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Sexual differentiation, gonad development, and spawning seasonality of the Hawaiian butterflyfish, *Chaetodon multicinctus*

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Synopsis

The reproductive biology of the coral reef butterflyfish, *Chaetodon multicinctus,* was investigated by histological examination of gonads sampled over an 18 month period from a shallow inshore population on Oahu, Hawaii. Most gonads developed directly from previously undifferentiated tissue. Ovarian development (the structural formation of lamellae and primary oocytes) was observed in fish ≥44 mm and testicular development (the formation of spermatogenic crypts) in fish ≥62 mm standard length (SL). In addition, testis formation was identified within the ovarian lamellae of several differentiated but immature fish. It is hypothesized that prematurational sex change may facilitate monogamy within the highly competitive social structure of this site attached species. Oocyte development in mature females was marked by distinct phases of primary growth, the formation of yolk vesicles, and vitellogenesis. Spawning activity was histologically identified by the maturation and hydration of fully yolked oocytes, and presence of postovulatory follicles. Recently spawned females from field collections and experimental gonadotropin-treatments exhibited postovulatory follicles that were estimated to persist at least 24 h after ovulation. Atresia of yolked oocytes was classified into four stages of cell degeneration and resorption. Monthly analyses of oocyte development and atresia within the sample population show that *C. multicinctus* has a protracted annual spawning season with a major peak during the early spring and evidence of spawning activity among some individuals in the fall. Histological analyses of spawning activity provide more accurate and unambiguous information than do traditional gonadosomatic assays in this and probably other coral reef fishes.

Introduction

Sexual maturation and spawning are important aspects of the life history of coral reef fishes, yet our understanding of the reproductive biology of this group is remarkably limited (reviewed by Thresher 1984, Walsh 1987). The majority of studies on reproductive activity have employed gonadosomatic indices (GSI) (e.g. Ralston 1981), macroscopic classification of gonad ripeness (e.g. Munro et al.1973, Nzioka 1979), or direct observations of spawning (e.g. Neudecker & Lobel 1982, Robertson 1983, Moyer 1984). With the exception of studies exclusively devoted to sex change (e.g. Moyer & Nakazono 1978, Ross 1984) only a few provide histological analyses of gamete development (e.g. Bouain & Siau 1983, Hourigan & Kelley 1985). This latter method may be preferred over more traditional GSI analysis for the study of spawning seasonality since the relationship between ovarian

weight and body size often changes with the stage of oocyte development (de Vlaming et al. 1982). Histological classification should be especially advantageous to identify reproductive activity in tropical reef species during the non-peak periods of a protracted spawning season.

Butterflyfishes of the family Chaetodontidae are among the most conspicuous members of coral reefs. However, detailed reproductive data exist for only one species, *Chaetodon miliaris,* studied by Ralston (1976, 1981), and no published analyses of sexual differentiation, gonad maturation, or oocyte development are available for any chaetodontid. The banded butterflyfish, *Chaetodon multicinctus* Garrett, is endemic to the Hawaiian Islands and Johnston Atoll (Burgess 1978) where it occurs on most coral reefs. It is a small obligate coral feeder and forms monogamous pairs that defend permanent feeding territories (Reese 1975, Tricas 1985, 1988, 1989). It is closely allied with the congeners *Chaetodon punctatofasciatus, Chaetodon guttatissimus,* and *Chaetodon pelewensis* that occur regionally in the Indo-Pacific (Burgess 1978) and thus is an excellent model species for comparative study.

This paper presents the first histological analysis of the reproductive biology of a member of the family Chaetodontidae. Patterns of gonad differentiation, oocyte development, and atresia are described. Histological analyses are shown to be preferred over GSI methods, and reveal that *C. multicinctus* spawns over a protracted annual period with a major peak in the spring and activity among some fishes in the fall.

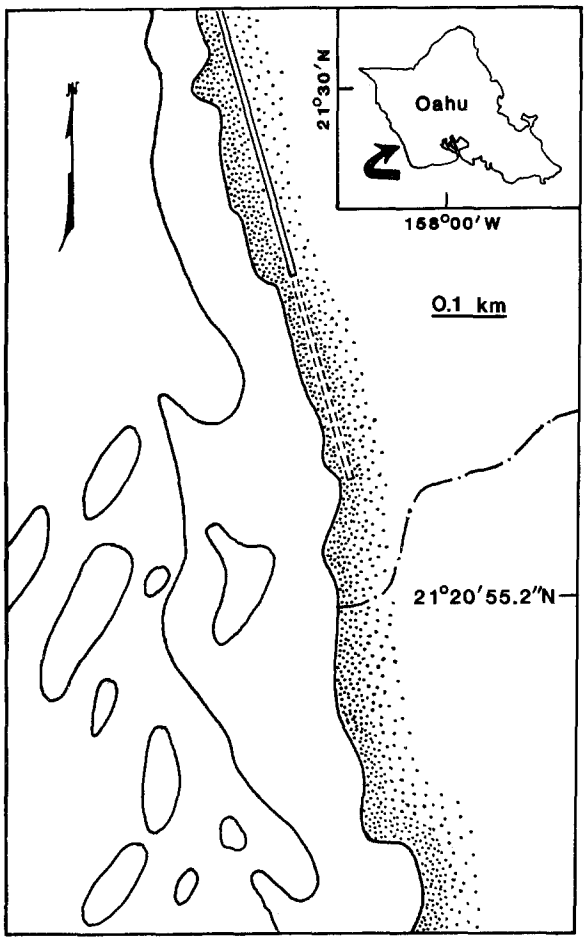

Fig. 1. Patch reef collection site at Kahe Point on the leeward coast of Oahu, Hawaii. Some collections made on patch reefs 0<0.5 km to the north of area shown. Submarine relief shows approximate 4 m contour of reef. After U.S. Geological Survey Map: Ewa, Hawaii 1983.

