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Gonad morphology, sexual development, and colony composition in the obligate coral-dwelling damselfish *Dascyllus aruanus*

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Abstract Gonad morphology and colony composition support the existing supposition that the obligate coral-dwelling damselfish *Dascyllus aruanus* has a protogynous hermaphroditic sexual pattern. Adults had either an active ovary containing vitellogenic oocytes, an ovotestis, or a spermiated testis and were classified as adult female, hermaphrodite, or adult male, respectively. Among individuals having male function, the testis (or testis portion of the ovotestis) takes the form of an unrestricted spermatogonial lobular testis. Among hermaphrodites having an ovotestis, a small proportion of individuals had a gonad in which both the ovarian and testicular portions were inactive (inactive hermaphrodites), whereas the majority had a predominantly testicular ovotestis that contained spermatozoa (male-active hermaphrodites). The size range of individuals within gonadal classes indicates that all *D. aruanus* first develop an ovariform gonad. Some individuals then undergo ovarian maturation to become adult females while others develop testicular tissue to form an ovotestis and become male-active hermaphrodites. Subsequently, progressive loss of ovarian tissue results in the development of a secondary testis from an ovotestis with the retention of a residual, afunctional lumen among adult males. The wide size range of individuals having an ovotestis suggests that some hermaphrodites function as adult females before developing testicular tissue while other individuals do not pass through an adult female stage. If this is the case, *D. aruanus* exhibits a diandric protogynous hermaphroditic sexual pattern. The apparent prolonged retention of an ovotestis with both healthy oocytes and an ovarian-type lumen in a spermiated ovotestis, as well as a functional sex ratio of 1:1 for adult females:adult males plus male-active hermaphrodites also raises the possibility that *D. aruanus* may be capable of bidirectional sex change during the hermaphroditic stage. Such a capability would be highly adaptive for a species having limited mobility and unpredictable recruitment of new colony members resulting in unpredictable mating opportunities.

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Introduction

The Indo-Pacific damselfish genus *Dascyllus* contains nine species comprising three species complexes (Randall and Allen 1977). A shared trait of all *Dascyllus* species is the close association individuals form with branching corals at some stage of their life. Among larger species, individuals use branching corals or, in the case of *D. trimaculatus*, sea anemones (Allen 1991) as a retreat during juvenile life stages but shift to other habitats as adults. In contrast, smaller species including *D. aruanus*, *D. reticulatus*, *D. melanurus*, and *D. marginatus* maintain an obligate relationship with one or more species of coral throughout life. Individuals of these latter species form close, frequently species-specific associations with branching forms of acroporan, pocilloporan, and stylophoran corals (Sale 1970, 1972; Coates 1980a, b, 1982; Shpigel 1982; Shpigel and Fishelson 1986). The benefits of these close associations may accrue primarily to the resident fish, which use the host corals as a refugium from nocturnal and diurnal predators. At night, groups of *Dascyllus* retreat deep into the interstices of the branches of their host coral whereas during the day, these same fish form feeding groups that forage for plankton up in the water column (Sale 1971; Coates 1977; K.S. Cole, personal observation). In habitats where branching corals are closely spaced or continuous, individuals may move relatively freely between coral colonies (Barash 1980; Coates 1982; Godwin 1995 – referring to *D. flavicauda* and *D. albisella*). However, where colonies of branching corals are widely spaced and relatively isolated from one another, freedom of movement by individuals may be much more restricted (Fricke 1980; Shpigel and Fishelson 1986; Schwarz and Smith 1990) for smaller, obligate coral-dwelling *Dascyllus* species.

Among the obligate coral-dwelling *Dascyllus* species, hermaphroditism has been reported for *D. aruanus* (Fricke and Holzberg 1974; Coates 1982), *D. reticulatus* (Schwartz and Smith 1990), and *D. marginatus* (Shpigel and Fishelson 1986) as well as for the larger *D. flavicaudus* (Godwin 1995). However, descriptions of gonad morphology and colony composition in terms of sexual function are limited and there are no descriptions for gonad ontogeny and associated sexual developmental pathway(s) among reported hermaphroditic species. In some instances, although gonadal features are suggestive of labile sexuality, verifying hermaphroditism has been problematic (J. Godwin, personal communication). Assessing sexual patterns on the basis of population demography in *Dascyllus* species has been further complicated by a lack of sexual dimorphism (Schwartz and Smith 1990), which makes visual determinations of colony composition, in terms of juvenile/adult ratios and functional sex ratios, extremely difficult. It has been hypothesized that no direct-development males are present in putative hermaphroditic coral-dwelling *Dascyllus* species (Coates 1982; Shpigel and Fishelson 1986; Schwarz and Smith 1990) but this remains to be verified.

D. aruanus, the humbug damselfish, is one of the most common of the obligate coral-dwelling *Dascyllus* species. It is one of two species, the other being *D. melanurus*, that make up the so-called aruanus species complex within *Dascyllus* (Godwin 1995). The remaining two species complexes include the reticulatus complex, consisting of *D. reticulatus*, *D. carneus*, *D. marginatus*, and *D. flavicaudus*, and the trimaculatus complex, consistsing of *D. trimaculatus*, *D. albisella*, and *D. strasburgi* (Godwin 1995). The first two complexes are predominantly made up of small *Dascyllus* [maximum length 70 mm standard length (SL) except *D. flavicaudus*, 90 mm SL, with *D. aruanus* attaining a maximum of 65 mm SL] while the trimaculatus complex consists of larger species (maximum length 110 mm SL) (Allen 1991). It has been suggested that the aruanus complex is ancestral to both the reticulatus and trimaculatus complexes (Godwin 1995), a supposition supported by recent mitochondrial DNA sequence analysis (Bernardi and Crane 1999). Therefore, an investigation of the sexual pattern and sexual ontogeny in *D. aruanus* should both characterize ancestral sexual patterns in *Dascyllus* and provide an evolutionary context for sexual patterns in other *Dascyllus* species. This study examined gonad ontogeny, colony composition based on sexual function, and developmental pathways for males and females in the humbug damselfish, *Dascyllus aruanus*, to investigate the nature of its sexual pattern and sexual ontogeny.

Materials and methods

Collections of 13 reproductive colonies of *Dascyllus aruanus* were made in Madang Lagoon, located on the northeast coast of Papua New Guinea, in January 1994 and of a single colony of *D. aruanus* in the vicinity of the Coral Reef Research Foundation located on Malakal Island, Palau (7°20′N, 134°28′E) in August 1999. In all instances, foraging groups of *D. aruanus* were first followed and observed in the field to assess group cohesiveness and to establish shared and repeated use of the same coral colony for refuge. Such groups of *D. aruanus* that were well isolated from other conspecific groups, both in terms of foraging area and occupied coral colony, were targeted for collection.

Once such a cohesive group of *D. aruanus* was identified, the individuals making up the group were followed until they took refuge in their host coral. After all individuals had retreated into the coral branches, the coral head was completely surrounded with a large plastic bag, detached from the substrate, carried up to the boat and shaken vigorously over a seawater tub to dislodge the sheltering *Dascyllus*. After careful inspection to ensure that all individuals had been shaken free from the coral, the coral head was returned to its former location and re-anchored to the substrate with surrounding coral rubble.

For each collection effort, all individuals removed from a single coral colony were placed in a separate container to maintain group integrity, transported to the laboratory at the Christensen Marine Station, and immediately euthanatized with an overdose of the fish anaesthetic, quinaldine sulfate. Individuals making up each colony were then measured (SL), after which a small abdominal incision was made in the thin portion of the lateral abdominal wall. Each individual was then placed in Dietrich's fixative (10 ml 37% formaldehyde; 30 ml 95% ethanol; 2 ml glacial acetic acid; 60 ml distilled water) for rapid preservation of internal tissues.

