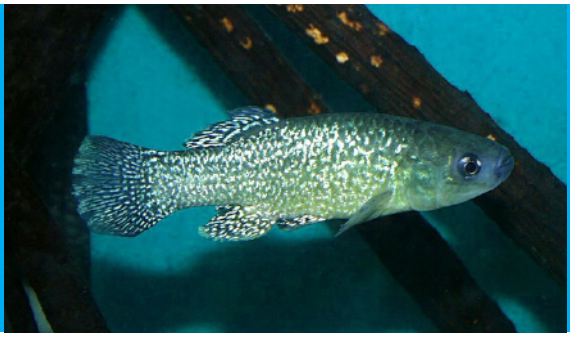
RECOVERY PLAN FOR KILLIFISH IN BERMUDA

(*Fundulus bermudae, Fundulus relictus*)



GOVERNMENT OF BERMUDA
Ministry of Public Works
Department of Conservation Services

RECOVERY PLAN FOR KILLIFISH IN BERMUDA

(*Fundulus bermudae, Fundulus relictus*)

Prepared in Accordance with the Bermuda Protected Species Act 2003

Funded in part by:

OTEP

Primary Authors
This recovery plan was prepared by:
Mark Outerbridge[1] and Samia Sarkis[2]
[1]Bermuda Zoological Society
Bermuda
[2]Protected Species Coordinator
Department of Conservation Services,
17 North Shore Road, Hamilton FL04
Bermuda

Contact: Samia Sarkis, scsarkis@gov.bm

Cover Photos were taken by Mark Outerbridge and Jennifer Gray
All other photos in the document by Mark Outerbridge
Maps were prepared by Mark Outerbridge

Published by

GOVERNMENT OF BERMUDA
Ministry of Public Works
Department of Conservation Services

To conserve and restore Bermuda's natural heritage

CONTENTS

LIST OF TABLES

LIST OF FIGURES

DISCLAIMER

Recovery plans delineate reasonable actions that are believed to be required to recover and/or protect listed species. We, the Department of Conservation Services, publish recovery plans, sometimes preparing them with the assistance of field scientists, other government departments, and other affected and interested parties, acting as independent advisors to us. Plans are submitted to additional peer review before they are adopted by us. Objectives of the recovery plan will be attained and necessary funds made available subject to budgetary and other constraints affecting the parties involved. Recovery plans may not represent the views nor the official positions or approval of any individuals or agencies involved in the recovery plan formulation, other than our own. They represent our official position only after they have been signed by the Director of Conservation Services as approved. Approved recovery plans are subject to modifications as dictated by new findings, changes in species status, and the completion of recovery actions.

Literature citation of this document should read as follows: M. Outerbridge and S. Sarkis, 2011. Recovery Plan for Killifish in Bermuda (*Fundulus bermudae, Fundulus relictus*). Department of Conservation Services, Government of Bermuda. 47 pages.

An electronic version of this recovery plan will also be made available at www.conservation.bm

----
Director
Department of Conservation Services
Government of Bermuda

11/1/12

Date

ACKNOWLEDGEMENTS

This investigation was initiated by the Bermuda Biodiversity Project, and funding was provided by Ernest E. Stempel Foundation, Mr. and Mrs. Anthony Jonklaas of the Kenridge Fund, the Bermuda Government Department of Conservation Services, the Bermuda Fry-Angle Aquarium Society, and Mr. and Mrs. David Cox. The support of these sponsors is gratefully appreciated. Thanks are due to Dr. Sarah Manuel and Anson Nash of the Department of Conservation Services Applied Ecology Unit and also to Evan Outerbridge for providing technical assistance in the field, as well as to Dr. Brian Luckhurst and Tammy Trott at the Department of Environmental Protection for granting access to their laboratory. The drafting and publication of this recovery plan was supported in part by OTEP (BDA 202), for which we are grateful.

EXECUTIVE SUMMARY

Current Species Status:
This recovery plan addresses the need for actions to conserve two endemic species of killifish, *Fundulus bermudae* and *Fundulus relictus*, in Bermuda. Both species are listed as Endangered (EN, B1a, biii) as per IUCN criteria, under the Protected Species Act 2003. Currently, there are no conservation measures in place for Bermuda's killifish species. These species represent up to 50% of the ichthyofauna inhabiting some of Bermuda's marine and brackish ponds. There is a dearth of information regarding the health and status of these *Fundulus* populations. As of 2006 estimates range from 436 to 11,325 mature individuals per pond. Those killifish inhabiting ponds located in parks or nature reserves are protected in part by Bermuda Parks Regulations. There is a possibility that an additional endemic *Fundulus* species exists, resulting from its prolonged isolation in one known pond.

Habitat Requirements and Threats:
These euryhaline species are highly adaptable and capable of living in water that has wide ranges in temperature and salinity. In Bermuda as of 2006 they were found in nine isolated marine and brackish water ponds, although historical distributions included marshes, canals, muddy bays and coastal mangrove communities. It is thought that the principle factor that has led to their limited distribution is loss of habitat through fragmentation of the wetlands in Bermuda. This restriction in habitat is due to both human development and natural processes. Pollution of ponds has also contributed to the decline in available habitat, as ponds and marshes were historically used as garbage disposal sites. Finally, predation by grey snappers, the American eel, and birds has been suggested as affecting killifish populations.

Recovery Objective:
The main goal of this plan is to increase population levels within each pond to ensure self-sustainability, and increase the area of occupancy for all *Fundulus* species in Bermuda, while maintaining genetic diversity.

Recovery Criteria:
Down listing for the two species of killifish in Bermuda will be considered when:

- The taxonomic status of all extant *Fundulus* populations in Bermuda is certified
- The current population status is assessed
- All potential habitats suitable for growth, reproduction and survival are identified
- Each species of *Fundulus* inhabits at least three separate ponds
- Population levels in each pond are >5,000 mature individuals

Actions Needed:

1. Identification of genetic make-up of existing populations for all ponds
2. Protection of species and pond habitat through legislation
3. Identification and restoration of protected wetland habitats suitable for killifish reintroduction
4. Population enhancement through captive breeding
5. Expansion of area of occupancy through translocation of known killifish species
6. Development of research programmes on reproduction of wild populations

Recovery Costs:

The total cost of recovery actions cannot be defined at this point. Funding needs to be secured through non-Governmental Organizations (NGO's), overseas agencies, and other interested parties for implementing the necessary research and monitoring studies on the biology of the killifish. Developing budgets for each action are the responsibility of the leading party as outlined in the work plan.

Date of Recovery:

Meeting the recovery objectives in Bermuda will depend on the restoration and protection of available habitats. Down listing will be considered following 10 years of implementation, once evaluation of conservation efforts is complete.

PART I: INTRODUCTION

A. BRIEF OVERVIEW

The killifishes, *Fundulus bermudae* and *Fundulus relictus*, are endemic to Bermuda. 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). Killifishes 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 marine and brackish pond environments (Outerbridge, 2006). There has been a dearth of information regarding the health and status of these *Fundulus* populations. Knowledge of the reproductive ecology as well as 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.,* 2007a & 2007b). Consequently, work on these species was initiated in 2004 by Mark Outerbridge of the Bermuda Zoological Society. Although this species has a fast doubling time, there have been few opportunities for range increase, due in great part to the restriction in habitat availability. The fragmentation of the wetland habitat in Bermuda makes these species vulnerable to human impact. Due to these unresolved threats, both species of killifish are listed under the Protected Species Act, 2003 as Endangered.

This recovery plan discusses threats and conservation efforts for the *Fundulus* species of Bermuda, summarizing current knowledge of the taxonomy, distribution, habitat requirements, biology and threats. The plan first recommends the identification of the genetic make-up of the species for all ponds, as there is some uncertainty regarding the existence of a third *Fundulus* species. The recovery of the populations depends on the availability of suitable habitat, hence the restoration of selected ponds is a priority in this plan. Finally, in order to ensure sustainability of the populations within Bermuda, an increase in the area of occupancy for each species, as well as in population levels is recommended and deemed possible through translocation and captive breeding. Should this be realized, it may be possible to down list *Fundulus* species of Bermuda to a lesser threatened status and/or remove it from the Protected Species list.

B. CURRENT PROTECTION STATUS

Killifish are not considered to be a marine species, therefore no protection is provided to it by the Fisheries (Protected Species) Act 1972. Most of the ponds which killifish inhabit occur in nature reserves and parks. These areas are protected under the Bermuda National Parks Act 1986, which prohibits the taking of any flora or fauna within the park. Both known species of killifish in Bermuda, *F. bermudae* and *F. relictus*, are protected under the local Protected Species Act 2003 as Endangered (EN, B1a, biii as per IUCN criteria).

Legal Protection

The Protected Species Act 2003 considers as an offence the willful destruction, damage, removal or obstruction of a habitat, and the taking, importing, exporting, selling, purchasing, transporting or having in possession a protected species. Offenders are liable to a fine of up to $25,000 for continuing offences.

Habitat Protection

Populations found in existing nature reserves are also protected to some degree by Bermuda Parks Regulations. These include the East Walsingham and West Walsingham Ponds, Lover's Lake, Bartram's Pond, the Blue Hole Bird Pond and Warwick Pond.

Furthermore Lover's Lake is designated as a RAMSAR site, providing additional protection for habitat of *F. relictus*.

C. TAXONOMY AND DESCRIPTION OF SPECIES

Class: Osteichthyes (bony fishes)
Order: Cyprinododontiformes (topminnows and carps)
Family: Fundulidae (killifishes)
Genus: *Fundulus*
Species: *bermudae* and *relictus*
Common name: Bermuda killifish, mangrove minnow and mangrove mullet (Beebe & Tee-Van, 1933)

The global killifish family consists of five genera and approximately 48 species. Bermuda's killifishes are believed to be descendants of the *Fundulus heteroclitus —Fundulus grandis* species 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). *Fundulus bermudae* was first described in 1874 by Albert Günther, who named it in reference to its origin. In 1988 Able & Felley described a new species of *Fundulus*, recording *F. relictus* from a single pond location in St. George's Parish (Lover's Lake). This latter species is believed to be a relict form that was formerly more widely distributed. Males and females of both *F. bermudae* and *F. relictus* have a deep vertical body profile. The body is rather short and robust, with posterior placed dorsal and anal fins of approximately equal size, a deep caudal peduncle, and a somewhat rounded caudal tail. The head is short and the eyes are large. The pupils are black and, during courtship and spawning, the rest of the eye also goes black as well. Males are usually dark olive-brown dorsally and pale yellow ventrally, from the head to the anal fin, including the pectoral fins. However, during the breeding season, spawning males tend to have an orange tinge on the mouth, lower snout and head, as well as a dark ocellus ringed in white on the posterior half of the dorsal fin. In both sexes 6–10 dark olive, irregular, vertical bars occur laterally from behind the head to the caudal peduncle. These bars are typically narrower and shorter in females than in males. Females are more plainly coloured, being light tawny yellow dorsally and nearly white ventrally, with lighter pigmentation in the fins and lacking the ocellus on the dorsal fin. They also possess a sheath of tissue on the anterior edge of the anal fin that functions as an oviduct during egg laying (Able & Felley, 1988).

