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Reproductive seasonal periodicity of the endemic Bermuda killifish *Fundulus bermudae* in an anchialine pond

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A total of 245 individuals from a population of the endemic Bermuda killifish *Fundulus bermudae* in an isolated anchialine pond (Mangrove Lake) were trapped between November 2004 and November 2005. Laboratory analysis of gonad development allowed determination of the seasonal reproductive cycles of both females and males. A distinctive annual pattern was evident, with female and male gonadal cycles synchronous throughout the study period. The results indicate that the *F. bermudae* males and females began their spawning season in February, but reached primary peaks in May and June respectively. Gonadal indices abruptly fell after June and continued to fall at a steady rate until September, marking the end of the spawning season. Gonad recrudescence, as indicated by basal gonad indices, occurred in September and lasted throughout the autumn and early winter months.

INTRODUCTION

Bermuda consists of over 120 named islands of various sizes, with a total land area of 55 km located in the north-west Atlantic Ocean at 32°18′N and 64°46′W, 960 km from the USA coast. *Fundulus bermudae* is endemic to these islands and is one of at least two killifish species presently found there. Both species are believed to be descendants of the *Fundulus heteroclitus–Fundulus grandis* group originating from populations on the east coast of North America (Able & Felley, 1988) whose colonization of the islands occurred thousands of years prior to human habitation (Smith-Vaniz et al., 1999; Grady et al., 2001).

Despite having had a broader historical distribution across the islands of Bermuda, recent surveys of these species indicate that they are currently restricted to nine small, isolated anchialine ponds (saline, land-locked bodies of water with subterranean connections to the ocean) (Outerbridge et al., in press). Bermuda has no permanent surface fresh water streams or lakes and fewer than 20 anchialine ponds. Temperatures and salinities vary from pond to pond, showing predictable seasonal patterns, and are dependent upon the amount of seawater that enters from the ocean. These ponds show many features which differ from those of other anchialine ponds elsewhere in the world and several have persisted for a long period of time with relatively constant characteristics (Thomas et al., 1991). Competition and predation are generally less severe in these isolated pond environments, thus favouring the growth and continued existence of some species that are rare or non-existent elsewhere in Bermuda. This enabled species like the killifish to survive and evolve to the degree of endemism.

Despite the isolation and age of Bermuda (110 million years old) the overall endemism rate is rather low (3%), having been greatly affected by the species extinction events associated with Pleistocene sea level fluctuations (Sterrer, 1998). Killifish represent 25% of the extant endemic ichthyofauna of Bermuda (Smith-Vaniz et al., 1999) and up to 50% of the ichthyofauna inhabiting some of the brackish pond environments (Outerbridge, 2006). There has been a dearth of information regarding the health and status of these *Fundulus* populations. Knowledge of basic population estimates and their structure is necessary for local conservationists to make informed management decisions and was deemed critical for species recovery plans, prompting recent investigations into the biology and ecology of Bermuda's *Fundulus* species (Outerbridge et al., in press). Similarly, information is limited regarding the reproductive ecology of these *Fundulus* populations. The purpose of this study is to describe the reproductive seasonal periodicity of an isolated population of *Fundulus bermudae* inhabiting Mangrove Lake.

Mangrove Lake is the largest and most productive of the anchialine ponds, over 12 Ha in area, and is believed to have formed during the last 11,000 years (Watts & Hansen, 1986). It is a simple basin fringed by red mangrove trees *Rhizophora mangle* and characterized by shallow depths, averaging only 1.4 m, fairly even contours and a gently sloping shoreline. The pond bottom comprises deep deposits of highly organic sediments and is often subject to large changes in oxygen, redox potential, temperature, salinity and nutrient levels (Thomas et al., 1991). Annual water temperatures range between 14°C and 31°C, salinities between 26 and 33 (Outerbridge, 2006).

MATERIALS AND METHODS

A recent assessment of the *Fundulus bermudae* population in Mangrove Lake (Figure 1) indicated that the total estimated population was 15,200 (±2,220) and that males and females occurred in approximately equal proportions