After fixation, specimens were trimmed by removing the head and tail, as well as much of the dorsal and lateral musculature. For each specimen, the remaining portion of the body consisting of the abdominal cavity and internal organs (all still in their original configurations) extending from the pectoral region to the anal region was decalcified (Fisher Cal-Ex), taken through a dehydration series, embedded in paraffin (Paraplast Plus), and sectioned (7 μm) in toto from the genital papilla to the anterior end of the gonad. All resulting sections were mounted on microscope slides, stained with Harris hematoxylin and eosin, and viewed with a light microscope to sex individuals, characterize gonad structure, and identify any gonadal features indicative of hermaphroditism. Based on observed features of gonadal tissue, each individual was classified on the basis of (1) presence or absence of male and female gonadal tissue, and (2) the presence (or absence) and developmental stage of male and female germ cells in order to determine sexual function.

In addition, in 1998 a single large colony of *D. aruanus* was collected from an isolated coral head located in the vicinity of the Coral Reef Research Foundation, situated on Malakal Island, Palau (7°20′N, 134°28′E). The collection methodology was the same as that for *D. aruanus* colonies collected in Madang Lagoon. Following transport of the large colony to the laboratory, the largest individual was removed, euthanatized with an overdose of quinaldine sulfate, and the body cavity opened to examine the gonad and identify the sex of the individual. Removal of the largest individual was done repeatedly until the first two consecutive females were found. All of the remaining individuals ($n = 30$), hypothesized to be predominantly female (as suggested by findings outlined in the Results section), were divided into five groups of six fish each, with each experimental group having roughly the same size distribution of individuals. Each of the five experimental groups was placed in a separate outdoor water table (approximately 122×244 cm and 15 cm deep) having live corals, flow-through seawater, and exposure to natural photoperiod conditions. After 6 weeks, the surviving individuals in each group were removed, euthanatized with an overdose of quinaldine sulfate, and the three largest fish from each group histologically examined for gonadal state.

Results

Ovarian features of *Dascyllus aruanus*

Histological analyses of gonads serially sectioned in their entirety were carried out on 93 *Dascyllus aruanus*

collected from 13 isolated corals in Papua New Guinea. Sixteen individuals had gonads that were not sufficiently differentiated as to assign sexual classification and were classified as undifferentiated, in terms of gonad development. Fifty-seven of the remaining individuals had completely ovariform gonads and were classified as females. The overall architecture of the ovary was the same in all of the examined females. The ovary was divided anteriorly into paired lobes that extended dorsally along the abdominal cavity. Each lobe had an internal cavity, or lumen. Posteriorly, the two lobes united to form a common structure and the two internal lumina also united to form a single, common cavity referred to here as the common genital sinus. From the common genital sinus, an oviduct extended posteriorly to the tip of a short genital papilla.

The wall of each ovarian lobe was moderately robust, consisting predominantly of smooth muscle, fibroblasts, fibers, and blood vessels and was lined internally with a simple squamous epithelium. Internal to the ovarian wall, each lobe consisted of a lining layer of ovigerous tissue (i.e., tissue containing oogonia and developing oocytes) surrounding an open region, the ovarian lumen into which mature ova could be released during ovulation. In cross section, approximately 30–60% of the inner perimeter of the ovarian wall was lined with a contiguous layer of ovigerous tissue. The ovigerous tissue formed extensive folds, or lamellae, which projected into the central portion of the ovarian lumen giving the latter a branched appearance. The remaining 40–70% of the inner ovarian wall had no direct contact with ovigerous tissue and formed the outer boundary of the lateral region of the ovarian lumen. In females having moderate to large numbers of vitellogenic oocytes, the outline of the lumen was obscured and compressed by the enlarged ovigerous lamellae.

Oocytes in all developmental stages were most common along the inner periphery of the ovigerous tissue layer just underneath the epithelium lining the ovarian lumen. In addition, stromal cells, capillaries, venules, and arterioles were abundant in this region. In larger lamellar folds, a prominent core of stromal tissue was also present. In contrast to the ovarian lobes, the common genital sinus had little or no ovigerous tissue associated with the ovarian wall.

Oocyte development and terminology equivalents

For the sake of consistency within existing literature related to *Dascyllus*, staging of oocytes follows that of Schwarz and Smith (1990) for *D. reticulatus*, with some additional descriptions and equivalent terminologies provided here to facilitate comparisons with existing (and sometimes conflicting) literature on developmental stages of teleost oocytes. The ovigerous tissue lamellae of *D. aruanus* typically included numerous oocytes in various developmental stages, with the exception of small females in which only early (i.e., stages I and II) oocytes were present. Both stage I and stage II oocytes were small cells surrounded by a thin stratified layer of follicle cells and having intensely hematoxylin-staining cytoplasm and a paler-staining, granular-appearing nucleoplasm in the nucleus. In stage I oocytes, as defined by Schwarz and Smith (1990), the cytoplasm in cross section formed a layer that was thinner than the diameter of the nucleus (Fig. 1A). In contrast, in stage II oocytes the cytoplasm formed a layer that was thicker than the diameter of the nucleus (Fig. 1B).

The nucleus of the smallest stage I oocytes viewed in *D. aruanus* contained a single, prominent, usually centrally located nucleolus; hence these oocytes were in the chromatin nucleolar stage of development described for a number of teleost species (e.g., Yamamoto 1956; Yamamoto and Yamazaki 1961; Wallace and Selman 1981). The nucleus of larger stage I oocytes, as well as all stage II oocytes observed in *D. aruanus*, contained numerous, prominent nucleoli situated close to the nucleus periphery, making these cells equivalent to the early and late perinucleolus stage (Yamamoto 1956;Yamamoto and Yamazaki 1961), or perinucleolar stage (Wallace and Selman 1981) or perinucleolar phase (Takashima and Hibiya 1995) described elsewhere.

Stage III oocytes as defined by Schwarz and Smith (1990) and so classified here were larger than stage II cells and differed considerably in appearance from the latter. The cell cytoplasm was considerably paler than observed in stage I and II oocytes (although still hematoxylin staining) and numerous clear vacuoles of varying size surrounded the nucleus (Fig. 1B, C). These vacuoles, an artifact of lipid fallout during the xylene step of dehydration, reflected the presence of developing oil droplets. In addition, small, roughly spherical, pale-staining vesicles were visible located directly below the cell membrane, reflecting the first formation of cortical granules (sensu Selman and Wallace 1989; referred to in various reports as yolk vesicles; Fig. 1C). Small, uniformly staining, spherical bodies called vitelline granules [also incorrectly referred to as yolk granules, yolk globules, yolk platelets or vitelline vesicles elsewhere, as reviewed in West (1990) and Takashima and Hibiya (1995)] were visible in much of the remaining cytoplasm. The nuclear cytoplasm was also different, having a fibrillar rather than granular appearance due, in part, to the possible presence of lampbrush chromosomes (Wallace and Selman 1981; Nagahama 1983; Takashima and Hibiya 1995; Fig. 1C).

Stage III oocytes were also surrounded by a well-defined, acellular, strongly eosinophilic layer, the chorion (also variously referred to as the vitelline membrane, vitelline envelope, zona radiata, or zona pellucida; Fig. 1C). In addition, stage III oocytes exhibited the first visible presence of microvilli (i.e., adherent filaments – Takahashi 1978) extending through the chorion, which became more prominent in stage IV oocytes (Fig. 1B, D).

