Life history of a simultaneously hermaphroditic fish, 
*Diplectrum formosum*

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Sand perch *Diplectrum formosum* were collected in the Atlantic Ocean waters off the south-eastern U.S. for life history analyses between April 2001 and July 2004 and ranged in size from 63 to 236 mm standard length ($L_S$) and 0–8 years of age. *Diplectrum formosum* are simultaneous hermaphrodites that reach 50% sexual maturity at 12.2 months and 122.8 mm $L_S$ for testicular tissue and 13.6 months and 129.3 mm $L_S$ for ovarian tissue. The gonad contains ovarian and testicular tissue separated by a thin basement membrane, with no means of internal self-fertilization. Spawning females were found between March and January with a peak of spawning activity in May and a realized spawning periodicity of 2 days, equivalent to a maximum of 168 spawning events per year. Fish with testicular tissue in spawning condition were obtained throughout the year in every month sampled with a maximum frequency between June and September. There were also trends indicating that testicular tissue was more likely to be in spawning condition in the presence of hydrated oocytes within the accessory structure. This may indicate the use of the accessory structure as a storage site for hydrated oocytes until a mate can be located.

Key words: accessory structure; maturity; ovotestis; Serranidae; spawning.

INTRODUCTION

Simultaneous hermaphrodites are faced with issues that do not pertain to gonochoristic or sequentially hermaphroditic fishes. These issues are due to physiological and energetic stresses incurred in production and maintenance of both sex tissues (Roff, 1983), as well as behavioural dilemmas, such as self-fertilization, and in which sex role each fish will spawn to successfully utilize this strategy (Leonard, 1990). Because fecundity and number of spawning events are directly proportional to size of females (Petersen, 2006) and longevity in iteroparous fishes, respectively, the reproductive output of simultaneous hermaphrodites is expected to be limited by their relatively short longevity and small maximum size compared to gonochorists and sequential hermaphrodites in the same family. By understanding the life history

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of simultaneous hermaphrodites, more knowledge is gained that can be applied to examine how this reproductive strategy can be successful in fishes.

The sand perch *Diplectrum formosum* (L.) is a simultaneously hermaphroditic serranid commonly found along the western Atlantic from Virginia, U.S.A. to Uruguay including the Gulf of Mexico and Caribbean Sea (Briggs, 1958). High abundance of *D. formosum* in important fishery locations (Wenner et al., 1979) and its appearance in stomach contents of numerous fish species (Manooch, 1977; Overstreet & Heard, 1978; Branstetter, 1981) indicate that it is a prey item and has ecological significance for economically important fishes. While *D. formosum* may have value ecologically, life-history variables of it and other simultaneous hermaphrodite fishes have been lacking due to their relative unimportance economically. Previous studies conducted on simultaneous hermaphrodite serranids predominantly deal with the genus *Serranus*, while focusing on the behavioural component of this strategy (Fischer, 1984; Hastings & Petersen, 1986; Petersen, 1995). Few studies have examined other genera, including *Diplectrum*. Bortone (1971) conducted a life-history assessment of *D. formosum* in the Gulf of Mexico, using less precise and accurate methods than those currently available to estimate age and describe the reproductive stages. The techniques used in the previous study combined with the relatively large amount of time elapsed (>30 years) and latitudinal variation of age, growth and reproductive traits observed in fishes (Conover & Present, 1990), warrants an assessment of *D. formosum* life history in the western North Atlantic.

Previous studies on *D. formosum* characterized the morphology of the ovotestis, which comprises mostly of ovarian tissue that is surrounded by a band of testicular tissue in the posterior portion (Bortone, 1971) capable of simultaneously producing mature egg and sperm, respectively. An accessory reproductive structure located posterior to the testicular and ovarian tissue present only in the ovotestes of fishes in the genus *Diplectrum* (Bortone, 1977a) has also been noted. This structure was later described in two *Diplectrum* species, but the purpose of the accessory structure for reproduction is still unknown (Bortone, 1977b; Touart & Bortone, 1980).