Study site and methods

Adult and juvenile pairs, and unpaired juveniles of *C. multicinctus* were collected over an 18-month period in 1981–1982 on shallow coral reefs (3–10 m deep) near Kahe Point on the west shore of Oahu, Hawaii (Fig. 1). All but one of the 1981 collections were taken semi-monthly from July through December, while those in 1982 were made every week. Fifty-eight separate samples were taken. Fish were speared between 0900 and 1200 h by divers using scuba, placed in plastic bags, and transported on ice to the laboratory. Whole individuals were weighed (nearest mg), measured (nearest mm) for standard length (SL), and their gonads removed then weighed (nearest 0.1 mg). In fish ≤60 mm SL, tissues along the medial mesentery between the gut and dorsal wall of the coelomic cavity were dissected when gonads were not visible. The GSI, computed as the ratio of ovary weight to gonad-free body weight × 100, was determined for sexually mature females.

Gonads were fixed in 10% buffered formalin for at least 5 days, washed, and preserved in 70%

isopropanol. Tissues were dehydrated and cleared in ethanol and xylene, respectively, or by a single series of ethanol-butanol washes. Gonad tissues were embedded in Paraplast, and 7 μm-thick sections cut on a microtome. Hematoxylin/eosin, hematoxylin/triosin, Mallory's, and hematoxylin/triosin/Mallory's were used to study tissue morphology. Feulgen's stain confirmed the presence of DNA-rich material in cell aggregates within ovaries suspected of spermatogenic activity. Carbohydrate inclusions characteristic of cortical alveoli in oocytes (Aketa 1954) were identified by aqueous PAS treatment. Ooplasmic lipids were identified by either a 1% osmium tetroxide stain (and hematoxylin/eosin counterstain) of preserved tissue or a Sudan Black B stain of 40–45μm fresh-frozen sections.

Ovarian sections were examined from fish in all size classes collected during all months of the study. Oocytes were staged and measured on a compound microscope with an ocular micrometer. Postovulatory follicles were measured along their major axis. Transverse histological sections were taken from the midregion of ovaries to maximize cross sectional area. Comparison of primary growth and vitellogenesis among oocytes from anterior, mid, and posterior regions of mature females showed that oocyte development was homogeneous throughout the ovary ($P>0.975$, 3-sample Smirnov test, $n = 5$ ovary pairs, Conover 1971).

Postovulatory follicles in recently spawned ovaries were examined by induced ovulation in captive fish. During February, March, and May 1982, and March, 1983, 16 adult females (71–94 mm SL) were collected with handnets by divers and transported to the Waikiki Aquarium. Each fish was given a single intramuscular injection of human chorionic gonadotropin (HCG Sigma) (approximately 10 IU/gbw) and held in large tanks with fresh flowing seawater (23.5–25.0° C). After 32–36 h, fish were stripped of ovulated oocytes by applying gentle finger pressure along the abdomen. Gonads were processed and stained with hematoxylin/eosin.

Results

A total of 628 fish were collected in the study. Histological sections of gonads were examined for 416 individuals between 29 and 95 mm SL. Ovary development (the formation of primary oocytes and ovarian lamellae) was recognized in fish as small as 44 mm SL (Table 1). Sections from the dorsal mesentery of smaller fish revealed only beds

Table 1. Sex ratios by size class for *Chaetodon multicinctus*. Fully differentiated but immature ovaries that contained spermatogenic crypts were classified as male and are indicated in parentheses.

Size class (mm SL)	n	Percent		
		Females	Males	Undifferentiated
<30	2	0	0	100
31–35	5	0	0	100
36–40	2	0	0	100
41–45	6	17	0	83
46–50	5	100	0	0
51–55	12	67	0	33
56–60	9	56	0	44
61–65	25	72	20 (4)	8
66–70	22	64	36 (14)	0
71–75	70	52	47 (1)	1
76–80	125	66	34	0
81–85	225	56	44	0
86–90	100	35	65	0
91–95	20	25	75	0

of connective tissue and primordial germ cells. The smallest fish with testis organization (formation of spermatogenic crypts in a structurally indifferent gonad) was 62 mm SL. All smaller fish either lacked differentiated gonads or showed structural ovarian development.

Ovaries

The onset of ovarian morphogenesis in *C. multicinctus* was marked by the proliferation of the stroma and gonia in the dorsal mesentery fold. This medial layer thickened and formed cell columns that developed into lamellae which projected laterally into the ovary lumen. Concurrent with gross structural development of the ovary was the appearance of primary oocytes (Fig. 2A). These cells formed in the lamellar folds and basal tissue layers, and were characterized by enlarged basophilic cells with large nuclei. Proliferation of the stromal layer abated as lamellae formation completed. Mature ovaries were paired, medially fused, and surrounded by a layer of connective tissue. Ovaries distended with yolked oocytes were anteriorly bilobed. During spawning eggs pass through an oviduct continuous with the lumen on the posterior aspect of the ovary.

Testes

In the majority of juveniles where testicular morphogenesis was observed, a thick bilateral stromal layer was formed within the undifferentiated dorsal-medial mesentery. Spermatogonia gave rise to aggregations of spermatocytes which formed spermatogenic crypts(Fig. 2B). Mature testes were elongate, paired, fused along the medial axis, and of the unrestricted spermatogonial testis-type (Grier 1981). Spermatozoa were only observed in crypts within fully-formed radially branched tubules. Sperm are released from crypts into the tubule lumen and collect into a large posteriorly projecting *vasa deferens*. Fully developed lobule luminae, sperm ducts, and liberated spermatozoa were observed in males as small as 65 mm SL.