Stage III oocytes sensu Schwarz and Smith (1990) are equivalent to several stages of oocyte development de-

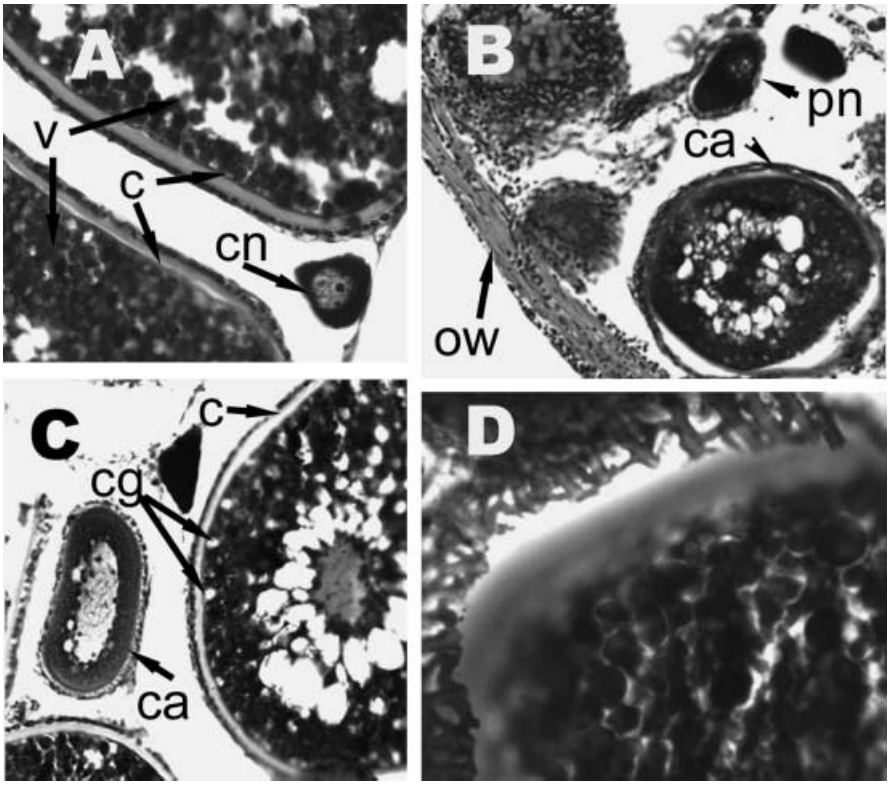

Fig. 1A–D Oocyte developmental stages and ovarian features in *Dascyllus aruanus*. **A** Chromatin nucleolar phase oocyte (*cn*) having a thin layer (relative to the size of the nucleus) of intensely hematoxylin-staining cytoplasm, flanked by two vitellogenic phase oocytes filled with vitelline granules (*v*) and surrounded by a conspicuous extracellular chorion (*c*). **B** Perinucleolar phase oocyte (*pn*) having increased amounts of intensely hematoxylin-staining cytoplasm relative to the size of the nucleus, and adjacent cortical alveolar phase oocyte (*ca*) having numerous, prominent oil droplets surrounding the nucleus, both located close to the well-developed ovarian wall (*ow*). **C** Early-stage cortical alveolar oocyte (*ca*) showing early development of oil droplets around the nucleus, numerous nucleoli and condensed strands of chromatin within the nucleus and less intensely staining cell cytoplasm, and adjacent later-stage cortical alveolar oocyte showing increased size, more oil droplets, and the presence of cortical granules (*cg*) just beneath the well-defined chorion (*c*). **D** Portion of a large vitellogenic phase oocyte filled with vitelline granules and surrounded by a network of microtubules situated immediately outside the chorion (slightly out of focus in this figure)

scribed elsewhere, including the cortical alveoli phase (Wallace and Selman 1990; Takashima and Hibiya 1995), and the combined yolk vesicle stage, oil drop stage, and primary yolk globule stage (described in Nagahama 1983). It should be noted that the term ''yolk vesicle stage'' as previously used referred not to the accumulation of vitellin in the cytoplasm that subsequently provides nourishment for the developing embryo, but to the first appearance of cortical granules near the periphery of the oocyte (which in early literature were referred to as yolk vesicles, e.g., Yamamoto 1956; Yamamoto and Yamazaki 1961).The term ''yolk vesicle'' has since been replaced with the more appropriate term cortical granule (Selman et al. 1988; Selman and Wallace 1989; reviewed in West 1990; Takashima and Hibiya 1995) and the yolk vesicle stage with the cortical alveoli phase to describe oocytes defined as stage III by Schwarz and Smith (1990).

Stage IV oocytes viewed in *D. aruanus* were much larger than stage III cells, exhibiting approximately a fourfold increase in diameter. The nucleus was similar in diameter to that of stage III oocytes. Consequently, the increase in oocyte size was primarily due to the formation and accumulation of eosin-staining vitelline granules that now made up the majority of the cell volume (see Fig. 1A, D). The chorion surrounding the stage IV oocytes was much more prominent (see Fig. 1A). In addition, microvilli arising from and extending between follicle cells and the oocyte plasma membrane were more prominent in stage IV oocytes, being both conspicuous and abundant, particularly in sections in which the plane of cut was parallel to the follicular layer (Fig. 1D). Stage IV oocytes as described here and in Schwartz and Smith (1990) are equivalent to primary, secondary, and tertiary yolk stage oocytes (Yamamoto 1956; Yamamoto and Yamazaki 1961; Yamamoto et al. 1965; Nagahama 1983) and vitellogenic phase oocytes (Wallace and Selman 1990; West 1990; Takashima and Hibiya 1995).

Stage V oocytes, as described in Schwartz and Smith (1990), are equivalent to the migratory nucleus stage (Nagahama 1983), pre-maturation stage (Yamamoto and Yamazaki 1961), or maturation stage or phase (Wallace and Selman 1990; Takashima and Hibiya 1995) and consist of large mature oocytes in which the vitelline vesicles have coalesced to form a sheet-like appearance.

No stage V oocytes were seen in ovaries viewed in this study.

Of 57 *D. aruanus* collected in Papua New Guinea and classified as female, 16 females (28%) had ovaries containing at least some vitellogenic (i.e., stage IV) oocytes. Ovaries containing at least some vitellogenic oocytes were classified as "active" and females having active ovaries were classified as adult, on the basis of inferred capacity for the production of mature ova. Among adult females, 7 had only a small number of early-stage vitellogenic oocytes and were likely in an early phase of the ovulation cycle. Another 4 females had moderate numbers of vitellogenic oocytes, many of which had considerable amounts of vitelline accumulations, while among the remaining 5 females, the ovigerous tissue predominantly comprised late-stage vitellogenic oocytes. The smallest female having vitellogenic oocytes was 19 mm SL, indicating that this is likely the lower size limit for first maturity. However, the majority of adult females ($n = 13$) were 25 mm SL or larger.

The remaining 41 (72%) females had ovaries having stage I–III oocytes, but no vitellogenic (i.e., stage IV or V) oocytes and therefore were classified as inactive females. Based on body size (see following section on colony demography), many were probably immature. Fully 21 of the 41 inactive females were less than 19 mm SL (Table 1). Another 17 females were 19–24 mm SL, while only 3 individuals were larger than 24 mm SL. Some or many of the larger females may have been functional adults but simply been at a resting stage in their ovulation cycle. For this reason, the term "inactive" was used to describe this gonadal state rather than "immature" to avoid inferences regarding capacity for sexual function.