Although very similar in appearance to *F. bermudae*, *F. relictus* can be distinguished by laboratory analysis of differences in certain body measurements and egg morphology; however, both species are indistinguishable in the field. It has been proposed that one, or possibly two, additional undescribed *Fundulus* species not previously documented from Bermuda occur (Smith-Vaniz *et al.,* 1999); however, it appears that no pond contains mixed populations. In 2001 researchers attempted to reconstruct the colonization history of Bermuda's killifishes using sequence variation in the mitochondrial cytochrome *b* gene in four extant Bermuda killifish populations. Their results suggested a prolonged isolation of the Evan's Pond population, which supported "the recognition of this population as an additional endemic species". Furthermore, the authors stated that this population is "an evolutionary significant unit within the *F. bermudae/F. relictus* lineage as well as a unique element in the evolutionary legacy of the entire *F. heteroclitus* group" (Grady *et al.,* 2001).

D. ECOLOGY

Habitat requirements

Killifish are extremely hardy and can tolerate widely fluctuating environmental conditions. They are a euryhaline species, easily surviving in salinities ranging from fresh water to full-strength salt water, as well as eurythermal, able to withstand temperatures from almost freezing to 58 ˚C (Griffith, 1974; Radtke, 1979). Another survival mechanism, proving once again the robust nature of the killifish, is the ability to survive in water that is severely oxygen depleted. In this condition killifish will gulp air at the surface in an effort to obtain oxygen by diffusion into the gills (Radtke, 1979). Additionally, killifishes are well known for utilizing shallow water sediments for refuge (Minckley & Klaassen, 1969).

Fundulus heteroclitus, also known as the salt marsh killifish, mummichog, or mudminnow, is native to coastal marshes, mudflats, river estuaries, tidal creeks, salt marshes, lagoons, and other shallow coastal habitats along the eastern coastline of North America from the Gulf of St. Lawrence to the Gulf of Mexico (Lee *et al*., 1980; Smith-Vaniz *et al.,* 1999). Conversely, Bermuda's extant killifish populations are currently found in the anchialine pond environment, except for those living in

Warwick Pond (which is seasonally fresh water). The present day saline, or anchialine, pools and ponds in Bermuda vary both in size and in structure. Nearly all date back in formation to the Holocene era (approximately 10,000 years ago.) The sporadic addition of fresh water into these ponds, either directly in the form of rainfall or indirectly as surface run-off, means that salinities vary throughout the year. They are generally slightly lower than that of pure seawater, but do show predictable seasonal patterns. The primary factor influencing salinity is the size and location of the underground connections each pond has with the ocean. Pond size, depth and volume, the size and nature of the connections to the ocean, the rate of fresh water inflow, and the tidal exchange of seawater all influence the hydrographic characteristics of each pond. Bermuda's anchialine ponds generally have a rich biota. Species richness increases with increasing physical stability and diversity of habitat. Thus ponds having submerged rock substrata, an abundant submerged mangrove root community along the periphery of the pond, and bottom sediment show greater diversity than ponds that feature sedimentary substrata only (Thomas *et al.*, 1992).

Physical Factors

The most important factor influencing physical stability is the amount of tidal exchange (Thomas *et al.*, 1992). Temperature and salinity are dependent upon the amount of seawater that enters from the ocean; for this reason, ponds close to the sea with relatively large connections have a higher flushing rate, with narrower ranges of salinity and temperature provide a more stable environment than that of ponds further from the sea. The mean ocean tidal range in Bermuda is only 75 cm, but is greatly reduced in the salt water ponds where there are more restrictions to tidal flow. While proximity to the ocean and the nature of the connections influence salinity level, the locations and sizes of these salt water inlets in relation to the tide level also affect the flushing rate. Salinity stratification can occur in poorly mixed ponds or where the connection to the sea is in the deepest part (due to the different densities of fresh and salt water), although this phenomenon is unlikely to occur in very shallow ponds. Thomas *et al.* (1991) described the physical characteristics of the six largest ponds in Bermuda after studying them over a 10-year period. The ponds are, in order of decreasing size: Mangrove Lake, Trott's Pond, Spittal Pond, Evan's Pond, Walsingham Pond, and Lover's Lake. Most possess a single connection to the ocean. Surface salinities ranged from 6.5 to 42.5 ppt, and the temperatures varied from 15.0 ºC to 37.5 ºC.

Biological Factors

Bermuda's marine ponds all have deep deposits of highly organic sediments and are subject to large changes in oxygen, redox potential, temperature, salinity and nutrient levels. Surface run-off from surrounding land transports particulate matter and plant nutrients into the ponds. Fringing mangrove trees are a common feature of marine ponds. These trees constantly drop leaves which slowly decompose, forming a highly organic detritus on the pond bottom that enhances the base of the food web. Due to their small physical size and accumulated sediments, the landlocked marine ponds are usually quite shallow, averaging depths of only 1.8 m. Because of this, ambient light levels at the bottom can be high, despite the fact that Bermuda's anchialine ponds typically are very turbid due to the high levels of suspended organic material. Plants, however, do not usually grow on the deeper bottoms of the ponds due to the unstable, anoxic environment created by the large amounts of sediment. The levels of dissolved oxygen also vary considerably between ponds in a diurnal cycle and at different times of the year. Daytime photosynthesis can saturate pond water with oxygen while the consumption of oxygen at night from fishes and microbial life on the sediment can reduce oxygen levels to zero, at least in patches, resulting in transitory nighttime anoxia. Anoxic events are routine in some of the poorly flushed anchialine ponds in summer and this is partly responsible for their low species diversity, which is typically more reduced than in open water marine habitats (Thomas & Logan, 1992). Competition, herbivory and predation are also generally less severe, thus favouring the growth and continued existence of some species that are rare or non-existent elsewhere. This coupled with the age of most of the ponds has in essence created refuges that had enabled species, like the killifishes, to evolve to the degree of endemism. Biotic characteristics of ponds are highly variable. Pond size, volume, and physical stability, as well as the stochastic nature of species colonization and the ability to of these species to adapt and survive in the ponds are all factors responsible for this biological variability. One of the curious features of the ponds is that there is great variability of biota among the ponds. Quite often a species is found in only one or a few ponds and very few species occur in all the ponds. This shows that despite the connections to the ocean, these ponds are relatively isolated. Without this isolation the killifish species would be homogenous in all ponds, whereas at least two species have evolved.

General Biology

Killifish in Bermuda appear to form into loose schools made up of equally sized individuals, feeding upon a wide variety of items that are both on the pond bottom and in mid-water. Preliminary studies indicate that they are opportunistic and omnivorous. Stomach content analysis has revealed that the killifish inhabiting Mangrove Lake eat filamentous green algae and plant material, mollusks, crustaceans and insects (Rand, 1981). Recent studies have shown that the various size classes of Bermuda's killifishes do not appear to be substantially different from the well studied *F. heteroclitus* populations along the east coast North America and in South West Spain. In fact the maximum sizes, as well as the mean sizes, in all of Bermuda's extant populations were larger than the maximum and mean sizes of fish caught on a salt marsh in North Carolina (Kneib, 1976). Bermuda's killifishes average 6.1 cm total length (TL) and rarely grow larger than 10 cm TL, although individuals have been measured up to 13 cm TL. Females are typically larger than males. Sex ratios among the Bermuda populations, where they were not 1:1, always biased towards females. In one instance (Bartram's Pond) this bias favoured females more than 2:1 (Outerbridge *et al.,* 2007a). Selective predation may account for this.

Reproduction
A study that examined the reproductive periodicity of the *F. bermudae* population in Mangrove Lake showed a distinct annual pattern in gonad development, with female and male gonadal cycles synchronous throughout the year. Spawning activity occurred over an eight month period starting in February (as indicated by the presence of ripe ova in the ovaries), continued into March and was then followed by a number of months of intense steady spawning activity, reaching a peak in May for males and in June for females. Gonadal indices abruptly declined after June, and continued to decline at a steady rate until September, which marked the end of the spawning season. Gonad recrudescence then lasted throughout the autumn and winter months (Outerbridge *et al.,* 2007b). It is unknown what the environmental cues are that elicit spawning in the Mangrove Lake population, however research into the reproductive biology of the North American species *F. heteroclitus* has linked spawning cues to environmental factors such as photoperiodicity, temperature, and tidal cycle (Hines *et al.,* 1985; Taylor, 1986).

Life Cycle
Based on observations of killifish in captivity, males and females have a brief courtship display ending with external fertilization. Females will deposit eggs individually on hard substrate (Able & Hata, 1984) and have been found carrying up to 108 ripe eggs at a single time (Outerbridge *et al.,* 2007b). These eggs are large in comparison to the overall body size of the female, and are quite sticky allowing them to easily adhere to solid surfaces. In the wild, Bermuda's killifishes begin to form breeding aggregations at the end of winter and continue to spawn throughout the spring and early summer months. The fry of the North American species *F. heteroclitus* typically hatch between 10–35 days depending on latitude, since temperature and salinity play a significant role in development (Kneib & Stiven, 1978). Numerous studies on *F. heteroclitus* have revealed that juveniles grow quickly, reaching sizes between 45–82 mm by the start of the next summer following hatching, and reach sexual maturity within the first year. They do not appear to live longer than four years (Fritz & Garside, 1975; Valiela *et al*., 1977; Kneib & Stiven, 1978). The lifespan of Bermuda's killifishes is unknown however they have been estimated to reach ages of five years, if not more.