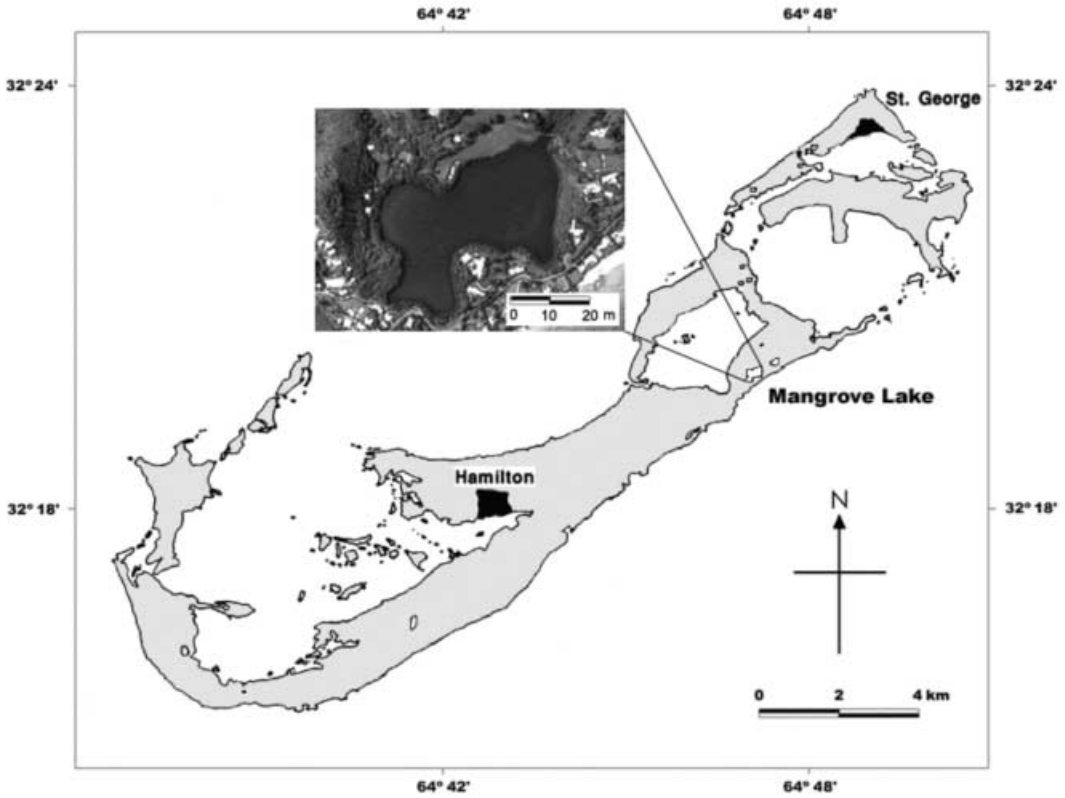

Figure 1. Map of Bermuda showing location of Mangrove Lake from which the study population of *Fundulus bermudae* was collected.

(Outerbridge et al., in press). This anchialine pond has the largest of all extant *Fundulus* populations in Bermuda and was chosen for the current investigation because it could withstand the modest levels of mortality associated with this study. Monthly trapping occurred on the morning of every full moon between November 2004 and November 2005 (inclusive). The cylindrical, Gee-type minnow traps used in this investigation were baited with a piece of cellulose sponge soaked in concentrated fish oil, and left to soak for one hour in the centre of the pond. Upon retrieval a maximum of 20 killifish (ten of each sex representing a wide range of sizes) were randomly selected, subjected to lethal doses of MS-222 dissolved in 0.5 litres of pond water, and taken to the laboratory for measuring,

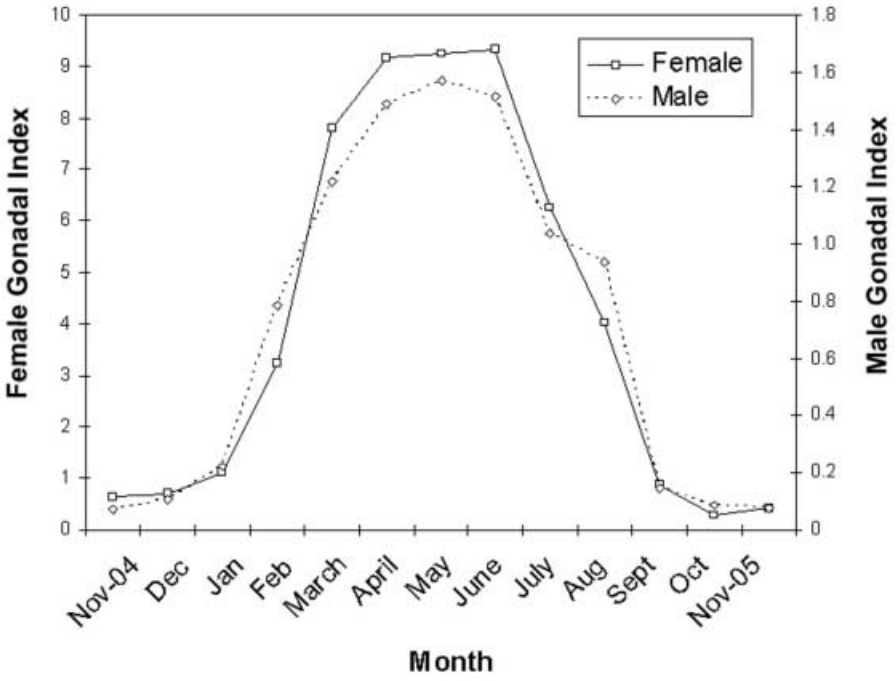

Figure 2. Seasonal variation in gonadal indices for male and female *Fundulus bermudae* in Mangrove Lake, illustrating the synchronous nature of both cycles.