By characterizing the age, growth and reproduction of *D. formosum*, this investigation provides life-history information on a simultaneous hermaphrodite belonging to a genus rarely studied. The objectives of the present study were (1) to describe life-history variables such as age, growth and reproductive seasonality, as well as size and age at maturity of *D. formosum* in the Atlantic Ocean waters off the southeast coast of the U.S. and (2) to describe the relationship between the ovarian and testicular tissue within the ovotestis and the possible role of the accessory structure in reproduction.

**MATERIALS AND METHODS**

**COLLECTION OF SAMPLES**

*Diplectrum formosum* were collected between April 2000 and July 2004 in coastal waters (6–47 m depth) of the Atlantic Ocean from Cape Lookout, North Carolina (34°08′ N) to Fort Pierce, Florida (27°26′ N). Fishery-independent sampling was conducted between April 2000 and October 2003. Sampling using chevron traps (Collins, 1990) followed protocol from Harris & McGovern (1997). Chevron traps were baited with cut clupeids and soaked for c.
90 min at depths ranging from 15 to 100 m. All specimens collected were kept. Chevron traps did not adequately sample small size classes, <140 mm standard length (Ls), due to mesh-size of the trap. Therefore, additional fish were obtained between July 2001 and July 2004 by stratified random trawls using two 22.9 m falcon trawls without turtle excluding devices (Collins et al., 1998). Trawls were towed for 20 min at depths from 6 to 14 m. Depth (m) and bottom temperature (°C) were recorded at each trap set and trawl location. Total mass (g) and Ls (mm) were recorded for each specimen collected.

Fishery-dependent samples were obtained by port sampling of fish caught by commercial fishermen that used hook-and-line gear (bandit reels) between October 2002 and July 2004. Samples from the commercial fishery supplemented the fishery-independent sampling, which was lacking in the late autumn and winter months. Commercial fishermen provided approximate location and time of capture of specimens. The effect of gear type (chevron trap, trawl and commercial hook and line) on mean size at age of captured fish was examined to determine if it was appropriate to pool data. All statistics, unless otherwise noted, were performed using JMP 8.0 (SAS Institute, Inc.; www.sas.com).

**AGE ESTIMATION**

Both sagittal otoliths were removed and stored dry in coin envelopes; the left sagitta was used in age estimation. Otolith sectioning followed the protocol used by Wyanski et al. (2000). The otolith was embedded in transparent epoxy resin and sectioned (0.3 mm thick) through the focus along a dorsal–ventral plane using a high concentration diamond wheel on a Buehler (www.buehler.com) Isomet-3000 low-speed cutting saw. Otolith sections were mounted on glass slides with Accumount mounting medium (www.rallensci.com).

Sectioned otoliths were examined with a dissecting microscope at ×10–40 magnification under reflected light. A video camera linked to a personal computer equipped with digital imaging software was used to measure increments. Otolith increments (one translucent and one opaque zone) were counted independently by two readers without knowledge of specimen Ls, mass, location or date of capture. The readers also classified the edge type, the distance between the last opaque zone and the otolith edge, on a scale from 1 (the opaque zone on the edge and was just deposited) to 4 (with a large space between the opaque and the edge, with a new opaque zone expected to be deposited soon). All estimates with an edge type of 4 were rounded up to the next age. The primary reader examined all otoliths and a secondary reader examined 35% of otoliths for each year. The secondary reader examined all the otoliths for the year if there was <75% absolute agreement with the first reader.

Marginal increment analysis was the primary method of verifying the periodicity of increment deposition. Otolith radius was measured along the ventral edge of the sulcus acusticus from the core to the outer edge. The distance from the core to the centre of each opaque zone of an increment was measured along the same axis. Otoliths that were not cut at the core or were unreadable were not used for measurements. All otoliths were measured for months in which <50 fish were collected (September to April) and random sub-samples of at least 50 fish per month were taken between May and August. Marginal increments were standardized to facilitate the use of multiple age classes and months.