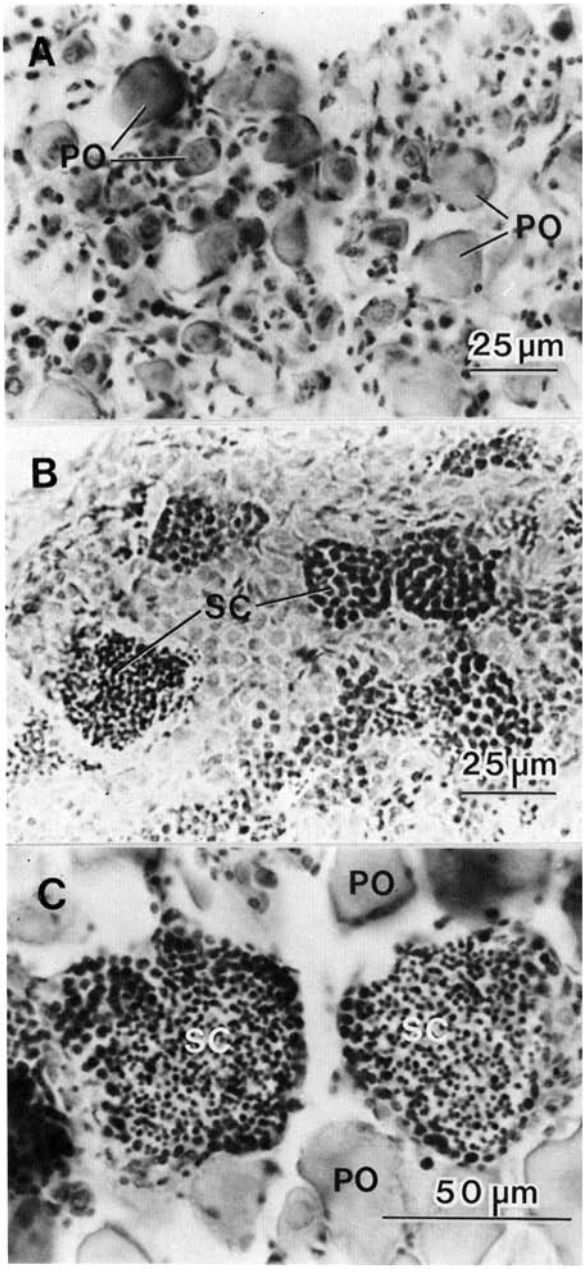

Fig. 2. Differentiation of gonad tissue in *Chaetodon multicinctus:* A – early ovary development from previously undifferentiated tissue in a 44 mm SL fish; B – early testis development from previously undifferentiated tissue in a 73 mm SL fish; C – spermatogenic crypt formation within lamellae of immature ovary from a 67 mm SL fish. All tissues stained with hematoxylin/eosin. PO = primary oocytes; SC = spermatogenic crypts.

A second distinct but less frequent pattern of testes formation was also observed in juveniles. Spermatogenic tissue secondarily developed within structurally differentiated ovaries that contained primary oocytes of the central nucleoli or more advanced perinucleolar stage (described below) (Fig. 2C, 3). Epithelial, stroma, and gonia (presumably spermatogonia) proliferated along the surface of the ovarian lamellae to form groups of mitotic cells that organized into basophilic crypts within the lamellae. Newly formed crypts contained aggregations of basophilic spermatocytes (Fig. 2C) that gave rise to central concentrations of spermatids. There was no difference in mean cell diameter of either primary spermatocytes or spermatids between adult testes and those of transitional juveniles (Two-way analysis of variance, $F = 0.054$, $P<0.90$). Like that observed in testes that developed directly from primordial germ cells in the dorsal mesentery, there was a predominance of spermatids but a lack of spermatozoa prior to tubule formation indicating that spermatozoa are not formed during testis differentiation. Although many immature individuals 61–70 mm SL showed stroma proliferation in the lamellae which might indicate spermatogenic activity, only five non-vitellogenic fish collected between February and May showed clear evidence of organized spermatogenic tissue. No remnant of an ovarian lumen or secondary development of *vasa deferentia* on the gonad surface, as occurs in many protogynous hermaphroditic fishes (e.g. Reinboth 1962, Dipper & Pullin 1979, Ross 1984), was observed in any mature testes. No evidence of degenerate testicular tissue, or oocytes in association with fully differentiated testes was found to indicate prematurational protrandry. The existence of spermatogenic tissue within a structurally developed ovary appears to be strictly prematurational since no crypt formation was observed in females with vitellogenic ova.

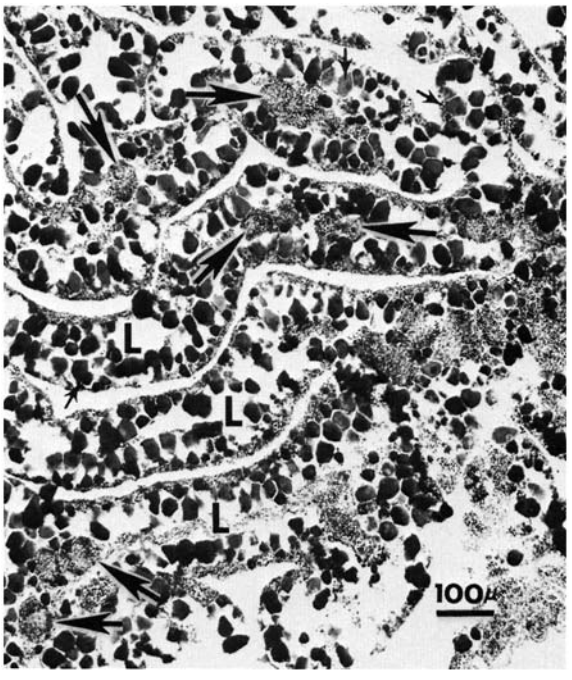

Fig. 3. Transitional gonad in a 67 mm SL *Chaetodon multicinctus*. Primary oocytes (small arrows) and spermatogenic crypts (large arrows) in ovary lamellae (L).

Oocyte development

Oogenesis involved distinct nuclear and cytoplasmic events, and rapid cell growth. Dimensions of various stages and associated structures are summarized in Table 2.

Primary growth (Fig. 2A, 4A). – Primary growth involved the enlargement of primary oocytes and appearance of nucleoli in a centrally located nucleus. The basophilic stain of cytoplasm adjacent to the nucleus indicated the presence of a 'yolk nucleus' (reviewed by Wallace & Selman 1981). During subsequent growth the cytoplasm appeared more heterogeneous and less basophilic. Lampbrush chromosomes were visible within the germinal vesicle (egg nucleus). Oil inclusions appeared as small transparent droplets under hematoxylin/eosin stain and black when fixed with osmium tetroxide. A perinucleolar stage (c.f. Kuo et al. 1974) was identified following migration of nucleoli to the nuclear membrane, and persisted in oocytes throughout vitellogenesis.

