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Distribution, population assessment and conservation of the endemic Bermuda killifishes *Fundulus bermudae* and *Fundulus relictus*

Mark E. Outerbridge[1,*], John Davenport[2], Anne F. Glasspool[1]

[1]Bermuda Biodiversity Project, Bermuda Zoological Society, PO Box FL 145, Flatts, FL BX, Bermuda
[2]Department of Zoology, Ecology & Plant Science, University College Cork, Distillery Fields, North Mall, Cork, Ireland

ABSTRACT: *Fundulus bermudae* and *Fundulus relictus* are endemic to Bermuda and are protected under the Bermuda Protected Species Act 2003. These killifishes were described as abundant and widespread in the wetland communities of Bermuda during the late 19th and early 20th centuries. Surveys were undertaken during 2004–2005 to determine the current distribution, as well as to estimate the size and structure of each *Fundulus* population. Killifishes are now found in only 9 isolated ponds. For 6 ponds, populations appear to be large enough to be self-sustaining for the foreseeable future; for 1 pond, the population is low enough to be regarded as vulnerable. Estimates were not feasible in the case of the remaining 2 ponds.

KEY WORDS: Anchialine ponds · Bermuda · *Fundulus bermudae* · *Fundulus relictus* · Killifishes · Mark and recapture · Visible implant elastomer

INTRODUCTION

Bermuda (32° 18′ N, 64° 46′ W) consists of over 120 islands, with a total land area of 55 km^2, 960 km from the USA coast. It lies on top of a volcanic seamount that rises 4000 m from the seafloor, capped by limestone and coral reefs. All islands are concentrated along the southern edge of the seamount, the larger ones forming a narrow chain linked by causeways and bridges. Bermuda has no permanent surface freshwater streams or lakes and fewer than 20 anchialine ponds scattered across the islands (Thomas & Logan 1992). These ponds (3 of which are man-made) are isolated, saline, land-locked bodies of water with permanent subterranean connections to the ocean. Natural freshwater ponds are few, and mostly man-made. Widespread drainage of freshwater marshes was employed as part of mosquito control methods in the first half of the 20th century, as the government attempted to prevent the spread of mosquito-borne diseases. In the 1930s, wetlands were used for the disposal of garbage, and after the 1950s this activity became the main feature of marsh reclamation. During this period, large tracts of marshland and numerous ponds were completely filled, leading to major losses in biodiversity. In contrast, the total acreage of saline ponds has remained unchanged since the end of the 19th century (Sterrer & Wingate 1981) and may function as refugia for endemic species. Thomas et al. (1991, 1992) reported detailed descriptions of the physical and biotic characteristics of the 6 largest anchialine ponds in Bermuda. Most of the ponds in Bermuda (irrespective of salinity) are small and shallow with low habitat complexity.

To date, 433 species of fishes have been recorded in Bermuda, of which 8 are currently recognized as endemic (Smith-Vaniz et al. 1999). Two of these 8 endemics belong to the killifish genus *Fundulus*: *F. bermudae* Günther 1874 and *F. relictus* Able & Felley 1988. These are believed to be descendants of the

*Email: mouterbridge@gov.bm

F. heteroclitus–F. grandis species group, and to have originated from populations on the east coast of the USA (Able & Felley 1988) at least 5000 yr before present (Smith-Vaniz et al. 1999, Grady et al. 2001). Uni- and multivariate analyses of adult morphology and isozyme analysis of natural populations indicate that both of the Bermuda forms are endemic and distinct from *F. heteroclitus* (Able & Felley 1988). The overall shape of both species is typical for the *F. heteroclitus–F. grandis* group. Both sexes have a deep vertical body profile, a rather short and robust body with posteriorly placed dorsal and anal fins of approximately equal size, a deep caudal peduncle, and a somewhat rounded caudal fin (Fig. 1). Bermuda's fundulids are sexually dimorphic: females are uniform olive to brown in colour, darker above, lighter below, without definite markings; males are darker in colour, usually dark green or olive with a yellowish underside, and when spawning have a dark spot towards the rear of the dorsal fin. *F. bermudae* and *F. relictus* are, however, indistinguishable in the field, though it appears that no pond contains mixed populations (Table 1). For more detailed physical descriptions see Able & Felley (1988).