Testis features of *D. aruanus*

Of 93 *D. aruanus* collected from Papua New Guinea, 5 individuals had completely testicular gonads, with no recognizable female germ cells (i.e., oocytes of any developmental stage) or ovarian stromal tissue, and were classified as male. In all cases, spermatozoa were also present, indicating that these were functioning adult males. Testis structure and internal features were similar for all 5 males. The paired testis lobes were roughly triangular in cross section (Fig. 2A). Each lobe consisted of a compact body of spermatogenic tissue that was directly apposed to the thin testis wall medially but separated from the remaining portion of the testis wall by a narrow, afunctional gonadal lumen along a substantial portion of the testis periphery (Fig. 2A, B). Large, irregularly shaped, interconnected sperm sinuses lined with a simple squamous epithelium and confluent with the testis tubules were present along the medial margin of the lobe on the opposite side of the testis from the afunctional lumen and typically were filled with free spermatozoa (Fig. 2A). Toward the midpoint of the medial sperm sinus region, a large gonadal vein and smaller-diameter gonadal artery were present (Fig. 2A). Based on their size, these were the principal incoming and outgoing blood vessels of the testis lobe. Almost immediately upon its exit from the testis lobe, the large-diameter gonadal vein united with the adjacent renal vein draining the trunk kidney.

Thin-walled channels making up much of the testicular tissue took the form of tubules radiating outward from the medial sperm sinus region to the periphery of the spermatogenic tissue immediately adjacent to the gonadal lumen (Fig. 2A, B). The cells of the tubule walls were cuboidal or polyhedral in cross section and in some regions the outer boundary was clearly delineated by a basement membrane (Fig. 2C), although in other areas boundaries between adjacent tubules were indistinct. In addition, some tubules had a distinct lumen whereas in others the lumen was either compressed and indistinct or not visible (Fig. 2C). Tubule lumina were most conspicuous in tubules having large amounts of free spermatozoa present but were also clearly visible in some regions having no contained sperm. In each histological section, some tubule lumina were continuous medially with a sperm sinus (Fig. 2B). At the peripheral margins of the testis, those tubules having distinct outer wall boundaries visible appeared to have a blind-ended terminus (Fig. 2A, B).

The tubules, referred to as testis tubules (Nagahama 1983; Grier 1993), contained developing male germ cells. These germ cells occurred in small clusters or groups enclosed by a surrounding membrane to form spermatocysts. All germ cells within a single spermatocyst appeared identical, indicating synchronous development. Spermatocysts were embedded within tubule walls but their distribution, based on developmental stage of contained germ cells, was not uniform (Fig. 2C) and spermatocysts at different developmental stages appeared at all locations along the length of the testis tubules. Spermatocysts containing early-stage spermatocytes were characterized by the presence of large, purple-staining nuclei spaced apart from one another (presumably by nonstaining cytoplasm) by a distance of approximately one nucleus diameter, giving the spermatocyst a scattered and granular appearance (Fig. 2C). Spermatocysts with early-stage spermatocytes tended to occur most commonly in the central region of tubules, midway between the medial and distal regions, but also could be found with less frequency close to the distal terminus as well as close to the medial sperm sinus region. Clusters of maturing spermatocytes were characterized by the presence of a larger number of smaller, purple-staining nuclei densely packed together (indicating reduced amounts of surrounding cytoplasm), giving the spermatocyst a densely granular appearance (Fig. 2C). Spermatocysts containing maturing spermatocytes tended to occur away from the middle region of the tubules toward both ends (i.e., close to the sperm sinus and also close to the distally located blind end) but could also be found in central portions of the tubule. Spermatocysts containing late stage spermatids or not-yet-released spermatozoa were characterized by the

Table 1 Size (standard length, in millimeters), within-colony size rank (in brackets), and gonad morphology classification (based on histostructure) for individuals making up nine *Dascyllus aruanus* colonies. M_a acTive male with spermatogenic testis; H_{Ma} hermaphrodite with spermatogenic ovotestis; H_i hermaphrodite with inactive ovotestis; F_a active female with oogenic ovary; F_i inactive female with inactive ovary; U individual with undifferentiated gonad (refer to text for detailed descriptions of gonad classes)

Colony	M_a	H_{Ma}	H_i	F_a	F_i	U
1	34 (1)	30 (2)	29 (3)	28 (4.5)	23 (7)	23 (7)
			25 (5)	28 (4.5)	23 (7)	14 (17)
					22 (10)	13 (18.5)
					21 (11.5)	12 (20)
					21 (11.5)	8 (21)
					20 (13)	
					18 (14)	
					16 (15)	
					15 (16)	
					13 (18.5)	
2		34 (1)			21 (4)	
		27 (2)			20 (5)	
		24 (3)			18 (8)	
		19 (6)			18 (8)	
					18 (8)	
					17 (10)	
					16 (11)	
3	33 (1)	20 (6.5)		29 (2)	18 (9)	19 (8)
				28 (3)	17 (10)	
				25 (4)		
				23 (5)		
				20 (6.5)		
4					18 (1)	13 (4.5)
					17 (2.5)	12 (6)
					17 (2.5)	9 (7)
					13 (4.5)	8 (8)
						7 (9)
5		22 (1)		19 (4)	21 (2)	11 (7)
		20 (3)			17 (5)	
					14 (6)	
6	39 (1)	32 (3)		34 (2)		8 (6.5)
				30 (4)		8 (6.5)
				26 (5)		
7	37 (2)	40 (1)		34 (3)		
		24 (6)		32 (4)		
				31 (5)		
8	39 (1)			28 (2)	25 (3)	
					20 (4)	
					19 (5)	
					18 (6)	
9		22 (1)	21 (2)		19 (3.5)	19 (3.5)
						11 (5)
10				34 (1)	30 (2)	
					27 (3)	
					23 (4)	
11					21 (1)	
					18 (2)	
					17 (3)	
					12 (4)	
12					20 (1)	
					19 (2)	
13					21 (1)	

presence of large numbers of closely spaced, intensely blue-staining nuclei and pale purple-staining flagella. Free spermatozoa present within the tubule lumina frequently were concentrated in the most distal portions of the tubules, as well as in regions adjacent to the medial sperm sinuses (Fig. 2B).

Ovotestis features of *D. aruanus*

Of the 93 *D. aruanus* examined, 15 individuals (19–40 mm SL) had an ovotestis containing both testicular tissue and either ovarian tissue consisting of oocytes embedded within a typical ovarian stromal tissue matrix and having an ovarian lamellar architecture; or stage I and II oocytes intermixed with testicular tissue. Ovotestes were further classified as early, intermediate, or late stage, according to the extent and degree of development of testicular tissue and male sex cells.

Among three individuals (21–29 mm SL) having an early-stage ovotestis, the architecture of the gonad was typically ovarian, with a lamellar configuration to the gametogenic tissue and a prominent gonadal lumen. In addition, the gametogenic tissue consisted of both oocytes and clusters of intensely staining male germ cells within developing spermatocysts that were typically