Cortical alveoli (yolk vesicle) stages (Fig. 4B). – Prior to vitellogenesis, PAS positive yolk vesicles (the

precursors of cortical alveoli) formed between the egg nucleus and oolemma. Under hematoxylin/eosin stain, previtellogenic oocytes with yolk vesicles were distinguished from primary oocytes by the former's transparent inclusions within the basophilic cytoplasm. Vitellogenic oocytes contained numerous cortical alveoli concentrated adjacent to the oolemma.

Vitellogenesis (Fig. 4A, 4B). – Yolk uptake rapidly accelerated oocyte enlargement. In early vitellogenesis, the vitelline membrane appeared as a thin eosinophilic band around the oocyte. Yolk spheres first appeared near the ooplasm periphery and migrated towards the cell interior. As cells enlarged, the vitelline membrane thickened and developed radial striations to form the zona radiata. In later stages of vitellogenesis, enlarged yolk spheres filled the oocyte interior and lipid droplets aggregated near the nucleus.

Maturation (Fig. 4C) *and hydration.* – Early oocyte maturation involved the coalescence of lipid droplets followed by migration of the nucleus to the cell periphery. The nuclear membrane then degenerated and its contents were released into the cytoplasm. Yolk fusion variably occurred during nuclear migration or after the breakdown of the nuclear membrane with the zona radiata still intact. Maturation and only early stages of hydration were observed among females taken during regular field collections. Later phases of hydration were found among females collected between 1500 h and dusk. Hydration involved the uptake of large amounts of water into the cell, yolk fusion, and cytoplasmic clearing. In late hydration, cells exhibited central concentrations of eosinophilic yolk, and peripheral concentrations of basophilic cytoplasm and liberated nucleoplasm. Striations of the zona radiata became less distinct prior to ovulation.

Ovulation and postovulatory follicles (Fig. 4D, 5A, 5B). – At ovulation, hydrated eggs are discharged from their follicular envelope into the ovary lumen. Newly evacuated follicles, termed postovulatory follicles, consisted of a hollow multilayered band of follicle cells attached to the lamellar surface (Fig. 4D). Older postovulatory follicles (probably ≥40 h

Table 2. Cell diameters and structural dimensions for stages of oocyte development in *Chaetodon multicinctus*. Measurements in microns. n = number of cells or structures measured.

Stages/Structure	n	Range	Mean ± SD
Primary growth			
Central nucleoli stage	201	31– 92	58 ± 12
Perinucleolar stage	220	51–154	92 ± 20
Cortical alveoli	23	123–179	142 ± 14
Early vitellogenesis	180	113–299	207 ± 34
Zona radiata (width)	32		3 ± 6
Yolk spherule (diam)	42		5 ± 1
Late vitellogenesis	331	227–453	338 ± 39
Zona radiata (width)	46		8 ± 2
Yolk globule (diam)	56		13 ± 2
Maturation	36	350–453	399 ± 25
Hydration	61	391–618	498 ± 56
Postovulatory follicles	84	52–206	101 ± 28
Ovulated eggs	4	464–639	525 ± 78
Atretic oocytes			
Alpha	21	258–381	316 ± 41
Beta	53	206–412	298 ± 50
Gamma	83	103–381	213 ± 75
Delta	162	21–484	104 ± 63