Fundulus bermudae and *F. relictus* are benthopelagic in the pond habitat, appear to form loose schools made up of equally sized individuals, and are omnivorous opportunistic feeders. Stomach content analysis has revealed that *F. bermudae* inhabiting Mangrove Lake, the largest pond in Bermuda, eats filamentous green algae and plant material, molluscs, crustaceans and insects (Rand 1981).

The annual spawning cycle for *Fundulus bermudae* is near-synchronous for both sexes. The spawning season for males and females begins in February, reaches a peak in May and June, respectively, and falls abruptly after June until September; the end of the spawning season (Outerbridge et al. 2007). Laboratory observations of spawning site preferences for *F. bermudae* and *F. relictus* suggest that both species may deposit eggs in algal mats or submerged vegetation (Able & Hata 1984). Such solid surfaces may include the roots and pneumatophores of the red and black mangrove tree *Rhizophora mangle* and *Avicennia nitida*, as well as widgeon grass *Ruppia maritima*, all of which are very common in many of the *Fundulus* ponds in Bermuda. The lifespan of Bermuda's killifishes is unknown; however, individuals have been kept in captivity at the Bermuda Aquarium, Natural History Museum and Zoo for 5 yr (J. Gray pers. comm.)

In 2003, *Fundulus bermudae* and *F. relictus* were listed as protected under the Bermuda Protected Species Act (www.bermudalaws.bm). Additionally, there are plans to submit an application for both species to be included on the IUCN (The World Conservation Union) Red List of Threatened Species. There have been few opportunities for these fishes to increase their range, largely due to the restriction in habitat availability and the fragmentation of the wetlands in Bermuda. Additionally, quantitative assessments of each pond population have been lacking and are limiting conservation efforts.

The aims of the present study were (1) to determine the current distribution of Bermuda's killifishes, (2) to estimate the size of each extant population, and (3) to describe the basic structure of these populations.

MATERIALS AND METHODS

A field list of 44 potential killifish habitats was drafted, including salt marshes, peat marshes, drainage canals, inland freshwater, brackish and fully marine ponds, mangrove communities, and sheltered,

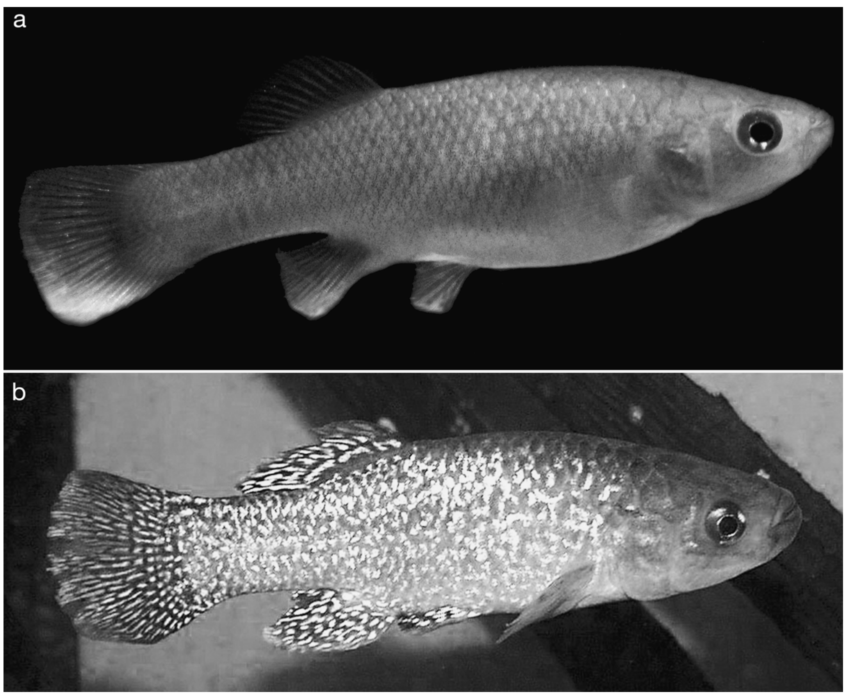

Fig. 1. *Fundulus bermudae*. (a) Mature female from Trott's Pond and (b) male from Mangrove Lake. Photos: Mark Outerbridge (a) and Jennifer Gray (b)