E. CURRENT THREATS

Habitat Loss
The main reason for the recent decline in abundance and distribution of Bermuda's killifishes is attributed to habitat modification during the first half of the 20th century. Historically, killifish were known from the brackish ditches at the back of Hamilton (Pembroke Marsh), the ditches around Devonshire Marsh, Paget East swamp, Stocks Point (St. David's Island), and the muddy bays and mangrove swamps about St. George's (Hurdis, 1897; Smith-Vaniz *et al*., 1999). Human activities have

caused nearly all of Bermuda's wetlands to decline, although some natural processes have also had an impact. Since the island's colonization humans have filled, dredged, drained, denuded, and polluted the ponds, marshes, and mangrove swamps in an effort to create more arable land, residential and commercial building sites, as well as waste disposal sites. During the period of marsh reclamation by garbage disposal (1920–1970), five ponds totalling 1.6 hectares were completely filled in. Widespread drainage of marshes was employed as part of the mosquito control methods in the first half of the 20th century as health officials attempted to prevent the spread of malaria. Records indicate that in the 17th century approximately 127.5 hectares of fresh water ponds, marshes and swamps existed, representing 2.4% of the total land area of Bermuda. It has been estimated that during the 1970s 100 tons of garbage was dumped daily into the Pembroke parish marsh complex (Sterrer & Wingate, 1981). By 1980 Bermuda's total fresh water wetland area had been reduced by 65% to only 58.9 hectares (Thomas, 2004). It has been suggested that the most concentrated destruction of Bermuda's wetland communities occurred between 1941 and 1943 when 32% of the island's total mangrove acreage was destroyed on Longbird and St. David's islands by the construction of the American-operated Kindley Air Force Base (Sterrer & Wingate, 1981). This single act of environmental damage forever altered the water quality and marine communities within the Castle Harbour and St. George's Harbour area, the latter having been mentioned as an area frequently containing killifish (Drummond-Hay, unpublished notes in Smith-Vaniz *et al.,* 1999).

Pollution

In the past, ponds were used to dump trash which filled many of them while simultaneously created toxic conditions. Ponds and marshes identified as possible locations for *Fundulus* introduction, which were historically used as garbage disposal sites, should have analyses of sediment samples for metals, total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) done prior to the release of any fishes. The effects of present day pollution (i.e. atmospheric deposition and road run-off) are unknown. Anecdotal evidence suggests that the population in Warwick Pond may be adversely affected by poor water quality conditions.

Predation

Documentation of predators to *F. bermudae* and *F. relictus* is largely restricted to observational notes. One published study reported that the American eel, *Anguilla rostrata,* was a predator of *Fundulus* in Bermuda (Boetius & Boetius, 1967), and anecdotal evidence suggests that killifish are being eaten by grey snappers, *Lutjanus griseus,* in the West Walsingham Ponds, and by birds in Warwick Pond and Mangrove Lake (Wingate, 1994; Outerbridge, 2006). The mosquito fish, *Gambusia holbrooki,* has been reported to be predatory towards a wide variety of fish species around the planet and have caused, or contributed to, the elimination of many populations of fishes with similar ecological requirements (Meffe, 1985; Page & Burr, 1991). *G. holbrooki* were deliberately introduced in 1928 to help control mosquitoes and are especially prevalent in nearly all of the wetland habitats throughout Bermuda. Another possible predator is the red-eared slider, *Trachemys scripta elegans*. This invasive species is found in Warwick Pond (as well as in all of the fresh water wetlands across Bermuda), and stomach content analysis of individuals caught from Warwick Pond showed that they were eating small fish at that location, some of which were identified as mosquito fish (Outerbridge, 2008).

Human Collection
The greatest threat concerning human collection of killifish comes from the inadvertent mixing of the different wild populations from collectors who are not aware of the unresolved taxonomic debate. A summary of the known killifish transfer history among the ponds of Bermuda is given in Appendix I.

F. CURRENT STATUS

Global Distribution
F. bermudae and *F. relictus* are endemic to Bermuda, located in the Atlantic Ocean at latitude 32° 19' N and longitude 64° 46' W.

Local Distribution
F. bermudae and *F. relictus* are presently found in Bermuda in only nine isolated, brackish water ponds totalling approximately 16.6 Ha. The largest of these ponds is Mangrove Lake at 9.9 Ha; the second largest is Trott's Pond at only 2.9 Ha, and the smallest pond is the Blue Hole Bird Pond measuring 0.09 Ha (Outerbridge, unpublished data). Table 1 provides detailed information on the estimated population within each pond.

Table 1. Petersen estimates of population for *Fundulus* in Bermuda (Outerbridge, 2007a).

Species	Location	Estimated Population	SE +/-
F. relictus	Lover's Lake	8,508	1,347
F. relictus	Bartram's Pond	1,793	224
F. bermudae	Blue Hole Bird Pond	5,394	480
F. bermudae	West Walsingham	2,202	178
F. bermudae	East Walsingham	unknown	—
F. bermudae	Trott's Pond	7,926	1,576
F. bermudae	Mangrove Lake	11,325	1,884
F. bermudae ?	Warwick Pond	436	13
F. bermudae ?	Evan's Pond	unknown	—

? Indicates a possible new *Fundulus* species (see Smith-Vaniz, W.F., Collette, B.B., & Luckhurst, B.E., 1999. *Fishes of Bermuda: History, Zoogeography, Annotated Checklist, and Identification Keys*. p. 178)

Fundulus species were found in the following locations during surveys performed in 2004 and 2005.

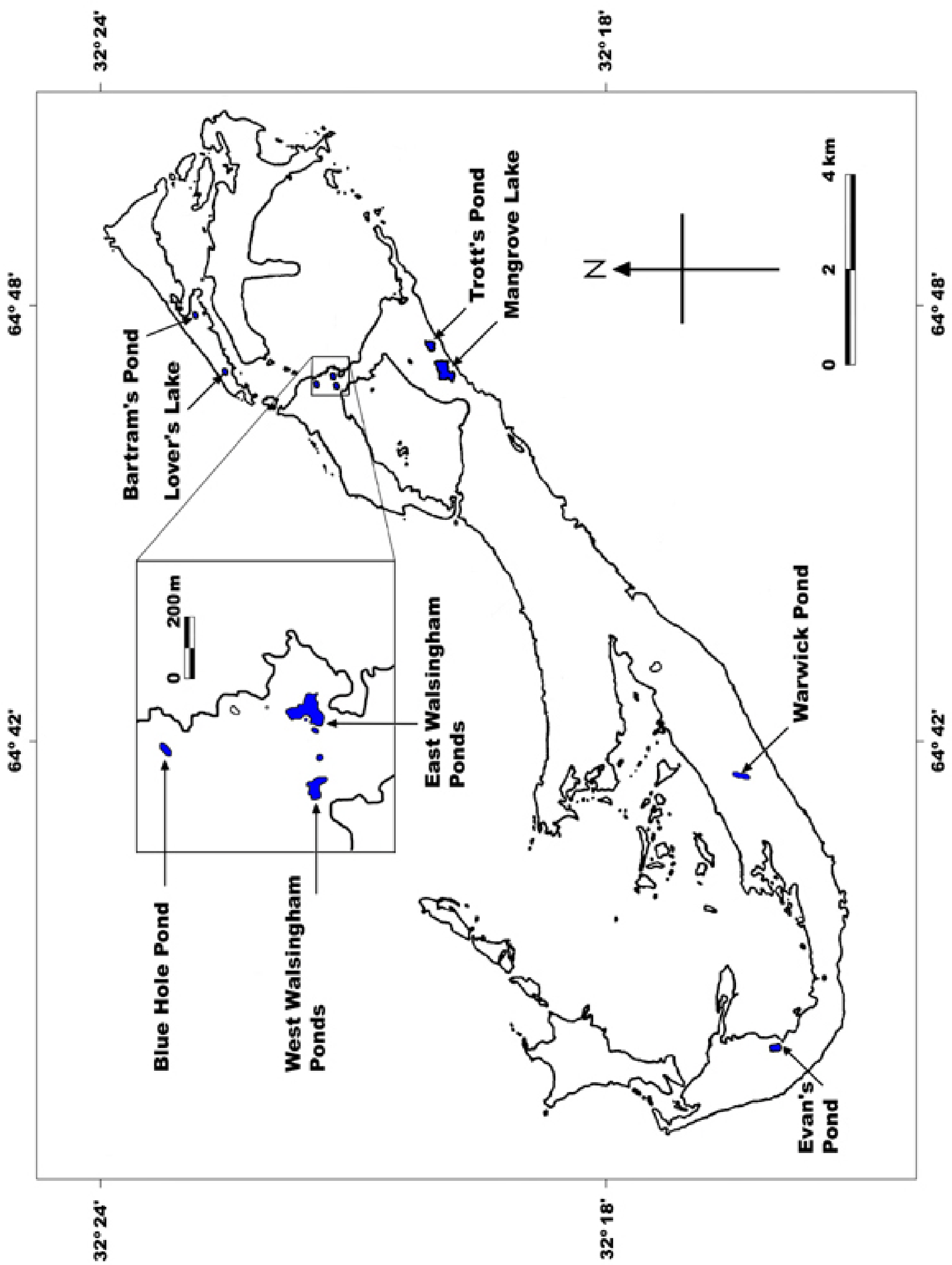


Figure 1. Map of Bermuda showing the pond locations where *Fundulus* was observed during 2004–2005 (Adapted from *Bermuda's Wetlands,* Thomas, 2002)

Lover's Lake

Lover's Lake is a 0.4 Ha marine pond situated in a government-owned nature reserve in St. George's Parish (Figure 2). It is a limestone basin, fringed by a dense growth of black mangrove trees, *Avicennia germinans*, which originally formed as an inter-dune valley that was further deepened by dissolution of the surface limestone by fresh water over a long period of time, as suggested by a very rocky shoreline and a number of exposed rock projections. It also has a shallow margin that quickly gives way to deeper water with two depressions located on the bottom, one of which continues on as a tunnel that connects to the ocean (Thomas & Logan, 1992). Appreciable amounts of sea water enters through this opening (resulting in > 60% flushing rate and a tidal range of 51.5 cm) that slowly mixes with the fresh water deposited during rain storms. The per cent flushing refers to the percentage of low tide volume added by tidal exchange on a mean tide. This index is an estimate of the exchange of pond water with ocean water by the tides. Higher percentages indicate a more stable marine environment. This situation has created a stable saline water layer in the deeper areas of the pond and a variable overlying layer of fresh, or brackish water, affected by evaporation and slow mixing (Thomas, 2002). The mean depth in Lover's Lake is 91 cm, although the maximum was recorded at 441 cm. Both of these figures are based on mean low tide level. Average annual surface water temperatures range from 18–29 °C (+/- 3.4 °C) and salinities vary from 22–37 ppt(+/– 3.7 ppt) (Thomas *et al.*, 1991).