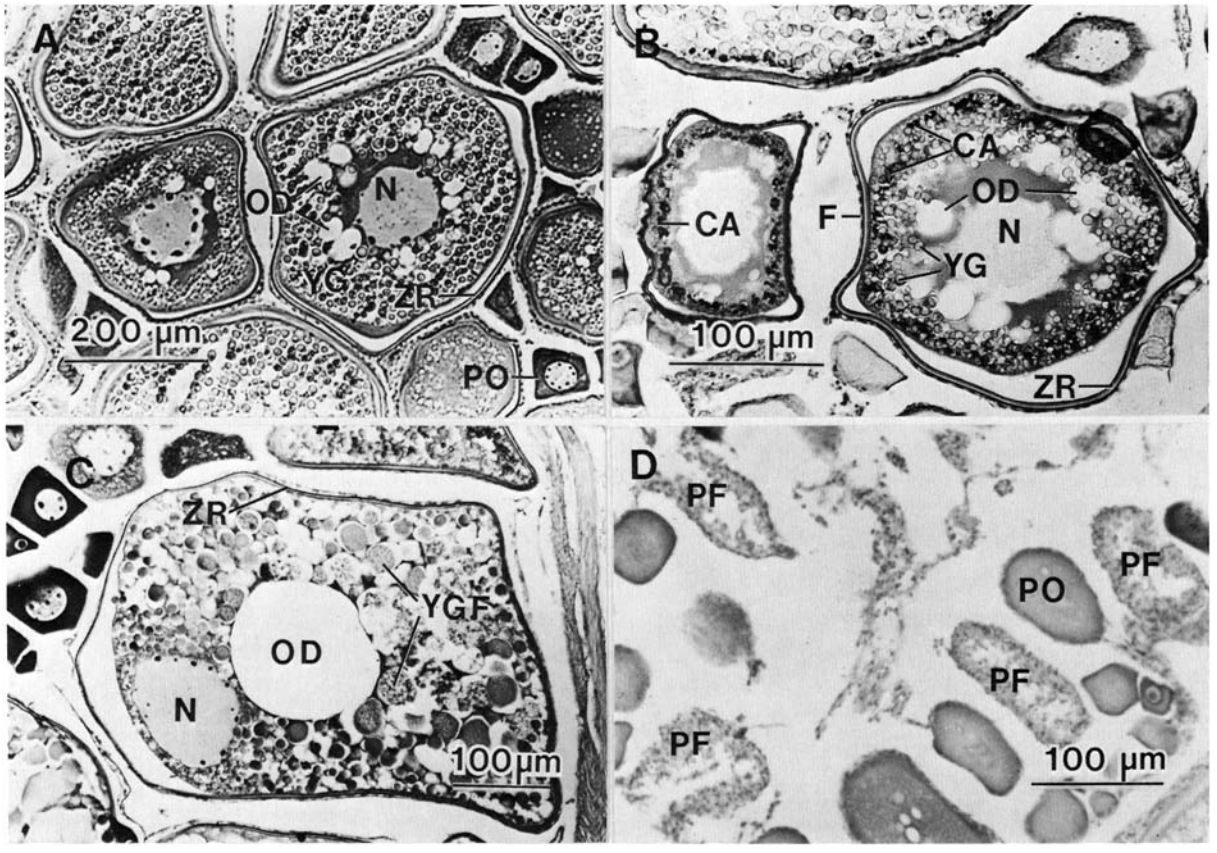

Fig. 4. Stages of oocyte development in *Chaetodon multicinctus:* A – primary growth and vitellogenesis (note lampbrush chromosomes and nucleoli in nuclei of oocytes); B – yolk vesicle stage (cell at left) and early vitellogenesis (cell at right) showing cortical alveoli (dark cytoplasmic inclusions); lighter eosinophilic bodies are yolk granules, PAS stain; C – maturation; D – postovulatory follicles from ovary of female (79 mm SL) collected in April 1982. All tissues except Fig. 4B stained with hematoxylin/eosin. CA = cortical alveoli; F = follicle; OD = oil droplet; N = nucleus; PF = postovulatory follicle; PO = primary oocyte; YG = yolk granule; YGF = yolk granule fusion; ZR = zona radiata.

of age) from field collections appeared as solid masses of degenerate cells of smaller size (Table 2) than atretic stage oocytes (described below). However, only fresh postovulatory follicles were used in analyses of spawning activity. Vitellogenic females injected with HCG exhibited postovulatory follicles (Fig. 5A, 5B) and produced clear buoyant eggs that could be fertilized. Recently formed postovulatory follicles were identified in gonad sections 36 h following injection of HCG.

Oocyte atresia

Not all fully developed oocytes were spawned. Some vitellogenic ova arrested development and showed distinct degenerative stages. Atresia of yolked oocytes in *C. multicinctus* was qualitatively similar to that described for other fishes (Bretschneider & Duyvene de Wit 1947, Lambert 1970, Khoo 1975, Saidapur 1978, Hunter & Macewicz 1985) although some differences exist.

Alpha atresia (Fig. 6A). – Alpha atresia was marked by convolutions of the zona radiata, nuclear disruption, early breakdown of yolk globules,

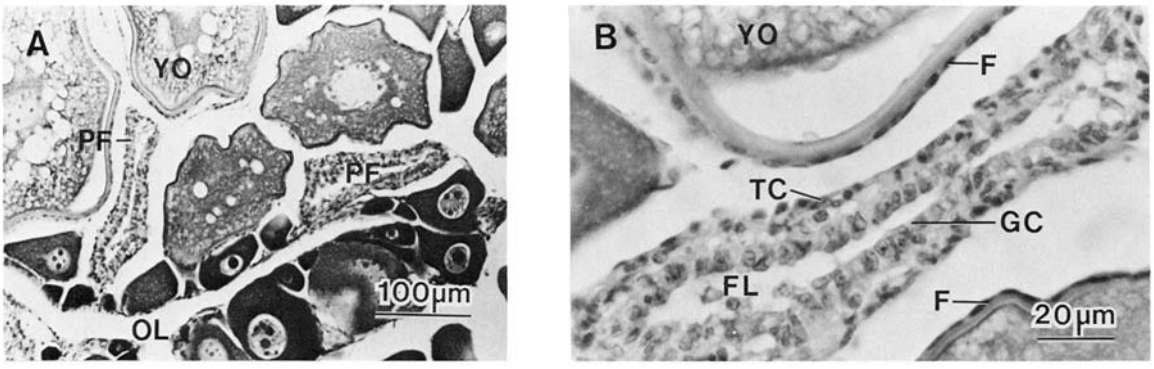

Fig. 5. Postovulatory follicles in ovary after induced ovulation in 94 mm SL *Chaetodon multicinctus:* A – photomicrograph showing two postovulatory follicles attached to lamellae wall; B – close up of follicle in Figure 5A. F = follicle; FL = follicle lumen; GC = granulosa cell layer; OL = ovarian lumen; PF = postovulatory follicle; TC = theca cell layer; YO = yolked oocyte.