Table 1. *Fundulus bermudae* and *F. relictus*. Summary of killifish distribution and transfer history among the ponds of Bermuda. na: not applicable

Pond	*Fundulus* species	Date of introduction	Source population	Estimated number of fish translocated	Status in 2004
Lover's Lake	*F. relictus*	na	na	na	Extant
West Walsingham Ponds	*F. bermudae*	na	na	na	Extant
East Walsingham Ponds	*F. bermudae*	na	na	na	Extant
Trott's Pond	*F. bermudae*	na	na	na	Extant
Mangrove Lake	*F. bermudae*	na	na	na	Extant
Warwick Pond	*F. bermudae*	na	na	na	Extant
Evan's Pond	*F. bermudae*	na	na	na	Extant
Nonsuch Island (saltwater pond)[a]	*F. bermudae*	1976	Trott's Pond	Unknown	Extirpated
Bartram's Pond[a]	*F. relictus*	1986	Lover's Lake	Unknown	Extant
Nonsuch Island (freshwater pond)[a]	*F. bermudae*	1993	Mangrove Lake	53[b]	Presumed Extirpated
Blue Hole Bird Pond[a]	*F. bermudae*	1995	West Walsingham	50[c]	Extant

[a]Man-made pond
[b]Jon Cotter (1993) Acclimation of brackish killifish *F. bermudae* to fresh water for introduction into Nonsuch Island, Bermuda (unpubl. paper in the Bermuda Aquarium, Natural History Museum and Zoo library)
[c]J. Madeiros (pers. comm.)

shallow bays dominated by seagrass beds in areas immediately adjacent to coastal mangroves (Outerbridge et al. 2006). Surveying used a combination of direct observation and baited traps.

Minnow traps made of 8 mm diagonal wire mesh were used. Each measured 425 mm long and 228 mm wide with a 165 mm long conical entrance at both ends that tapered to a 28 mm diameter opening through which fish could enter the trap. Each trap was baited with a small amount of canned sardines, securely fastened, deployed from a canoe and left to soak for 1 h at a random location within each habitat. Such traps have been standard materials in numerous investigations of *Fundulus* species in North America (Kneib & Stiven 1978, Meredith & Lotrich 1979, Sweeney et al. 1998, Kneib & Craig 2001). Kneib & Craig (2001) evaluated the efficacy of these traps for measuring relative abundance and density of killifishes and suggested that reliable estimates can be made if the traps are used in isolated pools where habitat complexity is low and killifish density remains constant during the sampling period — conditions satisfied in the present study.

Wherever extant populations were found, up to 12 traps were placed at random locations throughout each pond (12 in Mangrove Lake, Trott's Pond, Bartram's Pond and the West Walsingham Ponds; 7 in Warwick Pond; 4 in Lover's Lake; and 3 in the Blue Hole Bird Pond). Population censuses were subsequently performed based on the Petersen Index methodology of mark and recapture (Ricker 1958) using a visible implant elastomer (VIE) (Northwest Marine Technology). VIE effectiveness has been documented by Dewey & Zigler (1996), Frederick (1997) and Kneib & Craig (2001). The pale area on the abdomen adjacent to the proximal base of the pelvic fins was chosen as the tagging site. Application of elastomer to fish was accomplished by full anaesthesia (FINQUEL MS-222 Argent Chemical Laboratories) and injection immediately below the dermis so that the needle tip could still be clearly seen. Immediately after tagging, fish were transferred to a bucket of continuously aerated, untreated pond water and given 2 h to recover before being released at random locations within each pond.

Sex and total length, measured to the nearest 1 mm, of all killifishes captured were determined at the time of tagging. Whenever possible, sex was recorded based on presence or absence of a dorsal fin ocellus and an anal-sheath on the anal fin, as well as overall body colouration. Males were positively identified when they possessed the dark ocellus on the posterior half of the dorsal fin; females were positively identified if they possessed an anal sheath on the anal fin. Body colouration was used as a secondary identification technique.