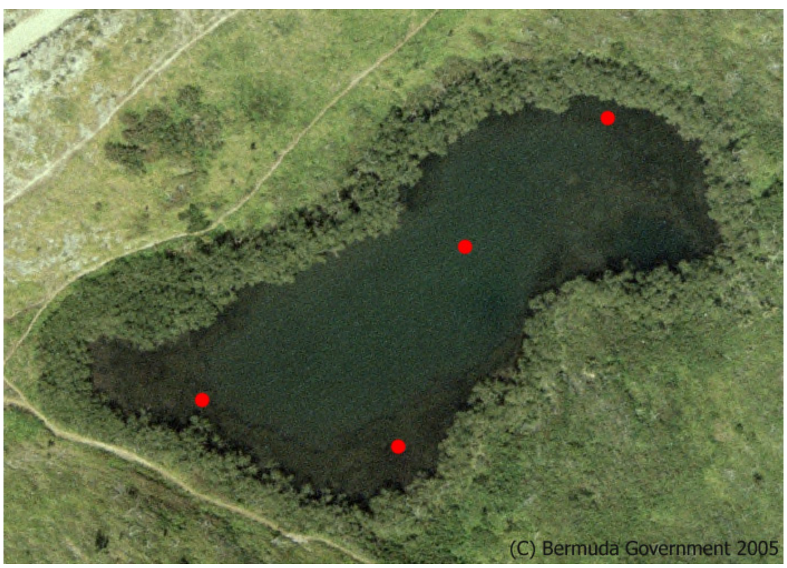


Figure 2. Aerial photograph of Lover's Lake showing previous *Fundulus* trapping locations

Bartram's Pond

Bartram's Pond (Figure 3) was originally about 0.3 hectares in size and bordered by mangroves, prior to being filled with garbage before 1945. Restoration began in 1983 with dredging, which resulted in the creation of a marine pond back to its former size and averaging 200 cm in depth. This new pond is situated in a one hectare nature reserve in St. George's Parish, and has two small islets intentionally created in the centre for water fowl. The mosquito fish, *Gambusia holbrooki,* was introduced in 1985, together with some invertebrate species that include the small gastropod *Batillaria minima,* and the sticky sea cucumber *Synaptula hydriformes*. *F. relictus* was introduced from Lover's Lake in 1986, and in 1987 red mangroves, *Rhizophora mangle,* were planted on the two islets which have since self seeded around the pond edges (Wingate, 1991). Other species currently found in the pond include widgeon grass, *Ruppia maritima*, transplanted from Spittal Pond, and the American eel, *Anguilla rostrata,* which have naturally colonized the pond. The underwater topology, as well as the annual variation in water temperature and salinity, for Bartram's Pond is unknown. Studies are presently being done.

Figure 3. Aerial photographs of Bartram's Pond showing previous *Fundulus* trapping locations

Blue Hole Bird Pond

The original pond was filled in the 1920s and 1930s. However, in 1991 a joint effort by the Bermuda Audubon Society and the Parks Department of the Bermuda Government resulted in the excavation of the site and eventual restoration of the former pond. The new 0.09 Ha pond (Figure 4), located on a government-owned nature reserve and park in Hamilton Parish, is marine, tidal and supports a population of *F. bermudae* and sparse widgeon grass deliberately introduced by Parks Department personnel from one of the West Walsingham Ponds. Red mangrove trees are also found growing in a dense thicket at the south west end of the pond, and there is visible evidence that this species is beginning to spread along the southern shoreline. The underwater topology, as well as the annual variation in water temperature and salinity, for the Blue Hole Bird Pond is unknown. Studies are presently being done.

Figure 4. Aerial photograph of the Blue Hole Bird Pond showing previous *Fundulus* trapping locations

East Walsingham Pond

The 0.8 Ha East Walsingham Pond, also known as Walsingham Pond and located in Hamilton Parish, is situated in a large tract of land designated as a nature reserve and managed by the Walsingham Trust (Figure 5). This pond is a partially flooded doline that originated from the collapse of a large cavern in the Walsingham Formation. It is fringed by red mangrove trees, has vertical cliffs that descend down to 5 meters into the water in some areas, and has two distinct basins separated by a shallow sill of rock submerged in thick sediment. The East Walsingham Pond is the deepest of all the ponds in Bermuda, and also contains the highest biodiversity. Oceanic connections are large, numerous and located close to the water surface in this pond thereby resulting in a comparatively large tidal range of 38.5 cm and a flushing rate of 9.7% (Thomas & Logan, 1992). This pond is well known for its high and varied biodiversity of marine fish species, algae and sponges and includes the endemic Bermuda sargassum, *Sargassum bermudense*, known only from this one area. The mean depth in the East Walsingham Pond is 392 cm, although the maximum was recorded at 618 cm. Both of these figures are based on mean low tide level. Average annual surface water temperatures range from 19–28 °C (+/– 3.4 °C) and salinities vary from 33–40 ppt (+/– 1.5 ppt) (Thomas *et al.*, 1991).

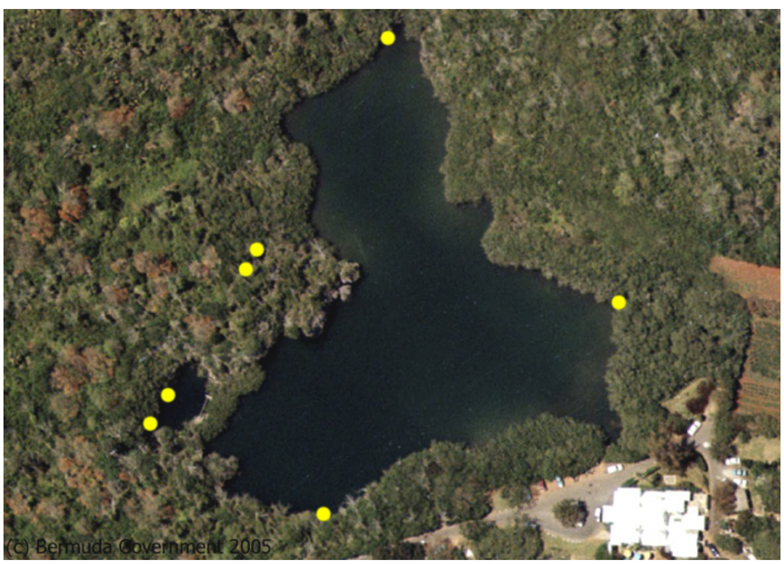


Figure 5. Aerial photograph of the East Walsingham Ponds showing previous locations of where *Fundulus* were observed

West Walsingham Ponds

The West Walsingham Ponds are also situated in a large tract of land in Hamilton Parish designated as a nature reserve and managed by the Wilkinson Trust (Figure 6). As with the East Walsingham Pond, they were naturally created when water accumulating at the surface in hollows and pools percolated through the porous limestone dissolving more and more calcium carbonate thereby creating subterranean pipes, tunnels and caves. Erosion thinning the rock over these caves then caused some to collapse forming the depressions, sink holes and ponds. Numerous exposed rock projections are common in the largest pond and around the edges. It is possible that part of the West Walsingham Pond complex also formed as an old inter-dunal low (M.L.H. Thomas, *pers. comm.)* The largest of the ponds has at least three surface connections to the sea, one of which is quite sizeable, accounting for the high tidal range observed in this pond. There is a substantial bed of widgeon grass growth in the shallow areas, and a number of marine fishes inhabit the ponds in addition to the killifish, that include grey snapper, *Lutjanus griseus*; mullet, *Mugil liza*; pin fish, *Lagodon rhomboids;* and the crested goby, *Lophogobius cyprinoides*. This pond area is only one of two killifish ponds that do not have any mangrove trees present, however it supports the best remaining salt marsh habitat in Bermuda. This marsh is heavily utilized by the killifish during high tides. The underwater topology, as well as the annual variation in water temperatures and salinities, for the West Walsingham Ponds is unknown. Studies are presently being conducted.

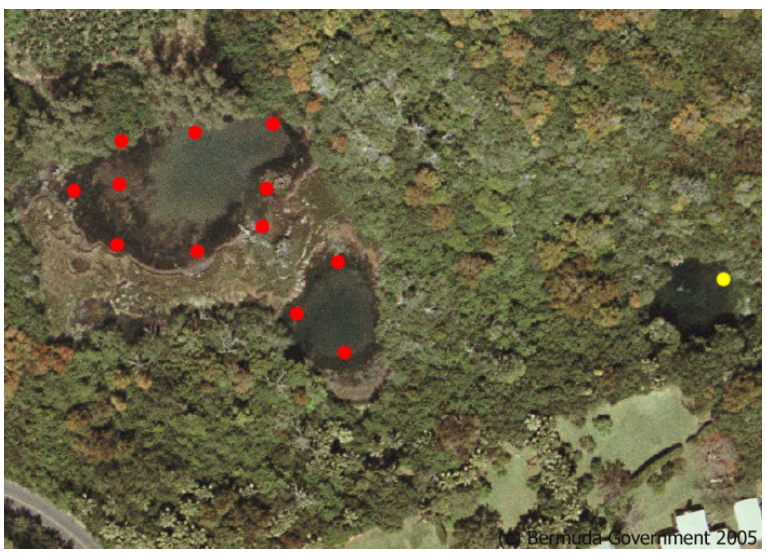


Figure 6. Aerial photograph of the West Walsingham Ponds showing previous *Fundulus* trapping locations (red) and locations where *Fundulus* were observed (yellow)

Trott's Pond

Trott's Pond is situated on a privately owned golf course in Hamilton Parish (Figure 7). It is 2.9 Ha and formed between low Pleistocene sand dunes which were inundated by postglacial seas. Over time, fresh water slowly eroded away from the depression creating fissures through which salt water entered from the south shore as the sea level rose around Bermuda. Trott's Pond is currently a simple basin fringed by red mangrove trees and characterized by fairly shallow depths, with the deepest part at its centre. It has fairly even contours and a gently sloping shoreline (Thomas *et al.*, 1992). The connection to the ocean is small and located at the surface. Not only does this connection give Trott's Pond a very low flushing rate of 0.5% and a small tidal range of 1.5 cm, but it also means that it has a yearly mean salinity very similar to that of the ocean. Rainfall and surface runoff from the surrounding area does not typically mix with the salt water below, but instead floats as a distinct layer on top, eventually draining off through the surface connection (Thomas, 2002). The mean depth in Trott's Pond is 269 cm, although the maximum was recorded at 320 cm. Both of these figures are based on mean low tide level. Average annual surface water temperatures range from 16–31 °C (+/– 4.8 °C) and salinities vary from 24–34 ppt (+/–2.6 ppt) (Thomas *et al.*, 1991).