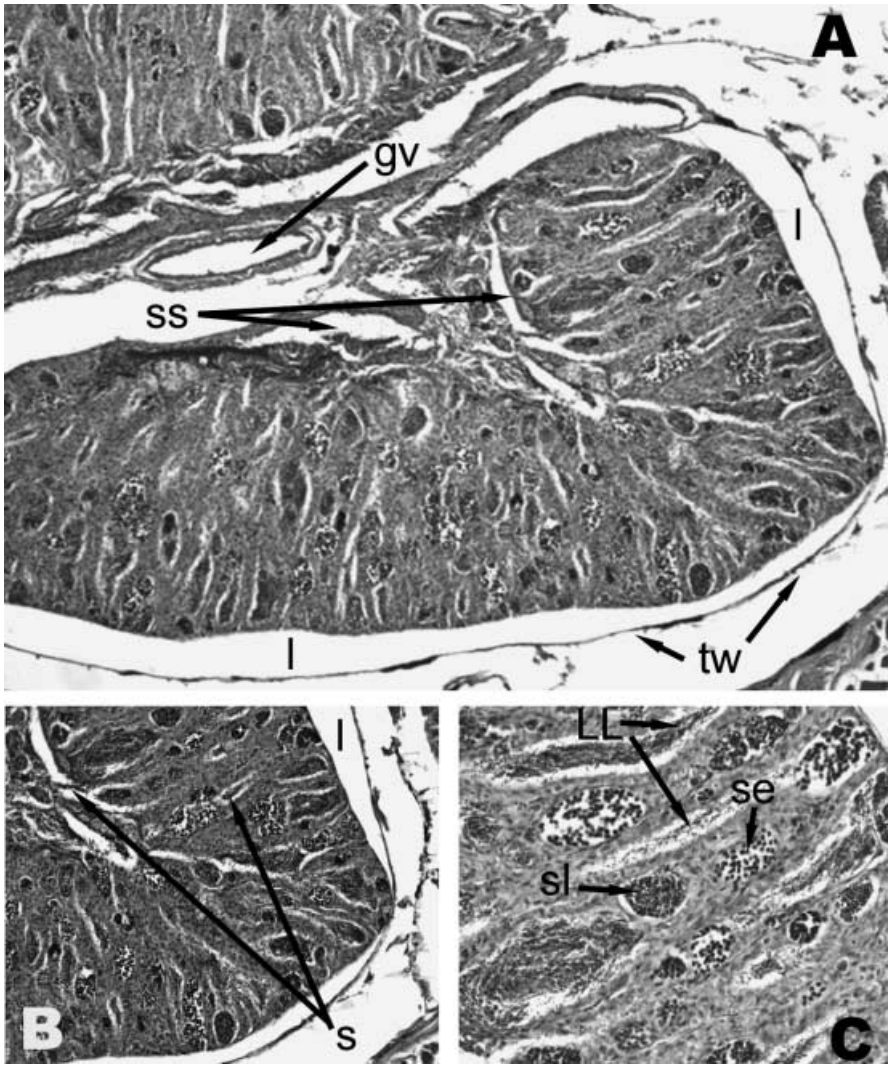

Fig. 2A–C Testis structure and features of *D. aruanus*. **A** Testis lobe showing prominent and extensive afunctional lumen (*l*) located directly beneath the testis wall (*tw*), radiating channels formed by testis lobules that empty into sperm sinuses (*ss*), and prominent gonadal vein (*gv*). **B** Lobule lumina filled with free spermatozoa (*s*). **C** Detail of testis lobules showing lobule lumina (*LL*) containing spermatozoa, and lobule walls containing spermatocytes, some containing early-stage spermatocytes (*se*) characterized by widely-spaced, strongly staining nuclei, and others containing late-stage spermatocytes (*sl*) characterized by larger numbers of more closely spaced sex cells

concentrated along the outer and/or inner margins of the gonad. No spermatozoa were present and no tubule or sperm sinus system was yet fully formed, although elongated openings or gaps were frequently visible in the locations and orientations typically occupied by testis tubules and/or peripheral sperm sinuses.

Among seven individuals (size range 19–27 mm SL) having an intermediate-stage ovotestis, the extent of testicular tissue making up the gonad varied but all stages of male sex cells, including free spermatozoa, were present. In one individual (24 mm SL), the small ovotestis still exhibited a central lumen and lamellar configuration to the gametogenic tissue and stage II (i.e., perinucleolar stage) oocytes made up the majority of the gonadal tissue. Spermatogenic tissue was confined to two relatively small peripheral regions, an inner medial region, and an outer lateral region. Small amounts of spermatozoa were also present (hence the designation of ''intermediate ovotestis'') but were confined to sinuses in the outer peripheral region. In another individual (22 mm SL), the ovotestis was small with developing spermatocysts and moderate quantities of spermatozoa in the sperm sinuses and some tubule lumina but also had a prominent afunctional (assumed to be previously ovarian) lumen and numerous opaque, stage I oocytes (Fig. 3A). In a third fish (20 mm SL) the ovotestis was unusually small in cross section. In anterior regions, peripheral sperm sinuses were filled with spermatozoa (although testis tubule boundaries were frequently poorly delineated due to the intermittent lack of a prominent basement membrane) and the central region of the gonad was predominantly composed of medium-sized opaque oocytes. In more posterior regions, spermatozoa-filled sperm sinuses and occasional opaque, medium-sized oocytes made up most of the ovotestis periphery, while an abundance of somatic cells, many of which did not appear to be organized yet into tubules, made up most of the central region. In another individual (27 mm SL), the ovotestis had a large central lumen surrounded on all sides by a developing radially arranged tubule system and peripheral sperm sinuses. Substantial numbers of spermatozoa were present in the peripheral sinuses and numerous healthy-looking stage I and II oocytes were also present. In the remaining in-

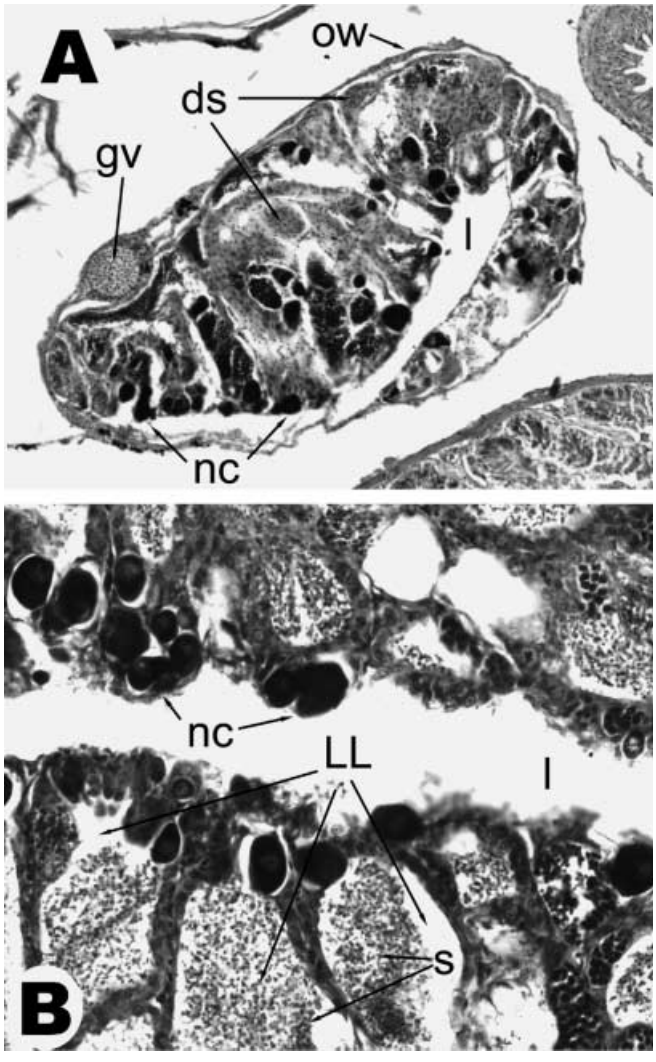

Fig. 3A, B Ovotestis structure and features of *D. aruanus*. **A** Intermediate-stage ovotestis with residual ovarian architecture including relatively thick ovotestis wall (*ow*), a centrally located afunctional lumen (*l*) bordered by chromatin nucleolar phase oocytes (*nc*), as well as developing spermatocysts (*ds*) and spermatozoa-filled sperm sinuses located close to the gonadal vein (*gv*). **B** Detail of late-stage ovotestis showing numerous chromatin nucleolar phase oocytes (*nc*) bordering the afunctional lumen (*l*), and distal ends of testis lobule lumina (*LL*) filled with free spermatozoa (*s*) and bordered by somatic cells and spermatocysts

dividual with an intermediate-stage ovotestis (22 mm SL), the ovotestis was predominantly made up of testis tubules arranged in a typical radiating configuration with tubules running longitudinally (anterior to posterior) in the central region and transversely (extending inward) along the lateral margins. Stage I and II oocytes were also present and were concentrated along the periphery of the afunctional gonadal lumen (Fig. 3B), and abundant spermatozoa were visible in both the tubule lumina (Fig. 3B) and the sperm sinuses. The elongated, apparently afunctional gonadal lumen located on the side opposite the sperm sinuses and found in the same position as the ovarian lumen in functional ovaries (based on placement relative to the gonadal vein and artery) is presumed to be a former ovarian lumen retained from either a juvenile ovariform gonad or possibly a previously functioning ovary.