Second, third and fourth recapture censuses were performed on each extant population based on the original marking event 1, 2 and 3 wk, respectively, after the initial tagging event. All trapped fish were examined for tags and the numbers recorded. Petersen population estimates — calculated as $N = [(n_1 + 1)(n_2 + 1) / (m_2 + 1)] - 1$, where N = the total estimated population, n_1 = number of fish marked in first sample, n_2 = total number of fish in the second sample, m_2 =

number of marked fish in the second sample — were averaged from the 3 separate recapture events to obtain a mean population size for each pond.

RESULTS

The 2004–2005 surveys confirmed the existence of populations in the following 9 locations only: Lover's Lake, Bartram's Pond, Mangrove Lake, Trott's Pond, Blue Hole Pond, both East and West Walsingham Ponds, Warwick Pond and Evan's Pond (Fig. 2). No other *Fundulus* populations were discovered in any of the peripheral wetland communities of Bermuda, including those described as having them at some time in the past 150 yr.

Petersen estimates (Table 2), mean lengths, size ranges (Table 3), and sex ratio data (Table 4) were collected for all ponds where extant *Fundulus* populations were found, except for the East Walsingham Ponds and Evan's Pond; no fish could be trapped for marking in these 2 ponds during the survey period. The population estimates (in order of decreasing size) were Mangrove Lake (11 325; *F. bermudae*), Trott's Pond (7926; *F. bermudae*), Lover's Lake (8508; *F. relictus*), Blue Hole Bird Pond (5394; *F. bermudae*), West Walsingham Ponds (2202; *F. bermudae*), Bartram's Pond (1793; *F. relictus*) and Warwick Pond (436; *F. bermudae*). Non-parametric Mood Median tests performed on all data sets were used to determine if a significant difference occurred between male and female median sizes (Table 5). Statistically significant differences were found only in Lover's Lake ($p = 0.006$), the West Walsingham Ponds ($p = 0.023$), and Warwick Pond ($p = 0.009$). In all 3 populations females were significantly larger than males. Additionally, females outnumbered males in all ponds surveyed, except Trott's Pond, where the sexes occurred in equal numbers. However, chi-square tests performed on the sex ratio data sets for Mangrove Lake, Lover's Lake, Trott's Pond, and Warwick Pond did not show statistically significant differences between female and male numbers ($p > 0.05$ in all cases), whereas significant differences were noted in the Blue Hole Bird Pond, Bartram's Pond, and the West Walsingham Ponds populations ($p < 0.001$ in all cases) (Table 5).

Table 2. *Fundulus bermudae* and *F. relictus*. Petersen estimates of population (mean ± SE, ind. pond^{-1}) for killifish in each of the ponds studied.

Pond	Petersen estimate
F. bermudae	
Blue Hole Bird Pond	5394 ± 480
Mangrove Lake	11325 ± 1884
West Walsingham Ponds	2202 ± 178
Trott's Pond	7926 ± 1576
Warwick Pond	436 ± 13
F. relictus	
Bartram's Pond	1793 ± 224
Lover's Lake	8508 ± 1347

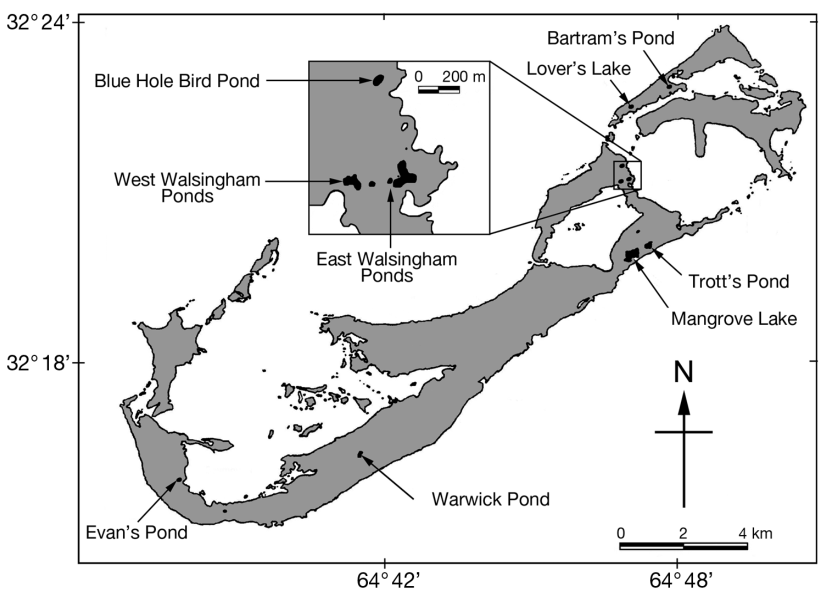

Fig. 2. *Fundulus bermudae* and *F. relictus*. Distribution of killifishes in ponds across the islands of Bermuda in 2004 and 2005