Figure 7. Aerial photograph of Trott's Pond showing previous *Fundulus* trapping locations

Mangrove Lake

Mangrove Lake is located partly on a nature reserve, but mostly on private golf course property and is immediately adjacent to Trott's Pond in Hamilton Parish (Figure 8). It is 9.9 Ha and may have formed through the action of dissolution of calcium carbonate from either rock or sand thereby creating a depression that gradually filled with salt water as the seas rose (Watts & Hansen, 1986). Mangrove Lake is presently a simple basin fringed by red mangrove trees and characterized by shallow depths, fairly even contours and a gently sloping shoreline. It also has a fairly continuous moat-like depression around the edges with a shallower centre forming a slight dome, resulting primarily from sediment accumulation. A few small subterranean fissures ensure that ocean water still enters Mangrove Lake from the south shore, however this pond has a very low flushing rate of 1% as well as a small tidal range of 1.4 cm (Thomas *et al.,* 1992). The mean depth in Mangrove Lake is 134 cm, although the maximum was recorded at 223 cm. Both of these figures are based on mean low tide level. Average annual surface water temperatures range from 20–29 °C (+/– 3.4 °C) and salinities vary from 27–33 ppt (+/– 2.3 ppt) (Thomas *et al.*, 1991).

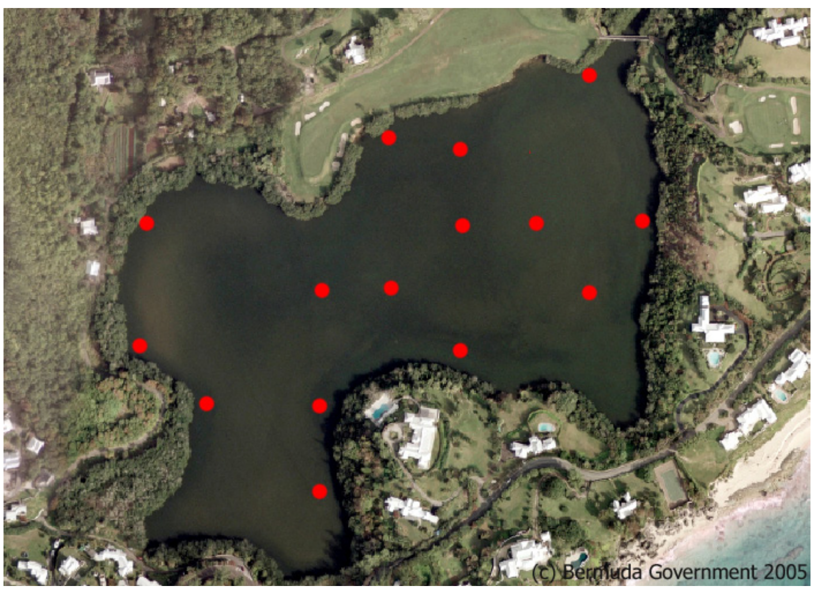


Figure 8. Aerial photograph of Mangrove Lake showing previous *Fundulus* trapping locations

Warwick Pond

Warwick Pond (Figure 9) is the third largest fresh water pond in Bermuda, measuring 1.3 Ha in area, and one of the few remaining seasonal fresh water wetland habitats. The land around the pond was once divided into many small, private holdings when land ownership gave extra voting rights, prior to the 1968 constitution. After 1968 these lots were gradually acquired by Graham Powell who offered the combined properties to the Bermuda National Trust. The Trust purchased the property in 1987, consolidating it with neighbouring land into a 3.8 hectare nature reserve. This pond is part of a long chain of wetlands that originally stretched from Southampton Parish to Spittal Pond in Smith's Parish. Much of the former Warwick Marsh basin has been lost to the effects of filling, drainage and ditching. The pond itself represents one of the few tracts of natural inland water that has survived and not been used for landfill. It is an important sanctuary for birds, and contains both killifish and large numbers of mosquito fish. Red-eared sliders, *Trachemys scripta elegans*, an introduced and invasive species, are also present. The pond originated from rain water collecting in an inland basin. Subsequent colonization of the area with various marsh flora occurred followed by the accumulation of dead plant remains over time. Water level fluctuations occur predominantly as a result of rainfall and run-off from surrounding hillsides and from evaporation, but also as a result of long-term tidal fluctuations raising and lowering the water table. The periphery of the pond is dominated by grasses and sedges. Distinct zones of native flora can be observed surrounding the pond including cattail, *Typha angustifolia*, the great American bullrush, *Schoenoplectus validus*, paspalum, *Paspalum distichum,* and *P. vaginatum*, and the invasive Brazil pepper tree, *Schinus terebinthifolia*. No mangrove species are present. There is very low biodiversity in the water, most likely resulting from a combination of factors that include high water temperatures in the summer months, unstable organic sediment, run-off pollution from surrounding land (it is bordered by a busy road and agricultural land), and the natural process of ecological succession whereby the pond is gradually filling with sediment and turning into a marsh (Thomas, 2002). This is evident by the pond's extremely shallow nature and the emergence of large parts of the muddy bottom during periods of drought. In the summer months, as evaporation increases, the water level decreases exposing more of the mud flats around the edges of the pond. The mean depth in Warwick Pond is 20 cm, although the maximum was recorded at 37 cm (Outerbridge & Thomas, unpublished data). The average annual surface water temperatures and salinities are unknown at this time. Studies are presently being done.

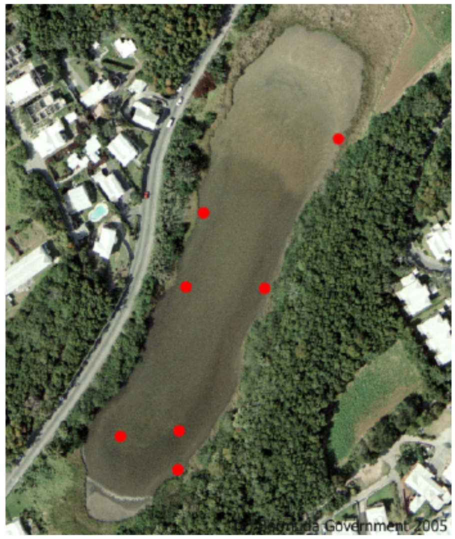


Figure 9. Aerial photograph of Warwick Pond showing previous *Fundulus* trapping locations

Evan's Pond

Evan's Pond, like most of the other ponds, is fringed by mangrove trees but in this case both species of mangrove are present. It is 0.7 Ha and is situated mostly on private land in Southampton Parish, but there is a nature reserve bordering the pond to the north (Figure 10). It formed between low Pleistocene sand dunes which were inundated by postglacial seas. Over time, fresh water slowly eroded away from the depression creating fissures through salt water entered as the sea level rose around Bermuda. This pond is a simple basin characterized by shallow depths, fairly even contours and a gently sloping shoreline. The pond is connected to the ocean in Evan's Bay by a large pipe-like opening through which marine fish and mobile invertebrates are known to regularly enter the pond. This opening also gives Evan's Pond a moderate tidal range of 7.5 cm and a flushing rate of 12% (Thomas *et al.,* 1991). These authors also stated that Evan's Pond had the second highest species count of the six largest anchialine ponds studied. The mean depth in Evan's Pond is 65 cm, although the maximum was recorded at 120 cm. Both of these figures are based on mean low tide level. Average annual surface water temperatures range from 17–32 °C (+/– 4.8 °C) and salinities vary from 28–41 ppt (+/– 3.3 ppt) (Thomas *et al.*, 1991).

Figure 10. Aerial photograph of Evan's Pond showing previous locations of where *Fundulus* were observed

G. CURRENT CONSERVATION ACTION

Post-mid-1960s restoration efforts have increased the open fresh/brackish water habitat such that the total area is now higher than at any time since the start of the 20th century. New ponds are continually being created which presents the unique opportunity to seed them with *Fundulus* rather than with *Gambusia*, the traditional choice of fish. Between 1976 and 1995 killifish of known identity were intentionally translocated from their native ponds to four man-made ponds as a precaution against possible extinction events. *F. relictus* from Lover's Lake were 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 were introduced to the dredged Blue Hole Bird Pond in the Blue Hole Park. In 1976 specimens of *F. bermudae* from Trott's Pond were introduced to an artificially created salt water pond on the Nonsuch Island nature reserve, while specimens of the same species from Mangrove Lake were later introduced, after adaptation, to an artificial fresh water pond on the same island in 1993 (D. Wingate, *pers. comm.*) (see Table 3 in Appendix I.) The successful translocation of *F. relictus* into Bartram's Pond and *F. bermudae* into the Blue Hole Bird Pond indicate that these species are ideal candidates for seeding newly created ponds, especially since they are very efficient predators of mosquito larvae.

Grady *et al.* (2001) used sequence variation in the mitochondrial cytochrome *b* gene in four extant Bermuda killifish populations to test taxonomic and phylogenetic hypotheses. Their findings did not resolve the taxonomic issues surrounding Bermuda's killifish species, however they did suggest that the Evan's Pond population is an evolutionary significant unit within the global *F. heteroclitus* group, and that the prolonged isolation of this population may support the recognition of these fish as an additional endemic species. In recent years many advances have been made in the field of conservation genetics. Consequently, a new study is currently underway at the Romberg Tiburon Center for Environmental Studies in San Francisco, California which once again attempts to clarify the number of different killifish species, or at least the degree of variation among various populations in Bermuda. Clippings taken in 2005 from the caudal fins of killifish caught from Lover's Lake (*F. relictus*), East Walsingham Pond, West Walsingham Pond, Trott's Pond, Mangrove Lake, Warwick Pond, and Evan's Pond (all presently named *F. bermudae*) have been sent to the laboratory for genetic analysis. The results are pending.

PART II: RECOVERY

A. RECOVERY GOAL

The principal aim of this Recovery Plan is to increase population levels within each pond, as well as increase the area of occupancy for all *Fundulus* species in Bermuda, while maintaining genetic diversity. If successful, this will ensure the sustainability of killifish populations in Bermuda, despite increasing pressure from human development.

The short-term goal (five years) is to continue research on the biology and ecology of Bermuda's killifishes, as well as assess the suitability of appropriate habitats and ensure their protection, in order to promote effective management.

The long-term goal (30 years) is to increase the population levels and range of Bermuda's killifishes, enhancing natural recruitment through captive breeding efforts, and restoring wetland habitats.

B. RECOVERY OBJECTIVES AND CRITERIA

Favourable conservation status will be achieved when:

- The taxonomic status of all extant *Fundulus* populations in Bermuda is fully resolved (i.e. when the total number of *Fundulus* species, or at least the degree of intra-population genetic sub-structuring, is known.)
- All potential habitats suitable for killifish survival, reproduction and growth are identified, assessed and restored.
- All *Fundulus* species and habitats are protected under legislation.
- Each species of *Fundulus* inhabits at least three separate ponds.
- Long-term, sustainable levels of killifish are reached in each pond (i.e. populations > 5,000).

These overall objectives translate into specific targets outlined below:

Short-term target (five years). To ensure that all studies necessary for development of effective management will be complete, and that both species and habitat will be protected under legislation. Habitats will be identified as "Critical Habitat" and designated as such under law, should they be considered crucial to the recovery of the species. This short-term goal includes re-surveying the extant populations, with a particular focus on Evan's Pond and Warwick Pond, and additional investigations to determine sources of predation. During this time, the identification and assessment of "health" status of current and potential habitats will be conducted.