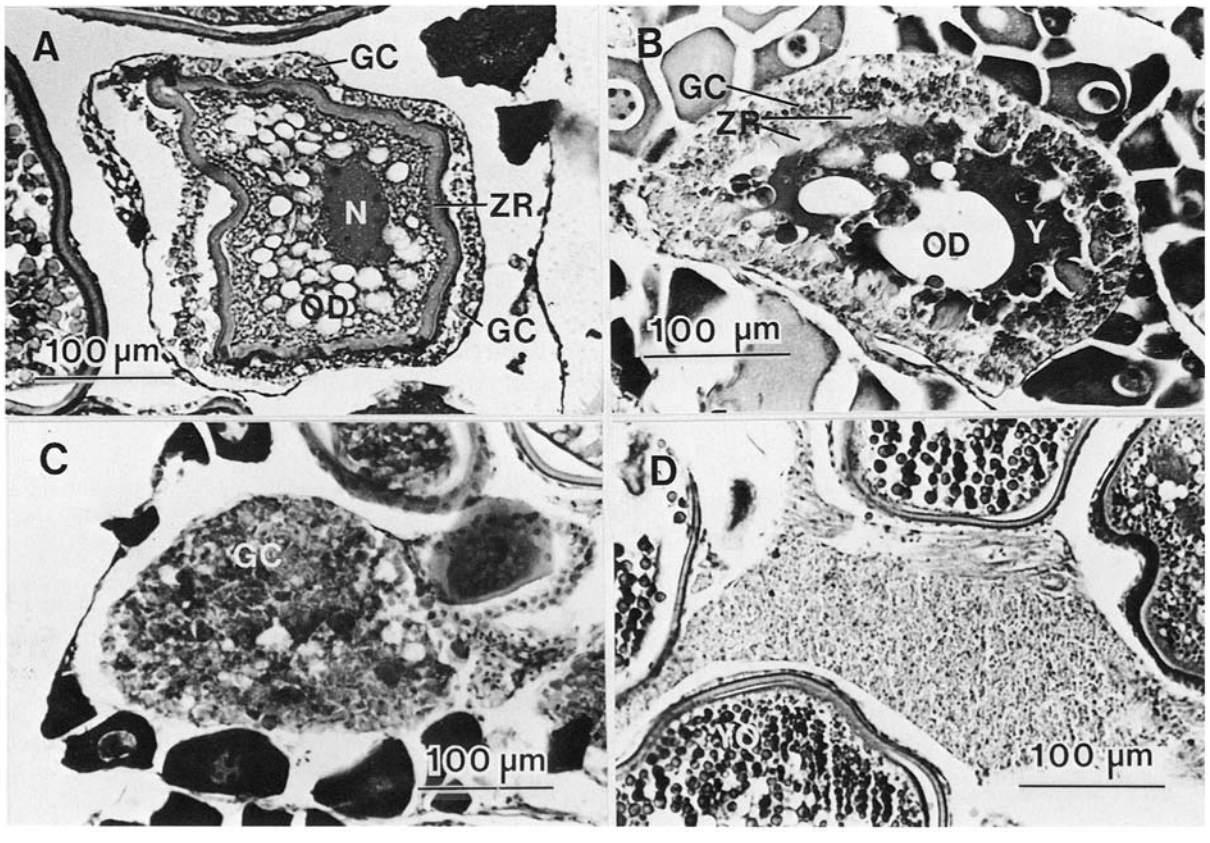

Fig. 6. Stages of atresia in yolked oocytes of *Chaetodon multicinctus:* A – alpha stage showing hypertrophy of follicle granulosa cells and coalescence of oil droplets; B – beta atresia indicated by disruption of the zona radiata; C – gamma stage in advanced resorption of yolk and cytoplasm by granulosa cells; D – delta atresia characterized by large brown body after full resorption of cytoplasm and yolk. GC = granulosa cells; N = nucleus; OD = oil droplets; Y = yolk; YO = yolked oocyte; ZR = zona radiata.

and hypertrophy of granulosa cells peripheral to the zona radiata. The end of alpha atresia was defined by disruption of the zona radiata following that described for the goldfish, *Carassius auratus* (Khoo 1975). This uncommonly observed stage apparently is of relatively short duration and signals the onset of subcellular reorganizations and breakdown prior to resorption.

Beta atresia (Fig. 6B). – During beta atresia, granulosa cells migrate into the cell to phagocytose yolk. Theca cells were not observed to invade the oocyte interior. The end of beta atresia was characterized by the full disintegration of the zona radiata (sensu Khoo 1975). Pronounced neovascularization associated with oocyte resorption often reported for other species (e.g. Bretschneider & Duyvene de Wit 1947, Lambert 1970, Hunter & Macewicz 1985, Saidapur 1978) was not observed.

Gamma atresia (Fig. 6C). – Resorption of oocyte contents continued during gamma atresia until completed. Small irregular shaped follicles (possibly lutein cells) were formed that stained orange-yellow in hematoxylin/eosin (Bretschneider & Duyvene de Wit 1947, Hunter & Macewicz 1985, Saidapur 1978). Theca cells of the follicle still surrounded the remnant oocyte.

Delta atresia (Fig. 6D). – The delta stage of atresia was identified by the residual cell components after resorption of yolk and cytoplasm. These oocytes contained light brown material under hematoxylin/eosin stain. No evidence for epsilon atresia, where cells of the corpus luteum differentiate into new oogonia cells (Khoo 1975), was found.

Seasonality of oocyte development

Gonadosomatic data show an increase in relative gonad weight during the spring months compared to that of the summer and fall (Fig. 7). The lowest monthly mean was 0.6% in September 1981 with an almost steady increase through May at 4.0%. This was followed by a dramatic decline in June to 1.5% which remained at about 1% for the rest of 1982. A

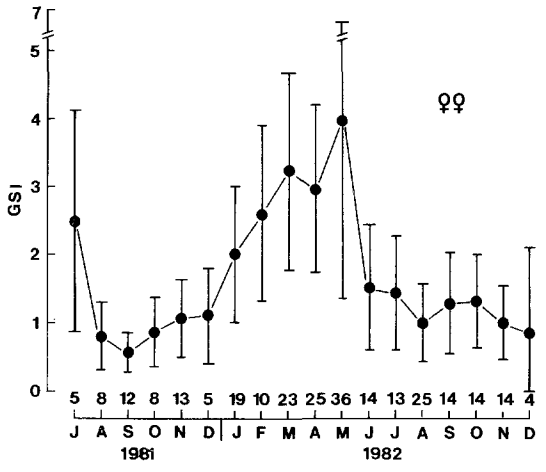

Fig. 7. Monthly gonadosomatic index (GSI) for female *Chaetodon multicinctus*. Vertical bars = 95% confidence limits. Numbers indicate monthly sample sizes.

similar drop is seen after July 1981. Visual inspection of these data suggest spawning occurred from January through May 1982, although the high variability of gonad weight among females within monthly samples precludes a clear statistical boundary for this period.