DISCUSSION

Distribution

The present Bermudian distribution of killifishes is very restricted. In the 19th century, killifishes were abundant and widely distributed throughout the marshes and ponds of Bermuda (Hurdis 1897, Smith-Vaniz et al. 1999). Beebe & Tee-Van (1933) described killifishes (referred to as the 'mangrove minnow' or 'mangrove mullet') as 'abundant in brackish pools and ponds.' Historical collections also indicate that killifishes were once extant in Stocks Point on St. David's Island, in Pembroke Marsh, and in what was once an area known as Paget East swamp.

During the 1990s local conservationists were aware of extant populations only in Lover's Lake, Mangrove Lake, Trott's Pond, both East and West Walsingham Ponds, Warwick Pond and

Table 3. *Fundulus bermudae* and *F. relictus*. Mean, median and range of total length (mm) of killifish in each of the ponds studied

Pond	N	Mean	Median	Range
F. bermudae				
Blue Hole Bird Pond	704	55.0	54.0	34–97
Mangrove Lake	664	71.9	67.0	52–126
West Walsingham Ponds	513	48.3	48.0	27–72
Trott's Pond	500	61.5	56.0	36–100
Warwick Pond	150	77.1	79.5	41–129
F. relictus				
Bartram's Pond	500	53.8	53.0	38–92
Lover's Lake	500	63.0	63.0	41–97

Table 4. *Fundulus bermudae* and *F. relictus*. Female to male sex ratios (F:M) of killifish in each of the ponds studied

Pond	N	F:M
F. bermudae		
Blue Hole Bird Pond	704	1.78:1
Mangrove Lake	664	1.17:1
West Walsingham Ponds	513	1.86:1
Trott's Pond	500	1:1
Warwick Pond	150	1.21:1
F. relictus		
Bartram's Pond	500	2.06:1
Lover's Lake	500	1.08:1

Table 5. *Fundulus bermudae* and *F. relictus*. Summary of the statistical tests for male (M) and female (F) killifish size and sex ratio (M:F) comparisons in each pond. Differences were tested with Mood Median tests and chi-squared tests (with 1 degree of freedom), respectively. Significant differences between the sexes are in **bold** ($p < 0.05$)

	N	F vs. M size	F:M sex ratio	
		p	χ^2	p
F. bermudae				
Blue Hole Bird Pond	704	0.285	54.2	**<0.001**
Mangrove Lake	664	0.062	3.46	>0.05
West Walsingham Ponds	513	**0.023**	47.71	**<0.001**
Trott's Pond	500	0.325	0	>0.05
Warwick Pond	150	**0.009**	1.31	>0.05
F. relictus				
Bartram's Pond	500	0.127	60.13	**<0.001**
Lover's Lake	500	**0.006**	0.8	>0.05

Evan's Pond. There were 4 other populations living in man-made ponds (created by dredging), where killifishes had been intentionally introduced as a precaution against possible extinction events. *Fundulus relictus* from Lover's Lake had been introduced in 1986 to Bartram's Pond on the Stoke's Point nature reserve, and in 1995 *F. bermudae* from one of the West Walsingham Ponds had been introduced to Blue Hole Bird Pond in the Blue Hole Park. In 1976, specimens of *F. bermudae* from Trott's Pond had been introduced to an artificially created saltwater pond on the Nonsuch Island nature reserve, while specimens of the same species from Mangrove Lake were later introduced, after adaptation, to an artificial freshwater pond on the same island in 1993 (Table 1) (D. B. Wingate pers. comm.). By 2001, it was suggested that 2 of the naturally occurring *Fundulus* populations extant in the 1990s might have died out (Grady et al. 2001), and in 2003 at least one population on Nonsuch Island had disappeared as a result of total destruction of a saltwater pond during Hurricane Fabian (J. Madeiros pers. comm.)