Among five individuals (20–40 mm SL) having a late-stage ovotestis the gonad was essentially testicular in both overall architecture, consisting of medial sperm sinuses and radiating testis tubules, and also in the predominance of various stages of developing male germ cells as well as an abundance of free spermatozoa. However, in all these individuals there was also the occasional presence of small opaque oocytes intermixed with the testicular tissue and the presence of an afunctional gonadal lumen in a location typically occupied by the ovarian lumen in functional ovaries.

Colony composition based on gonad function

Among the *D. aruanus* sampled in this study, 16 of 93 individuals collected had an undifferentiated gonad (Table 1), most of which were small individuals. Sexual differentiation appears to occur at 12 mm SL or larger since all fish < 12 mm SL had an undifferentiated gonad. However, a small number ($n = 3$) of substantially larger individuals also had an undifferentiated gonad (Table 1). Among the remaining fish having differentiated gonads, females made up the majority (74% of 77 fish). Of these, 72% (size range12–30 mm SL, mean 19 mm SL; $n = 41$) had an inactive gonad containing only pre-vitellogenic oocytes. Maturation, as judged by the earliest presence of vitellogenic (i.e., stage IV) oocytes, was most common among a small number of larger females (size range 19–34 mm SL, mean 28 mm SL; $n = 16$); 19 mm SL appeared to be the lower size limit for female maturity in this population (Table 1).

Only a small number of sexually differentiated individuals sampled (6% of 77) were functional males having a completely testicular gonad. These individuals were typically the largest in their respective social groupings (size range 33–39 mm SL, mean 36 mm SL; Table 1). However, not all functional males, as judged by the presence of free spermatozoa in the testis lobules and sperm sinuses, had a testis. The remaining sexually differentiated individuals (19% of 77) were hermaphrodites having an ovotestis. The majority of these (80% of 15) likely were competent to function as males, as judged by the presence of free spermatozoa in their ovotestes, and were classified as male-active hermaphrodites. The remaining three individuals had an ovotestis with no maturing sex cells of either sex and were classified as inactive hermaphrodites. Hermaphrodites tended to be similar in size range and size distribution to adult females (size range 19–40 mm SL, mean 26 mm SL; Table 1).

In terms of size rank, adult males were typically the largest within reproductive colonies [mean size rank (MSR) = 1.2, $n = 5$; Table 1]. This was followed by adult females (MSR = 3.7, $n = 16$), hermaphrodites (MSR = 4.9, $n = 15$), inactive females (MSR = 8.5, $n = 41$), and gonadally differentiated individuals (MSR =

9.4, $n = 16$). The lower MSR for hermaphrodites, compared to adult females, reflected the presence of some relatively low-size-ranked individuals (see Table 1) since 80% of the 15 hermaphrodites were either the largest, second-largest, or third largest individual within their respective colonies. Although individuals having an ovariform gonad made up the majority of fish within colonies, the functional (i.e., adult) sex ratio, across all collected social groups, was approximately 1:1 for females ($n = 16$) relative to males plus male-active hermaphrodites ($n = 17$).

Gonadal sex of experimental *D. aruanus*

The fish collected from Malakal Island, Palau, constituting one large aggregation, ranged in size from 16 to 50 mm SL. Once the body cavity of the euthanatized individual largest in the group was opened, the gonad could be inspected visually. In gross morphology, the testis of *D. aruanus* was white with an opalescent sheen to the thin testis wall and the spermatogenic tissue had a fine, grainy appearance. The ovary was more opaque, with a cream to yellow color, and had a smooth appearance in small ovaries, or a rough, pebbled appearance in more developed ovaries. The first 6 largest fish (size range 44–50 mm SL) serially removed were found to be male, based on gross gonadal appearance. The next 2 largest individuals (38 and 41 mm SL, respectively) were both female. The remaining 30 individuals, ranging in size from 16 to 38 mm SL, were separated into five groups of 6 fish, each consisting of 1 or more individuals measuring in the low to mid-20s (mm SL), the mid- to high 20s (mm SL), the low to mid-30s (mm SL), and the mid- to high 30s (mm SL).

At the end of 6 weeks, three groups still had six individuals, while the other two groups had only three fish left in each. Histological examination of the gonad in the three largest fish in each group showed that four of the five groups had two females and one male. The fifth group, which was one of the two groups in which only three fish remained, consisted entirely of females. Size rank of the four males varied from largest ($n = 1$, 39 mm SL) to second largest ($n = 2$, 33 and 37 mm SL, respectively) to third largest ($n = 1$, 35 mm SL). In all four males the gonad was entirely testicular.

Discussion and conclusions

Ovarian morphology in *Dascyllus aruanus* is similar to that of many other teleosts. The interior of each ovarian lobe consists of a medial lumen surrounded on three sides by numerous ovigerous lamellae. Once mature, oocytes are released into the lumen and pass through a common oviduct to the outside of the female's body, at the time of oviposition. As with other described teleosts, oocyte stages are characterized by a combination of features associated with the nucleus, the staining properties of the cytoplasm, the formation of a chorion and microtubules, and the relative size of the developing oocyte. Approximately three-quarters of all fish having a sexually differentiated gonad had an ovary, and of these, the large majority were sexually inactive and probably not yet mature.

Testis morphology tends to be more variable among teleosts. In the past, there has been some confusion regarding the classification of different types of teleost testes, due to the usage of similar terminology for differing testis morphologies. Currently, teleost testes generally fall into one of three categories. In one form of testis, spermatocysts make up part of the walls of narrow, elongate, roughly cylindrical structures surrounding a central lumen. The form of these elongate structures is tubular and the tubules extend from a localized sperm collection sites (usually located medially) out to the periphery of the testis, then loop back so that both ends of the tubule can directly discharge sperm into the sperm collection site. This testis type is referred to as an anastomosing tubular testis and appears most common in some of the lower bony fishes (Nagahama 1983; Grier 1993).

In the remaining two testis types, the tubular structures bearing spermatocysts, instead of being looped and continuous, are blind-ended at the testis periphery and therefore are more properly termed lobules (Grier 1993). One type of lobular testis is referred to as a restricted spermatogonial lobular testes (Grier et al. 1980; Grier 1981) and is equivalent to the so-called tubular testis of Nagahama (1983). In the restricted spermatogonial testes, the lobule lumen does not extend the full length of the lobule but, instead, is restricted to the proximal portion. The distal region of each lobule is completely cellular and consists of stacked spermatocysts that are arranged in a developmental series, also referred to as the zone of spermatocysts (Downing and Burns 1995). Spermatocysts containing spermatogonia are located most distally in the zone of spermatocysts, while spermatocysts containing spermatocytes, spermatids, and newly transformed spermatozoa, respectively, are situated progressively closer to the proximal region of the lobule, directly adjacent to the lumen. Therefore, in restricted spermatogonial testes, only spermatocysts containing spermatozoa are directly adjacent to the lobule lumen into which sperm can be shed following rupture of the spermatocyst wall (Grier 1993). In addition, in the proximal portion of the lobule of the restricted spermatogonial testis the lobule walls consist solely of somatic cells that are probably derived from bipotential Sertoli cells (Grier 1993). This region of the lobule, which contains no germ cells, is referred to as the efferent duct and functions to provide a conduit for shed sperm to be transported toward major collection channels of the testis (Grier 1993). This testis type appears to be restricted to the atheriniformes (Grier 1981).