The uncertainties of spawning activity from the gonadosomatic data can be more directly examined by histological analyses of ovaries. The majority of females sampled in every month of the study contained yolked oocytes (minimum = 58%, August 1981). Females spawned through most of 1982 with the most active period in late winter and early spring (Fig. 8). The broad spawning activity observed in the first half of 1982 peaked during March and was followed by a steady decline through August. The proportion of females spawning during the months of January–July (42%) was greater than females sampled in August–December (15%) ($P<0.01$, Mann-Whitney U-test) which indicates more reproductive activity for the population in the former period. The 1982 spring peak was followed by increases in the proportion of females that showed no vitellogenic activity during July (15%) and August (24%), indicating subsidence of vitellogenic activity. Further evidence for a decline in spawning activity was seen in the cycle of oocyte atresia that peaked in May and June 1982 at over

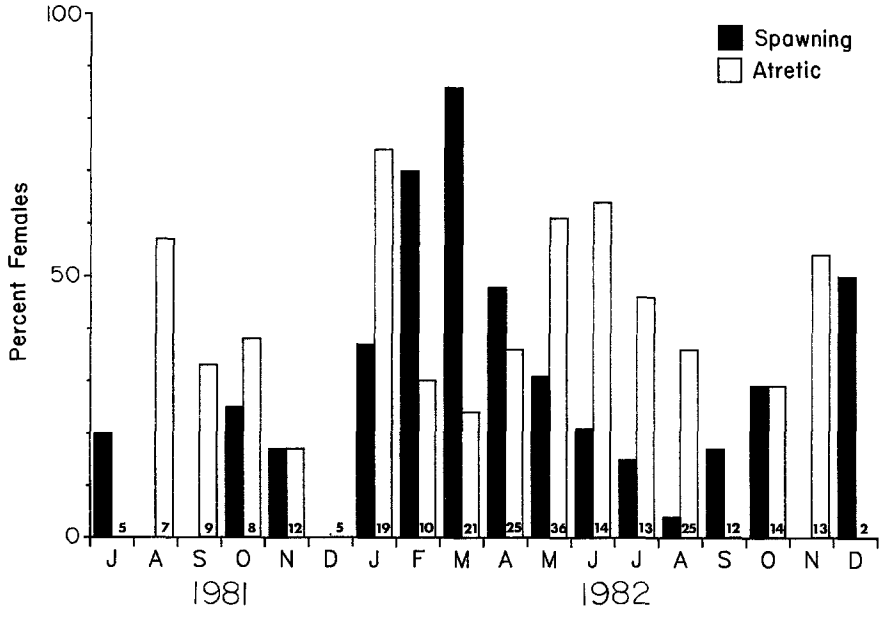

Fig. 8. Monthly summary of oocyte development and atresia in *Chaetodon multicinctus*. Ovaries with histological evidence of impending (oocyte maturation or hydration) or recent (postovulatory follicles) spawning activity indicated by dark bars. Atresia (alpha, gamma, or beta stages) indicated by open bars. Numbers indicate monthly sample sizes.

60% and lagged spawning peaks by 2–3 months (Fig. 8). Additional spawning is indicated during October–November 1981, and September–October 1982. Fall spawning differed from the spring cycle by its relatively short duration and low proportion of spawning females. Both bimonthly periods were followed by one month of spawning inactivity and indications of subsequent oocyte atresia.

Although atresia of yolked oocytes occurred seasonally, the percentage of atretic ova per female was highly variable over the study period and never reached 100% (range = 0 to 83%, n = 250). Advanced atresia (greater than 50% of oocytes in alpha, beta, or gamma atresia) is often used as an indicator of spawning cessation, but in *C. multicinctus* consistently occurred among only a low proportion of females (July–December 1981: $\bar{X}$ = 9.5%, SD = 8.6, January–December 1982; $\bar{X}$ = 2.3%, SD = 3.2). Further, atretic oocytes often occurred with postovulatory follicles within the same ovary. Thus, spawning may be reduced in frequency or intensity but not fully terminated during peak periods of oocyte atresia.

Discussion

Sexual differentiation

Juvenile *C. multicinctus* recruit to the reef at a small size and with undifferentiated gonads that may develop along one of three pathways recorded in this study. First, all ovaries differentiated from a structurally indifferent gonad. Similarly, the majority of differentiating testes also developed directly from previously undifferentiated tissue. Third, some testes developed within previously differentiated ovarian tissue. Unfortunately, we can not link the structural development of ovaries with sex determination especially since in many fishes all individuals pass through a female-like phase (Atz 1964, Yamamoto 1969, Takahaski 1977, Shimizu & Takahashi 1980, Matsuyama et al. 1988). However, since most males and females developed directly from previously undifferentiated tissue, the development of testes in structurally differentiated ovaries may present a case of prematurational sex change. Prematurational sex change

was reported in the parrotfish *Sparisoma* (Robertson & Warner 1978) but is apparently uncommon among coral reef fishes. Juvenile intersexes are generally thought to be a passive phenomenon and their potential function within the mating system of a species is unknown. One possible advantage unrelated to adjustments in relative measures of reproductive effort between the sexes (Ghiselin 1969, Warner 1975) is the assurance of obtaining a mate under severe ecological conditions (sensu Liem 1968) or existing social constraints. New *C. multicinctus* recruits spend their first few months on the reef at a restricted site within reef interstices to avoid fish predators and vigorous aggression from conspecific adult pairs. At sizes as small as 50 mm SL, fish with structurally differentiated ovaries often form pairs and begin to establish small feeding areas in suboptimal habitats or between existing adult territories. Because a mate is essential for defense of a feeding area and suitable coral habitat is often limited (Tricas 1985, 1988), homosexual 'protopairing' of juvenile females may represent an adaptation to enhance the early establishment of successful feeding territories in a highly competitive environment. Under appropriate conditions the subsequent differentiation of one individual into a functional male may occur after pair formation. This phenomenon may function to ensure a heterosexual mate in a monogamous mating system where social contact between sexes is constrained by strong selection for early site attachment.

Oocyte development and atresia

Oocyte development in *C. multicinctus* follows general patterns described for other marine and freshwater teleosts (reviewed by Wallace & Selman 1981, Nagahama 1983) but also exhibits notable differences that are important when using histological criteria to assess spawning activity. For example, in primary growth centrally arranged nucleoli migrate to a peripheral distribution within the nucleus similar to that in the grey mullet, *Mugil cephalus* (Kuo et al. 1974). Unlike grey mullet, however, nucleoli in *C. multicinctus* persist in that arrangement throughout vitellogenesis and on into final maturation until nuclear degeneration (Fig. 3C). Early or late vitellogenesis can be identified by the thickness of the zona radiata and diameter of yolk elements (Table 2). The striations of the zona radiata present throughout vitellogenesis form from associations between follicle cells and oocyte microvilli for transport of protein yolk precursors into the oocyte (reviewed by Laale 1980, Ng & Idler 1983) and increase in width during vitellogenesis in *C. multicinctus*. Measurement of the zona radiata and yolk spheres can eliminate the need to measure maximum oocyte diameters to identify vitellogenic stages.