The present survey results reconfirmed the presence of all the naturally occurring populations of *Fundulus* known to be extant in the 1990s, as well as 2 of the 4 translocated populations in the man-made ponds. Killifishes were not, however, found on the Nonsuch Island nature reserve, despite a recent (1993) introduction in one of the ponds. These results indicate that Bermuda's *Fundulus* species may have completely disappeared from the coastal mangrove and the inland marsh communities that may have provided a degree of continuity between isolated killifish pond populations. The extant populations were found living mostly in ponds of the eastern parishes of the islands, with 2 exceptions. Most of these ponds are small, isolated and anchialine in nature. Human modification of historical killifish habitats is clearly the single greatest reason why distribution is currently limited.

Site visits, direct observation and trapping proved to be effective in identifying extant populations; however, it remains possible that small killifish populations were overlooked in the present study. Other *Fundulus* species are well known for utilizing shallow water sediments for refuge (Minckley & Klaassen 1969). It is feasible that a very small population dispersed around a fairly large area, and living almost exclusively in the bottom sediment would create the illusion that a pond was uninhabited. This may have been the case in Warwick Pond, thereby leading conservationists to believe (erroneously) that the original population had disappeared by 2001.

Population and individual sizes

Prior to this investigation little was known of the health and status of Bermuda's *Fundulus* populations. Knowledge of basic population estimates and their structure is necessary for conservationists to make informed management decisions and is critical to the development of species recovery plans.

The Petersen Index method for estimating population size in the present study gave consistent results. Malone et al. (1999) showed that VIE tagged fish were not vulnerable to higher levels of predation when compared with unmarked fish, and VIE tags have high retention rates when implanted in fishes (e.g. Dewey & Zigler 1996, Frederick 1997). In addition, it was reasonable to assume that there was negligible recruitment or loss in the mark populations because of the geographic isolation of each pond and the short survey period (i.e. weekly samples for 4 wk)

Trapping intensity varied between ponds, as did the time of year when each survey was performed, so it was not possible to compare the sizes or sex ratios of each *Fundulus* population amongst ponds to determine whether significant differences occurred. Previous studies investigating the size of individuals of *F. heteroclitus* populations from North Carolina, USA, and Nova Scotia, Canada, report maximum ranges from 90 to 120 mm total length (TL) (Fritz & Garside 1975, Kneib 1976). In a comprehensive study of killifishes in a salt marsh in Tar Landing Bay (North Carolina), Kneib (1976) reported that the sizes of *F. heteroclitus* caught in minnow traps were in the range 16 to 72 mm standard length (SL) (approximately 90 mm TL), with an average size of 37.8 mm SL (or approximately 47.5 mm TL). Similarly Fernández-Delgado (1989) stated that the largest individuals sampled in a population introduced to the Guadalquivir marsh of southwest Spain were 100 to 120 mm TL, although larger fish measuring up to 132 mm TL were sporadically caught. In comparison, the populations of killifishes in Bermuda that were sampled with minnow traps during 2004 and 2005 ranged from 27 to 129 mm TL and the mean sizes ranged from 48.3 to 77.1 mm TL (Table 3). Only 2 individuals out of 3531 examined measured over 120 mm TL. Kneib (1976) used a combination of minnow traps and pit traps to collect fish of all age classes. Pit traps resembled small, water-filled depressions that occur on marsh mud surfaces at low tides. This trapping technique was most effective for fish of 5 to 40 mm SL, but was not usable in this study because Bermuda's ponds have a very limited tidal range (Thomas et al. 1991) and no exposed mud. Thus, the sample ranges and sample means (Table 3) are not fully representative of the entire population in each pond, leading to overestimation of the mean fish size of each pond population (as well as underestimation of population size). Kneib (1976) reported that young-of-the-year killifishes in their first growing season (Age 0, <30 mm SL) made up approximately 60% of the fish population in a month (August) represented by all age classes in Tar Landing Marsh. Fernández-Delgado (1989) reported a similar finding in southwest Spain. It is likely therefore that the population estimates (Table 2) for Bermuda's killifishes are conservative.