The formula to determine standardized marginal increment (M5) formation for each fish was: 

\[ M_5 = \frac{(O_R - I_L)(I_L - I_{L-1})^{-1}}{O_R - I_L}, \]

where \( O_R \) is otolith radius, \( I_L \) is the distance between the otolith core and the last increment and \( I_{L-1} \) is the distance between the otolith core and the next to last increment. Mean standardized marginal increments were plotted as a function of month to determine if the increments represented annual growth. If increments occurred annually, there will be a unimodal distribution of the marginal increments throughout the year (Manooch, 1987).

In an effort to produce a reliable conversion between Ls and age, a von Bertalanffy growth curve was fitted to the verified Ls-at-age data from fishery-independent samples and plotted to model *D. formosum* growth (von Bertalanffy, 1938) using the equation: 

\[ L_t = L_\infty (1 - e^{-k(t-t_0)}), \]

where \( L_t \) is Ls at age \( t \), \( L_\infty \) is the theoretical mean maximum Ls, \( k \) is the growth coefficient and \( t_0 \) is the theoretical age at which length equals zero.
REPRODUCTION

Whole gonads were removed, placed in Mega-cassette Tissue-Teks (www.sakura-americas.com), fixed in 11% seawater formalin buffered with marble chips for 2 weeks and transferred to 50% isopropanol for at least 2 weeks. A tissue processor was used to infiltrate the tissue with paraffin. Three portions of each gonad were excised and embedded in paraffin wax: one from the posterior-most zone, one slightly more anterior in the testicular tissue and an anterior section. Three sections of 7 μm thickness were cut from each paraffin block at an interval of 350 μm between sections using a rotary microtome. Sections were placed on a glass slide, stained with double-strength Gill haemotoxylin and counterstained with eosin Y, and a cover slip was affixed using Accumount mounting medium (www.rallensci.com).

Sections were viewed with a compound microscope at ×40–400 by two readers independently, without knowledge of specimen LS, mass, age, location or date of capture. Due to atypical gonad structure, both testicular and ovarian sectors of the ovotestis were examined and a maturity and reproductive stage [immature, developing, actively spawning (replacing the term ripe), spent and resting] was assigned to each fish based on histological criteria from Harris et al. (2004) with the female developing stage further subdivided to provide more resolution for spawning seasonality. Female sexual maturity was indicated by oocyte development at or beyond the cortical alveoli oocyte stage or if beta, gamma or delta stages of atresia of oocytes were present in the ovarian tissue of the ovotestis (Hunter & Macewicz, 1985). Male sexual maturity was defined as the development of cysts containing primary and secondary spermatocytes in the testicular tissue (McGovern et al., 2002).

Resting and immature ovarian tissues have similar characteristics and can be mistaken for one another, so to verify that these stages were correctly distinguished, size distributions of specimens containing ovotestes with resting or immature ovarian tissue were compared to the size distribution of active females (ovotestes containing developing, actively spawning or spent states). If resting tissue was correctly assigned, then there should be considerable overlap in size of specimens having active tissue with those in the resting stage and lack of overlap in size with specimens having immature tissue.

Length and age at first maturity, 50% maturity (L50 and A50, respectively), and 100% maturity were identified for ovarian and testicular tissue. L50 and A50 were determined with the PROBIT procedure in SAS (SAS Institute, Inc.; www.sas.com) with sex as an independent variable in the model. The LOGISTIC procedure was used to determine the most appropriate model (logistic, normal or Gompertz) to use in the PROBIT procedure. Because maturity occurs early in life, age was reported in months with a birth date in May, the month of annulus formation and the peak of the spawning season, with the LS reported in mm.