The dynamics of oocyte development that precede spawning in *C. multicinctus* occur rapidly. The onset of oocyte maturation and rare occurrence of hydrated oocytes in the morning collections indicate that final maturation begins approximately 6–8 h before dusk, and is consistent with the diel spawning periodicity of *C. multicinctus* (Lobel 1978). Final maturation also began in the morning for the related Caribbean pomacanthid angelfish, *Holacanthus tricolor*, and was completed in the afternoon for dusk spawning (Hourigan & Kelley 1985). The hydration and fusion of yolk globules increase egg transparency and buoyancy, characteristics that respectively reduce egg predation by planktivores and enhance offshore dispersal of zygotes.

The relatively uncommon observation of alpha atresia was due in part to early and rapid breakdown of the zona radiata. Beta and gamma atresia involve a longer process of cell resorption and therefore were more commonly observed. 'Brown bodies' of delta atresia occurred in >60% of ovaries and in all months which indicates a long-term persistence of that stage. For comparative purposes, alpha, beta, and gamma atresia in *C. multicinctus* can be pooled and related to the alpha stage described for some temperate species (e.g. Bretscheider & Duyvene de Wit 1947, Lambert 1970, Hunter & Macewicz 1985). Oocyte atresia in *Chaetodon* further differs from the above studies in that pronounced neovascularization was not observed during resorption. This may reflect the comparatively short duration and low intensity of oocyte

resorption in a tropical species with a protracted spawning period in which existent vascularization (or only minimal neovascularization undetected by our histological preparations) is sufficient.

Spawning seasonality

Although gonadosomatic data indicate spawning activity during the first 5 months of 1982 and a peak in May, the high variability among females within monthly samples make statistical confirmation difficult. The insensitivity of the GSI is due to the asynchronous and multiple spawning of females within monthly samples of *C. multicinctus* (Tricas 1986) and perhaps in part to the change in relationship between ovary weight and body size with stage of development (de Vlaming et al. 1982). Furthermore, the GSI method alone can not provide information on possible low levels of spawning activity that may occur in the summer and fall months. In contrast, histological analyses of oocyte development indicate that *C. multicinctus* has a peak in March and a protracted annual spawning period. The occurrence of oocytes undergoing primary growth and vitellogenesis during all months of the year indicates a constant recruitment of oocytes among females and lack of a distinct end to spawning in the population.

Although the spring spawning peak was followed by an increase in atretic females, the low proportion of females in advanced atresia does not indicate a clear postspawning period for the population as in temperate fishes with more gross postspawning atresia (e.g. Crossland 1977). Furthermore, alpha or beta atresia often co-occurred in ovaries with maturing oocytes or fresh postovulatory follicles. Thus, atresia in tropical species with protracted spawning periods may better indicate a decline in reproductive output by individuals or termination of spawning among a portion of females rather than the end of the spawning for the entire population.

Unfortunately, the static sampling of females makes it impossible to assess the spawning activity of individuals over the year. For example, a second but less pronounced spawning peak may also exist during the fall. From the data it is difficult to conclude that the fall spawning activity is a separate and distinct cycle rather than variability in reproductive output in which some females begin spawning earlier in the fall than others. Ralston (1981) reported peak spawning from January–May (based upon a GSI analysis) for the planktivorous Hawaiian butterflyfish, *C. miliaris,* but found no gravid or spawning ovaries in the summer or fall. Similarly Lobel (1989) reported peak GSI for *C. multicinctus* at Kona Hawaii from May–June. Spring spawning appears to be the general pattern for Hawaiian reef species although multimodal annual spawning peaks of unequal amplitude were reported for other Hawaiian species (reviewed by Walsh 1987).

Although the histological staging of oocytes provided important indications of spawning activities, the best evidence of recent spawning activity were postovulatory follicles. Postovulatory follicles, however, degenerate rapidly and can be difficult to distinguish from the latter stages of normal oocyte atresia (Khoo 1975, Hunter & Macewicz 1985). Furthermore, degeneration rates are probably an increasing function of water temperature and their value as indicators of recent spawning is limited to collections made immediately following spawning. In the temperate northern anchovy, *Engraulis mordax,* identifiable postovulatory follicles persist up to 48 h after spawning (Hunter & Goldberg 1980). Takita et al. (1983) reported the presence of postovulatory follicles in ovaries of aquarium held *Callionymus enneactis* (a tropical species) up to about 15 h after spawning. Assuming that *C. multicinctus* ovulated the day following HCG-treatment, 24 h would be a conservative estimate of the persistence of postovulatory follicles in this species since ovaries were fixed approximately 36 h after injection. The presence of identifiable postovulatory follicles in field collections (all collected before 1200 h) supports this minimum estimate since *C. multicinctus* spawns at dusk (Lobel 1978).

The coexistence of oocytes in different developmental stages indicates that females spawn multiply over a reproductive season. This dynamic distribution of oocytes termed 'group synchronous' development (Wallace & Selman 1981) indicates the existence of multiple batches that are shed seri-

ally over time. A more detailed analysis of the size-frequency distribution of ova shows that *C. multicinctus* spawns, perhaps multiply, during weeks prior to new and full moon phases (Tricas 1986). Asynchronous spawning activity undoubtedly introduces variability in the monthly proportions of spawning females by multiple sampling in the lunar month. In the present study this was partially controlled by the regular 7-day collection interval which sampled each lunar quarter within each calendar month. Further application of histological techniques to the reproduction of tropical species could provide valuable information on the growth rates of oocytes to help determine time intervals between spawnings.

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