Since Bermuda's killifish are now found in a few isolated populations, consideration has to be given to their viability in the short and long term. Population size is a major factor in the survival or extinction of populations: larger sizes provide insurance against unpredictable environmental events (environmental stochasticity) as well as stochastic changes in age structure, genetic drift and inbreeding depression (see Thompson 1991, Reed et al. 2003 for discussion). When considering the viability of vertebrate populations, there is a generally accepted 50/500 'rule of thumb' that a minimum of 50 adults is required to prevent an unacceptable level of inbreeding, and a population of 500 adults is required to maintain genetic variability (Franklin 1980, Soule 1980). If this is true, all of the Bermuda ponds currently containing killifish would appear to have sufficiently large populations, with the exception of Warwick Pond (estimated population size 436 individuals > 27 mm TL), and possibly Evan's Pond and the East Walsingham Ponds. Certainly, the killifishes would seem to be less threatened than the extensively studied desert pupfish *Cyprinodon* spp., some populations of which have consisted of <500 individuals for many decades (e.g. Chernoff 1985). However, more recent modeling exercises have taken stochastic events of demography and environment into consideration (see Reed et al. 2003), especially for fish (Nelson & Soule 1987), and suggest that long-term survival (>40 generations) requires minimum populations of the order of 5000. Given this criterion, the killifish populations in Bartram's Pond and the West Walshingham Ponds would be counted among those more vulnerable to extinction — and this does not take into account the fact that it is not feasible in the field to distinguish between the 2 endemic Bermudian killifish species, so that the pond–species attributions shown in Table 1 are tentative rather than definitive. Environmental stochasticity, associated with the frequent hurricanes that affect Bermuda, may have a greater influence on the probability of species survival than genetic issues (though the data of Grady et al. (2001) indicate some genetic bottlenecks); however, in either case, a minimum population size of 5000 seems a sensible target for insuring the survival of these isolated populations.

Sexual dimorphism, sex ratios, and predation

As with *Fundulus heteroclitus* populations in previous studies (Kneib 1976, Fernández-Delgado 1989), Bermuda's *Fundulus* females appeared to grow larger than males. In all pond samples except Bartram's Pond, female median sizes were greater than males, though this difference was not always statistically significant (Table 5). Fernández-Delgado (1989) found that overall sex ratios in *F. heteroclitus* on the southwestern Iberian Peninsula did not differ significantly from 1:1, whereas the sex ratio data of the *F. heteroclitus* population in the Tar Landing Marsh study (Kneib 1976) showed a seasonal trend with a bias towards females in late winter, spring and early summer in the larger size classes. Females in Bermuda outnumbered males in all ponds surveyed, except Trott's Pond, where the sexes occurred in equal numbers. It is possible that males are less likely to enter a baited trap during the breeding months, and male territoriality might affect local sex ratios. However, this explanation seems improbable, since females consistently outnumbered males in the survey samples, some of which extended past the spring and summer breeding months (e.g. Blue Hole Pond, Mangrove Lake, Bartram's Pond, Trott's Pond and Warwick Pond).

Selective predation may explain the observed sex ratios in Bermuda's killifishes. Males are more conspicuous due to their brighter colouration during the spawning months, thus making them more likely to be targeted by predators. Observations (M. Outerbridge pers. obs.) suggest that Bermuda's killifishes are eaten by piscivorous birds and fishes that include resident and migratory herons belonging to the family Ardeidae, gray snappers *Lutjanus griseus*, the American eel *Anguilla rostrata*, and probably the mosquito fish *Gambusia holbrooki*. The last species preys on a wide variety of fish species globally, and its introduction has contributed to the demise of many populations of native fishes with similar ecological requirements to killifishes (Meffe 1985, Page & Burr 1991). *G. holbrooki* was deliberately introduced to Bermuda in 1928 as a biological agent in mosquito control (Smith-Vaniz et al. 1999); it is prevalent in nearly all of Bermuda's inland wetland habitats, including 5 of the *Fundulus* ponds (Bartram's Pond, the East Walsingham Ponds, Trott's Pond, Mangrove Lake, and Warwick Pond). While eastern mosquito fish are not likely to be a threat to larger killifishes, they may be to eggs and fry. The combined effects of predation by this diverse suite of piscivorous birds and fishes on the *Fundulus* species of Bermuda are unknown at present.