Spawning season was described in D. formosum for testicular and ovarian tissue of the ovotestis in each fish. Spawning condition in ovarian tissue was defined by the presence of migratory nucleus oocytes, hydrated oocytes or postovulatory follicles. Testicular tissue was considered to be in spawning condition if it contained a predominance of spermatozoa in lobules and collecting sinuses with little or no occurrence of spermatogenesis. Spawning season was defined as the time between the dates of capture of the first and last specimens meeting the criteria of spawning condition.

Spawning periodicity as a female was estimated by dividing the number of fish in active condition (ovotestis containing oocytes undergoing vitellogenesis, migratory nucleus oocytes, hydrated oocytes or postovulatory follicles) by the number of fish showing imminent spawning (migratory nucleus oocytes or hydrated oocytes). To detect possible biases, spawning periodicity was also estimated by dividing the number of fish in active condition by the number of fish that possessed postovulatory follicles <24 h old. The age of the postovulatory follicles was estimated based on histological criteria developed for skipjack tuna Katsuwonus pelamis (L.) (Hunter et al., 1986), which spawn at similar ambient temperatures (23–24°C) as D. formosum. The maximum number of spawning events in a season was estimated by dividing the number of days in the spawning season by the spawning periodicity.

The accessory structure of the ovotestis was prepared using the same histological techniques. The appearance of hydrated oocytes within the accessory structure was noted in fishery-independent samples from 2003. Samples collected between 2000 and 2002 were not used for accessory structure assessment, because the posterior-most portion of the ovotestis,
which contained the accessory structure, was not consistently excised. Modifications in removal and sectioning of gonads from *D. formosum* in collections between 2003 and 2004 resulted in a more complete sample of the accessory structure.

RESULTS

**SAMPLING**

Between May 2000 and July 2004, a total of 1131 *D. formosum* were collected. Of these, 979 (mean = 176.6 mm *L*_5, range 72–236 mm *L*_5) were caught in fishery-independent sampling, with limited samples from the late autumn and winter months due to movement of the fish with the onset of colder water and a lack of sampling opportunities during these months. Bottom temperature ranged from 14.03 to 28.50 °C with a mean of 23.60 °C while salinity ranged from 35.26 to 37.09 with a mean of 36.36. Fishery-dependent sampling provided 152 fish between October 2002 and July 2004. Specimens ranged from 63 to 224 mm *L*_5 (mean = 179.2 mm *L*_5).

The *L*_5 distribution varied significantly between gear types (ANOVA, *n* = 1107, *P* < 0.001). The falcon trawl captured significantly smaller fish (mean ± s.d. 121.8 ± 19.3 mm) than hook and line (mean ± s.d. 185.6 ± 18.7 mm) and chevron trap (mean ± s.d. 188.9 ± 14.0 mm), but there was no difference in the size at age between gear types (ANOVA, d.f. = 2, *P* > 0.05) at the age of fish that all gears sampled (1 year old) therefore all data were pooled for analyses.

**AGE AND GROWTH**

Otoliths were obtained from 1131 specimens. Marginal increment analysis of 351 specimens ≥1 year of age showed significant differences by month (ANOVA, *n* = 351, *P* < 0.001) with increments being deposited annually in May (Fig. 1), as this was the only month in which the standardized mean marginal increment was significantly less than the preceding month (Tukey–Kramer HSD, *n* = 351; *P* < 0.01).

The primary reader assigned an age for 1054 otoliths, while the secondary reader estimated the age of 407 otoliths. The initial agreement on age was 82% (98% within 1 year). The percentage of agreement between both readers exceeded 75% each year. The age of fish ranged from 0 to 8 years old with a mean of 2.3 years. Otoliths of 77 specimens were considered unreadable, and these data were excluded from analyses.

The von Bertalanffy growth estimate model explained 63.3% of the variability in fishery-independent samples, with *L*∞ = 208.4 mm *L*_5 (95% c.i. = 204.7 to 212.2), *k* = 0.70 year⁻¹ (95% c.i. = 0.61 to 0.78) and *t*₀ = −0.75 (95% c.i. = −0.91 to −0.58) (Fig. 2).