Long-term target (30 years). In light of the habitat assessment, restoration of habitats will lead to the potential to increase the area of occupancy and population levels within each pond. Captive breeding and the engagement of the community will be necessary to achieving this long-term goal. Monitoring of efforts will be necessary to evaluate survival and growth of newly established populations, and determine their self-sustainability.

C. RECOVERY STRATEGY

The species addressed in this recovery plan are restricted to highly fragmented ponds, totalling less than 0.2 km^2. These ponds are furthermore easily impacted upon both chemically and physically by adjacent human activities, such as farming and maintaining golf courses which may result in fertilizer, pesticide and herbicide run-off, and ecologically by the encroachment of invasive species. The strategy for recovery revolves around the protection of wetland habitats, the assessment of their "health" status, namely water and sediment quality, their remediation in some cases, and in the active intervention required for increasing the species distribution to a greater range. Translocation of killifish of known species to new ponds is deemed necessary to ensure natural breeding and recruitment within all ponds. The uncertainty of the existence of a third species requires identification of the genetic make-up of the various pond populations, and a carefully thought out protocol needs to be developed to maintain genetic diversity through translocation.

The selection of ponds for translocation is critical as habitat quality appears to be poor in several areas, based on previous sediment and fish tissue analyses (J. Bacon, *pers.comm.*). This further drives the need for habitat protection of "healthier" ponds, controlling as much as possible input from external sources. It is believed that pollutants appear to be entering some of the ponds through groundwater, atmospheric deposition and/or road run-off (J. Bacon, *pers. comm.*). Based on this, stock enhancement needs to be focused on suitable ponds, further removed from potential contamination and success for growth and survival of the species further ensured via legislated habitat protection.

Mangrove Lake presently contains the largest population of *F. bermudae* (approximately 11,325 fish in 2004), while Lover's Lake contains the largest population of *F. relictus* (approximately 8,508 in 2004), and for this reason should be afforded high levels of protection. The populations in both Evan's Pond and Warwick Pond should also receive priority protection because there is evidence suggesting a prolonged isolation of the fish in these ponds; this supports the recognition of these Fundulus populations as an additional endemic species. Further evidence indicates that the Evan's Pond population is an evolutionary significant unit within the *F. bermudae/F. relictus* lineage as well as a "unique element in the evolutionary legacy of the entire *F. heteroclitus* group" (Grady *et al.* 2001).

D. TOOLS AVAILABLE FOR STRATEGY

There is a large amount of literature for *F. heteroclitus*. Based upon laboratory observations, Able & Hata (1984) described the reproductive motor patterns and spawning site preferences of five *Fundulus* species, including *F. bermudae* and *F. relictus*. They found that spawning site selection varied between these forms. The female *F. bermudae* individuals examined in this experiment were reported to deposit nearly 75% of their eggs on a spawning mop provided by the researchers, while the *F. relictus* females consistently ignored the spawning mop, preferring instead to use the glass surface of the aquarium, with an egg deposition rate of nearly 94%. Field observations are therefore strongly recommended for Bermuda's killifishes to determine natural spawning site preferences and time of day during spawning activity. Based on the research of Able & Hata (1984), it is quite possible that the *F. bermudae* and *F. relictus* populations in Bermuda's ponds are using submerged vegetation or algal mats to deposit their eggs. Mangrove roots and pneumatophores are present in all of the ponds currently inhabited by killifish except for the West Walsingham ponds and Warwick Pond, while widgeon grass *Ruppia maritima* was present in Bartram's Pond, West Walsingham Pond and Mangrove Lake. Due to the large biotic differences in Bermuda's ponds, it is not unrealistic to assume that the *Fundulus* species inhabiting them are opportunistic in spawning site selection, using whatever solid surfaces are available. Such surfaces might even include lost golf balls that can be found in great numbers in both Mangrove Lake and Trott's Pond. Studies are also warranted to determine the fecundity of Bermuda's killifishes and would be useful if any captive breeding programme is attempted. A reproductive characteristic typical of Cyprinodonts is the ability to spawn repeatedly in a single season. Foster (1967) found that female killifishes held in captivity under optimal conditions spawned almost daily throughout their breeding season laying up to 40 eggs per day, and Kneib (1976) stated that the mean number of ripe ova shed by wild female *F. heteroclitus* was 10–11 early in the season but later dropped to 1–2. Both authors also reported that the number of eggs a female is capable of producing is directly related to fish length.

Killifish are an ideal species for captive breeding and translocation since the population doubling time has been estimated to be 18 months. This means that the rate of growth of the reintroduced population will be high. Preliminary attempts on captive breeding were made in 2011, where eight "parent" killifish were transported to the Vienna Zoo, resulting in 90 F1 generation offspring. This first experiment indicates the potential for stock enhancement using controlled culture techniques, but at the time of writing the detailed breeding report was not yet completed. However, for further details on breeding techniques, please see Appendix II *Breeding Fundulus heteroclitus*.

If the results from the present genetics study show that there are only two species endemic to Bermuda, donor populations to a restored habitat need not be limited to the closest neighbouring pond. However, if there are more than two species, or significant genetic sub-structuring within species, then captive breeding programmes may be the only safe way to build up large enough stocks to seed new ponds while maintaining the health and viability of highly localized donor populations.

Additionally, there is information available on the levels of contaminants, such as metals, pesticides, pharmaceuticals, TPH and PAHs for some of the ponds, namely Warwick Pond, Lover's Lake and Mangrove Lake. Sediment and fish tissue analyses were conducted, providing data on suitability of selected ponds and health of fish (J. Bacon, unpublished). Necropsies on killifish of a number of ponds have also indicated abnormalities in reproductive tissue and should be taken into consideration when planning a captive breeding programme. Finally, the finding of cysts inside of killifish from Trott's Pond and Mangrove Lake further support a cautionary approach to translocation between ponds. All of this data is documented by Jamie Bacon (Bermuda Zoological Society), and should be duly taken into account for future translocation or stock enhancement programmes.

E. STEP-DOWN NARRATIVE OF WORK PLAN

The actions needed to achieve recovery are as follows:

1. **Identification of genetic make-up of existing populations for all ponds.**

   Actions proposed:

   - Analyses of collected samples
   - Protection of all species determined

   Work Team: Department of Conservation Services (DCS) and collaborative institution for analyses

   Team Leader: DCS

   Assistance: Graduate Student

   Outputs: Determination of taxonomic diversity of extant populations in Bermuda, Graduate thesis

   List of Equipment: Funding required for student stipend and laboratory fees

2. **Species and Habitat Protection**

   Actions proposed:

   - Integration of third *Fundulus* species (if determined) in Protected Species Act
   - Designation of Mangrove Lake, Lover's Lake and Evan's Pond (and possibly Warwick Pond as "critical habitat" for Bermuda's killifishes.

   Work Team: DCS

   Team Leader: DCS

   Assistance: Attorney General's Chambers

   Outputs: Legislation for habitat and species protection

   List of Equipment Required: GPS for boundary delineation, GIS mapping

3. **Identification and assessment of protected wetlands suitable for killifish reintroduction**
   Actions proposed:
   - Survey to identify suitable expansion habitats
   - Sediment and water quality analysis for all habitats, including sediment analyses for metals, total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs). *Gambusia* (mosquito fish) necropsy and tissue analyses for the same.

   Work Team: DCS, Environmental Health, Bermuda Zoological Society (BZS) and collaborative institution for sample analyses

   Team Leader: DCS

   Assistance: Bermuda National Trust, Department of Parks

   Outputs: A database for each pond will be established and kept up to date by team leader at DCS

   List of Equipment Required: Water quality testing kit, spectrophotometer and chemicals for colorimetric analyses

4. **Enhancing population numbers through captive breeding**
   Actions proposed:
   - Broodstock conditioning and egg incubation — captive breeding keeping known species separate (replacing broodstock every two months)
   - Growth of fingerlings
   - Fingerlings used for translocation programme (see Action 5)

   Work Team: DCS and Bermuda Fry Angle Association

   Team Leader: DCS

   Assistance: one full-time for six months broodstock conditioning (DCS) and coordination of egg distribution to Bermuda Fry Angle Association

   Outputs: Enhancing population size of natural stocks currently in low numbers contributing to translocation programme, report on culture techniques for Bermuda killifish, engaging community in preservation of threatened endemic species

   List of Equipment Required: Captive breeding equipment as suggested below:

   Two broodstock tanks (500 gallons), culture materials, supply of freshwater and seawater dependent on broodstock source, killifish feed, four tanks for egg storage (one gallon), egg distribution material (ziplock bags, water saturated cotton balls, etc.), trapping material and equipment for broodstock collection (see Action 3).

5. **Expand area of occupancy through translocation of known killifish species**

   Actions proposed:

   - Removal of eastern mosquito fish
   - Introduction of large numbers (minimum 500) of killifish in equal sex ratio, taking into account genetic make-up of different populations
   - Monitoring of introduced population by mark-recapture method
   - Multiple releases over time to ensure sustainability of population and optimizing genetic diversity
   - Control of invasive species management programme

A preliminary outline of the proposed translocation activities is given below in Table 2 and Figure 11.

Table 2. Summary of the proposed killifish transfers among the ponds of Bermuda.