The other lobular testis type, which appears to be most common in higher teleosts, is referred to as the unrestricted spermatogonial lobular testis (Grier 1992, 1993) and is equivalent to the lobular testis of Naga-

hama (1983). In this testis type, the central lumen extends the full length of the lobule and is surrounded by a single layer of spermatocysts containing germ cells in various stages of development. Unlike the restricted spermatogonial testis, there is no obligate pattern of distribution of spermatocysts depending on the stage of development of contained germ cells since all spermatocysts are directly adjacent to, and can release spermatozoa directly into, the lobule lumen (Grier 1992, 1993). The presence and extent of efferent ducts varies among teleosts having an unrestricted spermatogonial testis (Grier 1993).

In terms of lobule architecture and arrangement of spermatocysts, *D. aruanus* has an unrestricted spermatogonial testis in which the lobular component consists of both spermatocysts and somatic cells that surround a central lumen and are distributed along the full length of the lobular wall. In *D. aruanus*, the testis lobules exhibit a linear, nonanastomosing pattern of distribution in which radiating channels arise from the region of the sperm sinuses located on the medial side of the testis and extend toward the alternate side of the gonad periphery. The central, elongate lobular lumina are surrounded by a single layer of spermatocysts containing germ cells in various stages of development, with no rigid spatial arrangement of spermatocysts depending on their developmental stage. All of the spermatocysts are directly adjacent to the lobule lumen and upon maturation of spermatozoa, spermatocysts release sperm into the lobular lumen and on into an adjacent sperm sinus. However, there is no apparent distinction in *D. aruanus* between a germinal lobule region (i.e., where spermatocysts are intermixed with somatic cells) and an efferent duct region that, as defined by Grier (1993), contains no germ cells. The frequent presence of spermatocysts in portions of the lobule wall directly adjacent to its confluence with a sperm sinus suggests that no such distinction between germinal and nongerminal portions of the lobule system exists in *D. aruanus*. Such a distinctive testis feature may be a synapomorphy for the genus.

While many of the testis features in *D. aruanus* were similar to that of an unrestricted spermatogonial testis there was also an additional unusual feature in the form of a prominent, afunctional gonadal lumen located on the side of the testis opposite to the medial sperm sinuses. This lumen was quite extensive, ranging along a substantial portion of the testis perimeter (as illustrated in Fig. 2A). This feature appears to be a remnant of the original ovariform architecture of the juvenile (or possibly adult) gonad. Because of its presence, the distal ends of the testis lobules do not extend all the way to the gonadal wall as is the case for most teleosts exhibiting unrestricted spermatogonial testis morphology. Instead, most of the testis lobules terminate at the boundary of the afunctional lumen. This testis condition is similar to that described for secondary testis morphology in some protogynous serranids in which the lobules of testes in secondary male serranids, which take their overall arrangement from the former ovarian lamellar pattern, terminate along the surfaces of male lamellae (Grier 1993). This form of teleost testis has been termed an "inverted lobular testis" by Grier (1993). In *D. reticulatus*, which is also reported to be a protogynous hermaphroditic species, males also exhibit an afunctional gonadal lumen (Schwarz and Smith 1990) similar to that described for *D. aruanus*.

Within *D. aruanus*, the most variable gonad morphology was found among hermaphrodites having an ovotestis. Among these individuals, which encompassed a considerable size range, differences in ovotestis morphology was characterized by the progressive reduction of solid-bodied lamellae of ovigerous tissue and their eventual replacement by germinal lobules consisting of intermixed spermatocysts and somatic cells making up the lobule walls and surrounding an elongate central lumen. In early-stage ovotestes, healthy-appearing stage I and II oocytes were abundant along the margins of the former ovarian lumen, and the gonad architecture, including the placement of the former ovarian lumen, was still essentially ovariform. However, with the progressive development of testicular lobules, the central region of the lumen became replaced by a compact, uniform mass of gametogenic tissue consisting of testis lobules. In individuals having a late-stage ovotestis, the only indications of former ovariform morphology was the persistent retention of an afunctional gonadal lumen and the presence of small numbers of scattered, small, opaque oocytes.

Colony composition, based on collections of a number of cohesive social groups in their entirety and subsequent classification of individuals according to histologically determined gonad function, revealed a consistent pattern of population demography within groups. In large groups, the majority of individuals had an inactive ovariform gonad. These individuals were almost always smaller than individuals having gametogenic gonads (including mature ovaries and both spermatogenic ovotestes and testes) and many of these small individuals were likely juveniles. Over half of all sexually differentiated individuals fell into this category. However, the presence of some females having an inactive ovary that were substantially larger than some of the adult fish in other colonies suggests the possibility of some form of social regulation of gonad function within *D. aruanus* colonies. Such an absence of adult function in some large females having inactive gonads may reflect delayed maturation, or suppressed sexual function, or both. Among smaller fish having an inactive gonad, no juvenile males having a purely testicular gonad were found. The smallest individuals capable of producing sperm were hermaphrodites with a male-active ovotestis and were at least as large as the smallest mature females.

The size distribution of individuals having either an ovariform gonad, ovotestis, or testis indicates that sexual development in *D. aruanus* is hermaphroditic in gonad morphology, if not in gonad function. It is interesting to note that the functional sex ratio across all collected social groups was approximately 1:1 for females ($n = 16$): individuals with male function (i.e., males

plus male-active hermaphrodites; $n = 17$). The combination of these two features, namely an hermaphroditic sequence to gonad ontogeny coupled with a 1:1 functional sex ratio, suggests one of two possible sexual patterns for *D. aruanus*.

One possibility is that *D. aruanus* exhibits a modified diandric protogynous sexual pattern in which males develop either directly from a juvenile with an ovariform gonad, or indirectly following a functional female phase. Should the current classification of *D. aruanus* as a functional protogynous hermaphrodite (Fricke and Holzberg 1974; Coates 1982) stand, the absence of juvenile males, coupled with a predominance of pure females (juvenile and adult) in which there were approximately three females to every individual having adult male function (male and hermaphrodite) supports the possibility for a modified diandric functional hermaphroditic sexual pattern in *D. aruanus*. If *D. aruanus* is a diandric protogynous hermaphroditic species, the broad size range of hermaphrodites having ovotestes in varying stages of ovarian reduction and loss indicates that the development of testicular tissue may occur either shortly before maturation of female function, or at some time following the development of mature ovarian function. A similar finding has been reported for *D. reticulatus*, in which individuals having an ovotestis occupied varying size ranks within their respective social groups, and individuals developed ovotestes in the presence of larger, mature females (Schwarz and Smith 1990). Schwarz and Smith's (1990) conclusion was that neither size rank nor absolute size was important in *D. reticulatus* for determining when females might start to develop testicular tissue and transform into a male in what they assumed to be a functionally sex-changing species.

However, an alternative interpretation of the findings of Schwarz and Smith (1990) for *D. reticulatus* and the results reported here for *D. aruanus* is that approximately half of all juveniles develop testicular tissue and develop into males without ever functioning as a female, regardless of surrounding social conditions. Such an ontogenetic sequence in *D. aruanus* would be consistent with the finding for sex ratios of adults having female versus male function being roughly equal across all colonies. Moreover, if the transformation directly from an inactive ovariform gonad to a testis is accompanied by an unusually prolonged retention of ovarian tissue and architecture, this will result in a large number of male-active individuals also having an ovotestis. Under these circumstances, gonadal transformation from ovary to ovotestis to testis would not involve functional sex change. If this is the case, *D. aruanus* is functionally gonochoric and all individuals function either as male or female, but not as both. Under these circumstances, sexual ontogeny would involve one of two developmental pathways following the initial development of an ovariform gonad. Individuals would either retain the juvenile ovariform gonad and mature as adult females, or develop testicular tissue at some time following initial ovariform gonad differentiation, but prior to ovarian maturation, and subsequently function as a male without ever going through an adult female phase. Under these circumstances, hermaphrodites having an ovotestis are simply functional males that have not yet replaced or catabolized ovarian tissue.