**REPRODUCTION**

Maturity and reproductive stages were assigned to 1100 specimens. Both testicular and ovarian tissue, regardless of maturity, was observed in 950 specimens, with both mature testicular and ovarian tissue occurring in 856 specimens and immature tissue of one sex or the other occurring in 94 specimens. The overlap of *L*_5 distributions between specimens with ovarian tissue staged as resting and active as well as lack
of overlap of resting tissue with immature ovarian tissue confirmed that resting and immature stages in ovarian tissue were correctly assigned (Fig. 3).

The observations in this study of the structure of the ovotestis coincided with Bortone’s (1971) account. The ovotestis was compartmentalized, with testicular and ovarian portions separated from each other by a thin basement membrane [Fig. 4(a)]. The ovarian tissue made up the majority of the ovotestis from the anterior and middle thirds, while the testicular band was located on the posterior-most third of the ovotestis with the ovarian tissue running central to, without contacting, the testicular tissue. Mature sperm and eggs are released to the environment through separate ducts, with sperm transported through the sperm duct dorsomedial to the eggs that are transported through the lumen, to the accessory structure, and finally to the oviduct before release [Fig. 4(b)]. The accessory structure was located posterior to the testicular tissue in the ovotestis, with the wall of the accessory structure being composed of epithelial cells that formed highly convoluted, villi (Fig. 5).

When characterizing maturity between the tissues in the ovotestis, specimens with mature ovarian tissue and immature testicular tissue were less prevalent than specimens with immature ovarian tissue and mature testicular tissue. The smallest specimen with mature ovarian tissue was 117 mm $L_S$, with mature testicular tissue first appearing in a specimen of 106 mm $L_S$. The minimum age at maturity was 12 months for ovarian tissue and 5 months for testicular tissue. Using a logistic model, $L_{50}$ maturity was estimated to be 129 mm $L_S$ (95% c.i. = 124–134 mm) for ovarian tissue.

Fig. 1. Standardized mean ± 2 s.e. marginal increment ($I_{SM}$) by month (all years combined) for Diplectrum formosum otoliths. Numbers across top of plot are sample size. *, significantly different $I_{SM}$ from the previous month.
and 123 mm $L_S$ (95% c.i. = 118–127 mm) for testicular tissue (Fig. 6). There was a significant difference for $L_{50}$ between sexes ($\chi^2 = 8.35, n = 1020, P < 0.01$), with testicular tissue maturing in fish of a smaller size than ovarian tissue. Using a Gompertz model, $A_{50}$ was estimated to be 13.6 months (95% c.i. = 12.3–14.5 months) for ovarian tissue and 12.2 months (95% c.i. = 9.8–13.4 months) for testicular tissue (Fig. 7). There was a significant difference for $A_{50}$ between sexes ($\chi^2 = 8.84, n = 1016, P < 0.01$) with testicular tissue maturing in fish of a younger age than ovarian tissue. Length at 100% maturity was 179 mm $L_S$ for ovarian tissue and 173 mm $L_S$ for testicular tissue. Age at 100% maturity was 28 months for ovarian tissue and 18 months for testicular tissue.

Hydrated oocytes, migratory nucleus oocytes or postovulatory follicles, indicative of spawning condition as females, were present from March to January for c. 337 days per year including the months not sampled. There were no samples from November and December. The only month in which D. formosum were without ovarian tissue in spawning condition was February ($n = 6$). There was a peak of spawning in May with the highest percentages of spent females in September and resting females in February (Fig. 8). The average spawning periodicity as a female was 1.82 days when using the two methods of determining frequency [hydrated oocytes and migratory nucleus oocytes (1.55 days) or postovulatory follicles <24 h old (2.09 days)]. Assuming D. formosum spawn at one particular time of day, the realized periodicity of spawning events was 2 days and the maximum number of potential spawning events in a year was 168.
Actively spawning testicular tissue, indicating spawning condition of fish as males, was present throughout the year with a maximum frequency in the summer months (Fig. 9) and no distinct peak. Interestingly, the smallest proportion of fish in spawning condition as males was May, which is the peak spawning month of fish as females. There were no resting males collected during the present study.