Pond	Species	Source population	Date of introduction	Estimated number of fish
*Blue Hole Bird Pond	*F. bermudae*	West Walsingham	2008	250
*Bartram's Pond	*F. relictus*	Lover's Lake	2008	250
*Cooper's Island Pond	*F. relictus*	Lover's Lake	2008 & 2009	250 + 250
Shelly Bay Race Track Pond	*F. bermudae*	West Walsingham	2008 & 2009	250 + 250
*Wind Reach Pond	*F. bermudae*	Warwick Pond	2008 & 2009	50 + 250§
*Nonsuch Island FW Pond	*F. bermudae*	Warwick Pond	2009 & 2010	250§ + 100
*Devonshire Marsh (Freer Cox Pond)	*F. bermudae*	Warwick Pond	2010 & 2011	250§ + 250
*Paget Marsh (David's Pond)	*F. bermudae*	Warwick Pond	2010 & 2011	250§ + 250
*Seaswept Farm Pond	*F. bermudae*	Evan's Pond	2008 & 2009	50 + 250§
*Somerset Long Bay Pond	*F. bermudae*	Evan's Pond	2009 & 2010	100§ (†) + 150§ (†)
*Pitman's Pond	*F. bermudae*	Evan's Pond	2009 & 2010	100§ (†) + 150§ (†)
Seymour's Pond	*F. bermudae*	Evan's Pond	2009 & 2010	100§ (†) + 150§ (†)

* Indicates man-made pond

§ Indicates fish stock bred in captivity

(†) Indicates need to acclimate fish to salinity of the new pond prior to introduction

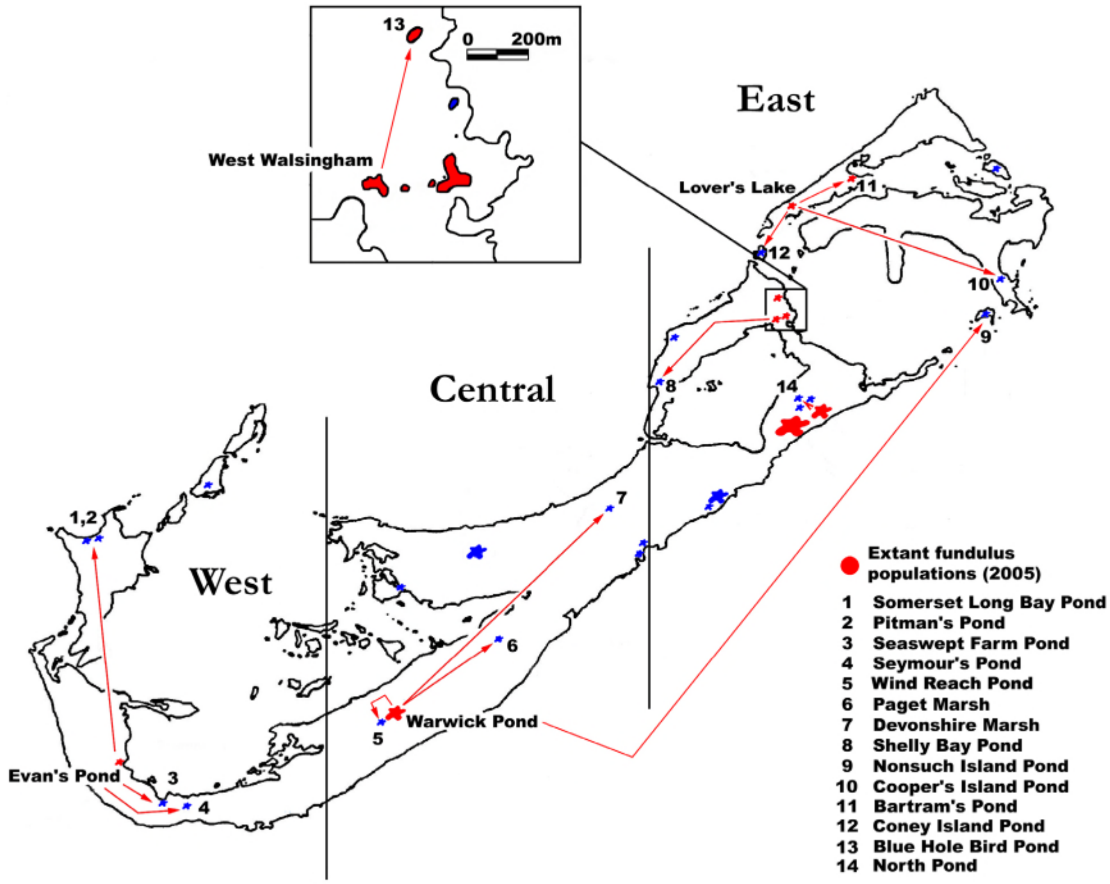


Figure 11. Schematic diagram of proposed translocation programme

Work Team: DCS and Environmental Health

Team Leader: DCS

Assistance: Bermuda Fry Angle Association, two part-time helpers for removal of mosquito fish for a 12-month period, one full-time and two part-time help for six months mark-recapture and release programme for a range of ponds, one full-time and two part-time help for monitoring of founding and donor population growth for six-month period following mark-recapture programme.

Outputs: Assessment of killifish population following translocation, increasing range of occupancy and optimizing survival of the species, data on killifish requirements for optimal growth and survival

List of Equipment Required: Truck, row boat, minnow traps, tagging equipment, life support system for fish (bucket, SCUBA tank, airstones, etc.

6. Scientific research on reproduction of wild populations

Actions proposed:

- Research into the breeding ecology of Bermuda's killifishes
- Determining the frequency of spawning in Bermuda and the mean number of ova shed per spawning episode would allow for estimates of the reproductive potential of each isolated population.
- Studies focusing on the factors affecting the incubation period of Bermuda's embryonic killifishes would also be beneficial for any future captive breeding efforts.

Work Team: DCS

Team Leader: Ph.D. student

Assistance: One volunteer

Outputs: Ph.D. thesis and valuable information specific to Bermuda

List of Equipment Required: To be determined— dependent on research focus

F. ESTIMATED DATE OF DOWN LISTING

It is anticipated that it will take five years to identify and restore key habitats for Bermuda's killifish species, and four years to complete a translocation programme for all populations. It is only once this goal is attained that down listing (or removal) of all species will be considered, following assessments of population distribution and water/sediment quality monitoring. Re-assessment of all species will be done in 10 years.

PART III: IMPLEMENTATION

Implementation schedule for work plan includes priority number, where:

Priority 1: An action that must be taken to prevent extinction or to prevent the species from declining irreversibly.

Priority 2: An action that must be taken to prevent a significant decline in the species population/habitat quality, or some other significant negative impact short of extinction.

Priority 3: All other action necessary to provide for full recovery of the species.

Priority #	Task #	Task description	Task Duration	Responsible Party
2		**Genetic make-up identification**		
	1	Analyses of collected samples	12 months	DCS
	2	Protection of all species identified	3 months	DCS
2		**Species and Habitat Protection**		
	3	Protection of third *Fundulus* species if needed	12 months	DCS
	4	Designation of selected sites as Critical Habitats	12 months	DCS
1		**Identification and assessment of wetland habitats for reintroduction**		
	5	Survey for suitable expansion habitats	100 man hours	DCS
	6	Sediment/ water quality and tissue analysis programme	12 months	DCS, Health, BZS
2		**Captive Breeding**		
	7	Broodstock conditioning and egg incubation	6 months	DCS
	8	Growth of fingerling	36 months	Fry Angle Association
	9	Translocation of fingerling to ponds	36 months	DCS, Fry Angle Association
1		**Translocation**		
	10	Removal of mosquito fish	12 months	DCS, Health
	11	Initial introduction of fingerlings	6 months	DCS/Fry Angle Association
	12	Mark-recapture programme	6 months	DCS, BZS
	13	Series translocation and monitoring programme	6 months	DCS, BZS
	14	Control of invasive species programme	36 months	DCS
3		**Research**		
	15	Breeding ecology	24 months	DCS
	16	Reproductive potential	48 months	DCS
	17	Captive breeding studies	48 months	DCS

REFERENCES

Able, K.W. & Hata, D. (1984) Reproductive Behaviour in the *Fundulus heteroclitus–F. grandis* complex. *Copeia*, 820–825.

Able, K.W. & Felley, J.D. (1988) Bermuda *Fundulus* (Pisces: Fundulidae) revisited: taxonomy of the endemic forms. *Proceedings,* **140**, 99.

Beebe, W. & Tee-Van, J. (1933) *Field book of the shore fishes of Bermuda and the West Indies*. G.P. Putnam's Sons, New York, 337 pp.

Boetius, I & Boetius, J (1967) Eels, *Anguilla rostrata*, LeSueur, in Bermuda. *Videnskabelige Meddelelser Fra Dansk Naturhistorisk Forening,* **130**, 63–84.

Foster, N.R. (1967) Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae). PhD dissertation, Cornell University, Ithaca, New York.

Fritz, E.S. & Garside, E.T. (1975) Comparison of age composition, growth and fecundity between two populations each of *Fundulus heteroclitus* and *Fundulus diaphanous* (Pisces, Cyprinodontidae). *Canadian Journal of Zoology*, **53**, 361–369.

Grady, J.M., Coykendall, D.K., Collette, B.B., & Quattro, J.M. (2001) Taxonomic diversity, origin, and conservation status of Bermuda killifishes (*Fundulus*) based on mitochondrial cytochrome b phylogenies. *Conservation Genetics,* **2**, 41–52.

Griffith, R.W. (1974) Environmental and salinity tolerance in the genus *Fundulus*. *Copeia*, 319–331.

Hines, A.H., Osgood, K.E. & Miklas, J.J. (1985) Semilunar reproductive cycles in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) in an area without lunar tidal cycles. *Fishery Bulletin*, **83**, 467–472.

Hurdis, J.L. (1897) *Rough notes and memoranda relating to the natural history of the Bermudas*. R.H. Porter, London: 408 pp.

Kneib, R.T. (1976) *Feeding, Growth and movements of killifishes on a North Carolina salt marsh*. Master's thesis, University of North Carolina, Chapel Hill.

Kneib, R.T. & Stiven, A.E. (1978) Growth, reproduction and feeding of *Fundulus heteroclitus* (L.) on a North Carolina salt marsh. *Journal of Experimental Marine Biology & Ecology*, **31**, 121–140.

Lee, D.S., Gilbert, C.R., Hocutt, C.H., Jenkins, R.E., McAllister, D.E. & Stauffer, J.R. (1980) *Atlas of North American Freshwater Fishes*. North Carolina State Museum of Natural History. Publication #1980–12. 867 pp.

Meffe, G.K. (1985) Predation and species replacement in American southwestern fishes: A case study. *Nature* **30,** 173–187.

Minckley, C.O. & Klaassen, H.E. (1969) Burying behavior of the plains killifish *Fundulus kansae*. *Copeia*, 200–201.

Outerbridge, M.E. (2006) *Distribution, population assessments and reproductive seasonality of Bermuda's killifishes*. MSc thesis. University College Cork, Cork, Ireland. 115 pp.

Outerbridge, M.E., Davenport, J., & Glasspool, A.F. (2007a) Distribution, population assessments and conservation of the Bermuda killifishes *Fundulus bermudae* and *Fundulus relictus*. *Endangered Species Research*, **3**, 181–189.

Outerbridge, M.E., Davenport, J., & Glasspool, A.F. (2007b) Reproductive Seasonal Periodicity of the Bermuda killifish *Fundulus bermudae* in an anchialine pond. *Journal of the Marine Biological Association of the United Kingdom*, **87**, 797–800.

Outerbridge, M.E. (2008) Ecological notes on feral populations of *Trachemys scripta elegans* in Bermuda. *Chelonian Conservation and Biology*, **7(2)**, 265–269.

Page, L.M. & Burr, B.M. (1991) *Peterson's Field Guide to Fresh water Fishes*. Houghton Mifflin Company, Boston.