However, if *D. aruanus* is developmentally hermaphroditic but functionally gonochoric, what makes this species unusual among fish species that have ovotestes at intermediate life stages is the prolonged period during which apparently healthy gonadal tissue of both sexes is present in the gonad, as evidenced by the large size of some hermaphrodites having spermiated ovotestes. Gonad morphology in *D. aruanus* has a number of features traditionally characterized as typical of functionally hermaphroditic teleost fishes (Sadovy and Shapiro 1987) including retention of oocytes (at least for a period of time) and the apparently permanent retention of an ovarian-type lumen. An examination of population structure revealed that gonad ontogeny initially follows an ovarian track such that all juveniles are gonadally female. The development of testicular tissue occurred no sooner than the size of first maturity for females. As discussed above, if all males develop an ovotestis prior to developing a mature ovary and therefore never function as a female, then *D. aruanus* is functionally gonochoric. However, two findings here suggest that this is not the case.

First, the broad size range of male-active hermaphrodites – including some very large individuals – having retained ovarian features, including oocytes, suggests that at least some of these individuals may have functioned as an adult female prior to ovotestis development. In addition, the presence of one and only one male among the three largest fish in four of five putatively initially all-female experimental groups supports the contention that *D. aruanus* has the capacity to shift from female to male function under favorable circumstances. Such a result is unlikely to have occurred by chance alone but rather supports the existing contention (Fricke and Holzberg 1974; Coates 1982; Shpigel and Fishelson 1986) that *D. aruanus* is a protogynous hermaphroditic species. The fact that the size rank of males within experimental groups was highly variable suggests that a variety of factors may play a role in determining which individual within a social group will ultimately change sex.

Hermaphroditism has been reported for a number of fish taxa that form obligate associations with corals or sea anemones. These include several species of *Dascyllus* (Fricke and Holzberg 1974; Shpigel and Fishelson 1986; Schwarz and Smith 1990), numerous species in the pomacentrid subfamily Amphiprioninae (Fricke and Fricke 1977; Moyer and Nakazono 1978; Fricke 1979, 1983), a number of species in the goby genera *Gobiodon* (Nakashima et al. 1996; Munday et al. 1997, 1998; Cole and Hoese 2001) and *Paragobiodon* (Kuwamura et al. 1993, 1994), and at least one species in the scorpaeniform family Caracanthidae (K.S. Cole, in preparation).

All of these species share a number of traits that may provide strong selective pressures for the evolution of hermaphroditism. Among small *Dascyllus* species, individuals form cohesive social groups and rely on the protection of host corals to avoid predation. When such corals are relatively contiguous over large areas, individuals are capable of extensive movements, but where such corals are patchily distributed and relatively isolated, individuals may be limited in their ability to move over long distances (Schwarz and Smith 1990). Under such conditions, mate availability may be limited to existing reproductive colony members and dependent upon the recruitment of new individuals to social groups through settlement events. Such recruitment may be highly episodic and unpredictable. For example, among colonies of *D. aruanus* and *D. marginatus* followed in the Red Sea, although recruitment of newly post-metamorphic juveniles to existing *Dascyllus* colonies was relatively high in the month following seasonal repro-duction, most new recruits disappeared, reflecting fairly high mortality rates (Sphigel and Fishelson 1986). For fish species having limited mobility and unpredictable recruitment of new colony members, flexibility in the development of male function, as suggested for *D. aruanus*, would be highly adaptive.

The apparent prolonged period of time over which ovariform architecture and healthy-appearing oocytes are retained alongside sperm-producing testicular tissue in *D. aruanus* suggests the further possibility that adults having an ovotestis may also have the capacity to reallocate between male and female function, depending on the current social conditions. Such a capacity for serial shifts between male and female function (i.e., bidirectional sex change) has been demonstrated in a number of fish taxa, including the gobiid genera *Trimma* (Sunobe and Nakazono 1993), *Gobiodon* (Nakashima et al. 1996; Munday et al. 1998), *Paragobiodon* (Kuwamura et al. 1994; Nakashima et al. 1995), and *Lythrypnus* (St. Mary 1993, 1996) and also in a species of anemonefish in the genus *Amphiprion* (Kuwamura and Nakashima 1998). A number of these include small, obligate coral-dwelling fish species such as *G. histrio* (Munday et al. 1998), *G. micropus, G. oculolineatus, G. quinquestrigatus*, and *G. rivulatus* (Nakashima et al. 1996) and the possibility of a similar sexual pattern has been inferred for *G. okinawae* (Cole and Hoese 2001). It has been suggested that a combination of limited mobility between corals and the restriction of reproduction to a single mated pair in any one coral colony has selected for bidirectional hermaphroditism in many of these species (Nakashima et al.1996; Munday et al. 1998).

In hermaphroditic fish species having unidirectional sex change, the sex ratio of adults tends to be biased toward the primary sex in many monandric fish species (Sadovy and Shapiro 1987) as well as among a number of diandric gobiid species (Cole and Robertson 1988; Cole and Shapiro 1992). However, among fish species having the capacity for bidirectional sex change the functional sex ratio has been found to be approximately 1:1 (Kuwamura et al. 1993, 1994; Nakashima et al. 1996; Munday et al. 1998). In this study, individuals having male function (i.e., males and male-active hermaphrodites) and female function (i.e., adult females) occurred with similar frequency. Consequently, in terms of functional sex ratio, *D. aruanus* conforms to a sex ratio pattern consistent with bidirectional sex change. In terms of ovotestis structure, the apparent prolonged period during which male-active hermaphrodites retain healthy-appearing oocytes and the former ovarian lumen is also consistent with a capacity to shift back and forth, at least during the hermaphroditic stage, between male and female function. Given that *D. aruanus* is currently placed in the basal species complex of the genus (Godwin 1995; Bernardi and Crane 1999) and, along with *D. melanurus*, another putative hermaphrodite (K.S. Cole, unpublished data), form close obligate associations with coral, it appears that labile development and/or expression of sexual function coupled with an obligate coral-dwelling lifestyle are ancestral traits within the genus. With increasing body size, (i.e., members of the reticulatus complex) obligatory associations with patchily distributed corals have become somewhat less pronounced, particularly for *D. flavicaudus* (Allen 1991) but hermaphroditic sexual patterns (or at least hermaphroditic gonad morphology) have been retained (e.g., Shpigel and Fishelson 1986; Schwartz and Smith 1990; Godwin 1995). Among the largest *Dascyllus* species (i.e., members of the trimaculatus complex), obligate associations with isolated corals or anemones are restricted to juvenile stages for *D. trimaculatus* and *D. albisella* (Allen 1991) and both species appear to be functionally gonochoristic (Godwin 1995). Unfortunately, little information on the ecology and sexual pattern of the third species in this complex, *D. strasbugi*, is available. The known patterns of lifestyle and sexual function for members of the trimaculatus complex suggests that reduced obligate associations with patchily distributed substrate is coupled with a loss of hermaphroditic expression and represents a more recent development within the genus (Godwin 1995; Bernardi and Crane 1999). While the exact nature of the sexual pattern of *D. aruanus* remains unclear, evidence here supports the current classification of sequential functional hermaphroditism (i.e., protogyny) with a possible intermediate stage in which sexual function may shift in either a female or male direction. Future studies linking sexual ontogeny and maturation processes, colony composition, and reproductive biology will be necessary to resolve the exact nature, and regulation, of hermaphroditic sexual function in this and other *Dascyllus* species.

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