Hydrated oocytes were found in the accessory structure of 22% of fishery-independent specimens from 2003. The proportion of specimens with hydrated oocytes in the accessory structure was highest at the earliest month (June) of sampling that year, near the peak of spawning season and declined in the months following. When hydrated oocytes were observed in the accessory structure, the reproductive stage of the ovarian tissue ranged from actively spawning with hydrated oocytes to resting. With the presence of hydrated oocytes in the accessory structure, the reproductive stage of the testicular tissue was developing or actively spawning. The actively spawning stage was the most prevalent in both sexes. When hydrated oocytes were not found in the accessory structure, the reproductive stage of ovarian tissue ranged from developing to spent. When hydrated oocytes were not present in the accessory structure, the testicular tissue was developing, actively spawning or spent. While the presence of actively spawning ovarian (z-test, \( n = 116, \ P > 0.05 \)) or testicular (z-test, \( n = 116, \ P > 0.05 \)) tissue stages were not significantly different regardless of the presence or absence of hydrated oocytes in the accessory structure, testicular tissue was nearing significance. Testicular tissue showed a trend of a larger proportion of fish containing actively spawning tissue when hydrated oocytes were present in the accessory structure than when they were absent.
Fig. 4. Transverse cross-sections of a mature ovotestis from *Diplectrum formosum* stained with haematoxylin and eosin-Y showing compartmentalization anteriorly between (a) t, ripe testicular and o, ovarian tissue and posteriorly (b) between sd, sperm duct with spermatozoa and as, accessory structure. ho, Hydrated oocytes are present within the ovarian portion. Magnification ×20.

**DISCUSSION**

**AGE AND GROWTH**

The size differences exhibited between the different gear types could be a function of multiple factors. The mesh-size of the chevron traps and trawls and hook size for fishery-independent sampling is size selective (Dalzell, 1996). Another possibility involves ontogenetic habitat use, which can play a role in location of juveniles

![Fig. 5. Villi-like projections (v) from the wall of the accessory structure in the ovotestes with the presence of hydrated oocytes (ho). Magnification ×400.](image)
Fig. 6. Probability of Diplectrum formosum maturity at standard length ($L_S$) by reproductive tissue type using probit analysis ($n = 878$). Testicular probit analysis ($\cdots$) was derived from observed maturity. Ovarian probit analysis (---) was derived from observed ovarian tissue maturity. The size of fish at 50% maturity was significantly smaller for testicular tissue than ovarian tissue ($P < 0.01$).

(Parrish, 1989). The smaller mesh of the trawl combined with a habitat preference in smaller and younger fish could explain the size differences observed between gear types, but because there were no differences in size at age, the results could be pooled for further analysis.

Fig. 7. Probability of Diplectrum formosum maturity at age by reproductive tissue type using probit analysis ($n = 878$). Testicular probit analysis ($\cdots$) was derived from observed maturity. Ovarian probit analysis (---) was derived from observed ovarian tissue maturity. The age of fish at 50% maturity was significantly younger for testicular tissue than ovarian tissue ($P < 0.01$).
Fig. 8. Spawning seasonality for ovarian tissue of *Diplectrum formosum* between 2000 and 2004. Reproductive stages: cortical alveoli (■), developing (□), actively spawning (●), spent (□), resting (□) and postovulatory follicles (POF) (□). Number above each bar indicates sample size.