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weighing and gonad removal. Total length was measured in millimetres (to the nearest 1.0 mm), wet body mass recorded to the nearest mg, and the gonads located and removed from the body cavity. Gonads (pair of testes in males and the ovary containing ova in females) were weighed wet to the nearest mg. Brief notes were made on the general condition of the gonads and samples were taken and preserved in 95% ethanol.

The involvement of gonad maturation was investigated by calculation of a gonadosomatic index (GSI), which normalizes gonad weight with respect to body weight and is calculated as the proportion by weight of the wet gonads to total wet body weight [(wet gonad mass/wet body mass) x 100]. Indices were calculated each month for both females and males and then plotted against time to show trends in seasonal changes of gonad development. The oocyte classification system developed by Vladykov (1956), later modified by Wagner & Cooper (1963) and Kneib & Stiven (1978), was used to determine oocyte ripeness as further evidence of spawning activity.

RESULTS

A subsample of 245 individuals (124 females, 121 males) from the *Fundulus bermudae* population in Mangrove Lake was examined in this study. Wet body mass and total length (TL) ranges for females were 1.8–21.4 g and 53–109 mm TL respectively, for males 1.6–17.0 g and 52–112 mm TL respectively. The seasonal patterns of male and female gonad development were near-synchronous (Figure 2). Both sexes show an index increase in February and March, marking the beginning of the spawning season, followed by three successive months of greater spawning activity. Gonadal indices abruptly fell after June and continued to fall at a steady rate until September, marking the end of the spawning season. Gonad recrudescence, as indicated by basal gonad indices, occurred in September and lasted throughout the autumn and early winter months. Ripe ova, i.e. those that were 2 mm in diameter and pale translucent with visible vacuoles at one pole (Vladykov, 1956), were first observed in February and last observed in September. The largest number of ripe ova found in a single female of *F. bermudae* (98 mm TL) in Mangrove Lake was 108, while the smallest female observed with a spawning ovary (defined by the presence of mature ova) was 56 mm TL.

DISCUSSION

Analysis using a gonadosomatic index has proven to be adequate for relative gonadal–body size comparisons in *Fundulus heteroclitus* populations in Delaware Bay (Taylor et al., 1979) and Chesapeake Bay (Hines et al., 1985) as well as the Gulf killifish *F. grandis* on the Alabama coast (Greeley & MacGregor, 1983), and was considered appropriate for the present investigation. One-way analysis of variance on the separate data sets for females and males in Mangrove Lake indicated that significant differences existed amongst monthly mean GSI values ($P < 0.001$). Tukey's pairwise comparisons determined that the mean values in February and September for both sexes were significantly different from the remaining months in the year, thus confirming the observation that February is the start of the spawning season, and September the end, for *F. bermudae* in Mangrove Lake. This annual periodicity closely resembles those reported by Kneib & Stiven (1978) and Fernández-Delgado (1989) for *F. heteroclitus* populations in the Tar Landing Bay salt marsh (North Carolina, USA) and for an introduced population in the Guadalquivir River estuary in south-west Spain. The Bermuda population, however, shows differences in monthly index continuity compared with the Tar Landing Bay population. The former maintained fairly constant spawning effort throughout the spring and early summer months whereas the North Carolina population showed a large primary spawning peak in the spring (April and May) followed by a smaller secondary peak in mid-summer (July). The results of the current investigation also indicate that the length of Mangrove Lake females at first sexual maturity approximated those reported by Fernández-Delgado (1989), but were larger than those reported by Kneib & Stiven (1978) and Taylor et al. (1979).

The annual breeding season of *F. heteroclitus* has been reported as typically beginning in the spring and summer months (March–May) and ending in the late summer or early autumn months (July–September), however, the length of the season appears to vary with latitude (Kneib & Stiven, 1978; Taylor et al., 1979; Hines et al., 1985; Kneib, 1986; Fernández-Delgado, 1989). Researchers studying the seasonal reproductive periodicities of *F. heteroclitus* and *F. grandis* populations in North America frequently obtained daily to weekly fish samples during the most active breeding periods (Taylor et al., 1979; Taylor & DiMichele, 1983; Greeley & MacGregor, 1983; Waas & Strawn, 1983; Hines et al., 1985), thus allowing them to document weekly reproductive rhythms. The mortality rate associated with such an intensive approach was deemed unacceptable on conservation grounds for the *F. bermudae* population in Mangrove Lake. Consequently, the monthly sampling regime was not rigorous enough to document the occurrence of lunar or semilunar spawning rhythms within the Bermuda *Fundulus* population. The results of the present investigation have shown that this population has a clear annual spawning cycle; however, it remains unclear whether spawning follows a lunar cue or some other environmental signal.

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