 mm 標準體長。個體孕卵數範圍從 63×10^3 個到 233×10^3 個，並且與標準體長成良好的直線關係。