The lack of samples in November and December and small sample sizes (<10) between January to March and October do not affect the age and growth variables established in this study. With increment deposition clearly occurring in May and the trend in \( M_S \) continuing on either side of the unsampled or poorly sampled months, the calculated age and growth were unaffected by the lack of samples.

*Diplectrum formosum* caught in this study attained a maximum age 0.33 times older with a larger maximum size than those in the Gulf of Mexico (Bortone, 1971). There are many factors that can cause this effect. The difference could be attributed to Bortone (1971) estimating ages of *D. formosum* using whole otoliths while this study sectioned the otoliths before reading. Sectioning otoliths provides more accurate estimates of age in fishes, which have high initial growth early in life, such as the *D. formosum*, due to the allometric change in otolith growth, which causes the outer increments to be obscured when read whole because of their proximity to each other (Beamish, 1979; Peltonen *et al.*, 2002; Lee *et al.*, 2009). Latitudinal and regional variation in age and growth could also be a factor (Conover & Present, 1990) between the western Atlantic Ocean, where fish for this study were collected and the Gulf of Mexico, where Bortone (1971) collected samples.

**REPRODUCTION**

Months with a small \( n < 10 \) sample size, October \( (n = 3) \), January \( (n = 2) \) and February \( (n = 6) \), may not be representative of spawning seasonality of the population and caution should be used if extrapolating data to November and December.
Fig. 9. Spawning seasonality for testicular tissue of *Diplectrum formosum* between 2000 and 2004. Reproductive stages: developing (□) and actively spawning (■). Number above each bar indicates sample size.

With the peak of *D. formosum* spawning occurring during the summer months, the lack of samples from those months does not affect the results obtained for reproduction. When analysing spawning season, the definition of spawning season only requires a single fish to be actively spawning, so small samples sizes did not affect this variable if actively spawning specimens are obtained. If anything, the length of the spawning season is underestimated due to the lack of samples from the late autumn and winter months.

Ovarian and testicular tissue differentiation occurred at sizes and ages prior to the smallest (63 mm $L_S$) and youngest fish (4 months old) that were obtained in the present study. Bruslé (1983) observed sex differentiation in the simultaneous hermaphrodite the brown comber *Serranus hepatus* (L.) at 20 mm $L_S$ and an age of 2 months, with ovarian tissue differentiating before testicular tissue. Using macroscopic observations, Bortone (1971) stated that development of ovarian and testicular tissue of *D. formosum* did not occur until fish were $>90$ mm $L_S$. In the present study, *D. formosum* $<90$ mm $L_S$ contained ovarian and testicular tissues that were distinguishable within the ovotestis. The observed difference in size at development between the present study and Bortone (1971) may be due to macroscopic observations being less accurate for smaller fish than observations using histological methods. Other reasons attributing to this difference are those listed above for maximum age discrepancies between the studies.

In previous studies of simultaneously hermaphroditic fishes, including *D. formosum*, the maturation of the testicular tissue preceded ovarian maturation within the
ovotestis (Bortone, 1971; Hastings, 1973). The present study supported these results with significant difference between male and female maturity within each fish, with testicular tissue maturing in slightly smaller and younger fish than ovarian tissue. Because *D. formosum* ovotestes contain both ovarian and testicular tissue, temporal and spatial differences in environmental conditions can be excluded as the cause of differing male and female differentiation, maturity and reproductive stages within each fish.

The decrease in growth rate of *D. formosum* as it nears $L_\infty$ coincides with the size and age at maturity of *D. formosum*. The growth coefficient $k$ in the von Bertalanffy equation indicates that this species grows at a fast rate initially and nears maximum size within the first 2 years. Future work would be needed to examine this relationship, but the stress of producing and maintaining both ovarian and testicular tissue could have an effect on somatic growth within this and other simultaneous hermaphrodites.