Environmental factors

Fundulus species have been reported to withstand a wide range of temperatures and salinities (Griffith 1974, Kneib & Stiven 1978). Waas & Strawn (1983) found that the reproductive activity of *F. grandis* ceased during hot midsummer months in shallow water ponds and that fish living in deeper tide marsh environments had heavier gonads and carried more ripe eggs than the pond populations. While it appears that the *Fundulus* populations in Bermuda's ponds recruit, despite the shallow nature of many of them (Outerbridge 2006), the small population found in Warwick Pond, and low population numbers (anecdotal evidence) in both Evan's Pond and the East Walsingham Ponds, is a cause for concern. High summer water temperatures caused by extreme shallowness, particularly in both Warwick and Evan's Ponds that have mean depths of 20 and 65 cm, respectively, together with low levels of dissolved oxygen (Thomas et al. 1991, M. E. Outerbridge & M. L. H. Thomas unpubl. data), may affect gonadal maturation and egg survivorship. Greeley & MacGregor (1983) suggested that constant high temperatures may slow the rate of ovarian rematuration in *F. grandis* sufficiently to limit the potential frequency of spawning, while Taylor & DiMichele (1983) found that extended periods of immersion with oxygen levels lower than 2.0 ml l^{-1} resulted in developmental arrest or death of *F. heteroclitus* eggs. Both factors could cause a failure in recruitment that, operating repeatedly over a time span of a few years, could cause a crash in numbers as an ageing population dwindles. This may explain why the population structure in Warwick Pond was dominated by large individuals, where the median size was 79.5 ± 16.5 (SD) mm TL (Outerbridge 2006).

Taxonomy

Subsequent to the colonization of Bermuda by the first founding population(s), isolating events, possibly in the form of sea level changes (Smith-Vaniz et al. 1999) followed by human induced habitat modification, have ensured that all extant *Fundulus* pond populations are now geographically and reproductively isolated from each other (Fig. 2). A possible exception may be the ponds in the Walsingham area — a tract of land with a network of ponds, subterranean caves and passages, most of which are flooded with seawater. As a result of the isolating events, genetic differentiation and speciation may have occurred within each population across Bermuda.

The taxonomic diversity of Bermuda's killifishes has still not been fully resolved, despite at least 2 efforts within the last 20 yr (Able & Felley 1988, Grady et al. 2001). Smith-Vaniz et al. (1999) have proposed that there may be an additional 2 endemic species present. Clarification of the number and distribution of Bermuda's *Fundulus* taxa is necessary for the implementation of management strategies. The latest taxonomic investigation of Bermuda's killifishes used sequence variation in the mitochondrial Cytochrome b gene from 4 extant populations (Grady et al. 2001). The results indicated that 'the populations did not appear to have accumulated intrapopulation variation or much interpopulation haplotype diversity', (p. 47); however, the authors suggested that 'more rapidly evolving genes…should be used to investigate both the taxonomic and conservation status of these populations', (p. 50). Consequently, new DNA analyses are currently being undertaken using fin clippings of all extant *Fundulus* populations.

Management and recommendations

Since the 1960s, local organizations including the Bermuda Audubon Society and the Bermuda National Trust have raised funds to purchase wetland habitats, holding them in trust as nature reserves, thus ensuring some protection against development. At the government level, protective planning legislation in 1983 designated all of the remaining wetland areas in Bermuda as nature reserves. Additional efforts have been made by a number of conservation agencies to raise public awareness regarding the ecological and aesthetic value of Bermuda's limited wetland habitats. Deliberate restoration projects have focused on the fresh and brackish marsh pond habitat, with the end result that a variety of ponds and marshes have been physically and biologically re-established island wide. The results of the present study provide crucial information that has informed the design of a killifish management and recovery plan, which is presently being drafted by the Bermuda government's Department of Conservation Services. Already it is evident that restoration of coastal wetland habitats, combined with transfer of killifishes from the larger extant pond populations, would be a sensible step forward — but should await more definitive genetic characterization of populations. The most immediate need is to evaluate the possibility of ameliorating the situation of killifishes living in small populations in very shallow ponds.

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