Radtke, R. (1979) The mummichog; a fish for all reasons. *Sea Frontiers,* **25**, 145–149.

Rand, T. (1981) *Comparison of the parasite fauna and diets of two species of Bermuda mangrove fishes; Part II Gut Contents*. Department of Agriculture and Fisheries Monthly Bulletin.

Scribner, K.T. (2006) Genetic considerations in reintroduction. *In Principles of Conservation Biology*, 3rd Edition. (eds. M.J. Groom, G.K. Meffe, & C.R. Carroll) pp. 567–568. Sinauer Associates, Inc., Sunderland, Mass.

Smith-Vaniz, W.F., Collette, B.B., & Luckhurst, B.E. (1999) *Fishes of Bermuda: History, Zoogeography, Annotated Checklist, and Identification Keys*. American Society of Ichthyologists and Herpetologists. Special Publication No. 4. 424 pp.

Sterrer, W.E. & Wingate, D.B., (1981) Wetlands and Marine Environments. In *Bermuda's Delicate Balance*. (eds. S.J. Hayward, V.H. Gomez & W.E. Sterrer), pp. 107–112. Bermuda Biological Station for Research, Inc. Special Publication No. 20. Island Press, Hamilton, Bermuda.

Sterrer, W.E. (1998) How many species are there in Bermuda? *Bulletin of Marine Science*, **62**, 809–840.

Taylor, M.H. (1986) Environmental and endocrine influences on reproduction of *Fundulus heteroclitus*. *American Zoologist*, **26**, 159–171.

Thomas, M.L.H., Eakins, K.E., & Logan, A. (1991) Physical characteristics of the anchialine ponds of Bermuda. *Bulletin Marine Science*, **48**, 125–136.

Thomas, M.L.H., Eakins, K.E., Logan, A. & Mathers, S.M. (1992) Biotic characteristics of the anchialine ponds of Bermuda. *Bulletin Marine Science*, **50**(1), 133–157.

Thomas, M.L.H. & Logan, A. (1992) *A guide to the ecology of shoreline and shallow-water marine communities of Bermuda*. Bermuda Biological Station for Research Special publication. No. 30. Wm. C. Brown Publishers, Iowa. 345 pp.

Thomas, M.L.H. (2002) *Bermuda's Wetlands: A Project Nature Filed Study Guide*. The Bermuda Zoological Society in collaboration with the Bermuda Aquarium, Natural History Museum and Zoo.

Thomas, M.L.H. (2004) *The Natural History of Bermuda*. Bermuda Zoological Society. Brimstone Media Ltd., Bermuda. 255 pp.

Valiela, I., Wright, J.E., Teal, J.M., & Volkmann, S.B. (1977) Growth, production and energy transformations in the salt marsh killifish, *Fundulus heteroclitus*. *Marine Biology*, **40**, 135–144.

Watts, W.A. & Hansen, B.C.S. (1986) Holocene climate and vegetation of Bermuda. *Pollen et spores*, **28**, 355–364.

Wingate, D.B. (1991) Bermuda Audubon Society Newsletter. Vol. 2, No 2.

Wingate, D.B. (1994) Bermuda Audubon Society Newsletter. Vol. 5, No 3.

APPENDIX I

Killifish transfer history in Bermuda's ponds

Table 3. Summary of the killifish transfer history among the ponds of Bermuda

Pond	Fundulus species	Date of introduction (if applicable)	Source population (if applicable)	Number of fish translocated	Status in 2012
Lover's Lake	*F. relictus*	—	—	—	extant
West Walsingham Ponds	*F. bermudae*	—	—	—	extant
East Walsingham Ponds	*F. bermudae*	—	—	—	extant
Trott's Pond	*F. bermudae*	—	—	—	extant
Mangrove Lake	*F. bermudae*	—	—	—	extant
Warwick Pond	*F. bermudae*	—	—	—	extirpated
Evan's Pond	*F. bermudae*	—	—	—	extant
*Nonsuch Island (salt water pond)	*F. bermudae*	1976	Trott's Pond	n/a	extirpated
*Bartram's Pond	*F. relictus*	1986	Lover's Lake	n/a	extant
*Nonsuch Island (fresh water pond)	*F. bermudae*	1993	Mangrove Lake	53[1]	extirpated
*Blue Hole Bird Pond	*F. bermudae*	1995	West Walsingham	50[2]	extant
*WindReach Pond	*F. bermudae*	2008	Warwick Pond	20	extant
*Sea Swept Farm Pond	*F. bermudae*	2008	Evan's Pond	103	unknown
*Cooper's Island Pond	*F. relictus*	2008 & 2009	Lover's Lake	400 & 334	extant
*Blue Hole Bird Pond	*F. bermudae*	2008 & 2009	West Walsingham	223 & 197	extant
*Bartram's Pond	*F. relictus*	2008 & 2009	Lover's Lake	365 & 401	extant
*Paget Marsh (David's Pond)	*F. bermudae*	2009	WindReach Pond	118	extirpated
*Shelly Bay Pond	*F. bermudae*	2009 & 2010	West Walsingham	242 & 477	extant
*Port Royal golf course (17th hole pond)	*F. bermudae*	2010	WindReach Pond	200	extirpated
*Seymour's Pond	*F. bermudae*	2011	WindReach Pond	400	extant
*Riddell's Bay golf course (15th hole pond)	*F. bermudae*	2012	Evan's Pond	49	extant
*BAMZ Madagascar Pond	*F. bermudae*	2012	WindReach Pond	<200[3]	extant

*Indicates man made pond.

[1] Jon Cotter (1993) "Acclimation of brackish killifish (*F. bermudae*) to fresh water for introduction into Nonsuch Island, Bermuda". Unpublished paper in the Bermuda Aquarium, Museum and Zoo library.

[2] J. Madeiros personal communication.

[3] R. Mariera personal communication

NOTE: Until the taxonomic diversity of Bermuda's killifishes has been fully resolved, the pond:species attributes in the above table should be regarded as tentative rather than definitive.

APPENDIX II

Breeding Reports

I. Breeding *Fundulus heteroclitus*

H. Fairfield
reprinted from *American Currents*, Dec. 1985

Prompting *Fundulus heteroclitus* (the mummichog) to breed is an easy matter, as this fish is very tolerant of its tank conditions. Normal fish keeping maintenance and care in a permanently set-up aquarium should give good breeding results. The following is an account of the conditions I provided to give the mummichog a chance to procreate.

The trio I purchased were placed in a standard ten-gallon aquarium provided with an undergravel filter; approximately two inches of coated, naturally colored gravel; several large, smooth river rocks; and several strands of hornwort. The set-up did not contain a light or aquarium heater. I did supply a full cover, because this fish, like other killifish, loves to jump. Rock salt was added to the aquarium water at the rate of 0.5 teaspoon per gallon as a normal maintenance additive. My particular water had a pH of from 8.0 to 8.5, and the hardness was 11 DH (183.3 ppm $CaCO_2$). I mention this only in passing, because the mummichog is quite adaptable, and in nature adjusts to many variable conditions. Every week 20 percent of the aquarium water was siphoned from the bottom and replaced with tap water treated with ten drops per gallon of Novaqua and 0.5 teaspoon per gallon of rock salt. I tried to provide a varied diet of live brine shrimp, Tetramin Staple food, crushed snails, and beef. You will discover that these fish will not turn down any food. In the early spring, as the breeding time approached, I started feeding chopped earthworms, and added a floating spawning mop (made from a bottle cork and acrylic yarn) to the aquarium. This mop provided a spawning medium and was long enough to reach the bottom of the aquarium.

The normal color of my *F. heteroclitus* is steel gray or brownish on the back and sides. This color gradually fades to white or yellowish on the stomach and breast. Most of the body is covered with a faint netlike pattern produced by the darkened edge of each scale. As the water temperature reaches from 68–70^{o}, and as the male and female mummichog achieve prime conditions, body changes take place—most noticeably in the tail. Small white or pale-blue spots, arranged in a vertical pattern on the males' sides, seem to glow on the steel-gray background of their bodies. The normally discrete spots in the caudal, dorsal, and anal fins become bright. A large, dark spot becomes very noticeable in the posterior parts of the dorsal and anal fins. In some specimens—though not, unfortunately, in mine—vertical blue bars in the posterior part of the body appear, and the fins take on gaudy yellow or yellow-orange margins. The color of the female changes little from the normal steel gray described earlier, but her body becomes fuller as it fills with eggs. I've noticed that the leading ray on the anal fin is long and quite opaque.

After a short courtship, the male drives the female to the spawning mop. If the male becomes too aggressive, she avoids the encounter, sending him into a frenzy. This is the reason two females are present in the breeding aquarium. Although the male can never be considered gentle in his breeding behavior, he soon calms down. With fin-stroking and bumpings, the pair align at the upper part of the spawning map, where quivering bodies produce an egg. The spawning mop should be removed, wrung out, and examined for eggs every other day. Mummichogs are avid spawn-eaters. The eggs are about 0.078" in diameter, clear and slightly adhesive. They can be easily removed from the spawning mop with your fingers.

I placed the eggs in a plastic margarine dish filled with water from the spawning aquarium and added enough acriflavine to color the water yellow. The acriflavine is a fungicide which protects the eggs for the first two days. I placed the covered margarine dish on top of the breeding aquarium to incubate the eggs. On the second day, the eggs were removed from the breeding aquarium (a length of airline used as a siphon is useful for moving eggs and fry). Replacement of the dish on the breeding aquarium and recleaning every two or three days is the norm. Incubation time varies with the water temperature. I found that most of the eggs hatch in two weeks (336-hr. average) at 67 °F. Warmer temperature hastens hatching, but too much heat seems to have a detrimental effect on the number of live hatches. The fry are free-swimming within 24 hours and can be fed live baby brine shrimp or microworms immediately. They can also be fed hard-boiled egg yolk as a substitute. For the first couple of weeks, I maintain the fry in the margarine dishes and replace their water every two days with fresh water from the breeding aquarium. Finely powdered Tetramin Staple food can be alternated with the baby shrimp.

After the two weeks of "intensive care", the fry can be placed in larger quarters with aeration, and later in a regular aquarium for final rearing. The rearing aquarium should have some type of filtration system and should be cleaned frequently. The fry grow quickly. Allowances should be made not to overcrowd to obtain optimal growth and health.

Before closing, I would like to relate some of the observations I have made while maintaining this species. I collected an average of six eggs every three days from the upper part of the floating spawning mop. The best spawning season seems to occur naturally in the early spring. I collected about 100 eggs from mid-February to early April. Eggs were also collected through the summer, but not in the quantities collected in spring.

I've enjoyed keeping and observing this fish because of its ease of care, willingness to breed, and independent nature.