The observed spawning season as a female *D. formosum* in the Atlantic Ocean waters off the south-eastern U.S.A., beginning in March with a peak in May, was similar to macroscopic observations by Bortone (1971) in the Gulf of Mexico. By utilizing histological techniques in this study, a larger number of reproductive stages could be observed compared to those seen macroscopically, thus giving a higher resolution in examining reproductive biology of *D. formosum*. *Diplectrum formosum* also do not aggregate seasonally to spawn as no aggregations were observed in sampling data and those fish in spawning condition were found throughout their range, with no distinct locations of spawning.

Hydrated oocytes frequently occurred within the accessory structure of the ovotestis in the present study. Bortone (1977b) hypothesized that the role of the accessory structure is for storage of hydrated oocytes until a mate can be found or for resorption of unused hydrated oocytes. While the lack of atretic oocytes within the accessory structure contradicts the theory of egg resorption, there is evidence to suggest that it utilized for storage of eggs until a mate can be found. The length of time the hydrated oocytes can remain in the structure is unknown, but estimates can be made based on the reproductive stage of the ovarian tissue in the fish. Ovarian tissue in spawning condition when hydrated oocytes are within the accessory structure indicates that the hydrated oocytes are $<36$ h old due to the time for degeneration of postovulatory follicles (present up to 36 h after spawning; Hunter *et al*., 1986). The occurrence of ovarian tissue in spent and resting condition when hydrated oocytes are within the accessory structure indicates that the hydrated oocytes have been stored for at least 36 h and probably much longer. The storage of oocytes in the accessory structure is also supported by the make up of the structure itself. The villi are composed of secretory epithelium, which suggests that the accessory structure has the capacity to maintain an environment for storage of eggs. The ability of the accessory structure to maintain hydrated oocytes for greater than 36 h as well as the presence of secretory epithelium composing the villi provides evidence that it is used for storage.

It is unlikely that self-fertilization occurs commonly in *D. formosum*, but spawning behaviour of simultaneously hermaphroditic fishes such as egg trading (Fischer, 1980, 1984) and monogamy with alternating sexual roles (Pressley, 1981) requires that both fish can spawn as both sexes in a relatively short time. This is evident in the *D. formosum* when both testicular and ovarian tissue within the ovotestis were in spawning condition concurrently. While this occurred frequently, there were many occasions...
in which the testicular and ovarian tissues within individual fish were slightly asynchronous, with the ovarian tissue in spawning condition before the testicular tissue, which would be detrimental to these strategies. In many of these occurrences, the testicular tissue was in spawning condition when the ovarian tissue contained postovulatory follicles. This indicates that the role reversal between sexes could still occur in a short period of time (hours to days) even with asynchronous tissues.

Observations by Bortone (1971) indicate that *D. formosum* are territorial and set up home areas. Because of this behaviour, *D. formosum* would be exposed to few conspecifics. This limits the amount of potential mates it encounters, making storage of hydrated oocytes in the accessory structure important to ensure that both fish are capable of reproducing at the same time during an encounter.

The potential storage capability of the accessory structure also provides a means of ensuring that the testicular tissue is in actively spawning condition and can be involved with egg fertilization. The ability of a fish to store oocytes in the accessory structure would also compensate for the asynchronicity observed and allow the fish to spawn as both sexes in a short period of time by maintaining the egg viability until the male tissue was in spawning condition. This would explain the trend of testicular tissue in spawning condition proportionately more in fish containing hydrated oocytes in the accessory structure than in fish without.

To accurately describe the reproductive life history in *D. formosum*, common life-history traits, such as size and age at maturity, age and growth and spawning seasonality should be examined. Secondarily, those factors not associated with gonochorists or sequential hermaphrodites, such as specialized structures or timing between tissues within each fish, need to be characterized to adequately describe the life history of a simultaneous hermaphrodite. The evidence from this study suggests that there are varying temporal differences between the development and seasonality of testicular and ovarian tissue within each individual fish and that the accessory reproductive structure could play an important role in *D. formosum* utilizing simultaneous hermaphroditism as a viable reproductive strategy.

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